Blackwater Fever in Waziristan 1938

by

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TABLE OF CONTENTS.

VOL. I

Vol II

PLATES.

Plates continued.

FIGURES.

Figures continued.

I N T R O D U C T I O N

In a military cemetery in Rawalpindi, in the Punjab Province of India, there is a stone to the memory of a British Sergeant killed in 1880 whilst fighting the "Wild Wazirs". We have been fighting them ever since. In fact, by 1935 there had been so many official campaigns that the Indian General Service Medal, referred to as "Evergreen Eve", had to be re-designed as it had been given so often.

In 1937 matters in Waziristan again came to boiling point when the Waziris made a savage and determined attack on a column of our troops on the march in the Khaisora Plain. From then on, until and after September, 1939, the fighting was dignified by the title of "Campaign". At that time the chief trouble maker was the Faqir of Ipi, Ipi being a small village in Waziristan. Ipi gathered around him renegades and malcontents; in short, any able-bodied Wazir who had some time and ammunition to spare. Time and again these bands became big enough to be a threat to security, for example by attacking isolated posts, sometimes with the aid of crude village-made cannon, firing solid shot. When such bands grew too large to be handled in comfort by the Waziristan Militia, Military forces had to be employed. The

method of using such forces followed a standard and effective pattern, namely an excursion in strength against the enemy, using pack animals and going across country to whatever area happened to be the headquarters of the band at the time. This taking of war into the enemy territory generally proved sufficient to break up a concentration of "badmashes" for a time.

In early July, 1938, Ipi had again become powerful in men, money, and to some extent in material. He had established his headquarters in a remote part of Waziristan, at Kharre near Wuzgai on the borders of Afghanistan. He represented a serious threat to security: accordingly a larger military force than usual was detailed to break up the concentration. The method was that a column of troops (hereafter referred to as "Razcol") numbering about 4,000 strong, moved out from the main fortress of Razmak; simultaneously a column about 3,000 strong moved out from the more Northerly camp of Damdil. (This column will be referred to hereafter as "Damcol"). These two columns met at Gardai Camp and marched in concert against Ipi. This move was noteworthy for two things. Firstly, it was through country only rarely traversed by military forces; the country itself was very difficult to fight in, although not more so than certain other parts of Waziristan: and secondly, it had been decided to

try out atebrin on this column as a method of mass prophylaxis against malaria, one company of one infantry battalion acting as a control by taking quinine instead of atebrin.

The move, which was against opposition throughout, and which will be described in detail later in the book, proved to be very trying, even for the highly experienced and thoroughly fit British and Indian soldiers of the military column. The column attained its objective however, routing Ipi's "lashkar" in a bitter little engagement. Sporadic resistance was encountered during the whole of the return march also.

It is necessary here to enlarge more fully on the conditions of such columns on the move in Waziristan. The roads are very few in number, and practically every move against trouble-makers is across country of a type perfectly designed for defensive purposes; there is on record in my experience a case when two fanatical tribesmen, tactically sited, held up 4,000 men for over two hours. Tanks and aeroplanes are surprisingly ineffective in a support role.

Each body of troops must travel self-contained, horse, foot, and guns. The only possible method of transportation is by pack animals, horses, mules and camels, which carry everything - food, water, ammunition, guns and wounded. Razmak

Column on the move generally had about 3,000 animals in the pack train, which took about 3½ hours to pass a given point. Obviously the kit that could be carried was minimal. In the case of officers it was limited to 5 lbs., which was carried on the officer's own charger. There could be no question of taking mosquito nets or tents. The routes generally lay along valleys, 3,000 to 5,000 feet high, along which the main body of the column would move - usually along a dry river bed. The only possible method of movement was by each battalion leap-frogging through the one ahead to seize and to hold the high points on each flank, and so permit the movement through of the main body. As the head of the column moved forward the tail folded in on itself by the flanking battalions falling back into the valleys from the high hills, ready to take up the same role again when required. It follows, therefore, that the infantry responsible for the safe movement of the troops would cover much more ground than the main body of the force, and in the course of the day would be called upon several times to clear opposition from the heights or the flanks. The mountain gunners also had a very trying time; their claim was that they marched at an average pace of five miles per hour. Movement was slow, laborious, and a severe physical strain, particularly as the temperature rarely was below 90 degrees in the shade, and water was

strictly rationed.

At night the column always formed a square, generally on the site of an older camp with low stone walls still standing. Space inside such camping sites was always riddled with what would now be called "slit trenches", and at night the whole force would require to go to ground in these as some method of protection against sniping, which usually continued throughout the night. The country traversed was always malarious, and no anti-malaria precautions were possible.

During the column under discussion, that in July, 1938, against Ipi's Headquarters at Kharre, the march, which was against opposition throughout, was made under unusually trying conditions. As has already been said, the opposition continued on the return journey. In fact, a final sortie by the enemy was actually made upon my battalion within one mile of Razmak Fortress as the troops closed up to go through the gates.

On the 21st July, 1938, three days after the column had returned to Razmak and Damdil, a soldier was admitted with malaria; he later developed haemoglobinuria. This was the first of six such cases, all of whom fell ill with malaria and haemoglobinuria within a period of ten weeks following the Kharre Column. It should be noted here that these patients

developed this condition whilst on a new regime for the treatment of malaria. The standard treatment at that time had been to give quinine sufficient to control the temperature, to continue with atebrin 0.3 grammes daily for seven days, to follow with three days rest, and then to give plasmoquine 0.02 grammes daily for five days. The latter dosage applies to Indian troops. The hospital accommodation available was not sufficient to handle all patients requiring treatment for malaria at that time, and accordingly it had been decided to modify the course as follows: namely, to give quinine as before, to give atebrin in the same dosage but only for five days, then to follow with only one day's rest prior to giving the plasmoquine course, which remained unchanged in dosage and in duration of administration. Furthermore, on the completion of the atebrin course, providing the patients were fit enough, each individual was transferred to convalesce in a barracks closely adjoining the hospital, set aside temporarily for that purpose. It functioned, therefore, as a convalescent camp. Such patients remained under medical care during that time and reported twice daily in the hospital, where they were given their plasmoquine. These points are all discussed in detail in the body of the book.

It is seen, therefore, that the onset of the disease in the six patients who developed haemoglobinuria while being

treated for malaria was related, chronologically at least, to a very trying march through country very rarely traversed; to the use of atebrin for mass prophylaxis during and immediately after the Kharre Column; to the introduction of the modified method of treating malaria; and to the discharge of patients following the atebrin course, the plasmoquine course being given to them as out-patients.

The above is a brief resume of a disease never seen before or since in Razmak, or for that matter in Waziristan itself. (We exclude one doubtful case previously reported and one very doubtful case previously reported. These will be discussed in detail later.)

Close consideration of these cases led us to the conclusion that the men were suffering from haemoglobinuric malaria, i.e. blackwater fever.

Now it has been truly said of blackwater fever that in certain parts of the World at least it is no clinical rarity but is one of the serious medical problems of the tropics, a disease feared by all European residents in areas where blackwater fever is endemic. A disease which has a mortality of twenty per cent is a disease to be feared.

Unfortunately, as Sir Rickard Christopher has said

..... "those whose work is directed to the more scientific investigations of tropical diseases are hampered by multitudinous but inconclusive literature ..... the putting forward of theories as to the causation of blackwater fever is a function inversely proportionate to the knowledge of the facts which are known about the disease ....."

Similarly, the late Warrington Yorke, in a review of the literature on blackwater fever, stated "..... Although during recent years a considerable number of papers dealing with blackwater fever have been published, very few of them are of scientific value and our state of ignorance regarding this important disease is almost as great as it was ten to fifteen years ago ....

..... Broadly speaking, recent communications on blackwater fever can be grouped into the four following classes, viz:-

(1) <u>Papers of an essentially clinical nature.</u>

Such papers appear unnecessary. They are, in the majority of instances, the efforts of those who have but recently visited the tropics - usually as a result of the war - and whose interest has been aroused by this most impressive disease. Such authors are simply repeating what has been written over and over again during the past twenty years.

(2) <u>Papers mainly concerned with the endemicity of the disease.</u>

These papers almost invariably fail to be of value

because the information they contain is deficient in one or more essentials.

(3) Papers dealing mainly with the treatment of the disease.

Such papers are, in the main, without any value and the claims made are quite unjustifiable.

(4) Papers dealing with the pathology of the disease.

A relatively small proportion of the total papers."

The author then deals with the last group of papers in detail.

And again, the late Professor J.W.W. Stephens considered that " ..... a complete and accurate epitome on blackwater fever cannot be written until a conflicting mass of statements in the literature is harmonised by more precise observations, by the use of modern methods of investigation, and by a much more rigorous use of the critical faculty than has hitherto obtained ....."

These are the opinions of men who had studied blackwater fever for many years. It behoves me, therefore, to justify this addition to the multitudinous literature on blackwater fever. My reasons are two-fold.

Firstly, I have attempted to harmonise the conflicting mass of statements in the literature, especially in view

of the recent work on the question of an alternative circulation through the kidney. Secondly, there is put on record what may be termed an "outbreak" of blackwater fever in Waziristan - a country previously thought to be free from this disease.

The plan followed in this work is as follows:-

We are considering the problem of six individuals who developed haemoglobinuria while undergoing treatment for malaria; as the title implies, I regard the disease in each case as blackwater fever. This diagnosis may not be acceptable to others, hence the somewhat full discussion on the problem. Chapters I and II give details of the circumstances surrounding the onset of infection and give detailed clinical notes. Chapter III deals with other causes of haemoglobinuria which require to be considered in the differential diagnosis of our patients. Chapters IV and V discuss in detail the laboratory findings and their significance in haemoglobinuria in general, and in intra-vascular haemoglobinurias in particular. These paragraphs stress the fact that the laboratory findings in blackwater fever do not differ in any essential respect from those in other haemolyses. I say this because for too long blackwater fever has been regarded as something "rich and strange" and it is only now

being brought into line with other haemoglobinurias. The three succeeding Chapters, VI, VII and VIII, deal, respectively with the possible parts played by quinine, by atebrin, and by plasmoquine in the production of haemoglobinuria in general, and in the production of haemoglobinuria in the cases under consideration.

As already stated, in my opinion the diagnosis in each of the patients was blackwater fever. In Chapters IX and X this diagnosis and the disease itself are discussed in detail. The subject matter is brought to a close in Chapter XI where a summary is given.

An appendix is added, giving clinical and other details more fully than in Chapter II. To lessen the heavy clerical work references are given grouped at the end of the book and not after each chapter. Certain of these have been read only as abstracts and as quoted by other workers. These, however, are given in full to facilitate reference purposes. This is not normal procedure but has been done deliberately, to give as full a list of references as possible, for the convenience of anyone interested.

Lastly, I should like to place on record my grateful thanks to some of the many people to whom I am indebted. Among them I particularly include Sir Rickard Christophers,

Sir H.H.Scott, Mister P.J.Shute, Doctor J.Trueta, the Librarians of the British Medical Association, the Royal Society of Medicine and St. Thomas' Hospital. I would also place on record my thanks for the help I have obtained from writings of such workers as N.H. Fairley, J.W. Field, H.Foy, B.J. Maegraith, G.R. Ross, J.A. Sinton, the late Professor J.W.W. Stephens, and the late Professor Warrington Yorke, whose papers are all of much value and much help to any student of the problem of blackwater fever.

C H A P T E R I

GENERAL INFORMATION.

TOPOGRAPHY OF WAZIRISTAN, WITH A DESCRIPTION OF RAZMAK FORTRESS

Probably the best short description of Waziristan is that found in the Encyclopedia Britannica (1929). I have been given permission by the authorities concerned to quote their work fully.

"Waziristan is a mountain tract in the North-West Frontier Province of India within the British sphere of influence, the boundaries with Afghanistan having been demarcated in 1894. Only a portion, consisting of the Tochi Valley, with an area of 700 square miles and a population in 1903 of 25,000, is directly administered. Northern Waziristan has an area of about 2,300 square miles, and South Waziristan has an area of about 2,700 square miles.

The Tochi and the Gomal Rivers enclose the central dominating range from North-East to South-West, geologically connected with the great limestone ranges of the Suleiman Hills to the South and dominated by the great peaks of Shuidar (Sheikh Haidar) and Pirghal, both of them between 11,000 and 12,000 feet above the sea. From these peaks

Westwards a view is obtained across the great slopes and cedar woods of Birmal and Shawal (lying thousands of feet below) to the long serrated ranges of the central watershed which shuts off the plains of Ghazni. To the East several lines of drainage strike away for the Indus and are, as usual, the main avenues of approach to the interior of the country. They are the Khaisora and the Shakdu on the North, which, uniting, join the Tochi South of Bannu; and the Tank Zam on the South. The two former lead from the frontiers of Pakistan to Razmak and to Makin, which is near Razmak and which is of some local importance, situated on the slopes of Shuidar; the latter valley leads to Kaniguram, the Waziri capital and the centre of a considerable iron trade. Kaniguram lies at the foot of the Pirghal mountain.

The Waziri tribes are the largest on the Frontier, but their standard of civilisation is very low. They are a race of robbers and murderers, and the Waziri name is execrated even by the neighbouring Mahomeddan tribes, who seem inclined to deny to the Waziris the title of belonging to the Faith. Their physique is said to be excellent.

Except on a few of the highest hills, which are well wooded, the Waziri country is a mass of rocks and stones, bearing a poor growth of grass and thinly sprinkled with dark evergreen bushes. Progress in every direction is obstructed

by precipices or by toilsome stony ascents, and knowledge of the topography comes only as a result of long acquaintance. The broken ground and tortuous ravines, by making crime easy and protection against attack difficult, have fostered violence amongst the people and developed in them an extraordinary faculty of prudence and alertness. The Waziri has developed into a raider and a highwayman. The blood feud is a national institution. (Here I may quote my translation of an old Pushtu saying:- "If you wait a hundred years then take your revenge your friends will say, 'What was the hurry?'")

Plate No. I, page 4, showing a picture of Razmak Fortress from a small supporting post on a North-Eastern hill, gives some indication of the nature of the country; the Waziris, who number 48,000 fighting men altogether, are divided into two main sections - the Darwesh Khel, about 30,000 (referred to as Wazirs) and the Mahsuds, 18,000. There are smaller sections and attached tribes who number 18,000 more.

The Darwesh Khel are the more settled and civilised of the two and inhabit the lower hills bordering upon Kohat and the ground lying on both sides of the Kurram River between Thal on the North and the Tochi Valley on the South.

The Mahsuds, who inhabit the tract of country lying

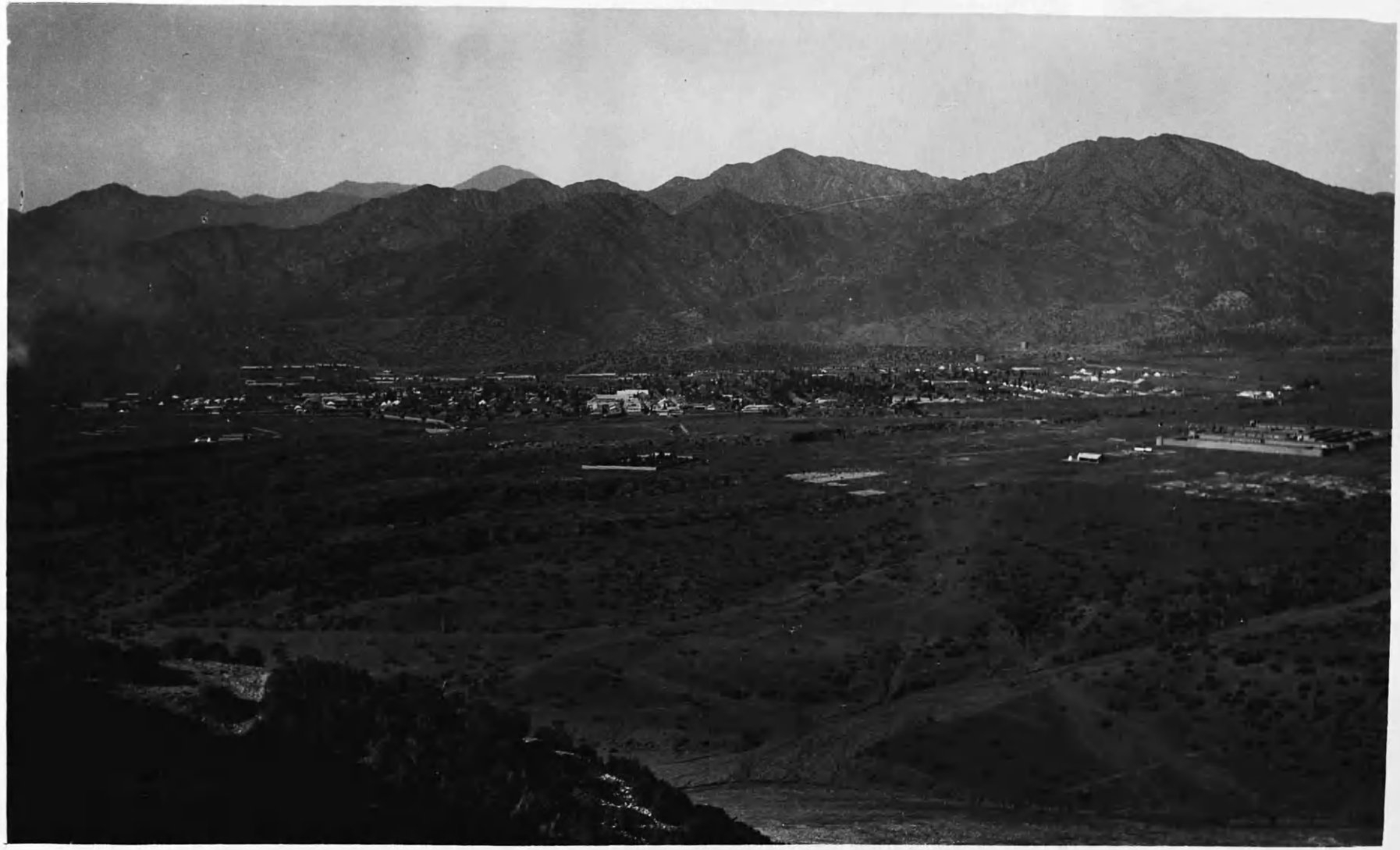


PLATE No. 1 RAZMAK FORTRESS from the NORTH-EAST

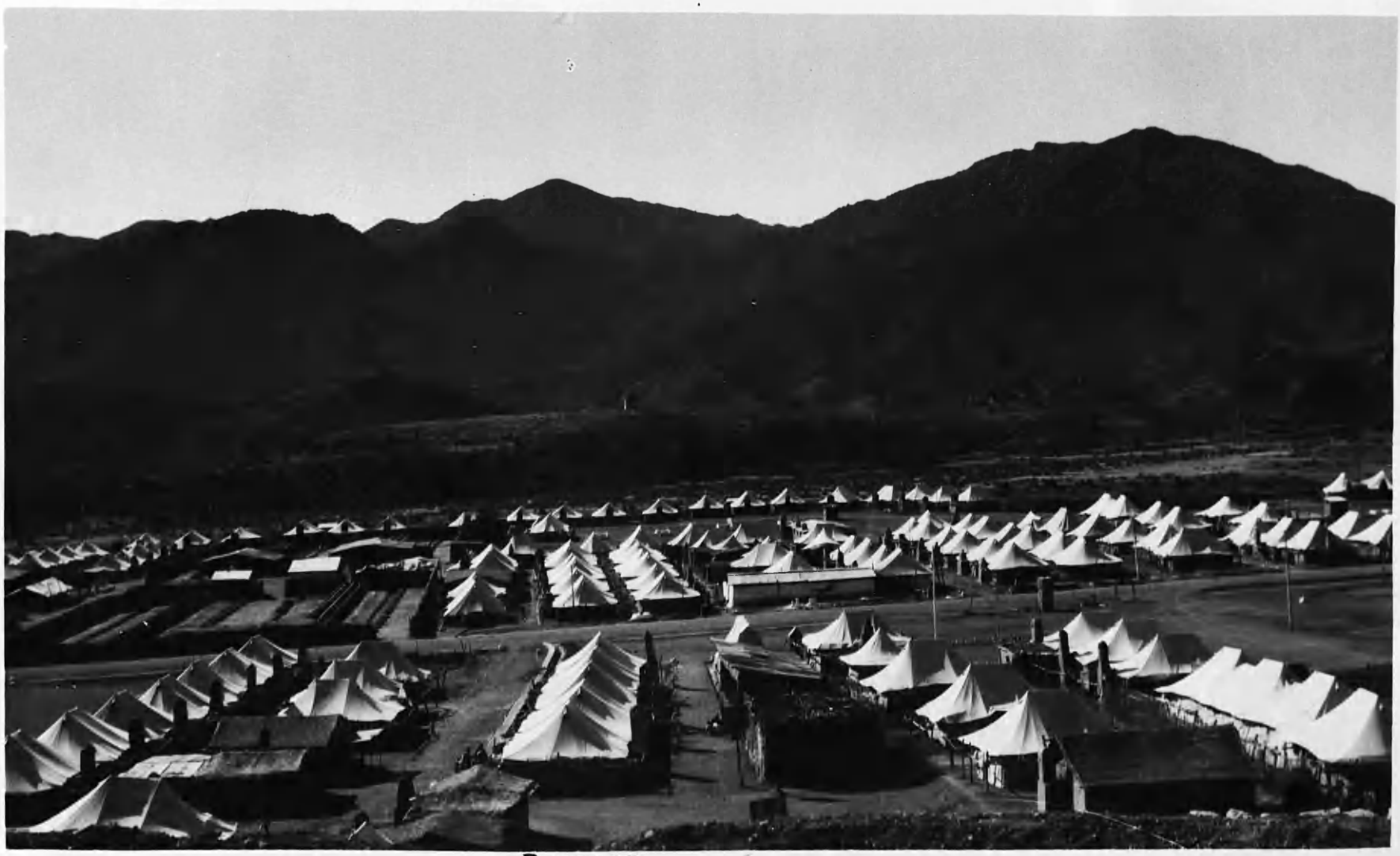


PLATE II

RAZMAK LOWER CAMP viewed from the UPPER CAMP

between the Tochi Valley on the North and the Gomal River on the South have earned for themselves an evil name as the most confirmed raiders on the borders. The Mahsud country, especially that part within reach of British posts, is more difficult even than Tirah."

Such is the opinion of the Encyclopedia Britannica. The following few notes are largely my own.

The position originally was that Waziristan was a "no man's land" where the Waziris could gather together uncontrolled and form large bands in the hills, then descend to raid the Plains. British expeditions were needed against various factions of the Waziris in 1852, 1859, 1860, 1880, 1881, 1894, 1897, and 1902. Finally it was decided to "Sandemanise" the country, but the Pathan is much more democratic and much less subject to the influence of his maliks than is the Baluchi to the authority of his chiefs. Lord Curzon finally reversed the policy and retired to the Indus, leaving two corps of tribal militia to replace the British troops. During the first World war the Mahsuds gave constant trouble, however, and during the third Afghanistan war sections of the militia went over to the enemy. A reversion of the previous policy, that is "The Forward Policy" was, therefore, decided upon and was first put into operation in the early 1920's. A small military cantonment, Bannu in the North of

Waziristan, was made the military base and rail-head, and the last link with civilisation. This cantonment was linked to the outside World by a good road through to Kohat in the North-West Frontier Province, and by a two-foot-gauge railway running ninety miles East across desolate country to link with the standard gauge lines at Mari Indus on the Indus River, "the Father of all the Rivers". The train which runs on this line normally covered the ninety miles in eight hours or so, except when it was attacked. The journey in it in hot weather was very trying, and the train well deserved its title of the "heat stroke express". A branch of this line runs due South from Laki Marwat junction - the junction being one little building which is periodically burned down by the Waziris - to Mansai, a small and unimportant secondary rail-head.

In the North-West Frontier Province the maintenance of law and order among the several Pathan tribes is the responsibility of the Constabulary of the Province. Their powers extend to and include Bannu but immediately outside the limits of Bannu is a barbed wire fence and a large notice marking the beginning of what was officially known as "tribal territory". In other words, Bannu on the Plains stood in relation to the hills of Waziristan, with their passes leading into Afghanistan, in exactly the same position as stands

Peshawar to the much better-known Khyber Pass.

In the periods between the official campaigns the individual responsible for the conduct of affairs in Waziristan was the Resident, an Officer of the Indian Civil Service, responsible to the Governor of the North-West Frontier Province. The Resident had his own staff of political officers and worked independently of, though in close co-operation with, the military authorities. To enable his orders to be enforced and for the carrying out of routine security duties the Resident had under his charge two corps of tribal militia, the Tochi Scouts and the South Waziristan Scouts. These two forces were military in training and were officered by Officers from the Indian Army. Each of the Scout forces operated from a large Headquarters, with smaller detachments at outlying forts. No praise can be too great for these men and their officers. They were continuously in danger. For example, in 1939 Colonel Campbell, Resident at that time, was ambushed and wounded in four places on the way to hand over his command. The Scouts were highly mobile and could live on very little. They knew the country intimately and they aimed to check trouble at the source, to keep the Resident fully in touch with the internal state of affairs, and to prevent the Waziris carrying out such acts as the blowing up of bridges carrying the few roads. Their

title of "Scouts" fitted their role. Their weapons were light, they had no artillery, and their hitting power was limited. But behind them stood the Army, slow and cumbersome to move but carrying tremendous hitting power. The principle of the "Forward Policy" was to establish military strong points at strategic sites throughout Waziristan, and so enable force readily to be brought to bear on any group of malcontents threatening the security of the peoples of the Plains. Even then, sometimes, instances would occur such as that at Bannu in 1939, when a gang got through the wire one night and burned the Bazaar, killing many Hindu shopkeepers.

Our role in Waziristan, therefore, was to protect the people of the more settled areas neighbouring the country. Even with a force ten times that which we had there could have been no possibility of imposing upon the Waziris the obedience to authority found in any civilised country. Among the Waziris themselves the law of Waziristan was still the law of the jungle. Each village was a fortified post, with armed men always on duty in the watch towers at each corner of the village walls. It is little wonder then that these people, near animal in many ways, scratching a bare sustenance from the poor soil, looked with hostility upon us, who barred their way to what they regarded as their legitimate prey, the rich

Hindu banias of the Bazaars, where "the valley sheep are fatter".

The main Army concentration of force was at Razmak Fortress, occupied first in 1922 by a force under the command of Sir John Maffey. Razmak is high in the hills, seventy two miles South West from Bannu and connected to it by a mountain road which could very easily be cut at any time by the blowing of bridges and by snowfalls. Razmak is discussed more fully later in this Chapter. Another smaller force was permanently stationed at Wana to the South of Razmak. The strength of the Wana garrison usually numbered about a brigade, with ancillary troops. Still smaller concentrations of military force were also to be found in camps such as Mirali, Damdil and Razani. Such camps were usually placed on intermediate points along the military roads, since places like Razmak could only be kept supplied with necessary stores by large road convoys, usually about seventy vehicles in each convoy moving up once weekly or fortnightly. During such moves the whole seventy two miles of road to Razmak from the base at Bannu had to be guarded by military forces. Except in times of serious trouble the total military force available in Waziristan was usually not more than one Division in strength, as against a potential Waziri force of close on 30,000, all of them experienced fighters, since in Waziristan,

among themselves, it was a case of "the quick and the dead". All of them were fighting in their own country - country perfectly suited to guerilla warfare; all had a wide knowledge of the country and all were capable of marching incredible distances; a Waziri in a hurry thinks nothing of marching forty miles or more in twenty four hours and can keep up this pace for days on end. Furthermore they were clever enough always to avoid, when possible, any trial of strength with the Army which, therefore, rarely got an opportunity to make the most of its far heavier equipment and strength. The odds were always heavily in favour of the tribesmen, but one great factor which operated in our favour was the character of the pathan, especially the Waziri. Among his own people he is constitutionally incapable of submitting to any form of discipline or command, although he makes an excellent soldier when properly trained in the Army. He is intensely proud and jealous and consequently Ipi was rather like Prince Charles Stuart, trying to make an efficient force out of groups of Highland clans, all bitterly distrustful of each other. Anything that operated in favour of Ipi always brought him large bands of recruits. For example, once when an aircraft flew over a group one day and circled, apparently to bomb, Ipi told his men to stand fast and he would turn the bombs into paper. He knew of our purpose always to give

warning before bombing by scattering leaflets, and he gambled on this. That instance alone brought him many supporters. Fortunately, however, these people could not be relied upon for more than a very few weeks. Ipi was in the position of any other dictator. Unless he could produce loot, and of course unless he could keep clear of any engagement with our military forces, his supporters soon quarrelled among themselves and dribbled back home. This factor was potent in preventing the Fakir of Ipi and other such trouble-makers ever from utilising to any important extent the potential force available to them in Waziristan. The force which might have been used against us was frittered away by the Waziris in feuds, with tribe against tribe, khel against khel, district against district, village against village, and man against man. These blood feuds are an important part of the code of the Pathan, and failure of any individual to carry out his part in a blood feud would lead to his immediate expulsion from the village. Feuds would go on for many years and were sometimes prosecuted with appalling ferocity, leading to the wiping out of every living thing in a family; men, women, children, and even the animals. In the lesser feuds killing is limited to males, including male children over the age of twelve, the women and the younger children being spared. The physique of the Pathan is generally described as excellent. This is not correct. The land is barren, and even with

financial help given by the occupying authorites the people live in a chronic state of malnutrition. Among the common disorders may be listed malaria, veneraal disease, tuberculosis, macrocytic anaemia, and helminthiasis. As I was one of the medical officers qualified to speak Pushtu I saw much of the tribesmen and do not consider that their physical condition was really good. There are no health services, although a few Pathan doctors and a few missionary physicians are to be found in Bannu, Kaniguram and in one or two other places. By far the greater number of tribesmen have no medical help available except what is given by the Missions and by a few military medical officers, who were attached to the Political Department. The tribes still employ largely the methods of treatment of their fathers, a subject which deserves a volume in itself; everyone, without exception, wears amulets strapped to their arms, each amulet containing an incantation "specific" against some disease. It is very common to find a tribesman wearing a whole collection of such amulets to protect himself against the "evil eye", against malaria, and against many of the commoner ailments.

This chronic state of poverty and ignorance explains much of the character of the tribesman and of his hatred of the troops who barred his way to the easy loot and to the rich lands of the Plains-people. An example of the Waziris at large is now shown by the incidents in Kashmir.

News has now come to this country that the Pakistan Government, lacking both men and money, has reverted to the policy of holding the line of the Indus, and in an operation well called "OPERATION CURZON" they have succeeded in extricating their troops from Razmak and other camps.

Life inside these camps, when we held them, was very monotonous. Necessarily the troops could not move outside the confines of the camp except when on road protection and other military duties. Going out on any military task was in fact generally regarded as a welcome break in the monotony. Between these military tasks the camp was in fact a prison. In times of trouble snipers were always in the areas around such camps and any injudicious move out of cover would certainly draw fire. For example, shots were fired into Razmak camp almost daily for one period of three months in 1938.

In 1936 a road building scheme was put into operation to complete strategic roads which would link all the important areas. This was difficult and costly, two Divisions of troops being needed to protect the workmen. Fortunately, by 1937 this work had been completed just at the time the tribesmen attacked out troops in the Khaisora Valley. It was from then on the situation deteriorated and increasing use had to be made of the military forces. This trouble

continued throughout 1938 and 1939, the main trouble being in the country lying between Razmak and the borders of Afghanistan.

R A Z M A K.

A more detailed description of Razmak may be of importance. The plates No. I, page 4 and No. II, page 5, show scenes of the country in which Razmak Fortress lay, plate II, page 5, being a view of the interior of Razmak. A large scale plan of the camp is also given (plate III, page 17).

Razmak is a camp less than one mile square. It lies on the Razmak semi-plain which measures roughly eight miles by three miles beginning at Razmak Narai and finishing at Tauda China. The valley slopes from North-East to South-West, the North-East end being 7,700 feet high and the South-West end about 5,800 feet. Excepting at the North-East end, and excepting a narrow gap at the Southern end, the plateau is surrounded by mountains rising to a height of eight to ten thousand feet. Razmak Camp itself is sited towards the South-East end of the valley at a distance of about one mile from the hills at the eastern wall. It is sited on a tongue of land between two large algids (nullahs), namely the Tauda China algid on the East, running under the Eastern mountain wall, and the Kabutar algid on the West. These two algids converge about half a mile below the camp at the South-West and continue as the Tauda China algid. At certain times of

PLATE III

PLAN OF RAZMAK CAMP

RAZMAK CAMP

SCALE 16 INCHES TO 1 MILE

FEET 500 200 100 0 500 1000 1500 2000 2500.

YARDS 100 50 0 100 200 300 400 500 600 700 800 900.

N

LINE OF PHOTOGRAPH.

KHASADAR'S SARAI

BANNU 12 MILES

WAZIR SARAI

DISPENSARY

NUR MOHD SARAI

AIR LANDING GROUND

RIFLEMANS TOWER

BRITISH BATHING TANK

RIDING SCHOOL

RESERVOIR

COOLIES CAMP

GATE NO·10·

SOMME LINES.

NORTH JAKDIL

GATE NO·1·

GATE NO·9·

MESSINES LINES.

NEW CEMETERY

GATE NO·2·

BDE. H·Q·

LASCAR LINES.

PRIVATE BUILDINGS

RESERVOIR

BORRETT'S TOWER

TIGRIS LINES.

BORRETT'S PARK

M·E·S DUMP

GATE NO·8·

BAZAR

BAZAR

NARAKAI TOWER

G·O·C's BUNGALOW

C·I·M HOSPITAL

CAMBRAI LINES.

YPRES LINES.

ARRAS LINES.

MULE STANDINGS

GATE NO·3·

AMARA LINES.

SUVLA LINES.

MEGIDDO LINES.

MILITARY GRASS FARM

GATE NO·4·

MILITARY DAIRY FARM

NULLAH

GATE NO·7·

SHAIBA LINES.

MILITARY VETERINARY HOSPITAL

SLAUGHTER HOUSE

GATE NO·5·

DESTRUCTOR

SHORA PICQUET

PISTOL RANGE

SIRDAR

HERDON LINES.

DHOBI GHAT.

GATE NO·6·

WOODED RIDGE TOWER

RIFLE RANGE

DERA ISMAIL KHAN 116 MILES.

the year they carry a heavy volume of water due to heavy rain and to melting snow.

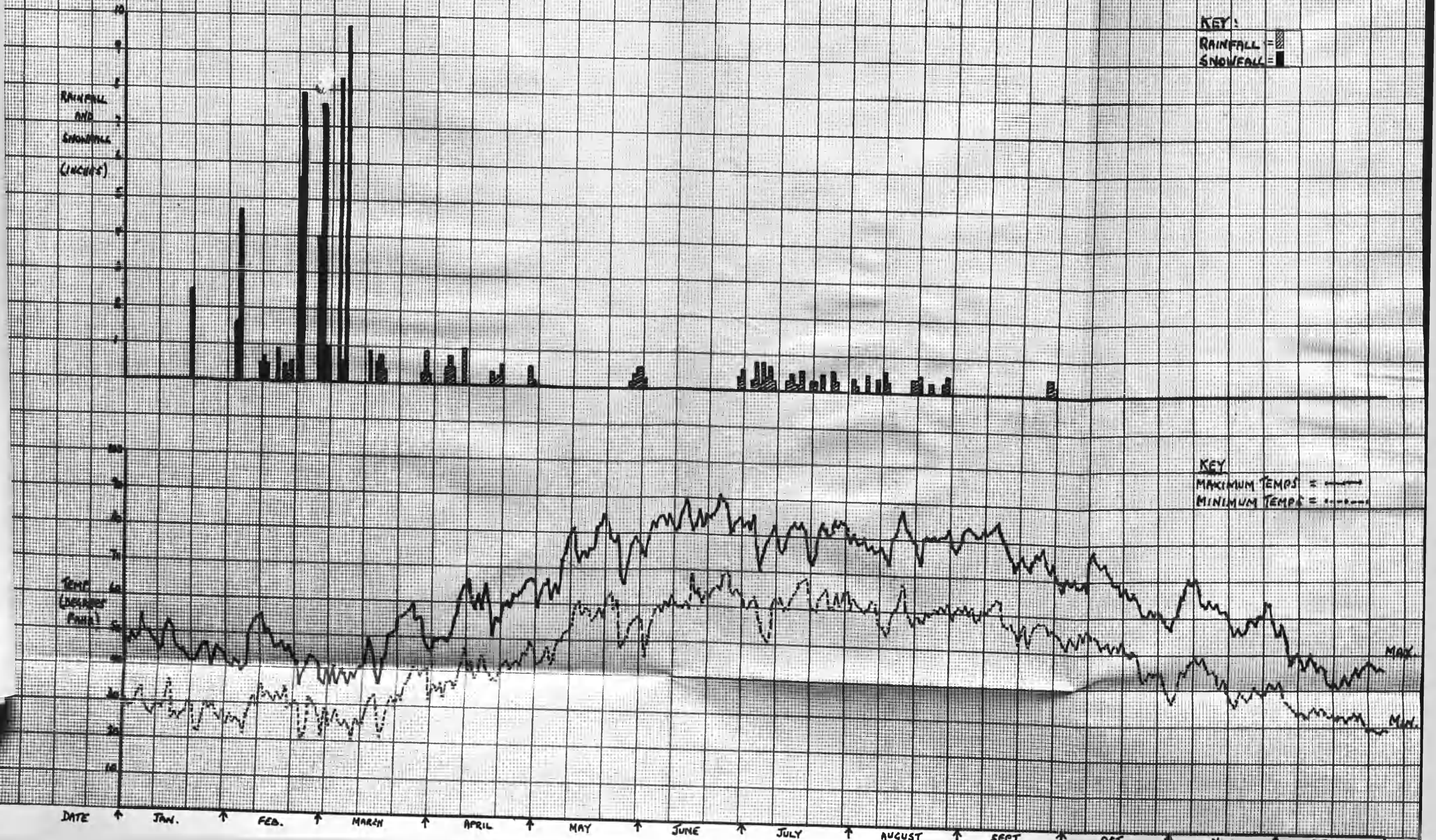
The hills surrounding Razmak have a covering of small shrubs. These range in type from conifers on the higher hills to the scrub of holly oak on the lower hills and in the plains immediately around Razmak. This scrub is thick in places and often has to be cleared to give a field of fire since it affords excellent cover for the enemy whilst not being so closely spaced as to impede progress.

The climate of Razmak is temperate, with cold wet Winters and heavy snowfall and rainfall, a dry Spring and warm Summer, with frequent thundershowers. Details of snowfall, rainfall, and temperature for 1938 are given on plate IV, page 19.

The soil itself is very thinly spread over outcrops of reddish stone, and owing to the destruction of grass and bushes by the continuous grazing of large herds of goats and cattle belonging to the Pathans there is much erosion. Such small amount of cultivation as there is must be protected by small stone-faced banks to prevent the soil and the crops from being completely washed away by the heavy rains and thunder-showers.

PLATE NO. IV

— RECORD OF RAINFALL, SNOWFALL, AND TEMPERATURES, RAZMAK 1938. —



Razmak Plain itself has been denuded of trees and also, to a large extent, of bushes by the wasteful and improvident habits of the local inhabitants, who cut down any living bush or tree as grazing for their animals, as well as for firewood. Never, by any chance, do they plant any young trees in their place. Even if they did plant young trees such would be destroyed by the large herds of starving animals. The result is that except in the late Spring and early Summer, when there is a certain amount of new growth of grass, which lends a greenish tinge to the hills and the plains, the actual Razmak Plain is bare, stony, and uninviting, and for most of the year is nothing but a desert of parched yellow grass. The Camp itself measures roughly $\frac{3}{4}$ square miles in area. It is lozenge-shaped, having a long axis of about one mile and a short axis of about half a mile. It is built on two levels, one being 500 feet above the other - that is the upper camp and the lower camp. It is surrounded by a wall with barbed wire outside which are various picquet posts dotted on the commanding sites around the camp. The walls are manned day and night, therefore the men are exposed to night chills etc. Anti-malaria precautions were not regarded as necessary, and in any case the wearing of veils etc. would have been highly dangerous against an enemy of such skill as the Waziri.

The normal strength in the camp was six battalions

of infantry, including one British battalion, a complete regiment of mountain gunners, one company of Engineers, a field ambulance, a hospital, animal transport companies and other ancillary troops. In addition to its serving soldiers each unit had on its strength a large number of followers, including sweepers, cooks, washermen and craftsmen of all descriptions. The actual military force, including attached personnel, usually numbered about 7,000; each battalion of Indian infantry served three years on a frontier tour, very often in one camp; British infantry served only one year.

A small bazaar of some twenty shops was permitted inside the camp, the majority of the shopkeepers being Hindus. These people were allowed to live inside Razmak and made up the bulk of the non-military personnel, among whom were also included the staff of the post office, the Garrison Engineer etc. The Resident also occupied the Residency inside the camp for several months in the year. His Pathan staff and any trusted Pathan on business to see him were housed in the Tehsils (the rest houses), one Tehsil each, at opposite ends of the camp of course, for Mahsuds and for Wazirs respectively. There could be no question of these two sections of Waziris sharing the same building; they were always at daggers drawn, with many feuds among them.

A large military medical staff controlled the

sanitation and health of the camp, including all civilian personnel. The civilians were completely under military control and as a result the general standard of sanitation of the camp was very much higher than any similar installation inside India. For example, no civilian could be employed or even be resident inside the camp until he had passed a physical examination, which included, among other things, the testing of his stools for intestinal parasites. This high standard inside the camp was largely offset, however, by two medical problems immediately outside, namely the coolie camp and the Wazir Serai.

The coolie camp, which is to be seen in plate I, page 4, and in the plan of Razmak Camp, plate III, page 17, was about 500 yards from Razmak Camp. It housed 600 coolies who were employed inside Razmak during the day, but for medical reasons and for reasons of security were housed outside the camp, which they always left before the closing of the gates at sundown. The standard of health among these coolies was very low, although the standard of sanitation of their camp was kept at a relatively safe level by a military medical officer detailed for the purpose.

The Wazir Serai, also seen in plate I, page 4, was owned by a leading Wazir, Noor Mohd. It was a fortified camp about 1,000 yards from Razmak Camp and served as a

hotel for Pathans; there was always much coming and going of Pathans to Razmak on business of various descriptions, and since none other than a few trusted Pathans could be allowed inside Razmak during the hours of darkness Noor Mohd was allowed to maintain his Serai for the convenience of Pathans. This camp, such a short distance from Razmak itself, was a continual source of worry from the medical point of view, and presumably also from the point of view of security. There seems little doubt that Noor Mohd's Serai represented a serious menace in more ways than one. It probably served as a useful clearing house for the relaying to the recalcitrant tribesmen information about troop or convoy movements, gathered from loose talk inside Razmak Camp itself.

Razmak Camp itself was well supplied with water, and there was no shortage of breeding places for mosquitoes in the broken ground, with many little nullahs both inside and outside the camp. Mosquito breeding was strictly controlled inside the camp but little could be done to control breeding outside. Consequently there was no shortage of mosquitoes, including many malaria-carrying types, inside Razmak itself. It was thought, however, that climatic conditions in the area, about 6,500 feet high, were such that malaria could not be transmitted, although there was no lack of mosquitoes. Records appear to

justify this opinion. The British battalions proved particularly useful as controls in that none of the reinforcements coming from England and arriving in the cold weather ever developed malaria in Razmak in the hot weather unless they had gone out on duty down into the malarious country at a lower level. Those people who did not leave Razmak during their tour of duty did not develop fresh malaria. This freedom from malaria was not due to any lack of gametocyte carriers. Many hundreds of cases of chronic malaria were always to be found among the population of the coolie camp, Noor Mohd's Serai, and even among certain of the personnel inside Razmak Camp itself. The distance from Razmak Camp to both the coolie camp and the Serai were well within the ordinary flying range of the Anopheline mosquito.

On humanitarian grounds the military authorities and the Resident permitted the treatment of civilians suffering from malaria, but in view of the numbers involved the only treatment possible was grains 30 of quinine daily for seven days, and plasmoquine 0.02 grammes daily for seven days.

It is to be regretted that the local conditions were such, and certain of our equipment was such, that a full investigation was not carried out to ascertain why Razmak

was free from malaria. Chopra (1936) states that "for the completion of the life cycle of the parasite inside the mosquito a mean temperature of 60°F. is necessary for sixteen days, and, therefore, elevation with consequent low temperature limits the prevalence of the disease". He quotes Kashmir as an example where, at an elevation of 6,000 to 9,000 feet, A.maculopennis, A.fuliginosis, and A.willmori are present in abundance, but no malaria exists. Neve (1941), who spent fifty four years as a medical missionary in Kashmir, states that .... "although there are indigenous Anophelines of the malaria-carrying variety to be found, malaria is unknown in the valley of Kashmir at a height of 5,200 feet, although this disease is common in places at a lower level down the Jhelum road ...." Wenyon (1926) states that ..."at a temperature of 18°C. - 25°C. P.vivax requires about seventeen days (to develop in Anophelines) and P.falciparum about nineteen days. At lower temperatures P.falciparum ceases to develop." Morgan (1947) can speak with authority on the malaria of Waziristan. He was for some years political medical officer in South Waziristan, and he moved over much of the country. He states that in South Waziristan Political Headquarters at Ladha, at a height of 5,500 feet, he did not find any fresh malaria, although just below Ladha, in the Waziri town of Kaniguram, height 4,300 feet, malaria was rife, and Sararogha, at 3,500 feet, a few

miles from Razmak, is the worst place in South Waziristan, with a spleen rate of ninety per cent. Morgan comes to the conclusion that it can be safely said that in Waziristan 6,000 feet and above is a safe height as regards malaria transmission, and probably somewhat lower may be fairly safe, for example at Ladha. On the other hand, the Malaria Institute of India (1947) stated that although it is generally thought that malaria transmission in the Himalayas region ceases at a height of 5,000 feet, yet in the A.superpictus area of Baluchistan transmission is encountered at a height of 6,500 feet. The Directorate of Malariology General Headquarters India (1947) considers malaria can be transmitted up to an altitude of 7,000 feet in certain areas of Baluchistan. This country borders Waziristan to the South and is closely similar, both in physical features and in climate. There are, of course, many instances on record of malaria transmission at a height even above those quoted for Baluchistan. For example, Garnham (1945) records an isolated epidemic at 8,500 feet in Kenya due to A.gambiae. Repeated tests carried out in Razmak showed the presence of important malaria carriers, A.stephensi and A.culicifacies to be the varieties of Anophelines most commonly present. Chopra (1936) states that A.funestus is commonly found in areas where blackwater fever is endemic. Whatever the correctness or otherwise of this statement, this variety was never encountered in Razmak. Ramakrishnan (1947)

states that the commonest malaria-carrying mosquitoes of Waziristan are A.stephensi, A.culicifacies and A.superpictus.

As may be gathered from the data, plate IV, the climate of Razmak is such that in June and July the mean temperature is high enough to allow of the completion of the life cycle of the parasite in the mosquito, and there is no lack of malaria-carrying varieties of mosquitoes nor of chronically infected individuals. The fact remains, however, that in spite of transmission being theoretically possible fresh malaria did not occur in Razmak Camp. It is necessary to stress this point since one of the problems which will require consideration is the question of where the individuals who showed haemoglobinuria had contracted malaria.

THE DIARY OF THE RAZMAK COLUMN.

At first sight this diary, and the accompanying large-scale map (plate V, page 29) appear to be redundant. Such an assumption is incorrect. We are attempting to establish the cause of lysis of red cells which occurred in six individuals, and malaria must rank high as a possible cause or contributory factor; therefore, since Razmak was non-malarious we must look elsewhere for the source of the malaria. Hence this diary, which is also of use in that it shows the severe physical strain to which the men were subjected over a period within days or weeks of the onset of the haemoglobinuria: these questions of source of parasite, and of severe physical strain will be seen to be of importance when we come to discuss in detail the problem of blackwater fever: this is done in Chapters IX and X.

In this expurgated record supplied by Waziristan District Headquarters, matters of purely military importance have not been included. The large scale 1 inch to a mile map (plate V, page 29) gives a plan of the country traversed and our route is shown on it. The contours of the map give height at all stages of the journey but, of course, cannot give any idea of the appalling nature of the country from the military point of view - a country perfectly designed for guerilla fighting.

The operation took place during the 9th/18th July, 1938, in the Tochi area in close proximity to Afghanistan. The object was to break up a lashkar of Ipi's. The diary in detail is given below.

9th July: Razcol moved out from Razmak along the central Waziristan road North-East to Gardai, a temporary camp. This was a fairly easy march of eighteen miles on a good road with practically no opposition. At Gardai Razcol was joined by Damcol which had moved up that morning from the North, also along the central Waziristan road.

The combined force, known as "Waztrike", harboured overnight at Gardai. There was little to report during the night but in the early morning, as the Force moved out, the rear-guard had some little difficulty in extricating itself from the enemy who were following up closely.

10th July: "Waztrike", with its rear-guard slightly engaged, moved out along the valley to Mami Rogha (Map Ref. 904422), another temporary camping site at a height of 5,500 feet - a spleen test once done in this area showed 14%. On the map the actual distance appears only about eight miles from Gardai to Mami Rogha, but the move was very difficult. The enemy stubbornly contested the high flanking hills the whole way along; this meant that the troops had to take and retake high

surrounding features; water was strictly rationed; the temperature was high - in the region of 104°F. - and there was no shade. The move took about nine hours to complete and at the end of it we met with resistance inside Mami Rogha Camp itself. The troops suffered from exhaustion on this part of the move but we had a quiet night with relatively little except fairly heavy enemy sniping.

11th July: Moved from Mami Rogha Camp to Degan camping ground (Map Ref: 905577). Degan was another crude camping site, unfortunately near a village containing hostile elements and containing inhabitants riddled with malaria. Anopheline mosquitoes were present in large numbers, Degan being sited at 3,500 feet on the South bank of the Tochi River. This march from Mami Rogha to Degan was a long one and very tiring, but fortunately opposition was relatively light and uncoordinated, and we were able to make harbour in good shape. Heavy sniping continued throughout the night in spite of much counter-fire, and undoubtedly much of the enemy firing came from the nearby village. The troops had very little sleep.

12th July: The next move, out from Degan, was likely to be very trying, probably with heavy enemy opposition, consequently the Force spent the whole of the 12th July resting at Degan Camp. The night of the 12th was a repetition of the previous one.

From the point of view of health Degan Camp was by far the worst of the halts. It was at 3,500 feet, with Anopheline mosquitoes breeding everywhere and no precautions possible against malaria; but military considerations were an over-riding factor, and no alternative halt was possible.

13th July: A very appropriate date. The move was made from Degan to a point, Wuzgai, ten miles North-West as the crow flies. Wuzgai (Map Ref: 810645) lay at a height of 5,000 to 6,000 feet.

This was the most trying day as yet. The route lay along the Duga Khulla Nullah, practically a dry river bed with a poor surface for marching. There was a tendency to slip back two paces for every pace forward. The animals also felt the strain. There was much heat exhaustion on the march but fortunately water was readily available. The heat was severe and the physical strain was much increased by the heavy opposition encountered. This opposition was carried on even in Wuzgai Camp itself, two of the Force - one a British officer - being shot dead at close range by a well-concealed sniper who opened fire some time after we had taken up positions. Sniping continued throughout the night. The only bright feature of the day was the linking-up with sixteen platoons of the Tochi Scouts, who materially assisted our move up the Duga Khulla Nullah. These fast moving

Scouts were of great help during the ensuing two days.

14th July: The sick and the wounded were temporarily left at Wuzgai, with two companies of British Infantry detached to protect them, and the remainder of the Force, together with the Scouts, moved up as light as possible towards its objective, Kharre, in the Riji Zawar area (Map Ref: 7873) where Ipi and his gang had their Headquarters. This was also a very hard day, with resistance and bitter fighting all day and all along the route, particularly at Barmand (Map Ref: 7968), Bazoma Narai (Map Ref: 7970) and Riji Zawar (Map Ref: 7972). These were all at heights between 5,500 and 7,000 feet. Fortunately we were able to inflict relatively heavy losses upon the enemy in some of the bitterest fighting seen for some time. It was not possible, unfortunately, to follow up our advantage as the enemy trickled through the border into Afghanistan. Ipi himself probably escaped in that direction.

The Commandant of the Tochi Scouts has been good enough to permit an extract being made from his records. This gives a picture of the engagement from the point of view of the Scouts, showing the demands made upon them. It reads:-

"14th July: The Scouts were split into two detachments of eight platoons each. On our arrival at Bazoma Narai we found it important to seize ground to the North of the Narai

to protect Waztrike and Razcol Headquarters. This was done by one platoon, which stayed in this position all day until retired by me at 20.00 hrs. Heavy fighting was going on on Razcol's left flank, with the Gurkhas trying to push through from Barmand to Kund Sar via Mazdak Punga. On arrival of the Scouts at Mazdak Punga we found the Gurkhas only a little way advanced along the Kund Sar ridge. We thereupon attacked on the east of the Gurkhas through a wooded ravine and captured a hil below Kund Sar. We then gave further support to the Gurkhas in the attack on Kund Sar and afterwards arranged a withdrawal from this foul country to the Southern slopes of Mazdak Punga. There we found it necessary to leave eight platoons of Scouts to help the troops hold that position for the night. We then returned back to the Bazoma Narai, where we received orders to occupy the Southern perimeter of the night camp at Barmand ....."

The military report then continues and gives detailed notes of the return march to Wuzgai, to Degan, to Mami Rogha, and to Gardai. The routes used were the same as those going out; only one night was spent at Degan on the return journey.

<u>19th July</u>: Damcol returned to Damdil and Razcol to Razmak.

The only points of note on the return were that the heat was very trying and that there was little opposition

practically the whole way through. The enemy had taken an unusually heavy beating and was licking his wounds. He did make one spurt towards the end; with tenacity that must be admired he crossed the high hills and laid an ambush for the troops as they entered Razmak, thus spoiling what was meant to be a triumphant return.

In the Column from Razmak the casualties inflicted by the enemy numbered fifty, including the dead, and the sick casualties directly from the Column numbered 169; this figure does not include approximately 100 cases of malaria occurring in the Razmak Column 4,000 strong.

DRUG PROPHYLAXIS AGAINST MALARIA USED DURING AND AFTER THE KHARRE COLUMN.

Although atebrin had been in use since 1930, the cost prevented it being freely tested in mass prophylaxis. By 1938, however, a few reports had appeared, all of which spoke favourably of the drug in this connection. The move down from Razmak to Kharre provided an opportunity for the testing of atebrin prophylaxis in the field. Literature then available showed that the method of obtaining the best results from atebrin used with this end in view had not been fully worked out; earlier reports had suggested that atebrin did have a definite prophylactic value when given during and for some days after infection, but the optimum dosage had not been fully established; at that time, of course, the question of an exo-erythrocytic phase in the life cycle of the parasite had not assumed that importance it is now accorded.

In this attempt to test the efficiency of atebrin in the field it was decided that during the Column the troops of Razcol would be given atebrin daily during and for seven days after the Column. At the end of each day's march each man was made to take 0.1 grammes of atebrin by mouth. This dosage was continued daily throughout the ten days of the Column and was carried on for seven days after the men had returned to Razmak. Each man, therefore, who had gone on

the Column received a total of 1.7 grammes of atebrin in seventeen days, beginning on the first day of the Column and finishing seven days after the Column returned to camp. It may be safely presumed that a very high proportion of the men did swallow the drug in the dosage mentioned. Discipline in the Indian Army was very high and the administration of the drug was carried out under the supervision of the officers of the units. The number of men who failed to take the whole course must have been negligible. This point was particularly gone into very shortly after the completion of the seventeen days course.

It should be stressed here that Damool, the body of troops from Damdil Camp, who had supported Razool throughout the move did not receive any prophylactic atebrin. It was hoped that they might serve as a control, and a further control was supplied in Razool itself, in which one company of the 7th Rajput Regiment, one of the infantry battalions taking part, was placed on quinine bisulphate, five grains daily on each day of the march and for seven days thereafter.

It is now realised, of course, that the daily dosage given was satisfactory but the drug was not given over a long enough period of time; the significance of a possible exo-erythrocytic phase of the parasite in man had not been realised, and furthermore the few previous reports had suggested that the

method we used should have been quite effective. More correct information was published in 1939 by Field whose work was a contribution of great value and who stressed the proper method of use of atebrin for prophylaxis, namely by low dosage maintained throughout the period at risk, and for some time thereafter. Field claimed that in the use of atebrin for mass prophylaxis protection seems to be related not only to dosage but also to the duration of administration, that is small doses continued well into the incubation period were more effective than large doses given at the time of exposure. Field, therefore, considered the action of the atebrin to be probably schizonticidal and the effect one of medico-curative prophylaxis. He further stated, however, prophylactic atebrin appears merely to prolong the incubation period to a point about three weeks after the withdrawal, a series of findings which has been partly confirmed by subsequent work. The dosage in use at that time was 0.4 grammes weekly, given in two successive doses of 0.2 grammes, Fairley (1945) carrying out further investigations confirmed that the effect of atebrin was probably medico-curative. He considered that with a daily ingestion of 0.1 grammes of atebrin the peak concentration of this drug in the blood is reached between the twenty-fifth and twenty-eighth day, that is after a total of 2.5 grammes of atebrin has been ingested, by which time there was usually a blood concentration of 21.9

micrograms per litre of blood. Fairley found that with m.t. malaria 0.1 grammes daily of atebrin virtually abolished recrudescences, whereas with b.t. malaria 0.1 grammes daily of atebrin merely suppressed the disease, overt vivax malaria developing with great regularity a few weeks after suppressive atebrin had been stopped. Fairley concludes that (1) a force of men can be employed for many months in hyperendemic areas of malaria without significant malaria casualties; (2) there should be no deaths from malaria, no blackwater fever, and no malaria carriers in the force; and (3) after cessation of atebrin suppressive treatment the residual problem would be exclusively that of benign tertian malaria.

From the information now available, e.g. the work of Fairley quoted above, it is realised that the course of atebrin given during the Column in July, 1938, was too short in duration though sufficient in daily dosage. The Column was of ten days duration; no one developed fresh malaria during the Column itself, as, of course, in the absence of any modifying factors malaria contracted during the Column would have been expected to become overt in the early days following the return of the troops to Razmak, that is during late July and during August, 1938. A complicating factor, however, had been introduced by our use of atebrin for mass prophylaxis. Admittedly the course of atebrin given was insufficient by present-day standards, but it was sufficiently

close in total dosage and in length of time to modify the resultant malaria in two ways. Firstly, in my opinion, the amount of atebrin was sufficient to suppress completely, or at least for a period of several months, attacks of malaria in some at least of the individuals affected during the march. This is particularly applicable to m.t. malaria. In certain other individuals who had become infected, especially those infected with P.vivax, the interval before which the disease became overt probably would be extended to a period of weeks instead of days, as is usual in uncomplicated cases. In other words, the most likely results to be expected from our attempted mass prophylaxis were a considerable reduction in the instance of m.t. malaria, and a prolonging of the incubation period in those individuals who had contracted b.t. malaria. Figure 1, page 41, illustrates our findings, showing, as it does, admissions for malaria to the Razmak Combined Military Hospital in 1938. Figures for 1939 and 1940 are given for comparison.

FIGURE I

Records of Malaria Admissions C.I.M. Hospital, Razmak, 1938-1940.

Rates are per thousand men per year; average strength all troops 9,000

	1938										1939										1940	
	B.T.		M.T.		Q.		MXD.		CLIN		B.T.		M.T.		Q.		MXD.		CLIN		B.T.	M.T.
	F.	R.	F.	R.	F.	R.	F.	R.	F.	R.	F.	R.	F.	R.	F.	R.	F.	R.	F.	R.		
Jan.		4			1							11		11							13	7
Feb.		5								1		7		2							8	2
Mar.		4		5						1		8									12	5
Apr.		13		16						2		17		2						1	12	1
May.		12								1	17	18	5								52	17
Jun.	16	53	8	13			1	1	7	12	24	33	7	2							109	37
Jul.	108	122	7	2			1		12	7	109	135	62	28							334	62
Aug.	178	155	8	15			1				149	220	84	52							264	200
Sep.	67	124	41	63			1	1	2		42	74	34	60			1				271	517
Oct.	30	37	39	40							16	60	23	41							127	209
Nov.	17	18	9	21							9	53	5	35	1						412	76
Dec.	3	18	1	1							7	24	8	25							84	245

Key: B.T. Benign Tert. M.T. Malignant Mal. F. Fresh attack. R. Relapse.

Notes: 1. After 1939 there was no attempt to classify cases into F and R attacks 2. The high figures for 1940 are probably due to the large numbers of new recruits, and the influx of State Force troops.

The information given in these tables may be accepted as correct. From May, 1938 to December, 1940, that is during the whole of the period reported upon, medical admissions to Razmak Hospital were under my care, and each diagnosis of malaria was counter-checked by one of the laboratory staff. In spite of this, however, it is very difficult to estimate the true significance of the data given above. Several factors serve to lessen the value of the figures; for example the data are based on the monthly admissions to the Combined Military Hospital at Razmak. This hospital served a total population of 9,000, made up of 7,000 of the Razmak Garrison and a total of about 2,000 from the many little outlying posts, such as Thal Fort. This means that the figures of malaria admissions for July, August, and September, 1938, cannot be taken as an indication only of the malaria from the 4,000 men of the Kharre Column. Furthermore, it is unwise to compare too closely the figures for 1939 with those of 1938. The total population at risk was practically the same, namely 9,000, but the composition was different, because in late 1938 and 1939 no less than three battalions completed their tour of duty on the Frontier and were replaced by three fresh battalions from the Plains, including one British battalion.. This introduces a fallacy which is even more strikingly shown by the figures for 1940, put in largely to illustrate this point. In 1940 the population was still the same, that is

about 9,000, but again there had been a change of three battalions, and the three new battalions were from State Forces, loaned to free the regular Indian forces for overseas expeditions. These soldiers of the State Forces were heavily infected with malaria, which accounts for the steep rise of the figures for this disease in 1940, and accounts also for the stopping of the analysis of malaria cases into "fresh" and "relapsed" categories, since this could not be easily done because of the lack of medical records of the State Force units. One other factor to be quoted is that the Razmak Combined Military Hospital did not treat the Damcol troops, except in a few special and difficult cases. Damdil Camp had its own Camp Hospital where conditions such as malaria received treatment.

It is considered therefore that the factors entering into discussion are so varied that from the data given alone very little definite information can be obtained of the incidence and type of fresh malaria among troops as a result of the Kharre Column. This was realised at the time, and with a view to obtaining information a scrutiny was made of the records of all individuals admitted to Razmak C.I.M. Hospital from the 18th July 1938, to the 30th September 1938. This period of time was to permit of inclusion of individuals with a prolonged incubation period due to atebrin.

In my opinion these records showed that the incidence of fresh malaria from the Kharre Column totalled 108 men. Colonel Warburton (now General), at that time commanding Razcol Field Ambulance, estimated the figure to be about 100, which is sufficiently close agreement with my figure. The details are:-

Total at risk during Kharre Column - 9th/18th July - 4,000

Admissions to Razmak Hospital with malaria, 18.7.1938 to 30.9.1938 - 230 fresh benign tertian and 39 fresh malignant malaria: Total fresh cases - 269

From a scrutiny of the records loss from the Kharre Column amounted to 104 fresh benign tertian and four fresh malignant - a total of 108 cases from the 269 admitted altogether.

One illustrative case is that of Captain R., the officer in sub-charge of the Razcol Field Ambulance at the time of the Kharre Column. He had lived and served in India practically all his life and he claimed that he had never had malaria; he was most careful to take his daily dose of 0.1 grammes of atebrin during and for seven days after the Column, yet on the tenth day after the Column had returned to camp, i.e. on the 21st July, 1938, three days after stopping the atebrin he was admitted to hospital with severe benign tertian malaria. His blood films showed only rings, and the spleen was not palpable. Some of these 108 men did not show overt

malaria until some weeks after the return to Razmak of the Column on the 18th July, 1938. Necessarily, however, these 108 individuals were infected during the ten days from the 9th to 18th July. This gives then the malarial incidence in the Kharre Column to be over 980 per thousand per annum.

In the reports on the results obtained when atebrin was used as a prophylactic among the forces engaged in the Solomons, New Britain, and Borneo, Fairley (1946a) shows that the incidence never rose above forty seven per thousand per annum, and generally was in the region of twenty per thousand per annum. This author later in the same article states that from past experience without the use of atebrin at least ninety per cent of the force would have acquired malaria. Our incidence of over 900 per thousand per annum is, therefore, very high - almost twenty times the incidence reported later among the fighting troops on daily atebrin in jungle areas, and is very near to the figure of ninety per cent suggested by Fairley to be expected among unprotected troops fighting in malarious areas. Therefore, the mass use of atebrin for prophylaxis on the Kharre Column failed to prevent a heavy incidence of malaria among the men at risk.

The next question to be considered is whether this atebrin modified the disease or the incidence of the disease in any way. As mentioned above two controls were available,

namely Damcol, of over 3,000 men, who were not given atebrin, and secondly a Company of Rajput Infantry in Razcol, who were given quinine daily instead of atebrin. Neither of these controls prove to be of any value. In the control group in Razcol itself numbering 150 men, the incidence of malaria among these men did not differ significantly from the incidence of malaria in the main body of the troops, i.e. the body who took atebrin; and the numbers used in this control (150 men) were too small for it to have any value.

The other control, i.e. the incidence of malaria in Damcol, also proved valueless because immediately on the completion of the Kharre Column Damcol moved back to its camp at Damdil, where it remained. This camp was near a malarious area at 3,500 feet, and it would not have been possible to have worked out the incidence of malaria from the Column as distinct from that acquired in the camp itself immediately afterwards.

From these controls, therefore, we cannot come to any conclusion as to the value or otherwise of the atebrin course in reducing the incidence of malaria in Razcol.

Two other ways in which the atebrin course may conceivably have affected the incidence are that the period of incubation of benign tertian cases may conceivably have

been prolonged to some degree and the incidence of malign tertian malaria may have been reduced, since this is the action usually found as a result of the use of prophylactic atebrin. No answer can be given to the first suggestion, namely that the atebrin may have prolonged the incubation period of the fresh malaria. All the documents available gave insufficient information to allow of any definite statement being made on this point. The last point is whether or not the incidence of malign tertian malaria was affected by the drug. (Quartan malaria and mixed malaria incidence were so low that they need not be considered.)

All records of malarial incidence available for Waziristan and for Razmak showed that P.falciparum was never responsible for less than thirty three per cent of all malaria admissions, and sometimes the figure rose to fifty per cent, but our figures for malign tertian malaria in July 1938 and August 1938 are striking exceptions to this previous infallible finding, because in these months the incidence of malign tertian malaria fell to 6% and to 4.4% respectively of the total; that is, there occurred during these months a very marked reduction in the incidence of malign tertian malaria, although the incidence of benign tertian malaria was within normal limits for that period of the year. Furthermore, if the figures for 1939 be compared it is seen that there was no such fall in the incidence of fresh malignant

malaria in July 1939 and in August 1939, such as had occurred in July 1938 and August 1938. For example, in July 1939 the incidence of fresh malignant malaria was sixty two per thousand per year, i.e. thirty six per cent of all the combined benign tertian plus malign tertian figures. This figure of sixty two per thousand compares strikingly with the July 1938 incidence of seven per thousand. Similarly, in August 1939 the incidence of fresh malignant malaria was eighty four per thousand per annum, which was also thirty six per cent of the combined malign tertian and benign tertian figures, and this similarly compares strikingly with the August 1938 incidence of malign tertian malaria, namely eight per thousand per annum. There is a strong probability, therefore, that the atebrin course given from the 9th July to 25th July, 1938, resulted in a much reduced incidence of fresh malignant malaria both for July 1938 and for August, 1938. Records extending back to 1930 had never shown such a drop in the percentage incidence of malign tertian malaria.

SUMMARY CHAPTER I.

In this Chapter a description is given of Waziristan in general and of Razmak Fortress in particular. This description is necessary in that Razmak Fortress, at a height of 6,500 feet, was held to be in a malaria-free area. A Column of troops operated from this fortress against the Fakir of Ipi in the Kharre area during the period 9th to 18th July, 1938; a diary of the move shows that it was a severe physical strain on the men; this Column was supported by a slightly smaller Column from Damdil Camp. For the first time in the military forces in India an attempt was then made to use atebrin for mass prophylaxis among troops. Only Razcol was given the drug, administered in daily doses of 0.1 grammes during the Column, and for seven days after their return to camp. It is held that this seventeen days course of atebrin completely failed to lower the incidence of benign tertian malaria resulting from infection during the Column, the total infection rate being 980 per thousand per annum. 96.3% of the cases admitted were found to be infected with benign tertian malaria. No information is available for comparative figures from Damcol, and no information is available as to whether the giving of atebrin lengthened the incubation period of the malaria. Data available, however, suggest that the atebrin course materially reduced the incidence of

malignant malaria in July and August, 1938. These figures are in keeping with the results obtained later by other workers.

C H A P T E R II.

PRELIMINARY DISCUSSION ON THE SIX CASES OF BLACKWATER FEVER RECORDED IN RAZMAK IN 1938

EXPLANATORY NOTE ON THE TREATMENT OF MALARIA CASUALTIES RESULTING FROM THE KHARRE COLUMN:

This has been sketchily dealt with in the previous chapter, but since the slight modification in standard treatment which occurred at this period must be considered as a possible contributory factor in the causation of the attacks of haemoglobinuria, a fuller note on this point is given below.

The operative movements of any troops in the lower valleys of Waziristan at any time during the malarial season always produced a crop of malarial casualties. The degree to which any Force was affected depended upon the area in which the troops had been required to fight. The medical information which was available about the country through which the Kharre troops would move was necessarily incomplete but did suggest that we should expect heavy malarial casualties.
We did not expect any marked reduction in malarial casualties from the use of atebrin which we were proposing to use as mass prophylaxis because our knowledge of the value of this drug and its method of use in prophylaxis were still insufficient for us to estimate the effect of this drug.

Any increased number of casualties had always strained the resources of Razmak Hospital, especially during the months of July, August and September, when malaria was rife; accordingly, Northern Command Headquarters at Rawalpindi authorised two modifications in the routine method of treatment of malaria at that time standardised throughout the Army in India. These two modifications were, the use of a Convalescent Depot, and secondly a reduction in the length of the atebrin course, with a reduction also in the length of the rest period between the finishing of the atebrin and the beginning of the plasmoquine treatment.

These two modifications in treatment came into operation immediately preceding the Kharre Column in 1938 at the beginning of July. Their adoption, therefore, is chronologically related to the "outbreak" of haemoglobinuria during malaria treatment; since these changes in treatment must be taken into account on the question of aetiology, a short note is given on each of the changes.

CONVALESCENT DEPOT.

The setting up of the Convalescent Depot followed an accepted pattern. Part of the troops' barrack accommodation in Tigris lines lying 200 yards from the hospital were taken over temporarily from the occupying infantry battalion by the hospital authorities (see Razmak plan, plate III, p.17).

The barrack accommodation consisted of brick huts which were of a standard type, cool, comfortable and weatherproof, quite satisfactory for the accommodation of convalescent patients in the warm weather of Summer, and early Autumn. The arrangement was that each patient suffering from malaria, and any other suitable type of case, was transferred to the Convalescent Depot from the Hospital proper as soon as he had become ambulant and fit to look after himself. In the case of patients suffering from malaria this was generally taken to mean that the patient would be able to move from the Hospital to the Convalescent Depot on the completion of the atebrin course, by which time he would have been, normally speaking, about five days free from fever. The full course of malarial treatment at that time lasted approximately seventeen days. This meant, therefore, that each patient spent not more than seven days in the Hospital instead of seventeen days. While in the Convalescent Depot the patient still remained a medical responsibility and still required treatment. To ensure continuity of treatment and of medical supervision, patients were required to walk morning and evening from their huts 200 yards to the Hospital, where the duty medical officer interviewed each man and made sure that each individual on the malaria course received and swallowed the plasmoquine tablet, plasmoquine being given twice daily. Apart from this exertion which was within

the capabilities of the patients sent to the Convalescent Depot such patients were not allowed to do anything else other than rest, and they were completely struck off all military duties. Had they been allowed to go back to their unit lines to complete the course they would undoubtedly have been caught up for some fatigue or other. On the completion of the plasmoquine course each patient was then allowed to return to his own unit, but remained off duty for one more week.

No person was transferred to the Convalescent Depot unless he appeared fit for it.

MODIFICATION IN THE STANDARD COURSE OF TREATMENT.

The second step in the easing of the strain on the hospital was by a modification of the standard malaria course as already indicated above. In 1934 the Director of Medical Services in India had ordered the adoption as a standard form of treatment of malaria a course as follows:- Quinine grains 30 daily to be given as long as thought necessary, followed by seven days of atebrin 0.3 grammes daily, followed by three days rest, followed by plasmoquine five days, 0.02 grammes daily. This course had proved much superior to preceding courses and remained as a standard from 1934. Some workers, however, felt that it was over-safe and could be

modified slightly without danger. This modification was decided upon as a necessary but purely temporary measure to ease the situation in Waziristan in 1938. The modified course was: Quinine as before, followed by five days of atebrin 0,3 grammes daily, followed by one day's rest, followed by plasmoquine as before, i.e. five days 0.02 grammes daily. This meant, therefore, that each individual's period of time of treatment was shortened by three days at least. In a station like Razmak, where a figure of 500 malaria casualties a month was not uncommon, this would represent 1,500 man days saved per month, a great help to an overworked military force and to an over-strained hospital.

As mentioned above, these two modifications in treatment had just been put into operation before the return of the Column from Kharre, and all malaria casualties resulting from that Column received this shortened course of treatment and, where possible, spent only a portion of their period of treatment actually in the hospital itself, the remainder of the period being spent in the Convalescent Depot.

Such was the position during the period when six of the patients under treatment for malaria developed haemoglobinuria during the course of that treatment.

On pages 57 to 81 a resume is given of each case,

together with the summary of all treatment and haematological and biochemical findings. The full case reports are given in the Appendix (pages 734 to 788).

RESUME OF CASE RECORD - PATIENT NO. 1.

(detailed Case Report, see Appendix, pages 734 etc)

No. 1862, LASCAR MOHD HUSSAIN.

27th Animal Transport Company - Royal Indian Army Service Corps, Razmak.

Aged 35 years.

Service: 8 years.

Plate No. VI, page 63 , summarises the treatment and the laboratory findings.

21/7/1938: Admitted to Combined Indian Military Hospital, Razmak, from unit lines in Razmak three days after the Kharre Column had finished. He complained of fever with rigors of two days duration: patient's previous history included two attacks of malaria, one being fresh benign tertian malaria (July, 1936) and one clinical malaria relapse (August 1936) - both thoroughly treated by the standard treatment - seven days atebrin, five days plasmoquine.

Also a history of attacks of bronchitis, 1931, 1934, 1935. One attack of broncho-pneumonia (February, 1938).

Physical examination showed thickening of the

pleurae both sides. Nil else abnormal.
Blood films showed ring forms and gametocytes of P.vivax.
Patient was put on quinine, grains 30 daily until temperature settled, which it did on the second day.
Atebrin course then given for five days at 0.3 grammes daily.
By that time (27/7/38) patient appeared fit enough to be transferred to the Convalescent Depot to complete his treatment. He had one day's rest from treatment then began his plasmoquine course, 0.01 grammes twice daily, the treatment being given morning and evening at visits to the hospital 200 yards away from the Convalescent Depot.

2/8/1938: At 13.00 hrs on 2/8/38, after he had received his ninth and second-last plasmoquine dose of 0.01 grammes the patient informed the medical officer that he had been passing red urine for the previous two days, and that he felt ill, with weakness, vomiting and fever. Plasmoquine stopped; re-admitted to hospital and found to have a temperature of 100°F., with blood pressure of 85/50. No cyanosis, and no abdominal pain;

marked pallor; blood film negative for malaria parasites; spleen and liver not palpable.

A diagnosis was made of blackwater fever - although blackwater fever had not previously been reported in Waziristan.

It was decided that intense alkalinisation was not indicated; on this day the treatment given essentially consisted of quantities of alkalies together with bland fluids given by the mouth, supplemented by rectal infusions, the total quantity of fluid ingested being balanced as far as possible against the urine output. This was our aim throughout the whole of the patient's illness. Other treatment given included measures to improve the blood picture, e.g. Campolon 4 ccs; hot applications were kept to the kidney areas throughout the whole of the patient's stay in hospital. The rise in temperature - undoubtedly due to haemolysis - was wrongly interpreted at that time to be due to persistent malaria, and 0.3 grammes of atebrin musonate was given intra-muscularly. This was repeated on the two succeeding days.

3/8/1938: On the next day (3/8/38) there was no improvement - the plasma was red and the urine was a deep rose

colour. A transfusion of 500 ccs. fresh blood failed to produce any obvious immediate improvement and slight jaundice was noticed on this day for the first time.

4/8/1938: By 4/8/38, i.e. the third day after re-admission to hospital and probably about the fifth day following the beginning of haemoglobinuria, the patient's condition was bad. He had become doubly incontinent, with marked jaundice; the amount of urine voided did not appear to be much; it stained the bed sheets red; the red cell count showed no change from the previous day, and in view of the fact that he had received a transfusion this suggested there had been a further lysis, which must have included some of the transfused cells.

On the afternoon of 4/8/38 patient showed signs of broncho-pneumonia (right lung).

5/8/1938: The next day the broncho-pneumonic condition had spread to the base of the left lung, and jaundice was severe in degree. The blood urea level was rising; at 18.00 hours catheterisation yielded 10 ccs. of pink mucoid slimy urine; this urine contained many casts and much amorphous pink-stained debris.

6/8/1938: By the seventeenth day (6/8/38) from the onset of the illness, i.e. the sixth day after the onset of haemoglobinuria, the patient's red cell count had fallen markedly; the plasma was pink in colour and the very small amount of urine passed on the bedsheets had stained them red in colour. Severe oliguria was present - the patient had probably voided only a few ccs. in the previous twelve hours. Another blood transfusion (500 ccs.) was given. Other treatment included a Campolon injection and fluids by mouth and rectum. The blood urea level by this time was rising rapidly.

7/8/1938: The next day (7/8/38) his condition was serious; no reaction to blood transfusion; red cell count unchanged; antivenene again tried in view of the obvious repeated phases of lysis.

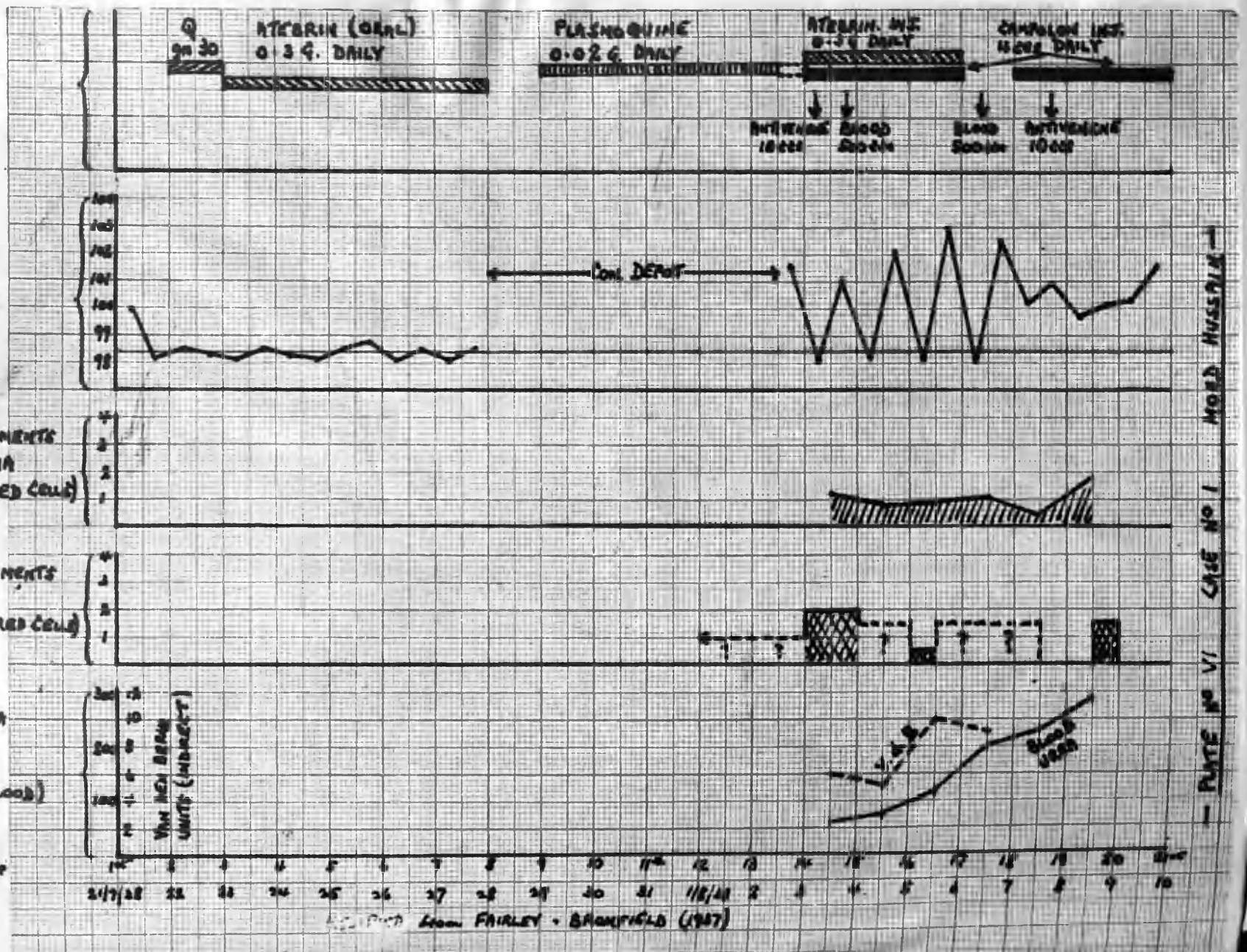
8/8/1938: By 8/8/38 the patient was dangerously ill with widespread involvement of the chest from pneumonia; rapidly-falling red cell count, with a rosy-red plasma; catheterisation yielded only 2 ccs. of deep red urine; the blood urea was 200 mgms. per 100 ccs. blood. On this day the rectal infusions were discontinued and the patient was given two pints of mixed glucose-normal saline solution intravenously.

9/8/1938: On the next day the patient died.

The post-mortem examination showed extensive plugging kidney tubules, which were severely damaged. The liver was also severely damaged.

SUMMARY.

This patient had several intravascular haemolyses over a period of nine days. He developed the dreaded complication of kidney damage which resulted in anuria and death. A significant finding was that normal transfused red cells apparently were destroyed equally with the patient's own cells.


TREATMENT
ATEBRIN (ORAL) 0.3 G. DAILY
PLASMOQUINE 0.02 G. DAILY
ATEBRIN I.M. 0.3 G. DAILY
CHLOROQUIN INT. 0.25 G. DAILY
TEMP (DEGREES FAHR.)
Cont. DEPOT
BLOOD PIGMENTS IN PLASMA (% SOL. RED CELLS)
BLOOD PIGMENTS IN URINE (% SOL. RED CELLS)
BLOOD UREA (MGMS IN 100 CC BLOOD)
VAN DEN BERGH UNITS (INDIRECT)
DAYS in HOSP
DATE
CASE No I MOHD HUSSAIN — PLATE No VI
Adapted from FAIRLEY + BROMFIELD (1937)

RESUME OF CASE RECORD - PATIENT NO. 2.

(detailed Case Report - Appendix, pages 748 to 755)

No. 13836, SEPOY DOST MOHD.

2/7 RAJPUT REGIMENT, RAZMAK.

Aged 22 years.

Service: 3 years.

Plate VII, page 67, summarises the treatment and the laboratory findings.

29/7/1938: Admitted to Combined Indian Military Hospital, Razmak, with complaint of fever and rigors daily for three days.

The patient's previous history included an attack of fresh benign tertian malaria (July, 1937). This had been treated by the seven days atebrin, three days rest, five days plasmoquine course.

The only abnormality detected on admission was the presence of ring forms of P.vivax in the blood films. Diagnosis was made of "fresh benign tertian malaria".

Patient was given quinine, grains 30, for one day and then was started on a five day course of atebrin 0.3 grammes daily, which was completed on 3/8/1938.

4/8/1938: Transferred to the Convalescent Depot to complete his treatment. He began there with one day's rest from treatment and then:

5/8/1938: Started a five days plasmoquine course 0.02 grammes daily, finishing on 9/8/38.

10/8/1938: Discharged to Unit for further convalescence of one week.

11/8/1938: Re-admitted to hospital in the morning. Stated that he had been passing red urine since the previous night and had marked weakness and vomiting. Temperature on admission 102°F.: pulse fast and soft: no cyanosis and no jaundice: patient fainted during medical examination: the spleen was not palpable and blood films did not show any malaria parasites; the blood picture showed severe anaemia; plasma rosy red; urine Burgundy-coloured with absorption bands of oxy-haemoglobin and methaemalbumen. Case diagnosed as blackwater fever.

Treatment was simple and symptomatic - hot packs to the kidney areas, much fluid by mouth, including sodium bicarbonate and sodium citrate solution. Iron tonics and marmite were also given from this day on until patient was discharged.

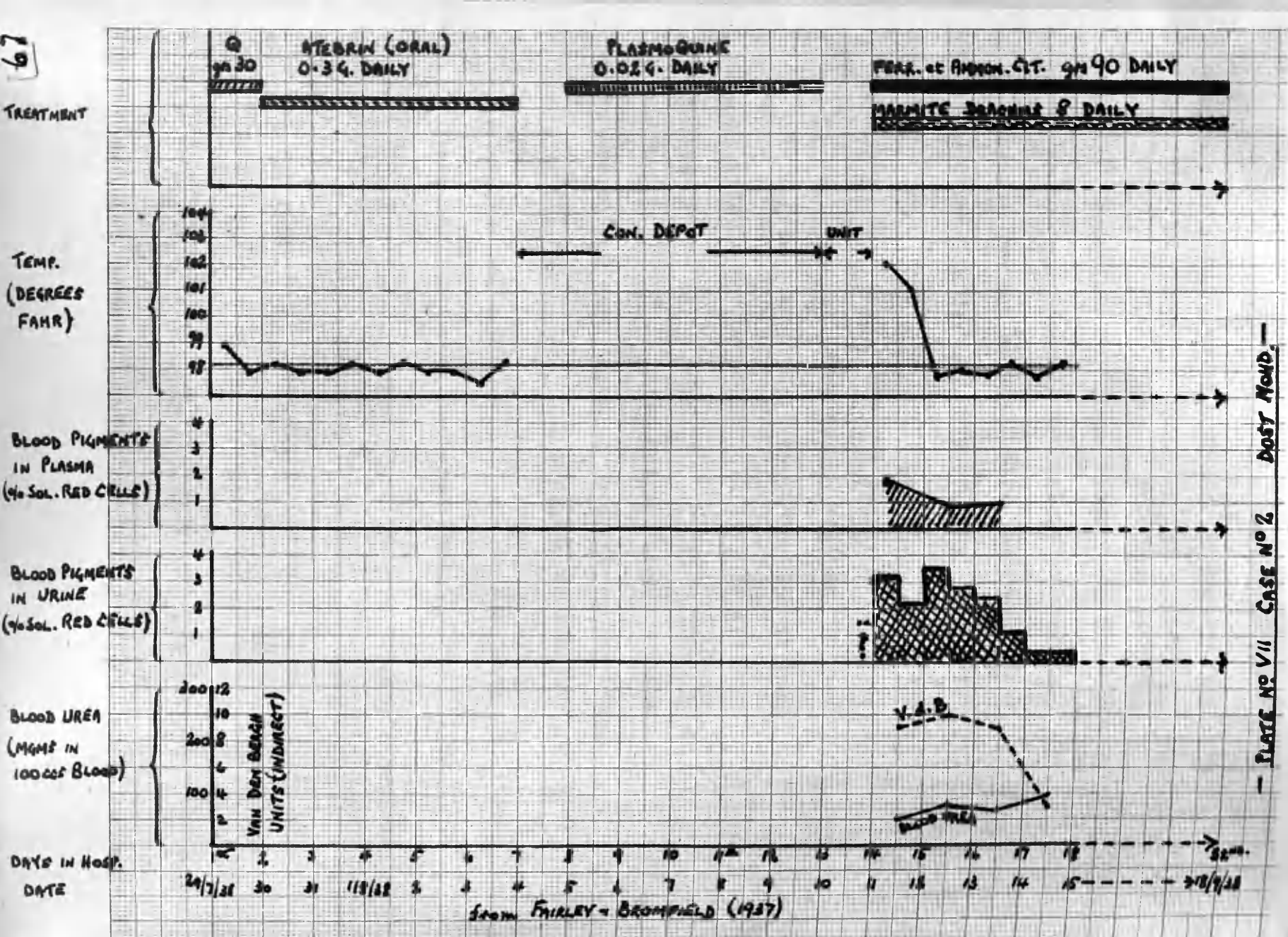
15/8/1938: In the succeeding four days (from 11/8/38 to 15/8/38) the patient's urine slowly cleared and the initially high van den Bergh reaction fell practically to normal and the plasma cleared in colour.

18/8/1938: By 18/8/38 the plasma was normal in colour and the only abrnomality in the urine was a heavy content of granular casts and masses of blood pigment mixed and free. The red cell count was still low.

18/9/1938: Discharged as cured with no abnormal findings. Red cell count six millions per cub.mm.

SUMMARY.

The condition seemed to have been a single intravascular haemolysis, or a very small number of haemolytic phases, the blood pigment taking some days to clear from the plasma and the urine.


97
TREATMENT
Q
gm 30
STEBRIN (ORAL)
0·3 g. DAILY
PLASMOQUINE
0·02 g. DAILY
FERR. et AMMON. CIT. gm 90 DAILY
MARMITE 3 DRACHMS 8 DAILY
TEMP. (DEGREES FAHR)
104
103
102
101
100
99
98
CON. DEPOT
UNIT
BLOOD PIGMENTS IN PLASMA (% SOL. RED CELLS)
4
3
2
1
BLOOD PIGMENTS IN URINE (% SOL. RED CELLS)
4
3
2
1
trace
BLOOD UREA (MGMS IN 100 CCS BLOOD)
200
100
12
10
8
6
4
2
VAN DEN BERGH UNITS (INDIRECT)
V.A.D.
DAYS IN HOSP.
DATE
24/7/28
30
31
1/8/28
3
4
5
6
7
8
9
10
11
12
13
14
15
3/8/28
PLATE No VII CASE No 2 DOST MOHD.
from FAIRLEY & BROMFIELD (1927)

RESUME OF CASE RECORD - PATIENT NO. 3.

(detailed Case Report - Appendix, pages 756 to 766)

No. 11577. L/NK. GURSARAN SINGH.

7TH INDIAN FIELD AMBULANCE, RAZMAK.

Aged 23 years.

Service: 3½ years.

Plate VIII, page 71, summarises the treatment and the laboratory findings.

1/8/1938: Admitted to Combined Indian Military Hospital, Razmak; complained of fever with rigors during the whole of the previous day.
Previous history (confirmed by Army records) showed that the patient had never had any illness in the Army except hepatitis. He said that previous to joining the Army he had never had malaria.
Blood films showed P.vivax rings; spleen not palpable.
Diagnosis - fresh benign tertian malaria.
The patient was given quinine for one day and was then started on the modified atebrin course - atebrin 0.3 grammes daily for five days. This treatment finished 6/8/38.

7/8/1938: Transferred to the Convalescent Depot to complete

treatment. Given one day's rest then began plasmoquine course, 0.02 grammes daily.

12/8/1938: On the twelfth day, after the second-last dose of plasmoquine, the patient was found to be severely jaundiced. He complained of passing "blood in the urine". His temperature was 101°F.; pulse fast; no cyanosis; no malaria parasites in the blood; liver and spleen not palpable; the red cell count found to be 2¾ million cells per cu.mm. with the plasma rose-coloured and the urine red due to blood pigment, confirmed by spectroscopic examination. Treatment as previous cases, namely hot packs to the loins, balanced fluid intake made up largely of glucose and alkaline solutions, given orally, with Campolon to stimulate blood regeneration. Antivenene given twice although with little hope of it helping the patient.

13/8/1938 to 18/8/1938: In these succeeding six days the patient showed a remarkable series of haemolytic crises as evidenced by the varying colours of the urine specimens passed. (Details are given on plate VIII, page 71.)

19/8/1938: By 19/8/38 no further colouration was detected in the urine which slowly also cleared of casts and

blood pigment masses.

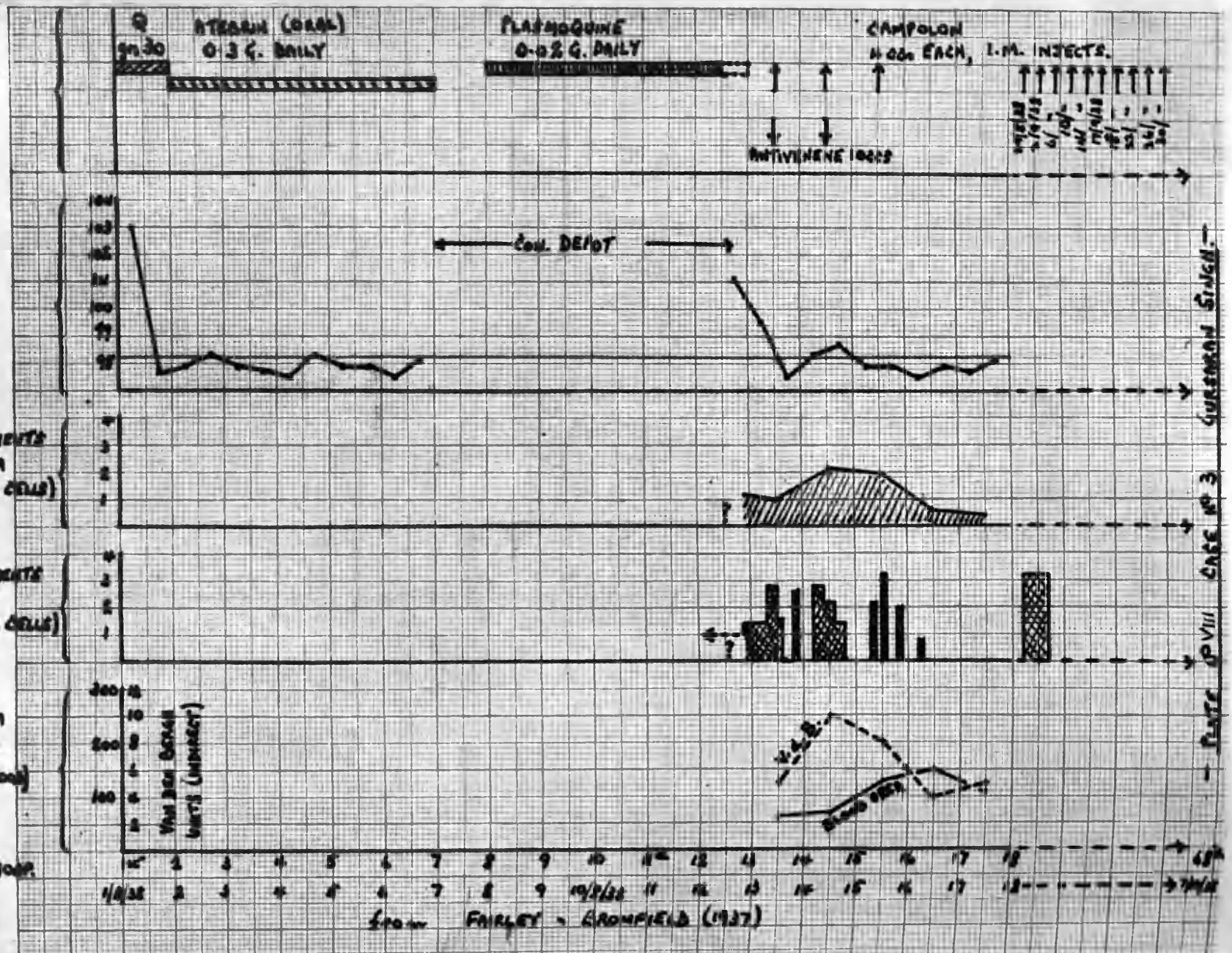
20/8/1938: Urine normal in appearance and content, and concentrating function of kidney also apparently normal (Fishberg's test). Campolon injections continued.

17/9/1938: Red cell regeneration slow. After 36 ccs. Campolon the blood count was very little changed. Campolon continued.

7/10/1938: Total 52 ccs. Campolon given. Blood picture and kidney function apparently normal. Patient discharged.

SUMMARY.

A series of haemolytic crises - at least eight in number - over six days. No permanent impairment of kidney function. The slow regeneration of red cells is not an uncommon finding in the Indian, who has to face so many demands on the haematopoeic system, due to chronic malaria, dysentery, etc.



From FAIRLEY & CROMFIELD (1937)

RESUME OF CASE RECORD - PATIENT NO. 4.

(detailed Case Report - Appendix, pages 767 to 774)

No. F.11, WATER CARRIER CHURU RAM.

3/17 DOGRAS - from THAL FORT.

Aged 35 years.

Service: 12 years.

Plate No. IX, page 76, summarises the treatment and the laboratory findings.

This patient was on the Kharre Column with Damcol - not Razcol - and therefore did not receive any prophylactic atebrin. On the return of Damcol to Damdil from Kharre this patient, in a two-company detachment of the 3/17 Dogras moved straight through to Thal Fort without halting at Damdil. Thal Fort was a small isolated strongpoint covering a vulnerable part of the Razmak-Bannu Road. Like Razmak, it was regarded as non-malarious. Quite often, and at the time that this incident took place, movement forward to Razmak from Thal was safer than movement back to the base at Bannu. All sick were evacuated, therefore, forward to Razmak instead of going back to Bannu. This evacuation of sick and casualties could only take place when the road was "open" to Razmak - not more than once weekly or once fortnightly at that time.

28/7/1938: Patient felt ill in Thal Fort with rigors and fever.

Stated that he never had any disease resembling malaria. Medical records showed that he had not had any illness during his twelve years of Army service.

29/7/1938: Reported to the Medical Officer in the Fort and was given such treatment as was available. Diagnosis of malaria was not made.

29/7/1938 to 1/8/1938: Kept in the Fort awaiting transport to Razmak. Had two rigors on alternate days. Clinically at that time the condition seems undoubtedly to have been malaria.

2/8/1938: Transferred to Combined Indian Military Hospital, Razmak.
Much underweight (99 lbs.). Blood films showed P.vivax rings and trophozoites. Spleen not palpable.
Diagnosis - fresh benign tertian malaria.
Given quinine for one day and then given a five-days course of atebrin, 0.3 grammes daily, finishing on 7/8/38.

8/8/1938: Transferred to the Convalescent Depot to complete treatment. Given one day's rest from treatment, then started on plasmoquine 0.02 grammes daily, as

an out-patient from the Convalescent Depot.

11/8/1938: (i.e. after two days plasmoquine 0.04 grammes total) Was found to be jaundiced and was re-admitted to hospital. This jaundice persisted without any change in degree for several days, but the urine remained normal until 14/8/38. There was marked tenderness of the liver and the spleen during this time and the patient also had a low-grade fever. The condition strongly resembled blackwater fever, sine black water, and the patient was treated as a definite case of the disease, i.e. hot packs to the loins, careful balancing of fluid loss and intake as far as possible with alkaline solutions.

14/8/1938: For the first time since re-admission four days previously the patient voided a specimen of red urine. The plasma was found to be pink-stained, due to blood-pigment; the red cell content was 2,350,000 per cu. mm.

14/8/1938 to 16/8/1938: On these three days the urine gave indications of four moderately severe phases of intra-vascular haemolysis with an indirect positive van den Bergh reaction. The urine showed absorption bands of oxyhaemoglobin and methaemoglobin, with casts and

masses of amorphous and granular blood pigment. Injections of Campolon began on this day, with continuation of the routine measures.

16/8/1938: Red cell count still low and icteric tinge still present. The last haemolytic phase occurred at 22.00 hours. From then on the urine was normal in colour, although it did not become clear of casts and blood pigment until 20/8/38.

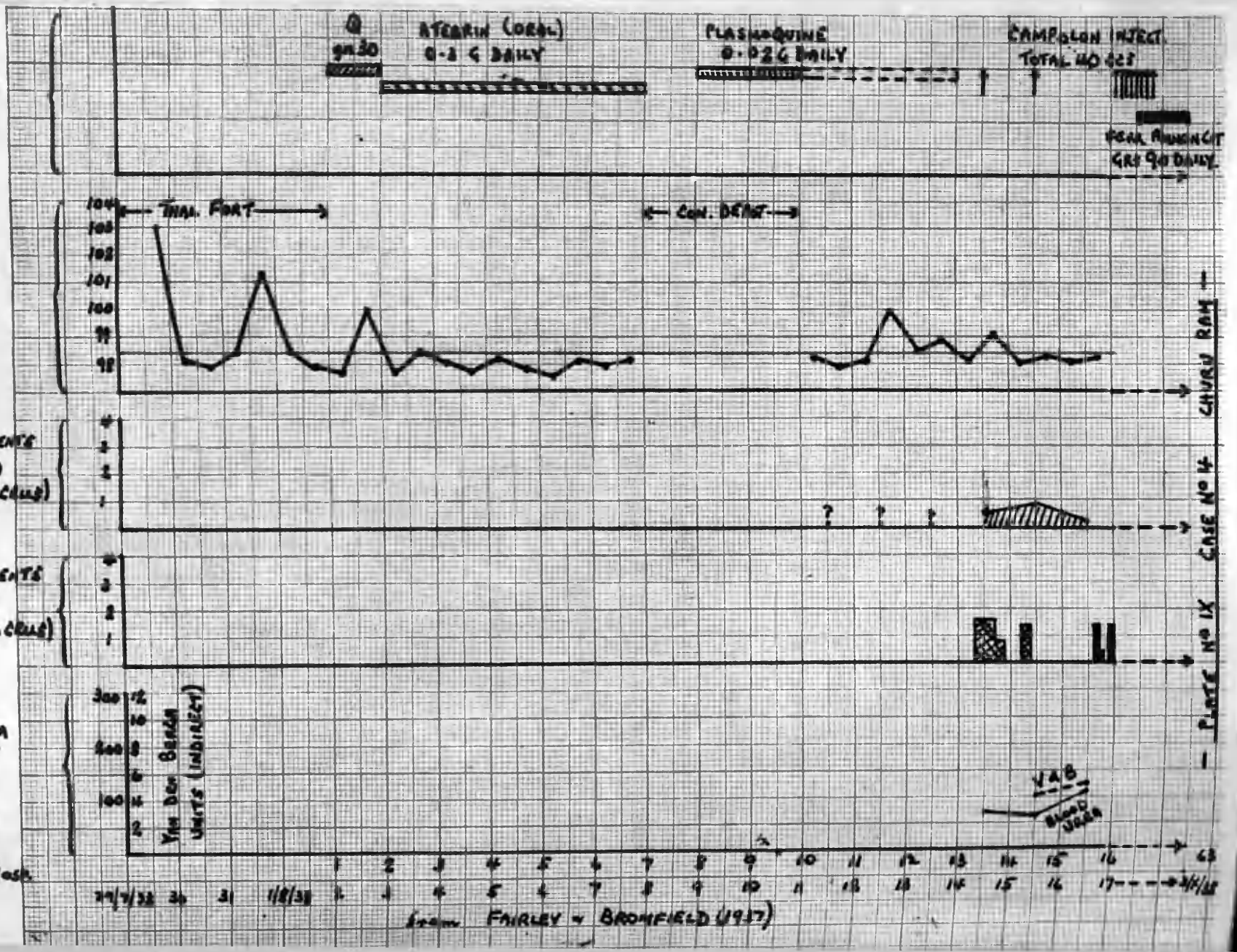
20/8/1938: Urine clear of blood-pigment masses and casts; urinary function normal as measured by Fishberg test. Icteric tinge and abdominal tenderness gone. Hot packs and alkaline mixture discontinued; Campolon injections continued.

18/9/1938: Since 14/8/38 the patient had received 40 ccs. intramuscularly of Campolon; blood picture almost normal now; Campolon stopped. Ferr. et ammon. citrate begun.

3/10/1938: Sent on leave as cured - weight 114 lbs.

SUMMARY.

A series of haemolytic crises - the first few not severe enough to produce haemoglobinuria, but the last four did.



from FAIRLEY & BROMFIELD (1937)

RESUME OF CASE RECORD - PATIENT NO. 5.

(detailed Case Report - Appendix, pages 774A to 781)

No. 5949. SEPOY DUNI.

3/17 DOGRAS, THAL FORT.

Aged 26 years.

Service - 7 years.

Plate No. X, page 82, summarises the treatment and laboratory findings.

With CHURU RAM (Case No. 4) this patient was on the Kharre Column with Damcol, not Razcol, and therefore did not receive any prophylactic atebrin. Also like CHURU RAM, Sepoy DUNI was in the two-company detachment of the 17th Dogras that went straight to Thal Fort for duty without halting at Damdil Camp. Details of Thal Fort are given on page 72.

25/9/1938: i.e. eight weeks after the Kharre Column, Sepoy DUNI fell ill in Thal Fort with fever and rigors. No previous history of any illness like malaria during his seven years service in the Army. (This was confirmed by his Army record sheet.) Patient also stated that he had never had malaria in civil life. He was detained in the Fort sick quarters until he could be transferred to Razmak for treatment. He was kept waiting only two days.

27/9/1938: Transferred to Combined Indian Military Hospital, Razmak.

His condition clinically was malaria, and the blood films showed ring forms, P.vivax. Spleen not palpable. Diagnosis - Fresh benign tertian malaria.

The patient was underweight, 119 lbs. (5 ft. 4 ins) and had a haemoglobin level of 90%. He was a vegetarian.

The only other abnormalities found were mild bronchitis and pharyngitis.

Heavy doses of Ferr. et ammon. citrate begun at once and continued throughout the stay of the patient in hospital. The malarial attack was difficult to control. Quinine, grains 30 daily, was given for four days before the symptoms abated and the fever settled.

1/10/1938: Atebrin course given 0.1 grammes three times daily for five days. Atebrin finished 5/10/38.

6/10/1938: Patient due to be transferred to the Convalescent Depot to complete his remainder of treatment as an out-patient, but his general condition was unsatisfactory, although he showed nil definitely abnormal. He was retained in hospital to complete

the plasmoquine course. Given one day's rest.

7/10/1938 to 10/10/1938 (Noon) Patient had completed 3½ days of the five days plasmoquine course (i.e. he had had 0.07 grammes of plasmoquine) when he complained of his urine being "high coloured". He would not admit that it was pink or red. He was not markedly jaundiced. The patient had been in bed throughout his stay in hospital.

10/10/1938: (12.00 hrs) Passed urine containing bile .

Examination showed fast pulse with a rising temperature, 100.5°F. at 18.00 hrs. Jaundice present; no cyanosis; no abdominal pain; spleen one finger enlarged. Blood count not estimated, plasma not examined. Blood films negative for ? malaria parasites. Findings strongly in favour of mild intra-vascular haemolysis without frank haemoglobinuria. Plasmoquine stopped.

11/10/1938: At 06.00 hours patient passed Burgundy-coloured urine; jaundice ++ with conjunctivae orange in colour. The liver area was tender; marked fall of the red blood cells to 2 million per cu.mm. with reticulocytes 15%; plasma red in colour; the urine showed absorption bands oxyhaemoglobin and methaemoglobin with a few hyaline and granular casts.

Obviously another and more serious haemolytic crisis had occurred at some time during the night. During this day the urine varied, red-orange-red-orange, suggesting other lyses. The output of urine was small in amount each time.

Diagnosis - blackwater fever, with repeated lyses, with oliguria and danger of anuria.

Treatment: as previously, namely hot packs to the kidney areas (also tried hot retention enemas). Fluid intake and urine output records kept. Glucose alkaline ½ pint every hour by mouth. Also given injections of Campolon and antivenene.

12/10/1938: In the previous twenty four hours the fluid intake was 100 ozs., the output only 14 ozs. Jaundice unchanged; liver less tender; plasma light red; urine port-wine first sample; throughout the day the colour of the urine again suggested repeated haemolyses.

Treatment as before including Campolon and antivenene.

13/10/1938: Fluid intake 66 ozs., output 20 ozs. in the previous twenty four hours. Jaundice slightly less; liver tenderness less marked; plasma pink colour; first specimen urine red in colour, fading

through pink in succeeding specimens until 16.00 hours, when the urine became yellow. Thereafter no obvious pigment staining of the urine. Treatment as before, including Campolon 4 ccs.

14/10/38: Fluid intake 60 ozs., output 33 ozs. Jaundice unchanged; no liver tenderness; plasma and urine colour normal; casts and haemoglobin masses in the urinary deposit.

15/10/1938: Improvement continuing; no haemoglobinuria; Campolon 4 ccs. given.

17/10/1938: Urine clear of casts and pigment masses; function not tested. Began marmite 1 oz. daily - continued until the patient was discharged - jaundice fading.

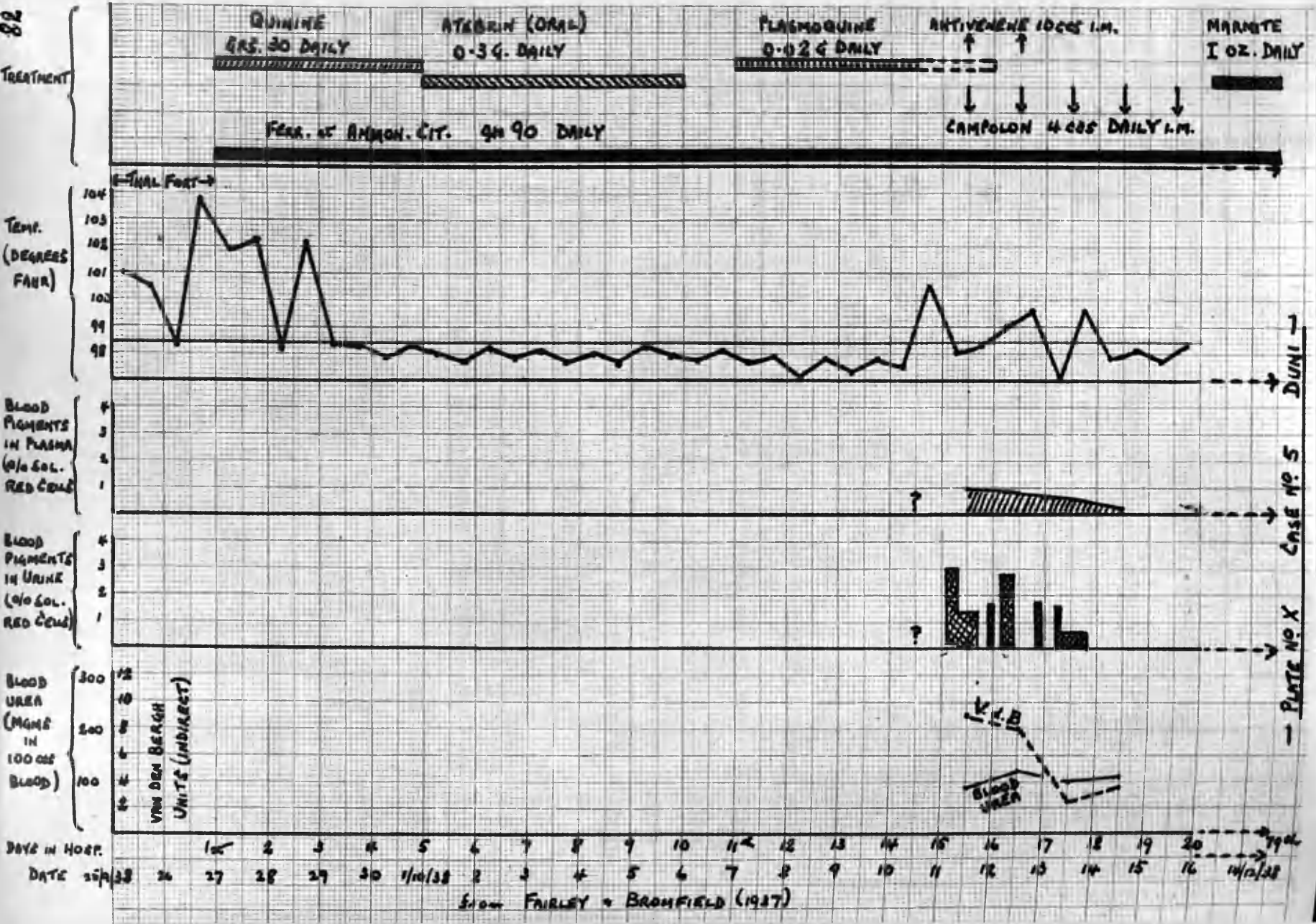
4/11/1938: Some subjective improvement; no jaundice; red cell count 3½ million cells per cu.mm.

27/11/1938: Patient very fit; red cell count 4.9 million.

14/12/1938: Discharged as cured. Weight 127 lbs. Red cell count 5.1 million. Blood pressure 120/80.

SUMMARY.

Patient suffered from a series of at least six haemolytic crises in four days. The first lysis was not sufficient to produce haemoglobinuria. No residual kidney abnormality.


TREATMENT
QUININE GRS. 30 DAILY
ATEBRIN (ORAL) 0·3 G. DAILY
PLASMOQUINE 0·02 G DAILY
ANTIVENENE 100cc I.M.
MARMITE I OZ. DAILY
FERR. ET AMMON. CIT. gr 90 DAILY
CAMPOLON 4 cc DAILY I.M.
TEMP. (DEGREES FAHR)
104
103
102
101
100
99
98
TRIAL FORT
DUNI 1
BLOOD PIGMENTS IN PLASMA (% SOL. RED CELLS)
BLOOD PIGMENTS IN URINE (% SOL. RED CELLS)
CASE No. 5
CASE No. X
PLATE No. 1
BLOOD UREA (MGMS IN 100 cc BLOOD)
300
200
100
VAN DEN BERGH UNITS (INDIRECT)
BLOOD UREA
DAYS IN HOSP.
DATE 1938
27 28 29 30 1/11/38 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
26 27 28 29 30 31 1/12/38
from FAIRLEY & BROMFIELD (1937)

RESUME OF CASE RECORD - PATIENT NO. 6.

(detailed Case Report - Appendix, pages 782 to 788)

SEPOY TARA CHAND.

3/17 DOGRAS, DAMDIL CAMP.

Aged 23 years.

Service 5 years.

Plate No. XI, page 88, summarises the treatment and the laboratory findings.

It is to be stressed that this patient was from the same Regiment as the two previous cases - Water Carrier CHURU RAM and Sepoy DUNI. Unlike them, however, Sepoy TARA CHAND did not go forward to Thal Fort after the Kharre Column. With the remainder of the battalion he returned from Kharre to Damdil Camp, where he continued to serve until he became ill.

It has been said in the early part of this chapter that no malarious cases from Damdil Camp were evacuated to Razmak Hospital, being treated in the Camp itself. This is true. This particular patient, TARA CHAND, was evacuated to Razmak, however, because he was supposed to be suffering from fever - cause unknown. Clinically it was quite unlike malaria and required full laboratory investigations, which could not be done at Damdil Camp.

The patient had never had malaria in the Army and claimed that he had never had it in civil life. The patient was found to be suffering from malaria, however. Occasionally fresh malaria would develop amongst troops in Damdil which was in a mildly malarious area. This introduced the probability that TARA CHAND contracted his malaria at Damdil Camp, not during the Kharre Column. The detailed history of the illness is:

27/9/1938: Eight weeks after the finish of the Kharre Column, Sepoy TARA CHAND was admitted to the temporary camp hospital at Damdil with a complaint of rigors, fever and shivering during the previous seven days. Plate No. XI, page 88, shows the course of his illness after admission. Clinically the condition did not resemble malaria, the fever being high and continued. It was decided, therefore, to evacuate the patient forward to Razmak for fuller investigation. This was done.

5/10/1938: Transferred to Razmak Hospital, by which time he had been ill for fourteen days with fever of unknown origin. On admission to Razmak Hospital the patient was free from fever. He was much underweight (99 lbs., 5 ft. 2 ins.). His red cell picture and his haemoglobin were both normal; his blood pressure was 110/75. The spleen was

enlarged two fingers and the blood films showed sexual and asexual forms of P.vivax. Nil else to report.

Diagnosis - benign tertian malaria (?)fresh.

Began Ferr. et ammon.citrate 90 grains daily. This was continued until the discharge of the patient.

NO QUININE GIVEN.

5/10/1938 to 9/10/1938 Atebrin course given 0.1 grammes three times daily for five days.

10/10/1938: Patient was due to be transferred to Convalescent Depot but his general conditinn was sub-standard. Therefore he was retained in hospital to complete the plasmoquine course as a bed patient. Given one day's rest.

11/10/1938: to 13/10/1938: In these three days the patient completed the first three days of his plasmoquine course, taking in all 0.06 grammes of plasmoquine, apparently without harm.

14/10/1938: Before the morning dose of plasmoquine was given the patient complained of weakness. Did not report any change of the colour of his urine. Plasmoquine stopped.

Re-examined 11.00 hrs. Pulse fast; no jaundice;

no cyanosis; no abdominal pain; spleen not palpable; blood pressure 110/70. Blood films negative for malaria parasites; blood count little changed; red blood cells 5 million; haemoglobin 90%; reticulocytes 2.5%. Plasma faintly tinged with pink, with faint absorption bands of haemoglobin; methaemoglobin bands not seen.

12.00 hrs. urine red in colour with absorption bands both oxyhaemoglobin and methaemoglobin; thereafter specimens normal in colour until 21.00 hrs. when urine was again red in colour; marked oliguria. Thereafter all specimens normal in colour. Pigment masses and casts present in the urine until 17/10/38. Treatment as before, i.e. hot packs to the loins; fluid output and intake chart; glucose-alkali and soda water alternately each hour, Campolon 4 ccs. daily and antivenene 4 ccs. daily.

16/10/1938: Patient felt improved. Pulse 88 per min. No jaundice; fluid intake 133 ozs., output 100 ozs. Blood films negative; plasma not examined. No biochemical tests carried out. Urine normal in colour since the previous day.

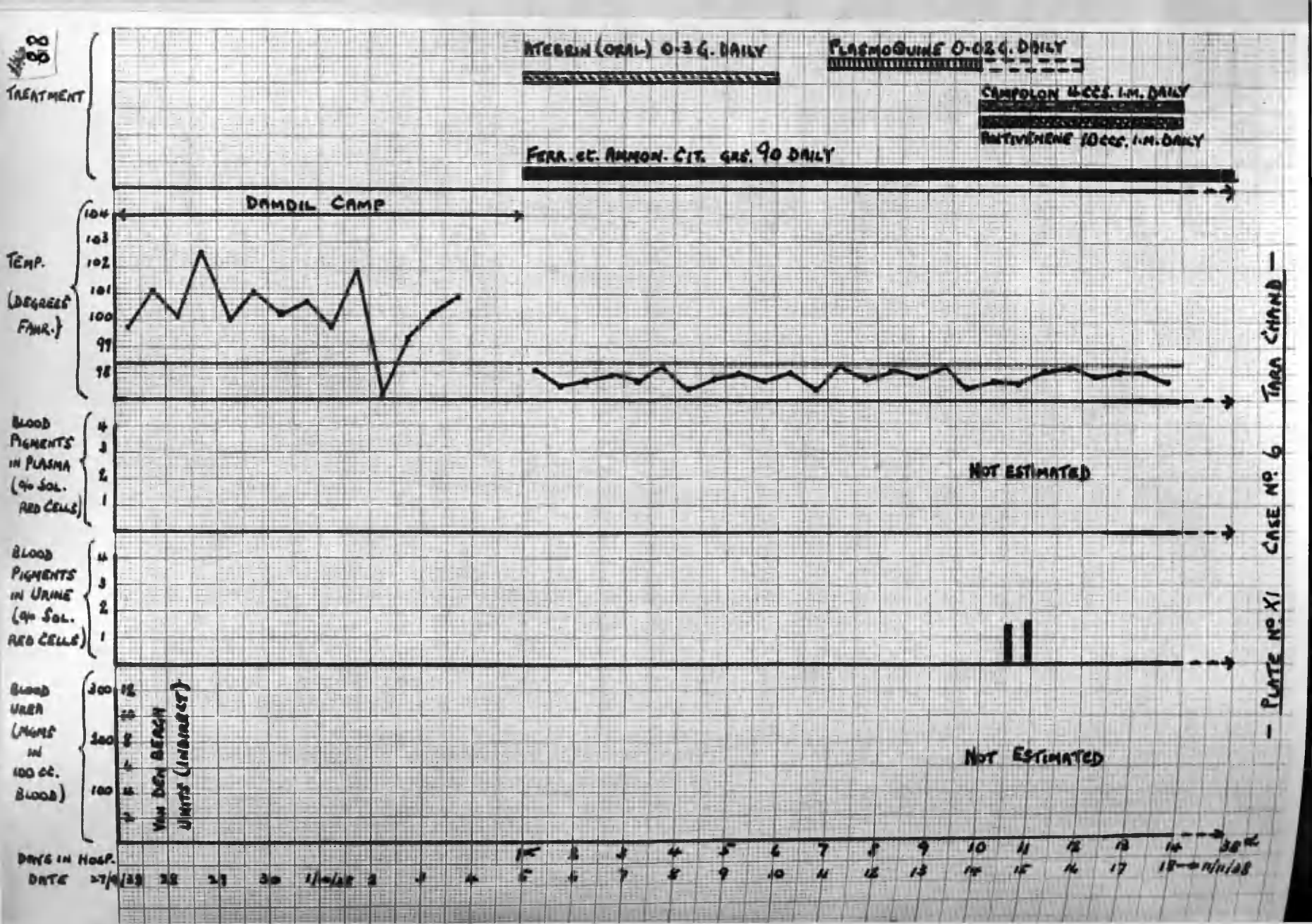
17/10/1938: Patient felt much improved; T.P.R. normal. urine free from casts and haemoglobin masses. All treatment stopped except the iron tonic. Patient had received 16 ccs. Campolon and 40 ccs. antivenene.

25/10/1938: Still felt weak, but blood picture satisfactory. Red blood count 5,300,000 per cu.mm. Haemoglobin 108%; blood pressure 115/78.

11/11/1938: Fit for duty. Weight 110½ lbs.

SUMMARY.

This patient's urine was coloured red on two occasions only, namely, 12.00 hrs. and 21.00 hrs. on 14/10/38. Lysis was mild in degree and in its effect on the red cell count. There was marked polyuria immediately after the attacks.


TREATMENT
NEOSALVARSAN (ORAL) 0·3 G. DAILY
PLASMOQUINE 0·02 G. DAILY
CAMPOLON 4 CCS. I.M. DAILY
ANTIVENENE 10 CCS. I.M. DAILY
FERR. ET AMMON. CIT. GRS. 90 DAILY
DAMDIL CAMP
TEMP. (DEGREES FAHR.)
104
103
102
101
100
99
98
BLOOD PIGMENTS IN PLASMA (% SOL. RED CELLS)
4
3
2
1
NOT ESTIMATED
BLOOD PIGMENTS IN URINE (% SOL. RED CELLS)
4
3
2
1
BLOOD UREA (MGMS IN 100 CC. BLOOD)
300
200
100
VAN DEN BERGH
UNITS (LINDEMEYER)
NOT ESTIMATED
DAYS IN HOSP.
DATE
27/8/28
28
29
30
1/9/28
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
n/n/28
38
TARA CHAND
CASE No. 6
PLATE No. XI

CONSIDERATION OF THE FINDINGS RECORDED IN PAGES 57 TO 88 i.e. THE FINDINGS IN THE SIX PATIENTS WHO DEVELOPED BLACKWATER FEVER IN RAZMAK IN 1938

On pages 57 to 87 a summary is given of each of the six patients who developed blackwater fever in Waziristan in 1938. The full case reports of each case are to be found in the Appendix, pages 734 to 788. Certain of the findings will be considered in Chapters III and IV. Other points requiring consideration are dealt with below.

Preliminary consideration must be given to factors which enter into all diseases in natives of India, namely race, religion, etc.

Briefly, to recapitulate, in the period July to September, 1938, there were admitted to hospital in Razmak large numbers of individuals suffering from malaria. Six of these individuals - all natives of India - suffering from malaria, during treatment for that malaria developed haemoglobinuria. One was a follower and the other five were soldiers. The highest rank amongst them was Lance-naik (Lance Corporal). Four of the patients were in their twenties, two were aged 35. One was a Sikh, two were Mussulmans, and three were Hindus. All of these patients had been in the Kharre Column, three with Razcol and three with Damcol. It is to be

noted here that on the Kharre Column Razcol consisted of one battalion Ghurkas, one battalion British Infantry, one battalion Rajputs, and one battalion of the Frontier Force Rifles. Also a high proportion of the officer class were Europeans. The composition of Damcol was equally mixed, except that there was no British Infantry battalion.

With this in mind we must consider more fully -

(1) RACE:-

The term "race" here also includes the sub-divisions by religion, since these two are often intimately co-related in India. The question of race is an important one from the point of view of diet, previous exposure to possibility of blackwater fever etc. None of the six patients were Europeans; their composition is given above. In the large number of men at risk the laws of chance are such that no significance can be placed on this combination of cases, and this question of race will, therefore, not be further discussed.

(2) DIET:

One patient was a vegetarian, the others were meat eaters. This is not significant.

(3) AGE:-

Four of the men were in their twenties, and two were aged 35. This age distribution also is not significant.

(4) PHYSIQUE:-

All patients were under-weight - four markedly so, but it is doubtful if this also is of any significance, as a very large number of equally sub-standard individuals, e.g. followers, had, and later did have, the same course of treatment without ill effects. Therefore this factor, per se, could not have played an all-important part.

(5) RANK:-

It is admitted that all the men were private soldiers except one Lance-Corporal. Again, this cannot be regarded as significant in any way. Such a distribution is well within the limits of chance.

One other factor of major importance is the question of the drugs themselves and the control of their administration. In 1938, at the time of the occurrence of this "outbreak", the attitude of the Army Medical Service towards atebrin and plasmoquine was that they were highly expensive drugs with powerful side-effects. The most stringent control was enforced in the administration of these drugs. All plasmoquine and atebrin in use in Razmak Hospital was stored in air-tight coloured bottles and kept in locked poison cupboards in my office. Tablets were administered to patients by a sub-assistant surgeon, who made sure that each man swallowed his

tablet. A register was kept of every individual receiving treatment. This register was checked frequently to ensure that there was no mis-use of drugs. The quinine solution was also checked frequently to ensure that it had not been diluted. These elementary details of hospital routine are recorded here to forestall any criticism that the patients did not receive the drugs, or that they may have received an over-dosage. Each patient received the treatment recorded in his case sheet.

The next point in the discussion of the drugs used is a possibility of an increased toxicity. This will be discussed more fully in the chapter dealing with the various drugs, i.e. Chapters VI, VII and VIII. It is merely to be noted here that at no time was there any obvious sign of colour change or deterioration in the atebrin and plasmoquine tablets, and the incidence of toxic effects was not higher during the period July-September than at any other time.

The atebrin used in the six cases came from three separate batches of the drug. The plasmoquine used came from two separate batches. One of the patients (No. 3) received 0.04 grammes plasmoquine from one batch and 0.05 grammes from the second. Some tests were carried out with the last batch of each drug to attempt to detect any clinical sign of

increased toxicity. These tests will be mentioned in a later chapter. They all gave negative results. The agent in India to the makers (Bayer & Company) was then approached and arrangements were being concluded for a conference on the question of carrying out tests in their laboratories in Germany. By this time (1939) the clouds of war were gathering and the tests had to be abandoned.

It is pointed out that one patient (No. 6) did not receive quinine.

DISCUSSION ON SYMPTOMS AND SIGNS.

In any discussion on intra-vascular haemolyses certain of the signs are so important that they require to be dealt with in full. Such include the blood picture, including blood pigment, van den Bergh reaction, blood urea, etc., character of the attack or attacks of intra-vascular haemolysis, jaundice, and abnormalities of urine output and contents. These are discussed in Chapters IV and V. Colic and cyanosis are discussed in Chapters VI to VIII.

Some information may be obtained from a consideration at this point of the findings on physical examination of our patients at the time of the intra-vascular haemolyses. Consider first, temperature. With the exception of Case No. 6, who suffered only in very mild degree, each patient at the

time, or in close relation to their lytic phase, developed intermittent pyrexia. The degree of temperature rise bore a rough direct relationship to the degree of haemolysis. Repeated haemolyses showed repeated rises in temperature. In our treatment of the first patient the common mistake was made of presuming that the rises in temperature were caused by persistent malaria, which, if present, also could have been regarded as a part factor in the production of the accompanying lysis; we were never able however to find any parasite in the blood films taken from the patient at the time of haemoglobinuria, and it is now realised that the temperature rises were due to haemolysis, not malaria. Our mistake - as it undoubtedly was - led to the first patient being given 0.3 grammes intramuscularly of atebrin musonate daily on each of the first three days following the re-admission of the patient to hospital. This treatment failed to influence the fever. It is not thought that this treatment played any part in the fatal issue of this case. It was then realised that the fever was one effect of the haemolysis, and accordingly no anti-malarial drugs were administered to any of the remaining patients after they had developed signs of intra-vascular haemolysis.

LIVER:- was not enlarged in any patient, and was tender at the onset of haemoglobinuria in only one case (Patient No. 4),

SPLEEN:- at the onset of black water was enlarged in two cases, (Nos. 5 and 6), and was tender in one case (No. 4). These abnormalities rapidly disappeared.

TREATMENT USED IN THE BLACKWATER FEVER CASES:-

The treatments recommended for blackwater fever are like the theories about its causation - they are numerous and generally of little value. If, as is usual at the time of the onset of blackwater, there is not present malaria severe enough to require administration of anti-malarial drugs, then there is no specific treatment for the black water. The condition is an intra-vascular haemolysis which does not differ, basically, in symptomatology and pathology from other intra-vascular haemolyses, e.g. incompatible transfusion.

The two most important problems arising with intra-vascular haemolyses are, firstly, anaemia; and secondly, changes in the kidney and its functions. Anaemia may be of such degree as directly to kill a patient. Anything up to four-fifths of the red cells may be destroyed within twenty four hours in a severe attack of lysis or in repeated attacks; and secondly as regards the kidney changes these are of prime importance since it is estimated that in blackwater fever fifty per cent of the deaths, i.e. about ten per cent of those attacked, are related to kidney damage.

Consider the anaemia. In a disease in which the

red cell count can fall rapidly and readily to values as low as or even lower than one million cells per cu.mm., the anaemia itself may be sufficient to kill the patient. This grave red cell loss must be made good, even at the risk of precipitating a further haemolysis, even although - as some workers suggest - the transfused cells from the donors are sometimes destroyed rapidly by the recipient patient. The problem of what happens to the red cell and its contents in blackwater fever haemolysis is a fundamental one. It requires to be studied in detail, and this will be done in Chapter IV. All that need be said here is that transfusion is not without risk, and should not be lightly undertaken, but in certain cases with severe anaemia, where the patient's life is in danger, risks must be taken and blood must be given.

The next question is that of changes in the kidney and in its functions. The problem here is two-fold, namely, treatment aimed at preventing kidney lesions, and treatment of established lesions of the kidney. In view of recent work, particularly the series of papers by Trueta and others, summarised in Chapter V, these two problems are not necessarily the same thing and, as with the problem of anaemia, can only be dealt with rationally following upon a consideration of the physiology and pathology of the kidney; these functions of the kidney are discussed in Chapter V.

SUMMARY, CHAPTER II.

By 1938 the Army course of treatment for the usual type of malaria attack had become standardised to seven days atebrin 0.3 grammes daily, three days rest, five days plasmoquine 0.02 grammes daily. Due to press of circumstances two modifications of this were introduced in Waziristan in July 1938; these were, firstly a cutting-down of the atebrin to a five days course, with a cutting-down also of the rest period to one day instead of three; and secondly the establishing of a temporary convalescent depot to which all suitable patients were transferred to complete their treatment after they had been five days afebrile. This meant that the malaria patients generally received the whole of the plasmoquine course as out-patients. The convalescent depot was under medical command and was only two hundred yards from the hospital. These temporary modifications came into operation at the beginning of July 1938 and were in use at the time the six individuals developed haemoglobinuria while undergoing treatment for malaria.

Pages 57 to 87 give summaries of the case records of each of the six individuals.

Perusal of the summaries shows:

(1) Only two of the six patients gave a history of having suffered from malaria previous to July 1938, namely

Case No. 1 who had had benign tertian malaria in July 1936 and clinical malaria in August 1936 both attacks being treated by the seven days atebrin, three days rest, five days plasmoquine course; and Case No. 2 who had had benign tertian malaria in July 1937, also treated by the seven days atebrin, three days rest, five days plasmoquine course.

In this connection it is to be remembered that Army medical documentation at that time was very carefully maintained. It is certain that none of the patients had had any malaria during the whole of their Army service, other than that recorded above.

In four of the patients, therefore, the attack of malaria following the Kharre move was apparently their first; certainly in all the four it was the first attack any of them had had since joining the Army. Only in Case No. 6 was the spleen enlarged, and that was after fourteen days of untreated malaria.

(2) Prophylactic atebrin had been given to three only of the six men; the other three were with the Damdil Column on the Kharre move and therefore were not among those given prophylactic atebrin.

(3) Three of the men first showed overt malaria in Razmak and two

in Thal Fort, both of which places were non-malarious areas; it is reasonably certain that these five had been infected during the Kharre Column; one patient, Case No.6, developed malaria in Damdil Camp after the move and it is admittedly possible that he was infected in Damdil which was mildly malarious.

(4) In all six patients the diagnosis was "fresh benign tertian malaria". The diagnosis was made by me and the blood-slide findings were confirmed by a member of the laboratory staff. After the development of haemoglobinuria the original slides were again examined; no trace was found of a mixed infection. These original slides were available fortunately because at that time all malaria slides were being kept until each patient had completed his treatment.

(5) Five of the patients received quinine; all received a full five day course of atebrin; and all were still receiving plasmoquine at the time of onset of the haemoglobinuria, excepting case No. 2.

(6) Four of the six patients, the first four, had been transferred to the Convalescent Depot after completion of the atebrin course and were receiving plasmoquine as out-patients at the time they developed haemoglobinuria. The remaining two patients were bed-patients in hospital at the onset of the black water.

(7) In each patient the number of attacks of lysis was multiple excepting Case No. 6, who had two attacks.

(8) Only one patient was given blood transfusions; he received two; the subsequent course strongly suggested that at least a large number, if not the whole of the transfused normal red cells were also destroyed in phases of lysis following each transfusion.

(9) One patient developed anuria from which he died.

An analysis was made of various factors which may have been of importance, e.g. race, religion, age, rank, dietary habits, etc. Nil significant was found. The distribution of these various factors was such as might be found in any chance selection from the population at risk.

The next factor considered was that of the drugs used. The first point was whether the dosages reported in the case-sheets were what the patients actually received; it was shown that every precaution had been taken and that the drug dosages were almost certainly as stated. The second point was whether there may have been increased batch toxicity of any one drug. This was shown to be very unlikely.

Two other questions raised in this chapter were temperature and anaemia.

In each case the temperature was raised with each attack of lysis. At first this was thought to be a recurrence of the malaria but after the first case had failed to respond to anti-malaria therapy it was realised that the temperature was due to the haemolysis. All blood films taken at the onset of lysis in each individual were negative for malaria.

The question of treatment of anaemia was briefly dealt with. Transfusion should not be lightly undertaken but may be required to save life in certain cases.

Other important problems, e.g. kidney involvement, were left for fuller discussion in later chapters.

C H A P T E R III.

INTRAVASCULAR HAEMOLYSES.

This work is concentrated round the problem of intra-vascular haemolysis which developed in Razmak in 1938 in six of a large number of patients undergoing treatment for malaria. The impression has been given in the preceding chapters that the diagnosis was blackwater fever in each of the six patients. There are many and varied other causes of intra-vascular haemolysis and of conditions closely simulating intra-vascular haemolysis; it is necessary therefore to consider at this stage whether the diagnosis of blackwater fever is the likely one or whether some other condition more adequately explains the "outbreak". This consideration of other likely conditions may also be of value in that it may throw light on the problem of intra-vascular haemolysis in general.

So far the only definite finding reported in the six cases has been that of haemoglobinuria, which of course may be produced by diseases other than those causing intra-vascular haemolysis. French (1936) gives a list of causes of haemoglobinuria as follows:

1. Oral administration of substances such as potassium chlorate, ether, phenylhydrazine, turpentine, pyrogallic acid, naphthol carbolic acid and various other

acids, glycerine, carbon tetrachloride, sulphonal and allied substances, tannin, saponin, strychnine, urotropine, impure aspirin and possibly quinine.

2. Inhalation of certain gases, e.g. carbon monoxide, naphtha vapour, arseniuretted and other hydrogens, etc.

3. Incompatible blood transfusions (this should also include high-titre plasma transfusions).

4. Intravenous injections of normal horse serum and of other sera such as anti-tetanic serum, etc.

5. Poisons from snakes, toads, spiders.

6. Ricin, abrin, cortin, phthallin, etc.

7. Poisonous mushrooms, toadstools, truffles.

8. Frost-bite and extreme exposure to cold.

9. Severe burns.

10. Large internal extravasations.

11. In a few cases of pregnancy associated with toxaemic symptoms.

12. Certain functional disorders of the vasomotor system, e.g. Raynaud's disease, factitious urticuria, and angioneurotic oedema.

13. Long-sustained physical exertion and fatigue.

14. Microbial toxaemia, e.g. malaria, less commonly syphilis, typhoid, anthrax and yellow fever.

15. Henoch's purpura.

16. Certain cases of nephritis.

17. Paroxysmal haemoglobinuria.

The above list is incomplete and unsystematic. A better approach to the problem is given in Whitby and Britton (1946) in a discussion on the larger problem of haemolytic anaemias which these authors regard as a group where the essential pathological process is an excessive intra-vascular destruction of red blood corpuscles. It should be noted that haemoglobinuria may be a symptom of almost any haemolytic anaemia. The classification of Whitby and Britton is on a causal basis; a modified version of it is given below.

CAUSES OF HAEMOLYTIC ANAEMIAS.

A. Toxic, infective, or poisonous factors: (exogenous)

1. Infections: malaria, streptococcal and staphylococcal infections, C.welchii, oroya fever.

2. Poisons: lead, phenylhydrazine, sulpha drugs, sulphones, benzedrine, potassium chlorate

arsenicals, toluyldiamine and allied drugs, potassium, saponin, ricin, snake venom.

3. Allergy: favismus, Baghdad Spring Anaemia, drug idiosyncracies.

B. Due to haemolysins (endogenous)

1. Incompatible blood transfusions; (also to be included in this group are the reactions with high-titre plasma transfusions where the red cells of the recipient may be lysed.
2. Rhesus-factor incompatibility.
3. Paroxysmal haemoglobinurias; a. syphilitic type
   b. march haemoglobinuria.
   c. paralytic myoglobinuria.
4. Acquired haemolytic icterus (acquired acholuric jaundice).

C. Due possibly to an anomaly of the erythron, congenital or acquired.

1. Haemolytic icterus (congenital).
2. Sickle-cell anaemia (African anaemia).
3. Mediterranean anaemia (Cooley's anaemia).
4. Nocturnal haemoglobinuria.
5. Physical factors affecting the erythron, e.g. severe burns.

D. Of unknown origin.

1. Acute idiopathic haemolytic anaemia. (Lederer's anaemia)
2. Subacute or chronic idiopathic haemolytic anaemia.

3. Symptomatic haemolytic anaemia.

This classification is a great improvement on the one given by French although this too is incomplete; it is well to remember that, as recently pointed out by Loutit and Mollison (1946), there are many cases of anaemia apparently haemolytic in character which do not come into any of the above categories.

Certain of the above causes of haemoglobinuria must enter into the differential diagnosis of the six Razmak cases and will be considered in detail therefore. To facilitate reference this discussion will follow the order given above, although it is not the order of importance as likely causes in our cases.

Firstly:

A. Toxic infective and poisonous factors:

1. Infections: Obviously malaria is the most important of this class. It will be discussed separately in Chapter IX. Other diseases listed above are also capable of producing haemolytic anaemia, for example the haemoglobinuria sometimes found in gas gangrene and in certain cases with coccal infection.

There was no trace of any infection other than malaria in the cases under

consideration, and accordingly the other infections do not require to be considered further.

2. Poisons. This is an unsatisfactory group; the list given above is very incomplete; there are many toxic substances which have not been mentioned. For example, there is no mention of the anti-malarial drugs although they are so important that Chapters VI, VII and VIII have been devoted to them. Another criticism of this portion is that it classifies together poisons which produce their effects in different ways; some produce anaemia by toxic effects on bone-marrow function; some drugs such as phenyl-hydrazine appear to have a direct destructive action on the red corpuscle; certain others act in some way or another to render the red cell more susceptible to ordinary destructive processes, well seen in chronic lead poisoning in which the abnormal red cells are destroyed in large numbers by the ordinary processes.

This question of poisons as a possible cause of the haemoglobinuria in our patients is one that had to be very carefully investigated. In India the great mass of the population is very ignorant, with a touching faith in the efficacy of drugs; furthermore there is no real control of the sale of any drug, no matter how dangerous; and, again, haemolytic drugs, e.g. the anthelmintics are freely used in every-day medical practice. Consequently in any case of haemoglobinuria developing in a patient in India, the possibility of self-medication must be seriously considered. Sinton (1936) gives a striking example of this. This possibility was always in our mind. Fortunately in Razmak the few traders allowed to live in the Camp were under military rule; and one instruction rigorously enforced upon them was our control over everything sold by these merchants. They were not permitted to sell any drug with any toxic properties. In 1938 I

was in medical charge of the bazaar shops. and am certain that our patients, no matter how anxious, could not have obtained any dangerous drug from the bazaar; the only other source of haemolytic drugs was the hospital itself. A description has already been given of the care taken to prevent unauthorised use of atebrin and plasmoquine and the same remarks apply to all other drugs with toxic properties, especially the sulpha group of drugs and the anthelmintic drugs which were in such demand in the open market. Both the sulpha group and the anthelmintic drugs were classed as poisons and were guarded as such. With such controls in operation it is reasonable to presume that the only drugs taken by the six patients were the drugs recorded in the case sheets. The compounds particularly important as regards haemolysis are quinine, plasmoquine and to a lesser extent atebrin; these are discussed in Chapters VI - VIII.

This mention of quinine opportunely leads to the next section, namely

3. Allergy: The first two types of allergy mentioned as capable of producing haemoglobinuria are favism (fabism) and Baghdad Spring Anaemia. The most important, drug idiosyncrasy, is left to the last. Neither favism nor Baghdad anaemia played any part in the production of disease in the six Razmak cases and it need only be said that both of these conditions are examples of a haemolytic anaemia due to acquired sensitivity to certain vegetables and pollens; in Baghdad anaemia the antigen is Verbena hybrida; and in favismus, which is fairly widespread on the Mediterranean littoral and in America the afflicted individual is sensitive to Vicia fava the bean plant; severe reactions have been reported even from inhalation of the pollen of the flower; some cases have proved fatal, with jaundice and with haemoglobinuria. It is proper here to ask whether favismus may reasonably be considered as a possible alternative diagnosis in the much-discussed cases quoted by Hippocrates. The last of the allergic conditions, and by far the most important, is that of drug

allergy. Allergy in general and drug allergy in particular will enter repeatedly into our discussions on the likely causes of the haemoglobinuria in the six Razmak patients. This is a suitable place, therefore, for a short preliminary discussion on the subject of drug idiosyncrasy; the particular type of drug hypersensitiveness to be discussed is allergy to sulpha drugs which is chosen because of the importance of this group and because this allergy is typical of drug allergies in general. It has already been pointed out that there is no suspicion that these drugs played any part in the causing of the disease in the Razmak cases.

Whitby and Britton, above, classify sulpha drugs under section 2, that is "poisons". Undoubtedly these drugs do have toxic side-effects but the important and dangerous reactions, e.g. haemoglobinuria, are manifestations of drug hypersensitiveness and are therefore discussed here.

In any discussion on drug hypersensitivity it is to be kept in mind, as Zinsser et alia (1944) have pointed out, drug idiosyncracies

are truly immunological forms of hypersensitiveness in that "..... antigenic compounds specifically oriented by non-antigenic groups may be formed in the body by combinations of the extraneous material with body protein". That is, the drug acts as a haptene.

Work on the sulpha drugs began following the introduction of prontosil by Domagk in February 1935. Almost immediately Trefouel et alia (1935) showed that the active principle was para-amino-benzene-sulphonamide; this was supported by Colebrooke et alia (1936). Since that time over three thousand new compounds have been produced from the parent molecule of sulphonamide. Of all these only a very few have been successfully utilised in treatment.

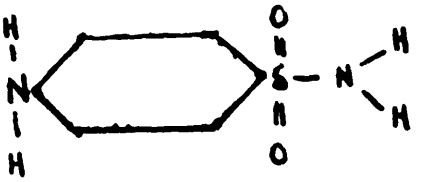
Five derivatives will be considered here, namely: sulphanilamide, sulphapyridine, sulphathiazole, sulphadiazine and sulphaguanidine; there are newer compounds of importance but information on them is insufficient to allow of discussion. In all these active compounds the effective nitrogen or nitro or amino or substituted amino group is in the

para position in the benzene ring. It is necessary to mention the chemical make-up since this furnishes an example of the remarkable way in which minor modifications in the molecule may markedly influence drug reactions. The five graphic formulae are given on plate No. XII, page 114. All of these substances are foreign to the body and are chemical poisons; the body therefore attempts detoxification; one stage of this process is acetylation. This has a bearing on one of the earliest recorded toxic effects of sulpha drug administration, namely haematuria which sometimes is followed by anuria; sulphapyridine and sulphathiazole are especially dangerous in this respect whereas sulphanilamide practically never shows this particular side-effect. The effect is a true toxic one and should more properly be discussed under section 2 above, but is dealt with here for comparison with other similar complications which are truly allergic in origin, e.g. haemoglobinuria which also may be followed by anuria. The explanation of the haematuria and the occasional related

PLATE No. XII

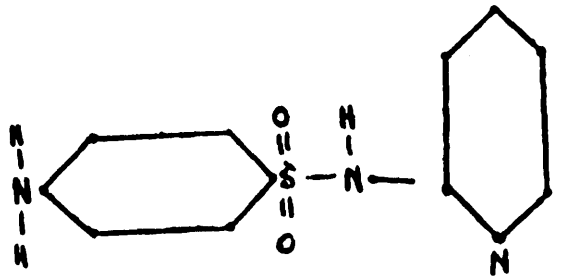
GRAPHIC FORMULAE OF CERTAIN OF THE SULPHA DRUGS.

SULPHANILAMIDE



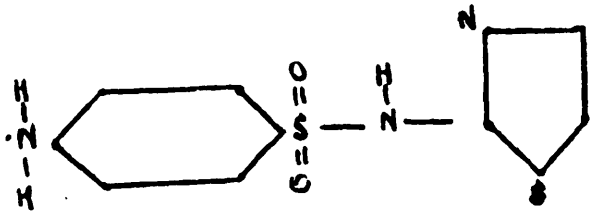
SULPHAPYRIDINE

i.e. 2 (PARA-AMINO-SULPHA) PYRIDINE



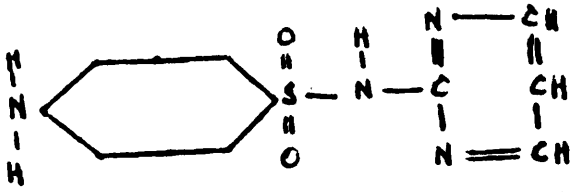
SULPHATHIAZOLE

i.e. 2 (PARA-AMINO-SULPHA) THIAZOLE



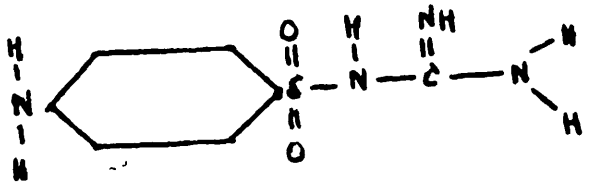
SULPHADIAZINE

(2 SULPHANILAMIDOPYRIMIDINE)



SULPHAGUANIDINE.

(SULPHANILYL GUANIDINE)



anuria is that in the process of acetylation crystalline compounds are formed, e.g. acetylsulphapyridine crystals, which have a low solubility in aqueous solutions; the excretion of the sulpha drugs is chiefly by way of the kidneys, by glomerular filtration with about seventy per cent re-absorption in the kidney tubules. During the passage of the solution from the glomeruli to the pelves the acetyl compounds crystallise out, causing haematuria from damage to the tissues and sometimes causing anuria from mechanical blockage of the urinary passages. These toxic effects are by no means uncommon; Klumpf and Weilerstein (1940) mention an incidence of six per cent of such effects due to precipitation of crystals in patients treated with sulphapyridine and sulphathiazole; this incidence is similar to that reported by Long et alia (1940); in a large series of cases they found haematuria in eight per cent of patients taking sulphapyridine and in two and a half per cent of patients taking sulphathiazole. These authors did mention also that anuria in sulpha drug administration may be due to a true toxic injury to the tubules of the kidney.

Tragerman and Goto (1940) also quote one of five fatal cases of sulpha poisoning in which the post-mortem findings supported the suggestion of Long et alia.

Although the crystals are precipitated in the tubules the danger of anuria appears to arise from plugging of the renal pelves and the ureters by concretions. Anuria, azotaemia, and even death may follow this plugging which is a mechanical process; at autopsy the kidneys show extreme dilatation of the capsules and tubules. It is important to note these typical post-mortem findings which are markedly dissimilar to those found in patients dying with anuria following haemolytic reactions due to sulpha drugs, or to any other cause of haemolysis. Another true toxic change due to sulpha drugs is the formation of intracorpuscular methaemoglobin the occurrence of which is directly related to the level of the particular compound in the blood. With a blood level of four milligrammes per cent most patients will show cyanosis; Harris and Michel (1939) reported a series of 476 patients of whom fifty eight per cent showed methaemoglobinaemia and

eight per cent showed sulphaemoglobinaemia.

The two complications mentioned above, haematuria with occasional anuria and methaemoglobinaemia, are due to simple poisoning; they are not allergic reactions and more properly belong to the section on poisons. They have been placed here as a comparison with those other effects of sulpha drug administration which more closely concern us, namely those due to idiosyncrasy to the group, or to any one of the group. Hypersensitivity reactions shown by affected individuals following ingestion of the offending sulpha drug include drug fever, skin reactions, agranulocytosis, and haemolytic anaemia. This last effect is the important one from our point of view; it is not a rare finding and strangely the drug usually implicated is sulphanilamide. Figures quoted by Long et alia (1940) are:

No. of cases.	1000	297	271
Complication	Drug. Sulphanilamide.	Sulphapyridine.	Sulphathiazole.
Drug fever	10%	4%	10%
Acute agranulocytosis	0.1%	0.3%	?
Acute haemolytic anaemia (1st-5th day)	1.8%	0.6%	?

Bennet and Ware (1941) reported twenty one cases of acute haemolytic anaemia in 522 cases treated with sulphanilamide; this is a much higher incidence than the averages found in several large series of cases. This haemolytic anaemia should not be confused with the anaemia caused by the depressant effect of the drug on the bone marrow.

Schnitker (1942) sums up the problem by saying that the haemolytic anaemia must be due to an idiosyncrasy, to a peculiar susceptibility. Points in favour of this are: the condition practically invariably develops within a few days after commencement of drug administration; the haemolysis which sets in tends to continue its course even after the blood and tissues are free of sulphanilamide; the condition usually recurs if another course of the drug is begun; and in certain of the cases reported the dosage sufficient to produce haemolysis has been remarkably small, e.g. De and Konar (1940) reported a case where one gram of prontosil apparently caused a rapid fall in haemoglobin, with methaemoglobinuria and death. In certain of the patients this haemolytic process may be severe, with a large number of red cells haemolysed; haemoglobinuria may occur, with

large quantities of methaemoglobin in the urine. In a few cases this may progress to anuria and to death. Gilligan and Kapnick (1941) claim to have found strikingly increased fragility of the red corpuscles during very acute phases of the haemolysis. In this connection Fox and Ottenburg (1941) have shown that the blood serum after acute haemolysis contains more methaemoglobin than was present in the remaining intact red blood corpuscles, suggesting that it was the methaemoglobin-containing cells which had lysed. This isolated finding calls to mind the suggestion by Thomson (1924a) that the "brassy"corpuscles of malignant tertian malaria can act as an antigen. The plasma also shows oxyhaemoglobin and methaemalbumin in such cases of haemolytic anaemia of any marked degree.

In those patients with haemoglobinuria in whom the condition progresses to death the post-mortem changes in the kidneys are the same as those in other haemoglobinurias, e.g. blackwater fever; these changes are very different from those reported above as being found in individuals dying of anuria following blockage of the urinary channels. It was to establish this important

difference that sulpha drug sensitivity has been so fully discussed.

B. Haemolytic anaemia due to haemolysins (endogenous)

1. Incompatible blood transfusions.

The general problem of auto-agglutination has been discussed by Lubinski and Goldbloom (1946) in their report on a boy with acute haemolytic anaemia associated with auto-agglutination over a thermal amplitude of 0-37 degrees Centigrade. These authors consider that agglutination may be subdivided into:

a. Auto-agglutinins active only at refrigerator temperature; these occur in many normal sera.

b. Auto-agglutinins active only at room temperature; usually found in diseased individuals, but may be found in normal individuals.

c. Auto-agglutinins active only at body temperature; this last type appears to be confined to a very small group of individuals who show haemolytic anaemia; the authors found only four cases in the literature in which auto-agglutination had been clearly shown to be active at 37°Centigrade. They add one

further case. Obviously this condition is one of the greatest rarity and is of no practical importance.

There are other conditions however which are associated with naturally-occurring agglutinins and which are of much practical importance. As is known, following a normal compatible transfusion the donor's cells are only slowly destroyed by the recipient at a rate approximately that of the destruction of the patient's own cells, that is about one per cent daily; there is therefore no sign of increased blood destruction following a compatible transfusion, in which the essential condition is that the donor's corpuscles should be inagglutinable by the recipient's serum; there is certainly a possibility of agglutination of the recipient's corpuscles by the donor's serum; in actual practice the chances of the latter reaction are almost negligible because of the dilution of the donor's plasma by the much greater volume of that of the recipient, and because of the great disproportion that exists between the volume of the injected plasma and the volume of the recipient's red cells. Very occasionally however a severe reaction can occur from this cause, e.g. Aubert et

alia (1942) from experiments with exceptionally high-titre incompatible agglutinins point out that the injection even of plasma containing such will cause intra-vascular agglutination and haemolysis of the recipient's cells. This would especially be true of course if a rapid massive transfusion from an incompatible high-titre donor is made into an exsanguinated recipient with a greatly reduced blood volume. This is academic from our point of view; the important thing is the reaction following upon an incompatible transfusion. The sequence of events in such cases has thrown light on other intra-vascular haemolyses. When incompatible blood is transfused the majority of the donor's cells are usually eliminated within a few hours. There are also changes in the titre of the agglutinins in the patient's serum; firstly there is a reduction for one-two days after transfusion, suggested by Mollison (1943) to be due to absorption by the incompatible red cells; this is followed by a rapid increase which reaches its highest level ten to twenty days after transfusion. Mollison records an unusual and striking example of this. A patient showed a reaction after the first five ccs. of a transfusion; she was thought to be

neurotic and was given omnopon and five hundred more ccs.of incompatible blood within an hour and a half; she then had a rigor but was given another five hundred ccs. within three hours. The patient failed to develop either jaundice or urine changes! Later she was found to have an anti-A titre of 262,000. Haemagglutinins therefore exhibit the features of true antibodies.

Agglutination is progressively more rapid and complete as the temperature is lowered to $0^{o}C$. Like other agglutinins they are found in the globulin fraction of the serum and are present in low titre in the body fluids rich in protein, e.g. milk and lymph. As with true antibody reactions the administration of even a small amount of incompatible blood, even ten ccs. may precipitate severe symptoms with development of what Whitby and Britton termed "haemolytic shock". The most important feature of the problem is that the terminal stages of those surviving the immediate reaction are sometimes characterised by renal failure. This finding of renal failure is common to many similar haemolyses, e.g. blackwater fever, and is considered in detail in Chapter V. In passing, the remarks of Whitby and Britton (1946)

may be quoted; they state that a widely-held opinion is that the kidney damage is due to arterial spasm and ischaemia, this view being supported on clinical grounds by the striking early symptoms of lumbar pain before pigment deposition. They think that the spasm is due to release of depressor substances from broken-down red blood corpuscles; this depressor substance is also thought to cause dilatation of the capillary bed and a sharp fall in blood pressure. This point of view is in line with the suggestion that the kidney changes in crush injuries are caused by liberation of a toxic substance from the damaged tissues. This question of ischaemia is dealt with in Chapter V.

B. 2. Rhesus factor incompatibility.

Landsteiner and Wiener (1940) were the first to identify this Rhesus factor. It was identified during investigations of unusual reactions in certain persons after multiple transfusions, and of unusual reactions in pregnant women, and in women recently delivered. This agglutinin is now recognised to be the cause of the erythroplastic anaemia of infants in the neo-natal period, when the infant is Rhesus positive and the mother is

Rhesus negative. Roughly fifteen per cent of the population is Rhesus negative; administration to these individuals of blood containing Rhesus factor, or in the case of females the production of a child whose blood is Rhesus positive, will lead in about ten per cent of such cases to the production of anti-Rhesus antibody, and thereafter any further administration of Rhesus positive blood will cause intra-vascular haemolysis similar to that seen in incompatible transfusions.

This condition of Rhesus sensitiveness played no part in the production of the haemoglobinuria in our cases since none had previously had a blood transfusion and all were males. We need consider this condition no further.

B. 3. Paroxysmal haemoglobinurias.

This is a heterogeneous group of conditions classed together only on the basis of the paroxysmal nature of the haemoglobinuria.

It is necessary to discuss these seriatim, firstly to consider whether our cases of haemoglobinuria may conceivably have been the result of any one of the group, and secondly to obtain information on the problem of intra-vascular haemolysis.

a. Syphilitic paroxysmal haemoglobinuria:

In a small proportion of individuals with established syphilis, congenital or acquired, cooling of the blood to 0°C. for ten minutes followed by warming of it to 37°C. for a short time causes lysis of a certain proportion of the red cells. Donath and Landsteiner (1904) and Eason (1906) showed that the condition was due to an autohaemolysis in the blood of the patient. Haemoglobinuria may result in such affected individuals following exposure to cold. The presence of this haemolysis can be illustrated therefore <u>in vitro</u> and <u>in vivo</u>.

The <u>in vitro</u> test, known as the Donath-Landsteiner reaction, was made in all our cases and was negative in each case. Serological reactions for syphilis were also negative in each case.

This condition of syphilitic paroxysmal haemoglobinuria therefore did not cause the haemoglobinuria seen in the six Razmak cases.

B.3. b. March haemoglobinuria (exertion haemoglobinuria)

This is said to be a rare condition in which increased haemolysis and, more rarely,

haemoglobinuria may follow exhausting exercise in the upright position. The condition was first described by Fleischer in 1881. The maintenance of the upright posture seems to play a definite part in the production of signs and symptoms; as long ago as 1914 Porges and Strisower claimed to have produced haemoglobinuria by exercising the patient for a few minutes in a lordotic position. Witts (1936) also stated that lordosis may be a contributory factor. Similarly Gilligan and Blumgart (1941) stated that if a man marches wearing a plaster cast to keep him from straightening he does not develop symptoms.

Other workers who claim to have proved that the erect posture is necessary for the production of haemolysis are Palmer and Mitchell (1943) and Lowbury and Blakeley (1948). The condition has been described at the beginning of this paragraph as a rare one but actually there is a remarkable difference of opinion on this point. Palmer and Mitchell (1943) found only four cases among 75,000 admissions to Canadian

Military Hospitals but Feigl (1916) claimed to have found haemoglobinuria in seventy per cent of twenty-six people who had marched about twenty-two miles, and Gilligan et alia (1943) found haemoglobinaemia in ten out of twenty-two men after a short cross-country run, and in eighteen out of twenty-two after a long cross-country run.

The answer probably is that mild haemoglobinaemia is possibly not an excessively rare finding in individuals after really strenuous exercise but it is very rarely so great in amount as to lead to haemoglobinuria. One interesting feature from the point of view of certain other causes of haemoglobinuria, particularly blackwater fever, is the suggestion by Witts (1936) that in march haemoglobinuria the lysis is a local one, in the renal vessels; Whitby and Britton (1946) supported this suggestion on the grounds that the patients do not show haemoglobinaemia. This suggestion of a local haemolysis in the kidneys is exactly that brought forward by certain workers to explain the haemolysis of blackwater fever. The theory of Witts is

not accepted by the majority of workers and there are apparently authentic reports in literature of the finding of blood pigments in excess in the plasma, which would support the theory that the haemolysis is intra-vascular, e.g. Lowbury and Blakeley (1948) claim to have demonstrated oxyhaemoglobinaemia in their case.

There is nothing to support the suggestion that this condition played any part in the causation of the haemoglobinuria shown by our six cases; admittedly these six men had undergone considerable strain in July 1938 but there was a time interval of days and even weeks between that strain and the developing of the black water. The only justification for this rather full consideration of an obscure condition is that it may throw light on the processes at work in other intra-vascular haemolyses. The close relationship of this disease with exertion has led to the theory that the pigment may be from breakdown of muscle such as occurs in the rare condition of paralytic myoglobinuria and in the relatively common condition of

crushed kidney. There is no evidence however to support this; muscle pigment has never been found in the blood or urine and Lowbury and Blakeley (1948) aspirated a small amount of fluid from a muscle of one such patient during and after exercise and found nil abnormal. Further they exercised an arm with the circulation cut off and blood from the arm after exercise showed nil abnormal. One other suggestion which has a bearing on other haemolyses is that brought forward by Porges and Strisower (1914) that the haemolysis is a result of the contraction of the splenic vein, which would occur particularly in the lordotic position. Certainly the factor of posture appears as important as exercise, for example the patient quoted by Lowbury and Blakeley (1948) developed severe haemoglobinuria after pulling upon a rope whereas vigorous swimming and "cycling" on the back with the hands behind the buttocks failed to produce any haemoglobinuria. These authors suggest that the explanation of orthostatic albuminuria may be somewhat akin to the

explanation of march haemoglobinuria. In this connection the British Medical Journal (1942) suggested that stasis with consequent haemolysis occurs in some organs when exercise is taken in the erect posture.

The aetiology of this condition is not yet established; the above suggestions are speculative and have been recorded only as a possible contribution to the solving of the problem of the renal lesion common to so many conditions with widely-differing aetiologies, e.g. crush syndrome, cholera, etc.

B. 3. c. Paralytic myoglobinuria.

This is an excessively rare condition. Only five cases have been reported in literature. This disease certainly need not be considered in the differential diagnosis of our six cases. There are two reasons why it is discussed here. The first reason is that given for other discussions in this chapter, namely that possibly a consideration of this condition may conceivably throw light on the obscure processes of intra-vascular haemolysis. The second reason is that a study of this condition is imperative in view of the

relatively recent establishing of the so-called "Crush syndrome", a condition regrettably common in the earlier years of the war and one of much importance. It requires some consideration in this paragraph also.

It should be said here that neither crush syndrome nor paralytic myoglobinuria are considered in the differential diagnosis of the Razmak cases; our discussion of these two conditions is meant to be a step towards obtaining a broad general background to the many problems of intra-vascular haemolysis and those other conditions which are capable of producing renal lesions identical with the lesions found in certain individuals after intra-vascular haemolysis.

A condition which throws some light on the problem of paralytic myoglobinuria is that of equine myoglobinuria, a condition of horses which is characterised by rapid pathological changes in certain voluntary muscles and by discolouration of the urine with myoglobin. This disease attacks healthy horses which have been rested for a

few days on full working diet; the onset of the condition occurs when the horse is taken out for the first time after the period of rest; there is hardening of certain muscles with loss of muscular power and the passing of coffee-coloured urine. There are two theories as to causation; the first is that the condition is a compensatory reduction of excessive number of red cells with the reduction taking place in the capillaries of the affected muscles. The second theory is that during the period of rest, muscle glycogen is deposited in excess; when the horse is exercised there may occur accumulation of sarcolactic acid with secondary degeneration of muscle and liberation of muscle pigment. Another condition, found this time in man, which resembles paralytic myoglobinuria is that called "Haffkrankheit", a disease found in the German town of Königsberg; it has been traced to the eating of fish which have been poisoned by trade effluents discharged locally; the affected individuals show myoglobinuria with post-mortem changes similar to those seen in

equine myoglobinuria. Those two conditions provide us, therefore, with different aetiologies giving the same clinical picture, one disease being a metabolic disorder of some description and the other a true poisoning. With these possibilities in mind we may now discuss the excessively rare condition of paralytic myoglobinuria. The muscles affected in this condition are the striped muscles as was the case in the equine disease. Voluntary muscle contains two pigments, namely cytochrome and myoglobin (muscle haemoglobin, myochrome). This myoglobin has a different absorption band from that of haemoglobin and it is myoglobin which is found in the blood and urine of patients suffering from this rare disease. This pigment myoglobin is the muscle respiratory pigment with functions resembling those of haemoglobin. One important practical point is the statement by Yuile and Clark (1941) that due to the smaller size of the molecule of myoglobin, it is cleared from the blood much more rapidly than is haemoglobin and consequently neither myoglobin nor its breakdown products occur in the blood.

Bywaters and Dible (1943) state that there are on record only seven cases of this excessively rare condition and they add one more to the literature. Their list includes the cases reported by Meyer-Betz (1910-1911) who reported male aet. 13, Paul (1923-1924) reported one female aet. 24, Gunther (1924) reported one case male aet. 54, Hittmair (1925) reported one case female aet. 41, Debré et alia (1934) reported one female aet. 3, Huber et alia (1938) report one case male aet. 4, Millikan (1939) reported one case adult male.

Certain of these references have not been seen by me.

Shortly afterwards Louw and Nielsen (1944) reported another case in a boy of ten years; they claimed that the history went back four generations, always in males.

Death may occur with uraemia; in the fatal case reported by Bywaters and Dible, post-mortem examination showed patchy degeneration of the pectoralis major muscle and also severely-damaged second convoluted tubules in the kidneys. The microscopic picture in the kidneys was identical with that

found in crush syndrome kidneys. It is sufficient to close this discussion with the note that this disease has provided us with an example of a condition where pigment closely related to haemoglobin is released in quantity in the body and is excreted through the kidneys, in which lesions may afterwards be found which are indistinguishable from those found in certain intravascular haemolyses.

Paralytic myoglobinuria therefore is a naturally-occurring example of processes now so well-known in "crush syndrome" which advantageously may be considered at this point. In 1941, when grave and extensive crush injuries were common among air-raid casualties, Bywaters and Beall (1941) reported four cases of crush injuries with secondary renal impairment. This report was soon followed by other similar papers. It was seen that the condition in such cases followed a fairly uniform course in which, following upon a severe injury generally of a crushing nature with much muscular contusion, secondary shock develops with a fall in blood pressure and haemoconcentration; when this

is treated the patient rallies, only to succumb some days later with anuria. When they reported this syndrome Bywaters and Beall then thought it to be an entity previously not reported; one suggestion was that this syndrome had not been seen in pre-war industrial and traffic accidents of crushing nature possibly because the patients died of secondary shock without getting active resuscitation, or that early amputation of extensively injured limbs had prevented the development of this condition.

In 1942 Bywaters and Dible stated that kidney lesions similar to those seen in "crush kidney" are found in such unrelated conditions as incompatible transfusion, blackwater fever, etc.

It was incorrect to presume that this was a newly-discovered entity. Although nothing had appeared in English literature this syndrome had been fully reported during the first World war; its significance escaped notice then; in 1923 Minami again drew attention to the syndrome and gave the credit for the first work to Hackradt (1917); I have not seen this last

which is in the form of a thesis.

The important aspect of this problem is the question of the cause or causes of the renal lesion. In this condition there is present a pigment, closely allied to haemoglobin, circulating in the body and being rapidly excreted in the kidneys; the kidney lesion which is found in this condition is indistinguishable from that of blackwater fever and many other conditions; substances toxic to the body are set free at the site of injury. This resemblance of the kidney lesion to that of blackwater fever and other haemolyses requires that this condition of crush kidney be considered in some detail. This will be done in the portion on kidney changes, Chapter V.

B. 4. Acquired haemolytic icterus (acholuric jaundice). This is the most common of the haemolytic conditions in this country (England). The division of the disease into congenital and acquired types was first made by Widal et alia (1908). The teaching was that in both types of the disease the destruction of the

cells takes place in the reticulo-endothelial system, particularly the spleen. Recent work by Loutit and Mollison (1946) suggests that while this view is correct in the cases of the congenital disease there is evidence that the acquired type is due to a haemolysin; this accounts for its being considered in this portion.

Loutit and Mollison concluded from their experiments that in the acquired type of the disease the red blood corpuscles are sensitised in the patient by an antibody; when normal red cells are transfused into such patients they too are eliminated at once. Admittedly an argument against the view of the above authors is their finding that when red cells from the patients are transfused into normal individuals these cells show normal survival time although in theory they are sensitised. To explain this, the authors question the validity of the widely-held conception that sensitised red cells are destroyed when normal complement is present. The authors suggest that the process of sensitisation may be a reversible one <u>in vivo</u>. Their hypothesis that

in acquired acholuric jaundice the haemolysis is intra-vascular is supported by the finding of methaemalbumin in the plasma of such patients. This finding, together with that of destruction of normal red cells in the patients does suggest that the causative agent is a haemolysin. This disease is a most unlikely cause of the Razmak cases of haemoglobinuria; it is not profitable or necessary to discuss this disease further.

C. Anaemias due possibly to an anomaly of the erythron, congenital or acquired.

1. Congenital haemolytic icterus (acholuric jaundice)

Discussion of this condition conveniently follows the discussion, immediately above, on the acquired type of this disease.

It is generally accepted that in the congenital type that the cause is an inborn defect of the red cells, shown by their behaviour in saline solutions. Unlike the cells from the acquired type of disease these abnormal cells are rapidly destroyed when transfused into a normal individual. And red cells from a normal individual do not show abnormal destruction rates when transfused

into a patient with this condition. Until very recently it was held that destruction of these abnormal cells takes place in the reticulo-endothelial system of the patient and consequently there is no sign of increased intra-vascular haemolysis. A few isolated findings by some workers suggest this may not be correct, e.g. the work of Josephs (1938). Such work is as yet unsupported.

This condition played no part in the causation of our cases; it need not be considered further as the fragility in saline of the red cells in all our cases was normal.

C. 2. Sickle-cell anaemia.

This is due to an inborn abnormality of the red cell, characterised by development of sickling when the red cell is examined under the microscope. The condition was at one time thought to be confined to negroes but has now been reported in whites and in animals. The abnormal red cells are readily destroyed by the normal processes of the body; this may rarely be so severe as to produce haemoglobinuria, first reported by Evans,R.W. (1945). Robertson and Findlay (1947)

confirmed this with a report of twelve fatal cases. I have never heard of a case of this disease occurring in India and an examination of the red blood corpuscles of the Razmak cases showed that these cells did not have any tendency towards sickling.

C. 3. Mediterranean anaemia.

This anaemia of infancy and childhood shows as part of the picture an abnormal appearance of the red blood cells. The cause is a congenital anomaly of the erythron. It is akin to sickle-cell anaemia and need not be considered further.

4. Nocturnal haemoglobinuria (Marchiafava-Micheli Disease).

In the classification of Whitby and Britton (1946) this disease is placed among the paroxysmal haemoglobinurias due to a haemolysin. The essential abnormality would appear to be in the red cell however and accordingly the disease is considered under this subdivision.

The fragility of the red cell is not abnormal. The disease was first reported by Marchiafava and Nazari in 1911. Patients

suffering from this disease show paroxysms of haemoglobinuria usually precipitated by sleep, whether this sleep be taken by day or by night. The cause is that the red cells are unusually susceptible to slight changes in the blood pH; and it has been suggested that the reduced pulmonary ventilation during sleep may sufficiently depress the pH value of the blood as to cause haemolysis in susceptible persons; blood pigment is present in the urine passed following sleep. The heamolysis is intra-vascular, therefore the problem is not simply one of destruction of abnormal red cells by the reticulo-endothelial system. The finding that blood transfusion has an inhibitory effect on the haemolysis has led Josephs (1938) to suggest that this effect of transfusion is due to the presence of a naturally-occurring antihaemolytic factor in the normal transfused blood. A full report of the condition is given by Scott et alia (1938).

This rare disease is another example of the difficulty in analysing the factors

responsible for haemolysis in haemolytic anaemias.

We can safely assume that this rare disease need not enter into the differential diagnosis of our cases.

C. 5. Physical factors.

This includes severe burns, frost-bite, etc. Whitby and Britton (1946) class these with the poisons but other workers consider that the haemoglobinuria with secondary renal damage is the result of direct injury to the red cell at the site of injury, e.g. Brown (1944) reported alterations in the saline fragility of the red cells in burns cases. Shu et alia (1943) investigated eleven cases of haemoglobinuria occurring in forty individuals suffering from severe burns. In seven of the eleven there was increased red-cell fragility to hypotonic saline. In some of these cases blood films showed poikilocytosis and spherocytosis. The authors further showed that injection of heated blood into an animal of the same species caused haemoglobinuria.

This cause of haemoglobinuria is noted

here merely to illustrate the complexity of the problem.

D. Haemolytic anaemias of unknown origin.

Acute idiopathic haemolytic anaemia (Lederer's anaemia).

Mackintosh and Cleland (1902) were the first to describe this entity, later re-discovered by Lederer (1925). This is another of the rare haemolytic diseases and the cause has not yet been identified. There is some evidence to suggest that a haemolysin may be a factor; a leucocytosis is always present however, suggesting an infective factor. Transfusion is remarkably successful in curing such cases.

Our patients did not show a clinical picture in any way resembling that of patients with Lederer's disease.

There are other diseases and conditions in which haemoglobinuria may occur as a rare and incidental symptom; these need only be enumerated as none of them had any part in the causation of the disease in our patients. They include: Raynaud's disease, angioneurotic oedema, internal haemorrhage, specific fevers, e.g. typhus, pernicious anaemia, etc.

One interesting point in the last of the list, i.e.

pernicious anaemia is the suggestion by Loutit (1946) that one factor in the disease may be faulty protoplasm in the red cells.

SUMMARY.

This chapter has been devoted to a consideration of some of the many causes of haemolysis. Mention has also been made of certain other conditions, e.g. crush syndrome, in which there sometimes is found a renal picture identical with that found in certain of the individuals suffering from any one of the haemolyses.

The first of the aims of this chapter was a consideration of the more likely possible causes of the haemoglobinuria which came upon the six individuals whose case-sheets have been summarised in Chapter II. This discussion of possible causes is very incomplete. Five of the most likely factors have not been mentioned, namely malaria, blackwater fever, quinine, atebrin and plasmoquine. This is because these are so important that a chapter has been devoted to the study of each of them.

The conditions which have been considered in this chapter include:

1. Exogenous Factors:

This includes infections:

e.g. malaria, oroya fever, etc.; the only infection which may have played a part is malaria which will be discussed in Chapter IX.

and poisons:

here the most important and most likely factors are quinine, atebrin and plasmoquine which will be discussed in Chapters VI, VII and VIII respectively.

One other very likely possibility was that of self-medication; from the facts it is submitted that this could not have happened.

and allergy:

the only important one of the possibilities under this heading was that of drug idiosyncrasy which was considered in a little detail because of the importance of this in general and in particular; sulpha-drug hypersensitivity was taken to illustrate the discussion. From this it was learned that drug allergy may cause haemoglobinuria and it was learned that the kidney lesion which may result is identical with that seen in other haemoglobinurias, but is very different from the kidney lesion seen with that

anuria, found sometimes with sulpha drugs, which is due to mechanical blocking of the urinary passages by acetylated derivatives of the sulpha drugs.

Sulpha drug allergy, like other drug allergies, shows all the findings of true antigen-antibody reactions.

2. Endogenous Factors:

This includes A. Haemolysins: this includes:

1. Incompatible blood transfusions.

Excluding the rare cases of auto-agglutinins, the haemolyses found in blood transfusions are due either to the giving of incompatible blood or to the giving of Rhesus blood to a patient sensitised to Rhesus factor either by pregnancy or by a previous transfusion. Neither of these causes of haemolysis need be considered in the differential diagnosis of the Razmak cases, all of whom were males and none of whom had ever had a blood transfusion previous to the onset of the black urine.

2. Paroxysmal haemoglobinurias.

Grouped together in this class are syphilitic paroxysmal haemoglobinuria, march haemoglobinuria,

and paralytic haemoglobinuria; none of these need be considered in the differential diagnosis of the six cases under review; in these six cases the serological reactions for the syphilis were negative, the Donath-Landsteiner reactions were all negative; and in no case was there any immediately-preceding severe physical exertion.

One point of interest was that in the causing of march haemoglobinuria posture is as important a factor as is exertion; this is in favour of the theory that the haemolysis is in the renal vessels. This last suggestion is not generally accepted but is recorded for discussion with a similar theory of blackwater fever causation, which will be considered in Chapter IX.

Paralytic myoglobinuria was of importance in that, while excessively rare, it is a naturally-occurring example of the processes at work in the "crush syndrome". This condition of crush syndrome was therefore discussed at this stage; this was done not because it played any part in the production of the haemoglobinuria in the Razmak cases but because

a study of the processes at work in this condition shows that the picture does not differ fundamentally from that seen in the intra-vascular haemolyses, excepting that in crush syndrome the pigment set free is myoglobin not haemoglobin. The character of the kidney lesion is identical in both types of lesion in spite of the differences in the freed pigment; this may be a point of much importance when consideration is given to the part played, if any, by free haemoglobin in the production of the kidney lesions in haemoglobinurias.

2. A. 3. Acquired haemolytic icterus:

This was considered under the heading of haemolysins because of recent work which suggests that the cause of this condition is a circulating haemolysin as distinct from the red cell abnormality which is the cause of the haemolysis in the congenital type of acholuric jaundice.

Acquired haemolytic icterus does not enter into the differential diagnosis of our cases; it is quoted as one more example of the complexity of the factors entering into the

causation of the naturally-occurring haemolytic anaemias.

B. Haemolysis due to an anomaly of the erythron.

In this class are included:

The congenital type of acholuric jaundice, sickle-cell anaemia, Mediterranean anaemia, and nocturnal haemoglobinuria; in all of these there is apparently a congenital anomaly of the red blood corpuscle.

Physical factors such as severe burns were also included here, the suggestion being that the haemolysis following such physical effects is due to a destruction of the damaged red cells.

None of the conditions which are classified above need be considered in the differential diagnosis of the Razmak cases.

3. Haemolytic anaemias of unknown origin.

The most important of this heterogeneous group is Lederer's anaemia; the cause of it is unknown.

Haemoglobinuria may also occur in such varied conditions as Raynaud's disease, angioneurotic oedema, in fevers such as typhus, in pernicious anaemia, and in many

other conditions. In all such cases however the haemoglobinuria is a rare symptom occurring incidentally in the course of the particular disease.

This draws to a close Chapter III which consists of a very brief and incomplete review of the more important of the many causes of haemolytic anaemia. Mention has also been made of certain other conditions which can produce a renal lesion like to that seen in the haemoglobinurias.

Much of the chapter may appear to have very little bearing on the subject of this work, namely that series of six cases of haemoglobinuria which occurred in Waziristan in 1938. In the course of Chapter III the reasons for this short general review have already been given. The two most important of those reasons are:

1. The diagnosis of the six cases was said to be blackwater fever; there are many other causes of haemoglobinuria, however, especially in the Tropics; it is necessary, therefore, to consider in greater or lesser detail the more likely of other, alternative diagnoses. This has been begun in this chapter where several likely alternative diagnoses were discussed. None of these was thought to have been a likely cause of the haemoglobinuria and therefore with the coming to an end of this chapter

the diagnosis stands as haemolysis, intra-vascular, ? blackwater fever.

2. It is proposed to discuss in detail in later chapters the pathological changes, particularly the kidney changes, that are found in intravascular haemolyses and to discuss these changes as found in the six Razmak cases. A few preliminary remarks in this connection have been made in this chapter.

CHAPTER IV.

The Laboratory Findings in Intravascular Haemolysis.

Chapter III was devoted to a consideration of the more important of the many causes of haemolytic anaemia. From this consideration it was seen that conditions markedly dissimilar in aetiology produce clinical pictures very similar to each other; e.g. the jaundice and anaemia of congenital acholuric jaundice and of chronic malignant malaria may be very similar clinically, although in the one case the haemolysis is intra-vascular and in the other it is mainly in the reticulo-endothelial system.

This question of the site of the haemolysis is the first of the problems which confronts us with the Razmak cases; we have said that the diagnosis is haemolysis, intra-vascular, ? blackwater fever, but have still to show the grounds on which the statement is made that the haemolysis was in point of fact intra-vascular and not elsewhere. To establish the site of the haemolysis it is necessary to discuss in detail the laboratory findings in the six Waziristan cases.

This chapter will deal with the blood pigments and cells. The next chapter will deal with the kidney changes and the urinary changes.

All the laboratory investigations discussed in the

succeeding pages were carried out in the District Laboratory, Waziristan, by Major T.C. Puri, the Deputy Assistant Director of Pathology at that time.

Firstly let us consider the blood pigments, beginning with a brief account of the physiology.

Without a free continuous supply of oxygen to the tissues the body would not survive for more than a very few minutes. This carriage of oxygen is the function of haemoglobin. The study of the blood pigments is therefore the study of haemoglobin and its derivatives.

The haemoglobin molecule is a conjugated protein, a porphyrin-iron portion (haem) plus a protein of the histone class (globin). This protein globin has about fifteen amino-acids and forms about one-third of the total plasma protein. Keilin (1944) states that in haemoglobin the protein portion is linked both to the porphyrin and to the iron. The globin forms about ninety-six per cent of the haemoglobin molecule. Whitby and Britton (1946) picture the molecule as composed of a small but relatively heavy porphyrin-iron portion floated by the large protein fraction.

The iron in the haemoglobin molecule forms 0.336% of the whole; since the atomic weight of iron is 56 then, even if each haemoglobin molecule contains one atom of iron (see Haem,

plate XIV , page 164, the molecular weight of haemoglobin must be at least about 16,700. But haemoglobin behaves generally as though it has four atoms of iron in it, therefore it must be a compound molecule with a molecular weight roughly 68,000; this compound molecule is easily broken down.

Human blood is estimated to contain roughly fifteen grammes of haemoglobin per 100 cubic centimetres of blood, which means that the total iron in the body which is engaged in the transport of oxygen is roughly about four grammes. The iron in the haemoglobin molecule is held by nitrogen atoms, partly by extra valencies (see formula for haem, plate XIV). The synthesis of haemoglobin outside the body was first carried out by Fischer (1929) who did so by first synthesising haematin and then combining this with globin; haematin is the pigment portion of haemoglobin; it is a porphyrin-iron compound. The synthesis of haemoglobin by the body is not yet fully understood. Whitby and Britton (1946) suggest that it is formed in the bone marrow, apparently within the red cells, from porphyrin precursor substances. These porphyrins are derivatives of a parent skeleton porphin the graphic formula for which is given in plate No. XIII, page 160, This substance porphin is made up of four nitrogen-containing pyrrole groups linked together by four

methinyl links (CH-) to form a stable ring. The presence of these methinyl links adds certain specific properties to the compound.

Substitutions may be carried out at positions 1 to 8 as shown in the abbreviated formula for porphin, plate No. XIII, page 160. For example, see the abbreviated graphic formula for protoporphyrin III, plate No. XIII, page 160. It is these substances so derived which are given the name "Porphyrins". The porphyrins possess to a marked degree the property of combining with metals and proteins. It is this property which accounts for porphyrins being found in practically all forms of plant and animal life, their function being that of respiratory pigments. Among such compounds in nature are: haemoglobin, haemochromogen, cytochrome, protoporphyrin, coproporphyrin, and uroporphyrin.

One set of substitutions in the porphin molecule which has a bearing on our subject is that substitution in which two hydrogen atoms in each of the four pyrrole groups are replaced by four methyl groups and four ethyl groups to form aetio-porphyrins. With these substitutions there are four such possible combinations; an example of one such is given on plate No. XIII, page 160. Each of these four aetioporphyrins is potentially capable of giving rise to a series of porphyrin derivatives. In nature, however, only two of

the possible four are found to occur; these give rise to a series derived from aetioporphyrins I and III.

Series I are tetramethyl porphyrins with the methyl groups in positions 1, 3, 5, 7.

Series III are tetramethyl porphyrins with the methyl groups in positions 1, 3,5, 8.

Compounds derived from series III are the important ones. They include haemoglobin and its derivatives. Compounds derived from series I are found in quantity only in certain pathological conditions. Interconversion from one series to the other does not occur in vivo; breakdown of haemoglobin cannot produce a series I porphyrin.

In nature the porphyrins seemingly are synthesised from simple substances.

During the formation of porphyrin III in the process of synthesis of haemoglobin some porphyrin I is also formed as a side product and is excreted in the urine, faeces, and bile, as coproporphyrin I.

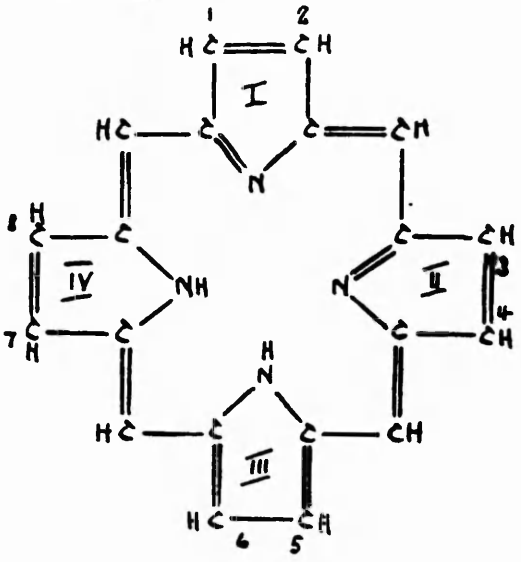
Coproporphyrins, mentioned above, are also derivatives of series I and III. The formula for coproporphyrin I is given in plate XIII, page 160. An important practical point is that the coproporphyrin normally excreted in the bile and faeces is almost entirely coproporphyrin I. Normally the

urine excretes 20-50 micrograms daily of coproporphyrin; this is a mixture of approximately equal parts of series I and series III. The amounts of porphyrin ingested with the food and the amount produced by bacterial activity in the intestines cannot account for the total output of coproporphyrin I, which supports the suggestion that coproporphyrin I in small amounts is a by-product of normal erythropoeisis and its measurement may be used therefore as an index of erythropoetic activity.

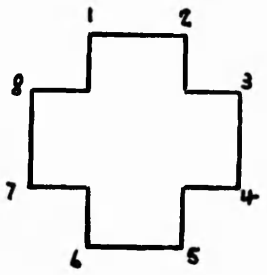
Porphyrin III, unlike porphyrin I, does not change before excretion; it is passed out as porphyrin III, and in excess only if there is some toxic action on the blood. It does not form a stage in the degradation of haemoglobin. When haemoglobin is destroyed as part of normal metabolism no free porphyrin is found, therefore Whitby and Britton (1946) suggest that when porphyrin III is being excreted its excretion is due to a blocking of a stage in the production of haemoglobin, and not to destruction of haemoglobin. Coproporphyrin III, corresponding to porphyrin III, is said to be found being excreted as a degradation product of certain abnormal blood pigments; there are two such pigments of much importance, namely methaemalbumin and methaemoglobin. It is held that once haemoglobin has been oxidised to these, then the normal conversion of the pigment to bilirubin does not take place and is replaced by degradation to coproporphyrin III.

GRAPHIC FORMULAE OF SOME SUBSTANCES DISCUSSED IN CHAPTER IV

PORPHIN

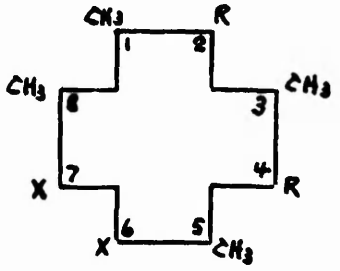


ABBREVIATED FORM

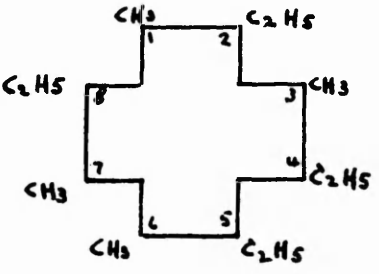


COPROPORPHYRIN I = substitute $-CH_2 \cdot CH_2 \cdot COOH$ in positions 2, 4, 6, 8.

PROTOPORPHYRIN III: where R = $-CH \cdot CH_2$
X = $-CH_2 \cdot CH_2 \cdot COOH$



RETIOPORPHYRIN (OF A TYPE NOT USUALLY FOUND IN NATURE)



There are three abnormal conditions which are characterised by the finding of increase of coproporphyrins in the urine; these are:

1. Congenital porphyry: a rare inborn defect of metabolism.
2. Acute idiopathic porphyry: need not be discussed here.
3. Porphyrinuria due to drugs and to toxins: causes include liver abnormalities, pellagra, sulphonal group poisons, alcohol, and metals.

None of these adds anything of value to the discussion.

The most important of the series III porphyrins is protoporphyrin III, formula plate XIII. When this substance is treated with a ferrous salt it forms haem, the pigment portion of haemoglobin. And haem plus globin forms haemoglobin. Haem, formula plate XIV, is therefore an iron-containing pigment, a porphyrin-iron compound with the iron partly held by extra valencies. Haem is a constituent of other respiratory compounds, and it is also capable of uniting with proteins and nitrogenous substances to form haemochromogens. Best and Taylor (1943) summarise the various substances as follows:

(Pyrrole nucleus) 4 = porphyrin compound.

Porphyrins plus metals = metallo-porphyrins.

Protoporphyrin plus ferrous iron = haem.

Haem plus various nitrogenous substances = haemochromogens of various respiratory pigments, e.g. cytochrome.

Haem plus globin = haemoglobin.

Haem plus denatured globin = haemochromogen of haemoglobin.

Oxidised haem (ferric iron) plus denatured globin = cathaemoglobin.

Oxidised haem (ferric iron) plus globin = methaemoglobin.

These various relationships are shown on plates Nos. XV and XVI, pages 170 and 171 .

Keilin (1944) summarises the types of compounds which proteins may form with haem; these are:

a. haemochromogens - usually formed by denatured protein linked to iron.

b. haemalbumin - native albumin linked only to the porphyrin of haem.

c. haemoglobin - in which the protein is bound both to the iron and to the porphyrin of the haem.

The above is a brief and incomplete discussion of the substances which may play a part in the metabolism of haemoglobin. Certain of the substances are of practical importance in that quantitative and qualitative changes of them may aid diagnosis in blood dyscrasias; this will be seen later in the chapter.

Free circulating extra-corpuscular haemglobin does not occur in the blood except in minute quantity; when present in amounts above this minute normal amount, the pigment is treated by the body as a foreign substance. Plasmolysis accounts for the minute normal amount in the blood and the value is increased by trauma when blood is taken for testing. Fairley and Bromfield (1934a) suggest 0.12% red cell solution as the upper limit of normal; in a series of fifty three cases these authors drew blood without special precautions against trauma and found the plasma in forty six patients free of any trace of haemoglobin when examined with a spectroscope. This figure of Fairley and Bromfield is in close agreement with that of Barratt and Yorke (1909) who gave the upper limit of normality for haemoglobin in the plasma to be from 0.1% to 0.15% red cell solution.

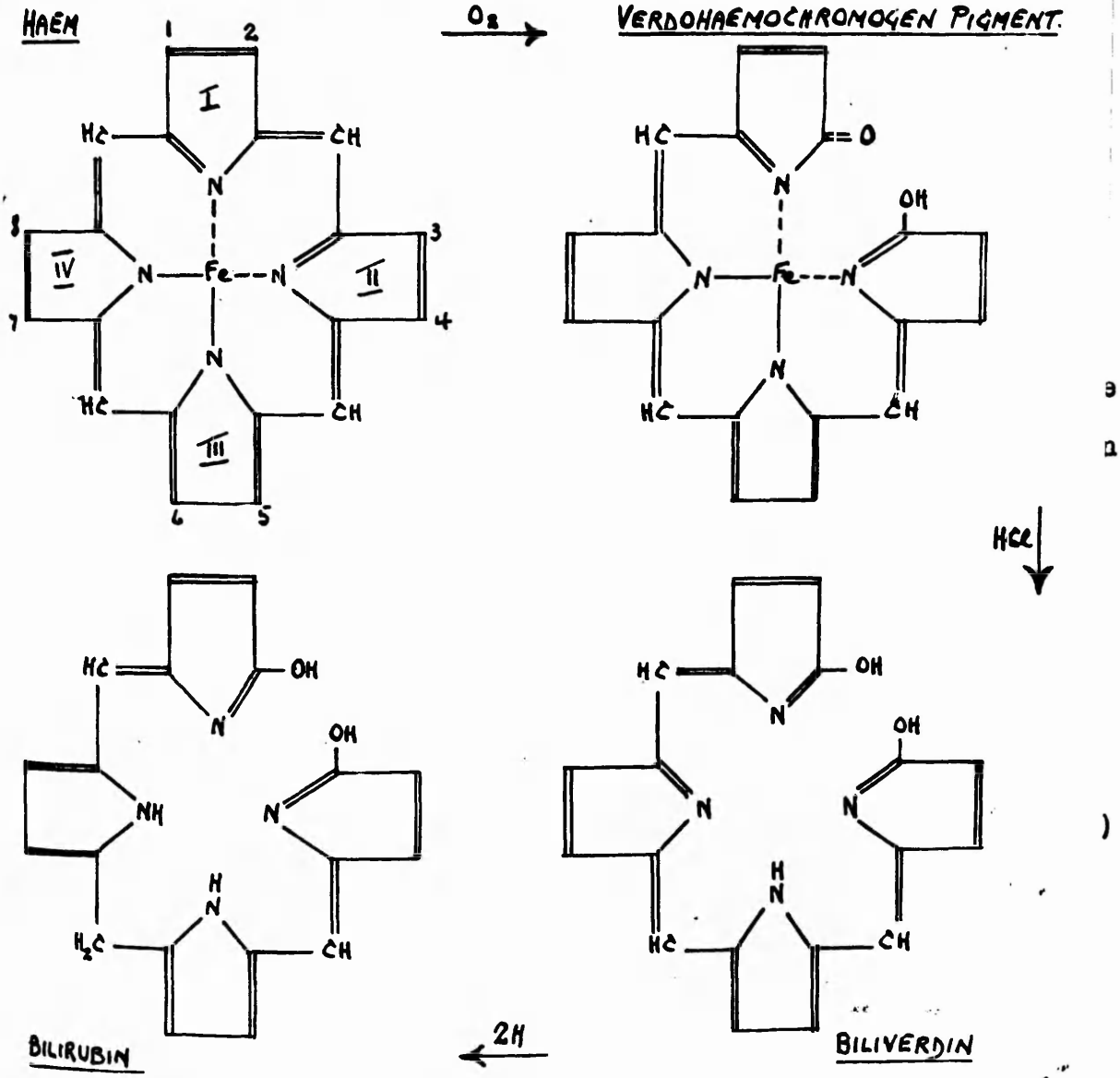
The function of haemoglobin is to carry oxygen to the tissues. It does so by forming a loose union with the oxygen, i.e. oxyhaemoglobin. In this oxyhaemoglobin the oxygen is very loosely held in combination with iron in the ferrous state. The combination is certainly according to the law of definite proportions, i.e.

haemoglobin 4 + 4 O O = haemoglobin $_4$ O8.

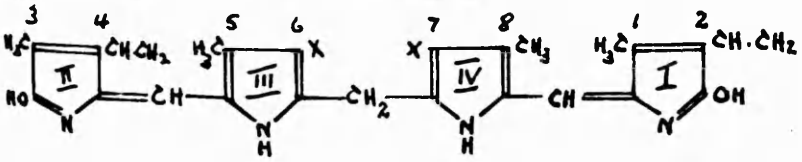
Blood pigments normally present therefore in venous blood

PLATE No. XIV

SKELETON GRAPHIC FORMULAE OF SOME SUBSTANCES DISCUSSED IN CHAPTER IV.

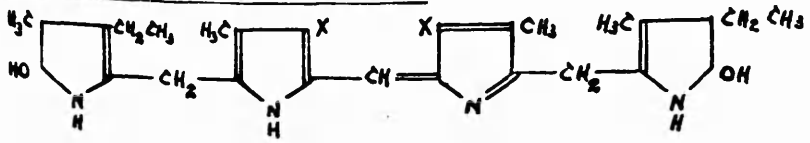


BILIRUBIN (STRAIGHT FORMULA) $C_{33}H_{36}O_6N_4$



$X = -CH_2.CH_2.COOH$

UROBILIN (STERCOBILIN) $C_{33}H_{44}O_6N_4$



(with acknowledgements to W.V. THORPE "BIOCHEMISTRY" LONDON 1944)

are oxyhaemoglobin and reduced haemoglobin. Both of these are"active" haemoglobin, fully capable of carrying oxygen to the tissues. Other related compounds which are found in minute amounts in normal individuals and other compounds found in abnormal conditions, are "inactive compounds", i.e. incapable of carrying oxygen to the tissues. Of these "inactive" compounds some are "potential" carriers of oxygen in that they can be re-transformed into haemoglobin and oxyhaemoglobin, e.g. methaemoglobin. Others of them cannot be re-transformed or can only be so with great difficulty, e.g. sulphaemoglobin.

In oxyhaemoglobin, the loose combination of haemoglobin with oxygen, the iron is in the ferrous state (Fe++) and the oxygen is readily available. In the other oxide of haemoglobin found in the body, i.e. methaemoglobin the union of the pigment with the oxygen is a stable one, a true oxide being formed with one atom of oxygen to one atom of iron and with the oxygen not available; the iron is in the trivalent state. This pigment, methaemoglobin, is of great importance in certain pathological conditions; it is also found in traces in the normal body; e.g. Peters and Van Slyke (1931) suggested that methaemoglobin is constantly being formed in the blood of normal persons, and Marshall (1932) supported this; the last-named thought that a minute

amount of methaemoglobin exists in equilibrium with haemoglobin in normal blood. More recently Gibson and Harrison (1947) have supported Marshall's suggestion and say that in normal blood the equation haemoglobin ⇌ methaemoglobin is very far to the left. They think that in normal persons the reduction of the methaemoglobin depends on certain enzymes in the red blood corpuscles. It is of interest here to note that in the last five years there have been reported two series of cases with individuals suffering from familial idiopathic methaemoglobinaemia. In both the latest two series the patients were Irish; the series were that of Deeny et alia (1943) and of Gibson and Harrison (1947). As already mentioned the last named authors think that this rare condition arises from an inability of the enzyme systems in the red cell to reduce the methaemoglobin to haemoglobin, and they suggest that the rapid removal of methaemoglobin from the blood which occurs after administration of methylene blue is probably due to the catalytic action of the dye.

If this disease were our only contact with methaemoglobin then the above short mention of this pigment would suffice, but this is not so; methaemoglobin requires full consideration because it is commonly found in large amounts in the body in many pathological conditions, especially after administration of the newer chemotherapeutic agents, e.g. plasmoquine. It is convenient however to postpone this

fuller discussion on methaemoglobin to somewhat later in the chapter, and to continue at this point with a discussion on the normal physiological processes connected with the metabolism of blood pigments.

So far our discussion has been limited to a consideration of those substances which may play a part in the synthesis of haemoglobin. It is now proposed to discuss processes involved in the degradation of haemoglobin in the normal body.

It is again stressed that the function of haemoglobin is to provide freely-available oxygen to the tissues. To do so it needs transport; this is supplied by the red blood corpuscle which forms about forty-five per cent by volume of normal blood. So far we have no satisfactory explanation of the fundamental problem of how the red cell carries the haemoglobin. The amount of haemoglobin in each red cell is too great for the answer to be one of simple solution. One suggestion is that the haemoglobin is adsorbed to the stroma of the red cell. Discussion on this point will be deferred to the section dealing with the red cell. Only a few relevant notes will be given here.

In the course of its duty the red cell takes a tremendous thrashing; its life is a short one, probably not more than one hundred days. It is not a living cell of

course. Technically there are three ways in which the cell may be destroyed as it becomes effete, namely: destruction by the cells of the reticulo-endothelial system; destruction by haemolysins; and destruction intra-vascularly by the simple mechanical factors of wear and tear. Until lately the view was widely held that the destruction of effete red cells was a matter for the macrophages of the reticulo-endothelial system, the spleen being particularly implicated. This view may require modification. Best and Taylor (1943), for example, basing their views on the work of Rouse, consider that the disintegration of the red cells takes place in the blood stream as a result of "fair wear and tear". In other words the function of the spleen and the other parts of the reticulo-endothelial system is that of a graveyard instead of a slaughter-house. Other workers do not agree, e.g. Maegraith et alia (1943) have isolated from normal animal tissue a lytic substance which is normally inhibited by factors present in the serum and in washings from the tissues. Those authors suggested that the rate of haemolysis normally occurring in an animal may be a function of the balance between tissue lytic agent and its inhibitor. We cannot proceed further along this interesting by-path in this chapter. It can only be said that at present there is no clear-cut proof for any one of the three possible methods of destruction of the red cell in the normal individual.

It is again stressed here that normally the degradation of haemoglobin does not cause free porphyrin to be produced. The normal urine contains only a trace of coproporphyrin and at least half of that trace is coproporphyrin I. And, furthermore, the coproporphyrin found normally in the bile and faeces is almost entirely coproporphyrin I.

Degradation of haemoglobin is carried out by the cells of the reticulo-endothelial system, especially the bone-marrow. The process is not yet fully elucidated; the principles are as follows. It is suggested that firstly on the downward path of haemoglobin the porphin ring, plate No. XIII, page 160, is opened by oxidation at one of the methinyl linkages while still retaining its iron and protein (see "verdohaemochromogen", plate No, XIV, page 164). The degradation continues with the removal of the protein and a re-arrangement with loss of iron, the iron being conserved in the reticulo-endothelial system as haemosiderin which is mainly ferric hydroxide in organic combination. The resultant product following upon this re-arrangement with loss of iron is most probably biliverdin, see plate No. XIV, page 164. Biliverdin then undergoes reduction to become bilirubin, plate No. XIV, page 164. Bilirubin is, of course, the major bile pigment. This suggested method of breakdown differs from that generally held, where the first process is thought to be

PLATE Nº XV

TABLE OF HAEMOGLOBIN DERIVATIVES. (from BEST & TAYLOR "PHYSIOLOGICAL BASIS OF MEDICAL PRACTICE)

(see also PLATE Nº)

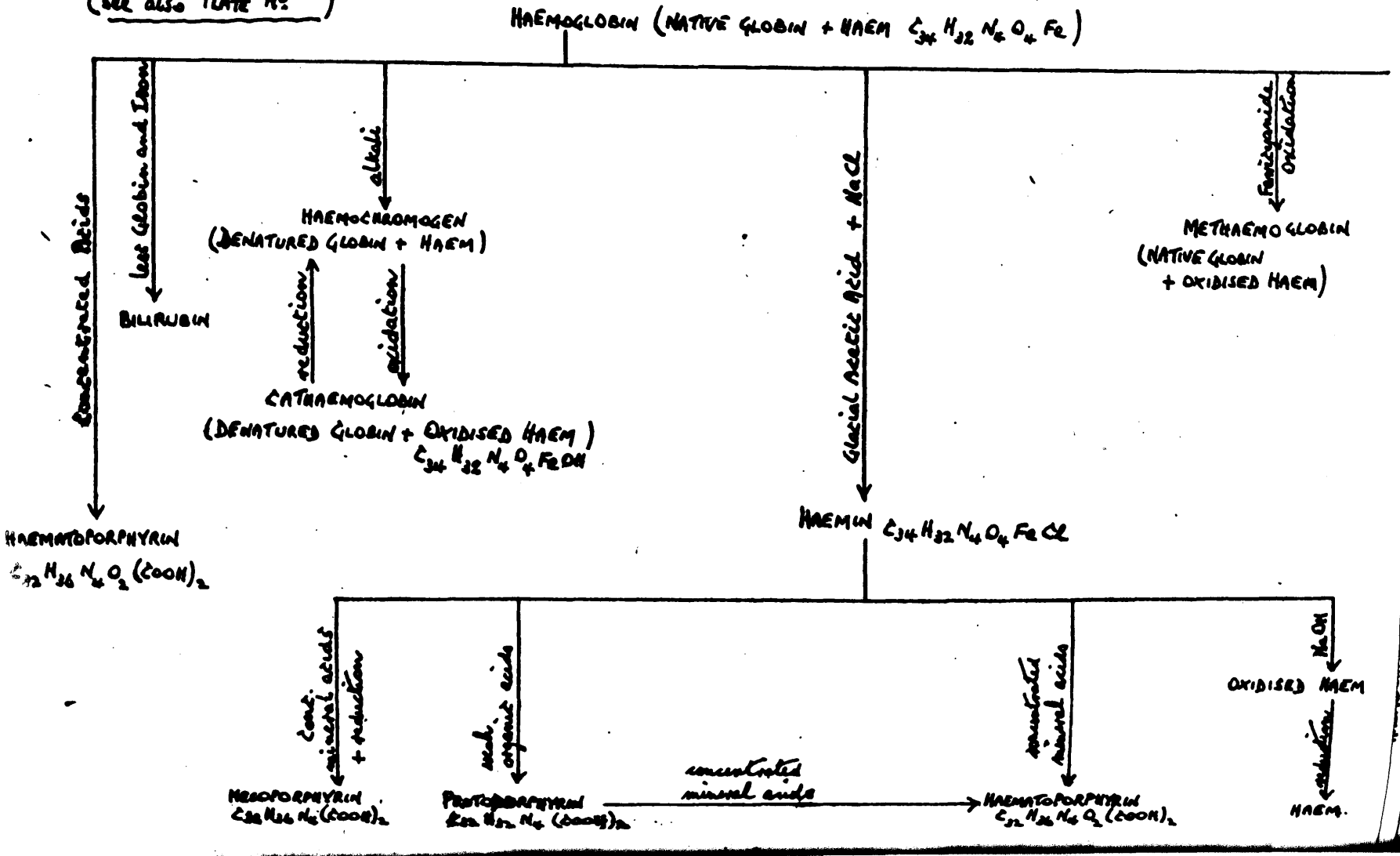


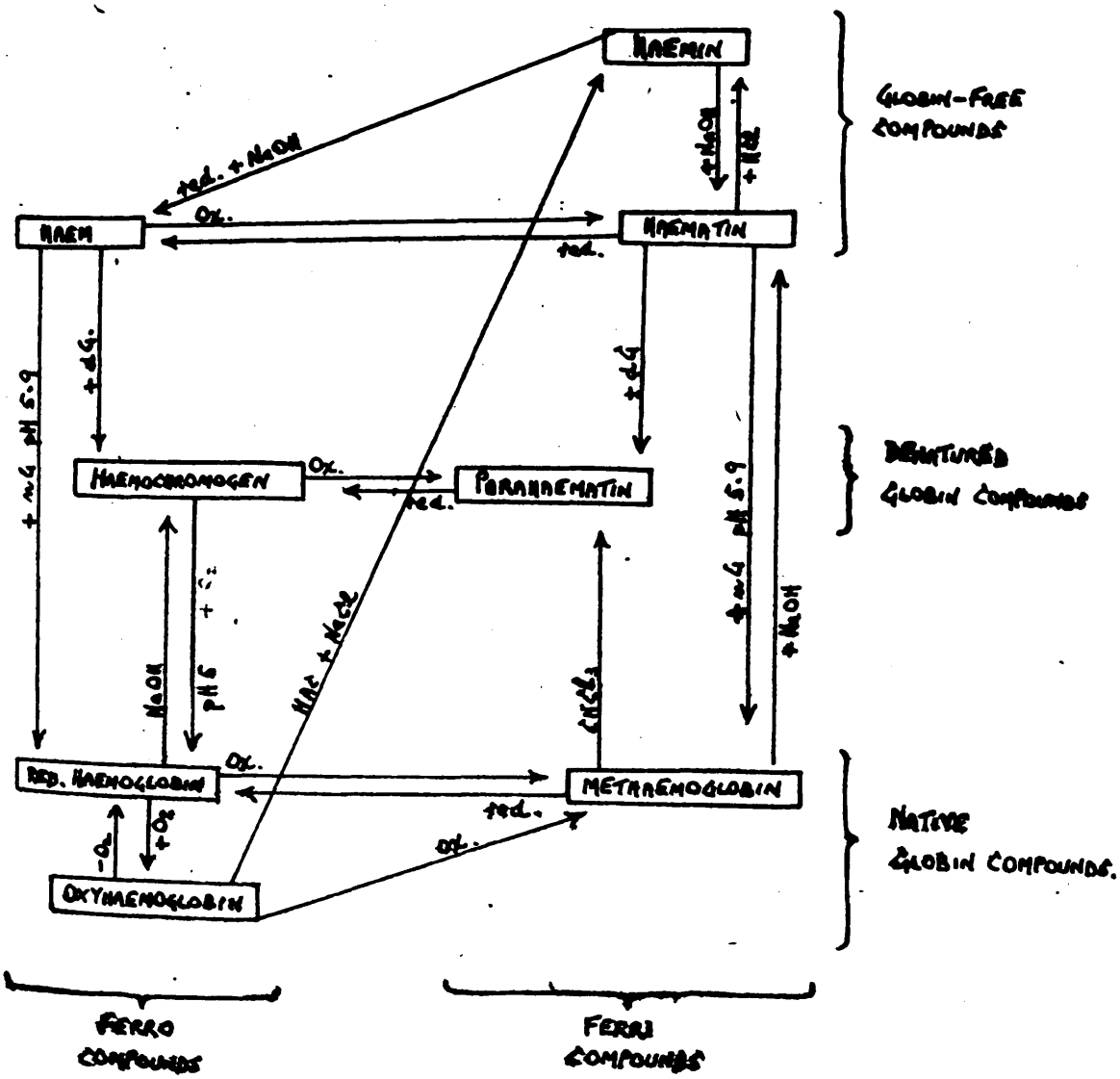
PLATE No. XVI

HAEM DERIVATIVES CONTAINING IRON.

(from "BIOCHEMISTRY" by THORPE)

KEY.

dG = DENATURED GLOBIN
nG = NATIVE GLOBIN
Ox = OXIDATION
Red. = REDUCTION



ALTERNATIVE NAMES

HAEM	HAEMATIN	PARAHAEMATIN.
REDUCED HAEM	OXIDISED HAEM	CATHAEMOGLOBIN
REDUCED HAEMATIN	OXYHAEMATIN	
HAEMOCHROME	PROTOHAEMATIN	

hydrolysis; this, if correct, would yield haem by the splitting-off of the protein. The hydrolysis would then continue and so produce bilirubin from haem. Experimental work is in favour of the first and is against the simple second explanation; for example, conversion of haematin into bilirubin is said not to take place in vivo; Rimington (1939) states that injections of haematin cause an increased faecal porphyrin level, also seen to a lesser extent in the urine. These findings are not a feature of normal haemoglobin breakdown. Apropos of this it has been noted that substances which produce methaemoglobinaemia on administration, e.g. the sulpha group of drugs, also cause increased porphyrin excretion; this last is also a finding in the breakdown of methaemalbumin, a compound similar to methaemoglobin. This means that there is an alternative route of breakdown, via the porphyrins, which is applicable to the abnormal oxidised pigments. The existence of this alternative route may explain the small quantities of series III pigment excreted by normal individuals. Another point in favour of an alternative breakdown via haem is that the intravenous injection into man and monkeys of haematin produces methaemalbumin.

Let us continue our study of the pigments found in the faeces and urine as the result of the normal breakdown of haemoglobin. A simple diagrammatic representation of the

processes is given on plate No. XVII, page 179. Here we have reached the stage where the degradation of haemoglobin has been completed; the resulting compound is recognised to be bilirubin, the formula for which is given on plate No. XIV, page 164. This formula shows bilirubin to be a chain-type pigment, that is an opened porphyrin ring allied to porphyrin, plate No. XIII, page 160. This bilirubin is mainly a waste product, manufactured by the intra-cellular activity of the reticulo-endothelial system. After the compound has been produced in the reticulo-endothelial system it is passed into the blood-stream for excretion. One gram of haemoglobin yields about forty milligrams of bilirubin. The excretion of the bilirubin is practically all by the large bowel via the liver, hence its name of "bile pigment". Obviously any condition which for any reason causes haemolysis necessarily causes increased production of bile pigment.

The daily destruction of effete red cells is considerable. Best and Taylor (1943) suggest a figure of ten million red cells destroyed every second. This means that the production and excretion of bilirubin is quite a considerable problem. Normally the blood level of bilirubin is about a maximum of 1.2 mgms. per 100 ccs. serum though the level may be considerably increased in sallow individuals. This bilirubin, together with a small amount of biliverdin, is excreted by the

secretory cells of the liver. The pigments then pass via the bile to the intestines, being accompanied by the bile salts sodium glycocholate and sodium taurocholate; the bile salts are true products of the liver cells whereas the bile pigments are not, hence the excretion of the salts and the pigments do not necessarily parallel each other. Their association is largely fortuitous. Even though the function of the liver cells as regards bile pigment is largely excretory, some type of change does occur in the pigments; e.g. before bilirubin passes through the barrier of the liver cells it cannot link with diazo reagent except with the assistance of alcohol; but after its passage through the liver cells, bilirubin is able to form this linkage without alcohol. Various explanations have been suggested. One explanation is that the change is in the physical state of the bilirubin, the suggestion being that the passage through the liver cells changes the bilirubin from a colloidal to a crystallisable state. Ehrlich demonstrated this in 1884 but it was forgotten until re-discovered by van den Bergh (1904) who was the first to put the finding to practical use by the introduction of the test which bears his name. In this test he utilised the characteristic reaction of bilirubin with Ehrlich's diazo reagent, and showed that the reaction may be "direct", that is without the assistance of alcohol, or it may be "indirect"; in the latter the reaction occurs only in the presence of alcohol. This

test, therefore, shows whether the bilirubin has passed the barrier of the liver cells or not. It therefore should enable a distinction to be made between a high bilirubin blood level due to over-production, as in haemolysis, and that due to a damming-back of the bilirubin into the circulation because of a failure of excretion. A high bilirubin blood level, no matter its origin, produces jaundice, which is simply a staining of the tissues by bile pigment. In the first type of high bilirubin blood level, that due to over-production, the reaction to the test is indirect since the pigment has not passed the liver barrier; the resultant jaundice is therefore called "retention jaundice". In the second type the reaction to the test is direct, since the pigment has passed through the liver cells and has been re-absorbed into the circulation. The resultant jaundice is called "resorptive jaundice", or regurgitant jaundice. There is another important practical difference between the two types of case. When the bile pigment is thrown back into the circulation due to failure of excretion, the cholesterol and bile-salts are thrown back with it; it is the bile-salts which are responsible for so many of the phenomena generally thought to be allied to jaundice, e.g. the itching and the bradycardia. In jaundice due to over-production of pigment these distressing features will not be present as there is no increase of bile salts in the blood, hence the term "dissociated

jaundice".

The van den Bergh test is also used quantitatively; the value arbitrarily chosen is 0.5 mgms. bilirubin per 100 ccs. plasma; this value of 0.5 mgms. per cent is one unit. The quantitative use of the test led to the interesting and important discovery that not only do the two states of the pigment differ in their reaction to diazo, but that the kidney has a different renal threshold for each. In patients with resorptive jaundice bile begins to appear in the urine when the quantitative van den Bergh level in the plasma has reached eight units or more. The bilirubin arising from over-production has a much higher renal threshold. Boyd (1944) goes so far as to suggest that true unchanged bilirubin never passes the renal threshold.

It is necessary at this point to consider the question of jaundice in more detail. The yellow colouration of the tissues is from the yellow bilirubin; biliverdin is green. It has already been pointed out that jaundice may be the result of over-production of bile pigment or the result of a failure in excretion. Clinically jaundice is not fully established until about twenty-four hours after the serum has reached the critical level; this time lag is the time required for the staining of the tissues. In many cases of haemolytic jaundice the reaction is biphasic, i.e. gives

both types of reaction. This is generally explained by the suggestion that the liver cells have become involved in the disease process and are allowing to regurgitate back into the circulation a small amount of changed bilirubin which is then found to be present in addition to the excess of unchanged bilirubin. In such cases the hyperbilirubinaemia is accompanied by pleocholia with bilious vomiting and dark-brown stools, all indicating the efforts made by the reticulo-endothelial system to cope with the over-production of bilirubin.

Another, and simpler, quantitative test is the "icterus index" introduced by Meulengracht (1920). In this test the standard is a 1/10,000 solution of potassium dichromate; the result of the test is expressed as a simple figure, which is the number of times that the serum required to be diluted to produce a colour matching the dichromate standard. In practice it has been found that normal serum will match with not more than a six-fold dilution; with latent jaundice the dilution lies between six-fold and fifteen-fold, i.e. gives icterus index values of between six and fifteen. With overt jaundice the dilution factor is any value above fifteen. This test depends on colour matching and is therefore of no value in intra-vascular haemolyses during the active phases.

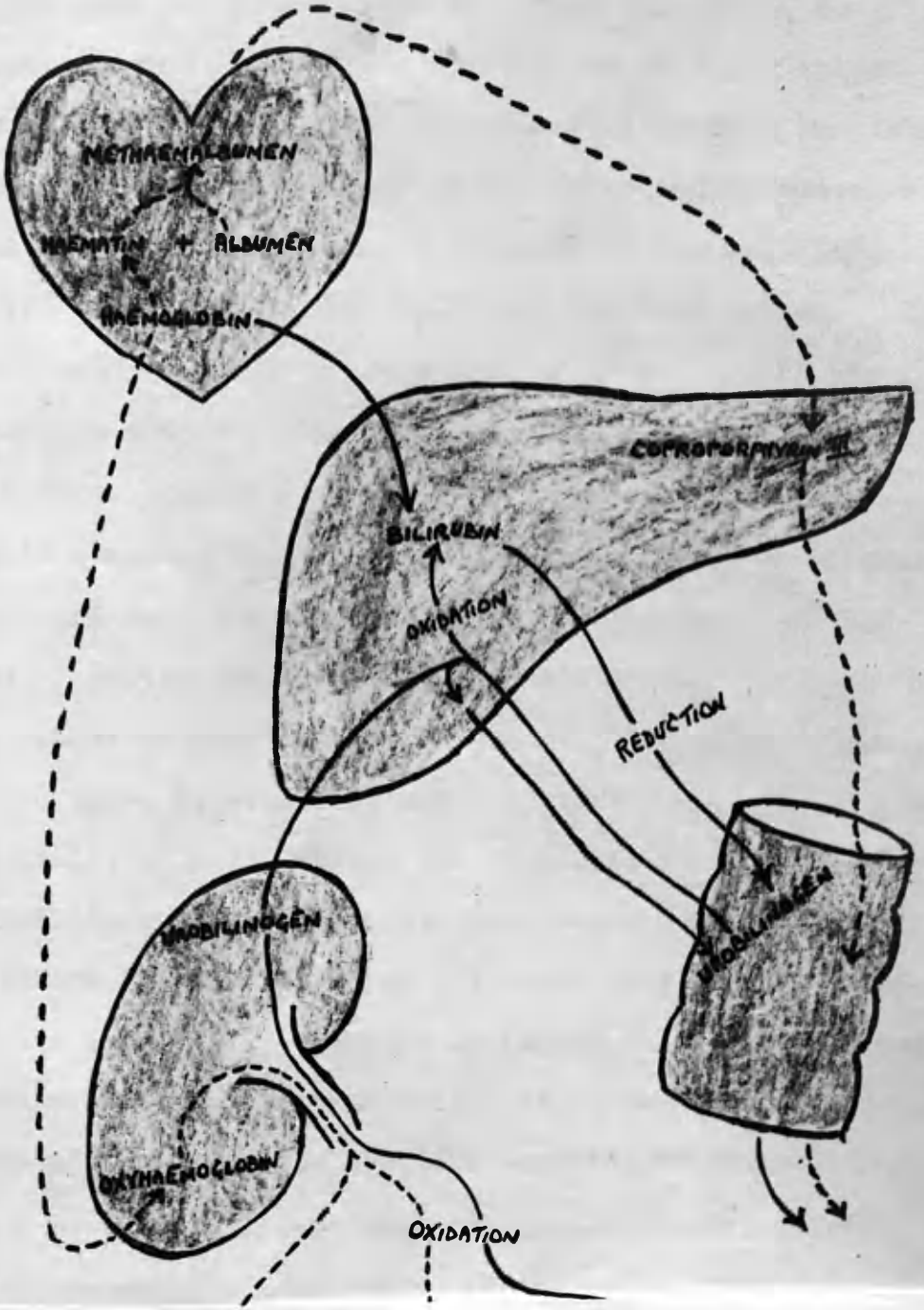
The practical applications of the above remarks will be made evident when we come to discuss the laboratory findings in intra-vascular and other haemolyses. In the meantime we must continue our observations on the excretion of bilirubin, illustrated in plate No. XVII, page 179.

Bilirubin, accompanied by a small amount of biliverdin, passes from the gall-bladder into the gastro-intestinal tract. The pigments pass unchanged as far as the large bowel where they undergo reduction by bacterial action and produce a colourless chromogen called urobilinogen (or stercobilinogen). Further chemical changes cause the stercobilinogen to be changed into the brown pigment stercobilin (or urobilin), see plate No. XIV, page 164. Both stercobilinogen and stercobilin are found in the faeces, the brown colour of which is due to stercobilin. The darkening which the faeces show on standing in air is probably the result of a change to stercobilin of any stercobilinogen which may be present.

The story is not yet finished; on its way down the large intestine, part of the bile pigment is re-absorbed and passes back to the liver via the portal circulation, probably as urobilinogen. Much the greater part of this reabsorbed pigment is again taken up by the liver cells and is passed out

– PIGMENTS IN THE BILE, BLOOD, AND URINE IN INTRAVASCULAR HAEMOLYSIS. –

(CONTINUOUS LINES INDICATE NORMAL PHYSIOLOGICAL PROCESSES
BROKEN ~ ~ ABNORMAL PATHOLOGICAL ~)



with the bile as urobilinogen or, after oxidation, as bilirubin again! In normal individuals, a small amount of the urobilinogen, which had been re-absorbed from the large intestine, escapes excretion by the liver and is excreted as urobilinogen by the kidney. A change of the urobilinogen to urobilin takes place after the urine has been voided. Bilirubin itself does not normally appear in the urine, the colour of which is due to other pigments. In normal individuals the urobilinogen found in the urine amounts only to a trace. It is again stressed that bilirubin is essentially an intermediate product and does not normally appear in the urine or the faeces. Whitby and Britton (1946) state that the total normal daily output of urobilinogen in the urine amounts to not more than 2.0 mgms. generally excreted in spurts two to three hours after meals. In the faeces the 24-hourly output of pigments, stercobilinogen and stercobilin, may reach 280 mgms. Any significant increase of serum bilirubin, e.g. after haemolysis, keeps the liver fully occupied in trying to clear the heavy concentration of blood bilirubin; as a result, when urobilinogen appears, having been absorbed from the intestine, it is ignored by the liver and passes in increased concentration to the kidneys whence it is voided in the urine; the finding of increased urinary urobilinogen is to be expected in such cases. On the other hand, even when bilirubin is in excess in the

serum, as long as the liver cells are healthy no bilirubin will appear in the urine; as already explained, this is because the renal threshold is very high for that state of bilirubin which has not passed through the liver cells. Consequently haemolysis must be very severe before even a trace of bilirubin appears in the urine, unless there is present a secondary change in the liver cells. In obstructive jaundice, however, bilirubin is a common finding in the urine since this type of bilirubin is freely passed out through the kidney at low concentrations. In the latter type of case bile salts will almost certainly also be found in the urine. Many cases of intra-vascular haemolysis show a biphasic reaction and show bilirubin present in the urine. In such cases there is probably accompanying liver damage; the bilirubin which gives the direct reaction with diazo and which escapes through the kidney is that portion which has been allowed to regurgitate through damaged liver cells. Rich (1930) suggests liver anoxaemia to be the cause of the liver damage; Hills (1946) suggests that the cause is a flooding of the liver with pigment. Whatever the explanation, the biphasic reaction, with secondary bilirubinuria is quite commonly to be found in haemolyses.

This ends the short discussion on the physiological

processes taking part in the metabolism of haemoglobin.

It is now possible to proceed to the consideration of the findings in certain pathological conditions and processes.

It must not be forgotten that the main object of this investigation is the study of six cases of haemoglobinuria which were seen in Waziristan in 1938. So far all that we are entitled to presume is that there had been a destruction of red cells so severe as to cause haemoglobinuria. It is meet, therefore, now to turn our attention to those changes in blood-pigment metabolism which are found in patients suffering from increased red cell destruction. Generally speaking, in such cases, the departures from the findings in normal metabolic processes are both quantitative and qualitative.

Consider first the qualitative changes; these are important, since the most striking finding in patients with increased blood destruction is the appearance in the blood of a pigment methaemalbumin which has not been mentioned in the findings in a normal metabolism of haemoglobin. Another abnormal pigment sometimes found in individuals showing intravascular haemolysis is methaemoglobin; this pigment is also usually found after the ingestion of certain drugs, e.g. the sulpha drugs. It is convenient to consider these two

abnormal pigments together, especially since they so closely resemble one another. There has already been mention of methaemoglobin, see page 165, and plate No. XVI, page 171. This pigment is an oxide of haemoglobin, with the iron in the trivalent state. We have seen that it is found in minute amount in the blood of normal individuals and is found in significant quantity in certain pathological conditions; it is especially to be found in the blood after the ingestion of certain poisons, e.g. coal tar derivatives. Methaemoglobin imparts a dark-brown colour to the plasma and to the urine when present therein. It has physical properties closely similar to those of methaemalbumin; this accounts for the fact that the identification of the latter pigment is a relatively recent occurrence. Previous to this identification, methaemalbumin had always been mistakenly identified as methaemoglobin. The cardinal difference between the two pigments is that methaemoglobin is essentially an intracorpuscular pigment, appearing only transitorily and rarely free, in the plasma, whereas methaemalbumin is extracellular in origin; it is never found inside the red cell. One other important difference is that methaemalbumin is never excreted by the kidney; so far as is known it undergoes degradation in the body, possibly in the liver, with coproporphyrin III as one of the products. Methaemoglobin,

on the other hand, is readily excreted by the kidney.

Methaemoglobin has been known for a long time, e.g. it is reported that Hoppe-Seyler (1865) found that methaemoglobin can be spontaneously formed from haemoglobin; all that is necessary is haemoglobin in the presence of an oxidising agent. Fairley and Bromfield (1937) found that when a concentrated solution of haemoglobin is incubated with plasma for forty-eight hours methaemalbumin results. But when a solution of oxyhaemoglobin is incubated for forty-eight hours in the absence of plasma then methaemoglobin results. Again, they found that if well-washed red cells are incubated for four days at 40°C. it is possible to produce intracorpuscular methaemoglobin. The important point of these findings is that plasma or serum has the power of producing methaemalbumin directly or indirectly from extra-corpuscular haemoglobin. Another important finding by Fairley and Bromfield is that methaemalbumin can be formed from methaemoglobin.

van den Bergh and Engelkes (1922) claimed that there are two types of methaemoglobinaemia, namely haemolytic methaemoglobinaemia and intracorpuscular methaemoglobinaemia; the latter more properly should be termed methaemoglobincythaemia. By "haemolytic methaemoglobin" the authors meant the presence in the <u>plasma</u> of this pigment; it has already been

said that such a finding is rare, having been reported in a very few cases of anaerobic sepsis and eclampsia. This class is of no practical importance and need not be further considered. The other class is of much importance, namely methaemoglobincythaemia. This is found in two pathological conditions; the first is the excessively rare condition of idiopathic enterogenous cyanosis, already discussed on page 166; it is of no importance. The other cause is the important one, namely methaemoglobincythaemia due to toxins. Many of the newer chemotherapeutic substances have the property of producing methaemoglobincythaemia, e.g. plasmoquine. Drugs capable of producing this pigment in the red cells are also capable of producing sulphaemoglobincythaemia. The former condition is much less serious than the latter, because methaemoglobin, although "inactive" (see page 165) is a potential oxygen carrier in that it reverts to haemoglobin in a few days; this recovery to active pigment is markedly accelerated by the administration of methylene blue. Sulphaemoglobin cannot be broken up in this manner, In sulphaemoglobincythaemia the skin is leaden-coloured, whereas in methaemoglobincythaemia the skin is dark brown. The former condition need not enter further into our summary.

In methaemoglobincythaemia, as the name indicates, the pigment is intra-corpuscular, which is in marked

contradistinction to methaemalbuminaemia. The generally-accepted explanation of the occurrence of methaemoglobincythaemia with administration of certain drugs is that such drugs are mild oxidising agents. It is advantageous to consider this problem further, taking as a typical and possibly relevant example the methaemoglobincythaemia which results from the taking of plasmoquine. Fischer and Weise (1927) were among the first to investigate this side-effect of plasmoquine administration. They consider that the condition can generally be recognised to be present when the blood concentration reaches 2½%, and it certainly can be detected when the concentration in the blood reaches four per cent. The authors reported that plasmoquine dosages of 0.05 grams intra-muscularly daily produced methaemoglobincythaemia on the fifth to sixth day and that this remained for five to six days. Daily doses of 0.1 grams intra-muscularly produced methaemoglobincythaemia on the third to fourth day, with the condition persisting for seven to fourteen days. These are dosages much in excess of the present-day therapeutic doses. Of much more importance to us is the finding of Fischer and Weise that doses of 0.02 grams plasmoquine daily did not produce methaemoglobincythaemia except after long-continued administration, namely about the seventeenth day; with such a dosage the changes in the blood were so small as to be un-noticeable. The importance

of this finding will become manifest later. The findings of Fischer and Weise have recently received support from Dimson and McMartin (1946) who failed to find methaemoglobincythaemia in fifty patients who had each received a total dosage of 0.1 grams of plasmoquine. The latter also failed to find methaemoglobin in the laked cells of patients whom the authors diagnosed as suffering from "plasmoquine haemoglobinuria"; more probably those patients were actually sufferers from true blackwater fever. One practical point to be mentioned here is that if haemolysis should occur in a patient with methaemoglobincythaemia then it seems reasonable to presume that there is a period when methaemoglobin is free and present in the plasma, even although this period may be very short because of removal of the pigment by the kidney and because of its conversion into methaemalbumin. Some workers do not agree.

In patients showing methaemoglobincythaemia from toxins the degree of change of haemoglobin into methaemoglobin varies in each cell. There is no uniform standard of change; this accounts for the occasional reports that, when haemolysis does occur in persons suffering from methaemoglobincythaemia, the cells lysed first and in greatest number are those with a heavy methaemoglobin content. Admittedly it is conceivable that such cells are less able to resist haemolysis than are

normal cells. It is doubtful, however, whether this is any justification for presuming that the presence of methaemoglobincythaemia is related to the haemolysis which sometimes occurs in patients so affected. It will be seen later that this is not an academic point.

The spectroscopic appearance of methaemoglobin is very similar indeed to that methaemalbumin; this will be discussed in the remarks, to follow, on the latter. It is necessary to stress that when methaemoglobin occurs in a patient it is practically always as the result of drug administration, and is practically always intra-cellular; practically speaking, it is only found free in the plasma as a transitory phenomenon in an individual who develops haemolysis when suffering from methaemoglobincythaemia. This, of course, excludes the minute amount found in normal blood.

The next pigment to be considered is methaemalbumin. The first point to note is that this pigment is never found in the blood of normal individuals, even in the most minute traces. The presence of this compound in any amount whatever is an indication of a pathological process at work. For many years, since the beginning of the systematic study of haemolyses, this pigment had been seen to be present in the plasma of individuals suffering from any marked degree of intra-vascular haemolysis, yet it had always been mistaken

for methaemoglobin until 1934. Admittedly the differentiation is difficult, well-illustrated by the story of its discovery. Fairley and Bromfield (1934a) were investigating certain laboratory findings in nine cases of blackwater fever. In one markedly-cyanotic case they found in the plasma a brown pigment resembling methaemoglobin but not reduced by Stoke's reagent. This new pigment was never found in the urine. Samples were sent to Professor Keilin at Cambridge who considered that the substance was a peculiar haemoglobin derivative with a normal prosthetic group, but with the globin portion of the molecule modified, i.e. the new pigment had the general appearance of methaemoglobin but the absorption bands are shifted about 60Å units to the short-wave end of the spectrum; it was easily reduced by sodium hyposulphite. It was not realised at the time that the protein moiety was, in fact, albumin; this accounts for the name originally given to it, namely "pseudomethaemoglobin". Later, after experimentation, the pigment was recognised as a compound of haem with albumin and was given the name "methaemalbumin" by Fairley (1938). This substance, when present in the plasma, imparts to it a brown colour. In any case of intra-vascular haemolysis, therefore, the colour of the plasma may be compounded of not less than three pigments, i.e. oxyhaemoglobin, imparting to the plasma a rosy-red colour; methaemalbumin, imparting to the plasma a dirty-brown colour; and bilirubin, imparting to

the plasma a yellow colour. Also, as already explained, methaemoglobin may make a fleeting appearance in the plasma under certain circumstances; this pigment also has a brown colour which is indistinguishable from that of methaemalbumin.

The identification of the new pigment by Fairley and Bromfield was the first step in the solving of certain puzzling findings in intra-vascular haemolyses. As early as 1914 Barratt and Yorke, working with rabbits, found that even when they increased the haemoglobin content of the plasma four hundred times, the resulting bile-pigment increase was only six-fold; thereafter it was repeatedly confirmed by various workers that there is no uniform relationship between the degree of haemolysis and the amount of bile pigment excreted. As has been discussed, the normal method the body has of dealing with haemoglobin is the reduction of it to bilirubin by the reticulo-endothelial system, the bilirubin then being excreted by the liver. What seemed to emerge from the work, quoted above, of Barratt and Yorke and of others, is that this normal method of dealing with haemoglobin, i.e. by the conversion of it into bilirubin, has only a relatively limited capacity and can only meet demands reasonably near the normal. It seems that even a minor degree of haemolysis presents to the body a problem quite beyond the normal functioning capacity of the reticulo-endothelial method of dealing with free haemo-

globin. One other obvious method remains whereby the body can rid itself of excess free haemoglobin, namely the method of excretion through the kidney with resultant haemoglobinuria. To Nature this must be a serious and extreme measure since it represents a heavy loss of iron, that very necessary metal which is so laboriously come by; for example, even although the body regards free circulating haemoglobin as a foreign substance, the kidney excretes it only after the level in the plasma has reached a threshold level for the kidney.

Pearce et alia, as long ago as 1912, stated that a concentration of 0.06 gms. haemoglobin per kilogram of body weight was required to produce haemoglobinuria in dogs.

Lighty et alia (1932), working with dogs, suggest that the level of haemoglobin in the plasma necessary to produce haemoglobinuria was, on the average, 84 mgms. of haemoglobin per kilogram of body weight. This was the level they found after repeated injections, however, which is an important point.

Fairley (1940a), using stroma-free haemoglobin placed the renal threshold level between 37-102 mgms. of haemoglobin per 100 ccs. of plasma. He did not claim this as definite, however.

Gilligan et alia (1941) found haemoglobinuria did not begin in patients until the plasma level was 130-150 mgms. of haemoglobin per 100 ccs. of plasma (this is roughly

the same level as that suggested by Lighty et alia). Gilligan et alia did note one other point, also noted by Lighty et alia, namely that once haemoglobinuria had started, it would persist until the level in the plasma had fallen much below the initial threshold level, sometimes until the plasma figure was as low as 30-50 mgms. per 100 ccs. plasma.

As is to be expected the available evidence suggests that tubular re-absorption is responsible for the threshold phenomenon. Yuile (1942) supports this view. He also agrees with other workers that, following repeated injections of haemoglobin sufficient to cause haemoglobinuria, the renal threshold for haemoglobin may be lowered sixty per cent or more.

And, furthermore, even taking into account the haemoglobin voided in the urine in such cases, there still remains a large amount of haemoglobin unaccounted for, which certainly had not been dealt with by the usual channels. Stephens (1927) provides a simple quantitative illustration of this. He found that the total amount of haemoglobin passed in the urine in a severe case of haemolysis amounts to some 100 ccs. of red cells. But in such severe cases four-fifths of the cells may have been destroyed, e.g. a fall in the blood count from five million red cells to one million red cells per cub.mm. of blood is not unusual. Now if the

average total volume of blood in the body be taken as about 5,000 ccs. then, since the red cells form approximately fifty per cent of the blood volume, the total volume of red cells in the body may be taken to amount to 2,500 ccs. We have seen that destruction can occur of four-fifths of this; therefore about 2,000 ccs. red cells had been destroyed. But only 100 ccs. of red cells have been accounted for in the urine. This has left no less than 1,900 ccs, unaccounted for, only a small proportion of which could have been dealt with by the reticulo-endothelial mechanism. The rough figures of Stephens are supported by Yorke et alia (1930) who claimed that not more than ten per cent of the liberated haemoglobin is excreted in the urine even in cases showing polyuria.

The identification of the new pigment by Fairley and Bromfield in 1934 went far towards solving the fate of the remaining haemoglobin. In short the formation of methaemalbumin by the body appears to be a process of conservation of iron. We have said that when haemoglobin is set free in the plasma, the body treats it as a foreign substance and attempts to eliminate it as soon as possible; with the identification of the new pigment methaemalbumin, it was realised that there are three ways in which the body tackles the problem of haemoglobin circulating free in the plasma; these are:

1. The normal; i.e. absorption by the reticulo-endothelial system; the haemoglobin is degraded to bilirubin.

This method of excretion may be sufficient to handle slight degrees of haemolysis, e.g. Fairley (1940a) suggests it can handle injections of small amounts of haemoglobin, say five grams. This mechanism, therefore, is probably able to cope with the haemolysis in the average case of malaria. The result will be hyperbilirubinaemia, but the Schumm test will be negative. (For a note on the Schumm test see below.)

2. Intra-vascular katabolism of haemoglobin; this is shown by hyperbilirubinaemia, Schumm test positive, and formation of methaemalbumin, essentially an extra-corpuscular pigment. The possible method of formation of methaem-albumen is discussed below.

3. Renal excretion of haemoglobin; this, the third mechanism, is an extreme measure. It represents the loss to the body of iron. This only comes into operation when the concentration of extra-corpuscular circulating haemoglobin reaches

the renal threshold. One suggestion is that this occurs when about seventeen ccs. of corpuscles have been laked.

Ross (1927b) estimated that if the haemoglobinaemia is such that it can directly be represented by a bilirubinaemia of less than six indirect van den Bergh units then the renal mechanism of disposal is not called into play. Actually however the most important of the factors determining the haemoglobinuria is the rapidity of the haemolysis and not the degree.

There are some differences of opinion as to the degree of haemoglobinaemia which may be found in a severe lysis. Among the earlier workers Christophers and Bentley (1908) estimated the figure usually to be about one per cent, although they reported one patient with a plasma value of 3.75 per cent haemoglobin. Barratt and Yorke (1909) found plasma values much the same range. Generally the opinion is that the values of haemoglobin in the plasma of patients with severe haemolysis are between one per cent and three per cent.

One point to be kept in mind is that, since jaundice takes about twenty-four hours to develop, in any severe lysis haemoglobinuria will appear some time before jaundice.

It is repeated that the formation of methaemalbumin is essentially a conservation process, preventing the iron

being lost by passage through the kidney. Fairley and Bromfield (1937) suggested that there may be two stages in the formatinn of this methaemalbumin in the body; firstly a splitting-off of the globin from the circulating haemoglobin by which haematin, haem, is left available; (this is not the usual method of degradation of haemoglobin, see page 169.) and, secondly, combination of this porphyrin compound (trivalent iron) with plasma crystalbumin resulting in the production of methaemalbumin. Certainly this pigment methaemalbumin can be synthesised in man and monkeys after intravenous injection of alkaline haematin.

This pigment differs from methaemoglobin in having an absorption band at 623 μμ. This band is not altered by Stoke's reagent; it is dispersed by strong ammonium sulphide with the production of a haemochromogen band at 558 μμ. The reaction to ammonium sulphide is the basis of Schumm's test. In practice, however, even with equipment available the differentiation of methaemalbumin and methaemoglobin is not easy. Furthermore, methaemalbumin has to be present in fairly high concentration in the plasma before it can be detected with any certainty by spectroscopic examination. The only equipment of this type available in Waziristan at that time was a difraction-type hand spectroscope by which it would have been impossible to differentiate the methaemoglobin band

at 630$\mu\mu$ from the 623$\mu\mu$ band of methaemalbumin. The differentiation in our cases was made by a chemical change, Schumm's test. This is a useful and delicate method of identifying the presence of methaemalbumin; it was originally meant to serve for the detection of haematin. The test is a simple one; to the serum to be tested there is added a layer of ether, then a measured quantity of concentrated ammonium sulphide, and, later a few drops of ammonia. The result is the formation of ammonium haemochromogen, with an easily-identified band at 558$\mu\mu$. Unlike methaemoglobin, methaemalbumin is not affected by Stoke's reagent. To summarise the qualities of methaemalbumin; it produces a brown serum; it is never found in the corpuscles; it is formed from haemoglobin, oxyhaemoglobin, and methaemoglobin after the liberation of these pigments from the red cells; it cannot function as a respiratory pigment, and it never appears in the urine in demonstrable quantities. The production of methaemalbumin is evidently a stage in a process by which the body disposes of circulating extra-corpuscular blood pigments; its appearance is to be anticipated in any intravascular haemolysis of sufficient magnitude; its appearance is entirely beneficial, because it conserves iron.

The brown pigment found in the <u>urine</u> in haemoglobinuria is methaemoglobin, which is derived from oxyhaemoglobin; the transformation takes place in the tubules of the bladder;

this methaemoglobin is not the result of methaemoglobinaemia.

Methaemalbumin is formed fairly rapidly in the body, having been found within four hours of the beginning of haemoglobinuria. (It should be remembered, of course, that the beginning of haemoglobinuria is not necessarily the same as the beginning of the haemolytic process.) The methaemalbuminaemia is dependent on the extent and duration of the haemolytic process, e.g. if the process is of small degree and of short duration the liberated haemoglobin will be dealt with by the reticulo-endothelial system as explained above. But if the haemolysis has been large, or acute, or long-continued then the body will form methaemalbumin; in the latter type of case hyperbilirubinaemia will also be present since the two mechanisms are not mutually exclusive. The first-fruits of this discovery of methaemalbumin by Fairley and Bromfield was that it provided a method of establishing whether or not any particular haemolytic anaemia of any degree was intra-vascular or not, because any degree other than a very minimal one of intra-vascular haemolysis leads to the production of methaemalbumin which is not found in other types of haemolysis. Typical of the findings in intra-vascular haemolysis are those quoted by Fairley and Bromfield (1938) in some Macedonian cases of blackwater fever. The pigments in the plasma were:

Total cases	oxyhaemoglobin positive	oxyhaemoglobin and methaemalbumin positive	methaemalbumin positive	methaemoglobin positive.
14	2	10	2	nil.

The two patients who showed oxyhaemoglobin only were two children in whom the haemolysis had been mild and of less than twenty-four hours duration.

One other result of the finding of the new pigment was that it explained puzzling features of certain cases of haemolysis in which the fall in the red cell count continued and in which there were repeated attacks of fever, but no haemoglobinuria. Investigation of such cases showed that the bouts of fever and the continued fall in red cells were accompanied by a rise in the value of the plasma methaemalbumin, or a re-appearance of this pigment in the plasma of those patients whose plasma had become free of it. This finding indicated that there had been a recurrence of the haemolytic process, but not of such degree as to produce haemoglobinuria. In such cases transfusion of blood may be a life-saving measure. Blacklock (1923) had previously put forward a similar theory on clinical grounds.

The best summary of the value of this finding by Fairley and Bromfield (1934a) is that given by Fairley himself. Talking of the discovery in connection with the haemolysis

of blackwater fever he said that the discovery had illuminated the mechanism at work even though it has nothing to do with the fundamental cause of the haemolysis.

It has been seen that the mode of formation of methaemalbumin is not clear; similarly the mode of degradation of the pigment is also obscure. The breakdown follows a somewhat different course from that of haemoglobin, with production of coproporphyrin III which is excreted via the liver. This was adumbrated by Yorke and Nauss in 1911. The matter will be dealt with at more length in later chapters.

This completes the brief summary of the metabolic processes of haemoglobin, and of the quantitative and qualitative changes in the pigment derivatives which are found in haemolysis.

Jaundice.

This is a convenient stage at which to discuss jaundice, a problem intimately bound up with the subject-matter of the preceding paragraphs, and a problem which will require our attention later in this chapter.

In man the bile pigment is bilirubin, yellow in colour and normally present in human serum in small amounts,

usually not more than 1.0 mgms. per cent. When excess of this pigment appears in the blood-stream for any reason, then it may escape into the tissues of the body; after some hours of this, usually within twenty-four hours, the tissues may become yellow, i.e. there will appear the clinical condition "jaundice". From previous paragraphs it may be gathered that excess of bilirubin in the blood-stream may arise either from over-production of bilirubin, typically seen in haemolysis, or from a damming-back of bilirubin because of malfunction of the liver. In practice, clean-cut division into the two types is rarely encountered; some workers, for example, claim that icterus rarely occurs with blood destruction alone, and consider that when blood destruction is accompanied by jaundice there must be a superadded element of liver malfunction, e.g. due to anoxaemia (Rich, 1930) or to a flooding of the liver with pigment (Hills, 1946).

An important point, and one already mentioned, is that the degree and onset of jaundice differ markedly in the two types of hyperbilirubinaemia. For example, in the jaundice found with liver malfunction not only does the bilirubin spill over more readily into the urine (said to occur with a serum bilirubin concentration of anything over eight units) but it appears to diffuse more readily into the tissues. On the

other hand, in jaundice primarily the result of blood destruction the bilirubin escapes into the urine only when its concentration in the serum is relatively high, said to be about twenty van den Bergh units, i.e. this slow escape into the urine is paralleled by an apparently slow escape of the staining pigment into the tissues. As a result, the jaundice in the latter class of patient is later in onset and very much less in degree than that found in individuals with the same concentration of bilirubin in the serum but in class I, i.e. individuals with obstructive jaundice.

Jaundice in Malaria.

This is relevant to the discussion on the haemoglobinuria of the Razmak patients.

The disease malaria well illustrates the remarks in the above paragraph on jaundice. In malaria there is haemolysis, both intra-vascular and in the reticulo-endothelial system. The intra-vascular haemolysis may range in degree from mild to severe, e.g. in severe attacks of malignant malaria as many as half a million red cells per cub.mm. may be destroyed in a single paroxysm; Voigt and Voigt (1938) examined the level of plasma haemoglobin in seventeen cases of malignant tertian malaria and found that in four of the cases the plasma haemoglobin levels were as high as 22-60 mgms. per cent. As a result of this considerable destruction of red

cells it is common to find muddy-yellow discolouration of the skin and the sclerae: van den Bergh himself, in 1924, was among the first to refer to an increased value of the indirect reaction in malaria. Ross (1932) also reported that in malignant tertian malaria he invariably found the indirect van den Bergh reaction to be positive. This opinion was generally accepted that malaria causes intravascular haemolysis of such a degree that there is hyperbilirubinaemia. With the giving of modern chemotherapeutic remedies early and in adequate dosage, the disease is nowadays brought much more rapidly under control, with blood-destruction reduced to a minimum; as a result the muddy-yellow appearance of chronic malaria is less commonly to be seen in malarious areas. An example of this is seen in the paper by Hills (1946) who opens his article with the statement that jaundice is uncommon in malaria; presumably he was only in contact with individuals treated early and adequately. Hills was reporting the findings in an investigation of the incidence of jaundice in 8837 cases of malaria treated in an American general military hospital in Assam over a period of twenty-eight months. The patients treated were all American and Chinese soldiers. The author was investigating individuals with a raised van den Bergh quantitative reaction, or with a raised icterus index, or showing overt jaundice. He took as his standards of minimal

abnormality, a standard of not less than 0.6 mgms. per cent for the van den Bergh reaction, and an icterus index of ten. He collected 321 patients from the 8837. The author concluded that only in twenty four of those cases could the changes be blamed upon malaria, i.e. in all the 8837 individuals treated for malaria only twenty-four showed "malarial jaundice"; this is an incidence of 0.27 per cent. In twelve of the twenty-four cases the method of investigation was the icterus index, in seven the method used was the van den Bergh reaction, and in five cases both methods were used. The van den Bergh values were low, which may be explained by the fact that the author reports a direct reaction; this direct reaction was confirmed by the finding of liver damage in three fatal cases who came to autopsy.

This discussion on jaundice and malaria will later be seen to have some bearing on the problem before us, namely the establishing of the cause or causes of the haemoglobinuria in the six cases of malaria in Waziristan in 1938.

For easy reference we shall abstract and discuss at this point those laboratory findings in the six Razmak cases which are relevant to the above paragraphs; such findings to be discussed will include the results of the van den Bergh reaction, jaundice, icterus index results where applicable, spectroscopic analysis of the plasma, spectroscopic analysis of the washed lysed red cells.

(spectroscopic analysis of the urine will be discussed in Chapter V), and the depth of colour of the blood pigments in the plasma; this last will be estimated by comparing the plasma with solutions containing varying quantities of lysed red cells.

i.e. Relevant laboratory findings in the Razmak cases.

The first of the laboratory findings to be discussed is the van den Bergh reaction, and the values shown by our patients. The findings are tabulated in figure No. 2 below. Where jaundice was found this has also been shown in the same table.

Figure No. 2.

Quantitative and Qualitative Results of the van den Bergh reaction.

Key. Qualitative: B = biphasic positive (± mild; + = present)
D = direct positive (++ marked; +++ = severe)
I = indirect positive
Quantitative: figures in brackets give the v.d.Bergh values. (All quantitative reactions were carried out using indirect technique.)
J = Jaundice. -ve = Test negative.
+ve = Test positive.
N.A. = Test not carried out.

Case No.	Number of days after coming under observation with ?lysis R.B.C.								
	1st	2nd	3rd	4th	5th	6th	7th	8th	1 month.
1	B+(6) J±	B++(5) J++	D+(10) J++	D+++(9) J+	N.A. J++	N.A. J++	Death		
2.	B+(9) J-ve	B+(10) J±	B+(9) J±	B+(3) J+	J+	J±	J±	J-ve	v.d.B. neg
3.	I (5) J++	I (10) J++	I(8) J++	I (4) J+	I (5) J+	J+	J+	J±	ditto

Figure No. 2. (contd.)

Case No.	Number of days after coming under observation with ?lysis R.B.C.								
	1st	2nd	3rd	4th	5th	6th	7th	8th	1 month
4.	I (4) J±	I (5) J+	J+	J+	J+	J+	J± till 12th day		v.d.B. neg.
5.	B (9) J++	D (8) J+++	D (2) J+++	B (3.8) J++	J++	J+	J± till 18th day		ditto
6.	I J -ve	J±							ditto

Cases Nos. 3 and 4 were actually first detected by the appearance of overt jaundice when they reported for plasmoquine treatment.

The above table shows that there is no close direct relationship between the onset and degree of jaundice and the height of the van den Bergh quantitative reaction. It has already been pointed out of course, that when the hyperbilirubinaemia has an indirect van den Bergh reaction, then the jaundice is of relatively slow development.

In the Razmak cases the quantitative van den Bergh findings, given above, were surprisingly low, and yet the patients developed jaundice; in some cases, also, that jaundice was quite marked in degree. This finding of jaundice consistently present, sometimes in severe degree, in individuals

showing low quantitative values for the van den Bergh test suggested that the circulating bilirubin might be, at least in part, that type of bilirubin which had been changed by passage through the liver and had then been re-absorbed into the blood-stream because of malfunction of the liver. This probably is the correct answer; the one patient who came to post-mortem examination certainly did show acute necrosis of the middle two-thirds of each liver lobule. Furthermore, as will be seen later, traces of bilirubin were found in the urine of some of the cases at times when the blood bilirubin levels were below the level at which unchanged bilirubin passes the kidney threshold, although they certainly were above that level at which changed crystallisable bilirubin can escape through the kidney.

Therefore the van den Bergh results, quantitative and qualitative, found in our patients do support the claim that haemolysis had occurred; they do not give any indication, however, of the site of the haemolysis or of the cause. The quantitative findings were low, very little above those found in uncomplicated malaria; those low values, in the presence of marked jaundice of some of the cases, and in the presence of positive direct and biphasic qualitative reactions, are in keeping with the suggestion that part of the circulating bilirubin had been regurgitated into the blood-stream after passing the liver barrier.

Jaundice is the next of the findings which requires discussion; this naturally follows upon the study of the van den Bergh findings.

From the table of results given above it will be seen that every patient showed jaundice at some time or another after the beginning of haemolysis. In fact this was the first symptom in two of the patients. The degree of tissue staining varied considerably, as did also the duration of the staining. Both the degree and the duration of the jaundice bear only a very approximate direct relationship to the severity of the haemolytic processes as measured by the haemoglobinuria, e.g. the fatal case, No. 1, was not the one that showed the most severe degree of staining. At the other extreme the least severe of the haemoglobinuric processes was found in case No. 6 and here the jaundice was fleeting and minimal. The jaundice was most marked and persistent in case No. 5 even though this case was by no means the worst as measured by the course of the haemoglobinuria. In this patient the jaundice was found to be well-established within a few hours of the patient's reporting that he had passed urine of a suspicious colour; the staining became intense by the second day, and persisted until the eighteenth day after beginning of haemoglobinuria, i.e. it persisted for fifteen days after the cessation of haemoglobinuria. It is realised that this persistence of jaundice

does not necessarily indicate continued haemolysis.

In case No. 4 the jaundice was also persistent; it lasted for twelve days, disappearing nine days after the last attack of haemoglobinuria.

After the discussion on urinary changes, Chapter V, it will be of interest to correlate the jaundice with the finding of bilirubin in the urines.

Icterus index is the next point for discussion. Actually, however, there is little to be gained in discussing this test further; as has already been explained, page 177, this is a rough method of estimating the bilirubin content by a method of colour matching. Haemoglobinaemia invalidates it, consequently it was used only on one of our cases and that after the haemoglobin had cleared from the plasma. This was in case No. 2, in whom the test was carried out one month after haemoglobinuria had stopped, and twenty-four days after the jaundice had faded. As might have been expected, the reading was within normal limits, namely four units.

Spectroscopic analysis of plasma and of washed red cells (including evaluation of the plasma colour change, using equivalent percentage solutions of red cells): so far, on the evidence submitted, it has been possible only to say that haemolysis had occurred in our six patients. In this

paragraph it is hoped to settle the next important question, namely whether or not the haemolysis was intra-vascular.

It has already been pointed out that intra-vascular haemolysis is marked by haemoglobinaemia and methaemalbuminaemia, both of which give specific spectroscopic findings. With these facts in mind it is a suitable time to review what the findings were when spectroscopic analyses were carried out on the plasma and on the washed red cells of the six Razmak cases of haemoglobinuria. To recapitulate briefly, plasma normally contains two pigments in small amount, namely bilirubin and haemoglobin; the latter is part oxidised and part reduced. It is possible that methaemoglobin is present in normal plasma but in amounts so minute as not to colour the plasma. In mild haemolyses, whether intra-vascular or reticulo-endothelial, there is an increase in the value of the plasma bilirubin which causes a deepening in the colour of the plasma; this is the basis of the icterus index test.

If the haemolysis be intra-vascular and of a degree such that the reticulo-endothelial system cannot cope with it, then the level of plasma haemoglobin also shows an increase; this latter pigment lends to the plasma a tint which varies from light pink to burgundy depending on the amount of haemoglobin present. In such cases, however, a third pigment makes it appearance, namely methaemalbumin;

this last pigment has a brownish tinge and its presence in the plasma in any demonstrable amount leads to a further deepening of the colour which may go as deep as very dark brown. Lastly, the situation may be further complicated by the presence, in certain cases, of methaemoglobin in significant amounts. In such cases the methaemoglobin is intra-corpuscular and therefore is not found in the plasma except for a very short period of time, as the result of lysis of red cells containing methaemoglobin; this has been explained already, on page 185. The colour of methaemoglobin is the same as methaemalbumin, therefore it lends no characteristic tinge to the plasma.

On pages 205 and 206, Figure 2, it was shown that hyperbilirubinaemia was present in our patients; it now remains to consider the findings on spectroscopic analysis.

Our patients were all receiving plasmoquine at the time of onset of haemoglobinuria. As will be seen later, plasmoquine is a drug with a narrow margin of safety, with a marked tendency to form methaemoglobincythaemia even in doses only a little above the therapeutic level; haemolysis is also to be found with such doses. In all our patients, however, the dosage was small and the total given in each case was not sufficient to produce cyanosis according to the

standards set out by Fischer and Weise, see page 186. This problem of the part played by plasmoquine is discussed in Chapter VIII.

The problem at this stage is two-fold; firstly, it is necessary to establish the site of the haemolysis, and secondly it is necessary to identify the pigments present in the blood. Before continuing with this problem, we shall interpolate a short note on cyanosis, which has a practical bearing on this problem of pigments. A few notes on methaemoglobinaemia are to be found on page 184. It was seen that when any drug is administered which is capable of producing methaemoglobincythaemia, then cyanosis is usually to be found when the methaemoglobin reaches a concentration of 2½%. In none of our patients was there ever any trace of cyanosis, which was to be expected in view of the very small dosage used of plasmoquine. The appearance of cyanosis after the onset of haemoglobinuria is of little differential importance since this will occur with methaemalbuminaemia. For completeness sake, however, we give a record of the findings of cyanosis in our patients, see figure No.3 below.

Figure No. 3.

Record of the findings of cyanosis in the Razmak cases.

Case No.	Number of days after coming under observation with ?lysis R.B.C.						
	1st	2nd	3rd	4th	5th	6th	7th
1		+	+ +	+ +	+	+ +	+ +
2			? ±	±			
3		±	+	?			
4					+	±	
5		+	±				
6							

Key: ± trace of cyanosis. + cyanosis present.

+ + cyanosis moderate.

From the above it is seen that case No. 1 was the only one who showed any degree or persistence of cyanosis; it is probable that in that patient much of the cyanosis was secondary to a pneumonic condition of the lungs. In the other uncomplicated cases cyanosis was found only occasionally and in mild degree, and in such cases was of course due to the presence of methaemalbumin. The heavy natural pigmentation of the Indian probably masked several cases of minor degrees of

cyanosis and our findings should therefore be taken as only approximately correct. If any cyanosis was present before the onset of the haemoglobinuria then it was so minor in degree that it escaped detection; this would indicate that any plasmoquine poisoning which might have been present was of slight degree and would support the contention, to be made later, that in fact our plasmoquine dosage was well within the limits of safety and that plasmoquine poisoning was not the major factor in the production of the haemoglobinuria in the six cases under consideration.

The presence or absence of cyanosis really helps very little, however, in the identification of abnormal blood pigment; the only satisfactory method is by spectroscopic examination. So far the only pigment identified with certainty is bilirubin. But since the patients were receiving plasmoquine, then with any haemolysis at that time theoretically at least three more pigments may have been present in the blood at the time of the haemolysis. These are: haemoglobin with oxyhaemoglobin, methaemalbumin, and possibly transitory methaemoglobin. It was hoped to establish which of the three were present in our patients; unfortunately the methods of examination at our disposal were insufficient for our purpose. We were working in a remote part of the country, several days' journey from large centres of medical research, and the only type of spectroscope available in Waziristan, or for that

matter the only type available in North India, was a hand-type spectroscope; this instrument could not differentiate methaemoglobin and methaemalbumin by direct examination. The only alternative was to use Schumm's test, which was done. This provides a more easily identifiable substance when the pigment under test is methaemalbumin. Using this method, our findings were such as to suggest that in all the patients the brown pigment present in the plasma was methaemalbumin, and we have reported it as such in our summary of spectroscopic findings. On no occasion were we able unequivocally to identify methaemoglobin in the plasma. The results of the spectroscopic analyses of the plasma specimens are given in figure No. 4.

In the interests of accuracy, however, it must be stressed here that at the time the examinations were made our experience was limited, consequently I cannot deny the possibility that methaemoglobin may have been present on occasion and may have escaped identification.

The foregoing remarks apply to examinations carried out on specimens of plasma. Similar spectroscopic examinations were also made in each case on solutions made by the lysing in water of washed red cells. The results are tabulated in figure No. 4 below.

Figure No. 4.

Results of spectroscopic analysis of plasma and of washed lysed R.B.C.

Case No.	Material examined.	No. of days following onset of lysis							
		1st	2nd	3rd	4th	5th	6th	7th	1 mnth
1*	Plasma	m-alb	m-alb	m-alb	m-alb	m-alb			
	Sol.washed R.B.C.	HbOO	HbOO	HbOO	HbOO	?HbOH*			
2.	Plasma	m-alb	m-alb		m-alb				Trace HbOO
	Sol.washed R.B.C.	HbOH	HbOO		HbOO				HbOO
3.	Plasma	m-alb	m-alb	m-alb	m-alb	m-alb ?			HbOO
	Sol.washed R.B.C.	HbOH	HbOO	HbOO	HbOO	HbOO			HbOO
4.	Plasma	m-alb	m-alb	m-alb					Trace HbOO
	Sol.washed R.B.C.	HbOO	HbOO	HbOO					HbOO
5.	Plasma	m-alb	m-alb	m-alb	m-alb				Trace HbOO
	Sol.washed R.B.C	HbOO	HbOO	HbOO	HbOO				HbOO
6.	Plasma	HbOO	m-alb						HbOO
	Sol.washed R.B.C.	HbOO	HbOO						HbOO

Key: m-alb = methaemalbumin present.

HbOO = oxyhaemoglobin present.

HbOH = methaemoglobin present.

* See remarks on case No. 1, page 217.

Remarks:

Case No.1. Showed the presence of methaemalbumin in his plasma until his death. Examination of the washed lysed red cells showed oxyhaemoglobin except on the fifth day, two days before death, when he showed what was interpreted to be small amounts of methaemoglobin but may actually have been methaemalbumin adhering to improperly washed red cells. In theory, differentiation between the methaemoglobin and methaemalbumin should have been easy by the use of Schumm's test, but in this case the results were equivocal.

Case No. 2. In this case the plasma showed methaemalbumin in the three specimens taken on separate days. On examination, the lysed washed red blood corpuscles showed the presence of oxyhaemoglobin absorption bands on the second and fourth days; on the first day, however, the absorption bands appeared to be those of methaemoglobin. This may have been methaemoglobin originally formed inside the corpuscles, or may have been methaemalbumin adhering to improperly-washed cells.

One month later, the plasma showed traces

of oxyhaemoglobin which is, of course, a normal finding.

Case No. 3. Methaemalbumin was present in each of the plasma specimens examined. The results were doubtful in the fifth-day specimen.

The same remarks apply to the specimens of lysed washed red cells as in case No. 2, i.e. the specimens all showed oxyhaemoglobin bands except on the first day when the pigment found may have been methaemoglobin or adherent methaemalbumin.

Case No. 4. In this case each sample examined of plasma showed methaemalbumin present, and each sample of red cells showed oxyhaemoglobin.

Case No. 5. Findings as in case No. 4.

Case No. 6. In this patient the lytic process was very short-lived and of mild degree. Only one specimen of plasma showed methaemalbumin, and all specimens of lysed cells showed oxyhaemoglobin.

The results of the spectroscope examinations are of much importance; they enable us to take the diagnosis of our cases one step further in that they show that in each case

the haemolysis was intra-vascular.

This at once narrows considerably the possible causes of the haemolysis.

The next table, figure No. 5 shows the degrees of colouration of the plasma by circulating blood pigments. The estimate is a crude one, the standards being solutions of varying quantities of red cells.

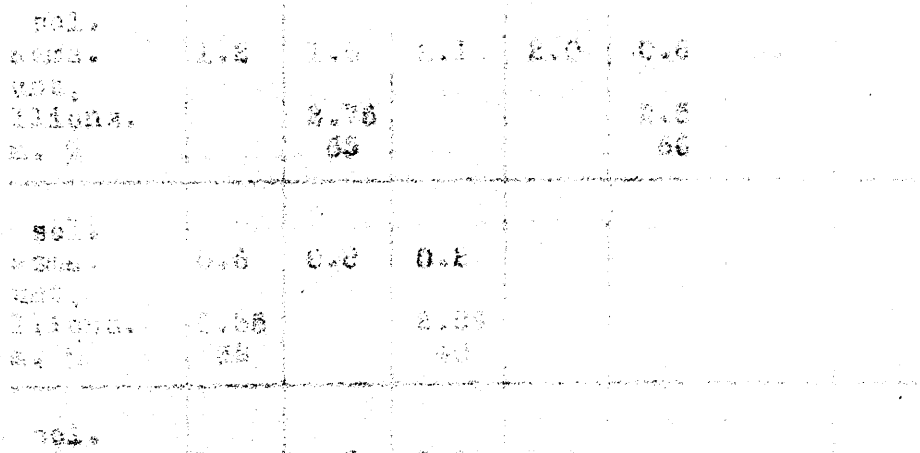


Figure No. 5.

Plasma colour changes, measured against % equivalent solutions red cells.

Case No.	Investigations made	No. of days after coming under observation with ? lysis R.B.Cs.						
		1st	2nd	3rd	4th	5th	6th	7th
1	% equiv.sol. RBCs plasma	1.2	0.8	0.9	1.1	0.5	1.8	?
	Blood count, RBCs. millions	2.64	2.74 ✱	2.46	1.96	2.04 ✱	1.32	1.45
	Hb. estim. %	48	48	47	40	41	30	27
2	% equiv.sol. RBCs plasma.	1.8	0.9	1.0				
	Blood count, RBCs. millions.	2.8		1.9				3.22
	Hb. estim. %	64		49				72
3.	% equiv. sol. RBCs. plasma.	1.2	1.0	2.1	2.0	0.6	0.4	
	Blood count, RBCs. millions.		2.76			2.5		
	Hb. estim. %		65			66		
4.	% equiv. sol. RBCs. plasma.	0.6	0.8	0.2				
	Blood count, RBCs. millions.	2.35		2.89				
	Hb. estim. %	38		40				
5.	% equiv. sol. RBCs. plasma.	1.0	0.8	0.6	0.2			
	Blood count, RBCs. millions.	2.17	1.8	1.7				
	Hb. estim. %	50	43	40				
6.	% equiv. sol. RBCs. plasma.	?						
	Blood count, RBCs. millions.	5.0						
	Hb. estim. %	90						

✱ Transfusion 500 ccs. fresh blood on the day preceding this red cell count.

For ease of reference the red cell estimations and the haemoglobin values are given in figure No. 5 also.

The figures in table No.5 for the haemoglobin levels in the plasma near to the times of lysis show that the values found by us are much lower than those reported by other workers. This may be explained to some extent at least by one difference in technique. From the reports of other workers it seems that the great majority used solutions of red cells lysed in distilled water as the gauge for estimating the haemoglobin content of the plasma. The use of distilled water would seem to give too high a value for the haemoglobin content of the plasma, because such a method fails to make any allowance for the other substances colouring the plasma, e.g. bilirubin. To obviate this error, the red cell solutions for comparison were made up by lysing normal washed red cells in a solution of tartrazine of strength equivalent to a serum with an icterus index of fifteen, i.e. a colour which would eliminate the part played by bilirubin in the colouring of the plasma. The tartrazine solution was matched against a 1/700 solution of potassium dichromate, which is roughly that of an icterus index of fifteen. Varying amounts of red cells were then added to this substratum to provide a range of coloured solutions against which our specimens of plasma were matched. This test cannot be regarded as anything other than approximate. The matching was by direct comparison; no colorimeter was

available.

In view of the wide margin of error which is likely in tests such as this, our values in figure No. 5 are very approximate, although they were made as carefully as possible with the equipment available.

It is to be seen from figure No. 5 that, roughly speaking, the rises in the values of free blood pigment in the plasma run parallel with the phases of lysis as indicated by falls in the red cell counts. This will be further confirmed when we correlate the phases of haemoglobinuria and plasma haemoglobin levels; this will be done in Chapter V.

It is necessary at this point to discuss, in brief, the red blood corpuscle, since any consideration of the laboratory findings in haemolyses necessarily includes the question of the red cell.

The Red Blood Corpuscle.

Compared with those of the living cells of the body, the functions of the red cell appear simple; yet there is still much that is not clear about the cell and its functions.

The opinions of Thorpe (1944) are typical of those of the majority of workers. Thorpe states that chemically it is convenient to picture the red cell as essentially a droplet of a red sol enclosed in a membraneous bag, although

no limiting membrane has been demonstrated histologically. He suggests that the surface membrane may be formed by surface concentration of a molecular layer of protein and lipide (lecithin-cholesterol); this cell boundary behaves like a membrane which is normally impermeable not only to colloids but also to crystalloids, although it is readily permeable to solutions of gases. It is further suggested that the shape of the cell is determined by the stroma.

Another suggestion is that the red cell is not a cell in the generally accepted sense, but is a gel.

Other workers who have discussed this problem of the corpuscle and its functions include: Evans, C.L. (1945) stated that the red corpuscle has an external limiting pellicle resembling that found in most living cells, being formed by a lecithin-cholesterol compound, and it is the solvent power of this which determines the permeability of the red cell by foreign substances. Zinsser et alia (1944) hold much the same opinion, stating that the permeability of the cell membrane may change in various conditions and that part of the process of hypersensitivity may be an increased ability of the injurious substances to get into the cell. Whitby and Britton (1946) support the gel theory, when they state that when red blood corpuscles are placed in normal saline they may be cut into several pieces without the escape of haemoglobin; therefore

according to this the mechanism of haemolysis is not necessarily a simple destruction of the cell envelope. Also, as long previously as 1929 Christophers was thinking along the same lines when searching for a common explanation of the haemolysis produced by acids and bases. He regarded the haemoglobin in the red cell to be present normally as a gel, and suggested that acids and bases produce haemolysis by an effect on the iso-electric point of the haemoglobin and not by their effect on the stroma. Haemoglobin is normally a gel at the iso-electric point, and any charge, either positive or negative, tends to produce haemolysis; this theory, therefore, holds that it is the haemoglobin rather than the stroma which is chiefly concerned in ordinary haemolysis. Whatever the explanation, "ghost" cells are found in the blood-stream following any intra-vascular haemolysis; these "ghost" cells appear to be haemoglobin-free protein remnants of lysed cells. These cell remnants will be more fully considered later in this work, as some workers hold them to be a possible casue of some of the more serious results of intra-vascular haemolysis.

In the absence of more precise methods of investigation, in any search for abnormalities in red cells, we are limited to crude and unsatisfactory investigations like the following: the behaviour of the red cell in a wet preparation, the appearances of the red cells in a stained film,

the red cell count,

the haemoglobin estimation,

the fragility of the cell in hypotonic saline,

the sedimentation rate,

and to certain related phenomena as bleeding time and coagulation time.

These examinations were carried out on all of our patients; the results are tabulated and discussed seriatim, see below.

Our object in carrying out these tests and investigations was to establish whether or not any abnormalities were present in the red cells of our haemoglobinuric patients which would account for the development of this haemoglobinuria. A full haematological investigation was carried out on each of our patients only after the beginning of the haemolysis, and one serious criticism which we cannot ignore is that if any abnormality is found in the haematological examinations, then there can be no guarantee that this abnormality is not a cuase but an effect of the haemolytic process. This will require to be kept in mind.

Red cell appearances in wet films: the only abnormality seen was a mild degree of clumping into masses of the red cells, i.e. auto-agglutination. Spherocytosis is said to occur, but was not seen in any of the

specimens examined.

This auto-agglutination cannot be taken as indicating any abnormality existing in the red cells previous to the onset of lysis, because such auto-agglutination is seen in many haemolyses in which the red cell is not abnormal. Important work has been produced in the last few years by Knisely and various co-workers, e.g. Knisely et alia (1945) and Knisely et alia (1947); these authors have found that in many unrelated conditions there is found to be present an aggregation into masses of the red cells; as a result of this aggregation the blood ceases to flow readily and becomes what the authors have termed "circulating sludge". Even trauma is capable of producing this sludging of the blood. Apropos of the last point, the authors have suggested that in the case of crush injuries, the crushing of the smooth and striped muscle releases substances capable of diffusing in through the vessel walls, and capable of reacting with the constituents of the blood. This does not explain the phenomenon of sludging in all the conditions in which it has been found to occur, e.g. in malaria where in certain monkey experiments a thick glassy precipitate was seen to form around the red cells all through

the body simultaneously within twenty minutes. This precipitate was thought to be fibrin.

Whatever the cause, we are justified in presuming that in our cases the forming of masses was not necessarily an indication of any abnormality in the red cells which had existed previously to the onset of haemolysis and which could have been the cause of the haemolysis.

Red cell appearances in stained films: from stained blood films it is possible to identify in the red cells changes in the shape, the size, the staining reactions, and to identify any nucleated or other early forms of red cells. Leishman's stain was the one used in all the films. In each case the original blood films, on which the diagnosis of malaria had been made, were available for comparison with the stained films made at the time of lysis.

Our findings were that each blood film showed abnormalities in the red cells except in case No. 6 in whom the lysis was very mild. In the remaining five patients, during and immediately after the time of lysis the blood films showed much irregularity in the shape of the red cells, the picture resembling that seen in a severe untreated case of pernicious anaemia.

This abnormality of shape was not found in the original blood films of the patients.

The size of the red cells also showed changes which had only come on at the time of the lysis; some of the cells appeared larger and some appeared smaller than normal. There was no equipment available for the measurement of the cells, and therefore no figures can be given of the actual sizes of the cells. The terms "larger" and "smaller" are relative.

Figure No. 6 gives a summary of the findings so far discussed; it also includes the results of other examinations which are discussed later.

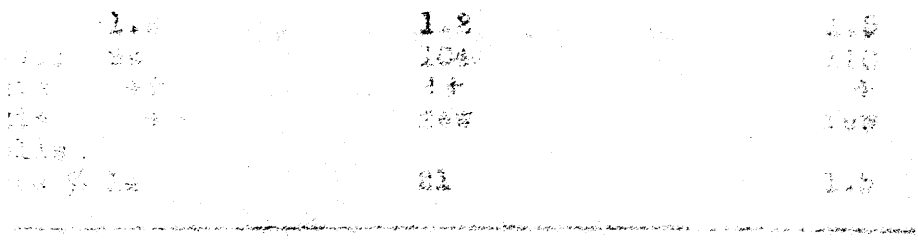


Figure No. 6.

Results of haematological examinations. (Red cells only.)

Case No.	Examinations made	No. of days after coming under observation with ? lysis of RBCs.							
		1st	2nd	3rd	4th	5th	6th	7th	one month
1	RBCs mllns/cmm	2.64	2.74	2.46	1.96	2.04	1.32	1.45	
	Hb. % (Sahli)	48	48	47	40	41	30	27	
	Col.Index.	0.92	0.92	0.9	1.05	1.02	1.1	1.1	
	Mean corp.vol.	102	98	100	100	98	101	102	
	Macrocytosis	+	++	++	++	++	++	++	
	Microcytosis	+	++	++	++	++	++	++	
	Immat. cells	few n	n +	n ++	n ++	n +	n +	n ±	
	Reticulocyte %	30	35	41	35	21	12	7	
2.	RBCs mllns/cmm	2.8		1.9				3.22	6.0
	Hb. % (Sahli)	64		49				72	110
	Col.Index	1.1		1.3				1.1	0.9
	Mean corp.vol.	114						111	91
	Macrocytosis	+						+	
	Microcytosis	+						few	
	Immat. red cs.	few n							
	Reticulocyte %	6						8	0.5
3.	RBCs mllns/cmm	2.76			2.5				3.0
	Hb. % (Sahli)	65			66				90
	Col.Index.	1.2			1.2				1.5
	Mean corp.vol	96			104				110
	Macrocytosis	++			++				+
	Microcytosis	++			few				few
	Imm.red cells								
	Reticulocyte %	14			21				1.5
4.	RBCs mllns/cmm	2.35		2.89					4.45
	Hb. % (Sahli)	38		40					87
	Col.Index	0.8		0.7					0.98
	Mean corp.vol			75					89
	Macrocytosis			+					
	Microcytosis			++					
	Imm.red cells								
	Reticulocyte %			24					11

Figure No. 6 (continued)

Case No.	Examinations made	No. of days after coming under observation with ? lysis of RBCs.							
		1st	2nd	3rd	4th	5th	6th	7th	one month
5	RBCs mllns/cmm	2.17	1.8	1.7					3.52
	Hb. % (Sahli)	50	43	40					80
	Col.Index	1.18	1.3	1.3					1.14
	Mean corp.vol	100							104
	Macrocytosis	+							+
	Microcytosis	few							few
	Imm.red cells	few							
	Reticulocyte %	15							13
6.	RBCs mllns/cmm	5.0							5.3
	Hb. % (Sahli)	90							108
	Col.Index	0.9							1.0
	Mean corp. vol.	90							96
	Macrocytosis	few							
	Microcytosis	few							
	Imm. red cells								
	Reticulocyte %	2.5							0.5

Key: ± = very few ; + = abnormality present

++ = abnormality marked ; n = normoblasts.

* blood transfusion 500 ccs. fresh blood given in preceding 24 hours.

It should be noted that polychromasia has not been mentioned in the above table. It was present immediately after the onset of lysis in every case, and disappeared within a few days of the stopping of lytic process.

It has been noted in the preceding paragraphs that

auto-agglutination and poikilocytosis were present in relation to the lytic phases but that such appearances could not be taken as proof of a pre-existing abnormality of the red blood cell. This interpretation of the abnormalities, that they were an effect and not a cause of the haemolysis, is also the interpretation put upon certain abnormalities noted in the above table. Abnormalities to be discussed in detail are: variations in size of the red cells; immature red cells; red cell counts; the percentage value of the haemoglobin; the colour index, and the mean corpuscular volume.

Variations in size of the red cells: it has already been noted that variations were found in the shape of the red cells. With any significant degree of poikilocytosis there is always to be found some degree of anisocytosis. This was so in the six cases under review. All the patients showed deviations from the normal in the size of their red cells. In all cases both macrocytosis and microcytosis were present. Exact measurements could not be made.

Neither macrocytosis nor microcytosis were so consistently or predominantly present to permit of our drawing any conclusions; these abnormalities in the size of the red cells were not in themselves of any significance.

Immature red cells: the same remarks apply to the presence of normoblasts as were applied to the variations in the size of the red cells. There was no form of red cell seen more immature than the normoblast. Normoblasts were found in small numbers in certain of the blood films of four of the six patients; the normoblasts when present were in numbers within that to be expected immediately following any haemolysis, especially with the marked degree of reticulocytosis that was present in our patients at that time.

Four examinations remaining to be considered are: the red cell count, estimation of haemoglobin, the colour index, and the mean corpuscular volume.

It is proposed to discuss these as a composite picture, patient by patient, rather than value by value. Before we do so it is necessary to give a few notes on the techniques used since there is much variation.

The red cell count was estimated in each case and on each occasion by means of a standard Thoma type haemocytometer using standard red cell pipettes; the blood was drawn at the bedside and was counted within five minutes of being drawn.

Estimation of the haemoglobin was made by means of a Sahli haemoglobinometer; this method was introduced by

Sahli (1931): in it the haemoglobin is reduced to acid haematin, then the solution is measured against a glass standard after about half an hour. We were never in a position to check the readings of our glass standard. We can only say that in a very large series of blood estimations made on routine hospital patients we found the average haemoglobin values to be ninety five per cent. We were then living at a height of seven thousand feet; it is presumed that our glass standards used in haemoglobin estimation gave readings that were too low. The error is of little importance in this investigation.

The colour index in each case is obtained from the two above measurements, i.e. the red cell count and the haemoglobin value. The colour index is only an indicator, and if either of the two measurements concerned are inexact then the colour index is also inexact.

The last of the four measurements is the mean corpuscular volume. This is obtained by calculation, using the formula given here:

$$\text{m.c.v} = \frac{\text{Volume packed cells in ccs/1000 ccm. blood}}{\text{Red cell count/millions per cmm.}} \quad c\mu$$

The figure for the top line was obtained by the method of Wintrobe (1933). The result is expressed in cubic microns, i.e. $c\mu$. The normal average mean corpuscular volume is

about 86 c.μ , with a range of from 80-95 c.μ .

Let us now consider our findings for these examinations.

Case No. 1. Our results show that the continued lysis over several days led to a steady fall in the number of circulating red cells, this fall being unchecked by blood transfusion. The red cell fall was accompanied by a parallel fall in haemoglobin percentage; as a result, the colour index varied only slightly from normal, with an initial value of 0.92 and a value at death of 1.1. This mild degree of swing towards macrocytosis was confirmed by a rise in value of the mean corpuscular volume, which reached a maximum of 102 c.μ . Some remarks on this will be made after the findings of other cases have been reported upon.

Case No. 2. Showed a picture similar to that of case No. 1, namely a marked fall both in the red cell count and in the haemoglobin values, with the colour index slightly above unity, and a mean corpuscular volume significantly above normal; the highest value for the mean corpuscular volume was 114 c.μ .

Within one month the red cell picture had

returned to within normal limits.

Case No. 3. The haematological findings here were as case No. 2, excepting that the blood regeneration was poor, e.g. one month later, and after heavy parenteral dosage of liver the blood picture showed only 3.0 million red cells per cmm. with 90% haemoglobin, and with a mean corpuscular volume of 110 c.μ . This is more fully discussed below.

Case No. 4. The findings in case No. 4 differ from those in the three preceding cases. Here we see that the blood picture following haemolysis showed a marked microcytic anaemia, with a low colour index and with a mean corpuscular volume as low at one time as 75 c.μ .; even after one month's treatment this tendency was still present to a mild degree.

Case No. 5. Like the first three blood pictures, case No. 5 is that of a mild macrocytosis, with the colour index above unity and with a high mean corpuscular volume. Like Case No. 3, this patient proved refractory to treatment; one month after re-admission he still showed only 3.52 million red

red cells, with 80% haemoglobin and with the mean corpuscular volume 104 c.μ .

Case No. 6. In this patient the lytic process was mild in degree and there was no significant change in the red cell findings.

With the exception of the mild case, No. 6, and of case No. 4, in each patient the lytic process produced an anaemia which was associated with large heavily-pigmented red cells which had a mean corpuscular volume above normal. The type of picture produced, therefore, was fairly uniform; this must lead to a consideration of whether this fairly uniform change, with macrocytosis, is indicative of a pre-existing abnormality of the red cells which abnormality may have played a part in the production of lysis, or at the least may have rendered the abnormal red cells more easy to lyse. Such a possibility requires careful consideration because of the prevalence of blood dyscrasias in the Tropics. A similar finding was reported by Fairley and Bromfield (1937) who found "megalocytic" anaemia in certain cases of Macedonian blackwater fever; the authors then presumed that the cause was a deficiency in foods containing the extrinsic factor.

Both macrocytic and microcytic anaemias are very common throughout India, and were found to some extent in the pre-war

Army; this question of anaemia in the Indian Army was the subject of a two-years investigation by me in Waziristan; as a result of that investigation marmite became an issue in Waziristan. In that investigation, from 1938, the large majority of my patients were found to have macrocytic hyperchromic anaemia, a finding which has recently been confirmed by Thomson and Freedman (1947), by Walters (1947) and by Passmore (1947). The reasons for this "tropical" anaemia occurring in the Army are well brought out by Thomson and Freedman, who describe conditions in a camp near Razmak. In the passing it should be emphasised that the deficiencies mentioned have now been supplied and that the diet in the Indian Army now is satisfactory. Patwardhan (1947) of the Indian Nutrition Research Laboratories describes the present-day diet as "adequate". This change in the diet, which took place in 1945, may explain why Hynes (1945) reports findings at variance to those of other workers.

It would seem from the above, then that tropical anaemia was quite common in the Indian Army at the time of occurrence of the haemolyses in the six patients. But even taking into account the fact that anaemia was not an uncommon disease, we cannot presume, on the evidence given, that any or all of our six patients were suffering from such anaemia

before the onset of lysis. In fact the macrocytosis and the other related red cell findings in the four cases are within the limits of the abnormalities to be found in any acute lytic anaemia with fast regeneration of red cells; the reticulocyte percentage estimations showed that fast regeneration of blood cells was occurring in our patients.

Confirmatory evidence that marked anaemia was not present in at least two of our patients before the onset of lysis is found in patients Nos. 5 and 6 in whom blood counts taken before the onset of lysis were found to be within normal limits.

We may proceed with the discussion still of the opinion that on the evidence as yet produced we are not entitled to assume that there was any abnormality present in the red-cell picture before the onset of lysis.

Other observations dealing with red cells which still remain to be considered are: the sedimentation rate, Donath-Landsteiner reaction, bleeding time, coagulation time, and the fragility of the red cells in hypotonic saline. In these investigations any deviations from the normal will provide less direct evidence of pre-existing red cell abnormality than deviations in the haematological investigations already considered, but if found may serve as a pointer to further work.

Figure No. 7 tabulates the results of the remaining haematological investigations.

Figure No. 7.

Results of further haematological examinations.

Case No.	Examination made	No. of days after coming under observation for ? lysis R.B.Cs.							
		1st	2nd	3rd	4th	5th	6th	7th	1 mnth
1	Sed.Rate, mms. per hr	17	46	67	72	70	67	59	
	D.L. test	Neg.			Neg				
	Bleeding time (Duke) mins.	1.9		4					
	Coag.time (Wright)mins.	3.4		8					
	% saline to lyse R.B.Cs.	0.35		0.3					
2	Sed. Rate	66						46	2
	D.L.test	Neg.						Neg.	Neg.
	Bleeding time	2.6						3.5	4.5
	Coag. time	3.9						6	6
	% sal. sol.	0.4						0.4	0.3
3.	Sed. Rate	45			26				25
	D.L. test	Neg							Neg.
	Bleeding time	2.4			3				5
	Coag. time	4.6			6				5
	% sal. sol.	0.4			0.4				0.3
4.	Sed. Rate			78					14
	D.L. test			Neg.					Neg.
	Bleeding time			1.5					4
	Coag.time			3.2					5
	% sal. sol.			0.35					0.3
5.	Sed. Rate	69							9
	D.L. test	Neg							
	Bleeding time	2.0							1.5
	Coag.time	5							2.6
	% sal.sol.	0.35							0.3
6.	Sed. Rate	25							4
	D.L. test	Neg							Neg.
	Bleeding time	2.0							3.5
	Coag. time.	4.5							3.0
	% sal. sol.	0.35							Not done.

These findings are most easily considered seriatim.

Sedimentation rate: The rate of sedimenting of the red cells serves as a rough method of measuring the rate of destruction of tissue, e.g. in tuberculosis. This sedimenting of the cells is also accelerated by anaemia. The investigation is not a measure of any abnormality of the red cell itself, but the findings have been recorded here for completeness sake. The method used was that of Wintrobe (1933), i.e. the figures given above are the rate of fall of the red cells, the measurement being made at the end of one hour. With this method the upper limit of normal is accepted to be about 15 mms. fall in one hour.

The sedimentation rates of the six patients are considerably above normal. This marked acceleration in fall is only partly explained by the anaemia present. A second factor explaining this acceleration is the process of lysis which was present at or immediately before the measurements were made. During this lysis there had been much destruction of body cells. It is seen that the sedimentation rate had returned to normal within one month, except in patient No. 3 in whom the persistence of the fast sedimenting is explained by the persistent anaemia.

The high sedimentation rates are, therefore, explained partly by the anaemia and partly by the lytic process. The high rates do not indicate any pre-existing abnormality of the red blood corpuscles.

Donath-Landsteiner reaction: This test was introduced by Donath and Landsteiner (1904). The test has already been mentioned, see page 126; when the result of the test is positive it indicates the presence in the blood of an autohaemolysin resulting from infection by syphilis.

In our cases the reaction was negative on all occasions.

Bleeding time: Estimates of the bleeding time of individuals are made to detect whether or not any scarcity of platelets is present. The method used was a simple one, that of Duke in which the test is that of finding the time taken for the patient to stop bleeding from a needle puncture of the ear; in normal individuals the time taken varies from three to five minutes.

In our patients the bleeding always stopped within the normal limits, suggesting that there was no shortage of blood platelets in the six patients.

Coagulation time: Like the previous test, this investigation is not a measure of red cell abnormality; it is one more of the necessary investigations of any full haematological examination. The measurement is that of the time taken for blood to coagulate after being drawn from the body. The method used was that of Wright, using a water bath with water at 37°C. By this method the normal time taken for blood to clot is not more than nine minutes. Lengthening of the time taken is found in several conditions, e.g. in haemophilia, vitakin K shortage due to liver disease, abnormal stability of the platelets, etc.

In each of our patients the time taken for the blood to coagulate fell within normal limits.

Fragility of the red cells in solutions of hypotonic saline: Some of the above tests do not have much application to the problem under discussion, and have been included to ensure that no avenue of investigation was overlooked. This last test, red-cell fragility, is important however, in that it has a practical application to the matter in hand.

The principle of the test is a simple one, namely that of ascertaining how far dilution of a saline solution requires to be carried before it is

hypotonic enough to lyse any red cells placed therein. With normal red cells, lysis in hypotonic saline generally begins in a 0.5% saline solution; lysis generally is found to be complete in hypotonic saline of strength about 0.3%

The importance of this test lies in its use in detecting whether or not there is abnormal fragility of red cells in a patient, e.g. as is found in acholuric jaundice and following burns; by "abnormal fragility" is meant that the cells are lysed in saline solutions which do not lyse normal red cells.

Figure No. 7 results show that the fragility of the red cells was normal in each one of the six patients tested.

It is pointed out that, as is customary, we tested only the fragility of the red cells in hypotonic saline. Recently some authorities have claimed that in certain conditions the fragility of the red cells may be normal when tested against saline solutions, yet may be abnormal when tested against other substances; for example Foy and Kondi (1943) claim that in blackwater fever the patient's red cells show increased fragility to lyso-lecithin although they are normal when tested

in hypotonic saline. This finding, if confirmed, may later be linked with a lytic substance reported by various authors to be present in the tissues of the body and possibly to be concerned with normal cell lysis. This problem is more fully discussed in Chapter IX. It is only mentioned here. We did not test the fragility of the patients' red cells to anything other than saline and cannot express any opinion on lyso-lecithin fragility.

This brings to a close the summary of these investigations which directly or indirectly give information about the red-blood corpuscle. A similar summary will now be given of the white cell findings.

The white-cell picture: So far in this chapter we have dealt only with pigment changes and with red cell changes in certain conditions. It is now proposed to close the chapter by recording the total white-cell counts and the differential white-cell counts, and to see whether or not the figures show any significant deviations from the accepted normal standards, deviations which may act as a guide to the aetiology of the condition which produced lysis in our six patients.

Figure No. 8 summarises the findings.

Figure No. 8.

White cell counts, total and differential.

Case No.	Examination made	No. of days after coming under observation with ? lysis RBCs.							
		1st	2nd	3rd	4th	5th	6th	7th	1 mnth
1	Total WBCs per cmm.	10000		18600		16000		12600	
			18000		17000		13400		
	DLC. neutrophils %	61	88	90	88	79	80	81	
	eosinophils %	3	1		1	1	1.5	1	
	basophils %	2			1	1	0.5	1	
	lymphocytes %	19	7	4	5	15	13	11	
	monocytes %	15	4	4	5	4	5	6	
	Immat. cells	Meta	Myel	Myel	Myel	Myel	Myel	Myel	
2	Total WBCs per cmm	7800						8200	7600
	DLC. neutrophils %	60						67	79
	eosin. %	6						5	1
	baso. %	1						1	2
	lymph. %	24						19	14
	mono. %	9						8	4
	Immat. cells								
3.	Total WBCs per cmm	8400			6000				7200
	DLC. neutrophils %	80			66				61
	eosin. %	1			2				7
	baso. %	1			1				1
	lymph. %	14			28				23
	mono. %	4			3				8
	Immat. cells	Meta			Mono				
4	Total WBCs per cmm			7200					8600
	DLC. neutrophils %			73					50
	eosin. %			1					4
	baso. %			1					2
	lymph. %			20					38
	mono. %			5					6
	Immat. cells			Meta					
5.	Total WBCs per cmm	6600							7000
	DLC. neutrophils %	61							64
	eosin. %	3							2
	baso. %	1							2
	lymph. %	33							28
	mono. %	2							4
	Immat.cells								

Figure No. 8 (continued)

Case No.	Examination made	No. of days after coming under observation with ? lysis RBCs.							
		1st	2nd	3rd	4th	5th	6th	7th	1 mnth.
6.	Total WBCs per cmm	8000							8600
	DLC. neutr. %	67							63
	eosin. %	1							2
	baso. %	1							1
	lymph. %	25							30
	mono. %	6							4
	Immat. cells								

Key: D.L.C. = differential leucocyte count.

Meta. = metamyelocytes

Myel. = myelocytes.

Mono. = immature monocytes

± = few in number

\+ = present

\+ + = moderate numbers.

In each differential count not less than 200 white cells were counted.

The figures given in the above should be compared with the normal values. The figures given by Osgood et alia (1939) are typical of those generally accepted. The values are given in figure No. 9 below.

Figure No. 9.

Normal values, total and per cent. of the white cells of the blood.

Type of cell	totals per cub.mm.	percentage
Polymorphonuclear neutrophils	1500 ---> 7500	33 ---> 75
" eosinophils	0 ---> 400	0 ---> 6
" basophils	0 ---> 200	0 ---> 2
Lymphocytes	1000 ---> 4500	15 ---> 60
Monocytes	0 ---> 800	0 ---> 9

Figure No. 9 shows the findings in the usually-accepted nomenclature of the white cells, i.e. the granular cells, with its three subdivisions, and the lymphocytes and the monocytes.

Patient No. 1 is the only individual of the six in whom the white cell findings showed a significant deviation from normal. In this patient, the only fatal one, there was found a marked increase in the total white cells; the marked increase began almost immediately after the lysis had started and persisted throughout the last days of the patient. In addition to this absolute increase in the total count there was a relative increase in the neutrophil leucocytes of the blood; in other words the white cell picture showed a polymorphonuclear neutrophil leucocytosis from the beginning of lysis until the death of the patient. This type of picture is one commonly

found in sepsis; the only explanation for its being found in this patient, and in none of the others, is that this particular change was probably produced by the pneumonic process which was found to be present in this patient in the last days of his life.

In the other patients the findings were within the limits of normal; this in itself may be significant; each of the six patients was under treatment for malaria at the time of onset of the haemoglobinuria, and one question to be discussed later is whether or not malaria was still present in an active form, and whether the malaria played any part in the production of the haemoglobinurias. Apropos of this, there is general agreement that one of the findings in any attack of malaria is a monocytosis, e.g. Stephens and Christophers (1904) consider that a figure of over fifteen per cent mononuclears in the absence of kala-azar is diagnostic of malaria, and this is the general opinion in later reports. The function of these monocytes is thought to be concerned with the engulfing and destroying of malaria parasites, e.g. Nocht and Mayer (1937) confirm the very old observation that monocytes may engulf whole parasites. Whatever the function of the monocyte, its presence in a markedly-increased degree appears to be a constant finding in active malaria. But in our patients, as shown in the differential counts in figure No. 8 there was no

monocytosis; this point should be noted for future reference.

From all the above investigations two important findings seem to be evident; these are:

1. In each patient the haemolysis was intra-vascular. This considerably narrows the field of likely causes of the haemoglobinurias.
2. There is nothing to suggest that there had been any pre-existing abnormality of the red cells which may have played a part in the production of the lytic process.

SUMMARY. CHAPTER IV.

At the end of Chapter III the diagnosis of the six cases of haemoglobinuria had not been advanced beyond the establishing that in each case there had been haemolysis; there still remain the problems of the site of the haemolyses, e.g. intravascular or reticulo-endothelial, and the cause or causes of the haemolysis.

The next step, therefore, in the establishing of a diagnosis is that of establishing the site of the haemolysis, since by doing so we should thus greatly reduce the number of conditions requiring to be taken into account in the differential diagnosis.

In the establishing of the site of the haemolysis the laboratory findings are of primary importance, and it is to a consideration of certain of those findings that Chapter IV is devoted. The particular laboratory findings which have been dealt with in this chapter include those related to the blood and to the blood cells. These are summarised below:

Blood pigments: the study of the blood pigments is the study of haemoglobin and its derivatives; The function of haemoglobin is the transport of oxygen to the tissues, and haemoglobin possesses certain distinctive properties which enable

it to do so. It is a conjugated protein, made up of a porphyrin-iron portion (haem) plus a protein of the histone class (globin); this protein moeity makes up more than ninety-five per cent of the haemoglobin molecule; the reactions of the haemoglobin molecule suggests that it is a compound molecule, with molecular weight about 68,000, made up of four molecules of 17,000 in which the iron is held linked to nitrogen atoms.

This blood pigment haemoglobin is apparently synthesised in the body from simpler substances which include a porphyrin compound. Porphyrins are substances with a marked ability to combine with metals and proteins, hence their presence in the haemoglobin molecule. The porphyrin derivatives of importance are the series III derivatives, so-called because they are the third of four possible derivatives; these series III derivatives are tetramethyl porphyrins with the methyl groups in positions 1, 3, 5, 8, see plate XII., page 113. Porphyrins of series I are tetramethyls with the methyl groups in positions 1, 3, 5, 7. This latter type is found in small amount as a side-product

in the synthesis of haemoglobin, and is found in excess in certain pathological conditions.

Porphyrin III, although a stage in the synthesis of haemoglobin, is not a stage in the degradation of this pigment; therefore when found in the excreta in anything more than the normal minute amount it probably indicates a blocking of the synthesis of haemoglobin. On the other hand a derivative, coproporphyrin III, is excreted as a product of the breakdown of certain abnormal blood pigments, e.g. methaemalbumin.

The most important of the series III porphyrins is protoporphyrin III; when treated with a ferrous salt this substance forms haem, the pigment portion of haemoglobin; the formula for haem is given on plate XIII, page 160.

The types of compounds which protein may form with haem are:

haemochromogens; i.e. denatured protein linked to iron.

haemalbumin ; i.e. native albumin linked only to the porphyrin of haem.

haemoglobin : i.e. protein bound both to the iron and the porphyrin of haem.

The various relationships of the pigments are shown on plates XIV and XV, pages 144 and 170.

Free circulating haemoglobin is not found in the blood except in minute amount; when present in any amount in pathological conditions it is treated by the body as a foreign substance.

Oxygen is transported to the tissues by the haemoglobin in the form of oxyhaemoglobin, a loose union of oxygen and haemoglobin with the iron in the ferrous state. There is another oxide of haemoglobin found in very small amount in the blood of normal individuals, namely methaemoglobin; in this, however, the union is a stable one, with the iron in the ferric state; this small amount of methaemoglobin is thought to be in equilibrium with haemoglobin in the normal individual; this equilibrium is upset in the rare condition of familial idiopathic methaemoglobinaemia.

Transport for the oxygen-bearing haemoglobin

is provided by the red cell; the method of carriage of the haemoglobin in the red cell is not yet fully understood. The red cells undergo severe buffetting in the performance of their duties; they are thought to have a life of about one hundred days. There are three ways in which the red cell may be destroyed; destruction by the cells of the reticulo-endothelial system:

destruction by haemolysis; and

destruction intra-vascularly by wear and tear.

The first was thought to be the method, but recent work suggests that the reticulo-endothelial system may be the graveyard and not the slaughterhouse; this problem is not yet settled.

In the metabolism of haemoglobin in the normal body the degradation of it follows a different pathway from that of the synthesis, e.g. as already mentioned, porphyrins are not produced during the degradation of haemoglobin; it is suggested that this degradation of haemoglobin (which takes place in the reticulo-endothelial system) begins by opening of the

porphin ring, see plate XII, page 113; the next stage is removal of the protein and a rearrangement with loss of iron. The iron is conserved as haemosiderin; the end-result of the degradation is bilirubin, plate XIII, page 160. There is, however, an alternative route of breakdown, via the porphyrins, but this route is only brought into operation to deal with abnormal pigments such as methaemalbumin.

Bilirubin, the end-product of the degradation of haemoglobin, is withdrawn from the blood-stream by the liver cells, and is excreted in the bile; a small amount of the allied pigment biliverdin is also present in the bile. During this passage through the liver cells the bilirubin undergoes some change, probably physical, and the two types of bilirubin, that which has not passed through the liver and that which has passed through but has found its way back to the blood, can be differentiated by the van den Bergh reaction. This reaction, therefore, enables a distinction to be made between the high blood level of bilirubin due to over-production (producing

"retention jaundice") and the high blood level due to liver malfunction (producing "resorptive jaundice"). In the second type of jaundice the high blood bilirubin is accompanied by a high level of bile-salts, which is not the case with the first type of jaundice. The van den Bergh reaction may be used quantitatively, the unit being 0.5 mgms. bilirubin per 100 ccs. plasma; the use of this quantitative estimation shows that the kidney threshold for unchanged bilirubin is about 20 units, compared with the low kidney threshold of from 5-8 units for bilirubin which has passed through the liver and has been regurgitated into the blood-stream. This finding has some practical importance.

In a short note on jaundice which was interpolated at this point, it was seen that jaundice is the result of a staining of the tissues by yellow bilirubin; this takes about twenty-four hours to appear after the serum bilirubin has reached the critical level.

The icterus index is another quantitative test for the measurement of blood bilirubin

level; this estimation is by a matching of the serum against a colour standard, in which the standard is a 1/10,000 solution of potassium dichromate; the index of the serum is the number of dilutions of the unknown serum which are required before the colour of the serum matches the standard; the index of normal serum is not more than six; overt jaundice is found with icterus index values of more than fifteen. This test is seriously interfered with by any intra-vascular haemolysis and therefore was not of any use in our investigations.

The bilirubin passes to the large bowel without incident; there it is reduced by bacteria with the production of urobilinogen (stercobilinogen), plate XIII, page 160; in turn this pigment changes to brown urobilin by oxidation. It is urobilin which colours the stools. Part of this urobilinogen is re-absorbed in the large bowel, and reaches the liver; again the liver cells pass this out, either as urobilinogen or as bilirubin; a small amount of this re-absorbed urobilinogen escapes excretion by the liver and is

excreted in traces as urobilinogen by the kidney. This urinary urobilinogen becomes urobilin on standing. Bilirubin does not normally appear in the urine. Any significant increase of serum bilirubin keeps the liver so fully occupied that much larger quantities of the re-absorbed urobilinogen escape re-excretion by the liver, with resultant marked increase in the level of urinary urobilinogen; even bilirubin may appear in the urine when the serum bilirubin level is high, especially when some of the bilirubin is of the regurgitated type. Hence bilirubinuria occurs much more readily with resorptive jaundice than with retention jaundice.

Increased red cell destruction leads both to quantitative and qualitative changes in haemoglobin derivatives. The two abnormal blood pigments which may be found in such cases are methaemalbumin and methaemoglobin.

As already recorded, methaemoglobin is found in minute amount in normal individuals; it is commonly found to be markedly increased in the red cells after the ingestion of certain toxic substances. This pigment is brown in colour, with physical properties very similar to those

of methaemalbumin; it is essentially an intra-corpuscular pigment, however, whereas methaemalbumin is never found inside the red cells. Another important distinction between the two is that methaemalbumin is never excreted by the kidney, in contra-distinction to the ready excretion of methaemoglobin by the kidney. Plasma and serum have the power of producing methaemalbumin from methaemoglobin or from haemoglobin. In this discussion it was noted for future reference that although plasmoquine is one drug with marked ability to produce methaemoglobincythaemia it does not do so in the very small dosages that were administered in our six cases; none of the six patients showed cyanosis except No. 1. In patients with methaemoglobincythaemia the pigment conversion is not uniform throughout all the red cells; it is a moot point whether cells showing methaemoglobin are more readily laked than normal cells.

Methaemalbumin is formed only when haemoglobin is circulating free in the plasma; this former pigment is never found in the blood of normal individuals. In physical properties it

so closely resembles methaemoglobin that it was not identified until 1934. It is always formed in the plasma in any intra-vascular haemolysis of other than minimal degree. The discovery of this pigment was the first step bowards solving what happens to the haemoglobin which is freed in haemolysis of red cells. Previously much of this freed haemoglobin had been unaccounted for; only a fraction of it had been identified in the urine, and only a fraction of it had been converted into bilirubin, Obviously excretion of freed haemoglobin by the kidneys represents loss of iron, hence although the body regards free circulating haemoglobin as a foreign substance, the kidney has a high threshold value for it; this is a line of defence against the too-ready escape of iron. And the formation of methaemalbumin is one more form of defence against iron loss, since it cannot be excreted by the kidney. Methaemalbumin is probably formed by the haemoglobin splitting into globin and haem, with a linking-up of the haem with plasma albumin. The easiest method of differentiation of methaemalbumin and methaemoglobin is

by Schumm's test.

It has already been noted that the mode of formation is not yet fully established; this can also be said of the mode of degradation. This degradation follows a different course from that of haemoglobin, and in it coproporphyrin III is a product.

At this point a short note was given on jaundice. It was emphasised that the degree and onset of jaundice differ markedly in the two types of hyperbilirubinaemia, occurring more quickly and more deeply with the resorptive type of bilirubin; as an example of a blood-destroying disease which produces jaundice, chronic malaria was quoted; in this disease there is both intra-vascular and reticulo-endothelial haemolysis, and as a result such patients show low-grade jaundice with muddy complexions; in fact, with falciparum malaria the plasma haemoglobin levels may be as high as 50 mgms. per cent. This haemolysis and secondary changes can be prevented by early and adequate treatment, e.g. one author reports only twenty-four cases of malarial jaundice in 8837 cases of

malaria.

This finishes the summary of the general discussion on blood pigment changes in the normal individual and in patients with intra-vascular haemolysis. The relevant findings in the Razmak cases are discussed seriatim below.

a. van den Bergh reaction: the Razmak cases showed surprisingly low values for the van den Bergh quantitative estimations, although jaundice was present in each of the patients and was marked in some; this suggested that some of the circulating bilirubin was of the resorbed type, due to liver damage.

b. Jaundice: every patient showed jaundice at some time or another after the beginning of haemolysis; there was no direct relationship between the degree of the jaundice and the degree of red cell lysis.

c. Icterus Index: this test depends upon a colour-matching and is invalidated by intra-vascular haemolysis.

d. Spectroscopic analyses:

1. plasma: in all cases the plasma showed methaemalbumin to be present.

2. washed red cells; when washed red cells were lysed, cases Nos. 1, 2 and 3 showed ? methaemoglobin; this may have been adherent methaemalbumin.

<u>The results of the spectroscopic examinations enable us to state that in our six patients the haemolysis was intra-vascular.</u>

e. Estimation of the degree of plasma colour change due to haemoglobin: solutions of red cells in tartrazine were used as standards, with which were matched the colours of serum specimens. This was a crude test and the results were of doubtful accuracy. They showed that the plasma contained haemoglobin in solution in amounts never more than that equivalent to a two per cent solution of red cells.

f. The red blood corpuscle: in each of our patients certain investigations were carried out on the red cell and on various related problems; the aim was to establish, if possible, whether or not any abnormal findings were present such as would suggest that pre-existing red cell abnormality had been the cause of the haemoglobinuria in our six patients. Among the

investigations were:

1. Red cell appearances in the wet films; in each of the patients the wet preparations showed clumping of the red cells; this appearance is also found, however, in patients suffering from various conditions in which the cause of the disease is not an abnormality of the red cells. Accordingly, the agglutination which was seen in the red cells of our patients is not of itself an indication that red cell abnormality had existed previous to the onset of the haemoglobinuria.
2. Red cell appearances in the stained films; with the exception of patient No. 6, stained films of all the patients showed much poikilocytosis and anisocytosis which had not been present in the original blood films which had been retained after the original diagnosis of malaria. Polychromasia was also found, together with small numbers of normoblasts.

In all the cases however the stained film appearances were not different from that which would be found immediately following

upon any severe intra-vascular haemolysis; these appearances certainly could not have been taken to indicate that there had been any pre-existing abnormality of the red cells

3. Red cell count, haemoglobin estimation, colour index and mean corpuscular volume; are all considered together because of their inter-relationship. With the exception of patient No. 6 in whom the lytic process was short and mild, each patient showed an anaemia as the immediate result of the haemolysis. This anaemia was always associated with large heavily-pigmented red cells, and with a high mean corpuscular volume. This type of picture is to be found in patients suffering from intra-vascular haemolysis of any aetiology and therefore is not necessarily indicative of red cell abnormality present before the onset of the haemolysis.

8. Related investigations:

1. Sedimentation rates; markedly increased in all patients, due probably to the lytic process and to the severe anaemia present.

2. Donath-Landsteiner Reaction; negative in each case proved also by the negative serological reactions for syphilis.
3. Bleeding time; normal in each patient.
4. Coagulation time; normal in each patient.
5. Fragility of the red blood corpuscles; the fragility of the red cells was tested against hypotonic saline; in each of the patients the cells did not show any increased fragility to the saline solutions. Unfortunately the fragility of the cells to lyso-lecithin was not tested.

The above investigations, direct and indirect show that there is no proof that there existed in any patient any abnormality of the red cells which may have played a part in the production of the haemoglobinurias.

g. White cell picture: in the total and differential counts of the six patients the only abnormality noted was a polymorphonuclear neutrophil leucocytosis in patient No. 1. This "septic" type of white cell picture in this patient was probably the result of a broncho-pneumonic process present during the last days of the patient's life.

The only other significant finding was that there was no increase, total or relative, in the monocytes

of any of the patients. This negative finding is one pointer suggesting that active malaria was not present in any patient at the time of onset of the haemoglobinuria. This is noted for future reference.

From all the investigations in this chapter it is presumed therefore that:

1. The haemolysis was intra-vascular in each patient.
2. The lysis in the six patients was not due to a pre-existing blood dyscrasia.

CHAPTER V.

Renal Lesions in Intravascular Haemolysis.

The Physiology of the Kidney:

Gamble (1937) sums up the performance of the normal kidney by saying that the description of the kidney as an organ of excretion is very inadequate. If removal of waste products were the kidney's only function a much simpler mechanism would be sufficient. Gamble stresses that the complicated functioning of the kidney, with its consequent complex design, is necessary for the production and maintenance of the extra-cellular fluid which is of such vital importance for the continuation of intra-cellular processes. This "constancy of the internal environment" is obtained by selective re-absorption from the glomerular filtrate. Best and Taylor (1943) give details of this complicated structure necessary for the maintenance of the internal environment. The functional unit is the nephron of which there are estimated to be about one million in the human. The components of the nephron are an invaginated glomerulus and the renal tubule. The renal tubule has several clearly demarcated divisions, these being the proximal convoluted tubule about fourteen millimetres long; the loops of Henle with descending and ascending portions, total length about sixteen millimetres; and the distal convoluted tubule about five millimetres long.

These component portions differ in the character of the component cells, and on the basis of the fact of structure usually subserving function, the components are held to differ in their functions. The blood supply to the kidney is unusual in certain respects, e.g. the blood is delivered to the glomeruli at high pressure and by large afferent vessels, whereas the efferent vessels are small. Gamble (1947) suggests that about one-quarter of the total cardiac output per minute passes through the kidney per minute. Smith, H.W. (1943) estimates the amount of blood passing through the kidney each minute to be about twelve hundred millilitres which is close to the estimate of Gamble's; John Shaw Dunn (1940) suggested a conservative estimate of 1080 litres of blood to pass through the kidney in twenty-four hours. The autonomic nerve supply to the kidney does not appear to give any secretory fibres to the kidney cells. Such effects upon urine formation as do occur on stimulation of the renal nerves are probably the result of vascular changes. This question of the blood flow through the kidney has recently come into prominence, and is more fully discussed below.

Hormones also play some part in the control of kidney function but cannot be discussed in this section.

Modern theories on the control of kidney function are largely based on the work of Cushney who considered the two

major factors operating to be blind physical force, glomerular filtration, and the vital activity of selective re-absorption of the kidney tubules. In Cushney's opinion the process at work in the glomeruli is that of simple filtration; variations in pressure cause alterations only of volume NOT variations in the composition of the filtrate. The diastolic is the important pressure; much of this pressure is expended in overcoming the antagonistic effects of the plasma protein pressure and of the intra-capsular pressure; generally speaking a pressure of forty millimetres of mercury remains as the effective available pressure. The strongest evidence in favour of the above is that by special technique, e.g. the work of Richards (1938), it has been found possible to draw off fluid from Bowman's capsule and to analyse this fluid; the fluid so obtained is found to have the composition of protein-free plasma. Foy, Altmann, Barnes and Kondi (1943) consider that one of the most important factors in the production of urine is the body fluid intake. The average daily output of waste by the kidneys is about thirty-five grams; the authors suggest that the minimum fluid to excrete this, even when the urine is strongly concentrated, is about 500 ccs.; this is probably an under-estimate, the figure being nearer 900 ccs.; as has already been said, the chief factor in the transudation through the glomerulus is the hydrostatic pressure in the capillaries; where this exceeds that of the colloid

pressure of the blood, fluids will pass out of the capillaries; it follows that changes in the hydrostatic pressure and in the osmotic pressure will cause changes in the amount of filtrate. This effect of the colloids can be shown by the escape of fluids when protein is deficient; the colloid osmotic pressure is largely due to the proteins.

Tubular re-absorption is the second of the two factors at work; this is the vital function of the kidney and can only be explained by specific activity on the part of the tubule cells. Obviously there is re-absorption of fluid and of contents since the filtrate becomes greatly reduced in volume in its passage down the tubules, and the composition changes by selective re-absorption of certain substances in whole or in part. Certain of the substances are classed as "high threshold substances" and others as "low threshold substances". The high-threshold substances are those of use to the body, e.g. amino acids, sugars and bases, which only "spill over" into the urine when the concentration in the blood is abnormally high. The low-threshold substances are largely excretory products of little importance to the functioning of the body; such include urea. It is estimated that ninety-nine per cent of the filtrate is re-absorbed in the tubules. The cells of the tubules are also reported to have the power of excreting certain substances into the lumen of

the tubules, shown best by experiments with diodrast; this last function of the tubules is of little practical importance. One important problem is whether there is selective re-absorption at different divisions of the tubule. Best and Taylor (1943) consider that there is active re-absorption of sugar and sodium in the proximal tubule, together with what they call "passive re-absorption" of about twenty per cent of the water. Active re-absorption of most of the water is said to take place in the ascending part of Henle's loop and in the distal convoluted tubule; this question is more fully discussed below. Chlorine and base are also absorbed along this portion of the tubule.

One other important problem is that of the plasma proteins. Plasma proteins are molecules of relatively large size, yet in certain conditions they are found to be present in the urine, very often in amounts which vary inversely to the size of the molecule. One suggestion is that certain of the pores of the glomerulus are large enough to allow the passage of relatively large molecules, e.g. Monke and Yuile (1940) state that three per cent of the glomerular pores are large enough to allow the passage of undissociated compound haemoglobin molecules which have a molecular weight of 68,000. This is also the molecular weight of serum albumin. These workers further state that the passage through the pores is

governed by an electric charge on the pores; this charge normally prevents the escape of protein. One finding in favour of the theory of pores is that normal urine contains minute traces of protein. As long ago as 1895 Moerner demonstrated this minute amount of protein, and suggested a limit of 78 milligrams per litre in normal urine; such amounts are not detectable by ordinary clinical methods. Proteoses are sometimes also found, e.g. in anaphylactic and other conditions as suggested by Oriel and Barber (1930). The minute traces of protein in normal urine, like the great bulk of the proteins in proteinuria, enter the tubules via the glomeruli. This seems fully proved by the interesting work of Bieter (1931) who failed to produce proteinuria in those fishes whose kidneys do not contain glomeruli. Fishberg (1934) agrees that the evidence is very strongly in favour of molecular size being one of the important determinants of whether or not a circulating protein passes through the healthy kidney into the urine. He also considers that the evidence equally favours this view in cases of albuminuria, e.g. "the predominant mechanism in the causation of albuminuria in renal disease is an increase in the permeability of the kidney to blood protein which results from injury to the glomerular membrane"; it is not known, however, just how injury to the glomerular filter actually produces increase in the permeability. The above notes do not apply, of course,

to the cases of "false" albuminuria where the protein is added to the urine after it leaves the kidney, e.g. as in inflammation to the urinary passages. As yet there is no definite proof to support the theory that a greater or lesser number of pores are big enough to allow passage of molecules up to about molecular weight of 68,000. Neither is there any support yet for the refinement which suggests that the permeability of such special pores is controlled by an electric charge.

One other theory which does not attempt to explain the passage into the urine of proteins in general but is concerned only with the passage of haemoglobin in the urine is that which suggests that a certain proportion of haemoglobin molecules may dissociate from haemoglobin$_4$ to haemoglobin$_1$; this would bring the much smaller components practically within the limits of glomerular permeability of about 15,000 as suggested by Yuile and Clarke (1941).

At present the position is that there is no satisfactory explanation of the phenomena of proteinuria.

The last part of this short note on kidney function and structure is by no means the least important part; in fact it may well prove to be the most important; this portion is that dealing with the circulation of the blood through the kidney. This has been brought sharply into focus in recent

years by Trueta and various co-workers; the more important relevant references are given below. These workers themselves point out repeatedly that for over one hundred years there have been recorded isolated observations adumbrating their work, but in each case the significant observation or finding was not followed up. It is interesting to look back over that century and see how the significance of certain observations appears to have escaped notice. Trueta and his co-workers report a number of such observations; the first group are those suggesting that there is a central nervous system control or partial control of production of urine.

e.g. Laycock (1838) talks of hysterical anuria.

Charcot (1877) considered hysterical anuria to be the result of a central nervous system inhibition of urine production.

Plehn (1903b) claimed that in blackwater fever the anuria is certainly of cerebral origin induced by the toxic properties of metabolic and retention products.

Porak (1918) strongly supported Plehn's view.

Neuwirt (1922) reports "reflex anuria" in relation to a left-sided renal colic.

Barrenscheen and Glaesner (1923), talking of a twenty-four hours anuria following quinine administration in a patient, suggested that this anuria could be explained as a reflex

spastic condition.

There are many other similar reports; Fishberg (1934) for example suggested that the albuminuria following epileptic fits may be the result of angiospasm.

Hints of the possible implication of altered blood supply to the kidney in the production of certain cases of anuria might also have been obtained from the reaction of such cases to certain lines of treatment, the rationale of which obviously was not understood at the time; e.g.

Wallace (1921) suggested rectal irrigations in the treatment of blackwater fever;

Neuwirt (1922) produced diuresis by splanchnic block in a case of reflex anuria following left-sided renal colic;

Ross (1932) suggested lavage of the renal pelves in the anuria of blackwater fever;

Peters (1942) described a case where splanchnic block was deliberately used in the treatment of anuria following haemolysis; this was repeated by O'Sullivan and Spitzer (1946); spinal anaesthesia as a treatment was suggested by the results obtained by Dobbs (1947);

Lyons and Raines (1945) suggested that the success got with decapsulation of the kidneys for anuria,

a treatment known since the early nineteen-twenties, may be due not only to the relief of tension but also to the removal of tonic nerve fibres; Abehouse (1945) supported this view.

Still further observations of significance included certain findings concerning kidney structure and blood supply; such findings go far back,

e.g. Bowman (1842) noted that in the human the juxta-medullary glomeruli are larger than the cortical glomeruli;

Bernard (1858) was the first to describe red blood in the renal veins.

More recent findings are those of:

Dunn and Montgomery (1941) in discussions on a heterogeneous group of cases showing renal necrosis, noted that the arrest of the circulation was incomplete in the proximal part of the interlobular arteries, and they went on to make the significant remark that this is due to the easier escape of blood through the deep glomeruli whose efferents pass not to the cortex but by short routes direct to the medulla; Maegraith and Findlay (1944) supported this, and suggested that

> one factor which may play a part in the production of oliguria in blackwater fever is a redistribution of the intra-renal blood flow, with depression of glomerular filtration;

Maegraith and Havard (1946) went further when they claimed

> that in certain conditions producing renal syndromes similar to blackwater fever there is a reduction of the renal blood flow and a redistribution of the blood flow within the kidney, with resultant failure of the glomerular flow and with resultant anuria.

By this time, however, Trueta (1945) had decided to investigate the renal anoxia found in certain pathological conditions. In his initial communication on this problem the author mentioned the possibility that the kidney lesion is the result of over-stimulation of vascular nerves; during his work as a surgeon, with all the opportunities for observing living pathology, he had often remarked upon the effect of stimulation of the vascular nerves, and in the preliminary note mentioned above Trueta stated that a team of investigators had already begun a detailed study of the problem. By 1946 Trueta et alia were in a position to give a short review of their findings to the Physiological Society, and lastly Trueta et alia (1947) published a full report of the

work they had done. In a foreword to the 1947 publication Ellis truly says "one of the most dramatic and disconcerting medical emergencies is the development of anuria due to 'acute interstitial nephritis' ... which may occur in blood transfusions, obstetrical shock and accidental haemorrhage, blackwater fever, and certain other conditions; in such cases those who die do not show changes in the kidney to explain the anuria. The most marked change is tubular epithelial necrosis, usually accompanied by a variable amount of pigment cast formation". Maegraith (1946a) described the condition as a renal complex of oliguria or anuria associated with nitrogen retention and inability to concentrate urine, and frequently accompanied by degenerative changes in the renal tubes, but not necessarily related to the passage in the urine of blood or muscle pigment. Ellis and Maegraith agree that the amount of pigment cast formation cannot explain the anuria.

It is these kidney lesions that Trueta attempts to explain; his work is a contribution to medicine in general and to the above-mentioned problem in particular; in view of the importance of this work I shall quote fully from it, with the kind permission of the senior author. I cannot improve upon the earlier paragraphs in the monograph, and give the extract herewith.

"The blood reaching the kidney has two potential routes through that organ and, according to circumstances it may pass almost exclusively by one or other of these routes or in varying proportion through each of them.

"The two routes diverge where the afferent arterioles of the juxta-medullary glomeruli leave the interlobular arteries. One route, the medullary, continues through the juxta-medullary glomeruli → the efferent vessels of these glomeruli and their derivative vasa recta → to the interlobular veins. The other route, the cortical, continues through the interlobular arteries → the afferent arterioles of the remaining glomeruli → these glomeruli themselves → their efferent vessels and the cortical intertubular capillary network into which these break up, and finally through the veins draining this network into the interlobular veins. The rest of both routes, like their beginning, is identical and is through ever larger venous trunks to the main renal vein.

"..... in extreme conditions the blood may pass either almost exclusively through one or other of the two pathways, or, in less abnormal circumstances, to a varying degree through both.

"The vessels making up the pathway of the greater

circulation are those associated with the cortical glomeruli; the channels of the lesser circulation are those associated with the juxta-medullary glomeruli."

The experimental work leading to the above conclusions was carried out on rabbits and rats, but the anatomical structures which provide a foundation for their concept are present in all the mammalian kidneys including those of the human.

It is reasonable to believe that the redistribution of the intra-renal blood flow, seen under experimental conditions in the animals, occurs also under certain conditions in the kidneys of other animals including man."

The authors had previously reported, in 1946, that the alternative circulation through the kidney could be well shown by clamping the hind limb of an animal. When this was done it was found that the renal circuit time on the affected side was much shortened; the blue colour of the blood in the renal vein of the affected side partially or wholly changed to red; red stream lines appeared in the renal veins after the cortex had paled and while it still remained pallid; and drugs injected into the arterial side of the circulation appeared in the renal veins but did not stain the surface of the kidney. This last finding was confirmed at autopsy, when

the cortex was found to be unstained, with the medulla deeply stained with dye. In unstimulated kidneys no difference was noted in the distribution of the pigment on repeating of the experiment. From these findings the only reasonable explanation was ..... a vascular short-circuiting; i.e. as a result of appropriate nerve stimulation blood may be diverted wholly or partly from the cortex, and short-circuited through medullary blood channels; it was suggested by the authors that the vessels involved are the vasa recta.

The authors then draw the further and very important conclusion that the appropriate nerve stimulation can be produced centrally or peripherally by a variety of noxious agents and that the picture seen in many loosely-related syndromes such as "sulpha kidney", blood transfusion kidney, etc., IS THE RESULT OF A DEFENCE DEVICE by which the cortex of the kidney is excluded from the circulating toxin or other noxious agent and is thus protected. Too-prolonged operation of the device results in permanent damage. The authors also suggest that the same mechanism may fulfil another role in haemorrhage and shock and conditions with decreased blood volume, by preventing the blood from reaching the filter of the kidney, and thus conserving fluid. Also, from the above, the mechanisms of hysterical uraemia, emotional anuria and post-abortion and post traumatic uraemia are readily explained,

as is also the response of the two last-named conditions to splanchnic block.

The Lancet (1946) pointed out certain important physiological and pathological implications from the work reported above, e.g. the physiological diminutions in urinary output may be adjusted (e.g. when the body is eliminating fluid in other ways) without greatly diminishing blood flow through the kidney ..... and this work may provide an explanation for a type of renal lesion common to a variety of conditions, including such important conditions as crush syndrome; incompatible transfusion, blackwater fever, etc. Maegraith (1946a) also had noted that many diverse clinical conditions show a renal complex of oliguria or anuria associated with nitrogen retention and inability to concentrate urine, and frequently accompanied by degenerative changes in the renal tubules, but not necessarily related to the passage in the urine of blood pigment or muscle pigment.

In view of the importance of the work it is necessary to mention at length certain of the findings quoted by Trueta et alia (1947) to support their contention of alternative circulations through the kidney. These findings are of particular importance to us in our attempts to establish the genesis of the kidney lesions in our series of patients, and in our attempts to establish a reasonable approach to the

the treatment of such patients.

The following excerpts will appear somewhat disjointed since they are taken from their context in different parts of the monograph.

The first point of importance made by the authors is that considerable parts of all loops of Henle, including in every case the thin segment of the loop, are supplied by blood circulating through the medullary pathway. The authors are not prepared to accept the generally-held theory that the function of the vasa recta is a nutritional one; instead they bring forward the interesting suggestion that the vasa recta are concerned with the re-absorption of water; this is in agreement with the generally-held view that the vasa recta do play some part in the re-absorption of water from the tubule into the blood. As has been said, a large number of vasa recta are adjacent to loops of Henle, and both the vasa recta and the thin segments of the loops of Henle are lined with flattened cells, suggesting that the transference of fluid is passive in nature. Such passive transference is within the bounds of possibility because of the proximity of the thin-walled vasa recta to the thin segments of the loops of Henle; it is conceivable that the pressure may be hydrostatic or osmotic or both. This question of whether water transference takes place at this level is not an academic point; in fact

it may well prove of considerable importance in our search for an explanation of the formation of pigment casts in haemolysis. The thick segments of the loops of Henle, and the convoluted tubules with their cells of secretory type are relatively ill-fitted for the role of water re-absorption, although they may play a minor part in this process. This diversion of blood flow in the kidney may also explain certain anomalous findings in the hypotension following haemorrhage; Corcoran and Page (1943) foreshadowed this explanation when they suggested that during hypotension the renal blood flow is distributed unevenly through the renal vascular bed, being greatest in the sites of least resistance; Trueta et alia point out that this supports their work in that the sites of least resistance are the ones found at the entrance to the medullary by-pass, because the efferent vessels of the justa-medullary glomeruli and the vasa recta into which they flow are much larger in calibre than the corresponding vessels in the cortex; if this opinion of Corcoran and Page is correct then part at least of the shunt may be physical. Trueta and his co-workers make it clear that the nervous system is an essential factor for the maintenance of a constant fluid balance in the body in all circumstances, and particularly under conditions of stress. This regulation may be affected by direct nerve control or through the intermediation of liberated hormones or both.

It follows that any significant degree of shunting of blood will lead to a reduction in the amount of glomerular filtrate and therefore of urine; one other factor also tending to produce such an effect is that of re-absorption of water from the tubules; it has already been pointed out that the most likely place for the re-absorption of water is at the area where the thin segments of Henle's loops are in close proximity to the vasa recta with their relatively large calibre and walls composed only of a single layer of pavement epithelium. One other point of importance in this connection is that the blood passing through the vasa recta does not traverse a capillary network since the smaller vasa recta constitute the "arterial components" with the larger vasa recta acting as the "venous components". Compare this with the situation of the blood supply to the cortical glomeruli; the efferent vessels are of small calibre and empty into a network of cortical capillaries of small calibre. Heggie (1947), the first to use the term "juxta-medullary glomerulus", estimates that in the rabbit these make up fifteen per cent of the total glomeruli in each kidney, and also considers that they can accommodate the whole normal glomerular capillary blood volume. To summarise the differences consider in brief again the route taken by blood which is diverted from the cortex through the medulla; the route is:

The most proximal parts of the interlobular arteries

- the afferent arterioles of the juxta-medullary glomeruli
- the juxta-medullary glomeruli themselves, in the deepest zone of the cortex
- the efferent vessels of the juxta-medullary glomeruli; these vessels lie partly in the cortical zone of the medulla
- the arterial components of the vasa recta, lying in the medulla
- the loops of the vasa recta, with offshoots to neighbouring capillaries.
- the venous components of the vasa recta system.
- the proximal parts of the interlobular veins.

The striking differences in the channels through which the two blood flows pass are paralleled to a certain extent by differences in the nephrons; this variation in nephrons was noted as long ago as 1909, resulting in an outer and an inner zone in the medulla; the outer zone was subdivided into an outer band and an inner band. In this connection Trueta et alia (1947) state that there are two types of nephrons, one with a long Henle loop and the other with a short Henle loop; the tubule with the long loop arises from a juxta-medullary glomerulus and this type alone passes into the

inner zone. Further, it is agreed that there is no difference in the vascular pattern of the capillary network surrounding the convoluted tubules of the cortical and of the juxta-medullary glomeruli; it can be inferred from this that with the short-looped tubules from the cortical glomeruli the greater part of the short loop of Henle lies in the cortex in a close-meshed capillary network, and only a short length of it reaches out into the outer part of the medulla in association with the vasa recta; compare this with the distribution of the tubules of the juxta-medullary glomeruli; with such nephrons the position as regards the loop of Henle is reversed, i.e. only a short length of the Henle loop lies in the cortex. The remainder of the extremely long loop, composed almost entirely of thin segment, lies in the medulla in close juxtaposition to the vasa recta. Here it is pertinent to stress again that Trueta et alia do not accept the view that the role of the vasa recta is nutritional and it is pertinent that it is the opinion of these authors that the vasa recta serve as the main conducting channels for the intra-renal blood flow when it is diverted from the cortex; we have stressed already their view that part at least of the function of the vasa recta is the absorption of water, even when the kidney is functioning normally and particularly when the blood is being "shunted"; as the authors repeatedly point out, in the medulla a large number of vasa recta lie in juxtaposition

with the thin segments of the long loops of Henle; in this area the walls of both are morphologically well suited for the transference of fluid. One inference from the variations in findings of experiments on individual animals is that made by Ellis (1947) who points out that sensitivity to stimuli varies greatly from individual to individual, and suggests it is possible that excessive stimulation of one type or another applied to persons unduly sensitive to that particular type of stimulus might result in excessive operation of the normal mechanism by which the blood flow is diverted from the cortex. Another paper with a bearing on this problem is that by Maegraith et alia (1947) who confirm earlier work and state that malaria may injure the liver; they found the injury to be mainly in the central zone of the lobules, and suggest that the cellular changes develop primarily from stagnant anoxia because of active obstruction to the venous outflow from the liver; they suggested that this obstruction arises from constriction of some part of the heaptic venous tree .... mainly by nervous reflexes. This would bring the processes at work into line with the kidney changes under discussion, but as yet Maegraith's work is not confirmed.

This ends our brief consideration of what may prove to be a fruitful contribution to medicine; it certainly is a fruitful contribution to part of our problem, namely the

problem of the kidney changes following upon severe intravascular haemolysis and other conditions producing a similar picture in the kidney.

It is relevant now to consider in some detail the pathological changes found in the kidneys in blackwater fever, and in other conditions giving a like kidney picture.

i.e. Pathology of Transfusion Kidney and of Similar Kidney Lesions.

This consideration includes kidney changes found in sulpha drug poisoning; in blackwater fever; in crush kidney, and in the various other conditions differing in aetiology yet producing much the same picture in the kidneys. This consideration is of importance because very often the kidney changes are the cause of death in the diseases enumerated above; e.g. fifty per cent of the deaths in blackwater fever are caused by the kidney damage. In blackwater fever the total death rate is about twenty per cent of individuals affected, therefore in this disease alone the kidney lesions are of great practical importance.

The kidney lesion found common to all the above conditions is well described by Maegraith (1946a) who describes the condition as a renal complex, with oliguria or anuria, associated with nitrogen retention and inability to concentrate urine, and frequently accompanied by degenerative changes in the renal tubules; many diverse conditions show this complex

which is <u>not</u> necessarily related to the passage in the urine of blood pigment or of muscle pigment. The renal function may become affected at any stage of the disease, with failure first being indicated by a reduction in the urinary flow ⟶ anuria. If the patient recovers the anuria is usually followed by polyuria which may last for several days; during this period the concentration of the urine is greatly reduced, indicating renal tubular damage.

Lucke (1946) reports the largest series of cases so far; he investigated 538 fatal cases of kidney failure collected at the United States Army Institute of Pathology during the war years; the renal lesion was essentially the same in all, namely a lower nephron nephrosis; the causes were:

battle wounds	221.	burns	48
sulpha intoxication	47	crush injuries	46
transfusion reaction	45	abdominal operations	36
poisons	20	heat stroke	19
blackwater fever	14	haemolytic anaemias	4
miscellaneous	38		

This realisation of a kidney picture common to a variety of unrelated diseases and conditions has largely resulted from work stimulated by a report by Bywaters and Beall (1941) of what they thought to be a specific and hitherto-undescribed syndrome, namely crush injury followed

by renal complications. Later however it was reported, by Bywaters among others, that Minami (1923) had previously described three cases of crush injury with secondary degeneration and necrosis of tubular epithelium; Minami, in turn, makes it clear that he is describing a syndrome which had been repeatedly seen in the hostilities of 1914-1918. It is surprising that this picture had not been seen in those cases of severe crush injuries found in civilians in motor accidents and in miners in crush accidents before the second outburst of hostilities in 1939. In this connection Bywaters and Beall suggest that such patients died of secondary shock or had had early amputation which prevented the onset of the kidney lesions. In the cases of Bywaters and Beall the findings were those of widespread cattarh of the tubules, with a granular deposit in the glomerular capsules; the proximal convoluted tubules showed desquamation and cellular mitoses with debris in the lumina (this is <u>not</u> confirmed by others). In Henle's loops the pigment appeared to be condensed and pigmented with eosinophilic granular casts which were later identified by Bywaters et alia (1941) as myoglobin. This report was later amplified by Bywaters and Dible (1942) who discussed renal changes in twenty-two fatal cases of crush syndrome. In brief their findings were:

Marked catarrh of the whole nephron, especially marked in the ascending limb of Henle and in the second

convoluted tubules;

first convoluted tubules showed in all cases a catarrhal condition with detritus in the limbs;

Henle's tubules showed changes chiefly in the wide part of the ascending limb (in the boundary zone of the medulla);

second convoluted tubules showed severe changes with pigmented casts and leucocytes.

The characteristics of the urine (fluid of low concentration, with practically no evidence of absorption of chlorides) agree with the interpretation of the histological picture, namely an aberration of tubular function allowing of excessive and unselective re-absorption of glomerular filtrate. It is interesting to read in 1948 that Bywaters is now of the opinion that in crush syndrome the lesions affect the distal convoluted tubule entirely. Another early paper on this problem was by Dunn who was so well-qualified to speak on renal pathology. With Dunn, Gillespie and Niven (1941) he further amplified the findings of the above authors and described one change, tubulo-venous anastomosis, which had not been described by Bywaters and Beall; the pathological changes found by Dunn et alia included:

Cortex: ..... general arrangement not disturbed, slight separation of the tubules as by oedema.

glomeruli: ..... normal, spheroidal masses of homogeneous material in many of the Bowman's capsules (albuminous exudates);

first convoluted tubules: epithelium of normal appearance (cf. the findings of Bywaters and Beall in the original paper)

second convoluted tubules: all show the same pathological changes; the degree of change varies greatly. In the majority of cases the alterations are slight with moderate dilatation of the lumina but in occasional tubules there are appearances which correspond to those seen in regenerated tubules after a mild tubular experimental nephritis.

Many tubules also show slight casts of orange-brown material like haemoglobin; the most remarkable histological picture is observed, however, in the region of the boundary zone where the straight descending limbs of the first convoluted tubules in the medullary rays of the cortex are changing to narrow descending limbs of Henle: at this site large numbers of severe focal lesions

are found, affecting the broad ascending limbs of Henle's loops, the segments which lead to the second convoluted tubules in the cortex. These lesions often occur in groups, and each group is surrounded by a definite recent growth of interstitial tissue; in some cases it is easy to ascertain that each focus implicates not only a tubule but also the thin wall of an adjacent venule; the tubule can be traced from comparatively normal conditions above and below to a level where there occurs an aneurysmal bulge of 40-80μ diameter, and this is usually filled by a dense mass of albuminous coagulum. The bulging part of the tubule indents the wall of the small venule and often protrudes into the lumen of it. It may actually rupture, i.e. the authors imply that the communication is formed by a break-through from tubule into vein. Bywaters and Dible (1942) agree with this view. The significance of this finding will be made manifest

later in this chapter.

There are also definite epithelial alterations which appear to be restricted to the ascending limbs of Henle and to the second convoluted tubules, very much like the appearances reported by Dunn and Polson (1926) in experimental uric acid poisoning of rabbits; in this experimental nephritis the lesion is determined by precipitation of the agent from a harmless soluble form to a destructive solid. Dunn, Gillespie and Niven then continue by making the highly significant remark that in the two cases of kidney damage under discussion by them the site of the most intense damage is at points where the lower nephron segments lie in close proximity to veins; they consider that the frequency of the association is so remarkable as to suggest a definite causal nexus.

In contradistinction to the findings of Bywaters and Beall (1941) and of Bywaters and Dible (1942), Dunn, Gillespie

and Niven failed to observe any significant change in the glomeruli or the first convoluted tubules, other than albuminous aggregates in many of the capsules of Bowman.

The findings of Dunn, Gillespie and Niven have been repeatedly confirmed by other workers, especially in regard to the absence of any serious abnormality in the glomeruli and the first convoluted tubules. Their report of the anastomoses sometimes to be found between the tubules and the veins has also been confirmed, e.g. McLetchie (1943) and Woods (1946). In the report by the latter author he records three fatal cases of carbon tetrachloride poisoning in which the kidney lesions bear a marked resemblance to those of the crush syndrome. The description of the pathological changes in the kidneys as recorded by Woods does not add anything to that of Dunn et alia given above, except that Woods remarks upon the great and uniform enlargement of the vasa recta, many of which contained portions of mural thrombosis, often closely continguous to adjacent renal tubules apparently at the ascending loop of Henle; the author describes these thromboses in detail, and confirms them to be the same as the anastomoses reported by Dunn, Gillespie and Niven. In Woods three cases only one showed these thromboses in any number; a second case showed only one such thrombosis, and the third case did not show any. Woods does not accept Dunn, Gillespie and Niven's theory of the genesis of such communications; his views will

be discussed later.

It was not long after the appearance of the paper by Bywaters and Beall (1941) before it was realised that the kidney lesions described by them are also produced by other diverse conditions. For example, such lesions had been repeatedly reported to be a common finding in the kidneys of individuals dying of anuria following an attack of blackwater fever. In fact we can go further and say that the tubulo-venous communications reported as a new finding in 1941 had been recorded certainly not less than twenty-five years previously as a finding in fatal cases of blackwater fever. In fact, as will be seen later, two of the earlier workers on blackwater fever used these self-same anastomoses as a basis of a theory to explain the mechanism of haemolysis in black-water fever; these workers were Plehn (1920) and Ravenport (1928). The changes in the kidneys, and the other relevant findings in blackwater fever have been studied so carefully over so many years that we may with advantage consider some of the more important work; e.g. Ponfick (1883) following experimental work on animals mentions that, following haemo-globinuria, the tubules of the animals' kidneys showed granular masses and precipitates; he thought the substratum of these masses to have been passed partly through the glomeru-li and partly through the epithelium of the convoluted tubules. The masses form within the labyrinth a quantity of

large casts, and the author then proceeds to voice a belief that has been accepted without question until very recently, namely that "it is in this that we meet the chief danger of any intense haemoglobinuria, that is in the blocking of the numerous renal tubules by semi-solid casts ..... the tubes are filled with wedged-in clots threatening a sudden occasionally inevitable standstill of the whole excretory process". This description was written over seventy years ago but could well serve for the beliefs of certain writers even today.

From that time on the literature of blackwater fever contains many papers on the pathology of the disease; e.g.

Ferrier (1896) in the examination of a fatal case mentions that the convoluted tubules and straight tubules contained exudate, granular detritus, and red cells agglutinated in the form of casts.

Barratt and Yorke (1909) discussed the composition of casts, and talk of large granules of fragmented material and epithelial casts.

Yorke and Nauss (1911) using laboratory animals, investigated the part, if any, played by casts in the production of anuria in blackwater fever; this subject will be dealt with below, but the observations of Yorke and Nauss are worth recording here. These authors injected

rabbits with intra-venous haemoglobin solutions which were stroma-free, and they succeeded in obtaining haemoglobinuria for the first time with such animals. Some of their animals died in convulsions a few hours after haemoglobin injections, before the onset of suppression. In such animals the only abnormalities found in the kidneys were brown plugs in the tubules.

In those animals in which the kidneys were removed following several injections of haemoglobin the findings were much more striking, with large numbers of the convoluted tubules and tubules of Henle filled with plugs of granular material intermingled with granular casts.

The next stage was found with animals dying of suppression; here the whole structure had radically altered, with some of the glomeruli enormously distended, and the tubules also enormously distended in the subcortical zone. Many of the tubules contained casts, with the epithelium in various stages of degeneration. The epithelium had disappeared in some cases. The findings of Yorke and Nauss of marked dilatation of the glomeruli and tubules have not been reported in the human and are a striking

example of the danger of applying to human disease the results produced in animal experiments. It is especially necessary to be careful in this respect in work dealing with intra-vascular haemolyses because the pigment methaemalbumin found always in man in haemolysis is not found in any animal other than the monkey.

Ross (1932) in discussing blackwater fever, suggests that in its passage down the renal tubules the foreign protein, haemoglobin, becomes solidified by extraction of water, and may cause stripping of the basement membrane; from the denuded surfaces so produced inflammatory lymph may exude and be added to the urine.

Fairley and Bromfield (1937) state that any severe intra-vascular haemolysis in man ordinarily leads to blockage of the lumina of a proportion of the collecting and other tubules with degenerative changes in the lining epithelium; they claim that their findings indicate that if a sufficient number of nephrons be involved then oliguria, anuria and nitrogen retention and uraemia all follow; where the haemolysis is small then the urea content of the blood causes diuresis with

polyuria.

There are many other papers on the pathology of the kidneys in blackwater fever but they add very little to the notes given above, and a fair summary of the relevant literature is given by Stephens (1937).

Kidneys; glomeruli .....often normal, but capsules may at times show distension and desquamation, and their contents may consist of granular material or blood debris or haemoglobin granules;

tubuli contorti ...the author does not specify which tubules are referred to, e.g. first or second convoluted tubules. He remarks that they may be dilated (this is of doubtful accurary as already noted) epithelium may be flattened, normal, or show degeneration or necrosis; this last change may be local or may affect large tracts. The contents of the tubuli may be

1. Albuminous matter tinged with haemoglobin.
2. Haemoglobin granules, blocks or casts.

3. Red cell debris, red cells.

4. Epithelial casts, granular casts, or hyaline casts.

tubuli recti ... some dilatation. Much of the epithelium may be shed, exposing the basement membrane; contents consist of granular matter.

interstitial tissue; intense hyperaemia may occur, with, here and there, actual haemorrhages; passage of blood into the tubules is described, due to fusion of the necrotic capillary and the tubular walls.

At this point it is relevant to report the kidney lesions found at autopsy of our fatal case; the full report of the post-mortem examination is to be found in the appendix, pages to .

Kidney changes: naked eye the kidneys showed no marked abnormality; the capsule stripped readily; no irregularity of the surfaces which were paler than normal. Structures of kidney not clear-cut; pyramids difficult to detect.

microscopic changes:

glomeruli; normal, few capsules showed some desquamation of cells with granular contents in the spaces

of Bowman.

convoluted tubules and loops of Henle; changes widespread. Much degeneration and disorganisation; in many places the lining epithelium is stripped or shows no nuclei; degree varies considerably. Many of the tubules and loops contain plugging with a mixture of lining cells, casts, red cells and amorphous debris; the amorphous material shows pigment staining.

The parts most affected are the two limbs of Henle, and the second convoluted tubule.

interstitial tissue; apparent hyperaemia with oedema. The hyperaemia is due to dilated vessels.

This completes the short note on the pathology of the kidney in conditions such as blackwater kidney, crush kidney, etc. Certain of the discussions on this syndrome claim that it is necessarily associated with nitrogen retention and acidosis. It is necessary to consider this in detail.

Nitrogen retention: this undoubtely is a very common finding in any degree of this condition; Lucke (1946) briefly sums up the findings as indicating "renal shutdown". Azotaemia is invariably present, with hypertension common. Low and Fairley (1942) consider that the marked rise in blood urea is derived

partly from destroyed red cells and partly from renal retention. The microscopical picture which such kidneys present fully explains this nitrogen retention. The table given below is indicative of the nitrogen retention so common in this disease.

Figure 10

Blood urea levels in the Razmak cases.

Case No.	Blood urea levels, milligrams per 100ccs blood Number of days after coming under observation.							
	1st	2nd	3rd	4th	5th	6th	7th	1 mnth
1	60 mmgs	80	120	200	230	290		
2	50 "	80	70	100				40
3	60 "	80	130	150				60
4	70 "	60	110					45
5	90 "	130	100	110				30
6	?	?						?

If the patient lives, regeneration even of gravely damaged tissues is remarkably rapid and complete. There is practically never residual nephritis and the nitrogen retention is quickly brought under control, i.e. the nitrogen retention is secondary to and fully explained by the kidney lesions.

Acidosis; is less definite a finding; as will be seen later, it is readily accepted that acidosis may occur, and some workers consider this physical state to be responsible for some of the phenomena of the crush

syndrome, etc. In point of fact however it is doubtful whether this condition is a constant finding; the fullest reports on this problem are found in the literature on blackwater fever, from which we may draw in this discussion; e.g. Fairley and Bromfield (1938) report a case in which the plasma carbon dioxide was 34.5 volumes per 100 ccs. They considered that this decreased bicarbonate reserve had resulted from an inability of the kidneys to secrete acid radicles adequately, with consequent decrease in the alkaline reserve and a tendency to renal acidosis. On the other hand other workers, e.g. Ross (1932) claims that in a large series of cases investigated by him the carbon dioxide combining power was normal in all cases. Similarly Maegraith (1946a) considered that available evidence does not support the claim that acidosis is common in blackwater fever. Unfortunately we had no equipment available to allow of our investigating this in our patients, and can offer no evidence in support of either point of view. There is no doubt that cases such as that quoted by Fairley and Bromfield, above, are to be found in any series of blackwater patients, but it is suggested that they are not a usual finding and, as will be

seen later, it is possible therefore that the excessive and uncontrolled alkalinisation so common a feature of the treatment of blackwater fever since 1926 may not only have been unnecessary; it may actually have been harmful.

We may now pass to a consideration of the quantitative and qualitative changes in the urine in patients showing the kidney lesions described above; here also we shall use the findings from the blackwater fever literature to illustrate our points, since such findings are of general application.

General remarks: Rogers and Megaw (1939) describe the urine of blackwater as first pink ⟶ bright red, soon changing to dark-red, brown, or even black, due to the presence of oxyhaemoglobin, methaemoglobin and urobilin. There is albuminuria which persists for some days after the pigments have disappeared from the urine. Brown granular debris and tube casts are found in the urine; usually these are abundant.

In this description the authors fail to mention quantitative changes.

One description states that the characteristic urine of blackwater is dark brown, generally acid, and that if it is

stored for some time it will separate into two layers, an upper of a clear though dark port wine tint and a lower one-third or one-half of a brown-grey colour and consisting of a sediment in which an enormous number of hyaline-haemoglobin tube casts are to be found together with a large quantity of brown granular material.

The above short general description of the urine in patients with intravascular haemolysis gives very little detailed information on each of the abnormalities. Since certain of the abnormalities may provide clues to the processes at work and to rational lines of treatment, it is essential to consider them in detail, and the easiest way to do so is to deal with them seriatim, a few lines to each; the more important abnormalities, and ones which shall be considered, include albuminuria, character of the urine (including the chloride content, colour, reaction and specific gravity), casts and deposits, urine output, pigments, and urea content.

albuminuria: this term is not correct but is of common usage. The proteins found actually are serum albumin and serum globulin. Fibrinogen is very rarely found. The results of the testing of the urines in our six patients are given

in Figure No. 11, below.

Figure No. 11.

Proteinuria in the Razmak patients.

Case No.	Number of days after coming under observation.							
	1st	2nd	3rd	4th	5th	6th	7th	Remarks.
1	+	?	+++	?	?	+++		Patient died.
2	++	+++	++	+ −	+ −			Traces of protein for 8 days.
3	+++ ± − ±	+ + −	− − −	+ ± + + ± − + −	+ −	±	+	" " 15 days.
4	+ + − −	+ + −	− ±	−				" " 8 days.
5	+ −	+ − +	+ + −					" " 8 days.
6	+ + −	−						" " 4 days.

Key: − = negative ; ± = trace : + = present;
++ = well-marked +++ = loaded

The routes whereby proteins may find their way into the urine have been discussed earlier in this chapter (see above). They pass into the urine via the glomeruli; it is not yet settled whether this takes place as the result of an increase in the permeability of the glomerular membrane or whether there

occurs some pathological change in the membrane, caused by some unknown factors. According to Fishberg (1934) the general opinion is that there are probably temporary changes in the glomerular permeability either through toxic action or through a need to eliminate undesirable protein products of metabolism.

Ross (1932) suggested that in intra-vascular haemolysis the passage of the foreign protein derivatives of haemoglobin injures the tubule cells which then become permeable to plasma proteins; This view of Ross may have been derived from Yorke (1922) who thought that the serum proteins which are found in the urine in severe cases of blackwater fever gain access to the tubule through the lesions produced by dislodgement of the epithelium a point of view which will be discussed in detail later in this chapter. It need only be said here that we may proceed on the assumption that the proteins do pass through the glomeruli.

It is generally agreed that the proteinuria is not restricted to the haemoglobinuric phase;

it may persist for several days after the cessation of haemoglobinuria, and in unusual cases may be permanent. The amount passed is considerable in quantity, e.g. it may "set solid" on boiling, with a content of about sixteen grams per litre.

In our cases there was no departure from the usual findings, namely a variable amount of protein for a varying period of days. This proteinuria is merely a secondary process and will usually disappear if the patient survives and the nephron returns to normal.

Character of the urine:

chloride content and specific gravity; Plehn (1920) claims that the urine in blackwater fever is always hypotonic, this hypotonicity being caused by an abnormally low content of sodium chloride, which substance normally plays an important part in the maintenance of the specific gravity of the urine.

Plehn was wrong; sodium chloride values as low as he claimed are not a usual feature of the urines in blackwater fever. We did not measure the sodium chloride content of the specimens of urine in our patients and there are few

references to such measurements in the literature of cases of intra-vascular haemolyses; Stephens (1937) does say that in blackwater low values are commonly reported; Bywaters and Dible (1942) say there is no evidence of absorption of chlorides by the tubules; in this connection we shall use the urinary specific gravity as a very rough guide to the level of the contained sodium chloride. It is realised that the sodium chloride is only one of the several substances which go to make up the specific gravity values of the urine, but it is such an important factor that it is permissible to use the specific gravity as a measure of the contained sodium chloride.

Some of the opinions as regards specific gravity in blackwater fever urines are as follows:

Stephens (1937) states that the specific gravity in the reports varies from 1002 to 1033.

Ross (1932) claims that in the blackwater type of intra-vascular haemolyses a low specific gravity is not an essential characteristic of the urine.

Lucke (1946) in talking of his series of 538 cases states that the urines had a fixed specific gravity of 1014. Although this statement cannot be accepted literally, it serves to show that the values obtained for the specific gravities, and therefore the values of the sodium chloride content were much higher than those obtained by Plehn.

Figure No. 12 below records the values of the specific gravities of the urines in the Razmak cases during and immediately following haemolyses.

Figure No. 12.

Maximum and Minimum Specific Gravity Readings of the Urines in the Waziristan Cases.

Case No.	Number of days after coming under observation for lysis.					
	1st	2nd	3rd	4th	5th	6th
1	-1024	?	-1030	?	?	?
2	1008 -1030	1006 -1008	1008 -1012	1012 -1014	1010	
3	1010 -1022	1010 -1016	1008 -1018	1008 -1010	?	1010 -1020
4	1010 -1026	1012 -1018	1004 -1016			
5	1024 -1030	1012 -1024	1008 -1022	1010 -1018		
6	1002 -1018	?				

The figures are given in detail in the case reports in the appendix, pages 734 to 788 ; from these, summarised above, there is no doubt that the values are consistently lower than would be the case with a range of normal urines, especially in view of the oliguria present in the majority of the cases during the period when the urine specific gravities were being recorded. It is to be seen from the detailed case reports in the appendix that the specific gravity values in our patients did tend to be grouped round 1014 immediately after the haemolyses; this is in rough agreement with the findings recorded by Lucke, noted above, and are sufficient to disprove Plehn's statement that in blackwater fever the urine is necessarily markedly hypotonic. And further, our findings strongly suggest that the content of sodium chloride in the urines is not greatly depressed as was claimed by Plehn, above; these points are not only of academic interest; they will come into prominence later in the chapter when we come to discuss Plehn's theories on the causation of the haemolysis of blackwater fever.

Another point mentioned in certain reports on the character of the urine in intravascular haemolyses is that in certain of the cases the urine may be almost like jelly; in contradistinction to this is the common finding that if the patient should survive then for several days after re-establishing of kidney function the urine is like glomerular

filtrate. We certainly confirm the first point, that in severe cases, e.g. our fatal case in this series, the urine may be like jelly; this has also been my experience in other cases not reported here. Unfortunately it is not possible to speak so definitely on the second point, that the urine of recovering patients may be like glomerular filtrate; our case records are not full enough to be of value. The only indicators we have are the records made of the specific gravity values.

colour: in any intra-vascular haemolysis the colour of the urine necessarily varies with the content of haemoglobin and its derivatives; the colour changes of the urines of the six Waziristan cases are given in detail in the appendix, pages to . Since these colour changes are not significant of anything other than changes in the contained pigments they need not be discussed in detail at this point

reaction: the reaction of the urine in patients with intra-vascular haemolysis is of more than academic interest. It is of practical importance, in that some workers believe that the reaction of the urine plays an all-important part in cast formation in the tubules of the nephrons.

Stephens (1937) summarising the literature, finds reports of pH values ranging from 5.8 to 6.8. Our rough findings in the Waziristan patients are given in figure No. 13 below; it is regretted that the only information available is that of the reaction, as measured by the use of litmus paper; more detailed records could not be made at the time.

Figure No. 13.

Reaction of urines during and immediately after haemolysis.

Case No.	No. of days after coming under observation for lysis.					
	1st	2nd	3rd	4th	5th	6th
1	alkal.	?	alkal	?	?	alkal.
2	alkal - neut	acid	neut - acid	alkal	alkal	acid
3.	acid	acid-alkal	acid-alkal	acid	acid alkal	acid
4	acid-alkal	acid-alkal	acid-alkal			
5	acid	acid - alkal	neut - alkal			
6	acid - alkal					

The detailed findings in the appendix show that the majority of the specimens of urine were acid

in reaction but that neutral and alkaline reactions were also quite common.

casts, including deposits: in this paragraph we shall still draw extensively from the literature on blackwater fever; there is no objection to this since the statements are of general application and the literature on this particular type of intra-vascular haemolysis is full and detailed.

From the beginning of the intensive study of the urine of blackwater patients descriptions of the deposits have been recorded, e.g. Ponfick (1883) talks of "peculiar flakes, forming a slimy sediment of a brown colour" which he likens to the material found in the tubules of the kidneys of fatal cases.

Plehn, F. (1898) stated that the sediment contained an abundance of swollen epithelial cells from the urinary passages, granular cells, detritus, haemoglobin masses, and haematin crystals.

Barratt and Yorke (1909) agree with the above, and mention particularly granular casts, hyaline casts, and epithelial casts.

Yorke and Nauss (1911) in experimental work, stated that after intra-venous injections of haemoglobin into rabbits, the later

specimens of urine showed much solid material, frequently soft masses composed almost entirely of casts and granular debris held together by mucoid material; later large numbers of renal epithelial cells appeared; still later, specimens showed much protein and many granular casts and enormous numbers of small degenerated renal epithelial cells and casts.

Plehn (1920) states that after re-establishment of function of the kidneys in those who survive the urine is loaded with albumen and is very turbid, with a large deposit about one-quarter of the volume, made up of granular casts, epithelial casts, granular debris and degenerated epithelial cells.

Ross (1932) states that microscopically the urine shows the sediment to be mainly of granular material, partly amorphous and partly casts, most likely derived from haemoglobin which has undergone precipitation during its passage down the urinary passages; the earliest specimens, Ross thought, usually show more amorphous

deposit than casts; casts become more numerous as the disease progresses, and they may persist after the haemoglobin clears from the urine. Epithelial and hyaline casts are also common.

Fairley and Bromfield (1937) agree that casts are not numerous at first but later in the course of the disease there are found small clots of mixed leucocytes and casts; these were identical with the casts plugging the tubules as observed in sections of the kidneys of blackwater fever patients.

Manson-Bahr (1940) states that the sediment in urines of blackwater cases is bulky, one third to one-half, brown-grey in colour and consisting of a sediment in which an enormous number of hyaline and haemoglobin tube casts are found together with a large quantity of brownish-granular material.

The above excerpts serve to show that the deposit in itself does nothing other than mirror what is happening in the nephron.

The casts are of all kinds; e.g. Plehn (1898) reports epithelial casts, granular casts, hyaline

casts, and pigment casts. These are the findings of many other workers. The casts also serve only to mirror what is happening in the kidneys; they disappear shortly after the restoration of the kidney function to normal in those persons who survive.

The relevant findings in our patients are given in detail in the appendix, pages 734 to 788 ; it is unnecessary to repeat them here as they add nothing to the excerpts given above.

In the five of our patients who survived casts persisted in the urine for several days after the apparent cessation of haemoglobinuria.

The only remaining point to note on this question of casts is that in crush injuries the pigments concerned in the production of casts are myoglobin and certain derivatives of myoglobin.

output of urine: here we shall do little more than record the outputs in our six patients; this is done in Figure No. 14 below. The significance of our findings will be the subject of discussion later; it is unnecessary, therefore, at this stage to quote extracts on this question of the output of urine in individuals suffering from intra-vascular haemolysis. Almost from the time of the recognition of blackwater

fever as an entity the marked changes in the output of urine of patients have focussed attention on the kidney changes in this disease; within very wide limits it is almost permissible to say that the degree of oliguria is directly proportional to the degree of kidney damage. The same might also be said of all forms of intravascular haemolysis and for those other conditions capable of producing lesions in the kidney similar to the lesions found in "transfusion kidney" etc.

In a few of the individuals showing mild lesions in the kidney there may be polyuria; case No. 2 of our series is an illustration of this, as shown in Figure No. 14.

Figure No. 14.

Showing daily urine output of Waziristan cases in the first days of lysis. Output given in ounces.

Case No.	No. of days after coming under observation for lysis.					
	1st	2nd	3rd	4th	5th	6th
1	3	?	½	nil	nil	nil
2	60	71	60	70	10	
3.	25	21	26	?	?	16
4	16	16	39			
5	14	20	33			
6	66					

With all our patients during the periods of haemolysis and immediately following haemolysis the fluid intake was balanced against fluid loss, therefore any variation in the amount of urine passed is not the result of abnormal deprivation or of abnormal supply of fluids to such patients.

The figures given above contribute nothing new to the problem.

As has already been said, the output of urine is of great importance in that it reflects the changes in the kidneys; in conditions capable of producing this type of kidney such as is found in blackwater patients the onset of oliguria is the first warning of that most dreaded of complications, the severe kidney lesion.

pigments in the urine: Fairley and Bromfield (1938) report that the pigments described as occurring in the urine of blackwater patients include oxyhaemoglobin, methaemoglobin, and urobilin and a brown pigment generally regarded as acid haematin; they point out however that the last pigment does not show the same characteristics as artificially-produced haematin and that it has not a typical spectroscopic picture.

They state that the oxyhaemoglobin amounts

range from 16 ---- 284 milligrams per 100 ccs. urine, and methaemoglobin 90 ---- 900 milligrams per 100 ccs. urine.

These authors found in an examination of fourteen cases that the urine showed oxyhaemoglobin in two, oxyhaemoglobin and methaemoglobin in ten. These findings probably equally apply to other forms of intra-vascular haemolysis.

In the case of crush injuries, of course, the parent pigment is myoglobin, but its characteristics so closely resemble those of haemoglobin that general remarks may be taken to apply to both.

Our findings of pigments in the urines of our patients are detailed in Figure No. 15 below.

Figure No. 15.

Abnormal pigments present in the urines of the Razmak cases.

Case No.	No. of days after coming under observation for ? lysis.						
	1st	2nd	3rd	4th	5th	6th	7th
1	HbOH HbOO.U	?	HbOH HbOO.U	?	?	B. HbOH HbOO. U	
2	HbOH HbOO.U	B.HbOH HbOO.U	ditto	HbOH HbOO.U	U		
3	ditto	ditto	ditto	ditto	U	HbOH HbOO.U	U
4	ditto	HbOH HbOO.U	ditto	U			
5	B.HbOH HbOO.U	B,HbOH HbOO.U	ditto	U			
6	HbOH HbOO.U	U	U				

Key to Figure No. 15:

B = Bilirubin. HbOH = Methaemoglobin.

HbOO= Oxyhaemoglobin. U = Urobilinogen.

Reference should be made to the detailed findings in the case reports, appendix pages 734 to 788 , where it will be seen that the relative amounts of haemoglobin and methaemoglobin vary according to the reaction of the urine, the oxyhaemoglobin being marked in amount in alkaline urine and the methaemoglobin being marked in amount in the acid urines. It is seen from our records in the appendix that we were never able to detect the brown pigment spoken of by Fairley and Bromfield (1938).

The origin of the blood pigments and the bile pigment has been discussed in detail in Chapter IV, pages 154 to 184 . For fuller information reference should be made to that chapter, and particularly to plate No. XVI, page 171. A short note is given below, however, on each of the above four pigments.

urobilinogen: is the only derivative of haemoglobin normally found in urine and even this pigment is minimal in amount in normal urines. Plate No. XVII, page 179, shows that the urobilinogen which appears in the urine is that which has escaped re-excretion in the bile and which has

passed into the general circulation. Best and Taylor (1943) state that the daily excretion of urobilinogen amounts only to 0.5 - 2.0 milligrams. Stephens (1927) states that this total is excreted in spurts, particularly related to meals. Urobilinogen is oxidised to urobilin after the urine has been voided; normally this pigment plays no part in the colouring of the urine which is due to urochrome, a compound of unknown constitution and of unknown origin. Ross (1932) says that increased urobilinogenuria is found in many pathological conditions, e.g. pyrexia, cancer liver, pernicious anaemia, and is a feature of conditions characterised by increased blood destruction, e.g. malaria. When present in such increased amounts urobilinogen may darken the urine. Ross certainly found increased urobilinuria in malignant tertian malaria, and quinine and plasmoquine administration caused an increase in the urobilinuria; in fact Ross states that he found the increase following the above drugs to be as much as that recorded in certain cases of blackwater fever! One suggestion to explain the increased output of urobilinogen by the body in haemolysis is that

the amount of bilirubin formed in such cases fully occupies the liver, which then ignores the intestinal urobilin and this latter is excreted by the urine; it must be remembered, however, that the actual amount of both pigments is markedly increased in haemolysis. Whitby and Britton (1946) agree that hepatic insufficiency plays some part in the increase of urinary urobilinogen. Nocht and Mayer (1937) state that this increased urobilinuria may continue for some days after an attack of intravascular haemolysis. Our findings are in agreement with this, i.e. we found increased excretion of urobilin during and after the haemolytic phase.

bilirubin: unlike urobilin, this pigment does not normally appear in the urine. In fact its absence from the urine has been repeatedly remarked upon by writers on blackwater fever, e.g. Plehn (1903b) remarks that in spite of severe icterus and bile pigment in the plasma, bile pigment in such cases is usually absent from the urine. It has already been pointed out in this work that the bile pigment which has passed through the liver cells appears much more readily in the urine than does that type which

has not done so; e.g. in the obstructive type of jaundice bile pigment appears in the urine when the bilirubin content of the blood rises above a certain low value, whereas in haemolytic jaundice a considerable degree of bilirubinaemia may be present without there necessarily being any bilirubinuria; for example, Beaumont and Dodds (1929) stated that the bilirubin which has passed through the liver, i.e. crystallisable bilirubin, which they term "bilirubin B" will appear in the urine with a serum value of over four van den Bergh units whereas the colloidal type of bilirubin found in excess in haemolytic disease has a kidney threshold value of not less than eighteen van den Bergh units. Whitby and Britton (1946) think that this high kidney threshold for the second may be because the pigment is linked with plasma proteins. Whatever the reason the facts would appear to be well-established, e.g. Ross (1932) tested eighty-six cases of blackwater fever and found only four to show even a trace of bilirubinuria; and in each of these four patients he also found a positive direct van den Bergh reaction of more than four units; each of the

four patients had anuria and we may reasonably assume that each had some degree of liver damage. One useful pointer in this connection is that of the presence or absence of bile-salts in the urine. The excretion of bile salts is independent of the retention or excretion of bile pigment, therefore in uncomplicated intra-vascular haemolyses bile-salts are absent from the urine; but in patients suffering from intra-vascular haemolysis with concomitant liver damage bile salts may be found in the urine and would then serve to confirm that any bilirubin present in the urine is of the crystallisable "B" type which had been regurgitated back into the circulation due to liver damage. Ross found bile salts in the urine of one of his four patients with bilirubinuria.

There is no question but that liver damage may occur in individuals suffering from intra-vascular haemolysis, and this is essentially likely to be found in blackwater fever since malaria itself may damage the liver. Maegraith, Andrewes and Gall (1947), and Most (1940) state that in severe cases (of blackwater fever) there is liver involvement and associated anoxaemia

with injury to many of the polygonal cells; obviously this would permit of the regurgitation into the blood of changed (crystallisable) bilirubin, and this would account for the positive immediate direct van den Bergh reaction and for the presence of bile in the urine. Some confirmation for this is found in the summary by Stephens (1937) who states that in blackwater fever bilirubin is absent from the urine as a rule, and that when present it may be associated with a direct positive van den Bergh reaction of the plasma. Low and Fairley (1942) also agree that it is only in the severest forms of blackwater fever that bile is found in the urine; one author does not agree with the opinions quoted above; he is Hills (1946) who discussed the question of malarial jaundice; He reports that no less than nine of thirteen patients showing "malarial jaundice" were found to have bilirubin in the urine, and he concludes "apparently bilirubinuria is the rule in this syndrome". Such statements are so at variance with the experience of others that they require to be confirmed before they can be accepted.

The findings in my patients have been

reported in Figure No. 15, page 323; it is seen from this record that in case No. 1, the fatal case, bilirubin appeared in the urine on the day before death; in patients Nos. 2 and 3 this pigment made a fugitive appearance in the urine on the second day only; and in case No. 5 it was found to be present in the urine on the first and second days; Figure No. 16 below, shows the van den Bergh reactions and the values present in patients Nos. 1, 2, 3 and 5 on the days when bile was found in their urines.

Figure No. 16.

Relationship between bilirubinuria and the van den Bergh values and reactions of the sera.

Case No.	Day of illness & bilirubinuria	v.d. Bergh qualitative	v.d. Bergh quantitative
1	6th	direct +++ (one day before)	not done
2	2nd	biphasic	10
3	2nd	"	10
5	1st 2nd	" "	9 8

Our findings, given above, do suggest some

degree of liver involvement, e.g. in case No. 1 the reaction was direct +++.

The Kazmak patients showed a higher incidence of bilirubinuria than that reported by other workers, four of the six patients showing bile pigment in the urine; in each case however this finding was fleeting in time; this high incidence of bilirubinuria may partly be explained by the suggestion of Ross (1932) that the tests usually employed for the detection of bilirubin in the urine depend on a colour change and that such tests are interfered with by the presence of haemoglobin; he claims that with the use of tests like those of Gmelin or Hippert, small quantities of bilirubin may be present and yet remain undetected. To eliminate possible criticism we used the iodine test suggested first by Smith (1876), and this may account for our higher incidence of positive findings. Unfortunately we failed to examine the urines for the presence of bile salts.

In the main, our findings would support the contention that bilirubinuria is not a common finding in the urine of individuals with intra-vascular haemolysis, and that when such is found it may indicate regurgitation of crystallisable bilirubin back into the blood-stream because of damage to the liver. In the one patient of our series who did come to autopsy, and who had shown bilirubinuria, the liver showed acute central necrosis involving the medial two-thirds of each lobule.

oxyhaemoglobin: again we use blackwater fever reports as the main source of material for discussion.

One interesting point is that for almost fifty years after blackwater fever became recognised as a syndrome, the colour change in the urine was thought to be due to haematuria. Among the first to recognise the real state of affairs were

Monestier (1873) using crystallisation, concluded that the urine contained dissolved blood, a mixture of

haemoglobin and methaemoglobin.

Louvet (1876), using sulphuric acid, claimed to have identified methaemoglobin.

Heinemann (1885) identified methaemoglobin by use of a microspectroscope.

It is now accepted that the oxyhaemoglobin found in the urine in haemolysis is derived from the same pigment circulating in the plasma; this has already been discussed and it has been pointed out that there appears to be a renal threshold level for this pigment, roughly 100 milligrams per 100 ccs. of blood (see page 191, Chapter IV). There are three obvious ways by which this pigment can get into the urine, through the glomeruli or through the tubules or both. Ponfick (1883) suggested both routes of excretion. Berthier (1896) reaffirms the French view that the oxyhaemoglobinuria results from quite a different process, namely that as the result of venous stasis and resultant multiple haemorrhages into the uriniferous tubules. This concept gained much

support from Plehn (1920) who was the first to report the occurrence of tubulo-venous anastomoses as already explained; Plehn considered that all haemoglobin in the urine finds its way there through these anastomoses. The generally-accepted view has long been, however, that haemoglobinuria is the result of escape of this pigment through the glomeruli. As already mentioned, Fishberg (1934) considers that there are probably temporary changes in the permeability of the glomerulus, possibly through toxic action.

The amount of blood pigment which does escape through the glomerulus is only a fraction of the blood loss as measured by the fall in the red cell count. This has also already been discussed, see page 192, Chapter IV. The principal factors governing the degree of haemoglobinuria are the rate of the haemolysis and the degree of the haemolysis; the rate of haemolysis is especially important, a rapid rate of destruction bringing the level of free circulating pigment quickly to the kidney threshold level before other more conservative measures can operate. Stephens (1937) states that no matter the degree or the rate of haemolysis the maximum concentration of pigment in the urine, as measured in

terms of blood, never exceeds ten per cent; he suggests that the amount of haemoglobin in the urine at any one time is equivalent to 200 ccs, blood. Fernan-Nunez (1936) considers that in an average case (of blackwater fever) the blood pigments in the urine are equivalent to that in 300 ccs. blood, although the actual blood destruction may be many times as great as the haemoglobinuria would suggest. These figures, however, can only be very approximate in view of the several factors involved, as mentioned previously in a much fuller discussion on this point (see pp.s 192 et sequ.). Very roughly speaking, the concentration of the blood pigment in the urine depends on the speed of the lysis, and the total blood lost through the kidneys depends on the length of time the lysis continues. In Figure No. 17 below, the values are given of the equivalent strengths of the pigment concentrations in the urines of the Razmak cases.

Figure No. 17.

% Haemoglobin derivatives present in the urines, measured against % equivalent solutions red cells.

Case No.	No. of days after coming under observation for ? lysis					
	1st	2nd	3rd	4th	5th	6th
1	2.5%	1.5%	? 1.5%	? 1.5%	? 1.5%	1.5%
2	3.2%	3.5%	2.4%	0.4%		
3	2.8%	2.8%	3.2%	0.8%	nil	3.1%
4	1.6%	1.4%				
5	3.0%	2.7%	1.6%			
6	1.6%					

The figures given above for each day are the highest values recorded on that day, and give no idea of the marked fluctuations that do occur in the values; this can only be fully appreciated by reference to the detailed records in the case summaries in the appendix.

In the above discussion the term "oxyhaemoglobin" has been loosely used to include other blood pigment derivatives,

e.g. the values given in Figure No. 17 above include all derivatives. It naturally follows that if the true value of oxyhaemoglobin be required this will be much less than the figures given above, which also include methaemoglobin.

We may at this stage again stress that in blackwater fever the duration of the haemolytic phase and the number of haemolytic phases vary greatly from individual to individual. Ross (1932) found that in 118 cases of this disease ten per cent of the patients had relapses; in this author's series of cases the duration of the haemolytic phase varied considerably from patient to patient; Ross gives the following times:

Up to 24 hours	.....	29 patients.
From 1-2 days	.....	28 "
" 2-3 "	.....	27 "
" 3-4 "	.....	16 "
" 4 days or longer	..	18 "

Ross's experience was that the haemolytic phase may last up to eight days; Manson-Bahr (1940) probably was incorrect when

he stated that the haemolysis may even continue for weeks. One factor which helps to determine the duration of the haemoglobinuria is the rate at which other methods of excretion operate to reduce the haemoglobin plasma level below the renal threshold. In this connection also, i.e. the content of the pigment in the urine of our patients, it is seen from the figures given and the records of duration of haemolysis as given in the appendix, that our results were much the same as those reported by other workers.

methaemoglobin: this pigment was first identified in the urine in the late nineteenth century; a few notes on the historical aspect are given above.

The characteristics of methaemoglobin have been discussed in Chapter IV, pages *184-200*. The physical characteristics of the pigment so closely resemble those of methaemalbumin that the latter pigment was not identified in the plasma until 1934. Previous to that identification

the brown pigment commonly reported in the plasma in intra-vascular haemolysis was thought to be methaemoglobin, and at that time it seemed reasonable enough to assume that the methaemoglobin in the urine of such patients takes origin from the methaemoglobin of the plasma, as is the case with the oxyhaemoglobin found in the urine. With the realisation that the pigment in the plasma is methaemalbumin opinions as to the origin of the urinary methaemoglobin had to be revised, because methaemoglobin does not appear in the plasma except fleetingly in those individuals in whom methaemoglobincythaemia is present at the time of haemolysis; methaemalbumin is never excreted via the kidney. The explanation now generally accepted for the source of the methaemoglobin in the urine is that it is formed from the oxyhaemoglobin. Until very recently it was not clear where and how fast this transformation occurred. Maegraith quotes a communication from Foy and Kondi which throws some light on

the above points. Foy and Kondi experimented upon a patient with paroxysmal haemoglobinuria; they had him empty his bladder then they immediately produced an attack of haemolysis by getting the patient to put his feet into cold water, and then immediately to pass urine. Methaemoglobin was found in the second specimen of urine, therefore conversion of the oxyhaemoglobin to methaemoglobin takes place, partly at least, in the urinary passages and in the tubules. Maegraith states that the conversion may go further, to acid haematin, and that this acid haematin is then taken up by the tubules or is deposited in the lumina. The last part of this statement should be carefully noted.

The prime factor governing the amount of methaemoglobin passed is therefore the amount of oxyhaemoglobin available, at least in part, for conversion. Time is another factor; probably the longer the urine is allowed to lie in the bladder the more complete will be the conversion.

There is one more factor to be considered,

namely the pH of the urine. This last point will be seen to be of some importance in the discussion below, on the cause of formation of casts in the tubules, e.g. Baker and Dodds (1925) laid great stress on the importance of urinary pH in determining the type of blood pigment present in the urine; these authors claimed that the highest pH value at which methaemoglobin was present was 7.0, i.e. this pigment would only be found in urines in which the reaction is on the acid side. Ross (1932) added that normally a certain amount of time is necessary for conversion, already mentioned above, and stated that the more acid the urine the shorter was the time necessary for conversion. In a series of 102 cases Ross found

oxyhaemoglobin alone in 56,

methaemoglobin alone in 6

and both in 40.

Wright (1928) puts the limits of conversion at much wider ranges, from pH 5.0 to 9.0; reference should here be made to our detailed urine reports, appendix pages 734 - 788.

This problem has already been discussed shortly, and may be summarised by our saying that using the very crude method of litmus-paper estimation of the pH values of the urines in the Razmak cases, then a change in the reaction of urine from acid to alkaline was always accompanied by a change in the predominant pigment from methaemoglobin to oxyhaemoglobin and vice versa; it must be stressed, however, that the reaction was never clean-cut and complete.

urinary urea; in the normal metabolism of the body there is much breakdown of amino-acids; this takes place practically wholly in the liver. The nitrogen released is converted into urea, which is therefore the chief end-product of protein metabolism. Arginine is now thought to be an important stage in the process of formation of the urea. This waste product urea is eliminated from the body through the glomeruli, and passes into the tubules. It is a substance of little value to the body, but a considerable proportion of it, possibly as high as forty per cent of the filtered urea, is returned

to the body; one explanation of this is that the process at work is one of simple diffusion. Nevertheless, in spite of the return to the blood of this considerable amount of urea, it is normally present in the urine at a concentration very much higher than that in the plasma, often showing as much as a sixty-fold increase.

In the type of kidney lesion now under discussion the characteristic of the lesion from the point of view of function is what Bywaters and Dible (1942) described as "aberration of tubular function" which allows of excessive and unselective re-absorption of glomerular filtrate; an immediate indication of this is a falling-off in the concentration of the urea in the urine, a finding well-marked in this type of lesion. The term "concentration factor" has been applied to this relation-ship urinary urea/blood urea. As already said this may have a value as high as sixty in health, whereas in this "lower nephron nephrosis" type of kidney lesion the concentration factor shows values much

below normal. Dunn, Gillespie and Niven (1941) stress this point and quote one case in whom by the eighth day the concentration factor was only 2.7; Georgopoulos (1933) had previously remarked upon the importance of this as a measure of the failure of efficiency. This change in the concentration factor is of interest in that it often serves to give a rough guide to the degree of damage the kidney has sustained. Unfortunately the urinary urea estimations were not carried out in my patients and therefore we are not in a position to give any information about the degree of reduction of the concentration factor in the Razmak cases.

This brings to an end the detailed consideration of the pathological picture and the urinary changes found in individuals suffering from that particular renal lesion common to many diverse conditions such as incompatible transfusion, blackwater fever, etc. etc.

This discussion may appear to have been over-detailed, but such detail was necessary since it is designed to serve as a basis for what may well prove to be the most important part

of the whole of this work, namely the attempt to explain the genesis of the various abnormal findings which go to make up the clinical and pathological pictures found in the condition of lower nephron nephrosis. The most important of these abnormal findings include:

1. Cast formation in the tubules;
2. Damage to the nephron, particularly marked in, and practically limited to, the loops of Henle and the second convoluted tubules.
3. The tubulo-venous anastomoses repeatedly reported.
4. Oliguria and anuria, with nitrogen retention.
5. Changes in the urine, which at first shows proteinuria and often blood pigment or muscle pigment, then later assumes the character of "glomerular filtrate" together with many casts.

Many theories have been put forward to account for the above. Some such theories attempt to explain only one or some of the abnormalities, while others attempt to explain the whole picture. As yet there is no one single theory which is generally accepted. The need is pressing in view of the gravity of the lesion with which we are dealing. Once we

fully understand the processes at work we shall be in a much stronger position to take the first hurdle, namely to find a satisfactory and efficient method of treating this particular kidney lesion.

With this end in view let us consider in detail each of the abnormal findings enumerated above.

1. Cast formation; the presence of the casts in the tubules has dominated the problem until very recently; the actual substances going to make up the casts are derived partly from muscle pigment or blood pigment, and partly from degenerated epithelium of the tubules. The relative amounts of each of the constituents will vary with the particular cause of the renal lesion, e.g. in carbon tetrachloride poisoning there is unlikely to be more than a very limited amount of blood pigment present. In such cases the formation of casts is necessarily related to the damage to the tubule, a problem discussed below, and therefore the only method of treating such cases is to treat the tubule lesion. The really important part of this paragraph lies in the discussion on the question of the pigment casts; in the great majority of cases of this type of renal lesion, e.g. in the intra-vascular haemolyses and in

crush syndrome, etc. a consideration of the formation of these casts is of great practical importance since it is claimed by some workers that it is these casts which cause anuria, and it is also claimed by some workers that the formation of pigment casts can be prevented. At this stage we may interpolate that some of the older workers, e.g. Plehn (1920) claimed that the pigment casts arise partly from the pigment secreted by the tubules and largely from a rushing-in of the blood into the tubule as a result of the formation of tubulo-venous anastomoses; these views are not generally accepted, although Woods (1946) has recently revived them.

We are basing our discussion on the generally accepted theory that the pigment escapes from the plasma via the glomeruli; if this assumption is correct, as it probably is, then we have in the first convoluted tubules a glomerular filtrate containing quantities of pigment, whether blood or muscle pigment, an abnormal constituent of the urine which has a kidney threshold value of about 100 milligrams per 100 ccs. plasma. As far back as 1903 Plehn (1903b) stated that the plugs were <u>secondary</u> to oliguria, a mere depositing as it were

from a super-saturated solution, and Yorke and Nauss (1911) indicated much the same when they said that the precipitation of casts was merely a factor of the concentration of pigment in the urine. This very important problem of which came first, the oliguria or the casts, remained in doubt for a long time; then in 1925 Baker and Dodds published results which seemed at the time to settle the matter beyond question. The interest of these authors had been stimulated by two fatal cases of incompatible transfusion in whom Baker and Dodds claimed death to have resulted from cast occlusion of the tubules. The authors carried out a few experiments with two rabbits and from their results they drew the following conclusions:

1. if the urine reaction is below pH 6.0 the blood pigment is converted into methaemoglobin, and possibly even to acid haematin.
2. if at least one per cent sodium chloride is present in the filtrate then this methaemoglobin is precipitated in the tubules to form casts.
3. the authors had previously concluded from their two fatal cases that death in such cases is the result of cast occlusion of the tubules.

On the basis of their results Baker and Dodds thereupon decided that the only proper treatment for all cases of intra-vascular haemolyses is alkaline diuresis.

Now in intra-vascular haemolyses of all descriptions the kidney lesion is the one that kills the majority of the patients, and here we have a theory that explains not only how oliguria arises, but also provides a method of preventing it. It is a reflection on the uncritical attitude of the Medical Profession that this paper met with wide and unquestioning acceptance, and that it led to the introduction of intense alkalinisation as the standard treatment in intra-vascular haemolyses. Hanschell (1926) was the first to apply to the human the findings of Baker and Dodds in rabbits. He claimed that his results supported those of Baker and Dodds, but his total number of cases was small, and he did not make allowance for the normal fluctuation of death rates in small groups. It was not for nearly a decade that doubt began to be expressed as to the correctness of the conclusions and the recommendations of Baker and Dodds. And it is only very recently that these doubts have crystallised into strong criticism,

reported later in the chapter. Ross (1932) should have credit for being among the first to refuse to accept the theory of 1926; he preferred the older theory of Yorke and Nauss (1911) mentioned above. Since then criticism has steadily grown, e.g. among the more recent critics Dunn et alia (1941) state that the presence of the casts in the tubules could easily be explained by a diminution of filtrate as a result of impaired circulation or from some other cause; it is to be remembered that this statement by Dunn et alia predates the statements by Trueta et alia (1946); this latter work, with its "mechanistic" explanation for the oliguria naturally lends great support to the suggestion that the casts are deposited from a saturated solution, a theory propounded in 1903 by Plehn. On the other hand, Dunn and Polson (1926) pointed out that the site of the kidney lesion in experimental uric acid poisoning, which also is the site of the lesion in lower nephron nephrosis, is the site at which there occurs a change in the reaction of the filtrate; Dunn and Polson thought that the damage done by the change of reaction of the filtrate was due to this change and concentration of filtrate serving to precipitate a toxic

substance at the site; this unrelated finding of 1926 is inserted here to show that the problem of cast formation in lower nephron nephrosis may not be a simple one. Whitby and Britton (1946) on the question of cast formation in crush syndrome etc. prefer the theory that the cause of the precipitation of pigment casts is primarily vascular and due to the failure of renal circulation. Yuile et alia (1945) after animal experiments, concluded that the precipitation of haemoglobin in the renal tubules is not primarily dependent on the reaction of the filtrate. Dunn et alia (1941) add the point that there is a possibility that the proximity of the venous blood - which we now know to be greatly increased by the engorgement of the vasa recta - may have an influence on promoting a greater swing towards an acid reaction in the particular area of the nephron. Foy, Altmann, Barnes and Kondi (1943) are definite that the evidence is insufficient to justify any claim that alkalinisation is effective either in preventing or relieving oliguria.

The question of the method of formation of casts in the tubules is not yet definitely settled therefore; undoubtedly they are formed from the free

blood pigment and to a lesser extent from the epithelium of the tubules. All that may be said at present is:

a. A swing in the reaction of the tubule contents to the acid side undoubtedly facilitates the conversion of pigment from oxhaemoglobin (or from oxymyoglobin) to methaemoglobin (or to metmyoglobin).

b. This second pigment, methaemoglobin, is less soluble than oxyhaemoglobin and is therefore more likely to produce casts.

c. There does appear to be evidence that the reaction of the tubule contents becomes acid at the level where the casts are most numerous, namely at the loops of Henle and the second convoluted tubules.

d. BUT, the site mentioned above is not only the site of change in the pH of the filtrate; it is also the site at which there occurs a great concentrating of the tubule contents due to absorption of fluid; this factor has especially come into prominence as the result of the work of Trueta and others (1947) mentioned above; these authors showed the engorged vasa recta to be lying in close proximity to the thin parts of

the loops of Henle, and have shown that in both structures the epithelium is admirably designed for the easy diffusion of fluids.

We can go no further than to say that cast formation in the tubules is due primarily to a simple deposition of material from a solution over-saturated with pigment; change in the reaction of the urine, with the resultant change in the type of blood pigment present, probably also plays some part. Further criticism of the theory of Baker and Dodds is given in the paragraph 4a below.

2. Nephron damage; the site and the character of this damage has been dealt with in pages 291 et seq. above. Essentially the lesions are limited to the loops of Henle and the second convoluted tubules; in these areas there is focal degeneration or necrosis with varying degrees of cellular reaction in the stroma around the more severely damaged portions of the nephron; the degree of focal degeneration may be severe enough to show stripping of the whole of the epithelium of the tubule.

Casts are present in large numbers where the damage is commonest:

the problems are what are the factors producing this renal damage, and what are the factors

determining the marked localisation of the damage. It will be remembered that at the area where the damage is common several things happen, namely:

i. change in the reaction of the filtrate to acid.

ii. concentration of filtrate contents due to much absorption of water from the tubules;

iii. precipitation of casts, probably largely as a result of concentration of filtrate;

Now this pathological picture of "lower nephron nephrosis" is common to many conditions markedly differing in the character of their aetiology, e.g.

intra-vascular haemolyses (including blackwater), where the kidney is excreting blood pigments, and possibly elements of the stroma of the cells;

crush injuries, where the kidney is excreting muscle pigment and possibly other breakdown products of muscle damage.

poisons, such as carbon tetrachloride which can cause this syndrome.

All of the above types of causes require consideration, as does another problem now causing much interest, namely the question of the supply of oxygen to the more distal portions of the nephrons.

What are the likely causes of this remarkably focal damage? Several causes have been suggested; among such are:

Casts: the question of casts has already been discussed and it has been decided that their formation is probably a secondary factor; they are unlikely to play any important part in the production of the tubule damage; like such lesions, the casts are an effect rather than a cause of the disease process. This is not necessarily the view of all workers in this field, e.g. Yorke (1922) thought that the degeneration in the tubules is a secondary matter and probably due at least in part to mechanical causes (in which case the presence of casts might conceivably play a part); this last view has been supported recently by Bywaters (1948) who now considers that the lesions in the second convoluted tubules are due to the precipitation in them of pigment, which produces failure of absorption of filtrate in the distal tubule with a rapid rise in pressure and resultant distension

of the more proximal parts of the nephron, and, finally, rupture; this suggestion of Bywaters is very similar to one made by Plehn (1920) which is discussed on page 374 below; this theory of Bywaters is so at variance with the microscopic findings that it cannot be accepted without much fuller proof, and in the absence of fuller proof it may again be said that it is unlikely that the casts are responsible for the tubule damage; it does not necessarily follow from this, however, the pigment itself is non-toxic. This aspect of the problem is dealt with immediately below.

concentration of filtrate contents; much of the absorption of water from the filtrate occurs at the level or immediately proximal to the level at which the damage is most concentrated; in view of this some workers consider that this water depletion alone may play a big part in the production of the tubule damage, e.g. Bakeman et alia (1952) consider the volume of the urine to be more important in this connection than the reaction of the urine;

Nicol (1940) states that in alkalosis dehydration is the most important factor influencing renal damage; Rigdon and Cardwell (1942) stated that they were able to produce necrosis of tubules with small doses of intra-venous sucrose only in dehydrated animals. It is conceivable that dehydration does play a part in the production of damage in that it allows to take place a concentrating of any toxic substances that may be present; another way in which it could play a part is by the production of a partial anoxaemia. Both of the above suggestions will now be considered;

concentration producing increase of toxin content;

a good example of how this is possible in other conditions is that already mentioned where Dunn and Polson (1926) produced uric acid nephritis in the parts of the tubules usually affected in lower nephron nephrosis; the authors considered that the damage in this experimental condition had been the result of the precipitation of the

substance as a destructive solid from its relatively harmless soluble form; the cause of the precipitation was thought by them to be the result of pH change with concentration of the filtrate. Dunn et alia (1941) also stressed that the localisation of the most intense damage to the nephron in crush syndrome is at points where the lower nephron segments lie in close proximity to veins, suggesting, as they said, a definite causal nexus. If we accept the work of Trueta et alia (1947) this is obviously the site at which the greatest absorption of water takes place; AND it is therefore precisely the site at which we may expect concentration of toxins in any conditions in which such are present and are being excreted from the body via the kidneys. This theory of toxin concentration has a great deal to recommend it from the theoretical point of view. Obviously however if toxin is present and circulating in the body in any of the conditions

producing this type of kidney lesion, then it must be present in very dilute concentration since there is little damage to be detected in the rest of the body at autopsy; therefore if toxin is responsible for the kidney damage then there must take place a concentrating of the toxin at or near the site of damage of the nephrons. This brings up the question of whether any poisons can be implicated in the conditions leading to the type of kidney damage we are discussing; let us consider these conditions in detail and see what, if any, poisons can be implicated;

firstly;

<u>intra-vascular haemolysis</u>; in this condition substances which are present and are capable of acting as toxins, at least theoretically, include the pigment derivatives themselves, and also products resulting from the breakdown of the cells, e.g. the stroma and derivatives of the stroma; probably also

there are others as yet not identified. There are very few experiments on record of investigations into the effects of injections of stroma-free homologous haemoglobin. It is well-known, of course, that large intra-vascular haemolyses can and do take place without development of a kidney lesion, e.g. Foy, Altmann, Barnes and Kondi (1943) state that within three-four days three quarters of the blood in a human may be lysed, liberating 600-700 grams of haemoglobin into the plasma, yet there may be no apparent ill-effects on the kidney; one other point, held by some workers to indicate that the haemoglobin is not the toxic factor responsible for the kidney changes, is the fact that precisely the same changes are to be found in the kidney in conditions in which there is no passage of blood pigment in the urine, i.e. the degenerative changes in the renal tubules are not

necessarily related to the passage in the urine of blood or muscle pigments. In contradistinction to this, however, there are on record some experiments in which the injection of homologous haemoglobin did produce the type of kidney lesion under consideration. On this problem Lucke (1946) considers that "pure" solutions of haemoglobin and of myoglobin are not nephrotoxic but that haematin may cause tubular damage; however he is merely expressing an opinion and produces no satisfactory evidence to support this viewpoint. Yorke and Nauss (1911) are among the few workers who have studied this problem experimentally, using injections of stroma-free homologous haemoglobin. Using experimental animals they were able to produce haemoglobinuria in rabbits. In those of their experimental animals which did not develop anuria they found brown plugs in some of the

convoluted tubules; in the animals which did develop suppression the authors found a picture not quite in keeping with that seen in the human in lower nephron nephrosis, e.g. in the experimental animals some of the glomeruli were enormously distended, as were also some of the tubules. This is the picture of a true "blocked kidney" which is not the picture seen in lower nephron nephrosis; we have already indicated the dangers of applying too literally the findings of animal experimentation to human disease, particularly in this type of condition. However, the findings of these authors do support the suggestion that injections of homologous haemoglobin are capable of producing kidney lesions. Yuile et alia (1945) also discuss the question of the genesis of the renal failure in the condition under discussion. They point out that all intra-vascular haemolyses

have at least several points in common, e.g. a circulating pigment; a factor of a "vascular or chemical" nature affecting the organism as a whole; and a specific kidney lesion with varying degrees of tubular degeneration and obstruction of tubules by pigmented casts in convoluted tubules, collecting tubules and the ascending loops of Henle; these authors also carried out experimental investigations into the problem, using rabbits into which they injected homologous haemoglobin; some of the experimental animals had acid urines and others had alkaline urines; others of the animals had had their renal tubules damaged either by temporary clamping or by poisoning with sodium tartrate; their findings were:

haemoglobin injections alone - no renal dysfunction.

haemoglobin injections after 15 minutes clamping renal pedicle -

haemoglobin casts in the urine, both acid and alkaline.

(haemoglobin injections after
(25 minutes clamping -

transitory rise in blood NPN with short periods of oliguria and anuria.

(haemoglobin injections after
(sodium tartrate -

degree of damage maximal after secreting acid urine.

The authors' conclusions were that precipitation of haemoglobin in the renal tubules is not primarily dependent on the reaction of the urine but is dependent on functional abnormality of the individual nephrons. From the scanty amount of evidence available therefore, it is not possible to say definitely that free circulating haemoglobin is capable of causing renal dysfunction unless some other factor is present; all that may be said at present is that it is possible that free circulating haemoglobin may act in two ways,

indirectly by stimulating production of a "vascular shunt", or directly in the nephron in combination with the vascular shunt to cause damage; this latter is purely hypothetical.

It would seem therefore that there is no proof that free haemoglobin acts locally as a toxin to the tubules, even when concentrated by the concentration of filtrate which occurs in the more distal parts of the nephron. This does not exclude the possibility that this substance may act indirectly by stimulating the production of a vascular shunt.

Also the above findings do not exclude the possibility of a toxic action on the part of the stroma, or a derivative of the stroma. It is held by many that the bouts of fever to be seen during each haemolytic phase are due to the liberation of stroma or stroma derivatives into the circulation but there has been little experimental work on this problem of the toxicity of

the stroma and we are not in a position to give a definite reply to the question. Much work still remains to be done on this problem.

Crush injuries; this condition may be a more fruitful source of information; in this condition also there is a pigment free in the plasma and later in the urine; in this case the pigment is myoglobin, very much akin to haemoglobin in its characteristics; one point is that it is extracted from the circulating blood much more rapidly than is haemoglobin because of its relatively small size, molecular weight 17,500. In addition to the circulating pigment there is theoretically present a circulating substance which is held to be derived from the autolysis of damaged muscle, e.g. Dunn, Gillespie and Niven (1941) in one of the early papers on the crush syndrome, say "it seems possible that a substance of special toxicity is produced in the presence of acid"; (this reference to acid is based on the belief that the

site of damage to the nephron is at that area where the reaction of the filtrate changes). Nothing is known yet of the nature or source of any toxic agent which may be responsible for the renal damage in cases of crush syndrome; the available facts, however, appear to support the view to which most observers have adhered, namely that after release of the affected tissue from a compressed and more or less ischaemic state toxin is absorbed from that tissue into the circulation as the vessels reopen, and that this toxic agent affects the kidney in the course of excretion; the authors go further and enter into the realms of speculation when they suggest that both uric acid and phosphoric acid may well come under review as possible toxic agents; they say that both of these molecules have a well-recognised origin in muscle tissue in the form of inosinic acid; they then make the further pertinent observation, based on experimental evidence, that renal

tubular damage can be most surely produced when the renal blood flow is normally abundant and the concentrating power of the organ is unimpaired, the reason being that there is a more intense concentration of toxic agent in that region than in other areas of the body.

No other author adds anything of value to the above summary of Dunn, Gillespie and Niven.

In this disease, also, therefore, we are not yet in a position to make a definite statement as to the method of production of the kidney lesion in crush injuries, although we are on surer ground than in the discussion on intravascular haemolysis, in that in crush lesions we can demonstrate a source of abnormal and toxic substances, the necrotic muscle. Dunn, Gillespie and Niven go as far as to suggest the identity of the substances involved; therefore the findings in crush injury are not inconsistent with the suggestion that it is a local concentrating of

toxin which leads to the lesions seen in the kidney in this condition. No definite proof is as yet forthcoming however.

Poisons, e.g. carbon tetrachloride and potassium chlorate; in this type of condition there is no free circulating pigment; presumably, however, the poisonous substance in some form or another is circulating in the body. Depending on the nature of the poison many of the tissues of the body show greater or lesser degrees of toxic change; for example in carbon tetrachloride poisoning practically all the important organs show toxic changes; this is in marked contradistinction to the findings with intra-vascular haemolysis, where the only tissue affected may be the nephron.

In this class of condition a toxic substance is necessarily present and there can be no objection to the suggestion that it is the excreting of this toxic substance which produces kidney damage.

Anoxia; this is the next of the factors suggested as playing a part in the production of nephron damage; Tomb (1941) claimed that the essential factor in the renal failure associated with crush injuries is lack of oxygen supply to the renal tubules. Rigdon (1942) claimed that the same is true in malaria in which there is a state of anoxia in the tissues. He suggested as possible causes either: losses of red cells or interference with their oxygen-carrying capacity; or alterations in the capillary blood flow through stasis or mechanical obstruction or general vascular constriction or collapse such as is seen in shock. Maegraith and Findlay (1944) are in favour of the above hypothesis, which is carried further by Maegraith and Havard (1946). Recently, however, this whole theory has been strongly attacked by Bywaters (1948) who first takes exception to the term "renal anoxia", suggesting that if the theory is correct the proper description of the process at work is "renal ischaemia";

he then goes on to say that in conditions in which renal ischaemia is definitely present it produces a patchy degeneration of the proximal convoluted tubules. He also attacks "reflex spasm" as a causal factor. However, the work of Knisely et alia (1947) on anoxia due to "sludged blood" may require us to consider this possibility of anoxia more fully than has been done. At present it is not possible to do anything other than to record the opinions of various authors as has been done above; there is not sufficient evidence on which we may come to a decision on whether or not this condition of anoxia does exist and does play a part in the production of the type of kidney lesion under consideration.

Anaphylaxis; Plehn (1920), talking of blackwater fever, claimed that the renal lesion is but one manifestation of a general anaphylactic reaction. There is no proof of this, and there are two serious objections to it; firstly such a suggestion places the kidney lesion of blackwater fever in a class

separate from all the others as regards aetiology, and secondly it is difficult to imagine an anaphylactic reaction in the human in which the damage is limited to the kidney. We cannot accept this theory of Plehn's.

In dismissing this theory of anaphylaxis as a cause of the kidney lesions of blackwater fever we most definitely do not wish to give the impression that anaphylaxis does not play a part in the lysis of red cells found in this disease; this possibility will be discussed in Chapter IX and is only mentioned here.

In this paragraph No. 2, several suggestions have been considered as likely causes of the tubular lesions found in the kidney in lower nephron nephrosis; such suggestions have included:

pigment precipitation;

concentration of circulating toxins; e.g. in intravascular haemolyses the suggested toxins included haemoglobin derivatives and/or stroma derivatives;

in crush syndrome the suggested toxin was from the

damaged tissue;

in poisoning the poison is self-evident.

If this theory be correct, and it is well worth serious consideration, then it is conceivable that the damage results from a concentrating of the toxin at the site of damage due to escape of fluid in that area, with resultant concentration of the filtrate contents.

renal anoxia; several suggestions but no careful experimental proof of this, except possible indirect proof of "sludging" of blood.

anaphylaxis; very unlikely.

Of all the above suggestions the one that most closely fits the facts is that of concentration of circulating toxin; this would explain the localisation of the lesions most satisfactorily; one indirect support is that in certain at least of the conditions producing this type of kidney lesion a vascular "shunt" is in operation, e.g. Woods (1946) reports great and uniform enlargement of the vasa recta; if this be the case it would seem that the "shunt" is originally brought into operation by the body to protect the kidney cortex from the circulating toxin, but unfortunately in doing so it increases absorption of fluid from the tubules with resultant concentration of the toxin in that area.

3. Tubulo-venous anastomoses; this finding has been reported in detail by several authors in the last few years, e.g. Dunn et alia (1941), McLetchie (1943), and Woods (1946); the impression given is that it had not been observed previously; this is certainly not so. There are several doubtful early references, e.g. Berthier (1896) who talks of multiple haemorrhages into the uriniferous tubules, but the first definite clear-cut report is that of Plehn (1920) who in discussing the renal lesions in blackwater fever, reported among other findings that he had found that the portions of the tubules above the plugs became enormously distended with fluid, a fact not accepted by any of the more modern workers except Bywaters (1948), whose opinion is given on page 355. Plehn claimed that this pressure so damaged the tubules that serum proteins and red cells could seep through into the urine. By this method he explained the proteinuria of blackwater fever; Plehn continued by saying that if the plugging cylinders are followed backwards towards the glomerulus a point is reached where the renal tubule <u>appears to communicate directly with a capillary</u> at a point where the epithelium has completely disappeared; the author added that it is easy to overlook such communications. Plehn was so sure of his findings that he based a theory of haemolysis in blackwater fever on such anastomoses, claiming that a direct admixture takes place between the contents of the blood vessels and the contents of

the renal vessels, and that owing to hypotonicity, because of low sodium chloride content, the renal filtrate has a haemolytic action on the blood which enters the tubule. Yorke (1922) agreed only to the extent of admitting that the serum proteins and red cells found in the urine in severe cases of blackwater gain access to the tubules through the anastomoses. This was an ingenious attempt on Plehn's part to link the microscopic and clinical findings; his theory rather reminds one of Procrustes' bed however. The theories of Plehn next received support from Rapoport (1928), who on a series of one case in Moscow confirmed Plehn's findings of anastomoses; in Rapoport's case many such tubulo-venous anastomoses were present; this author also supports Plehn's theory that the haemolysis of blackwater fever takes place in the tubules. It was not until 1941 that this tubulo-venous anastomosis again appeared in literature, in a report by Dunn, Gillespie and Niven. Dunn et alia inferred that the break-through was from tubule to vein; such is also the view of Bywaters and Dible (1942); McLetchie (1943) reports the same microscopic appearances in a male, aet. 33, with frequent vomiting due to pyloric stenosis; McLetchie suggested that the anastomoses are determined on a functional basis and depend on blood/filtrate readjustment which is particularly active at the areas where the break-through occurs. Woods (1946) reported three cases of carbon tetrachloride poisoning

in whom the kidney lesions were very localised and much like those found in transfusion kidney. He particularly stressed the great and uniform enlargement of the vasa recta; he did not agree with the suggested formation of the anastomoses as given above; in his opinion the lesions begin as thrombi in dilated veins and that in some parts these thrombi have broken through into the tubules. In the three cases of Woods one showed many anastomoses, one showed only one, and one patient showed none at all. Obviously, therefore the condition is not necessarily of severe degree in all kidney lesions of the type we are considering; in fact in many cases it may not be possible to identify any anastomosis. In view of this the anastomosis cannot play an important part in any lesion which is found; Woods does not agree with this; he considers that these lesions are to be regarded as of fundamental importance in the development of the renal picture as a whole. Woods accepts the theory of vascular "shunting", and claims that this defence device of a switch of the blood through the vasa recta leads to a sudden lowering of the relative pressures in the veins and tubules in the medulla, and rupture then occurs where the walls of these two structures are in close apposition. As a result blood enters the tubules; Woods claims that the casts in the tubules come from this blood, his theory being much the same as Plehn's; he holds that this would explain the position of the casts and would explain the immunity of the

first convoluted tubule. Professor Dorothy Russell assisted Woods in his interpretations of the kidney picture, therefore his theories must be recorded although they are at variance with those of the great majority of workers. Bywaters (1948) takes the opposite view to Woods; the former contends that the initial lesion is that of precipitation of pigment in the distal convoluted tubules; he claims that this produces failure of absorption of filtrate, with a rapid rise in intra-renal pressure, and distension of proximal parts; this distension finally results in rupture of the tubule (not necessarily into a vein) and a pouring-out of the glomerular filtrate into the interstitial tissue, lymph pathways, and veins. It is very difficult to accept Bywaters' opinions because in all reports of microscopic findings there is no mention of any indication of increased pressure in the more proximal parts of the nephron

Such is the position as it stands today; there is no doubt that in this type of renal lesion anastomoses are to be found. The percentage of kidneys so affected, and the number of anastomoses in kidneys so affected has not yet been worked out. Furthermore the mode of formation of these anastomoses is not clear, one group of workers claiming that the anastomosis is a break-through from vein to tubule because of increased intra-venous pressure, and another group claiming that the break-through is in the opposite direction as the

result of increased intra-tubular pressure.

Whatever the method of formation, it is quite unlikely that these anastomoses play an all-important part in the production of the specific lower nephron nephrosis as is the contention of some workers.

A final decision on the points above enumerated must be left until more exact information is available.

4. Oliguria-anuria, with nitrogen retention; one point of value, even though it is of negative value, which has already resulted from this detailed discussion is that we have been reluctantly compelled to accept the conclusion that the body of available evidence does not support the view that the formation of casts can be prevented by alkalinisation; had we been able to accept that view, which is still accepted by some workers, we should have been in a much stronger position to agree upon a line of treatment. This question of treatment brings us to the most-dreaded of the signs of kidney involvement, namely anuria. This problem of apparent cessation of kidney function in intra-vascular haemolysis is one which has long exercised the minds of all individuals responsible for the treatment of patients with this dread complication. Ellis (1947) rightly describes it as "one of the most dramatic and disconcerting of medical emergencies".

In the discussion of this problem we are again forced

to draw from the literature on blackwater fever for much of our information on the earlier approach to the problem. Unfortunately, tropical medicine has been so divorced from general medicine that this earlier work is not well-known, with the result that workers in other fields where the problem has become pressing are now tending in some cases to repeat the mistakes of the earlier workers on blackwater fever.

There is no lack of theories to explain the renal shutdown with resultant anuria; among the causes given for this shutdown are: mechanical blockage by casts;

low blood-pressure, with a failure to secrete urine;

damage to renal tubules; and

"shunting of the renal circulation".

This problem of the cause of anuria is of such paramount importance that it is necessary for us to consider the above possible explanations in detail; the simplest way to do so would be to consider them seriatim; consider

4a. Mechanical blockage by casts; to earlier workers the presence of casts in the urine and in the tubules seemed to provide a reasonable and simple explanation for the renal shutdown, e.g. Ponfick (1883) talks of the "blocking of the numerous renal tubules with semi-solid masses; the tubes are filled with wedged-in clots threatening a sudden

occasionally inevitable standstill of the whole excretory process". This view was generally accepted, e.g. de Haan (1905), and Werner (1907) agree. Since that time support for the theory of mechanical blockage has continued, although latterly criticism of this theory has grown. This theory of cast formation causing blockage in whole or in part received great support from Baker and Dodds (1925); their paper has already been fully discussed above, pages 348 et seq. Briefly, working with three rabbits these authors obtained results from experiments which led them to conclude that cast formation in the urine occurs only in acid urines with a sodium chloride content of not less than one per cent; they further concluded, from the findings in two fatal cases of transfusion kidney, that it is the formation of casts in the tubules which leads to renal failure and anuria. It was the uncritical acceptance of their theories which led to the adoption of intense alkalinisation as the standard method of treatment for this renal lesion, although in point of fact treatment by alkalies was not new. Wright (1917) had suggested bicarbonate and glucose because he thought acidosis to be an important factor in this condition. As

has already been said, criticism of this theory and of the treatment has steadily grown with the years, beginning with the criticisms offered by Ross (1932). For example, taking one point alone, Ross pointed out that in his large series of cases in Southern Rhodesia the chloride content of the urine was much below one per cent in almost all patients, partly due to restricted diet and partly due to pyrexia. Since then evidence of various types has accumulated which strongly suggested that Baker and Dodds were wrong in their assumptions. For example Bridges and Mattice (1940) point out that indiscriminate alkali therapy can produce alkalosis in a patient whilst the urine is still acid, therefore the pH of the urine is not necessarily an indication of the state of affairs inside the body. Similarly Ziegler and Brice (1937) suggested that phylogenetically the human kidney is adapted for excretion of an acid urine and that the kidney can more easily excrete solids in an acid than in an alkaline urine. Foy, Altmann, Barnes and Kondi (1943) are also highly critical of this theory of mechanical blockage by casts.

The most complete criticism of the theory so far

produced has been a paper by Maegraith (1944) who discusses the suggestion that renal failure is produced by mechanical blockage of the tubules, largely as a result of acid urine with a high salt content. Maegraith begins his criticism by pointing out that since the theory was introduced and treatment based on it was begun the blackwater fever mortality has increased from twenty per cent to twenty-five per cent; he considers that whether alkaline therapy is successful or not in preventing renal failure - and he thinks the evidence for that unsatisfactory - a decrease rather than an increase in the total mortality rate might have been expected. He then brings forward eight points for consideration; these are:

1. In many cases on record the urine is as frequently alkaline as it is acid before the onset of anuria.
2. The sodium chloride of the urine in the usual case of blackwater fever is about 0.5 per cent, not sufficiently high to help precipitate the blood pigment in the tubules.
3. Haemoglobinuria may occur without anuria, even with acid urines.
4. In some cases of blackwater fever oliguria, kidney failure develops after haemoglobinuria

has ceased.

5. Oliguria and anuria occur in conditions and in circumstances which exactly parallel the renal failure of blackwater fever but in which there is no passage of pigment in the urine.
6. The degree of blockage found in the kidney tubules at post-mortem examination is insufficient to account for the anuria.
7. If blockage were the primary factor it is to be expected that whatever urine is passed, coming as it does from unobstructed nephrons, would be normal in its constitution; in point of fact, however, it more resembles glomerular filtrate. Dunn, Gillespie and Niven (1941) had previously remarked upon the significance of this failure of the kidney to concentrate urine.
8. Although the urine passed in the immediate post-anuric and recovery phases occasionally contains massive deposits, it often is perfectly clear of casts and other debris.

Maegraith suggests that a better title for the kidney lesion would be "tubulo-vascular-renal syndrome".

I feel that in all criticisms of the theory of mechanical blockage not enough attention has been paid

to the histo-pathological findings in fatal cases. There is a surprising lack of unanimity in one important respect, namely the question of whether or not there is dilatation present in the affected tubules. Earlier reports occasionally speak of dilatation of the tubules; certainly Yorke and Nauss found it to be present in their experimental animals, and Plehn (1920) used the theory of increased intra-tubular pressure to account for the formation of tubulo-venous anastomoses. Bywaters agrees with Plehn. If such a finding of marked tubular dilatation is always present in fatal cases, such as is seen say in sulphonamide anuria due to a blocking of the pelves of the kidneys by deposition of crystals of the drug, then this would provide some degree of support for the theory of anuria due to mechanical blockage of the tubules, be the cause what it may. But the fact is that this description of enormously dilated tubules does <u>not</u> tally with the descriptions given by the great majority of workers, including workers of the standing of Shaw Dunn and Dorothy Russell; and it certainly has not been my experience. It would seem, therefore, that there is no microscopic appearance consistently to be found which would suggest that there is greatly increased intra-tubular pressure.

It is suggested, therefore, that there is no prima

facie evidence to support the mechanical blockage theory of Baker and Dodds; we have already refused to support their theories on cast formation. In discounting this theory of mechanical blockage I do not intend to suggest that the tubules plugged by casts are capable of functioning; it is feasible that they are functioning only very inefficiently or not at all. All that is suggested here is that cast formation by itself cannot explain the remarkable, sometimes complete, shutdown of the kidney which is to be found even with relatively few casts in the tubules.

4.b. Low blood pressure; this alternative explanation of the stoppage of urine production in "transfusion" kidney and similar conditions was probably first brought into prominence by Yorke and Nauss (1911); in their experiments with rabbits they concluded that a decreased filtering force in the glomerulus, resulting from a low blood pressure, may play a part in the causing of anuria in patients with intra-vascular haemolysis. Plehn (1920), who adhered to the theory of blockage by passage of haemoglobin, also said that the haemoglobin blockage is considerably facilitated by any factor which tends to lower the blood pressure of the patient. Foy, Altmann, Barnes and Kondi (1943) also say that in some cases of blackwater fever the blood pressure

sinks to a level incompatible with the proper secretion of urine, which is generally taken to be about 70-75 mms. of mercury; Maegraith (1944) states that in the renal lesions of blackwater fever a condition resembling shock is seen, the blood pressure generally being low, with oliguria developing, and with the kidney unable to secrete a concentrated urine. It is in this paper that Maegraith suggests the name "tubulo-vascular-renal syndrome" for the condition, to convey the idea of a vascular element in the causation of the disease. This is stated more definitely by Maegraith and Findlay (1944) who suggested that peripheral vascular atonic collapse occurs, with subsequent re-distribution of intra-renal blood flow and depression of glomerular filtrate. Whitby and Britton (1946) have already been quoted as saying that there is a failure of renal secretion.

The blood-pressure readings in our patients at the onset of lysis, first day usually, are given below:

	Case 1	Case 2	Case 3	Case 4	Case 5	Case6
Blood pressure.	88/50	92/60	85/55	100/60	100/60	Not done.

It is seen from the above figures that at the time of development of oliguria some of our patients had blood-pressure values relatively near the normal of the Indian sepoy. Furthermore in Case No. 2 the slightly

lowered blood pressure persisted for four days after the onset of haemolysis, yet this patient showed marked polyuria from the beginning, and lastly, it is not an uncommon finding that in intra-vascular haemolysis the blood pressure rises pari passu with the development of nitrogen retention yet anuria often persists in such cases. All we can say is that low blood pressure necessarily affects urinary filtration and if it is very low then, as suggested by Foy et alia above, urinary filtration cannot occur; but values as low as that are not commonly to be found in intra-vascular haemolysis, and even when present are very temporary. Another point which must be kept in mind is that there are other conditions, e.g. severe wounding, in which the circulatory depression may be relatively long-continued and more severe than in intra-vascular haemolyses yet renal lesions do not result.

The evidence, therefore, is such that this explanation of low blood pressure cannot be accepted as satisfactory to account for the onset and continuance of oliguria-anuria in the type of kidney lesion we are now discussing.

4. c. Damage to kidney tubules; it is conceivable that damage to the kidney tubules could play a part in the

production of oliguria; there are two ways in which this could happen; one way is by a gross escape of the glomerular filtrate through tubulo-venous anastomoses, suggested by Bywaters (1948) among others; the other way would be that inferred by Dunn, Gillespie and Niven (1941) namely, an ablation of the function of the tubular walls allowing of unrestricted absorption filtrate; that failure of tubule function does occur is of course well shown by the "glomerular filtrate" character of what urine is produced during the period when the kidney is labouring under difficulties. Bywaters and Dible (1942) also talk of an aberration of tubular function allowing of excessive and unselective re-absorption of glomerular filtrate. And Plehn (1920) had previously talked of a profound change in the renal epithelium which he thought to be the primary lesion, an anaphylactic reaction.

It is very unlikely, for reasons given above, that passage of glomerular filtrate through the tubulo-venous anastomoses plays any important part in the production of oliguria; on the other hand excessive re-absorption from the filtrate at the area around the loops of Henle probably <u>does</u> play some part in the cutting-down of the volume of fluid which has passed through the glomeruli; in fact this re-absorption of fluid is one point which

we are most definitely prepared to concede, though the method of re-absorption is not necessarily either of those suggested above. Other factors, however, probably are more important in the production of oliguria, for example more probably the fault lies in the total quantity of blood which is allowed to reach the glomeruli; what happens to the filtrate is probably a less important point.

4. d. Vascular shunt; on pages 278 et seq. above, a summary is given of a recent addition to our knowledge of the kidney. For long it had been known that central nervous system stimuli could influence the production of urine; examples of this have already been given, and Plehn (1903b) went so far as to state that in his opinion the anuria found in intra-vascular haemolysis is of cerebral origin, induced by the toxic properties of metabolic products; Yorke and Nauss (1911) also considered that one possible cause of the anuria in blackwater fever is a nervous inhibition of glomerular secretion. Porak (1918) took the view of Plehn that anuria is of central origin, death being due to anaphylaxis. Barrenscheen and Glaessner (1923) talking of anuria following quinine administration, also held that the anuria can be explained as a reflex spastic condition. In 1944 Maegraith and Findlay came near

to the current teaching when they suggested that one factor in the oliguria of intra-vascular haemolysis is a re-distribution of the intra-renal blood flow with depression of glomerula filtration. This was a laudable effort, but without experimental proof it could not be accepted, It remained for Trueta and co-workers (1946) to supply that proof; the work of the last-named has already been fully discusson on pps. 278 et seq. From that work we see that as a result of appropriate nerve stimulation a vascular short-circuiting may occur in the kidneys, by which the blood may be diverted wholly or partly from the cortex to pass through medullary channels, the vasa recta. The authors then draw the important conclusion that nerve stimulation may be produced centrally or peripherally by a variety of noxious agents, and it is concluded that the picture seen in many loosely-related syndromes such as sulpha kidney, blood transfusion kidney, etc.conceivably is the result of this "shunting" mechanism, the device being a defence mechanism by which the cortex of the kidney is excluded from the circulating toxin.

Herein, I submit, lies sufficient justification for the detailed consideration which has been given to the problem in the preceding pages; herein, also, conceivably lies an explanation of the problem of

anuria in lower nephron nephrosis; and herein lies an approach to treatment, the most logical and the most promising approach yet produced for this grave problem.

If this theory be correct, then the defence device must be a mechanism called into operation against circulating toxin or other abnormal substances; circulating toxins have, of course, already been held as a very likely cause of the renal damage.

In other words the oliguria and anuria are primarily the result of a by-passing of the great majority of the glomeruli by the blood being diverted through the juxta-medullary glomeruli to the vasa recta. It is here suggested that a second factor in the production of oliguria possibly is an increase in the transudation of fluid from the loops of Henle to the greatly dilated and engorged vasa recta.

Oblique support for this theory, and a guide to possible lines of treatment, is given by that measure of success which has been obtained in nephrosis anuria by certain apparently unrelated forms of treatment which, in retrospect, all probably owed their success to their having affected favourably the process causing the vascular shunt; examples of such lines of treatment are:

Stannus (1914) suggested splitting of the kidney capsule from pole to pole; conceivably this may have relieved the vascular shunt by its effects on the nerve supply to the kidney.

Neuwirt (1922) reported a case of reflex anuria in a patient with renal colic; bilateral splanchnic block in this patient was followed within a few hours by diuresis.

Wallace (1922) suggested high rectal double-current irrigations, using hot saline; in one case it seemed that irrigations were directly responsible for the beginning of urine flow.

The method of hot applications was the one used in the treatment of my six patients; in each case the hot applications were applied to the kidney areas posteriorly and in one case we used warm retention enemas.

Ross (1932) suggested lavage of the pelves of the kidney, using ureteric catheters. I have personal experience of only one case, not reported, during 1942 in a station near Baghdad on whom this

was tried, apparently without much success.

Peters (1942) described one case where he deliberately employed splanchnic block in the treatment of anuria and he reports that although his patient had been anuric for ten days he passed 700 ccs. urine within five hours of the bilateral block. O'Sullivan and Spitzer (1946) reported two cases similar to that of Peters.

Burkitt (1943) reported a treatment for blackwater, which he had used in Africa apparently with much success, namely massive doses of luminal by injection; using this treatment Gregory had thirty survivals in thirty patients; this is a significant difference from the usual figure of about twenty per cent deaths. Burkitt was not referring specifically to the problem of anuria in recommending this treatment.

Lyons and Raines (1945) thought the successes reported in the treating of anuria by decapsulation are probably due in part at least to removal of the sympathetic

tonic fibres.

The results of the treatment of the Waziristan series of patients were not such as would provide material for discussion.

It is considered that the experimental evidence produced by Trueta et alia (1947) makes it likely that the explanation of the anuria in lower nephron nephrosis is primarily a vascular shunt which acts by bypassing the cortex; a secondary factor of some importance may be increased transudation of filtrate from the loops of Henle into the dilated vasa recta.

In view of the gravity of the process, with its twenty to twenty-five per cent death rate, at least in the blackwater type of this process, any promising form of therapy must be fully investigated; there is as yet, however, no report of any series of cases in which the patients have been treated by methods designed to relieve the "shunting" of blood in the kidney if such shunting be present. The results of such a clinical trial are eagerly awaited.

If the answer to the cause of anuria is that it is the result of the operation of vascular shunting, then the next question is why certain individuals escape and others are picked out to develop this grave complication of anuria; the answer may be that suggested by Ellis (1947) when he points out that sensitivity to a stimulus varies greatly from individual

to individual and suggests that excessive stimulation of one type or another, applied to persons unduly sensitive to that particular form of stimulus might result in excessive operation of the normal mechanism by which the blood flow is diverted from the cortex.

5. Changes in the urine; the quantitative changes have been discussed in paragraph 4 above;

qualitative changes include proteinuria, blood pigment in the urine, and changes in the specific gravity. The proteinuria, haemoglobinuria, and casts in the urine, have been considered already.

Two changes in the character of the urine have previously only been mentioned and require further consideration, although they are not of primary importance;

one change often remarked upon is the thick type of urine which is to be seen in cases of severe oliguria; Stephens talks of one of his patients having urine like jelly, and this has been my experience on several occasions, not in the patients discussed here. The other type of change is that in which the urine has the characteristics of "glomerular filtrate".

It has always been difficult to explain the first type of urine, thick like jelly. Even the suggestion that there is much outpouring of mucus in the ureters and bladder cannot explain it, and one explanation is that

first propounded by Plehn (1920), and discussed above, that this is the result of a blood admixture as one result of tubulo-venous anastomoses. Yorke (1922) is less definite; he thinks that the serum proteins found in the urine, and causing the mucoid character of the urine, gain access to the tubules through lesions produced by dislodgement of the tubule epithelium. It is probable that some exudation of serum does take place into the tubules but it is unlikely that there is any important degree of frank bleeding through the anastomoses. The serum exudation probably is the answer, at least in part.

The other type of urine commonly to be found is that with the characteristics of glomerular filtrate; this type is found generally even after re-establishment of diuresis in those patients who survive. Dobbs (1947) states that urine for the first few days after establishing of diuresis is little more than a glomerular filtrate, indicating little concentration in the tubules. This strongly supports the suggestion that there is gross aberration. As Dunn et alia (1941) say "there is an extreme degree of ablation of one of the highest functions of the kidney". This damage is very extensive but fortunately it is remarkably reversible; repair, however, takes time especially since it is first necessary to get

rid of the cellular debris, casts and other solid materials in the urinary passages. Georgopolous (1933) agrees that there is ablation of function and suggests that the polyuria so common after establishment of diuresis is a forced diuresis due to the kidney having temporarily lost its power to concentrate the urine.

SUMMARY CHAPTER V.

This chapter began with a discussion on certain aspects of the pathological findings in transfusion kidney and other similar conditions.

Throughout the chapter there have been two principles consistently before us.

The first principle was to attempt to gain from the detailed discussion any information which might prove of value in the treatment of such conditions; it is submitted that we have at least indicated a method of approach to treatment which may yield valuable results.

Our second aim was to find whether there is any particular finding in this type of lesion under review which would effectively counter our suggested diagnosis of intra-vascular haemolysis for the Waziristan cases. It is submitted that the findings in our cases were fully in keeping with the findings which this chapter shows us to be expected in cases of intra-vascular haemolysis. The diagnosis remains therefore as "intra-vascular haemolysis".

In slightly more detail, this chapter deals with the following points.

The physiology of the kidney has been briefly summarised in

the light of the recent work on alternative circulations through the kidney. This finding of Trueta et alia (1947) is regarded as a most important piece of research. In the class of kidney lesion in which we are interested the detailed pathology has also been considered, using as subdivisions the questions of cast formation, of the nephron damage, of the tubulo-venous anastomoses, of the anuria, and of changes in the urine in this condition. A brief summary is given below of our findings:

1. Cast formation: it was concluded from the evidence that the casts are formed by deposition of pigment from a solution which has become supersaturated as a result of concentration of tubule contents at the level of the loops of Henle; change in the pH at the same site, with resultant changes in the predominant type of blood pigment present, probably also plays a part in the formation of the casts.

2. Nephron damage: several causes have been suggested to explain the remarkable localisation of the kidney damage, e.g.

   a. pigment precipitation in the more distal parts of the nephron;

b. concentration of circulating toxins, (e.g. haemoglobin or stroma in intravascular haemolysis, muscle breakdown products in the crush syndrome, etc), the concentration taking place just below the site of maximal absorption of fluid from the nephron into the neighbouring blood vessels and lymph channels.

c. renal anoxia.

d. anaphylaxis.

Of all the above, the theory of concentration of circulating toxins most adequatly appears to fit the picture, the localisation of the lesion being determined by the filtrate concentration, and therefore toxin concentration, which takes place at this point.

The particular toxic factor differs with the different conditions producing this type of kidney lesion.

3. Tubulo-venous anastomoses have repeatedly been reported as a finding in the kidneys of individuals dying of lower nephron

lesions. There are great variations present from patient to patient in the numbers of anastomoses; some show nil. The cause of the production of such aneurysims is not obvious. It is not even settled yet whether the break-through is from vein to tubule or from tubule to vein. Further the significance of such aneurysims is not clear. It would seem certain that they do not play an all-important part in the production of the kidney damage or in the production of casts, explanations for both of which have been given above. It is thought by some workers that these aneurysims are the cause of the thick jelly-like urine found in severe oliguria.

4. Oliguria-anuria; several theories as to causation,
   a. mechanical blockage by casts; not accepted to be the likely cause; there are many reasons why this is unlikely, and the histopathological picture is not in keeping with this theory.

b. low blood-pressure; also fails adequately to explain the facts, although it probably plays a minor part in the production of oliguria in a small number of individuals so affected.

c. damage to the tubules; two suggestions as to how this would act, either an escape of filtrate into the surrounding tissues, or excessive and uninhibited re-absorption of filtrate fluid. It is agreed that excessive re-absorption of filtrate fluid does probably take place, see 4.d. below, but not primarily because of renal tubule damage although this factor may assist.

It is considered that this is insufficient to explain the anuria.

d. <u>vascular shunt</u>; AN IMPORTANT PARAGRAPH IN WHICH IT IS

SUGGESTED THAT VASCULAR SHORT-CIRCUITING IS THE CAUSE OF THE OLIGURIA; THE STIMULUS PROBABLY IS A CIRCULATING TOXIN, A VIEW WHICH IS SUPPORTED BY THE CONCLUSIONS REACHED IN THE NOTES ON TUBULAR DAMAGE, SEE PARA. 2 ABOVE.

IT IS SUGGESTED THAT TREATMENT OF THE ANURIA SHOULD BE BASED ON ATTEMPTS TO RELIEVE VASCULAR CIRCULATORY CHANGES, E.G. BY SPINAL ANAESTHESIA.

e. urine changes; the only two urine changes which required further discussion at this point were, firstly a type of urine like jelly, and secondly a type of urine like glomerular filtrate. Both of those were thought to be merely indications of renal dysfunction; in the first the changes are probably due to seepage into the tubules

of serum from the lesions in the tubule walls; the second type of urine provides striking support for the theory of widespread toxic damage to the tubules; until this tubule damage has been made good, which takes place some days after diuresis, the kidney cannot concentrate urine, hence the polyuria, with the urine like glomerular filtrate.

CHAPTER VI.

Quinine as a possible cause of the Razmak cases of Haemoglobinuria.

This chapter properly should open with the closing findings of the previous chapter, namely that so far, all the evidence in the Razmak cases having been submitted and analysed, the diagnosis of the six cases now stands as "intra-vascular haemolysis, cause unknown".

It is essential that we now attempt to define the cause more exactly.

The first suspect cause is quinine. This drug was given to five of the six affected individuals; case No. 6 received no quinine. It is, therefore, not a constant factor, but in view of its reputation, probably greatly exaggerated, as a potent cause of haemoglobinuria, and in view of the possibility that case No. 6 had also been given quinine unknown to me - a very unlikely happening - it is necessary that we should devote one chapter to a consideration of whether or not quinine was the precipitating factor in the haemoglobinuria seen in Razmak. With the introduction of paludrine all that is written here may only be of historical interest but it is pertinent to our inquiry, our attempts to establish an exact diagnosis.

History of Quinine.

A chapter in the history of medicine so enthralling as this should not be allowed to pass unacknowledged, unnecessary though it may be to the main purpose of this paper; Scott (1939) rightly talks of the history of quinine as containing much of romance. In an introduction to the monograph of Duran-Reynals (1947) on this subject, Fulton correctly states that malaria has caused more casualties through the ages than has war; he might also have said that malaria has been a decisive factor in many military campaigns in the course of history. He estimates the figure of people infected in the World today to be about 300,000,000. This is probably an under-estimate. Only those who have worked in the Tropics, and especially those who have suffered from the disease, can appreciate how serious an obstacle is chronic malaria to the well-being and efficiency of a nation.

According to Chopra (1936) cinchona alkaloids occur in the barks of various species of two rubiaceous genera, Cinchona and Remijia, which are indigenous to the Eastern slopes of the Andes in South America between latitudes 10° North and 20° South; actually their distribution is wider than this; in a patchy manner they probably are to be found from the Atlantic to the Pacific.

Prior to the introduction of the cinchona alkaloids there was no specific treatment for malaria, which ravaged

unchecked great tracts of the earth's surface. Scott has said (1939) that the history of the treatment of malaria means the history of cinchona.

This state of complete helplessness against malaria continued until the seventeenth century. By that time the Spaniards had been in part possession of South America for close on one hundred years. There is no evidence definitely to prove whether or not they brought malaria as one of their gifts to the New World; the balance of evidence suggests that they did. Craig and Faust (1942) state that malaria was non-existent or of no great importance (unlikely) in America before the arrival of Columbus and before the arrival of the European colonists. The arrival of the latter, and especially the importation of negro slaves carrying virulent strains of the parasite from Africa, allowed the disease to become firmly established. This is the view of Scott (1939) who points out that prior to the Spanish Conquest the Gulf of Mexico was well-settled and prosperous, whereas in more recent times the area has been highly malarious. Peru was discovered in 1513 and within fifty years was firmly in the hands of the Spaniards; Scott points out that Peru was healthy until that time. Scott's opinion is that malaria was introduced from Europe or with the slaves from Africa. He feels that the only outbreak of disease even remotely likely to support the theory of pre-existing malaria is the condition recorded as "Modorra"

reported to have been a problem to the expedition of Pedrarius to Darien in 1514. There is one other report existing which suggests that malaria was present and a curse before the coming of the Spaniards; it is that report culled by Gray in 1737 from the notes of a Scottish surgeon, William Arrot, who had lived and worked in the Loxa area of Peru; in this report, as given by Stephens (1937) Arrot recorded that the people living at Loxa in his time there, were of the belief that cinchona bark had been in use before the Spanish Conquest, and that it had been used for the treatment of intermittent fevers which were frequent over all that wet and unhealthy country. However, Scott (1947) states again that he knows of no authentic record that malaria existed in America before the arrival of the Spaniards, although he does admit that it may have existed among the various fevers there. In answer to the query of how the natives so quickly discovered the efficacy of the quinine bark in the treatment of a new disease Scott suggests that the bark may previously have been in use as a more or less general febrifuge; this may be the explanation of what otherwise must have been a remarkable performance, namely that in a short space of time after the introduction of a new disease the natives were able to discover a naturally-occurring substance specific against the disease. There are on record various fanciful theories of how this discovery was made, for example Geoffrey (1736) tells of a

the powdered bark of which cures fevers. The next step in the story is highly romantic but unfortunately is probably apocryphal. It concerns the Countess de Chinchon, wife of one of the Viceroys of Peru. This noble, Don Luis, Count of Chinchon, was a Spanish Grandee who became Viceregent of Peru in 1629 (? 1631); the legend has it that the wife of this Regent was responsible for popularising the use of the drug in Europe after she had been cured of malaria by it while in Peru. This belief is perpetuated in the name "The Countess's Powder", and in the name "Cinchona" given to the tree. There is little historical fact to support this belief. Scott points out in the passing that the woman supposedly involved must have been the second wife of the Count, and not, as thought, his first wife, Ana de Osorio, who had died in Spain in December 1625; the Count later married Donna Francisca de Rivera in 1628 before he sailed for Peru. At this point Scott and Duran-Reynals differ in their record. Scott thinks that the second wife remained with her husband during the whole ten years of his tenure of office in Peru and that during that time she was cured of malaria by the bark which had been sent to her by the Corregidor of Loxa, Don Lopez de Canizares; on the return of the Viceregent to Spain in December 1639 the Countess brought home with a supply of the bark. According to Scott this was in early 1640, and the Countess died in 1641, said to be at Cartagena in Colombia; Scott thinks that this

is a mistake, the Countess actually dying at Cartagena in Spain. Duran-Reynals differs on several points; she agrees that the Viceroy held his post for ten years, but claims that he took up his post in 1631, not 1629, and the author thinks that there is nothing to support the theory that the Countess was cured of malaria or that she took any part in popularising the use of the drug. She further thinks that the Countess did die at Cartagena in Colombia of an epidemic disease when she and her husband were homeward-bound. However, Linnaeus, in his Systema Naturae of 1742, perpetuates the memory of the Countess. It is possible that the Count was aware of the properties of the bark because, after his death in Spain, large quantities of the bark were found in his castle; and apparently also there is a local legend to the effect that the people of the castle were in the habit of dispensing treatment for fevers. Duran-Reynals will have none of this; she claims that the link between the natives of Peru and the World at large was the Society of Jesus; certainly those missionaries are much more likely to have been in intimate contact with the natives than were the Spanish officials. The author thinks that a Jesuit missionary from Peru, attending a conference of the Society in Rome, brought the drug to the notice of John de Lugo,at that time one of the leaders of the Society; de Lugo obtained large quantities of the drug and attempted to popularise it in Rome and in Europe. From this the powder takes its

name of "Jesuits' Powder". de Lugo had a certain measure of success at first, but the hostility of the medical profession was such that before many years the drug fell into disfavour in Europe. Fortunately England was insulated to a large extent against the wild swings of opinion in Europe and attempts to popularise the drug in England still continued, largely through the efforts of business men. According to Baker (1785) around 1658 James Thompson (? Thomson) a merchant of Antwerp, was advertising the sale of Jesuits' Bark in London. The individual who did more than anyone, however, to keep the drug in use was that shrewd business man, Sir Robert Tabor (? Talbor); according to the Dictionary of National Biography, he was an assistant to an apothecary in Cambridge and he drew the attention of Professor Nott in Cambridge to a better method of preparing cinchona. Harvey (1683) refers to him as "a debauched apothecaries' apprentice"! So great was the feeling against the drug at that time that no physician could or would have dared to use it. Tabor had no such limitations; he realised the value of the drug and he also realised that he could not openly use it. Accordingly he prepared a "secret remedy", the active principle of which was a decoction of the bark; he proceeded to use this in the treatment of patients suffering from the ague. He went from success to success; he had to be protected by Charles II from the wrath of the College of

Physicians of London. His successful treatment of the Dauphin of France made him a Chevalier and a pensioner of France. He died in 1681 at the early age of forty-one; he described himself as "a singular physician" but actually he was a singular man of business. Stephens (1937) publishes some of the more pungent of Madame de Sevigne's remarks on the success of the Chevalier Talbot; according to her the only thing that Tabor could not do was "resusciter les morts".

Interest in the problem of the bark grew slowly, very slowly; the next step of importance was taken in 1735 when a group of French scientists proceeded to South America to investigate the problem. de la Condamine was one of the senior officers, with de Juissieu as one of the juniors. In 1738 de la Condamine wrote a sketchy appreciation of the genus, the first description of any note; it was on this that Linnaeus classified the tree. On his return de la Condamine attempted to bring back seeds which, however, were lost at sea. It is this paper by de la Condamine which lends support to the theory of the Countess and her malaria; he states that his information is based on a Spanish manuscript which had somehow strayed into the apothecaries' shop of the College of Jesuits in S. Paul de Lima; the author of that old manuscript had written it in 1699, and he claimed in it that part of the history is taken from individuals who were living in the areas

concerned at the time of the "discovery" of the drug by the Spaniards. de Juissieu, in the meanwhile, had been able to identify four species, each yielding barks differing in their content of effective alkaloids; de Juissieu carried on investigations for many years in the jungle, only to lose all his records by theft. He died insane after his return to France. The next in the field was a Spanish botanist, Mutis by name; he also worked under great difficulties for many years and was an old man before he received recognition. At the time of his death he had amassed great quantities of specimens which he left to his assistant to publish and classify. This assistant was shot as a traitor and the collection taken to Madrid. According to Duran-Reynals the collection still lies there unopened.

In the meanwhile much time had passed and the nineteenth century had begun; the opening-up of the Orient was being attempted, and malaria was proving to be the greatest stumbling block to this. By this time also Gomez, in 1816, had isolated cinchonine, and in 1820 Pelletier and Caventou had isolated quinine. The fact remained however that the only source of the alkaloids was the bark of the trees in Peru, the best species being Cinchona calisaya. This could only be obtained at a heavy price and with great difficulty from the depths of the jungles of South America. The obvious thing seemed to be to begin plantations in other

parts of the World, especially those parts governed by the great Colonial powers, the British and the French. Duran-Reynals claims that the first person to bring back seeds and to raise plants from them was Weddell, a French naturalist, sent out to South America in 1843. If this be true then nothing came of it; the next move was by the Dutch, who sent Hasskarl to the West; Hasskarl did succeed in getting certain samples out of the country and plantations sprang up in Java, then it was found that the samples he had brought back were useless, probably not even of the genus Cinchona. The British then sent out Markham; unlike his predecessors he was quite open and frank about his intentions, and surprisingly he did manage to get some specimens, largely due to the efforts of an Englishman resident there. The specimens so obtained were successfully transplanted to Travancore; the species obtained by Markham was not the best, being succirubra; The species by far the best in its yield of the more potent alkaloids was C.calisaya, which was limited practically to Bolivia and was carefully guarded by the natives. According to Scott the next person to take a hand in the game was Charles Ledger, a London Merchant, who was in South America on business. Ledger did obtain seeds and sent them to his brother who was unable to interest the British Government in them and was forced to sell half to a private buyer and half to the Dutch Government for a nominal sum. There is no need to go further into the

history of the transplantation of the trees.

For three centuries the cinchona alkaloids, particularly quinine, held their place as the only form of treatment for malaria, although they had many shortcomings. During all that time attention was focussed on efforts to find the best methods of administration of the drug; there is nothing now to be gained in examining the many types of courses recommended. We need only say that the last and most complete of such investigations were those carried out by Sinton over a period of years. In Sinton's opinion the best method of administering quinine was by intermittent dosage over a period of weeks, the patient being given alkalies at the same time. This standard course finally was abandoned in favour of treatment by synthetic drugs; this is discussed in Chapters VII and VIII. This change-over to the use of synthetic drugs took place in the Indian Army in 1934, and in the Army thereafter quinine had two secondary roles, firstly it was used to control symptoms, being more effective than the synthetic drugs for this purpose, and secondly quinine was given in a long combined course together with plasmoquine for the treatment of patients with chronic malaria. In the first method of usage the quinine was given as a short course before the beginning of the course of synthetic drugs.

From this interesting by-way of the history of quinine we must now return to the main body of the chapter.

The Pharmacology of Quinine.

In the above notes on the history of quinine it has been seen that it is one of a group of alkaloids which are present in the bark of two rubiacious genera, Cinchona and Remijia, originally indigenous to the Eastern slopes of the Andes around the level of the equator. Linnaeus was responsible for the name "cinchona"; the term "quinine" is taken from a native term meaning "bark of barks".

The discovery of the bark marked a great advance in tropical medicine giving as it did substances with a powerful action against a disease which had been responsible for more ill-health and deaths than almost all other tropical diseases combined. The later identification of the various active principles was the first step on that long road which has led first to plasmoquine, then to atebrin, and so to paludrine.

Field (1939) in his review of the drugs available at that time for the treatment of malaria, states that the only drugs of proved value in the treatment of malaria fall into two broad groups. The two groups were:

a. the alkaloids of cinchona bark,

b. synthetic compounds based on arseno-benzene, amino-quinoline, or amino-acridine.

Although we are discussing quinine a few notes on atebrin and plasmoquine will be given at this point for convenience' sake,

and for comparison.

The benzene ring forms a logical starting point. Its structure is well-known, with its closed system of six CH groups. Addition to the benzene ring of a second ring containing an atom of N provides a twin-ring system, the quinoline ring, which is the basis of both quinine and plasmoquine. Plate No. XVIII, page 430, shows the graphic formulae of quinine, plasmoquine and atebrin and illustrates the above remarks. Although it is not pertinent to the subject in hand, we may at this stage anticipate a little and mention that a three-ring compound of much importance is that formed by a pyridine ring flanked by two benzene rings as shown, the result being the acridine ring which is seen in Plate No. XVIII to form the basis of atebrin.

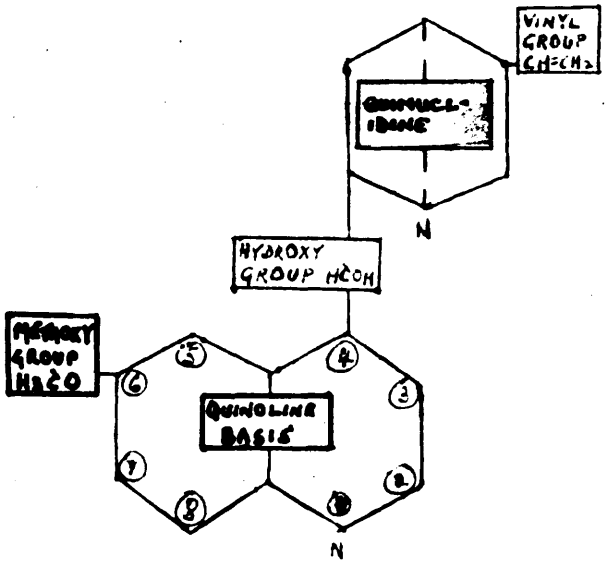
One or other of the quinoline, or acridine rings is the nucleus of practically every effective remedy know for malaria. Compounds derived from the benzene ring are of little importance; the quinoline ring is of much greater importance; as has been said, it is the basis for quinine and for plasmoquine. More exactly speaking, it is not quinoline itself but 6-methoxy quinoline which acts as the nucleus of quinine and of plasmoquine; this compound is formed by addition of a methoxy group (CH_3O) in the position seen in plate No. XVIII.

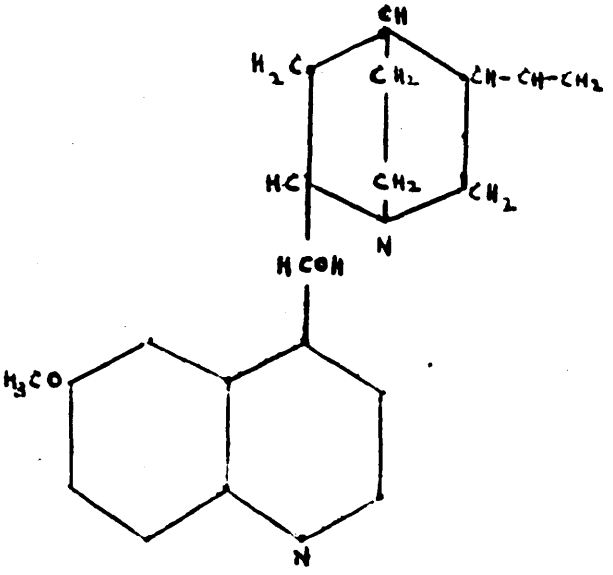
PLATE No XVIII

GRAPHIC FORMULAE OF QUININE, PLASMOQUINE AND ATEBRIN.

QUININE

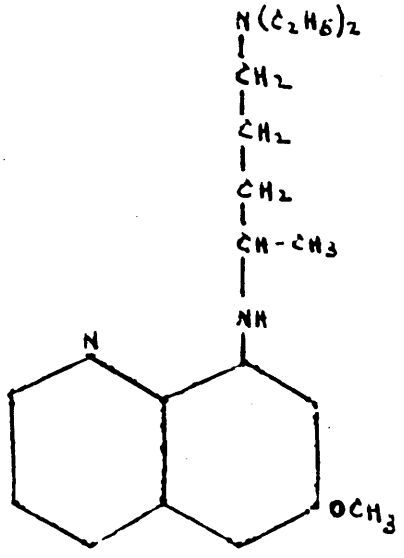
$C_{20}H_{24}O_2N_2$ (M.W. 324)





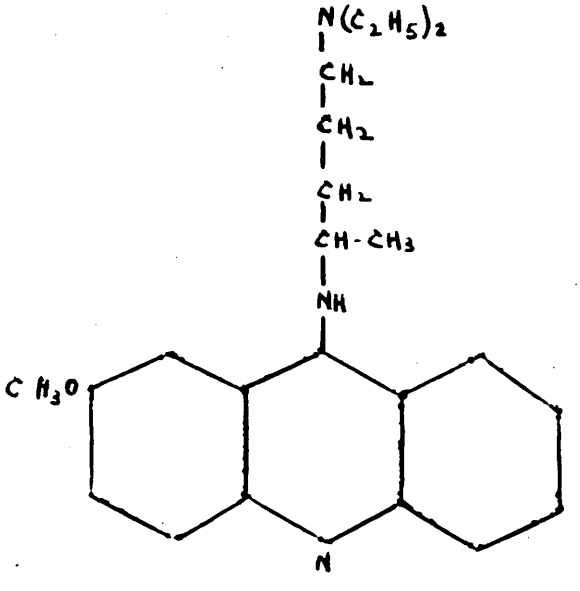
PLASMOQUINE

$C_{19}H_{29}ON_3$ (M.W. 315)



ATEBRINE (BASE)

$C_{23}H_{31}ON_3$ (M.W. 365)



At this point we may again anticipate a little, and point out some of the similarities and dissimilarities of the three drugs, factors which will be seen to be of practical importance; reference should be made to plate No. XVIII for information. It will be seen from the plate that quinine and plasmoquine have the same common nucleus, namely 6-methoxy quinoline. It is further seen that quinine is composed of this group and a quinuclidine ring (with a linked vinyl group) attached by a hydroxy group at position 4. Plate No. XVIII illustrates this clearly. Plasmoquine differs from quinine in that it has no quinuclidine ring; it has instead a long open-chain system which is attached to the quinoline base at position 8; the method of linkage also differs from that seen in quinine. One important point to be stressed here is that the supplementary open-chain system seen in plasmoquine is common to atebrin, in which it is attached opposite to the N atom.

The above points are not merely of academic interest. Structure subserves function, and does so in an amazingly specific fashion. One need only think of quinidine, the dextro-rotary stereo-isomer of quinine; this substance quinidine has the same empirical formula as quinine, with the same molecular weight, and yet in spite of this closest of relationships quinidine shows distinctive behaviour with polarised light and has pharmacological properties markedly

differing from those of quinine. Obviously, therefore, the points in common and the points of variance of structure of quinine, plasmoquine and atebrin require most careful consideration. Some of these will only be mentioned here; among these are:

The marked ability of quinine to induce hypersensitivity; this is not found with atebrin and plasmoquine;

The ability of quinine and plasmoquine apparently to reinforce the effects one of the other, and the apparent reduction in the toxic side-effects of both on their being given simultaneously.

Apparent enhancement of the toxic side-effects of atebrin and plasmoquine when they are given at the same time.

Quinine is generally administered as one of its acid salts, namely:

quinine hydrochloride	which contains	81.7% of base
quinine bihydrochloride	" "	81.6% " "
quinine sulphate	" "	73.5% " "

No matter which of the above substances is given by mouth, the salt is converted into soluble HCl form in the stomach; this soluble form is rapidly absorbed in the duodenum, then circulates in the blood as quinine base; according to Field (1939) the process is aided by bile. The maximum concentration of quinine to be expected in the blood even with large medicinal doses orally varies between 3-10 mgms. quinine per litre of

blood. A dose of not less than two grammes daily of quinine salt is necessary to get the above blood concentration. When the concentration of quinine rises above ten milligrams per litre of the blood then severe symptoms of cinchonism begin.

It is generally agreed that within a very short time, a few minutes at the most, ninety per cent of the quinine base disappears from the circulation. Field (1939) suggests that most of this quinine is taken up by vascular endothelium, to re-enter the circulation later; there is no experimental proof for this, and the suggestion is based apparently on the prolonged action of quinine. Chopra (1936) states that sixty per cent of the circulating quinine is taken up by the organs of the body, he infers the reticulo-endothelial system, where it is largely destroyed; Chopra thinks that the balance is to be found in the blood, some in the red cells and some in the plasma, and this portion which escapes destruction is eliminated unchanged in the urine. The process is rapid, the drug appearing in the urine twenty to sixty minutes after the taking of the drug by mouth. Ramsden et alia (1918) claim to have demonstrated the drug in the urine as early as eight to eighteen minutes after quinine had been taken on an empty stomach. They further state that after a single dose quinine continued to be excreted up to three days, and after multiple doses excretion continued for seven and a half days. By comparison with the findings of other workers the above are outside limits, for example Chopra

(1936) who speaks with authority on this subject, considers that after a single oral dose of quinine, excretion of the drug by the kidneys reaches its maximum in three to five hours, maintains this level for four hours and he considers that all the drug is excreted within sixteen to forty-eight hours. And Chopra considers that if the daily dose is not more than two grams elimination keeps pace with administration.

Let us consider here the details of the quinine courses given to those of our patients who did have treatment by this drug before the development of haemoglobinuria. This information is given in Figure No. 18 below.

Figure No. 18.

Relationship in time and dosage of quinine administration with time of onset lysis.

Case No.	Total dosage quinine given. (grains)	No. of days drug adminis.	No. of doses.	Minimum time interval in days between cessation quinine administration and beginning of lysis.
1	30 grains	One day	3 doses	at least nine days.
2	ditto	ditto	ditto	at least eleven days.
3	ditto	ditto	ditto	at least ten days
4	ditto	ditto	ditto	at least eight days
5	120 grains	4 days	12 doses	at least nine days.

Case No. 6 did not receive any quinine.

From the above table it is seen that in one case no quinine was given, in four cases quinine was given in minimal doses, only for one day, and that in all cases in which the drug was administered the time interval between the cessation of administration of drug and the beginning of lysis was outside the limits suggested as the time interval necessary for the body to clear itself of quinine even if we accept the findings of Ramsden and co-workers (1918) whose findings are much longer than those suggested by other workers. This point will be referred to later. This is the first reason why we are reluctant to regard quinine as the cause of the haemoglobinuria in the Razmak patients, no matter how grim the reputation of quinine in this respect.

A consideration of the action of quinine in the body and on the parasites of malaria may yield further information on whether or not quinine could possibly have been implicated in the production of the haemoglobinuria in our patients. It is necessary to begin this paragraph by stating that although the cinchona alkaloids have been employed the World over for three hundred years, and quinine has been employed for one hundred years, their mode of action is still unknown. There are several theories but none has a sound basis of experimental proof. As already mentioned, with a daily oral intake of thirty grains of quinine salt, the blood concentration does not

rise significantly above ten milligrams quinine base per litre of blood; actually the level is probably below this maximum level. Various authors suggest that with the dosage mentioned a nearer figure of blood concentration is a maximum of about seven milligrams per litre; Field (1939) puts the figure even lower, about 1/250,000. Speaking on the general action of quinine, Chopra (1936) claimed that it is a general protoplasmic poison, capable of destroying all cells when present in sufficient concentration; he thought that quinine, like saponins, tends to concentrate on the surface of cells to such an extent as to form almost a film; this film interferes with interface processes with the result that catalytic phenomena are hindered, with a resultant diminished metabolism; he stated that this surface action is shown by the arrest of Brownian movement, and a tendency to precipitate colloidal solutions such as proteins. According to Chopra it is this fact that makes quinine capable of producing anaphylactoid phenomena. He goes on to state that in vertebrates the toxicity of quinine is comparatively low. But he considers that the action of the drug on an organism like the amoeba is quite characteristic, causing retardation of metabolic processes, for example solutions as low as 1/50,000 may destroy amoebae and paramoecia in a few hours, and the author especially noted that when quinine and plasmoquine are mixed together their toxic action

on paramoecia is intensified. In the practical sphere he claimed that a similar effect is to be observed with certain pathogenic protozoa, including the plasmodia; in such the protoplasm in contact with quinine degenerated into granular detritus in a few hours; Chopra claimed to have found that solutions of quinine corresponding to thirty grains in the blood of a 150-pounds patient kill P.falciparum in five to twenty nine hours. It is possible that Chopra is basing his opinions on some of the findings of Bass (1922) who tried the effect of quinine upon the growth of malarial plasmodia in vitro; Bass took blood cultures of P.falciparum and added quinine hydrochloride in a concentration equivalent to that mentioned by Chopra, i.e. quinine in therapeutic proportions; with such concentrations of quinine Bass found that no growth had occurred after five hours incubation; some parasites had shrunk to small spheres and most stained badly; after twenty nine hours the plasmodia were evidently dead.

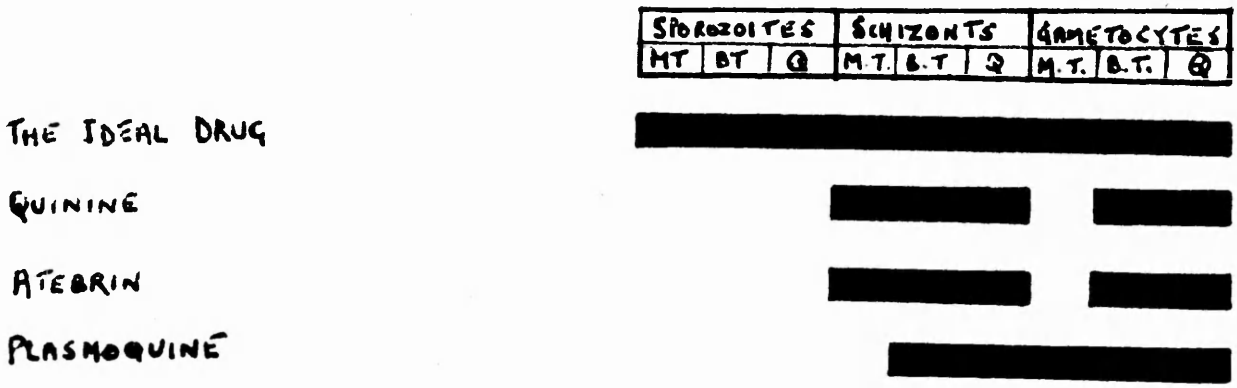
Other workers report widely-differing results. Kirschbaum (1923) mixed in vitro blood infected with P.vivax with an equal quantity of 1/5,000 quinine sulphate solution, thus reducing the concentration to half; he then incubated the mixture for five to twenty four hours at 37 -39°C. yet even with that concentration and treatment the blood was found still to be capable of infecting paralytics. Ciuca et alia (1937) dissected infected mosquitoes in a solution of quinine then

injected the sporozoites intra-venously into mental patients; from their experiments they concluded that under certain conditions malarial sporozoites are able to withstand the direct application of quinine (and of atebrin in 1/2,500 solution). Much of this type of work has been invalidated because of the failure of workers to realise that quinine has no apparent effect on certain phases of the parasite, and that it cannot be expected to produce sterilisation in vitro in less time than the duration of the schizogony cycle. The above are reports of in vitro experiments; what is the action of quinine on malaria parasites in vivo. Reference should be made to plate No. XIX, page 439; this plate is taken from the monograph by Field (1939), as is plate No. XX, which also is to be seen on page 439; plate No. XX shows the various types of prophylaxis possible in malaria. It will be seen from page 439 that the action of quinine is similar to that of atebrin in its effects on the malaria parasite; they are both effective schizonticides, and neither has any action on the crescents of falciparum malaria. In the passing it should be noted that, in contradistinction, plasmoquine has little or no effect on the asexual forms of P.falciparum but is active against the gametocytes of P.falciparum. It should be remembered that at the time Field drew up the diagrams seen on plate No. XIX, the existence of the exo-erythrocytic cycle had not been established.

Whatever the results of in vitro experiments, there

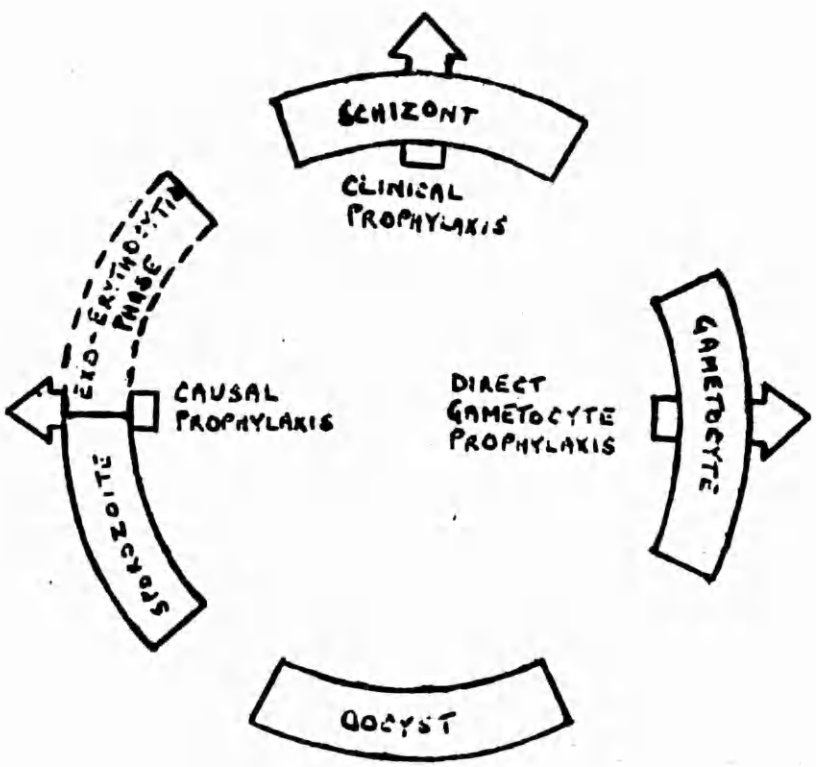
PLATE Nº XIX

ACTION OF CERTAIN DRUGS ON THE DIFFERENT STAGES OF THE MALARIA PARASITE.



KEY: M.T. = FALCIPARUM MALARIA.
B.T. = VIVAX MALARIA.
Q = QUARTAN MALARIA.
■ = EFFECTIVE IN NON-TOXIC DOSES.

PLATE Nº XX



from "CHEMOTHERAPY OF MALARIA" by FIELD (1939)

is no doubt that clinically quinine has a marked effect upon malaria parasites; this has been adequately proved for over three hundred years. And yet, as already said, its method of action is unknown. No one who has suffered from malaria which has been cut short by quinine would ever doubt its efficacy, at least in the control of the symptoms of the disease. In fact, as a means of control of the immediate symptoms and signs of a malaria attack, quinine in my opinion is superior to atebrin; this also is the opinion of the League of Nations Malaria Commission (see Fourth General Report of the Malaria Commission of the Health Organisation of the League of Nations 1938). One of the interesting records in support of the efficacy of quinine, certainly at least in the controlling of symptoms, is that record of the White Fathers of Africa, quoted by Thomson (1934); according to him, in the period 1878-1905 more than two hundred White Fathers died of blackwater fever in Central Africa, an appalling figure. In 1905 a system of daily prophylactic quinine was begun, the quinine administration beginning on the day of departure of the priests from Marseilles; the dosage given was five grains daily, doubled whenever anyone felt ill. Thomson stated that since the time of adoption of this scheme more than six hundred Fathers had served under severe malarious conditions, yet from 1905 to the time of writing in 1934 not one death had been reported from blackwater fever.

Quinine is effective, however it produces its results. It is not possible to record even a fraction of the number of theories on how quinine does act on the parasites. We can only quote the work of some of the modern authorities. The major problem is whether or not the quinine acts directly on the parasite, or whether indirectly, or both. Some of the opinions are:

Krishnan (1933), and later Krishnan and various co-workers in a series of papers, was of the belief that the mononuclear cell is the important factor in the control of malaria and that the action of quinine is indirect, stimulating the natural processes of immunity.

Chopra, Ganguly and Roy (1935) also consider that the action of quinine is not direct but is probably synergistic to other mechanisms set up in the body; and yet, however, Chopra (1936) states that quinine acts first on the infected red cell and haemolyses this, causing disappearance of the parasites from the peripheral blood; and, further, Chopra (1936) states that in malaria the action of quinine is to kill the parasite without injury to the host. He also quotes, without references unfortunately, the interesting fact that Binz, many years previously, observing the action of quinine on protozoa, had

prophesied from this that the organism causing malaria would be found to be one of that order. Obviously therefore Chopra would seem to support the suggestion that quinine acts both directly and indirectly.

Nocht and Mayer (1937) state that in their opinion the quinine treatment of malaria is not a <u>therapia magna sterilisans</u> and that no matter how large the dose of quinine, or how frequent the doses, relapses are inevitable. They did not think that there was enough proof to decide whether the action of quinine was direct or indirect. By "indirect" action they meant action by the production of changes in the tissues and/or mobilisation of antibodies; they think the action to be most probably a combination of direct and indirect effects, the direct most probably acting first. They say, and very rightly, that a direct action is suggested by the quick, often life-saving effect of intravenous quinine; no one can question this who has seen a man near to death being brought back practically to an apparent normal in less than an hour following the giving of adequate dosage of intravenous quinine. As regards the indirect action on the parasite, Nocht and Mayer think it

unlikely that such is due to a stimulation of antibodies by quinine; they consider that if this were the case why should quinine alone produce the influences which activate the protective mechanism in the case of malaria whereas in other infections the most diverse agents, e.g. non-specific proteins, can set the defence mechanism in action. Admittedly this last objection is not an insuperable one, nor is the statement by the authors that in ape malaria the action of quinine is not interfered with if the chief protective organs in the body are eliminated by extirpation of the spleen and by blocking the reticulo-endothelial system; such steps do not "eliminate the chief protective organs of the body".

Morgenroth (quoted by Field 1939) suggested that quinine concentrates as a film on the surface of the red cells and prevents the entry of the merozoites; this theory of a surface film of quinine has also been mentioned by Chopra (1936).

Bass (1922) suggests that the drug renders the red cell more permeable to the blood plasma with the result the parasites are exposed to its lytic action.

Yorke and Macfie (1924) consider that the action of quinine on the malaria parasite is a direct one, and that

the destroyed parasites then act as an antigen and cause the production of antibody which completes the cure.

Kingsbury (1926) thinks that quinine acts by haemolysing infected corpuscles; he claims that there is an initial rise in bilirubin values following upon the administration of quinine; Ross (1932) confirms the finding of increased bilirubin formation after giving quinine.

Chopra (1936) has already been quoted; he also thinks that quinine may act by haemolysing the infected red cells.

Nocht and Mayer (1937) have also been quoted already. Their opinion, in brief, is that malaria influences certain stages of the parasite, namely the free forms, and, in their opinion, the young parasites still attached to the surface of the red cells, i.e. the youngest forms, are the most susceptible and the gametocytes practically not affected.

These authors further say that if the drug be given over a long period in daily doses the therapeutic effect of quinine becomes progressively less marked; this is not accepted by many of the other workers.

Field (1939) discusses the problem at some length; he states

that quinine owes its dominant position in malarial therapy to its remarkable effects on the malarial parasite during the asexual cycle. He points out that the fever and other symptoms of overt malaria are associated with a rapid multiplication of parasites by schizogony, and that quinine arrests this multiplication in some way not understood, probably by destroying the parasites during segmentation when the host red cell is degenerated and pervious to the quinine in the blood stream, and also at the merozoite stage when even this protection is withdrawn. In other words, the theory of Field is much the same as that of Nocht and Mayer; the only time that the parasite is protected from the quinine is during the schizogony cycle in the red cell, and even this protection becomes less marked in the late stage of schizogony as the cell degenerates.

Field then points out a fact which has escaped the notice of so many observers, namely that blood infected with the malaria parasite may remain infected for some hours after exposure to strong solutions of quinine because there is some phase of the cycle which is quinine-resistant. Although effective destruction of merozoites may explain the therapeutic potency of quinine this alone can

hardly be expected to afford in vitro sterilisation in less time than the duration of the schizogony cycle. This failure of quinine to affect certain phases of the parasite is shown graphically by Field, see plate No, XIX, p. 439. Field also thinks that during periods of latency of malaria in the body of an infected individual, then even those phases of the parasite susceptible to quinine appear to lie deeply, sheltered from the effects of the drug; in other words Field agrees that not only does quinine fail to affect certain phases of the parasite; it may fail to eradicate from the body those phases which are susceptible to it. It is these factors which account for the high relapse rate following the treatment of malaria by quinine.

Field summarises the position as follows:

a. Quinine administration during the incubation period has little effect on the development of the parasite or on the time of appearance of the primary clinical attack. (When this was written Field had no definite knowledge of the presence of exo-erythrocytic forms of the parasite, forms which also are not affected by quinine. Field's diagram plate No. XX has been modified slightly by me to bring it into

line with recent discoveries.) This means, as said, that quinine has no effect on the exo-erythrocytic phase of the parasite.

b. Quinine given during clinical attacks causes the disappearance of asexual parasites from the peripheral blood.

c. Quinine given during periods of latency has no effect; certain residual parasites lurking in unknown sites appear to be unaffected by the quinine.

d. The persistent parasites, lurking beyond the action of the drug may again show schizogony towards the end of the period of latency, and multiply by schizogony to an extent sufficient to produce clinical recurrences of infection, i.e. "overt malaria".

This question of recurrence of the disease following treatment is, or rather was, a serious problem. One explanation offered by Nocht and Mayer (1937) is that given above, namely that the parasite becomes quinine-resistant; this explanation is not accepted by the majority of workers, for example Field (1939), Chopra (1936), and Fletcher (1921) agree that there is no such thing as quinine-resistance. There are certain individuals however, who at times appear not to absorb quinine, as shown by the failure to demonstrate the drug in their urine following

administration to them of quinine; these may account for a small fraction of the failures seen with quinine, but it is probably true to say that many if not practically all the cases of "quinine resistance" or of failure of quinine to act are due to the drug not being taken. All workers have had experience of that. Scott (1939) states that in the French Army in Macedonia urine tests showed only two per cent of the men to have quinine in their urine although all of them were supposed to be receiving the drug; when discipline was tightened it was possible to report a finding of 100% positive. The most likely causes of "failure" of quinine are: adulteration of quinine, deliberate avoidance of taking the drug; non-absorption of the drug from the gastro-intestinal tract due to the drug or to complicating diseases; and faulty preparation of tablets.

One other point to be mentioned in these notes on the pharmacology of quinine is that it appears established that quinine on administration causes well-marked contraction of the spleen.

Excepting a few incidental remarks no mention has yet been made of the toxic effects of quinine. The primary aim of this chapter, however, is to ascertain whether or not quinine is likely to have been the precipitating factor in the production of haemoglobinuria in the Razmak cases. All other problems of quinine and its administration are only incidental

to this, and the short discussion given above on the pharmacology of quinine had as its major aim the elucidation of any effect which may throw light on our problem of the role, if any, of quinine in the production of haemoglobinuria. The information that is available on this aspect of quinine administration is sketchy and incomplete, and as yet only one statement has been recorded which requires to be considered more fully, namely the statement that quinine causes haemolysis of parasitised red cells. This may conveniently be considered in the next paragraph.

Toxic side-effects of quinine, particularly haemolysis.

> Quinine is a poison; Chopra (1936) talks of it as a general protoplasmic poison and infers that the therapeutic dosage is one strong enough to kill the parasite yet leave the host unaffected. This happy result is not always attained. Even in the normal individual with no idiosyncrasy to the drug there seems only a narrow boundary between the therapeutically-effective dose and the toxic dose. The mildest toxic phenomena, those of cinchonism, appear in the majority of individuals taking therapeutic doses of quinine daily for more than a very few days. A blood level over ten mgms. per litre of blood will produce marked toxic symptoms.
>
> It is the aim of this chapter to ascertain if

possible whether or not toxic side-effects of quinine were the cause of the haemoglobinuria which developed in our patients; the diagnosis of those patients stands at present as "intra-vascular haemolysis, cause unknown".

In any discussion on the toxic side-effects of a drug there are two possible types of toxic effects to be considered, namely:

1. Those toxic effects due to simple over-dosage, found to develop in normal individuals after the concentration of the particular drug has reached a toxic level in the body. This level naturally varies with the drug concerned, and varies also to a much lesser extent from individual to individual. This toxic level in the patient may be the result of administration of one over-large dose, or the result of accumulation as the result of a series of doses, the latter being particularly to be found with drugs which are slowly excreted or slowly destroyed in the body.
2. Toxic by-effects produced in individuals hypersensitive to the drug; the actual dose of the particular substance may be minimal in amount, a dose which probably would not have the slightest effect on a normal individual. This

> hypersensitivity is a state of altered specific reaction capacity on the part of the body cells, the process being an antigen-antibody reaction in which the drug probably acts as a haptene. Only a relatively small number of drugs have this power of easily inducing a state of hypersensitivity, and only a small number of individuals are unduly sensitive to such stimuli.
>
> When a drug is administered to an individual hypersensitive to it, the reaction is quite different from that seen when the same dose of drug is administered to an individual not hypersensitive to it. The reaction is almost explosive in character, and the toxic phenomena may bear no relation to the toxic phenomena produced by a simple overdose of the same substance.

Which type of toxic effect are we discussing therefore when we talk of the "toxic effects" of quinine? Failure to differentiate has caused much misunderstanding. The answer is that quinine shows <u>both</u> types of "poisoning", and we must discuss both, beginning first with the toxic effects of quinine overdosage in normal individuals.

Toxic effects of simple quinine overdosage in normal people;

It has already been said that quinine is a general protoplasmic poison, and Chopra claims, see above, that the acid salts of quinine have a decidedly haemolytic action on red blood corpuscles in vitro, producing a faint tinge of haemolysis at 1/8,000; this finding of Chopra's has little bearing on the problem of the in vivo behaviour of the drug where the highest concentration in the blood with therapeutic dosage is not more than 1/100,000 at the most, and where the drug circulates as quinine base except in the first few minutes after intra-venous injection of quinine salts; Chopra did record that solutions of quinine base, even as strong as 1/2,000 produce no haemolysis in vitro even after twenty-four hours. Another point which will have a bearing on our findings is the question of whether quinine is cumulative when given in the ordinary therapeutic doses; Ramsden et alia (1918) infer that it is, and that it takes seven and a half days to clear the body of quinine after multiple doses had been given. Field (1939) and Chopra (1936) are of the opposite view; they consider that with the usual therapeutic doses of grains 30 daily there is no accumulation of quinine in the body, excretion and destruction keeping pace with ingestion; Field

considers that quinine is a drug with a powerful action and little risk, a drug of remarkable safety although he does say that it has its own clinical side-effects. Of course when Field talks of "remarkable safety" he is referring to remarkable safety as regards the production of serious toxic effects; quinine is a poison, but it is a poison with a wide margin between the production of mild toxic effects ("cinchonism") and the production of severe toxic symptoms and signs. The mild toxic effects of quinine are very common, being found in the great majority of individuals after three-four days of therapeutic quinine; I personally always develop cinchonism after two days of quinine thirty grains daily. These mild toxic effects include giddiness, ringing in the ears and tremor. They are seldom severe with the doses of quinine usually used, and they are not alarming; they rapidly disappear when the administration of quinine is stopped. In our series of six patients only one man received sufficient quinine to produce even the mild toxic phenomena of cinchonism.

It requires much heavier doses of quinine than the usual therapeutic doses to produce serious toxic effects in individuals who are not unduly

sensitive to this drug, i.e. there is a marked difference between the dosage sufficient to cause cinchonism and the dose sufficient to cause serious effects of poisoning. It is unnecessary to quote more than a few examples of the "remarkable safety" of the drug.

Steudel (1894), in Tanganyika, working on the assumption that the only way to treat blackwater fever is by collossal doses of quinine, sometimes gave as much as ten grams of quinine to a patient in twenty-four hours, and followed this with doses of six to eight grams daily for some time!

Most (1940) treated cases of cerebral malaria by intra-venous injections of 0.6 grams quinine dihydrochloride every four hours for at least twenty-four hours.

In my experience the most striking proof of the safety of quinine was what I saw in the Peshawar Mission Hospital. That part of the World is very malarious, and patients come long distances, even from the remoter tribal areas, for treatment for their malaria; both sexes and all ages are represented among the patients. The demand on beds for serious illnesses is such that malaria patients cannot be admitted as in-patients; they do want

and do require treatment for the malaria however and they want quick results. The giving of oral quinine to such patients proved a failure for several reasons, and by the early nineteen-thirties it had become customary to treat all malaria patients in the out-patient department, and to give them quinine intra-venously. This may seem heroic, but medical missionaries of wide experience stated that they had never had any serious mishaps from this method of treatment; during my stay there I certainly did not see any serious result from such methods in a two-months period. Chopra (1936) talking on this problem of the toxicity of quinine, thinks that a single dose of forty grains given to an individual will produce definite toxic effects but he places the fatal dose as much higher, about eight to fifteen grams by mouth, although as much as thirty grams has been taken without fatal result; a search of the literature suggests that the fatal dose may range from ten to one hundred grams by mouth, i.e. there is wide variation. This does not include patients in early pregnancy who seem to be especially susceptible, see below. On this matter of fatal doses Baills (1893) tells of two soldiers each of whom took twelve grams quinine sulphate in error. Both rapidly developed intense tinnitus,

total deafness and stomach cramps; one died in four hours and one recovered. Plehn, F. (1898) records several interesting cases of poisoning, e.g. one patient survived the taking of 41.0 grms. in a few days and another, an insane doctor, took very large doses of quinine daily for ten to twelve days; he had ingested 120 grams before he died. Raven (1927) quotes one of his cases, a fat woman, who took a total of sixteen grams quinine within a few hours; she died some hours later of heart failure.

On this same topic Dobreff (1934) stated that in Bulgaria in the previous four years the taking of overdoses of quinine sulphate had been the method chosen by one-third of all attempted suicides. There had been eighty-six such cases of quinine poisoning in ten years with no deaths.

A survey of the literature on this problem of quinine poisoning shows that in the great majority of individuals dying from gross overdosage of quinine the weight of the poisoning seemed to fall on the central nervous system. It is necessary for our purpose however to review any literature which suggests that quinine poisoning may cause haemoglobinuria. In this connection Chopra has already been quoted as saying that acid salts of quinine have

a decidedly haemolytic action on red blood corpuscles *in vitro*. This has little bearing on the problem of an *in vivo* action except possibly for a minute or two following an intra-venous injection of quinine salts, and this has never been reported as a problem. As regards the effect *in vivo* of the giving of quinine to patients suffering from malaria Chopra (1936) did think that the drug may act by destroying parasitised red cells, and Kingsbury (1926) had already noted a rise in bilirubin values following the administration of quinine. Nocht and Kikuth (1929) claimed that quinine has the power of suspending partly or wholly the effect of an antihaemolysin which, they claimed, is found in living animals; this theory of haemolysin-antihaemolysin in normal tissues has been strongly supported in recent years by Maegraith in a series of papers. Nocht and Kikuth claimed that they found this property of quinine marked in the presence of various haemolytic agents, such as cobra venom. Zylmann (1944) found quinine to have a lytic action on red cells in weak concentration. The practical importance of this is offset by the finding, by the same author, that atebrin has an even stronger haemolytic effect, while plasmoquine has little effect. Such findings illustrate the

lack of relationship of behaviour of drugs in *in vitro* experiments as compared with *in vivo* behaviour. The findings of Birnbaum et alia (1946) are a further example of this dissociation; they had previously claimed that red cells from malaria patients lake more readily in bile solutions than do normal cells, and in this paper of 1946 the authors report that quinine, atebrin and plasmoquine all accelerate the haemolysis in the presence of bile, and that atebrin shows a particularly noteworthy enhancing effect, whereas of course atebrin is the least haemolytic of drugs *in vivo*, as will be shown in Chapter VII.

On this problem of whether quinine overdosage can produce haemoglobinuria in an individual who is not unduly sensitive, the *experimental* evidence is not definite even in those patients who received an overdose of the drug while suffering from malaria. And records show that in the great majority of fatal cases of quinine poisoning there is no mention of haemoglobinuria. Petri (1930) reviews the literature on the toxic properties of quinine and concludes that haemoglobinuria can occur but that it does so only under very special conditions of the blood; he thinks that such conditions are observed occasionally

in blackwater fever, and in very rare cases in early pregnancy; it is this latter group which provides practically all the few cases of quinine haemoglobinuria occurring in individuals not affected by malaria. Petri considers that in early pregnancy this susceptibility to quinine may be due however to individual idiosyncrasy. This is discussed below. Terplan and Javert (1936) reviewed the literature on this problem of quinine haemoglobinuria in early pregnancy and could find only eight cases in the literature; these were:

Seitz (1927) reported three cases; in all the three the haemoglobinuria followed shortly after the administration of quinine; post-mortem examination was made only in one patient, and in one other of the three there was an associated abortion.

Kutz and Traugott (1927) report two cases; but both were aborting before the administration of quinine was begun.

Fromholt (1932) reports two cases but here also both were criminal abortions before quinine was begun.

Petri (1930) reports a very doubtful case; the dosage of quinine which had been given

was minimal, and at post-mortem examination no damage was found in the renal tubules.

Terplan and Javert then record a case of their own, the first in American literature.

This case is thoroughly unsatisfactory and cannot be in any way accepted as implicating quinine as the cause of the haemoglobinuria. These authors give the history that the patient had taken a total of 100 grains of quinine over a period of some days, and some time before death. Furthermore in this case, several weeks before the taking of quinine the patient had had an incomplete criminal abortion produced and had thereafter suffered for weeks from an incomplete septic abortion, for example the patient had a white cell count of 19,000,purpura was present, and at autopsy the patient's uterus showed necrotic material. Other drugs had also been given before the beginning of the haemoglobinuria. The drugs taken were intended to be

abortifacient; they included "six bile salt tablets and twenty-four tablets each containing 1/5 grains nux vomica". Also, the patient had been given blood transfusion before death. Lastly there was no definite history of haemoglobinuria, only one of haematuria.

Under the circumstances we cannot possibly accept this as a definite case of quinine producing haemoglobinuria, and some of the other cases recorded above are almost as doubtful.

Vartan and Discombe (1940) give a record which is much more satisfactory than any of the above. They report that their patient had never had malaria and had never left England. The patient was a married woman, aged 34; she became pregnant; she then bought quinine and took ninety five grains of quinine sulphate. On the same evening she developed a temperature of 102 and showed a scarlet rash all over the body. Jaundice and haemoglobinuria followed; haemoglobinuria and methaemalbuminaemia continued

for six days. The patient died of uraemia on the ninth day; at autopsy the tubules showed heavy damage.

This fatal case would support the suggestion by Petri that an idiosyncrasy is involved.

This case of Vartan and Discombe's brings the total cases of this type to ten; but it is only in three of these that we can exclude all other factors such as sepsis. It seems permissible to say, therefore:

1. In normal individuals the dosage of quinine necessary to produce serious toxic effects is much greater than the therapeutic dose; certainly the dose required is far above the amounts which my five patients were given; it will be remembered that the doses given to the Razmak cases were:

   120 grains given to one patient in four days, and four others received thirty grains each. Another point already stressed is that in all of the patients concerned at least eight days had elapsed between the stopping of quinine treatment and the beginning of haemoglobinuria.

2. In the majority of cases reported of fatal quinine poisoning the serious toxic symptoms and signs are limited to the central nervous system. Excluding malaria patients there are only ten cases in literature in which haemoglobinuria was noted; practically all of these ten cases occurred in pregnant women, and it is suggested that, in seven of the ten, other factors, e.g. sepsis, may have caused the haemoglobinuria.

It is concluded that the haemoglobinuria in our six patients could NOT have been due to simple quinine poisoning.

Toxic effects of quinine administration in <u>hypersensitive</u> individuals.

The conclusion come to above does not exclude the possibility unlikely though it may be, that the six patients who developed haemoglobinuria in Razmak did so because they were hypersensitive to quinine. This is the second possibility which we must consider.

Quinine idiosyncrasy, like other drug idiosyncrasies, is an immunological reaction. Zinsser et alia (1944) state that in the hypersensitiveness

of quinine seen in humans, such individuals have become sensitised by prolonged contact. They consider quinine hypersensitivity to be practically the same as the hypersensitiveness seen sometimes with other substances of low molecular weight, for example iodine. The authors think that the antigenic compounds are specifically oriented by non-antigenic groups, possibly formed in the body by combinations of extraneous material with body protein.

There is no doubt that quinine does possess the property of being able to produce hypersensitivity in certain of the individuals exposed to it. The statement by Field (1939) that very few individuals are excessively sensitive to quinine is correct; this is probably because relatively few individuals come into sufficiently prolonged or intimate contact with the drug. Quinine hypersensitivity shows the characteristics of hypersensitiveness in general, namely:

it is remarkably specific, e.g. such patients are not sensitive to quinidine, at least nothing like to the same extent;

the reaction is rapid following upon ingestion of the drug; sometimes it is almost "explosive";

the reaction may be provoked by minute doses, e.g. in one case of quinine sensitiveness a dose of 1/16 grains provoked a reaction.

Fortunately the number of such hypersensitive individuals is low. James (1931) quotes Fletcher and Travers to say that in thirty-five years experience in the Malay States they found only one case of quinine idiosyncrasy.

What are the symptoms to be expected following upon the administration of quinine to a person sensitive to it? Chopra (1936) does not mention haemoglobinuria as one of the manifestations of quinine idiosyncrasy, nor do Nocht and Mayer (1937); the latter authors mention as findings such abnormalities as urticaria, exfoliative dermatitis, cyanosis, kidney haemorrhage and nose bleeding. Field (1939) gives a similar picture, mentioning rashes (urticarial and erythematous), itching, haemorrhages into the skin, dyspnoea, oedema, and collapse. He does not mention haemoglobinuria.

References do exist in the literature, illustrating haemolytic phenomena in relation to the administration of quinine. Analysis of such references shows that in all such cases the patient was under treatment for malaria, or the patient was pregnant.

In view of this, consider the facts concerning the haemoglobinuria shown by the six patients in Razmak in 1938. The first point that must be made is that quinine hypersensitivity is not common; by the laws of chance it is highly improbable that six such individuals should have come together in one place at the same time; also, there was a time interval of not less than eight days in each case between the stopping of the quinine and the beginning of the haemoglobinuria. This is quite unlike the immediate reaction to the drug shown on its being administered to individuals hypersensitive to it. All the quinine had almost certainly been eliminated or destroyed before the lysis began; next, the five individuals who survived were given test doses of quinine before their discharge from hospital, and no case showed any sign of being unduly sensitive to the drug.

It is quite unlikely that quinine hypersensitiveness was the cause of the intra-vascular haemolysis in our patients.

There is one aspect of the problem, however, on which we have not yet touched, namely, the reaction of the patient <u>with malaria</u> to the giving of quinine.

In other words, if quinine played no direct part in the production of haemoglobinuria, did it act in an indirect fashion in that it caused the onset of malarial haemoglobinuria? Let us consider this problem.

Quinine and blackwater fever: Chapters IX and X are devoted to a consideration of blackwater fever. Here we shall only deal with the part, if any, that quinine plays in the production of this disease. Blackwater fever is a strange disease, a riddle still unsolved. It is necessarily related in some way to malaria, and until comparatively recently the only specific for malaria was quinine. It is easy to imagine the despair of the Profession when reports began to circulate that this new specific had a grim facility for precipitating the onset of blackwater fever; even as early as 1858 this belief appears to have had much support. In that year Antoniades reported that certain Greek doctors consider "haematuria" to have followed the administration of quinine to patients with intermittent fever, and that the doctors thought that this must be due to quinine poisoning or due to an idiosyncrasy to the drug. Antoniades goes on to say that it is criminal negligence to withold the drug on those grounds. Manson summed up the problem in his poser to Thomson when

when he said that we know that malaria is at the back of blackwater fever but "what pulls the trigger"? If we are able to establish that quinine has a powerful effect in the precipitating of attacks of blackwater fever then obviously such a happening will require serious consideration as a probable explanation of the outbreak of haemoglobinuria in Razmak. What is the evidence for and against the view that quinine causes attacks to develop of blackwater fever? A few excerpts on the views of various workers may be of interest.

Antoniades (1858) considered that it is negligence to withold quinine on the grounds that it may cause haemoglobinuria.

Steudel (1894) considered that the only way to abort blackwater fever was by the giving of heroic doses of quinine.

Koch (1898) gave the impression that he thought that blackwater fever was due to quinine poisoning, but individuals who had discussed the matter with him, e.g. Fleming of Southern Rhodesia, claimed that Koch believed in thorough quinine prophylaxis in the reduction of malaria and blackwater fever.

Crosse (1898) quotes Stanley the explorer who suggest-

ed likely causes of blackwater to be sun, fatigue, and damp, and claimed that blackwater fever yields readily to quinin. Stanley took for his own attack took 60 grains of quinine in one day. Thomson (1924) stated that at the time it was the custom in Southern Rhodesia to treat blackwater fever by large doses of quinine which were given intra-muscularly during and after the attack. We are not discussing the actual treatment during an attack of blackwater, and have put this reference in to indicate that in Southern Rhodesia at that time quinine could not have been regarded as one serious cause of starting attacks of blackwater. Thomson himself thought that quinine, properly administered, will prevent blackwater fever; he also says that quinine used improperly is dangerous in that it produces insidious chronic malaria. Thomson stated that he never saw a case of blackwater fever in a person regularly and properly treated with quinine. We have already quoted the striking effects of the giving of quinine prophylaxis to the

White Fathers of Africa.

Ross (1932) admits that there are many cases on record in which the relationship between the taking of quinine and the onset of haemoglobinuria is so intimate and dramatic that there would be no alternative to the assumption that quinine has acted as the precipitating agent of the attack. But there are also many, many cases in which quinine would appear definitely to be excluded as a causal agent. Ross feels that between the two extremes lie the great majority of cases, i.e. that the taking of quinine enters into the previous history but that the relationship between it and the occurrence of haemoglobinuria is not obvious. Ross thinks that it is a fairly generally accepted belief that quinine in certain cases is the causal agent responsible for the <u>onset</u> of haemoglobinuria but that in the majority of cases much of the evidence produced to incriminate quinine is of extremely doubtful value from the statistical point of view. He concludes that the belief that quinine is an exciting agent in blackwater fever is largely based on its

results in individual cases, and he feels that considerable doubt must remain as to whether such cases are to be regarded as affording an indication of a process that is common to all.

Chopra (1936) considers that neither clinical records nor animal experiments tend to show that quinine is the cause of blackwater fever. He also is one of the workers who consider that proper administration of quinine prevents blackwater fever, but goes on to say that once an individual is predisposed to blackwater fever by repeated attacks of neglected malaria full doses of quinine may act as an exciting cause. Chopra claims that blackwater fever was known in Europe long before quinine was introduced. Manson-Bahr (1940) also makes this statement that blackwater fever was known in Europe long before the introduction of cinchona bark. It is probable that such opinions are based on the series of cases reported by Hippocrates, but in Chapter IX it will be seen that it is doubtful whether those well-known cases of Hippocrates were

blackwater fever. There is no evidence which definitely proves that blackwater had been reported before the arrival of cinchona bark in Europe.

Foy and Kondi (1943) think that in the production of blackwater fever malaria is an important preparatory agent, although what exactly it does or how it produces its effect is unknown; but, the ground thus having been prepared, the trigger may be pulled by any one of several agents, e.g. anti-malarial drugs, or cold, or splenic contraction. In effect therefore the authors think that there is nothing specific about the action of quinine, which is merely one of several agents. This view is widely quoted.

It seems therefore that even those who do not consider quinine to be a likely cause of blackwater fever are of the opinion that it may not be altogether without blame.

There is no lack of support for the opposite view, an old one, that quinine has a powerful effect on the production of blackwater attacks. Among such supporters we find:

Veretas (1859) was one of the strong opponents of Antoniades and was strongly of the opinion that haemoglobinuric fever is caused by the taking of quinine; Karamitsas (1878) supported Veretas and spoke of "haemoglobinuria from quinine".

Tomaselli, in his three memoirs of 1875, 1877 and 1897, was one of the strong supporters of the quinine intoxication theory. The title of the first memoir is "On the question of quinine intoxication and malarial infection".

Pampoulis and Chomatianos (1888) produced a noteworthy point when they demonstrated the specificity of the effect of quinine in the production of attacks of blackwater in certain individuals. They quote one case in which their findings were:

Day.	Drug.	Haemoglobinuria	Time interval.
1	Quinine	+	One hour
4	Cinchinine	-	
5	Cinchonine	-	
9	Quinoidine	-	
10	Cinchonine	-	
12	Quinine	+	Three hours.

Murri (1896) also had a patient in whom it was possible

repeatedly to invoke haemolytic phenomena by the administration of quinine. The patient was a girl aged 19, suffering probably from chronic malaria, in whom it was possible to induce a whole series of phenomena → blackwater from as small doses of quinine as 1½ grains. This attack was provoked seven times in all by the deliberate giving of quinine.

Ziemann (1900) went further and reported one case in whom the type of reaction could be varied by variation in doses of quinine. He reported:

patient given quinine 0.01 g. → hburia.
" " " 0.005 g. → hbaemia.
" " " 0.004 g. → albuminuria

Moreschi (1920) had a patient aged six, no history of family intolerance to quinine; the patient developed malignant tertian and quartan infections; she was given a total of 4.0 grams quinine hypodermically and 8.0 grams orally over a period of thirty days.

The patient then developed quinine intolerance and thereafter as small a dose as 0.015 grams of quinine would precipitate haemoglobinuria. Treatment of the patient by cinchona was then started; the maximum dose given of

of this drug was 1.1 grams and the total dose was 9.0 grams in twenty six days. On the twenty seventh day the patient developed cinchona intolerance which was very marked. Thereafter, in turn, the patient developed intolerance to quinidine cinchonidine and optochin. The dosages required to produce a reaction are sometimes remarkably small, e.g. Thomson (1924) tells of a patient in whom 1/32 grains produced haemoglobinuria.

Marchiafava and Bignami (1900) held that the late appearance of blackwater (at the beginning of the nineteenth century if we exclude the doubtful cases of Hippocrates) was the result of quinine becoming more widely used at the same time; they overlook the fact that quinine was one of the principal ingredients in various preparations of bark which had been in use for two hundred years previously.

Nocht (1929) surprisingly supports the above when he claims that so far as he knows so long as one uses for the treatment of malaria <u>not</u> quinine itself but decoctions of the drug then blackwater fever is unknown, or at least very rare.

Osler (1935) states that it was the opinion of Deeks and James, with all their experience in Panama that

there are three causes superadded to the malarial infection to produce blackwater fever, namely: a. A renewed malaria attack, with production of toxins sufficient to destroy many red cells.

b. A lowering of body resistance.

c. Quinine.

Nocht and Mayer (1937) similarly suggested that there are three factors causing blackwater fever; they are: chronic malaria infection;

haemolytic disposition of the individual;

and medicinal toxicity due to quinine.

They further say, as regards quinine, that each individual liable to the disease has a threshold dose, in which amounts below the limit are tolerated, but amounts necessary to provoke the reaction may be as low as 0.01 grams or as high as 2.0 grams. They further say that in many cases the disposition to blackwater fever following quinine administration lasts several months or longer, and in such patients blackwater can be produced at any time. In other cases the disposition quickly wanes and vanishes permanently after the attack.

Christophers (1937) says that seemingly quinine enters into the picture of blackwater fever but to

what extent, how far an essential factor, and in what way it acts is completely a matter of hypothesis.

Rogers and Megaw (1930) say that in blackwater fever there is reason to believe that a dose of quinine is the usual precipitating factor, though quinine by itself cannot be the cause, and in fact regular mass treatment with quinine was said to have caused a great diminution in the frequency of the disease in the Cameroons. They consider two factors to be of importance, namely:

a. a history of more than one attack of malaria and

b. an irregular taking of quinine over a period of time.

They think that quinine and repeated malarial infections act together in some mysterious way to cause a blackwater attack.

The above-quoted authorities all agree therefore on quinine being implicated in the precipitation of blackwater fever. Some of the above reports include suggestions as to how quinine produces its effects. For example:

Cardamatis (1912) suggests the much too simple theory that quinine combines with the albumen of the stomach and is absorbed as quinine albuminate which in

certain circumstances acts as an antigen; this antigen combined with malaria toxin leads to the production of antibodies which induce quinine sensitiveness in the individual. This is a theory with nothing to support it but it is worth noting in that it introduces, probably for the first time, the idea of a compound antigen as a possible cause of blackwater fever; as will be seen in Chapter IX this theory of a compound antigen is now engaging a considerable amount of attention.

Nocht and Kikuth (1929) carried out a series of experiments on animals. Briefly they found:

Dog No. 1. haemolytic antibody = haemoglobinuria

" " 2 " " and

anti-haemambocepter = Nil.

" " 3 haemolytic antibody and

anti-haemambocepter and

quinine = haemoglobinuria.

i.e. quinine appears to destroy the action of anti-haemolytic ambocepter.

Nocht and Kikuth further claim that this property of quinine of suspending or considerably weakening the effects of antihaemolysin is also to be observed with other haemolytic agents, for example cobra

venom, and bile-acid salts. This may be related to some extent to the finding by Kritschewsky et alia (1923) that lecithin in high dilution activates a non-haemolytic mixture of quinine and red blood cells and that cholesterin has no inhibiting power on this mixture, although it has an inhibiting effect on snake venom haemolysis.

Nocht and Kikuth on their findings elaborated the suggestion that malarial infection produces haemolysin or other haemolytic agents and that quinine strengthens the haemolytic action of this substance.

Rogers and Megaw (1930) are obviously thinking along the same lines when, talking of blackwater fever, they say that in certain unknown conditions the red blood cells may become sensitised to the action of a haemolysin produced by the action of quinine on malarial parasites and that the sensitised corpuscles become dissolved when a further supply of haemolysin is brought into existence by the action of quinine on a new brood of parasites.

Petri (1930) may have given a lead on avenues to explore when he says that quinine can produce haemoglobinuria but only under certain physico-chemical

conditions of the blood, observed occasionally in blackwater fever.

Manson-Bahr (1940) suggests three factors necessary to produce blackwater fever: namely, a haemolytic factor, the result of malaria; lowered cholesterin content of the blood; and quinine. This theory has received little support and careful investigations show that there is no hypocholesterolaemia in blackwater fever.

The three best summaries on the problem are:

Chopra (1936) who points out the possibilities with quinine in blackwater fever and says all these possibilities have been mooted at one time or another.

1. That quinine causes blackwater fever by direct haemolytic action.
2. That quinine causes blackwater fever by sudden contraction of the spleen, which expels haemolytic toxin into the circulation.
3. That quinine causes blackwater fever by lowering the osmotic tension of the plasma, causing water to pass into the red blood corpuscles and burst them.

4. That quinine becomes highly haemolytic in the presence of bile.

5. That quinine becomes highly haemolytic in the presence of lecithin.

6. Blackwater fever is a form of quinine poisoning: the author states that this can only be the determining factor in those cases who have an idiosyncrasy to the drug. This very important remark should be noted.

Some of the above have already been discussed; others will be discussed in Chapter IX.

Nocht and Mayer (1937) also discuss a few of the theories.

e.g. 1. Special sensitiveness to quinine of red blood corpuscles in certain malaria patients. The authors point out that quinine is only haemolytic to red blood corpuscles in doses far above the therapeutic dose and say that neither haemolysis nor blackwater fever has been observed in healthy people after very large doses of quinine.

2. Liberation of a considerable amount of haemoglobin through the destruction, by quinine of parasites together with the containing red cells. The authors point out that the facts against this are that the dosage of quinine and the number of parasites bear no relation to the

onset of lysis, e.g. a person prone to black-water fever may develop haemoglobinuria after small doses of quinine even if the parasites are absent or present only in small numbers.

3. Haemolysins resulting from the malaria process, which circulate in the blood stream or are fixed in certain inner organs; in such cases the quinine is supposed to hasten or accelerate the activity of the haemolysin. Nocht and Mayer admit however that there is no experimental proof for this suggestion.

Stephens (1937) gives a broader summary: he talks of

1. Popular beliefs; he points out that the belief that quinine can provoke an attack of blackwater fever has prevailed in many countries since the introduction of the drug in 1820.

2. Personal beliefs; many patients, the subjects of blackwater fever, have attributed the onset of the attack to a dose of quinine taken shortly previously.

3. Experimental cases. In many cases these personal beliefs have been tested and quite frequently found to be correct; e.g. in some cases the outcome was fatal.

4. Diagnosis; there are no certain criteria in literature for distinguishing between what has

been termed "Quinine haemoglobinuria" and blackwater fever.

5. Minimal doses of quinine appear to be sufficient to induce haemoglobinuria in certain individuals.

6. Increase of dose. Patients appear to be tolerant of a certain dose of quinine but intolerant of an increase beyond that dose.

7. Time relationship between taking quinine and blackwater fever. In some cases the onset appears to be "immediately" after the taking of quinine but more usually it is after a few hours; in three quarters of the cases the onset is within about six hours.

8. No history of quinine. In some cases no history of the taking of quinine before the attack.

The problem is possibly a little less complex than at first it appears. The first thing is to correct the statement both by Stephens (1937) and by Scott (1939) that we may be dealing with a true quinine haemoglobinuria in certain cases as distinct from a malarial haemoglobinuria precipitated by quinine. As we have seen on page 456 et seq. true quinine haemoglobinuria is so exceptionally rare as to be dismissed: practically speaking, all cases of haemoglobinuria occurring during quinine treatment of malaria are cases of blackwater

fever.

In assessing the part played by quinine in the production of blackwater fever it has to be kept carefully in mind that at the time when practically all the above papers were written the only treatment and the universal treatment for malaria was administration of quinine. It is to be expected therefore that a history of taking quinine would be a very common finding in case reports at that time. Even taking this into account, however, and taking into account the fact that many individuals have developed blackwater fever without taking quinine, and taking into account that certain other factors such as fatigue and cold sometimes apparently are related to an attack of lysis there is still no gainsaying Ross's (1932) statement that there are many cases of blackwater fever on record in which the relationship between the taking of quinine and the onset of haemoglobinuria is so intimate and so dramatic that there would be no alternative to the assumption that quinine has acted as the precipitating agent. Nocht and Mayer rightly talk of the experimental certainty of the reaction in certain cases. In my opinion there are three factors which never have been sufficiently stressed in this problem, namely:

1. <u>The specificity of the reaction with quinine</u>, where even its isomeride fails to provoke any reaction. The cases quoted from Pampoukis and Chomatianos, page 473, and from Moreschi, page 474, are typical examples of this.

2. The dosage of quinine capable of precipitating blackwater fever in certain cases; not only is the reaction extremely specific but the dosage is sometimes so minute as also to indicate a specific reaction; take for example a case already reported, where a dose of grains 1/32 quinine produced haemoglobinuria; Ziemann, page 474, also gives an interesting report on the effect of dosage. This type of history must remind one of the so very similar reactions reported in individuals hypersensitive to quinine, pages 463 et seq. Another point to be remembered in the statement by Stephens (1937) that in three-quarters of the cases of blackwater fever developing in relation to the taking of quinine, the onset was within six hours of the ingestion of quinine.
3. There are two schools of thought, one claiming that quinine prevents blackwater fever and some of this school have used huge doses of the drug in the treatment of established blackwater fever, e.g. Steudel; the other camp however declares that quinine is the cause of blackwater fever.

The explanation is probably quite simple. The facts are correct but the interpretations are wrong. The answer probably is that quinine properly taken, in sufficient dosage and regularly every day, will prevent blackwater fever merely by suppressing any clinical manifestation of malaria which is

the one definite factor necessary for the production of blackwater fever. The example of the White Fathers is only one of many such proofs. But if quinine be taken irregularly so that it allows of the establishment of chronic malaria, particularly malignant malaria, or if it happens that quinine administration is begun in an individual already suffering from well-established chronic malaria, then that dose of quinine may quite easily precipitate an attack of blackwater fever. In such cases the quinine is not acting in any specific manner (this statement is not wholly correct and will be amplified below); it is merely one of the many factors capable of "pulling the trigger", factors like cold, injury, fatigue, plasmoquine, atebrin and the many others. Unfortunately it has an unhappy faculty of pulling the trigger.

I have stated that quinine is not acting in any specific manner when it pulls the trigger; the statement is not quite correct. The simple specificity of the reaction and the remarkably minute doses effective in certain cases leads me to postulate that while quinine as a factor is not specific, this reaction occurs in individuals specifically hypersensitive to quinine. In other words it would appear to be a quinine hypersensitivity reaction grafted unfortunately on to a malaria attack, with the resultant tragic outcome of malarial haemoglobinuria.

All this brings us then to consider again our chief end, that is, taking all the above factors into account do we consider that in the five of our six patients who all received quinine, the quinine administration precipitated an attack of blackwater fever, i.e. are we prepared to amend our previous diagnosis of "intra-vascular haemolysis" to "blackwater fever precipitated by quinine". The answer is No.

Reference to the table of dosage of quinine and the time interval elapsing, page 434, shows that the haemolyses all began not less than eight days after the cessation of administration of quinine, and furthermore, admittedly a point of lesser importance, none of our patients showed any hypersensitiveness to quinine on testing after an interval sufficiently long to allow of the re-establishment of sensitivity.

One must therefore presume that "blackwater fever precipitated by quinine" is not the diagnosis in the cases concerned. Our diagnosis therefore must remain, at present, as "intra-vascular haemolysis".

SUMMARY.

This chapter is necessary to exclude or confirm whether the administration of quinine played any part, direct or indirect, in the production of haemoglobinuria in these cases in Razmak in 1938.

The chapter opened with a short note on the history of quinine, tracing its origin from Peru; thereafter the pharmacology of the substance is discussed; it was not possible definitely to establish the method or methods, direct or indirect or both, whereby quinine produces its effects in malaria. It is thought that direct action must play some part; two of the actions claimed for quinine in this section were haemolysis of parasitised red cells and contraction of the spleen.

The toxic effects of quinine were then considered; such effects fall into one of two categories, namely, the toxic effects of overdosage in the normal individual and the toxic reaction to quinine in individuals hypersensitive to this drug. On investigation of the first part of the problem it was seen that normal individuals do not show serious toxic effects following quinine ingestion unless the dose is very high, much higher than that received by any of our patients in Razmak, and also it was noted in cases of overdosage in normal individuals not suffering from malaria that haemoglobinuria is practically

never found as a symptom; the only such cases on record, ten in number, were all pregnant women. Therefore if we presume our patients not to be hypersensitive to quinine, the amount of quinine they received and the long time interval between the cessation of quinine ingestion and the beginning of lysis definitely excluded any toxic effects of quinine as a cause of the haemoglobinuria from which they suffered. The next part of the problem was that of hypersensitivity to quinine; it was seen that in hypersensitive individuals even a minute dose of quinine may cause severe reactions. But again even in such cases haemoglobinuria is very rarely found as a symptom, the exceptions being in a very few individuals suffering from malaria. Our patients were suffering from malaria but the time lag, the law of averages, and the lack of quinine sensitivity on later testing all allow us to presume that quinine sensitiveness was not responsible for intra-vascular haemolysis in Rązmak in 1938, i.e. quinine apparently did not play a direct part in the production of the haemoglobinuria. This left for consideration the question of whether the cause of the intra-vascular haemolysis was an attack of malarial haemoglobinuria precipitated by quinine. Perusal of the literature on this last question of quinine in blackwater fever shows remarkable differences in opinion. The balance of evidence, however, undoubtedly incriminates quinine. I suggest in this paragraph that the explanation which links the two

apparently conflicting points of view is that quinine in adequate daily dosage will prevent blackwater fever by preventing chronic malaria, but if the quinine is given intermittently, so permitting the establishment of chronic malaria, or if administration of quinine is begun at a time when chronic malaria is already established, then quinine admittedly may play a part in precipitating an attack of blackwater fever; in doing so however it acts non-specifically excepting that the reaction is such as to suggest that it occurs in indiyiduals hypersensitive to quinine. Perusal of dosage, time intervals, etc. however, all show that our cases did not fall into this category of "blackwater fever precipitated by quinine".

We are now in a position to say therefore that quinine played no part, direct or indirect, in the production of haemoglobinuria developing in our six patients in Waziristan in 1938.

CHAPTER VII.

Atebrin as a possible cause of the Razmak cases of Haemoglobinuria.

Three types of antimalarial drugs had been administered to the individuals who developed haemoglobinuria in Razmak, namely quinine, atebrin and plasmoquine. The conclusion has been reached, in Chapter VI, that the administration of quinine had no part in the production of the haemoglobinuria; this leaves atebrin and plasmoquine for consideration. Logically plasmoquine should be considered first since it was the first of the synthetic antimalarial preparations to be produced. Chronologically it was the last of the three products to have been given; therefore we shall first consider what part, if any, was played by atebrin in the production of the haemoglobinuria.

History of the discovery of Atebrin.

As the first World war drew to a close with the Allies in possession of all the sources of quinine the Central Powers learned again the lesson of the effect of uncontrolled malaria on Campaigns. We would like to think it was in a pure spirit of science that so much of the energies of the Bayer combine were later devoted to attempts to synthesise substances effective against malaria. They succeeded, and it is poetic

justice that in the second World war, with quinine in the hands of the enemy, it was these synthetic preparations that saved the Allies.

The work was carried out in the I.G. Pharma Bayer-Meister-Lucius Works at Elberfeld in Germany, under the general direction of Schulemann Schoenhoefer and Wingler; they used as the basis of research compounds derived largely from methylene blue.

By 1928 they had so far succeeded that they were able to market a substance, beprochin by name, later called plasmoquine. This substance, the first, is discussed in Chapter VIII. Plasmoquine had certain real drawbacks, e.g. its toxicity and its lack of effect against malignant tertian schizonts; research was continued still under the general direction of Schulemann and Hoerlein. By late 1929 12,000 new compounds had been tested in the Research Department of I.G.Farbenindustrie. Mietzsch and Maus, working with Kikuth, produced among these a substance originally named Plasmoquine E or Erion and later named Atebrin. The acridine ring, see page 493 et seq., is the basis of atebrin and its successors. Plate No. XVIII, p. 480 gives details of atebrin, the base of which is 2 methoxy-6-chlor-9-alpha-diaethyl-amino-delta-methyl-pentyl-amino-acridin. From plate No.XVIIIit is seen that atebrin has affinities both with quinine and

plasmoquine, for example the side-chain of atebrin is identical with that of plasmoquine but is placed in the same position as the closed-ring system of quinine. The link NH is also the same as in plasmoquine. This may appropriately come into the next paragraph, namely:

The Pharmacology of Atebrin.

The graphic formula for atebrin has been given in plate No. XVIII, p. 430. In view of the like action to plasmoquine in some respects and to quinine in other respects, we shall have cause to refer to this formula later. Atebrin base is a yellow powder, somewhat bitter to taste and moderately soluble in water. It is marketed in two forms, namely tablets for oral use, 0.1 gram each (these are atebrin bihydrochloride with a little inert material) and atebrin for injection = atebrin-musonate = atebrin dimethyl sulphonate. The Bayer Company also produced an atebrin-plasmoquine dragee with 0.1 g. atebrin and 0.0033 plasmoquine; this is no longer made. Two obvious results of the broadening of the ring system from quinolin to acridin are a relative freedom from immediate by-effects and prolonged retention in the body.

Atebrin tablets when taken by mouth are absorbed from the small intestine. Nocht and Meyer (1937) say the absorption is rapid. Excretion begins a few hours after ingestion. When the drug is given by intra-muscular injection excretion

is said to begin within one hour. Some idea of the drug levels in the blood is to be got from the work of Fairley (1945) who showed that on a dosage of 0.1 g. atebrin daily the peak value was reached about the twenty-fifth to twenty-eighth day, i.e. after ingestion of 2.5 g. After this dosage over a period of time the average blood concentration was 21.2 micrograms per litre. And Maegraith (1946b) gives the following very interesting figures.

Atebrin whole blood concentration	= 208 micrograms	per litre.
plasma	= 60 "	" "
red cell layer	= 40 "	" "
white cell layer	= 9600 "	" "

In 1946 also the Armoured Medical Research Laboratory, U.S.A., issued a report on the problem of blood concentrations of atebrin and found that plasma levels rose gradually during the first three to six weeks, after which it is steady.

The mean plasma level was 30 micrograms per litre for each 1 gram taken per week, with a loss of 10% concentration each day. Actually Fairley (1945) was the first carefully to assess the levels and he had said then that the die-away averaged ten per cent daily of the level present, with considerable variations in values towards the end. As will be seen later, Chapter VIII, this may have proved of importance in the question of increasing plasmoquine toxicity.

One other finding was that of Thonnard-Neumann (1931) who carried out X-ray examinations of the gastro-intestinal tract after administration of (dragee) combined atebrin and plasmoquine and found a hyperperistaltic stomach, together with spastic contractions of the large and small bowel.

Atebrin is slowly excreted unchanged in the bile and in the urine which may become yellow with large amounts; the excretion begins, as was said, in a few hours and rises to a maximum in twenty four hours; elimination from the body continues long after the last dose. Field (1939) says that traces have been present in the urine several weeks after the last dose; he also claims that only 1/7 of the atebrin administered can be recovered from the excreta and the fate of the remaining 6/7ths is uncertain. There has been relatively little work done on this and the figures are difficult to confirm. In certain tests carried out by us in Razmak we did find traces of atebrin in the urine at least five weeks after the last dose in practically every individual tested. Tropp and Weise (1933) using colorimetric methods have shown that the atebrin is excreted in equal quantities in the stools and the urine, and that the excreted pigment is physically and biologically identical with atebrin. This stability of atebrin and its persistence in the body are qualities due to

the acridine ring and they are of importance since they allow of concentration being maintained in the body with intermittent dosage, as compared with the quick excretion of quinine. On this point Hecht (1933) has an interesting theory. He claims to have established that a large portion of the atebrin taken in by mouth is retained in the upper intestine, liver and bile, and claims that it undergoes a circulation inside the body rather like that of bilirubin, namely liver-intestine -liver-intestine. He considers that the drug circulates in this way before reaching the blood in any amount and that when atebrin does reach the blood and is found in it, this is the overflow from continued dosage during which the liver has become saturated. This is a tempting hypothesis and would explain why atebrin given orally has no immediate effect on the symptoms. It is generally taught that atebrin does have an immediate effect but this is incorrect and many workers still prefer to begin the treatment of malaria with quinine in order quickly to make the patient comfortable, then to carry on with atebrin. Hecht obviously accepted this when he says that atebrin given orally has no immediate effect upon the clinical picture, with the parasites and fever remaining uninfluenced up to one to three days. By his theory the first nine tablets of an atebrin course are supposed to be used up in saturating the liver and only the remaining six are available. There is no proof of this however.

We shall leave the discussion of the toxic effects of atebrin to a separate section and shall discuss the effects of the drug on the malaria parasite. Here also there is no definite information although there are several theories.

Field (1939) considers that atebrin has the virtues and defects of quinine; he summarises its effect as the same, see Plate No. XIX, page 439 , i.e. in human malaria it is an effective schizonticide but a poor gametocide especially against crescents. He thinks atebrin is more efficient than quinine in certain directions and in others is less efficient. Peters (1935) thought that the action of atebrin was direct in all probability, atebrin having a marked affinity for parasites, to which it becomes firmly attached. Chopra (1936) said that the destructive action of atebrin on the parasite appears exceptionally powerful.

One of the earliest notes on the dosage is given by Muehlens and Fischer (1930-31) quoted by Nocht and Mayer. They recommend 0.3 g. daily for seven to ten days. Chopra, Gupta and Sen (1936), quoting the makers of the drug, said that the usual effective dose of atebrin is 0.3 g. daily for five days. They treated fifty four cases of malaria, the majority being given atebrin-plasmoquine dragees. In two cases they found parasites in the peripheral blood after eight days of this treatment, and several more of the fifty-four

cases still had parasites in the blood on the sixth day. In one mixed case whom they treated benign tertian parasites disappeared on the eighth day and reappeared within one month.

In the same year Chopra, Das Gupta and Roy (1936) considered that atebrin had a more or less direct action.

Nocht and Mayer (1937) said that there was no definite proof whether the action of atebrin was direct or indirect and pointed out:

1. Removal of the spleen and blocking of the reticulo-endothelial system does not affect the action; this, of course, is a very crude and incomplete method of investigation.
2. Atebrin musonate, mixed with malarial blood and incubated for half an hour, apparently caused considerable prolongation of the incubation period when infected blood was injected into mental patients.
3. Testing with the fluorescence microscope of the blood of a patient with benign tertian malaria to whom an intra-venous injection of 0.2 g. atebrin had been given was said to have shown that selective combination of atebrin and parasite had taken place, and the combination was fast and durable.

Another report is the work of Ciuca et alia (1937) who dissected out mosquitoes on a slide in a solution of medicament, then injected the sporozoites intra-venously into mental patients. They concluded from their results that malarial sporozoites under certain conditions are able to withstand the direct application of atebrin in 1/2500.

If the effect of atebrin is direct then it is difficult to explain the persistence of parasites in the blood after several days of treatment with the drug unless there is some system of retention of atebrin in the organs of the body suggested by Hecht, see above. The makers originally suggested a five day course and the suggestion was supported at first by the League of Nations Commission, but this course is too short, e.g. Nocht and Mayer (1937) agree.

There is obviously little reserve when the patient is on a five day course and Nocht and Mayer consider that the course should be a seven day one of 0.3 g. daily; they especially stress the importance of this in malignant tertian malaria.

In the Fourth General Report (1938) the League of Nations Commission find that in a patient on an atebrin dosage of 0.3 g. daily:

P.vivax trophozoites disappeared from the peripheral blood on the 3rd day.

Malignant tertian trophozoites disappeared from the peripheral blood on the 4th day in 90% of the cases.

An investigation on this point was carried out by us in Razmak in 1938 and 1939. These results were surprising. The individuals examined were patients on atebrin plus plasmoquine in the dosage of 0.3 g. atebrin and 0.03 g. plasmoquine daily; both benign tertian and malignant tertian cases in this investigation showed 3.5% still to have parasites in the peripheral blood on the fifth day of treatment.

Similar tests were carried out using 0.3 g. atebrin alone for five days and both in malignant tertian and benign tertian cases parasites were found in the peripheral blood on the fifth day in twelve per cent of patients. Innes (1947) found that even on a dose of 0.6 g. atebrin daily patients with benign tertian malaria showed parasites present in the blood after two days.

Obviously therefore the five day atebrin course is not sufficient to sterilise the peripheral blood in a significant number of individuals. The Army in India was fully aware of the dangers of the short course as a routine, and as early as 1934 the D.M.S. India pointed this out when he said

that trials of the therapeutic value of atebrin had been tried for thirteen months and the findings were that even a seven day course is not perfect, e.g.:

Course No. 1. Atebrin 0.3 g. for five days ⟶ plasmoquine 0.03 g. for five days: 13.2% relapse.

" " 2 Atebrin 0.3 g. for seven days ⟶ plasmoquine 0.03 g. for five days: 12.1% relapse.

The longer atebrin course was then adopted as the official method of treatment of malaria in the Army, with the modification that only 0.02 g. daily plasmoquine should be given to Indian patients; atebrin and plasmoquine had not to be administered concurrently because of increased toxic symptoms. One year later, in 1935, the D.M.S. separated the atebrin and plasmoquine parts of the course by a three-day rest period further to cut down the incidence of toxic symptoms. It may also be inferred from this 1935 report of the Army authorities that the Army physicians had found atebrin inferior to quinine in controlling the symptoms at the beginning of treatment in spite of the general belief to the contrary. This may not be taken to infer that the final results of atebrin are inferior to those of quinine; in fact they are very much better. One very strong indication that a five-day course of 0.3 g. daily of atebrin is not sufficient is the fact that practically all modern types of treatment begin with a heavy initial dose, e.g. 1.2 g.

daily, gradually tapering off to 0.4 g. daily.

In 1939 Field confirmed that in Malaya quinine brought the symptoms and signs of malignant tertian malaria more quickly under control than did atebrin, and the 1938 report of the League of Nations Malaria Commission also comments upon the advantages of using quinine first. It has been our practice to do so since 1938. These clinical findings are of value in attempting an assessment of the function of atebrin in its attack on the parasite. This is possibly only of historical interest now, however, and may be brought to a close by a consideration of what was some few years ago one of the major malarial problems facing mankind, namely the effectiveness or otherwise of atebrin in prophylaxis against malaria. This has been discussed at short length in Chapter I where a description is given of its use in Waziristan. The earlier reports had given favourable figures, e.g. Soesilo and Gilbert (1934) who allowed mosquitoes to bite a patient carrying malignant tertian gametocytes then, later, allowed the mosquitoes to bite thirty three men. The men were divided into two groups, twenty-two of them were given prophylactic atebrin 0.2 g. daily for from four to six days; at the end of an eight-months observation period fifteen of the men were still free from malaria; eleven men served as controls and within two weeks ten of these had developed malaria. These figures are surprisingly favourable to atebrin in view of

later work. The best report on the subject is that published by Fairley (1946) who carried out a series of highly interesting experiments at Cairns in Australia. There is little to question in his conclusions that the prophylactic action of atebrin is probably schizonticidal, i.e. medicocurative to use Sergent's phrase, i.e. it is not a true causal prophylactic in malaria.

Fairley found that in tests with malignant tertian malaria 0.1 g. daily of atebrin virtually abolished recrudescences and if continued for twenty-eight days after the last exposure cure invariably resulted, but with benign tertian malaria, doses of 0.1 g. daily merely suppressed the benign tertian; overt vivax malaria develops with great regularity a few weeks after suppressive atebrin has stopped.

Fairley considered this to be due to the persistence of the, at that time, hypothetical exo-erythrocytic forms. He came to the conclusion that if daily atebrin is taken then a force can be employed in hyperendemic areas of malaria without significant malarial casualties and that there should be no deaths from malaria and no blackwater fever and that after cessation of atebrin suppressive treatment the residual problem would be exclusively that of benign tertian. This was our experience in 1938 as we have pointed out in Chapter I.

We can summarise this short account of the pharmacology of atebrin by saying that it is a drug easily absorbed and only very slowly excreted; it acts less fast than quinine in controlling signs and symptoms although the end results, e.g. relapse rate, are much better than those of quinine; and its action cannot definitely be said to be direct or indirect. The summary brings out two facts which are of importance in our particular problem, namely:

1. That the dose of atebrin 0.1 g. daily even over a period of some considerable time fails to prevent benign tertian malaria from becoming overt once the atebrin is stopped. This is in agreement with our experience in 1938 and shows that whatever strain of benign tertian malaria was picked up on the Kharre Column was not destroyed by the prophylactic atebrin begun during that Column. It is to be remarked that all of the six patients who developed haemoglobinuria had been suffering from benign tertian malaria.
2. That a five-day course of atebrin 0.3 g. daily in the treatment of malaria fails to clear the peripheral blood of parasites in a small percentage of cases; our experimental figures were about 12%. This means that the six patients who had been under treatment for malaria, all of them with benign tertian malaria, may conceivably still have had parasites in the peripheral blood at the end of their five-day course of atebrin. Tacit acceptance of

these facts is shown by modern types of courses of treatment which usually begin with a heavy initial dose of atebrin, e.g. about 1.2 g. on the first day, and tapers over a period of days to 0.4 g. daily.

With these facts in mind we may now consider:

Toxic side-effects of atebrin.

The discussion on the toxic effects of quinine, Chapter VI, began with a statement that such toxic effects fall into two categories, namely the effects of overdosage in the normal individual, and the effects of a dose of drug in individuals hypersensitive to quinine. Even up to a very few years ago a similar discussion on atebrin would have opened with the statement that unlike quinine atebrin induces hypersensitivity only with great rarity and that this aspect of poisoning need not be considered. This last statement cannot be accepted today. Evidence is now accumulating to show that atebrin can and does produce hypersensitivity even although the incidence is low and the reactions are less dramatic than is the case with quinine hypersensitivity.

We shall therefore open the question of toxicity of atebrin by dividing the problem into two sections, namely, the reaction of the normal person to overdosage, and secondly the reactions to the drug of individuals hypersensitive to it.

1. Toxic effects of overdosage of atebrin administered to normal individuals.

The most striking feature of a review of the literature on this point is the remarkable agreement on the safety of the drug; examples of toxicity are quoted but almost always as exceptions proving the rule of great safety. The dosage recommended of atebrin was 0.3 g. daily for a seven-day period and this was the daily dosage used by us. Details of the actual amount of atebrin taken by our six patients who developed haemoglobinuria are given below, Figure No. 19.

Figure No. 19. The relationship in time and dosage of atebrin administration and onset of lysis.

Case No.	Total dosage atebrin given (grammes)	No. of doses.	No. of days during wh. atebrin was administered	Min. time interval in days btwn. cessation atebrin and beginning of lysis.
1	1.5 g.	15	5	4 days.
2	"	"	"	7 "
3	"	"	"	5 "
4	"	"	"	3 "
5	"	"	"	4 "
6	"	"	"	4 "

This was the type of dosage until after the beginning of the second World War. Within the last few years the

the majority of workers have adopted courses of treatment with an initial heavy dosage, say about 1.2 g. on the first day and tapering off to about 0.4 g. on the third or fourth day; this would seem to be tacit acceptance of the fact that the older course of atebrin did not get a grip on the disease until the third or fourth day, and suggests that atebrin does not become effective until a certain blood level has been reached. This dosage of 1.2 g. on the first day is four times that previously used and four times that initially recommended. This would suggest that the drug has a wide safety margin between therapeutic dosage and toxic dosage, a fact well borne out by records. Naumann (1934) also talks of atebrin as the most neutral of all products; Barbosa (1934) gives details of five cases accidentally treated with very large doses of atebrin, e.g. one infant, aged 9 months, was given eight doses in thirteen days of one gram on each occasion - this is about forty times the maximum therapeutic dose for that age - and yet apart from yellow colouration only transitory symptoms were noted, namely slight diarrhoea and slight liver enlargement. In animal experiments Scudi et alia (1944) found that the L.D. 50 dose in rats was equivalent to about 300 tablets in man. Probably the best illustration however of the safety of the drug is that atebrin was used in millions of doses daily over a period of several years and yet without

significant evidenee of poisoning. Drew and Reid (1945) investigated 102 soldiers who had been on prophylactic atebrin 0.1 g. daily except Sundays for periods varying from four to eighteen months and could find no evidence of toxic action on the kidney, liver or the blood-forming organs; examples more striking than these are available however; I personally, and many of my men, took 0.1 g. atebrin daily without any break at any time for a period of over three years without the development of toxic effects. Other examples of the safety of the drug are given by:
Bispham (1941) who found that in almost fifty thousand individuals treated with prophylactic or therapeutic atebrin there were only thirty eight cases with severe effects; these strangely were largely abdominal upsets, namely symptoms of vomiting, diarrhoea, anorrhexia and epigastric pain; some of them had precordial pain and restlessness.
Also Markson and Dawson (1945) record a patient who took 0.7 g. atebrin weekly for sixteen months as a suppressive of malaria and then swallowed 25 gms. deliberately. After ten minutes he began to vomit, followed by diarrhoea, became weak and drowsy, then after three hours became stuporose, skin cold and clammy, poor pulse, contracted pupils. On that day the plasma concentration was 906 micrograms per litre but fell rapidly. The cerebro-spinal fluid never showed any atebrin and the urine remained clear. The patient recovered within

twenty-four hours.

Findlay (1947) while not giving details says that many surveys had been carried out on troops who had been taking atebrin for one to three years and that the surveys showed no evidence of damage to the cardiac, respiratory, haematopoeic and nervous systems.

Field (1939) said that in animals fifteen times the therapeutic dose did no harm. The only toxic effects sufficiently common to cause comment were mild gastro-intestinal symptoms. The Indian Medical Gazette (1935) talks of these as unimportant side-effects affecting 1 - 5% of people. One point of interest already mentioned is that the sumultaneous giving of atebrin and plasmoquine appears to increase the toxic action of both, especially the toxic effects on the gastro-intestinal tract. This is a well recognised phenomenon, to be discussed later, and no satisfactory explanation has ever been given for it. Thonnard-Neumann (1931) working on this problem of abdominal pain carried out X-ray examinations of the gastro-intestinal tract after administration of combined atebrin and plasmoquine and found a hyperperistaltic stomach with spastic contractions of the large and small bowel. Experimental poisoning of rats by Scudi et alia (1944) showed that huge doses were necessary to poison the animals and that food played a part in the keeping alive of the rats; fasting caused greatly-increased liver damage. In the fatal cases

there was no necrosis of the liver and the gastro-intestinal tract was distended with fluid of a serous character. In the great majority of fatal cases in the human no mention is made of these findings however. There is one other finding that is classed wrongly as a toxic effect, namely, a finding which is the most common of all the effects of atebrin - its skin-staining properties; it is a usual finding that all persons who are given atebrin treatment for more than a week develop a yellow discolouration of the skin; at first this was regarded as a toxic effect of atebrin upon the liver, the yellow colour being due to jaundice but this was soon proved to be wrong. Nocht and Mayer (1937) claim that the yellow colouration of the skin is a frequent secondary effect and that the whites of the eyes are not affected, the colouration being due to deposition of the dye in the skin and not a photosensitisation phenomenon such as is found with other acridine dyes. Field (1939) agrees and says that two to three per cent of individuals under treatment develop yellow staining which he claims also affects the sclerae. I consider this figure of two to three per cent is a gross underestimate and I consider that the sclerae are generally not stained. This staining is now accepted to be harmless. The question of whether or not it is really harmless will require to be reconsidered possibly in the future in view of the findings in recent years of marked skin reactions in

individuals taking atebrin.

On this question of affection of the eyes Findlay (1947) mentions that certain individuals in contact with atebrin dust have developed a curious corneal condition, a peppering of the whole surface of the cornea, this producing a difraction effect. Findlay (1947) also agrees that yellow staining of the skin is rarely seen on the sclerae; he also points out that this yellow deposit renders the tissues fluorescent.

Serious toxic effects in the human seemed, in the earlier reports very largely to be concentrated on the central nervous system. It is difficult to classify this and the other types of reaction to be reported; in a drug with such a very wide margin of safety as has atebrin the appearance of certain toxic effects in a very small number of people must necessarily raise the question of whether these toxic effects are an expression of low-grade hypersensitivity in the individuals affected. It is on this assumption that I proceed to discuss the remaining toxic conditions. I specifically exclude mild gastro-intestinal symptoms which are relatively common and may reasonably be presumed to be a true mild toxic effect in normal individuals, but the remaining toxic effects I class as under the heading of hypersensitivity reactions.

This qualification will almost certainly not be accepted but it is not really of primary importance that it

should be since we are interested in the problem only from the narrow viewpoint of whether or not atebrin is likely to have been responsible for the onset of the haemoglobinuria in our six cases, and the question of how the toxic reactions should be classified is only of importance in that if our theory of hypersensitivity be accepted then this type of reaction is obviously much more common than previously thought, and conceivably much increases the possibility of the onset of haemoglobinuria being a hypersensitivity phenomenon.

2. Toxic effects produced by atebrin in individuals hypersensitive to it.

Among the earliest of the more serious toxic effects associated with the giving of atebrin was that of toxic mania, i.e. "atebrin psychosis". We are classifying it here as a reaction found only in individuals temporarily sensitive to the drug - although the general belief is that it was a simple toxic reaction. According to Field (1939) Conoley was the first person to draw attention to this, mentioning it in an unpublished medical paper read at Kuala Lumpur in 1933. The condition appeared to be a true toxic mania developing shortly after the beginning of treatment and lasting at the most only a few days, leaving no residual abnormality. The suggested incidence was about 1 in 1,000 of individuals receiving therapeutic doses of atebrin. After the individual so affected

had recovered it was practically never possible again to produce mania, even by administration of heavy doses of atebrin. It seems to have been the experience of all workers, however, that this particular manifestation of toxicity of atebrin has become less common in recent years. The average incidence very roughly speaking was about one in a thousand before the outbreak of the 1939 war but since 1939 the incidance reported is very much less than this in spite of literally a millionfold increase in the use of atebrin from 1939 onwards. The references are mainly pre-1939, e.g. Nocht and Mayer (1939) who say that atebrin mania is a secondary factor, a condition of cerebral excitement similar to alcoholic delirium usually lasting a few hours. Among the few later reports are those of Bang et alia (1947) and of Findlay (1947). Bang et alia (1947) reported a series so unusual as to raise suspicions of some undetected and unreported factor at work. They report that of 110 patients treated with atebrin six developed cerebral symptoms on the fifth or sixth day of treatment. The treatment was, first day 1.2 g. atebrin, second day 0.8 g. atebrin, third to seventh day 0.4 g. atebrin daily. Four of the six patients had manic states with confusion and two had epileptiform convulsions. This series is quite out of keeping with anything ever reported in this connection and should be regarded with serious suspicion. Findlay (1947) does mention

toxic psychoses and also gives a surprisingly high figure. In a total of 89,168 cases quoted by fifteen authors he stated that the incidence was 1.4%. these apparently occurring during and after malaria and its treatment, never with prophylactic atebrin. Usually the onset is sudden, and manic in character, lasting one day to five weeks, and generally followed by complete recovery. In Findlay's series the dose previous to the onset varied, e.g. in one case as low as 0.5 g. This last statement is in keeping with a hypersensitivity factor in the causation of the condition. Findlay's figure of 1.4% is very high indeed in view of our previous remarks. Involvement of the nervous system in ways other than the production of mania is mentioned by:

Field (1939) who states that fatal doses of atebrin in two guinea pigs caused convulsions;

Markson and Dawson (1945) who mention coma;

Bang et alia (1947) who record two cases of epileptiform convulsions;

Findlay (1947) who mentioned that rarely one may find myeloradiculoneuritis and polyneuritis.

This last author thinks that these nerve changes and mental changes may be caused by atebrin interfering directly with the metabolism of the nerve cell but it is difficult to see how this can be so when so many of the cases developed such conditions after a very small amount of the drug.

The next type of reaction is dermatitis. We have mentioned how toxic mania was much discussed before 1939 and

is relatively little discussed or mentioned nowadays; this is the reverse of what has happened with skin lesions due to atebrin. Before 1939 it was a subject practically not to be seen in literature whereas today it is by far the most commonly discussed of the toxic effects of atebrin. There seems little doubt that since 1943 the condition has become apparently much more common. The picture generally follows a fairly definite course and produces a fairly uniform type of skin lesion; it is very unlikely that this was overlooked before; my own experience may be quoted. At one time I was specialist in charge of dermatological wards in Shaiba, in Iraq, and we had rarely less than thirty bed patients at any one time, but the type of lesion which one later came to associate with atebrin was not seen in any numbers in the skin wards. One of the few references to this particular type of toxic upset is one which has been overlooked and its significance has not been appreciated. It is that of Fields (1939) who reported that in Malaya even with prophylactic doses of atebrin there had been seen sometimes a dry irritable scaly thickening of the soles and the palms and he mentioned that Italian workers report drying of the nasal mucosae. This was significant when one considers the repeated reports of skin reactions in later years. Other workers who have vaguely mentioned the subject of skin abnormalities at that time were:

Nayadu (1937) who described two cases of giant urticaria thought to be related to the taking of atebrin, and Storey (1938) who reported one case of macules on a background like a typhus case.

Among the earliest of these later reports is that by Mohr (1944). He talks of reviewing the available literature (possibly the literature of the Allies was not readily available), and he concludes that with the usual dosages of atebrin evidence of true hypersensitivity is very rare; in his review of the literature which includes records of large numbers of German troops, he could find only three cases of "idiosyncratic allergic reactions" to atebrin; one feature was resistant urticaria following atebrin administration, with the skin tests positive to atebrin, and also to quinine. Custer (1946) was of the same mind in his report on aplastic anaemia thought to be related to the taking of atebrin. Twenty five of his cases of aplastic anaemia showed a preceding atypical lichen planus; he also thought that the condition may be due to a slowly acquired hypersensitivity with the antigen possibly being formed through inter-action of the drug and protein.

Kierland (1946) mentions dry eruptions resembling lichen planus developing in individuals three to six months after the beginning of atebrin prophylaxis; some showed aplastic anaemia with death.

Williams (1947) considers the syndrome to be an established fact and considers the incidence to be 1 in 2,000; in a series reported by him the initial symptoms appeared after mepacrine had been used for two to three months; he considers that the wide distribution of the disease in his cases, its appearance in different races, its independence of climate and its invariable association with the use of atebrin seems to prove the direct causative role of the drug.

Russell (1947) in discussing a case of skin lesions in England suggests that the condition is the result of cumulative action of atebrin and does not consider there is any evidence of idiosyncrasy.

Findlay (1947) discusses skin lesions associated with the taking of the drug; he particularly discussed lichenoid dermatitis. He says the complex has been seen in all theatres of war and thinks that climatic and geographical factors may have played a subsidiary role; the skin lesions assumed many forms, sometimes lichenoid and sometimes eczematoid; they even showed acute explosive generalised exacerbations, sometimes preceding a secondary exfoliative dermatitis; histological examination showed the lesion to be in the basal layer. He also mentioned several forms of dermatitis. In eighty per cent of the cases the lesion had developed within seven months of beginning the drug, but he considers that there has been much evidence that the drug is NOT the primary factor. He

thinks that the lichenoid eruption may be the result of action of various agents on tissues devitalised by long-continued dosage of atebrin but does consider that the eczematoid type of reaction is simply a drug sensitivity and claims that patch tests were positive.

Agress (1946) quotes five cases of hepatitis and exfoliative dermatitis in Chinese which the author thinks were the result of atebrin administration; this represents an incidence of 1 in 3,000; three of the five patients died. A striking feature was a rash from the second to the tenth day after as little as 0.1 g. There was no increase in urobilinogen.

In view of the dosage it is difficult to explain these cases on anything other than a basis of individual hypersensitivity to the drug.

This completes the few notes on the skin lesions associated with the taking of atebrin. The consensus of opinion appears to be that these lesions are a hypersensitivity phenomenon, not unlike the skin lesions which have been reported in individuals engaged in the handling of the drug. A factor on which information is lacking is the type of climate in which the lesion developed. This type of skin condition seems to have been rare in the Western Desert but was reported with significant frequency in the Burma jungles; there has not been enough information published on this point to enable one to express a definite opinion.

So far we have not mentioned what records exist of the effect, if any, of atebrin on the red cells. From our point of view this is an important question. Expert evidence of the effect of atebrin on red cells is meagre.

Hecht (1933) states that atebrin does not cause the formation of methaemoglobin either *in vivo* or *in vitro* and that it does *not* cause haemolysis.

Zylmann (1944) reports *in vitro* and *in vivo* experiments on the haemolytic properties of atebrin and other synthetic antimalarials, and reviews the German literature; he found that atebrin had a lytic effect on all red cells from 1/50 - 1/200 molar whereas plasmoquine had little effect. He also claimed to have found that when specific amboceptor and atebrin were injected together haemoglobinuria resulted and claimed that atebrin had a strengthening effect on the haemolytic action of amboceptor.

Birnbaum et alia (1946) report that red cells from malarial patients lake more readily in bile solutions than do normal cells and claimed that atebrin, in common with quinine and plasmoquine, accelerates this haemolysis in the presence of bile. They claim that atebrin exerts a particularly remarkable enhancing effect, e.g. hastens lysis in a dilution of 1 in 200,000 in association with bile; the authors however admit that the drug by itself is non-haemolytic *in vitro* even in

high concentration.

Mushett and Siegel (1946) were able to produce definite haematological changes in rats, mice, hamsters and various other animals, including monkeys. These take the form of basophilic inclusions both in the lymphocytes and in the red blood cells; the doses of the drug used however were about fifty times the therapeutic dosage.

These are the findings in experimental cases; there are certain records available showing the effect in human beings under treatment; among them are:

Naumann (1934) reports a case of paroxysmal haemoglobinuria in whom an attack of haemoglobinuria could be precipitated by quinine and plasmoquine but not by atebrin.

Indian Medical Gazette (1935) Editorial, points out a fallacy, saying that among immediate by-effects recently attributed to atebrin was haemoglobinuria. They point out that in such cases closer investigation has nearly always shown that plasmoquine was also being administered. This is quite correct. I have analysed several of the cases to be found in literature with titles of "atebrin haemoglobinuria" and the like, e.g. Moir (1933), and I have found that plasmoquine was also being given. The Editor concludes that in atebrin administration while one to five per cent may show unimportant side-effects, with ordinary dosage this

drug will not cause serious symptoms in more than 1 in 1,000. He does not say what he means by serious symptoms.

Chopra (1936) specifically mentions that atebrin has a wide margin of safety with the toxic symptoms mainly in the gastro-intestinal tract and that blood changes had never been observed.

Napier (1938), on the other hand, states that occasionally an idiosyncrasy is to be found to atebrin but here he was including yellow colouration of the skin which can hardly be regarded as idiosyncrasy. He claims that personal idiosyncrasy may produce, among other symptoms, haemoglobinuria but gives no support for his statement.

Custer (1946) quotes certain evidence which though not a support for the suggestion that atebrin does cause haemolysis, does certainly suggest that it may have an effect on the haematopoeic tissue. He found that aplastic anaemia was the cause of a disproportionately high death rate in areas where atebrin prophylaxis was in force. He considered as we have already said that an antigen-antibody reaction was involved.

Findlay (1947) also mentions aplastic anaemia and agranulocytosis among the rarer toxic manifestations associated with the taking of atebrin.

Maegraith (1946a) however considered that there was no evidence of any value that atebrin is a haemolytic drug or has

any effect on haemolysis in vivo; he considers the drug can be taken as a suppressive for very long periods without haemolytic effects and that it can be given in large doses even intra-venously without haemolysis.

This portion can be summarised by saying that atebrin is apparently capable of causing toxic effects in two ways, when given to normal individuals and when given to hypersensitive individuals. This latter category has only recently come to be recognised. Atebrin is seen to be a remarkably safe drug, the toxic dose being many times the therapeutic dose; in view of this we departed from the generally accepted theories and classed as ordinary toxic side-effects only the mild gastro-intestinal symptoms found in a significant proportion of normal individuals taking the drug - skin staining has not been included as a toxic symptom since it is merely a deposition of dye.

Other effects have been classed as examples of hypersensitivity, a classification not likely to be generally accepted. The first of such reactions, previously thought to be a simple toxic reaction, is atebrin psychosis, found at one time in about 1 in 1,000 cases though now apparently much less common excepting in certain series where the incidence is high. Another effect discussed is the type of skin lesion which has come increasingly to be reported following atebrin administration; the lesions are generally lichenoid in character; the

consensus of opinion is that these skin lesions are another illustration of low-grade hypersensitivity. The effect of atebrin on the red cells was then discussed, the conclusion being that there is evidence that atebrin in no way caused haemolysis in vivo and that it may cause aplastic anaemia, a rare manifestation of hypersensitiveness.

There is one other factor to be considered here, one which has already been mentioned in brief in Chapter II, namely the possibility that we had been dealing in Razmak with toxicity resulting from a batch of atebrin tablets of greatly increased toxicity. This batch variation in toxicity is not an uncommon feature in various organic arsenical preparations but it has never been reported to have occurred with atebrin. We have described the care taken to ensure that each man received only the dosage ordered, and we have said how the tablets were kept under lock and key, and in coloured bottles. Furthermore, no increase in toxic side-effects was noted to be a feature of the treatment of malaria casualties during the three-month period July to September 1938, the period during which the haemoglobinurias developed. The atebrin used in the six cases came from three separate batches of the drug which had also been used in large numbers of other patients. Tablets from the last batch of atebrin were the only ones available for testing when it was decided to do this; ten volunteers, the writer included, took 1.0 g. each

for seven days without ill effect except gastro-intestinal discomfort of mild degree; this of course is no real test and arrangements were made with the I.G. Farbenindustrie through the local representative in India for experiments to be put in hand to test the toxicity of the remainder of the atebrin; but 1939 had well advanced and events necessitated abandonment of the tests.

This likelihood of increased toxicity of one batch or rather one group of batches is so very unlikely and the remainder of the evidence submitted is so strongly in favour of the harmlessness of atebrin that we are entitled now to conclude that atebrin played no part in the causing to develop of the haemoglobinuria which developed in the six patients undergoing treatment for malaria in Razmak in 1938.

This does not, however, prove whether or not atebrin acted as an exciting factor to produce blackwater fever; this shall now be considered.

Atebrin and Blackwater Fever.

We have seen in the previous section that atebrin is a very safe drug with a wide safety margin and that while in a rare case it may produce phenomena of hypersensitiveness it has never been found to play a part in the production of haemoglobinuria per se.

The question then arises whether it may have precipitated an attack of blackwater fever in our six cases of haemoglobinuria, making the diagnosis "blackwater fever related to atebrin administration".

Atebrin has never had the grim notoriety possessed by quinine in this matter, e.g. Chopra (1936) considers that atebrin can be given to patients suffering from blackwater fever without ill effects and Nocht and Mayer (1937) say unequivocally "this much is certain", namely that atebrin is a perfect substitute for quinine as a prophylactic in cases of aversion or idiosyncrasy and in threatened blackwater fever.

Other workers are less sanguine, e.g. Foy and Kondi (1937a) say that atebrin is capable of firing-off an attack of blackwater fever but stress the point that the action is non-specific; as will be seen below they had some reason to make this statement. This was still the opinion of Foy and Kondi in 1943 where they repeat that atebrin may pull the trigger in blackwater

fever but in this respect it is only one of several substances capable of doing so.

Low and Fairley (1942) also claim that atebrin infrequently precipitates blackwater fever.

These are all expressions of opinion; what are the facts on which they are founded? The first few case-reports are not above criticism, e.g.

Nagelsbach (1933) reported a case of blackwater fever developing in a woman taking atebrin; one complicating factor is that she was in labour.

Moir (1933) and Murray (1934) both writing from West Africa each reported two cases who developed blackwater fever after atebrin but analysis of the information given shows that in Moir's cases the individuals were given atebrin plus plasmoquine, and in Murray's cases atebrin plus plasmoquine plus quinine; the authors give no reasons for picking on atebrin as the cause. Their cases cannot be accepted as examples of blackwater fever developing during the administration of atebrin. The next three cases are in a different category; they are the cases reported by:

Foy and Kondi (1937a). They began by saying that there is a general belief that atebrin administration is free from risks of precipitating haemoglobinuria in potential blackwater fever patients and that very

few such cases have been reported. They then reported three cases of haemoglobinuria occurring during or after normal standard atebrin treatment.

No quinine had been taken by any of the patients within two to nine months preceding the haemoglobinuria.

Case I. Male, aet. 5. P.vivax +++ , patient given 0.2 g. atebrin daily; after 0.7 g. (i.e. after three and a half days) developed haemoglobinuria. Died on the fourth day.

Case II. Male, aet. 26. P.vivax + , patient given 0.3 g. atebrin daily for five days; thirty-six hours after stopping atebrin, patient passed 800 ccs. urine, showing marked haemoglobinuria. This continued for four days, finally cleared up.

Case III, Female, aet. 38. P.falciparum ++++: 0.3 g. atebrin daily for five days; forty-eight hours after stopping atebrin passed red urine, once only; no recurrence.

The authors then concluded, as we have quoted, that atebrin is capable of firing-off an attack of blackwater

fever. They stress, however, that from this it should not be inferred that the drugs quinine, atebrin and plasmoquine are the direct cause of the haemoglobinuria.

The next case is that of Lucherini (1938) who reports what he claims to be the first case in Italy of haemoglobinuria associated with atebrin treatment of malaria. The patient was suffering from chronic malaria; during the three days preceding the attack the patient had been taking 0.3 g. daily of atebrin, i.e. after a total dose of 0.9 g. atebrin he developed haemoglobinuria; there is one point in Lucherini's case; the leucocyte count was 44,000 per cu. mm., and no reason is given for this.

Abbott (1946) quotes the latest of this type of case, and in some ways it is the most striking. The patient was an Italian nun, aet. 34, living in an area of hyper-endemic malaria; this woman was about to go on a long journey and began taking prophylactic atebrin. She began with an unusually heavy prophylactic dose of 0.3 g. atebrin daily for five days ⟶ 0.1 g. daily; previous to beginning atebrin she had been taking 5 grains quinine two to three times weekly. She had had one attack of mild fever in 1939.

On the seventeenth day after beginning prophylactic atebrin, after she had taken a total dose of 2.7 g. in twenty-seven doses, the patient developed what Abbott calls

"blackwater fever" with the temperature 102°F.; the blood films were negative. Next morning 100°F., pulse 108; skin and eyes yellow, spleen enlarged one finger, → 1000 hours urine black like blackwater fever; injection of 0.3 g. atebrin into the buttock plus phenobarbitone 2 grains. 1330 hours urine lighter, but still with haemoglobin → later specimens urine again dark red. Next day another 0.3 g. atebrin intramuscularly night and morning and urine clear of haemoglobin.

The points particularly to note are that the patient developed haemoglobinuria at a time when the blood level according to Fairley (1945) is optimum and when neither blackwater fever nor malaria should occur. Again, Abbott diagnosed the condition as blackwater fever but did not demonstrate the malarial parasite. The rise in fever can quite well have been explained by the lytic process itself. This case is not above suspicion even although the individual was a nun; certain cases are reported like this by Simeons (1936) whose notes are given below in some detail to prove the possibilities of other factors being involved. Simeons tried the effect of mass treatment of injectible atebrin. 5650 people were treated with two daily injections of atebrin musonate; each injection was given twenty-four hours apart.

The dosage of each injection was:

strong healthy adult males 9 ccs (9 ccs. = 0.3 g. atebrin)

" " " females 8 ""

After the second injection every patient was given 0.02 g. plasmoquine orally for three days. The only serious incident was four cases, two fatal, of haemoglobinuria developing one day after stopping the plasmoquine, i.e. four days after stopping atebrin. All four were Mussulmen and three belonged to the same household. The two fatal cases were found to have been taking treatment from a local hakim for syphilis. The others who survived also were cases of V.D. but claimed that they had not been taking treatment.

It must not be inferred that we suggest any literal resemblance between the two sets of circumstances, those cases of Simeon's and the case of Abbott's; Simeon's cases are quoted to show how easily other factors may lead to mistaken assumptions.

This brings to a close the discussion on atebrin as a possible precipitating factor in blackwater fever; we have seen that atebrin in this respect also is a very safe drug and there are less then ten cases on record in which it appeared to be related, in time of onset, to an attack of blackwater fever; when one considers the many millions of individuals who have been treated with atebrin then by the law of averages we should have expected many more cases of blackwater fever to have developed during atebrin treatment even as a chance relationship; the fact that we can find less than ten cases strongly suggests not only that atebrin does not favour an

attack of blackwater fever but that it actually serves to prevent development of blackwater fever.

In the few cases of blackwater fever on record who developed this disease during atebrin treatment, the blackwater fever developed in spite of atebrin, not because of it. Atebrin therefore played no part in the production of our six cases of haemoglobinuria.

Summary.

Atebrin is an acridine derivative and it is by virtue of this stable basis that it is less easily broken up in the body than is quinine; this also accounts for its slow excretion. Quinine has proved better in controlling the immediate symptoms of an attack of malaria but atebrin gives much superior late results. Its mode of action on the parasite is not known. Like quinine it has practically no effect on the gametocytes of malignant tertian malaria. The majority of courses e.g. the course for the Army in India was a seven-day course of atebrin 0.3 g. daily. After atebrin is given prophylactically it fails to prevent development of overt benign tertian malaria on the cessation of treatment. This confirms our findings, Chapter II, on the use of prophylactic atebrin, which means that individuals who were on the Kharre

Column and developed benign tertian malaria shortly thereafter, were suffering from the effects of disease acquired on the Column; again, a five-day course of atebrin 0.3 g. daily fails to clear the peripheral blood of parasites in a proportion of cases, twelve per cent in our experience. This means that the six patients of ours who developed haemoglobinuria shortly following a five-day course of atebrin for malaria may have had parasites still present in the peripheral blood at the completion of the course.

The toxic effects of atebrin were then considered, firstly the toxic effects of an overdose in normal individuals and secondly the effects of atebrin administration in hypersensitive individuals; atebrin was shown to be a remarkably safe drug with the toxic dose many times the therapeutic dose; it was because of this that we felt constrained to class only mild gastro-intestinal symptoms as true toxic effects in normal people. We have classed all other toxic effects as being examples of hypersensitivity, a classification which is not likely to be acceptable to the majority of workers; the first of such reactions, and one previously recorded as a toxic effect, is atebrin psychosis, found at one time in about 1 in 1,000 cases receiving treatment, though now apparently much less common, excepting in a very limited number of series where the incidence is remarkably high; no cause is known for this condition.

The next type of hypersensitivity reaction is the skin reaction to atebrin, a type of toxic effect recently given much prominence. These skin lesions are generally lichenoid in character but may resemble the lesions of iodism: the consensus of opinion is that the skin lesions are an expression of hypersensitivity.

The effect of atebrin on red cells was then discussed and the conclusion was that there is no evidence that atebrin in any way causes haemolysis in vivo although it may on very rare occasions be the cause of aplastic anaemia in hypersensitive individuals; from this we inferred that atebrin played no direct part in the production of the intra-vascular haemolyses we are considering.

The last section of Chapter VII deals with the part, if any, played by atebrin in precipitating blackwater fever. The general impression is seen to be that atebrin in this respect also is a remarkably safe drug, and few workers are prepared to say that it is capable of pulling the trigger. A perusal of the literature shows that there are on record less than ten cases of blackwater fever which appear to be related in time of onset to the taking of atebrin. When one considers the many millions of individuals who have taken atebrin, then by the law of averages we should have expected many more chance cases of blackwater fever developing independently of but during atebrin treatment. Therefore the few such cases that

we have found are not only NOT an argument in favour of the fact that atebrin precipitates blackwater fever but are in effect a very strong argument indeed in favour of suggesting that atebrin has a powerful effect in preventing the onset of blackwater fever, i.e. the few cases on record who developed blackwater fever during atebrin treatment developed the disease in spite of atebrin, not because of it.

We have the strongest grounds for stating that atebrin played no part, direct or indirect, in the production of the haemoglobinuria developing in six patients in Razmak; the diagnosis still remains as "intra-vascular haemolysis, cause unknown."

CHAPTER VIII.

Plasmoquine as a possible cause of the Razmak cases of haemoglobinuria.

In our cases under discussion quinine and atebrin have been shown to have had no direct part in the production of the haemoglobinuria from which our six patients suffered.

The diagnosis in the six cases of haemoglobinuria in Razmak remains at present as "Intra-vascular haemolysis, cause unknown", and it is now necessary to consider whether plasmoquine acted directly or indirectly as a causative factor to produce this intra-vascular haemolysis. Plasmoquine was synthesised before atebrin, and should have been considered before the latter but it was given after atebrin in the treatment of the patients and the order followed here is the chronological order of administration of the drugs, i.e. quinine, atebrin, plasmoquine.

History of the discovery of plasmoquine.

The hostilities of 1914-1918 brought home to the Germans the need for making themselves independent of quinine in their next campaign. Research was begun at the I.G. Farbenindustrie in Elberfeld; the two most promising lines were the organic arsenicals, which had been found to have an effect on *P.vivax*, and methylene blue which acted on *P.malariae*;

methylene blue was chosen as the starting point and so successfully was the search prosecuted that by 1924 Schulemann, Schoenhofer and Wingler were able to report synthesis of a substance, originally called beprochin, now called plasmoquine. This was a marked achievement marred only by the motives actuating the work. This was one of many compounds tested, Roehl being responsible for the testing which was carried out on canaries infected with P.relictum. Roehl found plasmoquine to be sixty times more effective than quinine; his results were reported in Roehl (1927). Sioli (1926) was the first to use the drug in humans after inoculating malaria into patients suffering from G.P.I.; even at this early stage in the use of the drug he mentions cyanosis and one of his patients apparently developed methaemoglobinuria. Muehlens (1926) then followed with a report of the effect of the drug in 134 patients naturally infected with malaria. These workers established the effectiveness of plasmoquine on P.vivax and P.malariae. They also noted its effects on the crescents of P.falciparum; Urchs (1928), the representative of the Bayer Company in India, talks of plasmoquine as N-diethyl-amino-isopentyl-8-amino-6-methoxy quinoline. We have already given the graphic formula for plasmoquine, plate No. XVIII, page 430, and in plate No. XIX, page 439, we see the effect of the drug in malaria. The graphic formula shows that plasmoquine has affinities, both with atebrin and quinine; the side chains of plasmoquine and atebrin are identical although placed in different positions on the

bases, and the basis of plasmoquine is the same as that of quinine, namely the quinoline ring, unlike the acridine ring which is the basis of atebrin. It may be noted that the formula, p. 430, is plasmoquine base, the salt used orally being 2 methylene-dioxynaphthoic acid.

The Pharmacology of Plasmoquine.

The graphic formula has been given on p.430. The dosages initially recommended were much too large; had atebrin been the drug under test this would not have been of any great importance, but plasmoquine is a very different substance.

Muehlens (1926) who was probably the first to use the drug therapeutically in humans, used daily dosages of 0.8 g. → 1.0 g. Sinton and Baird (1928) who were responsible for research on malaria in India at the time said that the original letter from the makers recommended the following course of treatment:

Plasmoquine 0.02 g. three or five times daily for not more than five days in succession. These authors recommended that after-treatment should be carried out much in the manner as had been recommended for quinine treatment, namely five days plasmoquine then four days rest → three days plasmoquine and four days rest → two days plasmoquine and five days rest → two days plasmoquine and five days rest → two days plasmoquine and five days rest, i.e. fourteen days treatment and twenty-three days rest; therefore

the course was thirty-seven days. The makers did recommend however that plasmoquine should be stopped immediately if gastric pains or cyanosis of lips developed and that plasmoquine should not again be administered until these toxic effects should have passed off. This recommendation was an indication that toxic effects were to be expected and to be treated with respect.

The tablets as first manufactured by Bayer each contained 0.02 g. plasmoquine; later, tablets of half this strength were introduced for use, i.e. 0.01 g. tablets; unfortunately 0.02 g. tablets were not withdrawn from manufacture and use until 1943 and there is no doubt that some at least of the toxic reactions reported following administration of plasmoquine were due to the accidental use of the 0.02 g. strength tablet in mistake for the 0.01 g. This was one suggestion offered to explain certain untoward effects of plasmoquine in the Western Desert.

It is interesting to know that from the beginning, and after experiments with patients, it was recognised that the giving of quinine with plasmoquine was useful in preventing cyanosis, e.g. Schulemann and Memmi (1927); these authors already recognised the methaemoglobinaemia to be due to plasmoquine.

Eicholtz (1927) experimenting with animals confirmed the effect of quinine in lessening the toxicity of plasmoquine.

This is discussed more fully later in this chapter. The makers recognised this by the introduction of compound tablets containing 0.01 g. plasmoquine and 0.125 g. quinine sulphate.

We have been using the term "plasmoquine" rather loosely and it is essential at this point to make clear that in our remarks above, and in the subject matter which follows, the term "plasmoquine" is used to denote the substance issued for oral use by the manufacturers; this in point of fact is not plasmoquine proper but is plasmoquine naphthoate, or more properly plasmoquine-2-methylene-di-oxynaphthoic acid, of which plasmoquine base is forty-five per cent. This has led to much confusion in literature where a small number of authors writing on the subject have quoted "correct" weights in describing plasmoquine dosage, i.e. the weight of the plasmoquine base present in the naphthoate compound; by far the most common method however is to quote the dosage given of the salt and this is the method followed here.

In contradistinction to the amount of literature available on quinine, and to a lesser extent, on atebrin the knowledge referring to the observation of, action in, and excretion of plasmoquine by the body is remarkably incomplete. It almost seems that after the synthesis of plasmoquine all efforts were then directed to finding a more efficient substitute, later produced in the shape of atebrin, rather than in research on plasmoquine itself.

From the meagre information obtainable it is not clear how and where plasmoquine is absorbed into the body when taken orally, nor is it known how the drug circulates. Chopra (1936) says that the fate of plasmoquine in the body is not known although there is little doubt that it is partly excreted in the urine; Edmunds and Gunn (1936) agree with this, and merely remark that the drug is disposed of slowly, some probably being destroyed in the body; Goodman and Gillman (1941) also remark that variable amounts of plasmoquine are destroyed in the body; the excretion is slow, and the continued administration will have a cumulative toxic effect. The last-named authors mention that the commonest gastro-intestinal symptoms following plasmoquine administration are nausea, epigastic pain and diarrhoea; Manson-Bahr (1927) had previously reported this even with small doses of plasmoquine and thought that rapid contraction of the spleen may be responsible; Thonnard-Neumann (1931) carried out X-ray examinations of the gastro-intestinal tract of individuals after they had been given combined atebrin and plasmoquine dosage and found spasm of the bowel with hyper-peristalsis of the stomach. This of course may have been due to the atebrin or to the combined treatment. Chopra, however, reported that plasmoquine in therapeutic dose had no action on the uterus although in large doses it does produce contractions of isolated uteri of laboratory animals. Field (1939) thought that the cause of the abdominal pains was unknown and that

plasmoquine has no effect on the intestinal mucosa and muscles of the gastro-intestinal tract. Chopra (1936) also stated that urobilinogen appears in the urine practically simultaneously with the administration of the drug and persists for several days. He also quotes certain blood changes but like some of the above findings this probably should be classed as expressions of poisoning; this aspect of the problem is discussed more fully below.

The method whereby plasmoquine produces its effect on the malaria parasite is not known either. Its effects on the various stages of the parasite differ from the other antimalarial drugs in that it has a lethal effect on the gametocytes of P.falciparum, yet strangely it has practically no effect on the schizonts of P.falciparum. This drug is a polyvalent gametocide and is schizonticidal in benign tertian and quartan infections. Very little work has been reported on the question of how plasmoquine produces its effects on the parasites, whether directly or indirectly.

Chopra (1936) suggests that both in benign tertian and quartan malaria a dose of about 0.05 g. will cause parasites to disappear from the peripheral blood from the second to the seventh day; with plasmoquine alone there is a high relapse rate however, even after prolonged dosage. In malignant tertian malaria the drug affects only the sexual forms. Sollmann (1936) states that in bird malaria the drug appeared to act by

interfering with the development of the plasmodia. Field (1939) has suggested that certain strains of P.vivax may be resistant to plasmoquine. There is little else in the literature except the question of plasmoquine dosage. Fletcher (1933) discussed this; the dosages used by Schulemann in 1926 were heavy, being 0.06 g. daily; this was soon recognised to be toxic - it is interesting here to note that a "modern" text-book of pharmacology published recently still recommends this toxic dosage and suggests it should not be given for more than fourteen days!

Two of the few modern papers on the problem of the effects of plasmoquine alone are those of Craige et alia (1947) and Innes (1947). In the paper by Craige et alia a report is given of experiments carried out on American volunteers inoculated with P.vivax malaria, using infected mosquitoes. The numbers of individuals treated were small and treatments used were two in number, one being plasmoquine alone for fourteen days, dosage varying from roughly 0.03 g. to 0.12 g. daily and followed immediately by eight days quinine sulphate, dosage 30 grains daily; with this course the patients relapsed. A combined course was also tried and is discussed below. In the article by Innes he discussed findings in a large series of benign tertian malaria patients. Several types of treatment were tried and in that in which plasmoquine was used alone, in dosages of 0.03 g. daily, the parasites persisted in the blood for 3.1 days. Plasmoquine was found to have a slow action but

was actively schizonticidal (the patients were suffering from benign tertian malaria). The results produced by this author are better than those produced by Craige et alia and are more in keeping with previous work. Long previously to the work reported above, more or less general agreement had been reached on the way in which plasmoquine should be used namely, given in one of two ways; as a five-day course, alone, at the end of an atebrin course; and as a longer course ten to twenty-one-days combined with quinine. The first method of administration, i.e. the five-day course of 0.02 g. (or 0.03 g. daily) was a public health measure, the idea being to utilise the gametocidal action of the drug in an attempt to sterilise the blood of patients under treatment for malaria. The Army method of treatment is an example of this. The second method, the long course, was that used in the treatment of chronic relapsing cases. This method of combining quinine with plasmoquine in the treatment of patients dates from the time of introduction of the drug, i.e. since 1927, the manufacturers producing a compound plasmoquine tablet containing 0.01 g. plasmoquine salt plus 0.125 g. quinine. The idea was that quinine should serve to lessen the toxicity of plasmoquine, e.g. the findings in this connection by Schulemann and Memmi (1927) and of Eichholtz (1927) are recorded, p. 538. They reported that the addition of quinine to plasmoquine is useful in preventing methaemoglobincythaemia. Manson-Bahr (1927)

confirmed this, and it was with this in mind that the combined dosage was recommended. It soon came to be recognised, however, that quinine when given with plasmoquine also did more than protect the individual; it seemed to increase the effect of the plasmoquine on the malaria parasite. Since our patients received plasmoquine alone at the end of an atebrin course this particular problem of the effect of combined quinine plus plasmoquine does not directly concern us, but is worth consideration in the hope that it may yield information of value since the information obtained, so far, on the effect of plasmoquine alone is very meagre. Sinton, working at the malaria treatment centre at Kasauli in India carried out intensive tests of this new drug plasmoquine and on 6.1.1929 issued an interim report suggesting that treatment of malaria with plasmoquine should be by the giving to patients 0.04 g. plasmoquine daily with grs. 20 quinine daily, both for a period of twenty-one consecutive days. This was put into immediate operation all over India and in 1931 Manifold published the results of this treatment in 3187 patients of whom 1298 were British soldiers. Three of the cases developed haemoglobinuria. By that time, however, Sinton (1930) had published a report recommending smaller doses still of plasmoquine. In this report Sinton concluded that the best method of treatment of malaria by plasmoquine was to give the plasmoquine in continuous oral treatment and small daily doses; he did say

that while the dose recommended was 0.04 g. daily with 20 grs. of quinine daily he was considering reducing the plasmoquine dosage to 0.03 g. daily, and in fact later in the same work he recommended for chronic cases of malaria a twenty-one-day course of 0.03 g. plasmoquine daily plus 30 grs. quinine daily. It is a pleasure to record that since that time the value of Sinton's recommendation has been realised in spite of the strong criticism initially evoked by his suggestions. The Army in India officially adopted the twenty-one-day course on 27.4.1933. The D.M.S. India ordered a further reduction in the dosage of plasmoquine to be given to the Indian troops. For them the dosage was reduced to 0.02 g. daily for twenty-one days; it remained at 0.03 g. daily for British troops. This type of course, long-continued administration of small doses of plasmoquine combined with quinine, has been used with little variation since then although it fell into disfavour, quite wrongly, for a time during the 1939-45 war. The treatment is effective, e.g. Sinton (1935) stated that since the introduction of the quinine-plasmoquine treatment throughout the Army in India the Kasauli Centre for resistant benign tertian malaria in British troops had had to be closed. It should specially be noted that the Indian Army authorities allowed the last fourteen days of treatment to be administered to individuals as out-patients.

Support was not long in forthcoming for the methods

suggested by Sinton, e.g. Fletcher (1933) said that the standard treatment of malaria in the United Fruit Company plantations in America was 0.02 g. plasmoquine daily plus grs. 24 quinine daily for six days; they record this treatment as safe enough to be given to a whole population with special medical supervision. Chopra also spoke strongly in favour of the combined treatment; he said that when quinine and plasmoquine are mixed they appear to reinforce each other's action, e.g. the toxic action on paramoecia is intensified. He said that not more than 0.04 g. daily should be given to ambulant cases and this dosage should not be given for more than six days, with daily medical supervision. He considered plasmoquine to be well tolerated in cases of quinine sensitiveness and of blackwater fever. The fourth general report of the League of Nations Malaria Commission (1938) considered the combined quinine-plasmoquine treatment to be one of the most efficacious methods of treating benign tertian and quartan malaria, even using plasmoquine 0.03 g. twice weekly; they considered this method greatly reduced benign tertian relapse. This was in marked contrast to previous opinions from this quarter.

Field (1939) was also in favour of the combined treatment which, he thought, should be for about fourteen days, with 0.03 g. plasmoquine daily and 1.0 g. quinine daily; this is the type of treatment used by Most et alia (1946) who treated

seventy two patients with quinine-plasmoquine for fourteen days with grs. 30 quinine daily and 0.03 g. plasmoquine daily except on the first day when the quinine dosage was grs. 45; they had no toxic effects. Craige et alia (1947) gave a small series of five patients 0.03 g. plasmoquine daily and grs. 30 quinine daily for twenty-eight days; three of the five relapsed. This difference is not significant in view of the numbers involved. Innes, dealing with much larger figures, gave many of his cases grs. 30 quinine daily and 0.03 g. plasmoquine daily for ten days with ten per cent relapse in six months.

One example of the safety of the combined treatment is the report of Willoughby and Aslett (1931):
They used beprochin compound (i.e. plasmoquine 0.01 g. plus 0.125 g. quinine) tablets and gave:

(1) Two tablets twice daily (i.e. 0.04 g. plasmoquine daily plus grs. 10 quinine daily) for seven days ⟶ four days rest.

(2) Two tablets twice daily (i.e. 0.04 g. plasmoquine/plus grs. 10 quinine daily) for seven days ⟶ four days rest.

(3) Two tablets twice daily (i.e. 0.04 g. plasmoquine daily plus grs. 10 quinine daily) for seven days ⟶ four days rest.

(4) Two tablets twice daily (i.e. 0.04 gl. plasmoquine daily plus grs. 10 quinine daily) for seven days ——→four days rest.

(5) One tablet three times daily for seven days (plasmoquine 0.03 g. daily and grs. 7 quinine daily)

i.e. this is a total dosage of plasmoquine 1.33 g. and quinine grs. 329) in 51) days.

To quote a personal experience I have used a combined treatment in many hundreds of cases of malaria and cannot remember any serious complication; this was particularly striking when I was in charge of 1200 beds for tuberculous patients for eighteen months and gave the combined treatment for ten days for chronic malaria which was very common, yet these patients, gravely ill and seriously under-weight, did not suffer from severe toxic effects.

This discussion on the pharmacology of plasmoquine is sketchy and incomplete; we do not know how the drug affects the parasite but it definitely is effective on the gametocytes and is the only one of the antimalarial drugs to affect the crescents of malignant tertian malaria. When quinine is added to plasmoquine it increases its effectiveness and this combined treatment formed the basis for the majority of methods of treatment of chronic malaria.

The toxicity of the drug should now be considered.

The toxic side-effects of plasmoquine.

In Chapter VII the discussion on the toxic effects of atebrin opened with the statement that atebrin is a safe drug with a wide margin of safety between the therapeutic and the toxic doses; it was also seen that atebrin has remarkably few toxic side-effects. This is in marked contra-distinction to the facts concerning plasmoquine. Plasmoquine is a dangerous and highly powerful drug, with a narrow margin of safety between the therapeutic and the toxic doses. It was this probably more than any other factor which stimulated the search that culminated in the synthesis of atebrin. In the two preceding chapters, VI and VII, the toxic effects of quinine and atebrin were considered under two headings, namely the toxic effects of overdosage of the normal individual, and the reaction resulting from the administration of the drug to hypersensitive individuals. This system will be used here also, beginning with:

Toxic effects of plasmoquine overdosage in normal individuals.

It has been suggested in the chapter on atebrin that true toxic effects of atebrin are few in number and apparently do not affect the red cells. This is very different from the position with plasmoquine.

Plasmoquine is a powerful drug, a point not sufficiently

realised; it is more than twenty times more powerful than atebrin, weight for weight. Plasmoquine is also a toxic drug, which has been recognised from the time of its synthesis. Unfortunately relatively little research has been done on the question of how plasmoquine produces its toxic effects. The great majority of the reports are from clinicians; among the few experimental reports we have those of Zylmann, of Sioli and of Birnbaum et alia. Zylmann (1944) carried out tests with quinine, atebrin and plasmoquine and surprisingly found that while quinine and atebrin had a definite lytic effect on red cells, plasmoquine had little effect; Birnbaum et alia (1946) found however that quinine, plasmoquine and atebrin were all capable of accelerating that haemolysis of malaria patients' red cells which is found to occur in the presence of bile. Naumann (1934) reported that in a patient with paroxysmal haemoglobinuria he was able to precipitate attacks of haemoglobinuria with quinine and with plasmoquine but not with atebrin. The dosages initially recommended for use were high by present standards and reports of toxic reactions soon began to come from clinicians. Sioli (1926) in his early tests and using the heavy dosage of 0.075 g. over periods as long as eight days reported abdominal pain as a usual feature and reported one individual who developed haemoglobinuria and cyanosis. The urine and blood showed absorption bands of haemoglobin.

The first toxic effect therefore to be discussed is abdominal pain: it is not definitely known how plasmoquine produces this pain; Field (1939) claims that the drug does not affect the intestinal musculature or mucosa; Manson-Bahr (1927) had said that administration of plasmoquine causes marked contractions of the spleen and this may be a pointer. There is no doubt of the realness of the pain; a small investigation on this point was carried out by us in Razmak in 1940. Indian troops on treatment for malaria were questioned, on the completion of the course, whether they had suffered from abdominal pain or discomfort during the taking of plasmoquine and if so on what day did the discomfort develop. Over 200 patients were questioned on this point. The Indian soldier has little sense of time, and is very open to suggestion, therefore the figures are only approximate but they serve to show that the pain and discomfort are directly related in time of onset - and in degree - to the dosage administered of the drug; figure No. 20, below, gives details; no attempt has been made to distinguish between discomfort and pain; the answers were unreliable. It is to be noted that even the slightest degree of discomfort was recorded.

Figure No. 20.

Percentage of individuals with abdominal pain and discomfort on taking plasmoquine.

Day of ingestion plasmoquine	% individuals complaining of abdominal pain	Total dosage of plasmoquine taken before onset of discomfort.
1st	nil	0.03 g.
2nd	3 %	0.06 g.
3rd	12 %	0.09 g.
4th	61 %	0.12 g.
5th	83 %	0.15 g.

The story is very much the same with all individuals; the pain comes on as a mild condition at first and increases with continued administration of the drug. The painful area is generally in the mid-line of the abdomen, between the umbilicus and the xiphisternum, does not move and does not radiate; the character of the pain has a "pressing" quality and it feels deep in the abdomen; some patients say it is as though there was a "knot in the gut"; there is slight tenderness but no guarding; it varies little in character except slowly to decrease or increase according as to whether the drug is withdrawn or increased; administration of stomachics did not influence it; hot applications gave slight relief.

From personal experience I agree with this description.

There appears no reason to doubt that this is a simple toxic effect, method unknown. The explanation cannot be splenic contractions. Another abnormality early noted, developing slightly later than abdominal pain is cyanosis. This was serious enough but a much grimmer complication was that of haemoglobinuria which so often and so readily followed continued administration of drug after onset of cyanosis: the haemoglobinuria would follow onset of cyanosis very often after one or two more days of drug administration; the order of appearance of toxic effects was therefore abdominal pain -- cyanosis and haemoglobinuria. The safety margin of the drug was very narrow indeed. Chopra (1936) carried out some tests on animals with the drug and found for example in cats that death occurred with dyspnoea, asphyxia, brachycardia and methaemoglobinaemia. He claimed that plasmoquine could form methaemoglobin <u>in vitro</u> with the blood of animals and that in man one third of the oxygen-carrying capacity of the blood may be lost as the result of this conversion. He confirmed that lethal and therapeutic doses in man are not widely separated; he considered that the maximum dosage that can be given to man without producing toxic symptoms was 0.03 g. daily. This question of cyanosis in humans has been considered previously by Muehlens (1926) who was the first to prove that the cyanosis came from methaemoglobin formation in the blood. Then Schulemann and Memmi (1927) agreed with this statement and

Manson-Bahr (1927) also confirmed it, using a Zeiss hand-screen spectroscope. Fischer and Weiss (1927) considered the methaemoglobin formation from a quantitative viewpoint. Their findings were that methaemoglobin in the blood can be proved with certainty at 4% and can generally be recognised at 2½%. They found that plasmoquine dosage of 0.05 g. intra-muscularly daily produced methaemoglobinaemia on the fifth to sixth day, which remains five to six days; and that plasmoquine dosage of 0.10 intra-muscularly daily produced methaemoglobinaemia on the third to fourth day, which remains seven to fourteen days.

They found individual differences in the time of appearance of the methaemoglobinaemia. The authors came to the surprising conclusion that if the use of the drug is persisted in, the cyanosis disappears.

They found that with a dosage of 0.02 g. plasmoquine daily, methaemoglobin did not appear in the blood until about the seventeenth day except in a few cases; they considered that on this dosage changes in the blood were so small as to be unimportant. They thought that pharmacologically the formation of methaemoglobin by plasmoquine is on a par with that due to aniline derivatives. These dosages found to be safe were less than twenty five per cent of the dosages originally recommended. The use of the bigger doses led to

the appearance of many reports in the literature of severe toxic effects following administration of plasmoquine.

We have already mentioned Sioli's (1927) case of haemoglobinuria following a total dosage of 0.62 g. plasmoquine. Manson-Bahr (1927) reported one case of haemoglobinuria following a total of 0.40 g. plasmoquine; in the same year the United Fruit Company (1927 report) reported a small number of serious complications following plasmoquine and adopted the use of quinine plus plasmoquine to combat this. In the next report of the United Fruit Company, i.e. the 16th Annual Medical Report, 1928, Brosius reported that with daily doses of 0.06 - 0.08 g. plasmoquine, fifteen out of 265 patients developed haemoglobinuria. Sinton, Smith and Pottinger (1929) continued the discussion and came to the following conclusions:

1. Combination of quinine and plasmoquine is better than either alone.
2. Plasmoquine should not be used except in combination with quinine.
3. Daily doses of quinine with plasmoquine should be NOT less than grs. 20.
4. Plasmoquine treatment should be stopped on the least suspicion of the occurrence of toxic symptoms.

Ross (1927a) said that plasmoquine had never produced haemoglobinuria in Rhodesia even in quinine-sensitive cases, and Majumdar (1929) treated two cases of blackwater fever with

a total dosage of 0.4 g. plasmoquine administered within seven days and had no ill results! Smith (1929) said at the same time that in his experience toxic symptoms regularly occurred between the sixth and tenth day - he was using 0.03 g. daily dosage, ten to seventeen days; he found that when he stopped the drug for one to three days immediately cyanosis appeared, the drug could be re-started without toxic effects. Sinton and Bird (1928) came to the conclusion that some patients are more susceptible to the drug than others and went on to say that there is a personal idiosyncrasy; they concluded that the safety margin of the drug is small and that the brunt of the toxaemia is borne by the liver.

Manifold (1931) gave the most detailed report up to that time of the effects of the use of plasmoquine in the treatment of patients. He was summarising the results obtained by the Army in India following adoption of treatment suggested by Sinton, see p. 544. Manifold remarked that with daily doses exceeding 0.06g.plasmoquine definite toxic effects appear in a high proportion of cases; he included as toxic effects cyanosis, jaundice, albuminuria and methaemoglobinuria. In the cases summarised by Manifold the treatment used had been 0.04 g. plasmoquine daily plus grs. 20 quinine daily, both for twenty-one days. The results came from hospitals throughout India. It is worth noting that it was the rule to keep the patients in hospital only for the first seven days of the treatment,

the remainder of the treatment, fourteen days, was given in the out-patient department. This twenty-one-day course was given only to cases of benign tertian malaria. 3187 cases received this treatment; 1298 were British troops and 1915 Indian troops; in twelve British and fourteen Indian troops the treatment had to be abandoned completely; owing to the appearance of toxic symptoms (severe abdominal pain, cyanosis) treatment had also to be abandoned for varying periods of time in 21.4% British patients, and

10.2% Indian patients.

The author states that in the great majority of cases the symptoms were never really severe; one remark that may be made here is that the figure of 10.2% complications for Indian troops is surprisingly low in view of the heavy dosage. This discrepancy is probably due to the difficulty in detecting cyanosis in the Indian patients. Manifold said that toxic symptoms appeared to be the result of a definite cumulative amount of the drug. This became evident after the sixth day. Of the twelve British cases in whom the drug had to be stopped completely, in eight the reason was severe cyanosis; surprisingly enough treatment was not stopped permanently in two British soldiers who developed jaundice; the treatment was continued after the jaundice had cleared, and apparently no ill effects resulted. There was one surprising fact in Manifold's record: he reports three cases of methaemoglobinuria and five cases of albuminuria in the

series; these were all among Indian troops and all came from one hospital in which the total number of cases treated in this fashion was sixty-four; these three haemoglobinuria cases are discussed under the section on plasmoquine in blackwater fever. The only point to make here is that these should never have been accepted on their face value without most searching investigation; to have three cases of haemoglobinuria in a series of sixty-four, and especially when these three were the only cases of haemoglobinuria reported among over 3,000 treated all over India must necessarily arouse strong suspicion that some undetected factor played a part in the development of haemoglobinuria. We have already given a striking example of this and we shall quote it again, namely the experience of Simeons (1936). Simeons decided to try mass prophylactic treatment of an isolated population, using atebrin musonate followed by plasmoquine; the dosages used were:

Two daily injections atebrin musonate, each injection given 24 hours apart; strong healthy males 9 ccs. (0.3 g. drug)
strong healthy females 8 ccs.
others pro rata.

Plasmoquine was given daily for three days after the second injection atebrin, plasmoquine dosage 0.02 g. daily for adult males; about one per cent patients reported giddi-

ness; the only serious incident was the appearance of haemoglobinuria in four individuals. This haemoglobinuria began one day after stopping plasmoquine. Three of these patients belonged to the same household. Two died; these were found to have been taking treatment, presumably arsenic, from a local hakim for syphilis. Two others lived; they also had syphilis but denied taking any recent treatment for it. The question of course is whether the condition was plasmoquine poisoning or atebrin-plasmoquine poisoning, or blackwater fever, or arsenical poisoning, or haemoglobinuria due to syphilis, or a combination of two or more factors. With evidence lacking the blame cannot be laid on plasmoquine as the causative factor.

Fletcher (1933) talking of plasmoquine poisoning said that although toxic symptoms may arise suddenly the onset is usually not abrupt, with cyanosis as the first warning sign; it was his experience that if the drug is stopped these signs pass off but if the drug is continued the cyanosis, originally seen in the lips, spreads and becomes deeper; thereafter an attack resembling blackwater fever develops, with red cell destruction, haemolytic jaundice and methaemoglobinuria. Fletcher considers that a dosage of 0.03 g. daily is reasonably safe.

Chopra (1936) supported Fletcher's statement on dosage after experiment with animals. This author agreed

that plasmoquine is a dangerous drug, and said that slight jaundice, cyanosis and abdominal pain are by no means rare. In fact Chopra said that urobilinogen appears in the urine simultaneously with the administration of the drug and persists for several days; he reported a marked drop in the red cell count and haemoglobin values with the serum giving a direct bilirubin reaction. Chopra found the intoxication symptoms developed as a rule after a total dose of 0.18 g. plasmoquine. This confirms his statement and that of Fletcher that treatment with 0.03 g. plasmoquine daily for five days should not be found to produce toxic effects.

During the 1939-1945 hostilities there were occasionally cases reported which threw doubt on the safety even of the apparently safe dosages of plasmoquine, i.e. 0.03 g. daily for five days in British troops and 0.02 g. daily for certain native troops, including the Indian Army; Smith (1943) reports a series of such cases. He was Consultant in Tropical Diseases to the Army in the Middle East at the time the incidents occurred. Details and a discussion are to be found in the D.M.S. Middle East Forces Memo on Medicine, number 16 of 10.7.1943, published by G.H.Q., Middle East Forces. Smith mentions fourteen cases of haemoglobinuria occurring in the Middle East over a period of eighteen months. Nine of the cases occurred in Egypt, two in Iraq, two in the Sudan and one in Palestine. Nationalities were: Indian 7, white Rhodesian 1, Greek 1, Polish Jew 1, Palestinian Jew 1, East

African 1, Basuto 1, Mauritian 1. Smith points out that all of these people had lived, usually for many years in endemic foci of malaria; it is noteworthy there was not one single British soldier among them although there were many British troops in the Middle East. In several cases the patients were still undergoing the plasmoquine moiety of the malaria course at the time they developed haemoglobinuria; the course in the Middle East at that time was:

grs. 30 quinine for the first three days.
→ 0.3 g. daily atebrin for five days.
→ three days rest.
→ 0.02 g. plasmoquine for five days.
(0.03 g. " " " " for British troops and other Europeans.)

Four of the patients had proved malignant tertian malaria, two had proved benign tertian malaria, one unknown, and seven had clinical malaria. Smith said he was uncertain whether these were examples of true blackwater fever or whether it was due to the toxic effects of plasmoquine.

Bedford (Consultant Physician with the Middle East Forces), writing in the D.M.S. letter quoted above, gave it as his opinion that plasmoquine must have played some part in the causation and orders were issued that a full three days must be allowed to elapse between the completion of the atebrin course and the beginning of the plasmoquine course. I was in the Middle East for three years during the war, in

Sudan, Egypt, Palestine, Syria and Iraq, responsible at the time for never less than 400 beds and I did not see any case similar to those quoted; these cases could not be properly investigated at the time of occurrence, e.g. no spectroscopic analyses were possible. One factor which in my opinion was not sufficiently considered in the fourteen cases was that we were still being supplied at that time with two sizes of plasmoquine tablets, one of 0.02 g. and one of 0.01 g.; it is not difficult to imagine that mistakes were occasionally made in the press of circumstances. This series of cases cannot be quoted as examples of haemoglobinuria developing in individuals receiving 0.03 g. plasmoquine daily; the information is too meagre.

Another similar series of cases is quoted by Dimson and McMartin (1946); the title of their article is "Pamaquine Haemoglobinuria"; the value of this paper is difficult to assess. The authors discuss a number of cases of haemoglobinuria developing in Indian troops during routine treatment for malaria and during "blanket" treatment of apparently healthy troops. At the time these occurred the routine treatment for malaria was quinine gr. 30 for two days → atebrin 0.3 g. for five days → two days rest → plasmoquine for five days in dosage of 0.02 g. for Indian troops and 0.03 g. for British troops. The control of the blanket treatment was the responsibility of Unit officers, an

to this scheme although they were quite over-shadowed by its great value to the troops as a whole.

The first drawback was that those in authority were not only "malaria conscious"; they had become "malaria over-conscious" by 1944 and it is my experience that the V.C.Os's, on whom the bulk of control invariably devolved, were such good and such careful soldiers that inevitably the danger was one of overdosage of the troops rather than under-dosage. This is proved by the fact that some of the men who developed haemoglobinuria while taking blanket treatment are recorded to have had doses of plasmoquine as high as 0.15 g. whereas the maximum supposed to have been given them was 0.09 g. plasmoquine. Furthermore it is impossible to believe that all men receiving treatment in unit lines remained at rest, e.g. one of the patients who developed haemoglobinuria during the blanket treatment was found to have been doing full duty during the whole period of plasmoquine administration; in fact the authors say that almost all of the patients who received blanket treatment were carrying out normal duties during the administration of the drug. Another possibility is that a very small number of individuals may have overdosed themselves in the hope of getting a little break in hospital from admittedly severe conditions. Lastly, other individuals may have avoided taking the drug. All these make Dimson and McMartin's discussion of less value than it might have been.

The authors are correct when they say that the problem was whether the patients were suffering from plasmoquine haemoglobinuria or blackwater fever; it is to be remembered that blackwater fever is rare in the Arakan.

The authors give the dose of plasmoquine previous to onset of haemoglobinuria as being:

1. Individuals receiving blanket treatment.

Plasmoquine dosage before haemolysis began:

Number receiving such dosage:

0.03 g.	0.06 g.	0.09 g.	0.15 g.	?g
2	3	5	2	1

There were three fatal cases in this group.

2. Individuals under treatment for malaria.

Plasmoquine dosage before haemolysis began:

Number receiving such dosage:

0.08 g.	0.10 g.
2	3

i.e. haemoglobinuria began with doses of plasmoquine varying from 0.03 g. to 0.15 g. It is significant that it was only in those individuals receiving blanket treatment that the lysis occurred among individuals in the smaller dosage group; this at once again raises the problem of whether the individuals had received greater dosages of plasmoquine than are recorded. The fact that the authors were able to find records of cyanosis

only in five of the eighteen cases is of little significance; to persons unaccustomed to working with native troops the finding of cyanosis is not easy. Dimson and McMartin stated that most of their patients denied ever having had malaria but they found positive blood films in three of the twelve examined. Several of the others showed splenomegaly.

They concluded that an essential cause in producing haemolysis was the toxicity of the plasmoquine in patients who were over-susceptible. They also conclude that there was no sign of batch toxicity; they found no skin reaction when they injected 0.1 g. plasmoquine intra-dermally into the individuals who had recovered from haemolysis.

A finding which has some bearing on the above is that of Craige et alia (1947). In an investigation carried out by them on a small group of cases, they found that with giving of 0.03 g. plasmoquine daily there were practically no toxic symptoms and methaemoglobinaemia was only three per cent of the total haemoglobin. With high experimental doses of 0.14 g. daily of plasmoquine there was much severe abdominal pain with vomiting, and the methaemoglobinanaemia was twelve per cent of the total haemoglobin.

It has been repeatedly remarked in the preceding paragraphs that while plasmoquine is toxic, its toxicity can be reduced to a surprising degree by the simultaneous administration of quinine. This combined treatment has also been proved

to be more effective than the administration of either drug given alone, e.g. Innes (1947) quotes fifty patients showing multiple benign tertian relapses who were given intermittent combined plasmoquine and quinine over thirty days; none of these patients relapsed. It has already been pointed out that the standard treatment in the Army in India given to thousands of patients over the time it was in use, was a twenty-one-day course of quinine and plasmoquine, and generally speaking during the last fourteen days of treatment the individuals attended as out-patients!

The experience with combined atebrin and plasmoquine is a different story and so far has been mentioned only incidentally. The effect of combined atebrin plus plasmoquine is in striking contrast to that of combined plasmoquine-quinine. The combination of plasmoquine and atebrin is definitely more toxic than is an equivalent dosage of both drugs given separately. Many authorities have written on this subject and all are agreed; the best example is that the treatment courses in use the World over may vary somewhat but to my knowledge there is no course in use where atebrin and plasmoquine are given together, in spite of the marked time-saving which would result. The administration of both at the one time was forbidden in the Army in India: furthermore in the great majority of courses suggested from 1933 onwards there is a break between the finishing of the atebrin course

and the beginning of the plasmoquine course, the idea presumably being to allow the concentration of atebrin in the blood to fall before beginning the plasmoquine, otherwise the result in effect would have been administration of atebrin and plasmoquine together. The standard Army course pre-war had a break of three days; during the war under stress of circumstances this was cut down to two days, and as has already been explained in Chapter I, in the latter part of 1938, again under stress of circumstances, the rest period was reduced to one day. It might be claimed that this is of little importance since it is acce ted that atebrin is a drug excreted only slowly from the body, taking about six weeks finally to clear after the last dose had been given. The Armoured Research Laboratory Report 1946 showed, however, that the loss is not evenly spread over six weeks; they found that the daily loss was roughly about ten per cent of the atebrine concentration, from which it may be inferred that the loss of atebrin is heavy in the days immediately following the stopping of the drug. This is in agreement with the findings of Fairley (1945) who also states there is an average daily die-away of ten per cent of the level present, although there is considerable variation in levels obtained during the die-away period; this variation however was more marked towards the end of the period. If we take an abstract figure of 100 units as the value of the atebrin present on the day the administration stopped, then it follows that on the first day

following stopping of the drug the value drops to ninety units; on the second day the value is theoretically eighty-one units, and on the third day it is seventy-three units; the difference in concentration of atebrin on the first and third days following cessation of treatment is therefore quite considerable, being a difference of seventeen per cent of the total present at the end of atebrin administration. As we shall see later this may have been of practical importance.

Before considering the plasmoquine dosage administered in our six patients with haemoglobinuria it is necessary to consider the question of:

2. Toxic effects produced by plasmoquine in individuals hypersensitive to it.

Hypersensitivity to atebrin came to be recognised late in the use of the drug and some reactions classed by me as hypersensitivity reactions, e.g. psychosis are not accepted as such by the majority of workers. This placing of reactions like psychosis among hypersensitivity phenomena was done because the margin of safety of atebrin is such that toxic reactions appearing in individuals who had taken only small doses of the drug were regarded as being in themselves an indication of hypersensitivity to this drug.

The classification of the reactions in plasmoquine is more difficult because of the narrow safety margin between the therapeutic and toxic doses. In the assessing of the toxic

dose of any drug the level taken is that at which a significantly large number of normal individuals develop signs of poisoning. With any drug, however, the inducing of toxic phenomena by deliberate overdosage of individuals will show that only a proportion of normal people develop poisoning at the level taken as the toxic dose for that drug; the remainder will develop poisoning at levels below or above the average. In other words there is necessarily a "beaten zone", a "scatter" of results round the bull's eye. In drugs which are not markedly toxic this beaten zone may extend over a considerable range whereas with very toxic drugs the values necessary to produce poisoning in a group of individuals will be found to be concentrated around the average dose; plasmoquine is a case in point; the safety level of dosage is thought to be in the region of 0.03 g. daily, e.g. Fletcher (1933), Chopra (1936) and Craige et alia (1947) all agree that with this dosage there are practically no toxic symptoms. Yet there are plenty of cases on record where even this dosage apparently produced severe toxic effects and certainly only moderate increase of this dosage will undoubtedly lead to poisoning in normal individuals. In other words there is little margin of safety. This being the case it is difficult therefore to distinguish between hypersensitivity in the true sense of the word and merely slightly increased susceptibility to the drug, this increased susceptibility not indicating hypersensitivity.

Several workers have supported the theory that certain of the toxic effects of plasmoquine are examples of hypersensitivity, e.g.

Sinton and Bird (1928) said they agree with other workers that some patients are more susceptible to the effect of the drug than are others and they think there is little doubt that there is personal idiosyncrasy to plasmoquine in some cases. When Sinton made this statement he was using dosages of the drug now recognised to be toxic even for the normal individual.

Similarly, Manifold (1931) said that in a certain small percentage of cases there appears to be a definite individual idiosyncrasy. This author also was discussing cases where the drug was used in excess, i.e. daily doses of 0.04 g.up to three weeks, admittedly given together with quinine.

Fletcher (1933) considered that certain individuals are peculiarly sensitive to the drug and yet he considers that drug idiosyncrasy is unknown. He obviously was using the term "sensitive" not in the same sense as "hypersensitive".

Dimson and McMartin (1946) consider that certain patients who developed haemoglobinuria were <u>oversusceptible</u> to plasmoquine. This presumably means "hypersensitive" as they carried out skin sensitivity tests.

If it is taken that the above authors meant by "idiosyncrasy" and "over-susceptibility" that in their opinion true plasmoquine hypersensitivity exists, then there is no proof of this. Hypersensitivity to a drug is a true antigen-antibody reaction with the characteristics of such a process, namely an explosive onset of signs of poisoning, generally following a dose far below that required to produce poisoning in the normal individual, e.g. in quinine-hypersensitive individuals a reaction may sometimes be evoked by a dose less than 1/100 of that required to produce severe toxic effects in the normal individual. Similarly with atebrin the dose producing hypersensitive reactions may be about 1/50th of the true toxic dose. Again, the reactions found in hypersensitive individuals are not necessarily or even usually the same as those found in the poisoning of normal individuals by the same drug; and lastly hypersensitivity to any substance is an acquired altered reaction capacity on the part of the body cells following upon continued or repeated exposure to that substance, e.g. hypersensitivity phenomena are not found in individuals on their first treatment with atebrin or with quinine. But the reactions which have been interpreted by some as hypersensitivity reactions with plasmoquine are always following a dose close to the therapeutic dose; among the few exceptions to this rule is the series quoted by Dimson and McMartin where eight patients developed haemoglobinuria following doses of 0.03 g. plasmoquine. For reasons given above,

however, these figures of Dimson and McMartin cannot be accepted and at present it is still correct to say that in all recorded cases of haemoglobinuria following plasmoquine the dosage of plasmoquine given was generally close to the maximum recommended for plasmoquine when given alone, i.e. 0.1 g. ——→ 0.15 g. This may be inferred from reports on the problem, e.g. Smith (1929) said that toxic symptoms occur regularly between the sixth and tenth day and Manifold (1931) agrees that there appears to be a cumulative action of the drug, becoming evident after the sixth day. The drug is slowly excreted from the body and it is not difficult to conceive of a mounting blood level finally reaching a toxic value; this much more closely fits the picture than any theory of hypersensitivity reactions; Fletcher (1933) considers that the onset of serious toxic symptoms is usually less than abrupt and only comes on if the drug is persisted in, in spite of obvious warnings such as cyanosis.

All agree that it is only rarely necessary to abandon use of the drug which is another point in favour of the toxic process being a reaction to simple overdosage, e.g. Smith (1929) states that if the drug be discontinued for one to three days on the appearance of cyanosis then, after this break, the administration of the drug may be continued, a finding reported by the majority of workers.

It is submitted then that, on the evidence at present

available, there is no undoubted incidence of true plasmoquine hypersensitivity; the serious toxic reactions quoted in the many reports available in the literature all could come within the class of simple overdosage, particularly when it is kept in mind that the toxic dosage is very near indeed to the therapeutic dosage; in fact in individuals with a low resistance to the drug these may be identical. In view of the close structural relationship between quinine and plasmoquine there is no reason to suppose that individuals could not be made hypersensitive to plasmoquine if exposed to it long enough. Its toxic properties are such as to prevent this; plasmoquine is never used for prophylaxis nowadays and individuals are not exposed to it long enough to develop a hypersensitivity.

The question is: what is simple overdosage? This is of importance in attempts to trace the cause of haemoglobinuria which developed in the Razmak cases.

The maximum safe daily dosage is said to be 0.03 g. but the drug is slowly excreted and the action is cumulative; yet nothing is known of the safe value of the level of the drug in the body, and it is this that seems to be the important factor. Figure No. 21 below shows the dosage of plasmoquine taken by our patients before they developed haemolysis.

Figure No. 21.

Dosage of plasmoquine taken before onset of lysis.

Case No.	Total plasmoquine ingested before haemolysis (grammes)	Period in days over which the plasmoquine adminstration was spread.
1	0.09 g.	$4\frac{1}{2}$
2	0.10 g.	5
3	0.09 g.	$4\frac{1}{2}$
4	0.04 g.	2
5	0.07 g.	$3\frac{1}{2}$
6	0.06 g.	3

The cases reported by Dimson and McMartin (1946) are the only ones in whom haemoglobinuria was found in relation to doses in any way comparably small, and as has been said, the figures of Dimson and McMartin cannot be relied upon. In other case reports of haemoglobinuria the dosages were much higher.

The top dosage ingested in our cases was 0.10 g., and the average over the six cases was 0.075 g. Admittedly in an individual with a low toxicity threshold cases one to three might have come within the toxic range in theory but the remaining three certainly did not, and it is unwise to postulate

one pathological process to explain one group and another pathological process to explain the other group. One point that must be considered in this connection is whether or not there was increased toxicity of the drug. Such is theoretically possible and in two ways, namely actual increased toxicity of a batch or batches of the drug, or relatively increased toxicity because of a high atebrin blood level.

Increased toxicity of the drug was put forward as a suggestion by Amy (1934) in attempting to explain an "outbreak" similar to the one in Razmak. However Bayer & Company state in a personal communication 16.5.39 that samples were sent to them by Amy at the time and were found to be normal in toxicity and that later Colonel Taylor (now Major-General Sir John Taylor) spent a week in the laboratories and was convinced that with the methods of control in use it was impossible for a batch of plasmoquine with a raised toxicity to leave the place. Bayer proceeded to point out that batch toxicity is very unlikely to be the explanation of the haemoglobinuria in my cases. Support is lent to their opinion by the fact that the drug used had come from two separate batches with which many other patients had been treated without obvious increase in the incidence of toxic side-effects. Samples from one batch only were available for testing and ten volunteers each took 0.04 g. daily for five days without any serious ill effects although all developed abdominal pain. Arrange-

ments were put in hand to send some of this batch to Bayer's in Germany but this had to be abandoned due to the change in the international situation (1939). We agree with Messrs. Bayer that it is highly unlikely that batch toxicity was responsible for the haemoglobinuria developing in our patients. "Relative toxicity" is a possibility, however. It will be remembered that pressure on hospital beds in Razmak in 1938 was such that the time interval between the finishing of the atebrin and the beginning of the plasmoquine was cut from the standard three days to one day; it will be remembered also that there is a considerable difference between the blood levels of atebrin one day after stopping atebrin treatment and three days after stopping atebrin treatment. The giving of atebrin together with plasmoquine markedly increases the toxic reactions and one obvious possibility is that the cutting-down of the rest period between the two drugs may conceivably have produced a state of affairs where the combined levels of atebrin and plasmoquine reached a dangerous level. Bedford (1943) stressed this point when he was Consulting Physician Middle East Forces.

I do not accept this view. Admittedly atebrin given with plasmoquine increases the toxic effects but the margin of safety is not nearly so low that the rest period could not be cut without danger; this shortened rest period has been used on many occasions without other than mild toxic symptoms

resulting; furthermore several hundred patients received the modified course and of them only six developed haemoglobinuria; lastly, as mentioned by Dimson and McMartin (1940) the rest period during the 1939-1945 hostilities was again cut down; in fact in the American forces at one time there was no rest period; this is sufficient proof that the safety margin is not strictly limited and that there was no "relative toxicity" sufficient to account for the development of haemoglobinuria. Another factor in favour of the reduced rest period not playing any part in the lytic process is that the haemolysis came in phases, spread in some cases over several days. Figure No. 22 shows the number of days over which the lytic processes were spread.

Figure No. 22.

Number of days over which the lytic processes were spread.

Case No.	1	2	3	4	5	6
Period of time (days) from the beginning to the end of the lytic phase.	At least 7	5	7	At least 8	4	?1

i.e. the phases of lysis were occurring at periods long after the combined atebrin-plasmoquine level had fallen to values far below the toxic level; e.g. in case No. 5, the lytic process

did not begin until one day after the plasmoquine course had finished, i.e. six days after the atebrin course had finished.

In problems like this, where the distinction is between plasmoquine haemoglobinuria and blackwater fever, it has been suggested that spectroscope analysis may be of use. Plasmoquine produces methaemoglobin in the red cells and the idea presumably was to examine the blood to find whether there had been a selective lysis of the red cells containing methaemoglobin with a resultant flooding of the plasma with this pigment; there are several factors which render this investigation of little use. The formation of methaemalbumin takes place within a few hours and can be distinguished from methaemoglobin only be special technique. Secondly the methaemoglobin is rapidly removed from the blood via the kidneys and circulates in the plasma only for a very limited period of time. This test is of no value in the differential diagnosis of plasmoquine haemoglobinuria and blackwater fever.

We may close this section with a few words on the question of the synergistic effect of quinine and plasmoquine, and of the antagonistic effect of atebrin and plasmoquine. This has never been satisfactorily explained and to discuss it would be to enter into sterile speculation but it is tempting and probably correct to suppose that the answer will be found in the structural make-up; plasmoquine and quinine have

a common basis, the methoxy-quinoline ring, but their side-attachments differ, with plasmoquine having a long open side-chain and quinine having a quinuclidine ring joined to the quinoline basis at the opposite side to the open side-chain of plasmoquine. The two side-chains appear supplementary to each other, and possibly synergistic; whereas in the combination of atebrin and plasmoquine the side-chains are the same and may be the toxic factor when the drugs are used together. This, however, is sterile hypothesis.

It is concluded that while poisoning by plasmoquine was seriously to be considered as a possible cause of the development of the haemoglobinuria in the Razmak patients, the balance of evidence is against this.

There remains one other possibility in connection with plasmoquine, that the plasmoquine "pulled the trigger" and produced six cases of blackwater fever. Let us then consider:

Plasmoquine and Blackwater Fever.

The part played by plasmoquine in the precipitating of blackwater fever is very difficult to decide. The striking feature in blackwater fever is the haemoglobinuria which gives it its name of "malarial haemoglobinuria". There is no specific test marking off the intra-vascular haemolysis of blackwater fever from any other haemolysis occurring in a patient with a

history of malaria. Unfortunately one of the most common features of plasmoquine poisoning is haemoglobinuria; it follows then that haemoglobinuria developing in a malaria patient under treatment with plasmoquine at once raises the question of whether the condition is one of plasmoquine poisoning or of blackwater fever; this is the problem that we are discussing. The methaemoglobincythaemia produced by plasmoquine is of no use in the differential diagnosis.

It was concluded earlier in this chapter that the cases of haemoglobinuria in Razmak were unlikely to have been due to plasmoquine poisoning, the main reason for this being the low dosage given of plasmoquine. This leaves the problem to be discussed of whether the plasmoquine could have produced haemoglobinuria indirectly by causing onset of blackwater fever. Opinion is sharply divided on this problem, as may be expected from the difficulties in differential diagnosis. Opinions vary between one extreme and the other, namely that plasmoquine does not cause blackwater fever and that plasmoquine definitely causes blackwater fever!

Majumder (1929) gave two individuals suffering from blackwater fever a total dosage of 0.4 g. plasmoquine in seven days without causing recurrence of haemolysis.

Manifold (1931) considers that in about 0.1% of Indian troop cases in the Army it is possible that an attack of blackwater fever may be precipitated in individuals

who have suffered from many attacks of malignant tertian malaria. Manifold was summarising the results of a twenty-one-day quinine-plasmoquine course of treatment for malaria, used for the first time in the Army in India. The full details are given in this chapter, p. 556; 3187 patients in all were treated and of them three developed haemoglobinuria. The details are:

Case No. 1. Indian follower; benign tertian malaria; given daily grs. 20 quinine with plasmoquine 0.04 g.; developed haemoglobinuria on the fifth day. Died on the ninth day. Methaemoglobinuria continued to the time of death.

Case No. 2. Indian soldier; benign tertian malaria; given daily grs. 20 quinine with plasmoquine 0.04 g. developed haemoglobinuria on the fourth day. This continued for five days. Patient survived.

Case No. 3. Indian soldier; benign tertian malaria; given daily grs. 20 quinine with plasmoquine 0.04 g.; developed haemoglobinuria on the fourth day; haemoglobinuria lasted for forty-six hours.

The dosage of plasmoquine was heavy, especially for Indian troops, and in these cases the possibilities of plasmoquine poisoning cannot be excluded. This series proves

nothing.

In 1934 Amy was faced with a problem similar to ours, and in countries adjoining Waziristan. Ten individuals developed haemoglobinuria over a period of one year; five of the cases were grouped, however, within a period of nine days, in Quetta. Amy was quoting from records submitted to him, always an unsatisfactory procedure and he admits his doubts on the problem of dosage of plasmoquine, etc., e.g. he states that he was doubtful whether a certain patient had received any plasmoquine at all, and he felt that with other cases there were "vague possibilities that some may have received an overdose". The same remarks therefore apply here as with Manifold; the series proves nothing as the figures are unreliable and would appear to be near to the toxic level for Indian troops.

Fairley and Bromfield (1934c) thought that blackwater fever may be precipitated by plasmoquine.

Chopra (1936) felt that plasmoquine is well tolerated in cases of blackwater fever, although he makes it clear in the same report that plasmoquine has strong haemolytic properties; he quotes one case of undoubted plasmoquine poisoning following a total dosage of 0.4 g. plasmoquine; he considers this to have been plasmoquine poisoning and not blackwater fever.

Nocht and Mayer (1937) said that, so far as had been reported, plasmoquine and atebrin do not cause blackwater fever and that plasmoquine can produce methaemoglobinaemia; they gave no facts to support their opinion.

Foy and Kondi (1937a) consider that plasmoquine, with quinine and with atebrin, is capable of "firing-off" an attack of blackwater fever, although they stress that the action is not a direct one and that these specific drugs are NOT a direct cause of haemoglobinuria.

Rogers and Megaw (1939) are much of the same mind; they say that the view that plasmoquine is safer than quinine in the treatment of blackwater fever is probably incorrect; they think the reverse is probably the case.

Manson-Bahr (1940) like Foy and Kondi thinks that any drug of value in the treatment of malaria may predispose to blackwater fever.

and Low and Fairley (1942) agree that plasmoquine can precipitate blackwater fever.

Mann (1943) records a case of haemoglobinuria following a total dosage of plasmoquine 0.12 g. in four days, following upon three days rest after a five-day atebrin course. On the details given the case may however have been plasmoquine poisoning or blackwater fever.

Smith (1943) discusses the paper by Mann and gives details of

fourteen cases of haemoglobinuria occurring in the Middle East. These cases have already been discussed on p. 560. Few details are given, and Bedford (1943) discussing the same cases considers that plasmoquine may have played some part in the production of the haemoglobinuria.

Maegraith (1946a) states that in blackwater fever there is evidence to show that plasmoquine may actively stimulate haemolysis, much in the same way as quinine. There is no such evidence incriminating atebrin.

Dimson and McMartin (1946) report testing of urine in 3,000 men every day they were taking plasmoquine. They report that jaundice was rare. All jaundice cases were investigated. They considered six to be "formes frustre" of blackwater fever although in another part they doubt the existence of blackwater fever as an entity. The authors quoted another investigation of 200 men of an Indian mobile workshop company receiving blanket treatment. 200 of the men were given blanket treatment 27.7.44 - 31.7.44; it is not clear quite what they were given. Thirty six cases reported sick; these showed:

Nine with jaundice, high coloured urine, abdominal pain, anorexia (two developed frank haemoglobinuria).

Eighteen with high coloured urine, abdominal pain, anorexia

Three with giddiness, and abdominal pain.

Two with diarrhoea and abdominal pain.

The authors then quote that the incidence of "formes frustres" of blackwater fever was much more than 0.2%; they obviously are confusing blackwater fever and mild plasmoquine poisoning, and therefore, it is difficult to accept their figures and their findings; stringent investigations of the dosage of plasmoquine taken would, in my opinion, have shown that much overdosage had taken place; this has already been pointed out. So very little is known about blackwater fever itself that it is difficult to come to a conclusion on the question of whether a particular drug or any other factor may precipitate an attack of blackwater fever. The disease itself will be discussed in Chapter IX and at present our evidence must be based on findings, some of which are negative.

e.g. 1. Blackwater fever is a disease arising out of malaria infection.

2. It does not differ in any feature other than the above from any other intra-vascular haemolysis.

3. Plasmoquine is a toxic drug with a narrow safety margin; there is no evidence that hypersensitivity plays any part in the ordinary case of poisoning by plasmoquine.

4. One very commonly found feature of plasmoquine poisoning is haemoglobinuria.

5. Serious toxic symptoms rarely arise with plasmoquine administration until a minimum of about 0.12 g. - 0.15 g. has been ingested, the action being cumulative. As we shall see later, many substances and conditions have the power to a very varied degree of "pulling the trigger" and producing an attack of blackwater fever. Quinine is particularly suspect in this respect. There seems no reason why plasmoquine should not also possess this ability to pull the trigger though it probably is much less liable to do so than is quinine. This is the consensus of opinion in the majority of reports on the subject.

That is the position as regards the relationship of plasmoquine to blackwater fever; how does this affect the particular problem before us? So far, we have failed to establish a definite diagnosis in our six cases of haemoglobinuria; at present the diagnosis stands at "intra-vascular haemolysis, cause unknown."

We have considered all the more likely causes of intravascular haemolysis, including poisoning by quinine, atebrin and plasmoquine and with the doubtful exception of plasmoquine have found no explanation for the haemoglobinuria.

This leaves only blackwater fever as the diagnosis; this is made admittedly on negative grounds but there are no specific findings in blackwater fever.

The conclusion therefore is that the individuals who developed haemoglobinuria in Razmak 1939 were true cases of blackwater fever. In each of these individuals the attack began during or immediately after plasmoquine administration. The dosage of plasmoquine taken was so small that it was ruled out as the primary cause of haemoglobinuria which is now held to have been blackwater fever. Plasmoquine, however, is a toxic drug, one easily capable of producing haemoglobinuria by itself, and one which has been found capable of precipitating attacks of blackwater fever. The diagnosis is then carried a stage further and is made "blackwater fever, precipitated by plasmoquine."

Summary.

Plasmoquine, the first of the synthetic antimalarials, is a substance with a side-chain identical with that of atebrin, and with a methoxy-quinoline basis exactly as quinine. Little is known of the pharmacology of this substance.

Plasmoquine has a lethal effect on the gametocytes of malignant tertian, benign tertian and quartan malaria. It has a lethal effect on the schizonts of benign tertian and quartan malaria only.

When the drug was introduced in 1926 the dosages

recommended were unnecessarily high and proved very toxic. Sinton worked out a twenty-one-day course with small doses of plasmoquine 0.02 g. daily, with the concurrent administration of grs. 30 quinine daily; this course became the standard for the Army in India. The place of plasmoquine in the treatment of malaria then became more or less standardised; firstly it was given to sterilise the blood of gametes after an attack of malaria, the idea being to utilise the markedly gametocidal action of plasmoquine; in such cases the course given was a five-day follow-up course; secondly it was used in the treatment of chronic relapsing malaria, generally benign tertian malaria, in which it was given as a long course, generally about twenty-one days, and always with quinine concurrently.

Even with the smaller doses which later came into use, plasmoquine proved to be a toxic drug; these toxic effects were considered in detail from two points of view; first, the toxic effects of overdosage in normal individuals, and second the reaction to plasmoquine of a person hypersensitive to it. It was seen in the first class of individual that even with normal individuals plasmoquine has a narrow safety margin; even therapeutic doses cause abdominal symptoms, sometimes severe, the cause of which is not obvious; one suggestion is splenic contraction. Abdominal pain, often with nausea, is very commonly found towards the end of each five-day plasmoquine course. A much more serious sign is cyanosis, due to formation

of methaemoglobincythaemia and this may be quickly followed by haemoglobinuria, even with doses very little above the therapeutic level.

The administration of quinine with plasmoquine much reduces the incidence of these more serious toxic effects, so much so that in the Army twenty-one-day course the patient was treated generally as an out-patient during the last fourteen days of the course.

The administration of atebrin concurrently with plasmoquine has the opposite effect, namely markedly to increase the toxic reactions. None of the commonly accepted methods of use of plasmoquine envisage its being given at the same time as atebrin.

As regards the second class of poisoning, namely hypersensitivity, the records in the literature do not support the suggestion that true hypersensitivity can be acquired to plasmoquine with the dosages and lengths of administration of the drug at present in use, e.g. in the courses in use today plasmoquine is only given for short periods; it is never used for prophylaxis. It is considered that there is no proof available to support the theory that any of the series of haemoglobinuria cases reported are due to anything other than simple overdosage of the drug.

The daily dosage of the drug generally accepted to

be safe is a maximum of 0.03 g. plasmoquine daily;. the drug is slowly excreted and the action is cumulative; this may account for the frequency of toxic phenomena on the fifth to sixth day. The dosage which our patients had been given before the development of haemolysis was:

Case	1	2	3	4	5	6
Dosage (g.)	0.9	1.0	0.9	0.4	0.7	0.6

It was concluded that this dosage could not have caused haemoglobinuria when all the factors were considered, e.g. dosages of cases reported in the literature are usually much heavier than the above.

Increased toxicity of one batch of drug was also ruled out both by the makers and by myself; the drug used came from two batches and these two batches had not produced an increased incidence even of minor toxic reactions in other patients, nor was it found to be specially toxic when administered to ten volunteers in a total dosage of 0.2 g. in five days.

It was also concluded that the cutting-down of the rest period to one day had not led to a state of affairs whereby the combined atebrin-plasmoquine level, in passing each other (one going down and the other going up) had produced an especially toxic combination dosage at one time.

It was concluded that plasmoquine poisoning, directly or indirectly, was probably NOT the cause of haemoglobinuria in Razmak.

No definite explanation can be given for the synergistic effect of quinine with plasmoquine, nor for the antagonistic effect of atebrin and plasmoquine; it was suggested that the side-chains played a part in these reactions of one drug with the other.

The last point considered was whether plasmoquine had been responsible for "pulling the trigger" to produce the six cases of blackwater fever. This was difficult to assess because plasmoquine haemoglobinuria may result from dosages of drug near to the accepted therapeutic dosage - many of the cases quoted in literature as examples of plasmoquine precipitating blackwater fever are much more probably examples of plasmoquine haemoglobinuria. The laboratory findings in plasmoquine poisoning and blackwater fever cannot be used in differential diagnosis of the one from the other, in spite of the methaemoglobincythaemia found after plasmoquine administration. There are conflicting opinions on the ability or otherwise of plasmoquine to precipitate blackwater fever but the consensus of opinion is that it can do so although it is not nearly so active in this respect as is quinine.

The diagnosis finally arrived at in our six cases was blackwater fever and it was held that blackwater fever had been precipitated by administration of plasmoquine.

CHAPTER IX.

Blackwater Fever.

In Chapter VIII it was decided that the haemoglobinuria occurring in Waziristan in 1938 was due to blackwater fever. It now remains to consider this disease more fully, beginning with the history of the condition.

History of Blackwater Fever.

The early history of blackwater fever, like that of the discovery of the effect of cinchona bark, is hidden in obscurity.

With the exception of reports on one group of cases and a few doubtful isolated passing references, there is nothing in the literature of medicine in any way suggestive of blackwater fever until we reach the early nineteenth century. The series of cases which is the exception is that reported in the Hippocratic writings. These writings deal with the diseases found at that time (450-350 B.C.) in the lands and on the islands bordering the Aegean Sea. At this time the Hippocratic school of priest doctors had its headquarters on the island of Cos, and the writings attributed to Hippocrates are compiled, as Scott (1947) says, from discourses in the temple of Asclepius; Hippocrates himself, "Hippocrates the Great" is reported to

to have been born in 460 B.C. and to have died in Thessaly when over one hundred years old. He practised therefore in Cos towards the end of the fifth century B.C. and in the fourth century. The collection of writings which bear his name are thought by Adams (1939) to represent the opinions held by the family of Hippocrates and his immediate successors. What is important about these writings is that they represent the beginning of the scientific approach to medicine, with many of the writings of the school based on records of facts rather than theories. Much has been made therefore of these cases, reported in the Hippocratic writings, which are thought by some to have been blackwater fever. Before we begin to discuss these highly controversial cases it is well to be sure that malaria itself was to be found in the areas where the cases occurred. There are many historical writings and many ruins of cities and temples which tell of the part played by malaria in the despoiling of centres of Greek civilisation in Greece itself and in the outlying areas. (It was on this evidence that Ross thinks there was no malaria in Greece before 500 B.C.) These writings and ruins do not tell however whether the disease was always present or whether it had come as a visitation upon a previously healthy area; areas are to be found today in Greece, Sicily and Italy so malarious that no one could live there, yet there are great and noble ruins, suggesting that malaria had come as a new factor. Hippocrates however had differentiated fevers in a way which suggested that

malaria was known in his time, e.g. Nocht and Mayer (1937) say that Hippocrates not only differentiated the intermittent malarial fevers from others but also divided them into three groups, Quotidian, Tertian and Quartan. Scott (1947) considers that there had been some separation before the time of Hippocrates; Scott talks of a possible reference to malaria in Homer's Iliad and in the works of several of his predecessors, e.g. Pindar in 470 B.C.; and reference to Adams's (1939) translation of the writings will show that some reports given are very suggestive of malaria, e.g. when the writer talks of "true tertian" and fevers with rigors and "regular quartans". The bulk of the evidence is that malaria was endemic in the Aegean at the time the Hippocratic school were recording their findings. This then raises the question of whether or not the disputed cases were cases of blackwater fever. These annotations are to be found in "Epidemics" Books I and III; incidentally Adams (1939) considers the Epidemics, Books I and III as undoubtedly genuine; a total of forty-two cases is reported, of whom fifteen showed black urine and three showed red urine. Certainly not all these cases can be considered to be possible blackwater fever cases, e.g. some were in women during the puerperium; in others of the series however from the scanty information given in Adams's translation there is no doubt that the description given is compatible with though not specific of a diagnosis of blackwater fever. Two examples

are "Philiscus who lived by the wall", and "Silenus who lived on Broadway". The most careful of the investigations into this problem of the Hippocratic writings was that reported by Foy and Kondi (1935); these authors carried out a painstaking investigation into the subject, e.g. they made a careful examination of the works of Hippocrates in the Greek and also examined the German, French, Italian, Latin and English translations. In their references to the Greek and Byzantine literature on the subject the authors had the expert help of the German, American and British Schools of Archaelogy. Foy and Kondi quoted some representative cases, including the two mentioned above, namely Philiscus and Silenus and they felt that if the cases quoted by Hippocrates are not blackwater fever then it is very hard to say what they can be, and they concluded that blackwater fever has been known to exist in Greece from the earliest times; they say that Greek opinion is almost solidly in agreement with this view.

In spite of the careful work of Foy and Kondi, the majority of workers remain unconvinced that the cases of Hippocrates were cases of blackwater fever, e.g. Stephens (1937) points out that bile and drugs may conceivably have coloured the urine. Other possibilities are favism and Weil's disease, both of which are found in Greece today and both of which conceivably were known in Greece and the neighbouring countries in the time of Hippocrates. The evidence is not conclusive enough to

permit of a diagnosis being made of blackwater fever. This is the opinion of the large majority of workers. Foy and Kondi (1935) also mention two vague references, one by Actuarios, a twelfth century medical writer and one by Protospatharios, a seventh century Greek anatomist (Foy and Kondi say seventeenth century); neither of these can be taken as evidence that the writers at that time were talking of blackwater fever. Another isolated reference is that in Stephens (1937) who quotes the translation of the work of Prosper Alpinus (1553-1617) who talks of haemoglobinuria.

With these few and very doubtful exceptions there is nothing in literature until the nineteenth century that may be a description of blackwater fever, e.g. Torti (1712) quoted by Stephens (1937) in his very exhaustive Therapeutice Specialis makes no mention of any condition like blackwater fever. Then in the nineteenth century from many parts of the world and apparently mainly independent of each other a series of reports make their appearance. Manson (1886) "confidently asserts" that before 1850 the cases of haemoglobinuria related to malaria were very few indeed whereas since then this disease had simultaneously appeared in America and Africa as a sporadic endemic and epidemic disease. There are minor differences of opinion as to who was the first author to report the disease in some or other part of the World but it is probably correct to say that between the beginning and the middle of the nineteenth century

reports had appeared from all continents, adequately describing the disease, (In all the earlier reports it was called "haematuria") e.g. from

Africa, Boyle (1831) quotes a report from a Mr. Tedlie about 1822 who described the urine in endemic fevers of the Cape Coast as having the appearance of bloody water. The next series of reports included those from Berenger-Feraud (1874) who traces the disease back definitely to about 1850 (e.g. by 1855 blackwater fever had appeared in the statistics of the Senegal hospitals) and with a fair degree of probability to 1820; he talks of it being known in Dakar from about 1820.

In America (North) the first doubtful reference is also in the early 1820s and by 1840 the condition was well established to be a clinical entity in the hot Southern States. In many of these earlier reports the authors talk of having known of the disease or having seen the disease a long time previously but no one dates the appearance anything much before 1820. Cummings (1859) produced the best of the earlier reports and speaks of "haemoglobinuric" fever; he reports six cases. Reports from South America begin to come in a little later, about 1840.

In Europe, Alibert (1809) mentions oliguria and black urine in his discussion on the treatment of intermittent fevers

but this is an isolated mention and it is not until 1850 that the condition came to be recognised in the two malarious black spots of Central Europe, namely Italy and Greece; at the same time cases were being reported from Madagascar and the island of Nosse Be near it.

The first definite report from Greece was by Antoniades and this was closely followed by a stream of papers from others, e.g. Veretas, (1859); not only do they all recognise the disease to be an entity but even in that short time they had realised the possibilities that quinine was the exciting agent.

The reports from India began to come in later, surprisingly enough not until 1878, but that may have been because of the general paucity of reports in English on the disease until about that time. Other individuals claim this late appearance is because of the late re-appearance of quinine in India.

Thomson (1924a) says that the apparently late appearance of the disease has led to the mistaken conclusion that the disease was of recent origin; he thinks however that the late recognition was due to several factors, the most important being the exploration and development of the Tropics by the non-immune white races.

Scott (1939) puts the matter correctly when he says that it is a subject for constant wonder that while malaria has been known for twenty-five centuries, blackwater fever, so far as is known at present, has been observed for little more than one century and no recognisable description of it has been discovered before the latter half of the nineteenth century. Scott does not think there is sufficient evidence to accept as blackwater fever any of the eighteen cases recorded in the Hippocratic writings. This author feels that the chief difficulty would be to explain the absence of any records of the disease for so many centuries, from the time of Hippocrates to the nineteenth century. The passing references of Protospatharios and of Actuarios are too flimsy to be taken as proof. In reply to Thomson's suggestion that blackwater fever only became known in the Tropics when the non-immune Europeans arrived there Scott asks what the explanation is of the disease not being reported from Greece before the nineteenth century when Greece is one of the plague spots of the disease. Scott thinks we cannot trace the disease back beyond 1822 with any certainty. He is not prepared to say whether the disease was a new condition or one of older standing which had not previously been recorded.

This preoccupation with dates is not without practical importance for, as we know, in 1820 Pelletier and Caventou isolated the alkaloid quinine which later acquired so grim a significance in the causation of blackwater fever. The coincidence in dates, for it is now so regarded, between the isolation of quinine and the beginning of reports on blackwater fever proved one more pitfall for workers and provided an apparent ready-made reason for the appearance of blackwater fever, although quinine had been given for 200 years previously as an admixture with other cinchona alkaloids in combined extracts from the bark.

Scott's opinions (1939) are probably representative of the great majority of workers, namely that the disease had not been reported prior to 1820, the cases of Hippocrates not being examples of blackwater fever, and that there is no satisfactory explanation as yet why the condition should begin to be reported at that time, whether it was a new disease sui generis or an older disease which had not previously been reported. The probability is that it had not previously been recognised, that it was not a disease sui generis, although admittedly its symptomatology is very striking. It is generally accepted that the apparent appearance of the disease for the first time at the same time as quinine had been isolated is a coincidence.

Easmon first used the term "blackwater fever" in English in 1884 and in 1885 the same author explained that he

had taken the name from the French term "fievre bilieuse melanurique".

General.

Eclampsia has been called "the disease of theories"; the same is very true of blackwater fever. One measure of the confusion may be seen in the multiplicity of names which, including French names, total more than fifty. The cause of blackwater fever is as yet still unknown; in fact in one report written recently by Dimson and McMartin (1946) the authors obviously do not accept the theory that the condition is an entity and state that all cases can be divided into plasmoquine haemoglobinuria, quinine haemoglobinuria or whatever happens to be the precipitating agent. However, this opinion is quite out of keeping with that of the large majority of workers who agree with the Editor, Indian Medical Gazette (1932) who claimed that the term "blackwater fever" should be confined to a specific symptom-complex and should not be applied to all forms of haemoglobinuria. Christophers (1937) rightly said "practically nothing is known of the ultimate causes concerned in the bringing about of destruction of red cells to which the disease owes almost all its features".

It is difficult even to find a satisfactory definition for the disease; Low and Fairley (1942) define blackwater fever as "an acute complication of chronic malignant tertian

malaria, characterised by one or more intra-vascular haemolyses of considerable severity, haemoglobinuria, fever, vomiting, jaundice, and anaemia ..... the disease invariably originates in an endemic zone heavily infected with malignant tertian malaria. The native population may enjoy immunity .... quinine is the factor precipitating the attack .... plasmoquine may also precipitate blackwater fever". This definition cannot be accepted; there are at least five statements in it which are not generally accepted. Probably the best definition of the disease is the simple one of "malarial haemoglobinuria"; nothing can be added to this description which would be accepted by all interested in this question. Blackwater fever first came clearly to be separated from haematuria some seventy years ago. By the beginning of this century the kidney lesions had come to be recognised; since that time, close on forty years ago there has been much sterile speculation on the problem but little fruitful research and the only advance has been the identification of methaemalbumen by Fairley and Bromfield in 1934. We do know that many substances can "pull the trigger"; Manson's poser now might be changed to "what charges the gun". Foy and Kondi (1943) convey the same idea when they say that in blackwater fever malaria would appear to be an important preparatory agent but what exactly it does or how it produces its effect is unknown. But, the gun having been charged, the trigger may be pulled by any one

of several agents, e.g. antimalarial drugs, or cold, or splenic enlargement. It is of advantage to enumerate these points which are not seriously controversial.

Nocht and Mayer (1937) said:

1. A malaria infection must be assumed in all cases of blackwater fever; blackwater fever invariably arises out of malaria. This is one of the few statements acceptable to all.
2. Blackwater fever always occurs in sporadic form in relation to individual malaria cases; it never occurs in epidemic form. To this may be added, however, a rider that on occasion the cases may be so grouped in time and space as to resemble an epidemic, e.g. Ceylon in the big malaria outbreak in 1936; this is further discussed below.
3. Most blackwater fever patients are infected with malignant tertian malaria but others have been observed following benign tertian and quartan malaria. Some authors claim that the relative frequency of the three species found in the blackwater fever cases is that of the relative frequency of the species in the particular area, hence the heavy malignant tertian preponderance in Africa. Foy and Kondi (1937b) basing their findings on 360 cases of blackwater fever in Greece actually conclude that the vivax species

is fully as efficient in the producing of blackwater fever disposition as is malignant tertian malaria.

4. In by far the majority of cases blackwater fever arises from a chronic infection, i.e. several relapses or new infections; this is widely accepted.

5. All investigations aiming at the discovery of a specific cause have failed, or remain unconfirmed.

Ross (1932) adds

6. Blackwater fever is uncommon in individuals with less than one year's residence in a malarious area and that the rate at which susceptibility to blackwater fever is acquired varies greatly in individual cases.

Scott (1939) puts the matter in the same way when he says that all over the World wherever blackwater fever exists, Europeans are the chief victims although it is also found to attack Bengalis working in the Assam Tea Gardens and Egyptians working in the Sudan.

Chopra (1936) also agrees that the disease only attacks susceptible migrants as the Bengali clerks coming into the Assam Tea Gardens. Chopra adds other points.

7. The presence, within half a mile, of an aboriginal population saturated with malaria. The author holds that the aboriginals themselves have become

immune or tolerant to the infection. The malaria strain concerned is of intense virulence.

The two remaining theories of Chopra are not generally accepted and need not be noted.

Rogers and Megaw have had long experience in India and their views are particularly valuable. Writing in 1939 they claim that:

Blackwater fever occurs in most places where severe endemic malaria persists through the greater part of the year and in such places is very much more common in migrants. In places where malarial infection is limited to a brief season in each year the disease is uncommon even when malaria occurs as severe epidemics. They hold that the disease is decidedly rare in places which are visited at intervals of several years by epidemics of malaria even when these are very severe, as in the Panjab; in their opinion the factors conducing to blackwater fever are:

1. Existence of intense endemic malaria.
2. Recurrent malaria; even the most severe first infections are rarely accompanied by haemoglobinuria.
3. Disease is rare in recent migrants; few cases occur within six months of arrival.
4. Nearly every case of blackwater fever is in individuals imperfectly treated for malaria; this may

explain why the blackwater fever attacks do not necessarily occur in the blackwater fever area.

5. A dose of quinine is the usual precipitating factor though quinine by itself cannot be the cause.
6. Cold, heat, and fatigue often precipitate an attack.
7. Where malaria prophylaxis has been effective blackwater fever is invariably controlled.
8. Malaria parasites are present in seventy to ninety per cent of cases; usually malignant tertian malaria.

Sinton (1927) also points out that in the Punjab in spite of the occurrence of intense autumnal malaria, largely malignant tertian and in spite of big periodic epidemics, there is no indigenous blackwater fever.

Stephens (1937) summarise the problem of the causation of blackwater fever by saying:

1. Aetiological importance has been assigned to bacteria, protozoa, etc. etc. The tests have always been negative.
2. Accessory aetiological factors listed include alcohol, chill, diet, disease, emotion, exertion, parturition, sunstroke, syphilis, etc. etc.
3. The mechanism of the haemoglobinuric process has been attributed to such diverse agents as haemolysins radiant energy, lactic acid, etc.

Or has been considered in a general way as an anaphylactic phenomenon.

But, the above opinions are largely speculative. Concerning this matter of aetiology, it is pertinent to discuss here the question of whether or not there is at any time a "pre-blackwater" phase in the disease.

Koch (1899) suggests such a phase of disease when he says that certain features point to the fact that blackwater fever does NOT arise suddenly but gradually. He quotes patients giving a history of strikingly dark-coloured urine and slight icterus for a long time before the onset of frank haemoglobinuria.

Blacklock (1923) is thinking along the same lines when he says that the importance of the pre- and post-haemoglobinuria states, which are inherent parts of the disease, is apt to be lost sight of because of the use of the term "blackwater fever" and he thinks that terms such as "occult" or "subliminal" blackwater fever may be used for these conditions.

Manson-Bahr (1927) holds that in some cases of blackwater fever there is a pre-blackwater stage with low fever, nausea, vomiting, persisting headache, large spleen and urine showing traces of albumen and urobilin.

Undoubtedly in blackwater areas one sees patients with yellow sclerae, large spleens, low-grade fever and dark urine.

These patients do give the impression of trembling on the verge of an attack of lysis, but that is not necessarily a "pre-blackwater" condition. It is merely a description of a patient with under-treated chronic malaria and such patients are the perfect examples of individuals who will possibly develop blackwater fever. Rogers and Megan (1939) also take this view when they say that such patients are suffering from "bilious remittent malaria" and that these people may have some clinical resemblance to blackwater fever. Some workers have shown that in such cases the bilirubin values in the blood and the urobilinogen in the urine may be higher than those found in the blood and urine of individuals with frank blackwater fever. This is not surprising when it is realised how heavy is the blood destruction in certain cases of malaria, e.g. Whitby and Britton (1946) talk of one million red cells destroyed in one malignant tertian paroxysm.

One other cause of confusion is the problem to be discussed below, namely occult blackwater fever.

This point was raised by Blacklock (1923) see above, when he talked of "subliminal blackwater fever". The picture often painted of blackwater fever is that of a disease with the individual showing severe intra-vascular lysis and heavy haemoglobinuria; this is not necessarily so; there is great variation in the degree of lysis of the red cells. This

variation in degree is found with different patients and even in the same patient at different times in those cases suffering from recurrent attacks of lysis. Some of these lytic phases may be extremely mild in degree, a fact only now recognised. These phases are of course the explanation of the type of happening where an individual recovering from a frank haemoglobinuric phase of blackwater fever shows repeated small spurts of temperature, continued jaundice and repeated falls in the blood count; this is a condition of blackwater fever sine blackwater as it were. Nocht and Mayer (1937) talk of rudimentary forms of blackwater fever in which there is no haemoglobinuria but where the urine shows much urobilinogen, with the patients jaundiced and exhausted.

There seems every possibility that careful investigation in blackwater areas, particularly investigations with spectroscopic analyses of the blood, and the checking of red cell counts, would show that occult blackwater fever, i.e. blackwater fever of minor degree, is more common, possibly much more common that is generally realised. It is felt that attention should be turned to this problem by workers in areas where the disease is not rare. These "rudimentary forms" of blackwater fever account for some of the individuals regarded at present by some authors as being in a "pre-blackwater" state.

The last of the minor general problems to be settled

at this point is that of whether or not "epidemics" of blackwater fever may occur. Nocht and Mayer (1937) state that blackwater fever occurs arbitrarily in sporadic form in relation to severely-malarious areas, being most frequent where malignant tertian malaria predominates; they state that the disease never occurs in epidemic form. If these statements of Nocht and Mayer's are correct and the disease is so readily limited, then we must question our diagnosis of blackwater fever being the cause of haemoglobinuria in the six individuals who developed this sign in Razmak in 1938. Cases of blackwater fever do occasionally occur, however, which do not fit into the rather rigid specifications given by Nocht and Mayer; there are many striking examples of this on record, e.g. reports of "epidemics" to be found going back to the latter part of the nineteenth century. Such"outbreaks" are particularly reported among prisoners, those making canals, etc. etc. and during epidemics of malaria in areas with little previous malaria, e.g. the Ceylon epidemic of 1934-5; when six cases of blackwater fever occurred although there had been only seventeen from 1913-34. Sutton (1911) gives an interesting report on the crew of the U.S.S. "Des Moines"; twelve men went ashore for thirty-six hours after having been off the West Coast of Africa for about five weeks. Within ten days of returning from the shore seven of the men had developed blackwater fever and three had developed malaria; there seems no doubt that "outbreaks" of blackwater fever can occur, even in areas previously thought

to be free of the disease. One such outbreak, which very closely concerns us is that reported by Amy (1934); at that time, according to Rogers and Megaw (1930) blackwater fever was to be found in India, East and South of the line Surat-Dehra Dun. This agrees with Gosh (1941) of the Malaria Institute of India who gives the same areas affected in India as does Stephens (1937), namely Assam, Bengal Duars, Bihar, Madras Presidency, Orissa, Bombay Presidency, Central Provinces, Coorg, Hyderabad, United Provinces; the areas most particularly affected are the foothill areas such as the Assam and Bengal Dooars, Jeypore and hill tracts of the United Provinces terai; i.e. the Punjab although so ravaged by malaria, does not appear in the list as a place where blackwater fever may be found; this is true also of the North-West Frontier, which has areas of very severe malarial incidence, e.g. Peshawar area. Amy (1934) stated that previously there had been no record of blackwater fever in India West of longitude 75°, with the area to the North and West of the line Surat-Dehra Dun free of this disease; yet between 30.8.32 and 13.8.33, a period of one year, there occurred ten cases of "blackwater fever" along the 250 mile line Peshawar-Fort Sandiman; this is practically the line of the North-West Frontier of India, far to the West of the 75° line and far to the North of the line Surat Dehra Dun. Five of these cases were grouped in location and time, namely Quetta, August 1933, where five cases occurred within nine

days. These cases were all in military personnel who, with one exception, were natives of the North-West Frontier Province and the Punjab; only one had been in a blackwater fever area (East Africa) in his service. The individuals ranged in rank from followers to officers, from all arms of the services, ages between eighteen and forty-five. There were six deaths. Certain of these cases occurred in country very like Waziristan and one of the cases occurred in Waziristan at Wana, near Razmak.

Amy sent samples of the plasmoquine used to the manufacturers, Bayer, who said (1939) that this was found to be normal in toxicity.

These reports of this disease in Army personnel had come to Amy in his capacity as a medical Staff Officer; he did not see any of the cases in person and had no hand in the investigation; they were poorly investigated and there is considerable doubt in one or two of the cases whether the dosages of drug reported had in fact been given. This type of report is always very unsatisfactory where reliance must be placed on the individuals who originally treated the cases. As said, all the cases reported by Amy were poorly investigated, e.g. he talks of "massive naked-eye methaemoglobinaemia", taking all the factors into account it seems most probable that the individuals concerned had been suffering from blackwater fever.

I have discussed this matter with medical missionaries with long experience on the Frontier and in the Punjab; I have also discussed it with officers of the Indian Medical Service who have had long service in these areas both in civil and military employ, and I have had some years of experience in these areas myself but I have heard of only one other case in these areas which may possibly have been blackwater fever. This was told to me by Major Duggal, I.M.S. This officer was a specialist in pathology in the I.M.S. and had served three terms on the Frontier - he was captured by the Pathans on his last tour - Duggal states that he was on the original expedition to open up Razmak in 1922-23 with Sir John Maffey, and at that time he saw in an infantry-man a case of what he took to be blackwater fever; the man's illness began below Razmak camp a few weeks after the patients had arrived from the plains; there is no other information available on the patient. Such information as is obtainable does not permit of a diagnosis being made.

If Amy's cases were blackwater fever they seem to be the only recorded cases of this disease on the Frontier although isolated cases may have occurred without being reported.

It seems then that sporadic outbreaks of blackwater fever may occur for reasons not obvious, even in areas regarded as free from this disease; the rarity of the condition in Waziristan therefore is not a valid argument against our

diagnosis of blackwater fever which is supported by that series of cases reported by Amy in the same region five years previously.

It is necessary at the close of this section to point out again that although we have solved the diagnosis we still have not solved the cause of blackwater fever. As Scott (1939) says "the problem still unsolved in blackwater fever is the cause. Malaria is at the back of it but what is the proximate cause, and why, among hundreds of endemic centres of malaria are there only a few centres of blackwater fever?" This lack of any definite knowledge explains the remark at the opening of this chapter that blackwater fever is a disease of theories; none of these is accepted by all to be the answer. It is necessary therefore to discuss the more important of such theories, with a consideration of the pros and cons in each case.

The first point requiring discussion is the part played by malaria in blackwater fever.

Malaria and Blackwater Fever.

It is proposed to discuss some of the current theories as to the causation of blackwater fever; before this can be done intelligently we require to discuss malaria itself, for the one definite thing that is known of blackwater fever is its relation to malaria; stamp out malaria and you stamp out blackwater fever. The mechanism whereby malaria produces blackwater fever is not known. The most surprising feature of the problem is that although malaria itself has been known for at least twenty-five centuries, blackwater fever has only been recognised for one century. Long before scientific proof was forthcoming there was a realisation that malaria in some way was connected with damp marshy places, and with insects. Scott (1939) quotes the remarkable observation of Columella in 100 A.D. who reports that marshes breed mosquitoes armed with stings and that these insects might convey disease in their bite. There are several theories on the origin of the name malaria; probably the earliest in the English language or in any language is the note by Walpole in 1740 on "a horrid thing called the malaria that comes to Rome every summer and kills one"; certainly the name had become established by the beginning of the nineteenth century.

The following classification of malaria parasites is widely accepted.

Class — SPOROZOA.

Subclass — Coccidiomorpha

Order — Coccidiida

Sub-orders — Eimeriidea; Haemosporidiidea

Family — PLASMODIIDAE Mesnil, 1903

Genus — PLASMODIUM

The term Plasmodium is probably the least suitable of the several names suggested but it is too firmly entrenched to be changed. Of the genus Plasmodium which attack man there are four species recognised, namely P.falciparum, P.vivax, P.malariae, and P.ovale. Malaria is the term applied to the symptom complex resulting from obvious infection of the human by these protozoan parasites. The parasites have a complex cycle of development, the asexual phase of which takes place in the body of man while the definitive host is the anopheline mosquito. Malaria is a chronic disease.

There are several phases in the life cycle of the organism and these various phases are not all equally affected by antimalarial drugs; at least one phase in fact is not affected at all, except by paludrine, see plate No, XX, p.439. In brief the cycle in the human is:

(a) The sporozoites are introduced into the patient by the bite of an infected mosquito. Thereafter there is a blank for eight to ten days; this blank was a source of the greatest difficulty in the consideration of malaria but has recently been cleared up, see discussion on the exo-erythrocytic phase, below. During these ten days the parasite is undergoing further development in the internal organs.

(b) This period is followed by the appearance of the parasites in the peripheral bloodstream. This is the phase of active multiplication by schizogony and when the parasites attain a certain concentration, symptoms are produced in the host, i.e. "malaria" develops. The figures necessary to produce overt malaria are surprisingly high, e.g. Whitby and Britton (1946) assumed there must be one parasite to every 100,000 red blood cells to produce symptoms. In this particular phase of schizogony the parasite is easily killed by all of the anti-malarial drugs excepting the malignant tertian schizonts, which are not affected by plasmoquine.

If drugs be administered to the patient these asexual parasites are rapidly overcome and disappear from the peripheral blood. If treatment be stopped at a stage short of thorough eradication or what passes at present for thorough eradication of the parasite from the body there will be freedom from obvious parasitism and from

symptoms for at least ten days; thereafter reappearance of the parasites and symptoms becomes more and more likely

After the initial phase of recrudescence there may be a trouble-free period and then, about nine months later, a second period of recurrence of the disease.

The terms used are:

Recrudescence	re-appearance of symptoms and parasites within eight weeks of recovery.
Relapse	re-appearance of symptoms and parasites within eight to twenty-four weeks of recovery.
Recurrence	attacks after twenty-four weeks.

One phase of the asexual cycle which has been the subject of much discussion and a phase of very great practical importance is the so-called "exo-erythrocytic phase" immediately following upon the biting of the host by the infected mosquito. James and Tate (1937), continuing the pioneer work of Raffaele (1934), discussed those forms of P.gallinaceum found in the cells in the reticulo-endothelial system of infected chickens, and showed that there is a phase of development which takes place in the reticulo-endothelial cells, particularly of the spleen, brain, liver, kidneys and in the cells of the

capillaries. Huff and Coulston (1944) using bird malaria also, recorded that the sporozoites take about three generations from the reticulo-endothelial cell to the red blood corpuscle. The search for these forms in human malaria has been intensive and since 1937 there have been several claims that the forms had been demonstrated in the human but these were never substantiated until Shortt, Garnham, Covell and Shute (1948) reported success. Following closely upon their demonstration of these forms in the monkey they reported the finding, in the liver of an infected human, plasmodial masses studded with chromatin particles. This step forward will no doubt materially assist in the synthesis of antimalarial drugs with a powerful action against this phase of the organism, i.e. antimalarial drugs producing causal prophylaxis.

One feature of the benign tertian infection is that the red cells enlarge. In all the species numerous brown-black granules (malaria pigment) appear in the red cells as the ring forms enlarge. This is a degradation product of digested haemoglobin, related to haematin.

Malaria is generally thought to be a self-limiting disease; Nocht and Mayer (1937) suggest that in the absence of treatment the following are the intervals of time required for spontaneous "cure":

Malignant tertian malaria usually one to two years.

Benign tertian malaria usually four to five years.

Quartan malaria usually longer.

These figures are approximate and are not generally accepted. This complete disappearance of the parasite probably may represent a stage in which the organism and the host reach a condition of seeming symbiosis which can easily be upset in favour of the parasite, e.g. Shute (1946b) considers that latency in benign tertian malaria may occur:

1. Under natural conditions.
2. With drug prophylaxis.
3. With mixed infections.
4. As a result of immunity to one particular strain.

In more detail:

1. Latency under natural conditions is shown by the latency during the winter, so often found with benign tertian malaria.
2. With drug prophylaxis: A course of quinine, atebrin or plasmoquine, taken for the first one to three weeks after infection may lead to considerable latency before the appearance of overt malaria.
3. With mixed benign tertian and malignant tertian infection benign tertian may appear after several months.
4. Strain immunity is seen in inoculation malaria (and by the immunity to the local strains so often shown by indigenous peoples).

Of considerable importance is the question of how cure

results, whether assisted or natural. One type of blood cell that has been repeatedly invoked to explain cures in malaria is the monocyte, now thought to take origin from the reticulum; the closely-related histiocytes are also thought to play a part. Monocytes are actively phagocytic for foreign particles and for the blood protozoa. McCallum (1898) claims to have seen this happen to the malaria parasite in bird malaria. Nocht and Mayer say that this may occur in serious cases of malignant tertian with whole parasites phagocytosed, and Whitby and Britton (1946) have seen marked erythrophagocytosis by monocytes in the circulating blood. Taliaferro and Taliaferro (1929) go so far as to say that the chief mechanism of defence against malaria is phagocytosis and destruction of the parasites by the large mononuclear cells. Krishnan, Lal and Napier (1933), talking of monkey malaria stated that the fate of the animal depends on whether or not the rise in the mononuclear count is maintained. Several authors have claimed that the beneficial action of quinine is due to its accelerating the natural processes of cure by monocytes. Nocht and Mayer (1937) also suggest that the liberated pigment, damaged red cells, etc. lead to a great increase of large mononuclear cells but point out that this effect is common to all protozoan diseases.

Whatever the reason there is no doubt that monocytosis does occur in malaria; this has been known for a long time.

Stephens and Christophers (1904) said that a figure of over fifteen per cent of large hyaline mononuclears in the absence of kala azar is diagnostic of malaria. Christophers and Bentley (1908) suggested a rise of over twenty per cent of mononuclear leucocytes is found in malaria.

Nocht and Mayer (1937) suggested the rise may be as high as thirty per cent.

Ross (1932) gives values of monocyte count in his large series of blackwater cases in Southern Rhodesia.

Eight cases showed less than ten per cent mononuclear cells.
Twenty cases showed ten to twenty per cent " "
Eight cases showed twenty to thirty per cent " "
Two cases showed over thirty per cent " "

Ross (1932) was probably right and in agreement with the later reports by Nocht and Mayer (1937) when he said that part at least of this mononuclear increase is a reaction to blood destruction and is not specific, e.g. Krishnan (1935) points out that during and after an attack of blackwater fever there is an increased number of mononuclear cells which he considers to be a simple reaction to red cell destruction and to the liberation of free haemoglobin.

This question of the function of the monocytes is of importance, i.e. whether these cells are merely a mechanical

response to debris or whether they have a specific function; Hicks and Opie (1942) suggest the function is more than mechanical; they claim to have shown that these cells liberate a proteolytic enzyme which they consider responsible for the destruction of red and white cells. The question has not yet been definitely settled.

These are a few of the more important facts known about malaria; what is not known is how malaria predisposes to blackwater fever, and what part it itself plays in the production of the lytic phase.

Scott (1939) quotes the opinion of Bastinelli who wrote in 1899 that "the preceding malaria creates the fundamental disposition, existing malaria the accidental disposition, and quinine the provocative agent". Almost all workers are agreed that the malaria undoubtedly acts to create some fundamental change. Scott (1939) says, for example, that a predisposition arising from chronic malaria is a necessary preliminary for blackwater fever.

Krishnan (1935) working with monkey malaria thought a damaged reticulo-endothelial system to be a pre-requisite to malarial haemoglobinuria.

Rogers and Megaw (1939) stated that malaria alone is rarely capable of causing blackwater fever; by this it is presumed they meant the actual lytic phase.

Ross (1932) from his experience in Southern Rhodesia confirms that infections with malaria is a necessary preliminary to the development of the susceptible state in which haemoglobinuria may occur, and proceeds to say that apart from its sensitising action malaria may also play a part in the precipitation of the actual attack. Ross continues by saying that even if malaria plays no part in the actual precipitation of haemoglobinuria, susceptibility to blackwater fever is greatest during the actual malarial attack. This last point is challenged, see Foy and Kondi below. Ross went on to say that in the production of susceptibility to blackwater fever chronicity or frequency of infection appears almost essential.

Yorke et alia (1930) using spleen puncture, found no evidence of heavy malarial infection before the onset of blackwater fever.

Similarly Foy and Kondi (1937b) also using spleen punctures, showed that it is very doubtful whether in blackwater fever there is a heavy infection with malaria before an attack of blackwater fever. These last-named authors state that it is abundantly clear that the patients they examined were not suffering from an acute attack of malaria before they developed an attack of blackwater fever. This fits well with clinical experience where

again and again patients give a history of an attack of blackwater fever beginning like a bolt from the blue when they felt very well.

Two other important facts which have been mentioned before and are again brought forward are:

1. The disease rarely occurs in individuals immediately upon their arrival in areas of endemic blackwater fever. Scott (1939) quotes an analysis of 1050 cases and found that in the majority of these the disease occurred in the second to third year of residence.
2. Blackwater fever is rare in indigenous natives though natives from other areas may develop the disease, e.g. Bengalis in Assam.

One last point is that the post-mortem changes in fatal cases of malaria and in fatal cases of blackwater fever do not markedly differ except when the kidney lesions of blackwater fever are present; in neither disease are the findings useful as a basis of explanation for the relationship between the two diseases;

It is useful here to recapitulate before proceeding to consider how our findings affect the diagnosis we made of blackwater fever in Waziristan; it may reasonably be said: that malaria is the essential pre-requisite though its method of function is not clear, a "sensitising" action being the most

widely accepted theory; that a history of chronic and repeated infections is commonly obtained, especially of chronic malignant tertian malaria, in areas where malaria is constantly endemic; that monocytosis is a constant feature of malaria and usually of blackwater fever though its function are as yet ill-defined; that the disease rarely immediately attacks susceptible migrants, and rarely attacks indigenous natives; that the disease may occur in almost "epidemic" form; that the onset of haemoglobinuria may come as a "bolt from the blue"; and that many different substances and factors may have the power to pull the trigger in the individual previously "sensitised" by malaria.

This description of malaria and blackwater fever was necessary for two reasons, firstly to see whether the description would provide any serious obstacle to our diagnosis of blackwater fever in the Waziristan cases, and secondly to give a foundation for the discussion on the theories on the causation of blackwater fever, since malaria in some way or another enters into so many of such theories.

Firstly then let us see if our diagnosis is brought into question by the above brief description of malaria. Undoubtedly there are certain general statements which are against our diagnosis though not entirely excluding it; among these are: that blackwater fever is usually related to chronic

or repeated attacks of malaria, particularly of malignant tertian malaria; and that this disease rarely occurs outside certain areas.

Figure No. 23 below summarises the relative information in our six cases.

Figure No. 23.

Relevant information regarding malaria in the Razmak cases.

Case No.	Information re previous attacks of malaria	Size of spleen on admission	Parasites recorded on admission	Army service (years)
1.	1. Fresh benign tertian malaria July 1936 2. Clinical malaria August 1936 (Second attack obviously recrudescence)	Spleen not palpable	Ring forms and gametocytes of P.vivax.	8
2.	Fresh benign tertian malaria July 1937	"	Numerous ring forms P.vivax.	3
3.	No report previous malaria.	"	Ring forms P.vivax.	3½
4.	" "	"	Asexual frms. P.vivax.	12
5.	" "	"	Ring forms P.vivax.	7
6.	" "	Spleen palpable 2 fingers enlargemt.	Gametocytes and ring forms P. vivax.	5

This is interesting information.

It is seen that, from the history,

One individual had suffered two previous attacks of malaria.

One individual had suffered one previous attack of malaria.

Four individuals stated they had never had malaria previously.

This last statement is a sweeping one; all the patients were Indians and all had homes in areas of endemic malaria, four being from the Punjab and two from the United Provinces. They obviously must have been exposed to infection until they joined the Army, and also while on home leave during the Army career. They all denied any history suggestive of malaria at home before joining the Army and it is unlikely that they did contract malaria while on leave because pre-1939 each man was examined on return from leave and if he gave a history suggestive of malaria or showed splenic enlargement he was given a twenty-one days course of quinine and plasmoquine; none of these men had any record of this in the medical history sheets; these medical history sheets were among the most carefully kept documents in the Army at that time and the entries could be relied upon as a true record of a man's health. It seems then that apart from the unlikely possibility that they had been infected while at home on leave and had not received

treatment on return, four of the men had never had malaria while in the Army (periods ranging from three and a half to twelve years), one had had one infection in three years and one had had two infections in eight years. These figures are to be relied upon. Here then we have a group of men of whom it may safely be said that they developed haemoglobinuria while undergoing treatment for malaria although they had never suffered from frequent or chronic attacks of malaria. In fact in four of the cases so far as the patients were aware it was their first attack. The spleens of all were not enlarged except case No. 6, who came into Razmak hospital after eight days untreated malaria.

Another important point is that in all of our six cases the parasites were P.vivax species. The blood films were checked and counter-checked by individuals with many years of experience of the microscopical appearances of malaria parasites. It is agreed that occasionally a mistake may be made and it is agreed that there is always a possibility that each case was one of mixed infection with the malignant tertian rings not seen but it is unlikely that either or both of these explanations fit all the six cases; it may be presumed that in some at least of the six cases the species of parasite causing the disease was P.vivax. At that time P.vivax was responsible for about two-thirds of all cases of malaria in Waziristan. No explanation can be given for these differences

from the usual findings but it is held that such differences in themselves do not necessarily make our diagnosis untenable; they are unusual findings but then blackwater fever is a strange disease; we need only think of the blackwater fever sometimes seen in inoculation malaria, e.g. Most (1940) reports two cases, and many others have been reported; I have had one case in my practice using the Madagascar strain of P.vivax. This developed in a hospital in Glasgow in 1936 but has not been reported except privately to Colonel James. Another point generally brought up in discussions on blackwater fever is that it is migrants who are susceptible. In this respect at least our findings do not conflict with those of the general run of patients for our cases certainly were migrants; their homes were on the plains of India many miles away from Waziristan and they had only come to Waziristan in the course of their military duties. The periods spent there had ranged from seven months to two and a half years before their attack of haemoglobinuria.

Ross (1932) held that acute malaria may play an immediate part in the precipitation of haemoglobinuria, whereas Foy and Kondi (1937b) and other workers had held that acute malaria is not necessarily present at the time the patient develops blackwater fever, a finding well in keeping with clinical experience. It is of interest to see whether our patients were suffering from malaria at the time of onset of haemoglob-

inuria. All of them had been suffering from acute malaria a very short time previously; after the onset of malaria however, five of the six patients had received a short course of quinine, all had received a five-day course of atebrin 0.3 g. daily, and each individual had received plasmoquine 0.02 g. daily for periods ranging from two to four and a half days. This considerably lessens the chance of the patients having been suffering from acute malaria at the time of onset of blackwater fever although it certainly does not exclude the possibility because the course of treatment given was a short one. At the time of onset of haemoglobinuria this possibility of persisting malaria was thoroughly investigated in all cases; all blood films were found to be negative, and intensive antimalarial treatment when tried had no effect on the fever, which later was regarded as probably the body's reaction to the freed stromata and other foreign protein. With one exception the differential count at the time of onset of the haemoglobinuria showed no monocytosis.

It has already been suggested in Chapter VI that the patients were not suffering from active malaria at the time of onset of haemoglobinuria. The probability is that in the majority at least the infection had come under control before the onset of haemoglobinuria.

It is concluded that in spite of the departures of the findings in our cases from those usually found in

blackwater fever these differences did not exclude the diagnosis, which still therefore remains as "blackwater fever".

It now remains for us to discuss the less unlikely of the many theories put forward as to the cause of blackwater fever.

We shall begin by discussing some of the less widely accepted of such theories, and group them under "Miscellaneous".

Miscellaneous Theories on the Causation of Blackwater Fever.

It would indeed be a formidable task to summarise all the theories produced on the causation of blackwater fever. At one period in the history of the disease it seemed to be a point of honour for each new writer to produce a new theory, and, since the cause is as yet unknown, this production of new theories has not yet ceased.

The fundamental facts of the disease were fairly well established early in the disease, e.g. Karamitsas (1882) says that the action of the pathogen is not primarily on the liver or kidneys but on the blood, leading to a considerable destruction of red cells and liberation of haemoglobin followed by

haemoglobinuria. The problem is, what is the pathogen; a list of suggested pathogens culled from various authors makes interesting reading; Scott (1939) discusses several theories but reiterates that all crash on the fact that new arrivals escape. Pellarin in 1865 suggested the haemolytic action of the urine to be the factor in the production of blackwater fever. This theory has been repeatedly resurrected, e.g.

by Plehn (1903) and (1920)

by Rapoport (1928)

Barthelemy-Benoit (1865): Disposition secondary to malaria; he believed the predisposing cause to be a bilious and sanguinous temperament.

Berenger-Feraud (1874) suggested sunstroke, diet, change of locality; in this last he obviously introduced the idea of migrants.

Karamitsas (1879): Familial disposition; later this is supported to some extent by Ross (1932)

Yersin (1895) suggested B.coli communis.

Sambon (1898) parasites allied to babesia, in view of the findings in the redwater disease of cattle.

Plehn, F. (1898) emotion.

Christophers and Bentley (1908) suggested haemolysin.

Ashburn et alia (1912) spirochaetes.

Leishman (1912) chlamydozoa.

Coles (1913) suggested that protozoa may be responsible.

Huntze (quoted by Scott)(1916) suggested light sensitiveness

may be a factor.

Nocht and Kikuth (1929) suggest destruction of protective action of haemolytic antibody by quinine; this was on experimental anaemia of dogs but they held the results to be applicable to blackwater fever.

Ross (1932) could not accept the theory of blackwater fever virus because blackwater fever does not attack migrants until they have been resident for some time in the blackwater fever area and it does not attack the native inhabitants; he could not accept the theory of physical variations such as chill, wetting, over-exertion and fatigue. He considers that a familial susceptibility may exist; this supports the view of Karamitsas (1879); Ross, however, was basing his information on repeated infections of individuals rather than on high incidence among members of a family; these two are not necessarily the same thing.

Naumann (1933) suggests disease of the liver.

Osler (1935) quoted the opinion of Deeks and James that one factor may be a lowering of the body resistance.

Krishnan and Pai (1936) working with monkeys claimed to have demonstrated a marked diminution in cholesterol in the pre-haemoglobinuric state; they further claimed that haemoglobinuria did not develop in animals in which the free cholesterol was normal or above normal.

Chopra (1936) has collected a long list which includes several

theories already given above, namely: these suggestions include,

1. Quinine causes blackwater fever by direct haemolytic action in individuals with intolerance either alone (this was one of the earliest of suggestions, e.g. Veretas (1859)) or in bile solution or in lecithin. Or by lowering the osmotic tension of the plasma with resultant destruction of the red blood corpuscles by osmosis.
   Or by inducing a sudden contraction of the spleen; this will be discussed later.
2. Increased lactic acid; this is the theory of Blacklock and MacDonald (1928) and will be discussed in a separate section.
3. Presence of a foreign protein derived from destroyed merozoites, this leading to "anaphylaxis", to be discussed in a separate section.
4. Hyperactivity of the reticulo-endothelial system due to repeated attacks of malaria; Foy and Kondi (1943) are among those who refuse to accept this.
5. An underlying chronic malarial infection.
6. Some undiscovered poison, like that causing piroplasmosis.

Krishnan and Pai (1936) consider an important factor to be a reticulo-endothelial system damaged by malaria in certain highly susceptible individuals.

Nocht and Mayer (1937) refuse to accept theories of abnormality of red cells or simple overflow haemoglobinuria from heavy destruction by quinine of parasitised red cells or the haemolytic effect of detritus on red cells. These authors consider hypocholesteraemia to be one possible explanation.

Scott (1939) agrees that predisposition resulting from chronic malaria is a necessary preliminary for blackwater fever. He groups theories of blackwater fever causes into four main classes.

a. That the condition is a pernicious form of malaria.

b. Quinine intoxication.

c. A disease sui generis.

d. An induced condition arising from repeated malarial infection.

Scott then tentatively mentions one factor worthy of further consideration, namely civilized conditions of living, e.g. Bengali clerks in the Assam Tea Gardens, and Egyptians in the Sudan, and Europeans in the Tropics. It is doubtful whether this need be considered further. So far as my experience goes the Bengali in the Assam Tea Garden and the Egyptian in the Sudan live as they would have lived at home.

Ham and Castle (1940) discussing haemoglobinuria in general put forward an unsupported theory of "normal cells

being destroyed by 'abnormal stasis' and 'abnormal cells' being destroyed by normal stasis".

Manson-Bahr (1940) considers blackwater fever to be the result of repeated attacks, or continuous infection with, malignant tertian malaria, but considers three other factors necessary, namely an anterior haemolytic factor the result of malaria, lowered cholesterin content and quinine.

His idea is that there is some derangement of metabolism associated with chronic malignant tertian disease and this is precipitated by quinine, the result being production of a potent haemolytic substance.

Maegraith et alia (1943) suggest the cause of blackwater fever to be the upsetting by malaria of a balance of a haemolytic substance and an antihaemolytic substance which they claim to have discovered in normal individuals. This had been adumbrated by Nocht and Kikuth in certain experiments in 1929.

Calewart (1944) suggested lowered tension of blood to be a factor in the production of the intra-vascular haemolysis of blackwater fever. He examined normal and blackwater fever bloods with the interfacial tensiometer of Lecomte du Nouy and claims that malaria parasites have the same haemolytic effect as adding a trace of soap to an isotonic solution of NaCl containing red corpuscles.

Hills (1946) suggests a theory similar to one previously criticised by Nocht and Mayer, namely that the haemoglobinuria of blackwater fever merely represents a malarial haemolytic mechanism so intense and acute that the reticulo-endothelial system cannot keep the plasma haemoglobin below the renal threshold, i.e. blackwater fever is merely a further stage in malarial haemolysis, a stage of pernicious malaria.

Dimson and McMartin (1946) give a list of suggested causes which includes several already quoted, and in addition they mention:

1. Hypertrophy of the reticulo-endothelial system, causing greater tendency to haemolysis in the chronic malaria cases.
2. Lysolecithin formation suddenly increased, formed from stagnant blood in an enlarged spleen.

This is an incomplete list but it serves to show how varied and numerous are the theories which have been produced to explain blackwater fever.

The majority of the theories mentioned above have been quoted only to be dismissed. Such are the theories which have been born of unsupported speculation or of single unsupported findings; included in such we may dismiss theories on the haemolytic action of the urine, of particular predisposing bodily dispostion, familial disposition, effects of

diet, specific organisms such as B.coli, babesia-like organisms, spirochaetes, chlamydozoa, protozoa, light sensitisation and civilised conditions of living. There is no evidence to support the isolated experiences which led to the putting forward of the above theories and it is pointless to discuss each of them in detail.

It is necessary to enlarge a little upon certain of the other theories put forward in the above list but first it may be profitable again to consider what precisely is the problem before us. There has been much muddled thinking in the past and a failure to grasp the essentials of the problem. Stephens (1937) was on the right lines when he talked of factors in three classes, namely:

1. Factors of aetiological importance.
2. Accessory aetiological factors.
3. The mechanism of the haemoglobinuria itself.

Manson was thinking on the same lines when he asked what pulled the trigger.

In my opinion there are at least two separate factors concerned, those producing the fundamental underlying disposition, and those actually pulling the trigger. Certain of the explanations which have been put forward in the past have been attempts to explain the creation of the underlying dispostion. Others have been attempts to explain the mechanism precipitating the actual lytic phase and some have

been attempts to explain both the fundamental change and the immediate effect.

Foy and Kondi (1943) made this clear when they said that in blackwater fever malaria is an important preparatory agent (although its mechanism is unknown) but the trigger may be pulled by any one of several agents; they included in these latter classes both physical changes and drugs. It is necessary to keep these divisions in our minds in any discussion on possible causes, i.e. what charges the gun, and what pulls the trigger. It is necessary at the beginning of the discussion to repeat that malaria undoubtedly is a major factor in charging the gun; it is not yet established that malaria is the only factor.

Among the factors which have been enumerated above and not yet discussed are:- change of locality (Berenger-Feraud); emotion (Plehn); haemolysin (Christophers and Bentley and several other workers); physical factors, e.g. chill and exertion (Barratt and Yorke, Nightingale etc.); climate (Parrot); haemolytic body balance (Nocht and Kikuth, Maegraith et alia); liver disease (Naumann); lowering of body resistance (Osler); hypocholesterolaemia (several workers including Manson-Bahr); quinine either alone or in combination with other factors; increased lactic acid; anaphylactic reactions; abnormal reticulo-endothelial system; underlying chronic malaria; abnormal red cells; change in interfacial tensions;

pernicious malaria; stasis; lysolecithin formation, etc. These are all very interesting though some are little more than vague speculations.

Discussions have been deferred to other sections, (see below) on the theories of abnormal red cells; special strains of parasites; special lytic substances; lactic acid; and anaphylactic phenomena. This leaves the following for consideration:-

1. Quinine, either alone or as a secondary factor; Chapter VI deals with this aspect of the problem and there is no need to say anything other than that quinine is well recognised as having power to precipitate blackwater fever but only in individuals hypersensitive to the drug; quinine therefore is merely one of the many substances and factors which may pull the trigger, either by itself or in company with other substances. It does not create the fundamental disposition.

2. Change of Locality: undoubtedly change of locality appears to play a part in the production of circumstances favourable to the onset of blackwater fever. We need only think of the susceptibility of Bengalis in Assam and other individuals safe enough in their own environment but equally in danger with migrant Europeans when they are moved to another part of their own

country. The explanation possibly is that the change of locality allows of the development of the fundamental disposition, presumably by attacks of malaria caused by strains to which migrants are not immune. The effect of change of locality therefore is simply that of production of chronic malaria.

3. Emotion. Plehn (1898) and later Cardamatis (1910) quoted cases suggesting this. In such cases the emotion must act by pulling the trigger although the exact mechanism is unknown.

4. Physical Factors. Probably must produce their effects in the same way as emotion; here also the mechanism is not clear.

5. Liver Disease: there is no doubt that liver damage is found both in malaria and in blackwater fever, e.g. Hills (1946) and Maegraith, Andrewes et alia (1947) reported damaged livers in post-mortem examinations of individuals who had malaria; or blackwater fever. In such cases however the liver damage is the result of the blackwater fever or of malaria and not the cause of the blackwater fever.

6. Hypocholesterolaemia: cholesterin in vitro has an inhibiting effect on the action of certain haemolytic substances, e.g. cobra venom; furthermore certain workers claim to have shown that animals fed with cholesterin

are less sensitive to haemolytic substances than are normal animals. Other workers claim to have shown that the blood cholesterol level is low immediately prior to the haemolytic phase in blackwater fever, e.g. Krishnan and Pai (1936) with monkeys.

Krishnan and Pai (1936) explained this by saying that repeated attacks of malaria have a tendency to lower the blood cholesterol level. Others support these findings but perusal of their reports show that in many cases they were quoting an opinion and not recording their own findings. Careful work on this problem by several workers, e.g. Ross (1932) shows that hypocholesterolaemia is NOT a common finding in blackwater fever; in fact it is so uncommon that it cannot be taken as the factor necessarily constantly present to produce blackwater fever.

Therefore since hypocholesterolaemia is not normally found before or during the lysis in blackwater fever it cannot be an essential factor in the production of this disease.

7. Abnormal reticulo-endothelial system. Certain authors, e.g. Krishnan (1936) suggest a damaged abnormally functioning reticulo-endothelial system is the cause of blackwater fever; others, e.g. Chopra (1946) suggest the haemoglobinuria possibly to be the result of an

over-functioning reticulo-endothelial system greatly hypertrophied by chronic malaria. These were again largely expressions of opinion with no foundation in experimental work. Foy and Kondi (1943) summarised the generally accepted opinion on this by saying that although the part played by the reticulo-endothelial system in the haemolysis of blackwater fever is admittedly not clear modern work suggests that even when this system is grossly hypertrophied it is unlikely quantitatively and qualitatively to account for the sudden blood destruction.

8. Chronic Malaria, Pernicious Malaria. These two have been suggested as causes of blackwater fever, e.g. Hills (1946) suggests that the haemoglobinuria of blackwater fever is a "spill-over" of haemoglobin from the plasma which has become overloaded with haemoglobin produced by severe haemolysis. It is quite common to get both chronic malaria and pernicious malaria and yet never to see blackwater fever, however, e.g. Chopra (1930) states that chronic malaria cannot be the whole answer, e.g. in certain districts of the Punjab where malarial conditions are ideal as regards malignant tertian infection blackwater fever is unknown. Similarly Whitby and Britton (1946) quote malignant tertian malaria so severe in certain patients that 1,000,000 red cells may

disappear in one paroxysm, yet we do not get haemoglobinuria. Undoubtedly malaria is necessary for the development of the fundamental disposition; but there is no proof that malaria plays any part in the actual precipitating of the lytic process. In fact there is no proof to the contrary, see Foy and Kondi (1937b).

9. Stasis. Vague theories dealing with stasis as a factor in the destruction of red cells have been put forward by Ham and Castle (1940) but the work is as yet much too indefinite to allow of any expression of opinion.

i.e. only malaria so far has been proved to play any part in the production of the underlying disposition to the disease, and the means whereby it produces this effect are unknown.

Among the many factors which may cause the precipitation of an attack of blackwater fever with the onset of lysis, there have been included emotion, chill, fatigue, wetting and quinine; there are many others, e.g. plasmoquine, phenacetin and other drugs; shock, trauma, severe illness; and sometimes no obvious cause at all, with the disease striking an apparently healthy individual living under good conditions, e.g. an individual on leave in England. All of these, far too lengthy to be listed, must be classed as substances or conditions that may pull the trigger.

So far malaria, and only malaria, has been proved able

to produce the fundamental change in the body.

One more interesting point is whether there may be some recognisable factor common to all the varying factors recognised to be capable of pulling the trigger. On this point several workers have implicated the spleen, e.g. Chopra (1936) talks of quinine causing sudden contraction of the spleen which expels haemolytic toxin into the circulation. Dimson and McMartin (1946) similarly talked of lysolecithin formation, dealt with below, formed from stagnant blood in an enlarged spleen; we may also infer from the article by Ham and Castle (1940) that they think that stagnation in the spleen may be responsible for increased blood destruction; Foy and Kondi (1943) talk of splenic enlargement as one of the factors which may pull the trigger, and Barratt and Yorke as long ago as 1909 had mentioned the possibility of splenic contraction playing a part in the production of blackwater fever; it will be seen later, e.g. in the section on anaphylaxis that other authors bring the action of the spleen into question to explain certain of the phenomena they encountered, e.g. Bergenheim and Fahraeus (1936) who suggested that an enlarged spleen produced larger quantities of lysolecithin; also Gear (1946) who suggested that the enlarged spleen holds quantities of antibody. This is a tempting hypothesis, that all of the varying factors found to be capable of pulling the trigger in blackwater fever do so by virtue of their common effect on the

spleen either by producing contraction of it or by producing stasis in it. One important point in this connection is the remarkable series of results reported by Burkitt (1943) who obtained gratifying results in blackwater fever by injection of 15 grains of sodium luminal; this drug may conceivably have produced its striking effect by stopping splenic contraction.

Unfortunately there is not enough proof to support this, but fuller investigation into this aspect of the problem is long overdue.

Again, it is necessary to go from the general to the particular and see whether any of the above suggestions has a bearing on the particular problem of the Waziristan cases. All that can be said in this connection is that each of the affected individuals had been suffering from malaria a few days before the onset of the haemoglobinuric condition. It has already been admitted that an initial attack of malaria is very unlikely to have produced the "sensitisation" necessary for the development of blackwater fever and a tentative suggestion was made that each of the individuals had had malaria some considerable time previously, at home in their villages. Admittedly this could not be proved; all that can be said is that each individual had malaria and each individual developed haemoglobinuria now diagnosed to be blackwater fever; the

fundamental disposition must therefore have been present, the gun must have been charged. The second point concerns "pulling the trigger". It should be remembered that blackwater fever may follow a primary attack of malaria, e.g. it is known in inoculation malaria. Several authors, e.g. Ross (1932) have suggested that malaria also plays a part in this pulling of the trigger but others, e.g. Foy and Kondi (1937b) disagree and certainly on the evidence active malaria did not appear to be present in our cases at the time of onset of the blackwater fever. Malaria probably did not help to pull the trigger, i.e. in my cases it took no part in the precipitation of the lysis. I have suggested, however, that plasmoquine most likely did act in this connection, i.e. plasmoquine pulled the trigger or was one of the important factors in this, see Chapter VIII.

The last problem is the part, if any, played by physical strain in the onset of the condition. Physical strain, chill, fatigue, wetting all are held to be possible factors contributing to the onset of an attack of malarial haemoglobinuria; it is felt that conceivably these on occasion may precipitate the catastrophe. In Chapter I details are given of a gruelling short sharp move against enemy opposition. This undoubtedly was a strain, even to the fit men taking part and some may claim that this played a part in the precipitation of the disease but figure No. 24, below, shows that the time

interval is so long that this is quite unlikely.

Figure No. 24.

Time interval, in days, between the ending of the Column and the beginning of haemoglobinuria.

Case No.	1	2	3	4	5	6
Time interval (days)	13	22	22	21	73	77

It is also to be remembered that while the Kharre march was severe, it was not more so than many other marches carried out week after week. Physical strain and exposure played no part in the precipitation of the haemoglobinuria which occurred in my six cases.

We can only say that so far as the Waziristan cases are concerned, malaria was the predisposing factor and plasmoquine was one of the factors, if not the only factor, which pulled the trigger.

The next theory to be considered is:

Abnormal red blood corpuscles as a cause of blackwater fever.

This is a theory which has attracted attention from the beginning of the study of blackwater fever, and is still attracting attention. The statement has been repeatedly made that the cause of the red cell lysis is abnormality of the red

cells, e.g. Ross (1932) makes the vague statement that accumulated evidence points to abnormality in the behaviour of the red cells as the cardinal factor in the causing of lysis, but remarkably little careful calculated investigation has been carried out on this aspect of the problem; practically all workers with the exception of Foy and Kondi have limited themselves to the crude method of testing the fragility of the red cells in saline solution; the results of such tests vary with the various workers, e.g. Stephens and Christophers (1900) claimed that the resistance of the red cells to lysis by hypotonic solution is raised, whereas Plehn (1903b) sometimes found a lowered resistance.

This crude test has been repeated many times by many workers and it is now generally accepted that there is no significant change in the reaction of the red cell to varying strengths of saline solutions. Nocht and Mayer (1937) said that certain workers hold that a special sensitiveness of the red blood corpuscles especially to quinine may play a part in the production of blackwater fever but these authors do not agree. Foy and Kondi are among the few workers who have attempted a planned examination of the problem of the red blood cell in blackwater fever. In 1941 Foyl Kondi and Moumjidis transfused 25 ccs. of blood from an actively haemolysing case of blackwater fever into a normal man; after eleven days the volunteer developed falciparum malaria but did not at any time

show any sign of haemolysis. The authors also transfused cells from normal blood into the blackwater fever patient and found these normal cells to be rapidly destroyed. They finally concluded that "it seems probable that a circulating haemolysin may be responsible for blackwater fever." This technique was not above criticism, but it was the attempt to investigate the matter on proper lines. Foy and Kondi (1943) continued the work and again repeated that the changes which lead to the haemoclastic crisis in blackwater fever cannot be in the red cells; they concluded that the fundamental defect is in the environment. At the same time, however, they found that in blackwater fever while the fragility of the red cells is normal in saline solutions the red cells have an increased fragility to lysolecithin. Birnbaum et alia (1946) working with red cells from malaria cases also report that such cells lake more readily in bile solutions than do normal cells; the enhanced fragility lasts for several weeks after the attack of malaria. Normal and malarial sera, however, both showed inhibitory action on this. Birnbaum et alia concluded that in malaria it is probably the red cell which is affected.

At present therefore the balance of evidence would appear to be against the suggestion that the cause of the blackwater fever is abnormality of the red cells but there are certain findings such as those of Foy and Kondi with lecithin which make it clear that the problem has not been properly

investigated and that further work is required. Loutit (1946) showed how the problem should be tackled in his work on certain other types of haemolysis; until similar work is carried out with blackwater fever cases we shall not be able to come to any decision on this point. It can only be said at present that there is no clear-cut evidence either way but that it seems that there is no obvious proof that the red cell is implicated in the causing of blackwater fever.

Findings in the Razmak cases are given in figure No. 25 below.

Figure No. 25.

Fragility red cells in the Razmak cases.

Case No.	1	2	3	4	5	6
% normal saline solution in which cells lysed completely.	0.30	0.40	0.40	0.35	0.35	0.35.

In this crude test therefore nil markedly abnormal was found in our cases.

The next theory requiring consideration is the lactic acid theory.

Sarcolactic acid as a cause of blackwater fever.

Blacklock and Macdonald (1928) pointed out that blackwater fever arises mainly in the course of prolonged infection with malignant tertian malaria and since there is no evidence that this species of parasite produces a haemolytic toxin then there must be some other explanation of the association. The authors turned their attention to the points differentiating P.falciparum from other species and they claim that these are:

1. The parasite of this species sporulates in the internal organs.
2. The cells containing the sporulating forms adhere to each other and to the vessel walls.

The authors then drew attention to certain other haemolyses, e.g. march haemoglobinuria and they claim that blackwater fever and these other haemoglobinuriae have one thing in common, namely anoxaemia. They then claim that a deficiency of oxygen from whatever cause leads to the production in excess of lactic acid in the blood. In experiments with animals they found this substance lactic acid to be haemolytic in vitro and in vivo; they concluded that lactic acid is the haemolytic agent responsible for lysis in blackwater fever. This theory has met with strong criticism, e.g. Ross (1932) considers the theory fallacious and points out that many facts known about the formation and destruction of lactic acid have been overlooked, e.g. anoxaemia and hyperlactacidaemia occur in a variety of

pathological conditions to an extent sufficient to produce symptoms yet there is no haemoglobinuria. Furthermore hyperlactacidaemia has never been demonstrated in blackwater fever. This theory of Blacklock and Macdonald is typical of many of the theories produced to account for blackwater fever, namely a theory produced by hypothesis alone, with little attempt made to prove or disprove it by careful investigation; it would not have been difficult to find out whether in point of fact there is present increased lactic acid related to blackwater fever. Ross pointed out that hyperlactacidaemia does not exist.

Recently this theory has found supporters in Dimson and McMartin (1946) who consider that the lactic acid theory has not been entirely disproved; here again, however, the authors do not produce careful investigations to support their claim which seems merely an expression of opinion.

Unless further and more definite proof is obtained to support this theory it must be regarded as quite unlikely to be the explanation of blackwater fever.

The next theory is:

Individual differences in species and strains of Plasmodia as a cause of blackwater fever.

This is a tempting theory and one that has attracted many workers by its simplicity. Ross (1932) was thinking along these lines when he said that the assumption of a hypothetical blackwater fever virus would admittedly clear up

several points. There are three great drawbacks, however, namely:

Why should the occurrence of blackwater fever usually be limited to a very few of the many areas of malaria?

Why the infrequence of blackwater fever in the first year of residence?

Why the immunity of the indigenous native?

It is necessary to consider the evidence in this matter. The problem divides itself naturally into two main questions.

1. Is there any difference in the individual abilities of the four species to produce blackwater fever, or rather the disposition to blackwater fever?
2. Are there special strains within the species with the power readily of inducing blackwater fever susceptibility?

Firstly consider problem 1; i.e. is any one of the four species of plasmodium more commonly and closely associated with blackwater fever development than are the others? This section can be opened with the statement that one of the most commonly accepted of the beliefs concerning blackwater fever is that it is related to infection with P.falciparum, particularly chronic malignant tertian malaria; but like so many of the beliefs about blackwater fever careful analysis shows that there is little to support this belief. It is unnecessary to quote large numbers of extracts from writings in which authors express the belief that malignant tertian malaria is the cause

of blackwater fever sensitivity. A few such reports are:

Thomson (1924a) states that when P.falciparum exists in a high percentage blackwater fever may occur if the other necessary factors are present. The higher the incidence of malignant tertian the more frequent blackwater fever becomes; he considered the high incidence of blackwater fever throughout Africa to be due to the intensity of malignant tertian malaria. He feels that P.falciparum is the species most commonly found in blackwater fever and he explains the other cases occurring with benign tertian infection by suggesting that they must have been mixed infections in which the malignant tertian infection has been masked by another species.

Blacklock and Macdonald (1928) go so far as to base their theory on lactic acid formation on the fact that malignant tertian infection is the one associated with blackwater fever; they claim that lactic acid is produced by P.falciparum much more than by any other parasite because of the biological characteristics of the cycle of this parasite in the body; the inference is therefore that malignant tertian is the species commonly responsible for blackwater fever.

Sayers (1927) claims that the home of the malignant tertian parasite is in the splenic pulp where schizogony

proceeds. A haemolytic toxin is liberated by this parasite but since the circulation in the splenic pulp is normally sluggish this toxin reaches the circulation in a fairly steady slow stream. Should a sudden contraction of the spleen occur, e.g. as is suggested with quinine ingestion a sudden large dose of haemolytic toxin is shot into the circulation, with resultant haemolysis and possibly haemoglobinuria.

Scott (1939) is somewhat more careful and only states that where malignant tertian malaria greatly preponderates blackwater fever is prone to occur.

Manson-Bahr (1940) however makes the sweeping statement, in talking admittedly only of therapeutic malaria, that no case of blackwater fever has ever been observed in association with vivax inoculation.

There are many other workers who appear to think the same but there are also many eminent authorities who think otherwise. Koch himself (1899) was ahead of his time in questioning this belief when he stated that the frequency of association of blackwater fever with malignant tertian malaria is NOT greater than that which arises from the ratio of the two forms of malaria (i.e. benign tertian and malignant tertian). He considered the preference of blackwater fever for tropical malaria (malignant tertian) to be only apparent. This is a point which has been

repeatedly overlocked, namely that Africa, from where so many of the reports of blackwater fever have emanated, is a country where generally speaking there is a high relative incidence of malignant tertian and this may give a false impression.

Ross (1932) then suggests that too much significance is placed upon the species of parasite present at the time of the attack as indicating the variety of malaria which has led to the development of susceptibility.

Amy (1934) gives actual figures of the type of parasite found in his small outbreak, which has already been discussed. There were ten cases of blackwater fever in one area previously free from this disease. The figures were:

Malignant tertian rings in four cases.

Benign tertian rings in four cases.

Benign tertian schizonts in one case and nil in one case.

Amy is inclined to support Thomson's theory and suggests that in the benign tertian cases there may also have been malignant tertian parasites which were not detected. There is no evidence for this belief however. The figures quoted by Amy are small compared with the very large series reported and discussed by Foy and Kondi (1937b) who investigated 360 cases of blackwater fever in Macedonia. Positive results were found in 144 cases, i.e. forty per cent of the 360 cases. The

low percentage is explained by the fact that many people had begun quinine before the blood had been examined; many of the forty per cent positive were diagnosed on splenic punctures which would have probably shown any hidden malignant tertian infection when present. The findings in the forty per cent positive were:

P.falciparum.	Forty seven per cent.
P.vivax.	Thirty three per cent.
Both.	Fourteen per cent.
Pigment only	six.

But in Macedonia falciparum malaria is one and a half times commoner than vivax malaria, therefore there appears little difference between the relative potency of vivax and falciparum so far as the production of blackwater fever is concerned; in fact the evidence is slightly in favour of vivax being the more potent in producing blackwater fever. The authors admit that it is not possible to rule out previous sensitisation with P.falciparum; even making allowance for this possibility, benign tertian alone must have been the factor in a significant number of individuals.

Stephens (1937) thought blackwater fever has been found with all four species and states that in some of the reports the relative frequency reported of the species found in blackwater fever cases is the frequency of the species themselves in that particular area.

Certainly there can be no doubt that blackwater fever can occur following infection with species other than P.falciparum. This has been well brought out in certain catastrophes with inoculation malaria, e.g. Schilling and Jossmann (1924) report blackwater fever following inoculation with vivax strains and Bamford (1934) records blackwater fever following therapeutic inoculation with quartan parasites in a case of G.P.I.; this particular strain had been used at the same time in three other patients all of whom showed no reaction. Unfortunately I am in a particularly strong position to confirm that blackwater fever may result from therapeutic inoculation with vivax malaria for I had a fatal case of this type in 1936 in a male Jew, aged 17, inoculated as part of treatment for disseminated sclerosis; there is no doubt as to the diagnosis; the strain used was the "Madagascar" strain. The authorities were informed at the time and Shute (1947) in discussing the matter again says that they could not understand what had happened as there never had been any such report of this complication although between 1925 and 1946 the number of patients infected ran into thousands. Lastly it is again pointed out that in our six cases of haemoglobinuria in Waziristan the species present was P.vivax in all cases. The diagnosis was made and the diagnosis was confirmed by individuals with long experience in the detection of species in blood films; it is admitted that there may have been a double infection but it was not

found and one cannot postulate double infections merely to fit facts into a preconceived theory; there must be more positive evidence before it is accepted that malignant tertian malaria is more likely to lead to blackwater fever susceptibility than is malaria due to other species.

The evidence is not sufficiently clear to implicate malignant tertian malaria more than other species of parasite.

What then is the position as regards the second of the possibilities, i.e.:

2. Are there special strains within the species with the power readily of inducing blackwater fever susceptibility?

It is possible to begin this section by saying that there is undoubtedly wide variation in the virulence and other characteristics of different strains of parasite.

Chopra (1936) states that there is wide variation in the virulence of different strains of parasite in different parts of the world and there is a wide variation in their reactivity to various drugs. Furthermore, the individual strains may vary from time to time, e.g. Nocht and Mayer (1937) state that resistance may set in to all medicaments. These authors in common with others mention remarkable sensitivity of inoculated malaria to therapy. Presumably they meant malaria inoculated by blood. At that time the significance of the exoerythrocytic phase in mosquito infection was not

known.

Shute (1946c) points out that in the evaluation of antimalarial drugs the strain of the species must be taken into account and he states that it has undoubtedly been proved that there are geographical strains within a species which vary in clinical virulence; he quotes experiments proving that the amount of quinine required to control attacks of certain Rome and Sardinian strains of malignant tertian malaria was eight times that required to control attacks of malaria induced by strains from India, Africa and Rumania. The studies were made on primary cases. The conclusion was that there are degrees of virulence and resistance within strains of the same species of plasmodium.

One of the most striking examples of naturally occurring variants was that which at one time threatened a campaign; this is reported by Fairley (1946) who reported in detail the finding at Aitape and Wewak in New Guinea of a strain of P.falciparum resistant to atebrin; this is the only strain ever reported to be resistant; it was not resistant to quinine or plasmoquine. The origin of the strain was never determined, whether it had arisen as a mutation or whether the resistance was a biological characteristic of a geographically limited strain.

The behaviour of the Madagascar strain of P.vivax maintained in this country for therapeutic use is an example

of a single strain varying from time to time. According to Shute (1947a) this strain was originally obtained from a lascar from Madagascar - actually he may have been infected at a port en route; it was first obtained in 1924 and has been maintained through man-mosquito-man since then. At the beginning this strain appeared very virulent, e.g. Hanschell, (1924) using it in disseminated sclerosis cases, had severe reactions; two of his patients nearly died. James (1924) said then that the Madagascar strain had become more virulent. Shute (1947b) does agree that in certain hands it may have a death rate of up to fourteen per cent and even in Horton the death rate for inoculated malaria may be two per cent. Also this is the strain that was followed by blackwater fever in one of my patients in Glasgow in 1936. Certainly this theory of local variation in strains is one which is firmly entrenched in the minds of clinicians who have had to treat malaria in various parts of the world; we need only think of the killing disease of the Burma Border compared with the mild type of disease in certain parts of the North of India. It is rightly said that malaria varies not only from village to village but almost from house to house. Another point supporting this is that natives living in areas of endemic malaria even in definitely virulent endemic malaria may appear immune to the disease, yet if they move to another area they will develop malaria. One must presume that they have an immunity

and that that immunity is limited to the strain of malaria found around their homes.

It seems then to be a fact that there may be wide variation in strains of species and wide variation in the character of the disease that they produce. This being so it was not then a far step to the suggestion that blackwater fever is the result of infection with a strain of parasite characterised by the power of producing this disease. This theory has received a lot of support, e.g. Cort (1929) suggested as the cause of blackwater fever a malaria parasite of a variety capable of elaborating a potent haemolysin. Nocht at first postulated the same theory; in fact he claimed to have been the first to have done so, though this is unlikely but later, in Nocht and Mayer (1937) Nocht admits he no longer believes in the theory of a special strain of parasite. James et alia (1932) gave indirect support for this theory when they proved the existence of biological strains of P.falciparum differing profoundly in virulence according to the country or area from which they came, especially when these authors produced two cases of blackwater fever in patients following inoculation with a special Italian strain.

Giglioli (1932b) favours the theory of special strains; he considers that blackwater fever must be related to certain strains of plasmodium, biological varieties of the commonly known parasites and more particularly of

the aestivo-autumnal species. He considers the haemoglobinuric attack to represent the culminating phase of infection with a haemolytic strain.

Krishnan and Pai (1936) agree that this is probable but think that the factor of host resistance to be of far greater importance.

Ross (1932) considers the possible explanation of blackwater fever to be exaltation of virus by constant passage through the relatively immune aboriginal tribes of the locality. He suggested that certain species of anophelines may be implicated; he quoted A.funestus and A.gambiae in Southern Rhodesia; it is not clear whether he infers that passage through these vectors causes changes in the ordinary strains. This is a point of view supported by

Chopra (1936) who says that among factors always found in blackwater fever areas are: firstly malarial strains of intense virulence; and secondly anophelines of the funestus species. Foy, Kondi and Moumjidis (1941) also said that one possibility to be considered as a cause of blackwater fever is infection over long periods with a special strain of malaria.

There are serious objections to this simple, all too simple, theory and the number of authors who could be quoted who do not agree with the theory is larger than the list given of those in favour. Practically all of those who can be quoted

against the theory are merely expressing opinions, however, without experimental evidence to support them; a more telling argument is the existence of three facts, namely if this simple explanation be the answer then:

Why should the occurrence of blackwater fever be limited to a very few of the many areas of endemic malaria?

Why the infrequence of blackwater fever in the first year of residence in a blackwater fever area?

Why the immunity of the indigenous population?

Furthermore there have been repeated experiments where volunteers have been inoculated with blackwater fever blood during active phases of haemolysis yet there never has occurred a case of blackwater fever in these volunteers, e.g. Foy and Kondi (1936) inoculated 106 mental patients from fifty eight cases of blackwater fever and failed in all cases to produce blackwater fever. These findings cannot be explained away by theories and unless they are explained away then there is no evidence to support the theory that blackwater fever is the result of infection with a special strain of parasite.

The only work done with animals which has a bearing on this problem is that of Napier and Campbell (1932) dealing with P.knowlesi discovered accidentally in Macacus rhesus

monkeys. This particular parasite was found to be intensely virulent against M.rhesus with sixty per cent of the red cells becoming infected, with finally haemoglobinuria. Haemoglobinuria much more commonly resulted from infections by strains repeatedly passaged.

This is an example of the fact that the host resistance is probably of as great importance as parasite strains in the production of grave types of disease, and possibly in the production of blackwater fever.

But does this finding affect the Waziristan cases? It will be remembered that five of those six individuals who developed malaria followed by haemoglobinuria almost certainly became infected during the Kharre Column when we were moving throuch country very infrequently traversed by our forces; details of this are given in Chapter I. Blackwater fever was unknown, practically speaking, in Waziristan before that time, and it is indeed tempting to suggest that the strain which these men had picked up was one particularly capable of producing blackwater fever. There is no positive evidence to support this, however, and the remaining men, almost 100 in number, did not show any similar lytic phenomena. It can only be said then that while the theory of a special strain would have fitted well with the happenings in Waziristan this cannot be postulated in the absence of any positive evidence in its favour, either in Waziristan or in any other area of

endemic malaria.

Two theories remain to be considered, namely the theory of a special lysin and the theory of anaphylaxis. These require full consideration which must be carried over to the next chapter.

A summary of this chapter is given below.

Summary.

The early history of blackwater fever is obscure; Hippocrates about 400 B.C. reported eighteen cases of "black urine" some of whom gave histories very suggestive of blackwater fever. Some distinguished workers accept this to have been true blackwater fever; generally speaking however it is thought that they were not cases of blackwater fever. It is now generally accepted that the disease first was recognised early in the nineteenth century; reports of it began to appear at that time from widely scattered areas of the world; there is no explanation to account for this and it is not yet known whether the disease arose at that time sui generis or whether these were the first cases recorded of a condition previously unrecognised. The term "blackwater fever" was introduced by Easmon in 1884.

There has been little advance in knowledge of the

condition since that time excepting in the recognition of the kidney lesions and in the identifying of the blood pigment methaemalbumen.

The few statements on blackwater fever which are generally acceptable are:
That malaria is the essential prerequisite.
That chronic infection with malaria is found very commonly to have been present before blackwater fever developed; there is no unequivocal proof that blackwater fever is more common with chronic malignant tertian than chronic benign tertian malaria.
And that migrants are most heavily attacked, but not usually in the first year of residence.

The problem originally was "what fired-off the attack of lysis". It is now recognised that many varying factors and substances may pull the trigger; the fundamental problem as yet unsolved, is how the gun comes to be charged, i.e. what is the underlying mechanism. In this discussion it was suggested there is no such state as the "pre-blackwater stage"; the majority of individuals so-called are probably suffering from chronic untreated malaria while others may be suffering from subliminal blackwater fever, which is probably more common that is generally realised; careful spectroscopic investigation would probably confirm this in blackwater fever areas.

These blackwater fever areas are suggestively those in which malaria is present practically all the year round, e.g. the Bengal Dooars, Madras Presidency, etc.

Blackwater fever may occur sporadically in areas normally free from it, and it may occur almost in epidemic form; therefore the rarity of the disease in Waziristan does not necessarily prevent the diagnosis of blackwater fever being made in our cases. This diagnosis of blackwater fever is supported by a similar "outbreak" near the same area, reported by Amy 1934.

Malaria and blackwater fever. Under this heading the relationship of malaria to blackwater fever was discussed. It was pointed out that malaria is the one definite factor known to be related in some way to blackwater fever but the mode of action of malaria in this respect is not known. Malaria is the result of infection with the genus plasmodium which has an asexual cycle in man and other animals; this asexual cycle begins with the exoerythrocytic phase which is not affected by drugs (other than paludrine) and then proceeds through schizogony.

If untreated, malaria is thought to be self-limiting but some of the cases may actually be examples of latency and not of cure. One marked change found in individuals affected with malaria is monocytosis. The monocytes are thought by

some to play a part in the production of cure and of immunity but many others think that the function of these cells is largely non-specific, to clear the debris found in the blood in every attack of malaria. None of the findings, post-mortem or otherwise, assist in explaining just how malaria predisposes to blackwater fever but the mechanism must be one of "sensitisation". The evidence available suggests that an acute attack of malaria is not a common finding in an individual showing frank blackwater fever.

The records of the Waziristan cases showed surprisingly that none of the individuals concerned had had frequent or chronic malaria. In fact in four of the cases the attack preceding the onset of haemoglobinuria was thought to be the primary attack of malaria. This seems to be confirmed from Army Records. All the individuals showed benign tertian forms in their blood on admission; some of the cases may have been examples of mixed infection but in some at least the species must have been P.vivax. These facts do not necessarily exclude the diagnosis of blackwater fever, e.g. blackwater fever may be found with benign tertian malaria following inoculation. It is held that at least the majority of the patients in the Waziristan series were not suffering from active malaria at the time of onset of haemoglobinuria; the cause or causes of blackwater fever not being established, it was decided to consider the more important theories under certain headings.

Miscellaneous theories on the causation of blackwater fever.

In this section were listed a few of the less unlikely of the many theories. Certain of these theories were dismissed without further discussion, e.g. haemolytic action of urine, familial disposition, effects of diet, specific organisms, light sensitisation, and civilised modes of living.

Before considering the remainder of the theories it was pointed out that the substances and factors may produce their action in any one of three ways, by leading to the production in the body of a fundamental disposition to blackwater fever, or by leading to actual production of the lytic attack itself, or both.

Among the causes postulated as producing the first (i.e. fundamental dispostion) only malaria has been definitely proved to be capable of producing the fundamental change; malaria may also fall into the third category; all the others fall into the second category, i.e. the category of those that pull the trigger; these are many in number and varied in character, e.g. drugs, emotion, chill, exertion, fatigue.

It was not proved that liver disease or hypocholesterolaemia or blood stasis play any part in the production either of the first or of the second state.

In several of the theories discussed above and in

several remaining to be discussed, the spleen is thought to be implicated in the production of the actual lytic phase either by the contracting of the spleen, with a flooding of the body with the particular substance responsible for the haemolysis, or by the spleen producing its action by holding large masses of blood in stasis. There is as yet no proof whether or not this theory has any foundation.

Lastly, in a discussion of the problem as it affected the Waziristan cases it was held that only two factors of those mentioned are implicated, namely malaria and plasmoquine; the malaria provided the necessary sensitisation and plasmoquine fired off the attack. Physical strain did not play any part in the production of the attack.

The next theory discussed was:

Abnormal red blood cells as a cause of blackwater fever.

There is no definite evidence at present either for or against this theory. The experimental evidence is insufficient but the balance is against the theory that abnormal red cells are responsible for blackwater fever. The Waziristan cases showed no abnormality in the reaction of the individual red cells to hypotonic saline solutions.

Theory of lactic acid as a cause of blackwater fever.

This theory was introduced by Blacklock and Macdonald who held that malignant tertian malaria produced anoxaemia which

in turn produced hyperlactacidaemia; they found lactic acid to be haemolytic *in vivo* and *in vitro* and claim that this substance is the active haemolysing agent in blackwater fever. There is no experimental or other evidence to support this, and the theory is regarded as very unlikely to be correct.

Individual differences in species and strains of Plasmodia as a cause of blackwater fever.

This necessarily must be considered under two headings: namely:

1. Do the individual species differ in their ability to cause the development of blackwater fever?
2. Are particular strains of parasite to be found which are capable of producing blackwater fever?

On the first problem opinion is fairly evenly divided but in the reports in which figures are given and not merely opinions, e.g. the large series of 360 cases reported by Foy and Kondi, there is no evidence that blackwater fever is more often related to malignant tertian malaria than to malaria caused by the other species. Furthermore, inoculation with benign tertian and quartan species has occasionally led to blackwater fever; one personal experience of this is reported; it is also pointed out that in the six Waziristan cases the only species detected was *P.vivax* in each of the six cases.

The second part of the problem is whether there exist special "blackwater" strains of any of the species. It must

be accepted that strains do vary widely in their characteristics and in the type of disease which they may produce; the striking report by Fairley from New Guinea is an example. It is a short step from this acceptance of wide variation of strains to the theory that blackwater fever is due to a special haemolytic strain of parasite. This short step however is not justified on the evidence available. Several workers have accepted this theory but have failed to produce any convincing evidence to support their contention; the little experimental work that has been done is strongly in favour of there being no special haemolytic strain; this theory of a special haemolytic strain would have well explained the findings in the Waziristan series where the cases of haemoglobinuria followed upon malarial infection acquired during a march through country infrequently traversed. In the absence of satisfactory proof this theory is held not to be acceptable.

The two remaining theories both requiring full discussion will be dealt with in the next chapter, a continuation of the problem of blackwater fever.

CHAPTER X.

Blackwater Fever (contd.)

Haemolysins as a cause of blackwater fever.

Like the theory of special strains of parasites as a cause of blackwater fever this theory of haemolysin is so simple that it has attracted much support in spite of the paucity of satisfactory experimental work on the subject.

Inside this major theory there is, as it were, a number of "sub-theories"; the simplest way of discussing the problem is to divide it into three sections, namely:

1. Discussion on the general belief of circulating haemolysin.
2. Discussion on lecithin and lecithin derivatives.
3. Discussion on the theory of lysin-antilysin balance.

1. The theory of haemolysin.

Haemolysins having been found to be the cause of haemoglobinuria in certain diseases, e.g. paroxysmal haemoglobinuria, it was inevitable that this should be postulated as an explanation of the haemolysis in blackwater fever. Before we quote the unsupported opinions of various workers it is of advantage to consider the small amount of experimental evidence available on this problem. A good example of the way the problem should have been tackled and a series of experiments from which we may draw valuable information relative to our

problem is the report of Loutit and Mollison (1946). This report throws light on the investigation of intra-vascular haemolyses in general. Their work was primarily on the question of acquired acholuric jaundice anaemias. They showed that the acquired type of this disease is due to a circulating haemolytic antibody. They established the findings which must be recognised as common to all conditions with circulating haemolysins, namely:

1. the individual so affected is capable of destroying normal red cells transfused into him.
2. his own red cells should show signs of being sensitised and easily haemolysed.

The authors reported one striking finding, namely that cells of individuals with acquired acholuric jaundice did not undergo lysis when transfused into a normal individual. The current conception of red cell sensitisation by an appropriate antibody is that once the red cells have become sensitised in this manner then haemolysis results from the action of normal complement. To explain the unexpected normal survival of transfused cells from their acholuric cases Loutit and Mollison suggested that the current conception is not necessarily the correct conception, and they suggest that the process of sensitisation is a reversible one <u>in vivo</u>. Experiments by Browning (1925) with brilliant green and red cells also suggested that all haemolytic phenomena are not to be explained in terms of

laws governing production and action of true serum haemolysins. In such cases there is obviously some other factor present which completes the destruction of the sensitised red cells, i.e. some potentiating substance in the plasma of such cases.

This work of Loutit and Mollison has been mentioned in some detail since it shows that in one disease producing intra-vascular haemolysis from circulating haemolysin the process is not a simple one obeying the normal rules governing antigen-antibody reactions; and this must be taken into consideration before we may condemn only on negative grounds any theories put forward of circulating haemolysins. In point of fact certain experiments by other workers suggest that red cells from haemolytic anaemia cases do not live in the blood of normal individuals as long as did normal cells, e.g. Brown et alia (1944) claimed that, while the survival time of normal transfused cells is fifty days, those from haemolytic anaemias only lived for seven days.

Foy and Kondi (1936) are among the few workers who tackled the problem by the making of experiments rather than the making of hypotheses. They were investigating the problem of haemolytic strains of parasite and/or haemolysins. They used in their experiments fifty eight cases of blackwater fever at varying periods of time after the first passage of black urine. From these they inoculated 106 mental patients, sometimes using

blood inoculations and sometimes using the indirect method of mosquito biting. In the blood inoculations 8-15 ccs. were given by immediate inoculation intra-muscularly into G.P.I. cases. In all experiments, i.e. direct inoculation, and passage through mosquitoes these authors failed in every case to produce haemoglobinuria. Sixty-eight cases were infected by the indirect method, through mosquito passage, the anopheline species being elutus, superpictus and maculopennis. In the sixty-eight cases the organisms came from thirty-five different cases of blackwater fever.

Furthermore in the cases who were given direct blood inoculation the test had been made even more approaching to "blackwater fever" conditions by giving blood inoculations to mental patients with a suitable "malaria ground", i.e. to individuals suffering from acute malaria of any of the three common species, or from mixed infection (in cases in whom the blood was loaded with parasites), or suffering from chronic malaria with low-grade infections.

The bloods used for inoculation were heavily loaded with vivax, or falciparum parasites or both.

All the cases were observed from nine to eighteen months after the blood inoculation or the infected mosquito feeding.

There is no comparable series in literature; the

numbers are large, far more than the majority of workers have seen, and the conditions were testing to a severe degree. This experiment must be regarded as one of the findings to which close attention is necessary.

Then Foy, Kondi and Moumjidis (1941) carried out the next logical step in the investigation. They pointed out that two questions remained to be answered, namely:

a. Are the red cells of blackwater fever patients peculiarly liable to haemolysis by specific autohaemolysins?

b. Are <u>normal</u> red cells lysed when transfused into blackwater fever patients?

They considered problem b.; they carried out transfusions into a blackwater fever patient, a woman, aet. 24 and claim that their results showed undoubtedly that this blackwater fever patient had the power to destroy normal cells transfused into her. They then transfused 25 ccs. of blood from the actively haemolysing patient into a normal healthy man who did not develop haemolysis but who developed malignant tertian malaria after eleven days.

At first sight these results appear contradictory, i.e. destruction of normal cells in blackwater fever patients, yet no destruction of normal cells by blood from the blackwater fever patient. The authors themselves point out the fallacy in the second part of the test. The fact that 25 ccs. of

blood did not produce haemolysis cannot be taken to indicate that it did not contain haemolysins, e.g. in the giving of blood in blood transfusions the serum of the donor may be and often is haemolytic to the cells of the recipient and yet, although such serum is given in quantities much greater than the volume used by Foy, Kondi and Moumjidis yet ordinarily no haemolysis results because of the great dilution of the donor's serum by the recipient's serum; therefore the fact that 25 ccs. of blood did not produce haemolysis in the healthy individual is no proof that haemolysins were not present; haemolysins may have been present and were swamped by the heavy volume of serum in the recipient. Foy, Kondi and Moumjidis came to the conclusion that a circulating haemolysin may be responsible for blackwater fever, and produce the important suggestion that continuous sensitisation is necessary before the red cells become liable to haemolysis. This would be explained, they think, by infection over long periods, possibly with a special strain of parasite.

Foy and Kondi (1941) still on the subject of intravascular haemolysis report a case of blackwater fever in a pregnant woman who gave birth to a child during her attack of blackwater fever when she was actively haemolysing. The child showed no sign of haemolysis. Zinsser et alia (1944) have confirmed what is well-known in epidemiological work, namely

that non-diffusable colloidal materials can pass across the placenta, i.e. foreign proteins may enter the foetus in utero, and both active and passive sensitisation of the foetus can take place. Foy and Kondi suggest two possibilities to explain why the cells of the child were not destroyed by circulating haemolysin which they think is the explanation of blackwater fever; their suggestions are:

1. The cells of the child were <u>not</u> susceptible to the mother's haemolysin. Or
2. The maternal haemolysins did not pass the placenta or the amount that did pass was insufficient to bring about haemolysis.

Still working on the same problem Foy and Kondi (1943) point out that any changes which the red cells undergo in the body are changes produced by interaction of the cells with the environment, i.e. with the medium which bathes them. They concluded that in blackwater fever the changes may be in the cells, or the environment or both.

But from previous work, reported above, it is seen that normal cells are also destroyed when transfused into blackwater fever patients. They conclude <u>that in blackwater fever the fundamental defect is NOT in the red cell but in its environment.</u> They give as an example congenital haemolytic jaundice where there is (1) Cell abnormality, (2) Some action in the spleen,

proved by the fact that the periodic haemolysis is stopped by splenectomy. The last part of their statement would appear wrong.

The authors introduced the question of the splenic factor merely to attempt to bring out a theory which has been discussed already, see p.637, namely that there is some fundamental factor common to all those haemolytic conditions in which the spleen is enlarged. We have already claimed however that this theory, while very attractive is still only a theory.

Foy and Kondi continue by saying that possibly several factors are involved, e.g. there may be more than one process that can prepare the body for the haemolytic crisis, and possibly all the processes may not work in the same way. They then bring out points which have been stressed several times previously in this work, namely, that in blackwater fever the preparatory agent is malaria - we might almost say malaria alone - and that, the ground having been prepared, the trigger may be pulled by any one of several agents, e.g. antimalarial treatment or splenic enlargement or cold; or the attack may appear without any obvious precipitating cause.

The work of Foy and Kondi, while the best and most carefully planned of this type of experiment, was not the first

of its kind, e.g.

Blacklock (1923) was one of the first to carry out such experiments, e.g. on the fourth day of lysis in a blackwater fever patient who died later this author took 1½ ccs. citrated blood and mixed it with ½ cc. citrated saline. He then injected ½ cc. of this mixture into a healthy European; the patient took quinine for two days. The result was negative, no malaria and no blackwater fever. This experiment may appear crude and ill-planned but in view of the possibilities and the lack of knowledge at the time it should be regarded as a courageous and commendable effort.

Thomson (1924b) was next; he inoculated ten guinea pigs intra-peritoneally with centrifuged deposit from ten cases of blackwater fever. Result - negative in all cases.

United Fruit Company (1926) report an experiment in which ten ccs. blood taken from a patient in his first paroxysm of haemoglobinuria were immediately injected into the vein of a negro; the patient did not develop either malaria or blackwater fever.

The above are a few of the experiments carried out on this problem.

Expressions of opinion include those of:

Cort (1929) who states that the cause of blackwater fever is a potent haemolysin liberated by a special variety of malaria parasite.

Fairley and Bromfield (1934c) first state that it is improbable that the cause of blackwater fever is true haemolysin but still obviously adhere to the theory of haemolysins by suggesting that there occurs a derangement of metabolism associated with chronic malignant tertian malaria and this derangement of metabolism gives rise to a potent haemolytic substance; this view was later confirmed by Fairley and Bromfield (1940).

Nocht and Mayer (1937) considered that the cause of haemolysis is that the production of haemolytic substances suddenly and spontaneously increases; they feel that all investigations support the theory that haemolytic substances play a part in blackwater fever and one very likely theory, they feel, is that of circulating haemolysins resulting from the malaria process, which haemolysins circulate in the bloodstream or are fixed in certain inner organs. They considered that we cannot exclude the possibility of circulating haemolysins merely on the negative evidence that the existence of such has never been demonstrated. They said that the haemolysins are present in the inner

organs only, not appearing in peripheral circulation.

Rogers and Megaw (1939) thought that under certain conditions, as yet unknown, red blood corpuscles appear to become sensitised to the action of a haemolysin.

Low and Fairley (1942) said that the lytic substance in blackwater fever is possibly the lysin normally contained in the reticulo-endothelial cells which have hypertrophied as the result of chronic malaria, this lysin suddenly being liberated into the blood. They suggest that such lysin may become immediately fixed to the corpuscles in the visceral vessels and therefore cannot be demonstrated in the peripheral blood. This theory that antibody is "bound" as soon as it is produced is supported by an isolated finding by Brown et alia (1944) that the survival time of cells transfused from patients with lysis into the blood of a normal individual is only seven days compared with the fifty days survival time of the healthy red cell.

The statements quoted above are little more than statements of opinion; few of the authors have any experimental support for their statements.

One theory which conflicts with the above is that of Ham and Castle (1940); this has been mentioned previously in passing. They do not accept the theory of circulating haemolysins, and suggest instead that the haemolysis in haemolytic

anaemias is due to intra-vascular stasis. This stasis may be normal in character, in which case only abnormal cells will be destroyed. On the other hand, normal cells will be destroyed by abnormal stasis; according to the authors, stasis leads to changes in red cells by which they are more readily destroyed. Their theory is not proved but must be considered for further investigation in view of the work on "sludged blood" recently brought into prominence by Kniseley et alia (1947). In the absence of any supporting findings we cannot do more than acknowledge this work of Ham and Castle.

This brings to a close the first part of the discussion on lysins as a cause of blackwater fever. It has been seen that few experiments have been carried out which give information on this point, and these few experiments do not give unequivocal results although they do distinctly show that if there be a haemolysin concerned in the production of blackwater fever then it is not a simple circulating substance for such has never been demonstrated in the blood of any individual tested. Admittedly, available methods of detection are crude in the extreme compared with the intricate processes which we are studying, e.g. there do exist diseases producing intra-vascular haemolysis in which the cause is undoubtedly a circulating haemolysin and yet such has never been demonstrated. Certain of the workers on this problem have

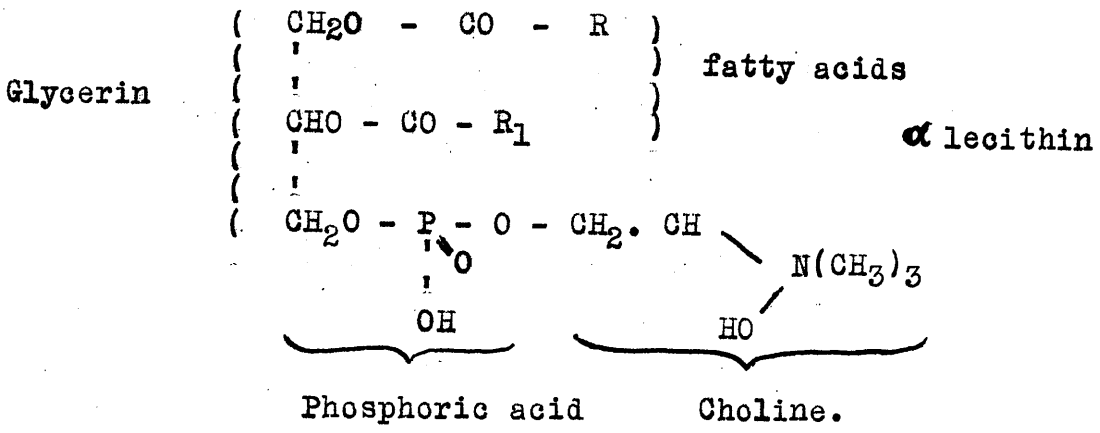
supported the theory of haemolysin and have explained the failure to demonstrate it by suggesting that the lysin is to be found only in the inner organs (Nocht and Mayer) or that the lysin as it is produced is taken up by and bound to the red cells (Low and Fairley) or that the whole process of haemolysis has a different explanation to that generally accepted (Loutit and Mollison); from our experience in the Waziristan series of cases we are not in a position to express an opinion on this matter as we did not carry out any experimental investigations.

Some of the workers have gone further even and have attempted to identify the substances concerned in the lysis of red cells in blackwater fever. This theory forms the subject of the next section, i.e.

2. Lecithin and Lecithin derivatives.

Evans, C.L. (1945) quotes Overton's opinion that the external limiting pellicle of the red blood corpuscles as in most living cells, is formed by a lecithin-cholesterol compound whose solvent power determines the permeability of the cell by foreign substances. Best and Taylor (1943) agree that all cells contain at least one of the phospholipids; in this class also are cephalin and sphingomyelin. The amounts of lecithin and cephalin vary in the various tissues, e.g. in the heart and lung the lecithin-cephalin ratio is two to one. Thorpe (1944)

gives the structure of α lecithin to be



About seventy per cent of the structure of lecithin is fatty acid, and this fact, coupled with the physical property of lecithin of being able freely to mix with water, suggests one important function of these phospholipids, namely the transport of fat and possibly action as intermediaries in fat embolism. It is thought also that lipids may have other functions but these have not yet been clearly established.

It is this substance lecithin, and certain derivatives, which is thought by some to be implicated in the haemolysis of red cells in certain intra-vascular haemolysis, e.g. Kritchevsky and Muratoffa (1923-24) claimed that lecithin in high dilution activates a mixture of quinine and red cells, the misture in itself being non-haemolytic. They further established that cholesterin has practically no inhibiting action on this haemolysis, a finding which is in marked contrast to

other haemolyses such as that produced by snake venom. Chopra (1936) also mentions that quinine becomes highly haemolytic in the presence of lecithin. One finding which suggests the mechanism of haemolysis is that of Schulman and Rideal (1937) who suggested that sodium oleate and taurocholate appear to attack the protein complex. In view of the important part played by phospholipids in the passage of substances into the cell itself it is not difficult to imagine that changes in the permeability may readily lead to destruction of the cell itself. Bergenheim and Farhaeus (1936) said that the haemolytic substance is lysolecithin, obtained by partial hydrolysis of lecithin. They suggested that this substance is produced in the "stagnating blood" of an enlarged spleen, and that it acts on the surface of the red cells so as either to haemolyse them or predispose them to destruction. Whitby and Britton (1946) support the above theories; they state that normal serum contains lysolecithin which has a direct lytic action on red cells. They point out that incubation of plasma for a few hours under static conditions causes a marked increase in lysolecithin, due apparently to serum lecithinase. They further agree that the normal serum content of the lysolecithin is increased by passage through the spleen. The particular application of the above to Blackwater fever is to be found in the work of Krishnan and Pai (1936) and of Foy and Kondi (1943); Krishnan and Pai

(1936) state that in malarial monkeys there is evidence of increased fatty acid production with rise in lecithin in pre-haemoglobinuria states ..... The authors think that unsaturated fatty acids or lysolecithin are probable agents of haemolysis. Foy and Kondi (1943) investigated the effect of lysolecithin on red cells and they claim to have found that cells from blackwater fever patients are more fragile than ordinary cells in a lysolecithin system; they thought that this substance may play a part in the haemolysis of blackwater fever because of the action on the lipoid-protein complex of the membrane. They suggest that the action of the lysolecithin is best explained on the basis of colloidal enzyme action. Their suggestion was that an enzyme lecithinase, acting on a substrate of red cells or serum containing lecithin splits the lecithin into unsaturated fatty acid and into lysolecithin which is powerfully haemolytic. These authors suggested three types of lecithinase, namely:

lecithinase	a	found in cobra venom.
	b	found in plant tissues. e.g. possibly the explanation of favism.
	c	found in Cl.welchii toxin.

All these lecithinases were found to have a disintegrating action on the cell membrane or intra-cellular lipoprotein complex whose integrity is dependent on the presence of

lecithin. Foy, Kondi and Moumjidis (1941) previously had thought that in blackwater fever the cause of lysis lay not in the red cell itself but in the red cell environment. In this newer work of Foy and Kondi (1943) the authors still claim that the fundamental change in blackwater fever is in the environment. They think that although the red cell has some abnormal features, e.g. increased fragility to lysolecithin, this is secondary to the change in the environment.

These suggestions that lecithin or a lecithin-derivative is haemolytic in nature would have little bearing on the problem of blackwater fever haemolysis unless either or both of two things can be proved, namely that the particular haemolytic substance has an especially marked effect on the red cells in blackwater fever, or that it is produced in greater quantity than normal in blackwater fever. As regards the first suggestion, we have already quoted the statement by Foy and Kondi (1943) that red cells from blackwater fever cases are particularly sensitive to the action of lysolecithin; this is an isolated finding and until more evidence is available it cannot be accepted as the explanation for blackwater fever lysis, particularly as the authors themselves still consider that the fundamental change is not in the red cell itself but in the environment. The other possibility is that the cause of blackwater fever is an increased production of lysolecithin

or some similar haemolytic substance. We have quoted Krishnan and Pai (1936) who suggested that there is a rise in lecithin in the pre-blackwater states of malarial monkeys, and Bergenheimer and Fahraeus (1936) who suggested increased production of lysolecithin in an enlarged spleen. Other workers, investigating on similar lines, have produced a theory which in part incorporates that of lysolecithin fragility, but which gives a different explanation for the production of this substance. This theory is discussed in the next section, namely:

3. Lysin-antilysin balance: this theory, briefly put, is that blackwater fever and certain other haemolyses may be the result NOT of production of a haemolysin normally absent from the blood but of the failure of the body to produce an anti-haemolysin normally present in the blood and normally responsible for the maintenance of a lysis-antilysis balance. This theory links with the theory of lysolecithin destruction in that some workers hold that the lytic substance said normally to be present in the body is lecithin or a lecithin derivative.

Among the first who suggested this possibility are Bijon (1915) and Ameuille et alia (1918); both groups suggested that the fault in blackwater fever is lack of antilysin to counteract lysin. Then Kritchevsky and Muratoffa (1923-24) concluded from experiments that substances are normally present

in human serum which check the haemolysis of a haemolytic quinine solution. Aguilar (1926) supported this view when he used haemostatic serum in the treatment of blackwater fever in the belief that in blackwater fever there is lack of an antihaemolytic substance present in the blood. Kikuth (1927) also considered that normal serum in small quantities can check the haemolytic action of a lecithin-quinine solution. Witts (1936) talks of a potential lysin present in normal serum; Scott (1939) states that both haemolytic and anti-haemolytic substances are present in normal blood and that there is deficiency of the latter in blackwater fever and paroxysmal haemoglobinuria.

The most careful piece of work on this problem is that of Maegraith et alia (1943) who discovered that normal animal tissue contains a heat-labile lytic agent which is normally inhibited by factors present in tissue washings and in serum. They suggested that the rate of haemolysis occurring in an animal at any one time may be a function of the balance between the tissue lytic agent and its inhibitors; the application of this to blackwater fever is their finding that sera from actively haemolysing blackwater fever cases apparently were much less active than normal sera in inhibiting the lytic agent, i.e. they think in blackwater fever lysis is simply a manifestation of excessive uninhibited activity of a normal lytic process. They suggested that the mechanism of reduction

of activity of the inhibiting factors in blackwater fever is unknown; possibly it is due to malarial infection or drug administration or both.

Ponder (1944) confirmed the above work and linked it with previous theories by suggesting the tissue lytic agent to be similar to lysolecithin. He thought that the substance is not species-specific.

Brückmann and Wertheimer (1945) confirmed also that washed tissue cells haemolyse washed red cells and agree that the lytic substance is not species-specific; they did not agree that the substance plays a part in normal or pathological blood destruction however and were of the opinion that the lytic phenomenon depends on autolysins and stasis.

Laser and Friedmann (1945) have isolated from human plasma a haemolytic substance which is nitrogen-free - and which therefore cannot be lecithin - they found that in vitro haemolysis by this agent is inhibited in a marked manner by antimalarial drugs. The suggestion of these authors is that the malaria parasites at some stage in their development produce or cause to be produced metabolite closely related to this natural haemolytic substance.

Maegraith (1946a) agrees with the findings of Laser and Friedmann.

Stone (1946b) found tissue extracts containing lipoids would agglutinate red blood corpuscles, and concluded from experiments that the haemagglutinating activity of saline extracts of animal tissues could be paralleled by a mixture of lecithin and cholesterol.

Enough evidence has now accumulated to suggest that normal tissue does contain substances haemolytic in character, and that normal serum contains substances which neutralise this haemolytic substance. Certain workers claim that the haemolytic substance is crystalline, without nitrogen in it and therefore is not lysolecithin. The work done on these haemolytic factors is sketchy as yet and is quite insufficient to allow of any decision on whether or not such a process plays any part in the normal blood destruction in the body.

Furthermore even if this balance should prove to be a physiological process of importance in the normal body, it by no means follows that imbalance of this process with lack of anti-haemolytic factor is the cause of haemolysis in black-water fever: such is quite unlikely when one remembers the most puzzling features of the disease, how it rarely attacks individuals during their first year of residence, and how the disease does not commonly attack indigenous natives.

The work in Waziristan can add nothing to this problem as investigations on this were not carried out.

Antigen-Antibody Reaction as a cause of Blackwater Fever.

This theory as an explanation of blackwater fever has tempted many workers through the years and it is a theory which is being repeated "re-discovered" by newcomers to the field of tropical medicine; there has been much written on the subject which deserves full attention.

It is best to open the subject with a brief consideration of a few of the principles of immunity in general and of immunity in malaria in particular. In the study of immunology an antigen has been defined by Zinsser et alia (1944) as a substance which gives rise to specifically reaction elements or "antibodies" in the blood serum or other body fluids of an animal to which these substances have been administered in a manner excluding their digestive disintegration; or more simply, a material with power to elicit a specific change in the cellular reaction capacity. A complex type of antigen is the compound antigen that can be separated into two components; in these the specificity depends on one of these components whereas the other component confers on the complex the capacity to elicit the production of antibody. The fraction associated with specificity cannot by itself cause antibodies to be produced within the normal body <u>but</u> by itself is capable of reacting with the antibody evoked by

administration of the compound antigen.

Landsteiner terms the specific fraction "haptene" or "partial antigen"; i.e. antigenic compounds specifically oriented by non-antigenic groups may be formed in the body by combination of extraneous material with the body proteins.

Normally the tissue cells of the higher animals are prepared only for metabolic processes concerned in the nutritional and excretory functions essential to the maintenance of life, i.e. the substances which reach the cells for nutritional purposes come into actual contact only after much preliminary digestion, e.g. proteins are reduced to amino acids, etc. Zinsser et alia (1944) suggest that most materials which are not in this class of predigested nutritive material give rise to a specifically altered state of reaction capacity on the part of the cells, hence relatively simple substances such as quinine can act as haptenes. Zinsser et alia further suggest that antibody formation may be connected in some way with non-diffusibility, the property of reacting only with cell surfaces.

Zinsser et alia (1944) subdivide hypersensitiveness into anaphylaxis, allergy and idiosyncrasy. In anaphylaxis circulating antibodies can be demonstrated; allergy is that form of anaphylaxis where as yet no circulating antibodies have been detected and which cannot be passively transferred;

and idiosyncrasy, the last type of hypersensitiveness, also cannot be passively transferred, and no antibodies have been demonstrated; it is typically found with compound antigens, e.g. drug sensitiveness. The important thing to note from this is that it is possible to have hypersensitiveness in an individual without being able to demonstrate it by laboratory tests.

Zinsser et alia consider that human hypersensitiveness depends for its development on two factors, namely sensitisation and heredity, i.e. a specific sensitising factor superadded upon a hereditary predisposition. They suggest that in the relatively few cases in which reactions occur upon first contact there probably has been a masked previous exposure, possibly intra-uterine. Obviously there must be predisposition, otherwise every individual would become hypersensitive to substances with which he comes into contact. These workers consider that in most protozoan diseases an attack of the disease confers a relative immunity which is in some cases considerable. They consider however that this immunity is resistance to superinfection rather than a true immunity and that where relative resistance persists the organisms are still latent within the body. In effect they mean that in protozoan diseases the immunity is an immunity of infection.

Whitby and Britton (1946) agree with the question of

a hereditary factor in cases of allergy; they also mention one other general point namely that of naturally occurring cold agglutinins in normal sera. This is mentioned here only to be dismissed since such agglutinins are of no practical importance, except in paroxysmal haemoglobinuria.

One problem of hypersensitiveness is whether the human can produce antibodies active against humans. This is possible and was first demonstrated by Landsteiner (1900) who showed isoantigens, the blood groups, to be present in the human.

Later Schwentker and Comploier (1939) showed that emulsions of homologous kidney and brain are autoantigenic - I have often thought in this matter that the nerve lesions which are rarely to be found with antirabies therapy are also a result of formation of an organ antibody, the reaction of which with the injected material causes damage to the recipient's central nervous system. Specially important relevant work on this point is that of Browning (1925) who investigated the antigenic power of haemoglobin. This author found haemoglobin to be antigenic but only very weakly so; antibody formation was found only in a small proportion of the animals treated. Similarly he found that globin is not a potent antigen. AND antiglobin serum did not react with homologous red cells. He quoted Muir and Ferguson's work to show that

the haemolytic antigens of the red corpuscle which, in combination with homologous antibody, lead to complement absorption reside in the stromata after corpuscles have been laked, e.g. addition of haemolytic antibody to haemoglobin solution after the removal of stromata did not fix complement. Similarly it has been pointed out already that globin possesses properties distinct from that of undissociated haemoglobin and does not contain receptors in common with the stromata of the red corpuscles.

These are general remarks and show that the body proteins are capable of acting as isoantigens; haemoglobin and globin are very weak in this respect but the stroma is much less weak.

The particular aspect of the problem of immunity is that concerning malaria, i.e. what is the character of the immunity, if any, which develops against malaria. It has been noted already that in the opinion of Zinsser et alia (1944) the immunity is one of infection. Nocht and Mayer (1937) stated that Koch in 1900, working in New Guinea, recorded one finding of importance in the question of immunity to malaria, namely that in districts heavily infected with endemic malaria children died early from malaria or gradually acquired a certain degree of immunity through repeated infections. By the time the native was twenty he was usually free of clinical signs of malaria.

Craig and Faust (1945) stated that as early as 1909 Craig, the senior author, had said that the evidence available on the subject of malaria was sufficient to show that immunity in malaria is due to the phagocytosis of the plasmodia by macrophages and other cells and by antiplasmodial and anti-toxic substances present in the blood.

Yorke and Macfie (1924) in discussing the problem of cure in malaria expressed some opinions which have a bearing on the question of immunity; these authors suggested that no form of quinine therapy destroys all the malaria parasites. The quinine destroys large numbers of parasites and thus sets free a considerable quantity of soluble antigen which leads to the formation of an immune body and this antibody finally produces cure by destruction of the remaining parasites. The authors considered that if there is no heavy production of antigen or if the host fails to produce antibody then infection is not completely sterilised and a relapse occurs with the setting-up of a state of equilibrium or tolerance. They concluded that the essential factor for the production of cures in malaria is the capacity of the host to produce antibody in response to the antigen formation resulting from the destruction of a considerable number of parasites by a medicament.

Thomson (1924a) in talking of blackwater fever, discussed more fully below, also talks of the formation of anti-

bodies. He goes so far as to claim that he had identified the antigen which he thinks is an altered red corpuscle in malignant tertian malaria, to which he gives the name "brassy corpuscle". In those cells the altered standing reactions of the red cells as the asexual forms grow older serve to indicate when the change takes place; he thought that these altered corpuscles act as foreign bodies capable of stimulating production of a specific haemolytic amboceptor; he further suggested, however, that the antibody produced acts only on these specifically altered corpuscles and not on the normal red cells; he thought that the change in the red cells was a chemical one induced by the contained asexual forms of P.falciparum. It will be seen later that this opinion of Thomson's forms the basis of theories produced by several workers on the causation of blackwater fever. It is introduced here to illustrate that even at this time the possibility of antibody formation against malaria parasites and/or altered corpuscles was fully realised.

Taliaferro writing in 1929 strongly disagreed with the findings of Yorke and Macfie above. Taliaferro pointed out that experimentation in malaria is difficult due to the rigid host specificity of the human parasite coupled with the undesirability of experimenting with humans - at that time there was no really effective antimalarial drug other than quinine. He considered however that the data available indicated that

resistance to infection, at least among certain individuals, exists in malarious districts. This author thought that the course of events was that an acute attack develops into a latent infection which confers an immunity to superinfection and that the complete disappearance of the parasite allows reinfection; i.e. the immunity is an "immunity of infection". In certain bird experiments the author also found that this latent infection could sometimes be set up without an apparent acute attack; from a study of these infections with bird malaria Taliaferro found the further interesting fact that although this immunity to superinfection was often of a high grade it was very labile; a large number of conditions which lower the resistance of the body can cause the latent attack to flare up into an acute attack. In 1929 the author concluded that the basis of the immunity is purely cellular and had no evident humoral basis. Taliaferro in 1941 considers the characteristics of malarial immunity are determined in the first place by the localisation of the parasites in certain tissues. This immunity shows itself in four ways, namely phagocytosis, production of new phagocytes, reparative proliferation, and the production of specific antibodies. Again, writing in 1944, he suggested that the rate of destruction of parasites is largely a phagocytic phenomenon which is greatly increased when there is an acquired immunity. The most reasonable explanation he thought for the influence of the

immunity is that macrophages and the parasites are influenced by a specific opsonin. He considered the immunity to be strain-specific and reiterated that the acquired immunity is largely if not entirely associated with a latent or low-grade infection. This author states that other serological reactions which occur during malarial infections include formation of antibodies (including precipitins, agglutinins and complement-fixing substances). He considered that complement-fixation depends on a genus antigen and is thus very different from acquired immunity which as he had said, is species and strain specific.

Chopra in 1936 pointed out that one of the oldest theories in the production of cure in malaria, far older than the work of Ehrlich, was that remedies stimulate resistance of the body cells in general and so help them to overcome infection.

Nocht and Mayer (1937) discuss the work of Koch in 1900, mentioned above, and agree that in tribes showing immunity the infants are as seriously affected as are European children. They agree also with much of Taliaferro's work when they say that in malaria as in many parasitic protozoal infections, the asexual development can be inhibited through the action of protective antibodies. Parasites remain viable, e.g. schizonts can be found in apparently healthy persons and

may increase and even reappear in the blood without increasing to such a degree as to cause clinical symptoms. They further agree that immunity is strain-specific and suggest that serious local epidemics of malaria are often the result of mass migration of persons from outside into endemic malaria areas. They also think that the immunity is labile. They think it is essentially an immunity of infection; the causative organisms do not disappear altogether after the disease has run its course but remain in the body where a balance is struck between the powers of resistance and the tendency of the parasites to increase. They quote the term "labile infection" given to this state by Schilling. (Schilling was hanged at Nuremberg for his anti-social activities during the second World War.)

Field (1939) has studied this question carefully and considers that this broad fact is beyond question, that persons who have had much malaria develop immunity, e.g. single infections, when allowed to run their course, burn out in time and may confer an immunity against the particular parasite to which they are due; repeated fresh infections with a single species of parasite has the same effect. Field claims that there is general agreement that the immunity so produced is specific, being limited to the infecting species and being strongest against the infecting strain, i.e. he considers it a strain-specific immunity. He thinks that the development of this species immunity is slow, possibly requiring a year or longer

to develop. The people of a highly infected area become more and more immune to the parasite, to which they are constantly exposed. The thinks that the antigenic stimulus which leads to the development of immunity is provided by the smouldering of old infections which run their course, and by repeated fresh infections which occur under hyperendemic conditions. It follows then that a general malarial immunity would be acquired only by independent immunisation against each of the three species and against each of the prevailing strains. Field considers that within each species there may be several strains antigenically distinct, hence the building-up of a composite immunity is a slow process, spread possibly over many years. In discussing the effect of chemotherapy on immunity Field points out that there are two schools of thought. One holds that immunity will result only if infection is given some measure of free play, the other being that immunity may develop from infection kept down by treatment. Field thinks the truth lies between the two. One point that none of the authors consider is whether all the stages in the life cycle of the parasite are equally antigenic. Field considers that on occasion effective drug prophylaxis may react unfavourably on the production of immunity, e.g. he observed 2,500 labourers continually for three years and found that after one year's drug prophylaxis there was insufficient immunity to prevent an early recrudescence of infection. He thinks immunity is a precious possession; and thinks quinine

administration limited to actual attacks affords little hindrance to the development of a parasite-host equilibrium, but as a prophylactic it may interfere with this process, e.g. Scott (1939) quotes an experience in Indo-China where the use of quinine as prophylactic led to such an increase in malaria that the land had to be abandoned. Scott also produces an interesting example of the effect of immunity when he quotes the Niger Expedition of 1841-2. There were fifty-eight negroes in the party and eleven of these were attacked by malaria; those eleven had lived for a long time in England; presumably they had lost their immunity. Craig and Faust (1945) in their consideration on the subject consider that natural immunity exists but is rare. They consider generally speaking that there is no race which is naturally immune to malaria but some races become largely immune through repeated infections. All native races in endemic malaria regions possess a relative immunity, usually limited to the particular strain of plasmodium present there; it is doubtful whether these authors are correct in presuming that native races have a relative immunity; the individuals of such races do have an apparent immunity but this is probably as the result of repeated infection in childhood. Craig and Faust consider that immunity is a most important factor in the epidemiology of malaria and that acquired immunity is actually a tolerance to infection. The authors consider that this immunity is largely homologous; heterologous immun-

ity is much less effective. These authors think that the mechanism of immunity in malaria is concerned with the destruction of plasmodia by the reticulo-endothelial cells which act as phagocytes, with the production of plasmodicidal bodies. This had been suggested many years previously by the senior author, Craig, see p. 699, whose final opinion is that it cannot be entirely explained by phagocytic action.

Ciuca et alia (1943) have studied immunity in malaria. They have observed forty-one patients for periods ranging from four to fourteen years. They consider that at present it is not possible to rule out humoral or cellular immunisation.

Clark (1944) agrees that immunity is never absolute. Fatigue, exposure, etc. or the introduction of a fresh strain of parasite can break any degree of tolerance.

One case on record which is suggestive of allergy to malaria parasites was reported by Golz in 1946. His patient had four attacks of generalised urticaria and angio-neurotic oedema at forty-eight hour intervals; the fourth attack was associated with a typically mild malarial rigor and P.vivax was found in the blood films. The patient gave a history of a similar attack at the onset of his previous attack of malaria eight months previously.

Mayer and Heidelberger (1946) investigated the problem

of antibody production in malaria using crude P.falciparum and P.vivax antigen and also more highly purified vivax antigen. They found that with sera from cases of chronic relapsing vivax malaria there was equally good complement fixation with soluble vivax antigen and with normal human stromata antigen; the antibody to the stromata was distinct from the specific malaria antibody. The same was found with P.falciparum antigen; in the immune response there was species specificity. They went so far as to say that if syphilis be excluded, then as a test for latent malaria the complement fixation reaction with either malaria antigen or with a preparation of normal human stromata antigen is specific for malaria. Huff and Coulston (1946) in dealing with avian malaria also supported the suggestion of specific antibody formation.

Clinically and experimentally it seems that immunity is developed to malaria. The likelihood is that this is an immunity of infection and that both cellular and humeral factors play a part.

At the beginning of this chapter a few of the more important details of antigen-antibody reaction were discussed, particular attention being paid to compound antigen. It was seen that the tissues in the body are capable of acting as antigens, the stromata of red cells being quoted as an example;

lastly it was seen that the malaria parasite is also capable of stimulating formation of antibodies. It was necessary to ascertain all of these facts before we were in a position to discuss whether or not immunological reactions play a part in the production of blackwater fever. We are in a position now to say that both the human red cell and malaria parasite, and possibly both together, are capable of acting as antigens.

Before beginning the detailed discussion on the question of whether antigen-antibody reaction is responsible for blackwater fever there are a few relevant findings to be reported which may have a bearing on the later discussion.

The first such finding is that reported by Browning (1925) who stated that red blood corpuscles sensitised by brilliant green are haemolysed by normal serum independently of the action of complement, i.e. all haemolytic phenomena are not to be explained in terms of laws governing production and action of true serum haemolysins. It has also to be remembered that, even with a simple haemolytic agent attacking all red cells equally and at the same rate, certain cells survive, presumably because they are more resistant to a destructive influence; a similar state of affairs is probably present with the more complex haemolysins such as serum haemolysins, i.e. they seem to lyse some red cells more easily than others. Loutit and Mollison (1946), reporting a series of observations on haemolysis in congenital and acquired haemolytic jaundice,

also suggested that not all haemolytic phenomena follow the laws governing the production and action of true serum haemolysins.

It is not possible to discuss in more detail the theories put forward on this question of antigen-antibody reaction as a cause of blackwater fever. It has been shown previously in this chapter that Browning (1925) has established that haemoglobin, globin, and stromata are all antigenic although the first two are only weakly so; it is also accepted that malaria is capable of provoking immunity. The possibilities that these processes do exist, and their implications, were fully appreciated even by early workers on the problem of blackwater fever. The earliest workers on this aspect of the problem are mainly British, e.g.Christophers and Bentley (1908) from their work on blackwater fever concluded that it was due to poisoning with a haemolysin ..... acting under special conditions. They did not consider it remarkable that no haemolysin had been demonstrated in blackwater fever, because in experimental work with dogs injected with haemolytic serum they had found it impossible to demonstrate the presence of the substance apart from its effects. They considered that production of the haemolysin is in some way the result of malaria ..... On the grounds that length of residence for a time is essential they refuse to accept the theory that the

haemolysin is a toxin derived from the malaria parasite, and they considered there must be changes in the body of the host; they suggested that haemolysins are thrown out as a result of the constant phagocytosis of red cells, with the endothelium the most likely agent. Later Christophers (1947) confirmed that they meant to convey that blackwater fever is probably due to a specific haemolysin and state that at the time they did not expect to demonstrate a haemolysin as it would be bound by receptors in the cells on which it was acting. This paper is among the first to convey the idea of a specific haemolysin being responsible for the haemolysis of blackwater fever.

Cleland (1909) concluded that blackwater fever may be the resultant condition, the evidence of anaphylaxis to plasmodium proteid.

Cardamatis (1912) suggested that quinine combines with the albumen of the stomach and is absorbed as quinine albuminate. He suggested that this substance, under unknown conditions, acts as an antigen which, combined with malaria toxin, causes production of antibodies which induce quinine sensitiveness. This author is obviously interested only in the part played by quinine but he foresaw the possibility of compound antigens in which substances such as quinine may act as specific orienting factors, the other part of the compound being the parasite or the red cell or both.

de Raadt (1917) held the same view; he considers the predisposition to blackwater fever arises at the same time as malarial immunity and consists in the development of haemolytic amboceptors in the blood of the individual concerned. He thought that the appearance of blackwater fever will depend on the complement content of the blood. He thought, as did Barratt and Yorke, above, that chill, exertion, etc. act by producing explosive development of complement.

Plehn (1920) stated that the German workers were wholly of the belief that the first step in the development of blackwater fever is that the patient becomes "super-sensitive" to the malaria parasite protein owing to repeated destruction of the parasites during the febrile attacks. The author believed that when this super-sensitiveness attains a sufficiently high degree a further solution of quite a small number of parasites may precipitate the crisis, although he thought that generally speaking this crisis only occurs when numerous parasites are destroyed. He therefore claims that in most races the decisive cause of the destruction of parasites is quinine. Other drugs have the same action, e.g. phenacetin salvarsol, i.e. he believes that the sole essential for production of blackwater fever is an acute dissolution of a certain number of malaria parasites.

This author then confuses the issue by saying that he considered the condition of super-sensitiveness to be transitory and to be compensated by antibody formation; in other words he would place the condition much in the same class as anergy.

Yorke (1922) considered that Plehn meant to convey that the primary lesion in blackwater fever is an anaphylactic shock.

Thomson (1924a) also pointed out that blackwater fever resembles the picture produced by the action of a specific serum haemolysin. He admitted all attempts to demonstrate autohaemolysin in blackwater fever serum had failed but did not consider that this necessarily excluded the suggestion of specific haemolysin as a cause of blackwater fever. In fact he went further and claimed that the prolonged action of the falciparum parasite so changes the character of the containing red cells that they alter in every way; he termed these "brassy corpuscles" and considered that they act as foreign bodies capable of producing a specific haemolytic amboceptor; he thought that this amboceptor was specific only to the altered "brassy" corpuscles; this was an over-simplification of the problem and was not accepted. Thomson (1927) repeated his claim that the brassy corpuscles are the antigens which lead in the

end to the production of blackwater fever.

Fairley and Bromfield (1934c) attempted to explain the varying picture found in blackwater fever by stating that the haemolytic agent may be present in variable quantity in different cases and at different stages in the same case, e.g. sometimes the substance is produced in large amounts and rapidly leads to the production of a lethal dose of haemolysed corpuscles whereas at other times it fluctuates in quantity, with sub-lethal haemolyses. Apparently following a haemolysis the haemolytic agent is decreased or entirely used up. Time is necessary for its production in a concentration adequate to produce another haemolytic crisis.

Ross (1932) also talks of blackwater fever being related to "sensitising action" of malaria infection.

Fernan-Nuñez (1936) also suggests that blackwater fever is due to antigen-antibody reactions: he considers haemoglobinuric fever to be an allergic phenomenon. He suggests that sensitisation rather than immunity may develop during the course of malignant tertian malaria; this is somewhat on the same lines as the suggestion by Plehn, p. 705. Fernan-Nunez claims that if the immunisation process does go wrong and sensitisation rather than immunity develops, then a subsequent attack of malignant tertian malaria will produce an allergic

reaction with destruction of the sensitised red corpuscles.

The author states that whether immunity or allergy will occur will depend to some extent on the general health of the affected individual. This allergy results from sensitisation by the protein antigens from the malaria parasite and allergic attacks, i.e. haemoglobinuria, will result from reactivation or reinfestation by the same species or strain of plasmodium. The author suggests that substances such as quinine provoke attacks of blackwater fever by destroying parasites. One interesting feature of this paper is that the author presents his ideas as original.

Chopra (1936) on the same subject says briefly that the simplest and most widely held theory is that as a result of repeated destruction of red cells by recurrent attacks of malaria, a haemolysin is formed; the products of chemical changes which are brought about in the corpuscles due to presence of plasmodia act like a foreign material; this is much the same theory as that of Thomson, see above.

Foy and Kondi (1937b) carried out spleen punctures on cases of blackwater fever and consider their results showed that the onset of blackwater fever is not necessarily related to the presence of large numbers of malaria parasites

in the body. If blackwater fever is a reaction to malaria parasites then it must be a reaction to small numbers of parasites.

Foy and Kondi (1937b) claim that in a population that has already been exposed to malaria for one to two years, the number of blackwater fever cases is significantly correlated with the number of malaria cases of the same year but this correlation does not hold in an unsensitised population. They also suggested that the high incidence of blackwater fever in Greece was probably not unrelated to migration of refugees from areas in Asia Minor where malaria was absent or of negligible proportions.

Nocht and Mayer (1937) ask why only a small portion of chronic malaria patients develop blackwater fever; they consider one important factor must be a special haemolytic disposition in the infected individual; this is a point far too often overlooked in any discussion on this aspect of blackwater fever; it is well recognised to be an important factor in immunological problems in general, see Zinsser et alia, p. 690. These authors point out that blackwater fever may be found in individuals infected therapeutically with malaria of a strain recognised to be safe ordinarily. I have previously discussed this point. They consider

another proof is the behaviour of P.knowlesi malaria in monkeys where untreated M.rhesus monkeys invariably die, usually with haemoglobinuria, whereas other more resistant strains develop a chronic non-fatal disease.

Nocht and Mayer also suggest another factor, an exciting cause, of blackwater fever is often medicinal toxicity which they thought is often due to quinine although many other drugs are capable of this.

Rogers and Megaw (1939) also talk of "sensitised corpuscles which become dissolved when a fresh supply of haemolysin is brought into existence by the action of quinine on a new brood of parasites or by parasites alone."

Foy, Kondi and Moumjidis (1941) found some slight experimental proof for the theory of haemolytic substances when they showed that normal cells are destroyed when transfused into blackwater fever patients. They considered it probable that a circulating haemolysin may be responsible for blackwater fever and thought that continuous sensitisation is necessary before the red cells become liable to haemolysis.

One of the most complete of the recent papers on this subject is the report by Gear (1946). Gear noted that the liver in a monkey dying of yellow fever was auto-antigenic, and that the antibody so produced was active against an

emulsion of normal liver, i.e. he suggested liver cell plus virus = auto-antigen capable of producing auto-antibody. Antigen plus antibody = a reaction causing degeneration of normal liver cells.

This finding led Gear to consider whether a similar mechanism might not be at work in blackwater fever. He suggested that in blackwater fever the system would be:

red blood cells plus malarial parasite plus and/or quinine = auto-antigen, leading to the production of haemolysin and this haemolysin will be effective against any part of the complex (i.e. typically the course of events with compound antigens).

Gear suggests that in response to this auto-antigen a haemolysin is produced by the reticulo-endothelial system, particularly the spleen and that the titre of this haemolysin is boosted by repeated malaria.

He then suggests that when the circulation through the spleen is free this haemolysin is mopped up by red blood corpuscles which are removed by the reticulo-endothelial system as soon as they became sensitised.

BUT if the circulation through the spleen is impeded and the spleen becomes congested as it does in an attack of malaria then the haemolysin accumulates and therefore factors

which cause a sudden contraction of the spleen, e.g. quinine, chill, exertion, will cause to be expressed into the circulation sufficient haemolysin to sensitise and cause to be lysed a large number of corpuscles, i.e. will precipitate an attack of blackwater fever. Obviously by this theory any factor causing contraction of the spleen will lead to expression of antibody, leading to an attack of blackwater fever, e.g. quinine, chill, exertion, emotion, etc. etc.; this is one other attempt to implicate the spleen, a point already discussed, p. 637.

There is no need to continue a recital of quotations supporting the view that blackwater fever is the result of an antigen-antibody reaction. The excerpts given above range over a period of forty years and include some of the most notable of workers in this field. In the extracts given above it is seen that all are agreed on the main principle, namely that it is possible that the cause of blackwater fever is an antigen-antibody reaction: there are minor variations in the details, e.g.:

Christophers and Bentley did not consider the parasite itself to be implicated: they thought that the haemolytic substance is a product of the reticulo-endothelial system.

Cleland thinks, in marked contrast to Christophers and Bentley, that the antigen is the plasmodium proteid.

Cardamatis suggests a compound antigen, with quinine albuminate plus malaria toxin.

Plehn suggests that the condition is not one of allergy but rather of anergy.

Thomson suggests "brassy corpuscles" of malignant tertian malaria to be the antigen.

Fernan-Nuñez distinguishes between sensitisation and immunity; he considers sensitisation to be "immunity gone wrong".

Foy and Kondi point out that large numbers of malaria parasites are not usually found in blackwater fever cases and that any theory based on the presence of large numbers of parasites is under suspicion.

Nocht and Mayer point out that whatever else is present there must be a special haemolytic predisposition of the individual affected with blackwater fever and that there is generally a medicinal toxicity.

Gear suggests that the antigen is a complex one, namely red cell plus parasite and/or quinine and also suggests that blackwater fever is the result of a sudden flooding of the antibody into circulation by contraction of a stagnant spleen. If circulation is free through the spleen then steady slow production of lysin is taken up by red cells which are destroyed by the reticulo-endothelial system.

These are the points on which the various workers differ.

Their theories all agree in one respect, however, namely the serious one that they are all expressions of opinion unsupported by any experimental work. This particulat type of theory is most popular only because it is one that most easily fits the facts known about the disease and not because it is the theory most fully supported by experimental evidence.

What criticism there is of this theory is based largely on the fact that there is no evidence in support of it, e.g.

Stephens (1937) states that opinions on blackwater fever as an anaphylactic phenomenon are largely speculative.

Maegraith (1946c) talking of Gear's work considers that it has "a frail foundation". He admits however that what evidence there is supports the theory as a possibility.

Very little careful experimental work has been done on this problem. A disease with a death rate of twenty-five per cent does not lend itself to human experimentation. Among the few experimental points in favour are:

Firstly the work by Foy, Kondi and Moumjidis (1941), p.710 who claim to have shown that when normal red cells are transfused into blackwater fever patients these normal red cells are haemolysed equally with the cells of the patients. Another paper of importance is that of Findlay and Markson (1947). These authors reported that European troops rarely developed

blackwater fever during the first nine months of residence in West Africa; it is suggested that this time was necessary for sensitisation. The authors also report another interesting finding, namely that among African troops the incidence of blackwater fever was low in 1941 and 1942 but rose in 1943, 1944 and 1945 although in African civilians there was no corresponding rise in incidence. The only difference between the two sections of the native population was that the soldiers had been protected against malaria. The authors suggest the soldiers thus lost their immunity to malaria to some extent, and suggest that in the subsequent infections the strains reacted with specific antigens, e.g. the African troops transferred to India did not show a similar rise in the incidence of blackwater fever since their reinfections were with different strains of parasite.

Another finding of these authors was that injection of normal blood into patients convalescing from blackwater fever produced no reaction but injections of blood from patients suffering from malaria due to the local strain of parasite produced haemolysis in three out of six patients although similar injections had no effect on normal individuals.

The authors therefore took the antigen-antibody theory one step further by postulating that it is the local strain of parasite which produces blackwater fever, i.e. not only is immunity strain-specific but blackwater fever is almost

strain-specific. This is in conflict with the findings where natives who removed to another district of the same country become non-immune to blackwater fever, e.g. Bengalis in Assam.

There is then as yet no satisfactory evidence which unequivocally supports this theory of antigen-antibody reaction causing blackwater fever. On the other hand there is as yet no evidence which rules out such a possibility, e.g. it must be remembered it is possible to have lysins in the blood without their being readily detectable. Among suggestions to explain this we have that of Fairley and Bromfield (1934c) who suggest that one reason for failure to demonstrate the lysins may be because of immediate fixation of the haemolytic agent by the corpuscle. Gear (1946) agreed with this suggestion of steady taking-up of antibody by the corpuscles or a storing of it in the spleen, a theory supported by Nocht and Mayer. Fairley and Bromfield (1934c) also suggested variation in production of the haemolysin.

The failure to demonstrate a circulating haemolysin does not rule out the possibility of an immunological mechanism being responsible for blackwater fever.

Certain of the workers who support this theory have suggested that blackwater fever follows dissolution of large numbers of parasites. This is not supported by the work of Foy and Kondi (1937a), p.708 , who did not find heavy infection

in individuals who had spleen punctures carried out during or before onset of blackwater fever. Again, this suggestion of heavy malarial infection is not an essential part of the theory of antigen-antibody reaction as a cause of blackwater fever, and therefore this finding of Foy and Kondi does not invalidate the theory. At this point we may with advantage weigh the theory against the well-recognised stumbling blocks. We have already mentioned that Scott (1939) pointed out that every theory crashes on the following:

1. Why do newcomers escape?
2. Why are indigenous natives practically immune?

A third query may be added, namely:

3. Why is the distribution of blackwater fever so patchy, being found usually only in a very few of the many hundreds of areas of endemic malaria in the World?

How does this last of the theories face these hurdles which bring down every other theory?

Consider point No. 1. That newcomers are immune to the disease, developing it only after a certain length of residence in the Tropics, although admittedly there are a few cases on record where certain individuals have developed blackwater fever after very short stays in the Tropics.

This is the most difficult obstacle to accepting many of the theories on the causation of blackwater fever, but it

does not invalidate the theory of antigen-antibody reaction: in fact it lends indirect support in that those in favour of the theory point to the time lag as being evidence of time necessary for the development of sensitisation, e.g. see the paper by Findlay and Markson (1947), p.714 who report that European soldiers did not begin to show blackwater fever until after a nine months' residence in West Africa. Therefore in so far as this theory is concerned the time lag becomes not a stumbling block but a stepping stone to acceptance.

2. Why are indigenous natives immune? It has always been thought, probably correctly, that indigenous natives are immune to malaria because they are suffering from it, i.e. the immunity is one of infection, an immunity to super-infection as it were, and an immunity which is strain-specific. Ross (1932) wonders just how real this "immunity" is but no one who has lived for long in the Tropics can doubt that somehow or other the native seems to come to terms with his infection although the protection which he has is very labile, e.g. many of our battle casualties showed a flare of malaria after being wounded. This question of immunity to malaria must have some bearing on the question more particularly affecting us, namely immunity to blackwater fever; before the relationship of these two are discussed it is possibly worth while to record other findings which may be

relevant. The first of these is that natives who leave their own territory apparently do become susceptible to blackwater fever, e.g. the often-quoted examples of the Bengali Babu in the Assam Tea Gardens and the Egyptian in the Sudan; the second point, and one which I feel has not had the attention it deserves, is that all those indigenous natives who receive treatment much like that given to the European do not show the same immunity to blackwater fever as do their "less fortunate"brethren. Scott (1939) has questioned whether "civilised conditions" may play a part in the developing of susceptibility to blackwater fever but the main differences between the so-called "civilised conditions" of the native in the Assam Tea Gardens and the primitive conditions of the native in his village do not lie in any change in living conditions or food but lie essentially in a change in the medical treatment available and given; in other words there is no real medical treatment available in the majority of villages excepting possibly a few grains of quinine occasionally, whereas in the large trading companies malarial prophylaxis has a high priority. Consequently it may be that the native loses his expensively acquired immunity. The two last points may have much in common, namely the fact that a native who moves into another area loses his immunity to blackwater fever (and to malaria) and that the man who receives treatment or is protected against continued re-infection loses his immunity to blackwater fever. This is well brought out in the article

by Findlay and Markson (1947) where natives protected against malaria showed a steady rise in the incidence of blackwater fever whereas civilian natives in the same area showed no such rise. Both in the case of those protected against further infection and those treated with drugs the factor in common must be the native so protected or treated loses his immunity to re-infection, presumably because of cure of his latent didsease. In losing his immunity to re-infection he apparently loses his immunity to blackwater fever. Findlay and Markson (1947) go so far as to say that once the native loses his immunity to the local strain he is in a worse position than the native transported to an area where the strain of parasite is different.

The problem then is to see whether it is possible that these findings can be satisfactorily explained by the theory of immunological reactions. It is easy enough to give more than one explanation of the findings which would not clash with the general principles of immunology and which would fit into the theory that blackwater fever is an immunological reaction; e.g. it can be postulated that the specific antigen which leads to the formation of haemolytic antibody and later to blackwater fever is a compound antigen in which the haptene is an indifferent drug, e.g. quinine, plasmoquine or any other such drug which had been administered at the time, and in which the antigenic element is the red cell

stroma and/or plasmodium; the native immunity could then be explained in two ways. One way would be that in the native the destruction of plasmodium is very low-grade, not sufficient to act as antigen, or secondly that in the native the haptene element is lacking, e.g. it has been roughly estimated that of the hundred million or so individuals suffering from malaria in India not more than eight million receive any treatment for the condition and it is just that section of the population, those fully treated and protected against infection which shows increased susceptibility to blackwater fever. There are several similar explanations. There is only one fault to find with all of them, that there is no proof they are correct. Immunity of the native to blackwater fever is bound up in some way with immunity to malaria and until the processes of the latter are clearly understood we cannot offer any satisfactory explanation of the former. All that can be said is that, in the present state of our knowledge, the fact that the indigenous native is generally immune to blackwater fever is not necessarily a stumbling block to the acceptance of the theory of antigen-antibody reactions as a cause of blackwater fever. On the other hand, there is as yet no proof that the fact of native immunity lends any support to the theory of immunological reactions causing blackwater fever.

The third stumbling block in all theories on the causation of blackwater fever is the patchy distribution of

the disease, which is found only in a few individuals in a few of the many areas where malaria is endemic.

The first part of the problem, the patchy distribution of disease among individuals, is probably in favour of an immunological reaction being the cause of blackwater fever; it is an accepted principle in immunology that one of the factors in the development of anaphylaxis is individual hyper-susceptibility; this is self-evident for were there no differences in susceptibility then all people would become anaphylactic to allergens instead of the unfortunate few as is the case. It is easy then to say that if the allergen theory of blackwater fever be found to be correct then the explanation of the patchy distribution of the disease among individuals is the same explanation as that given for the patchy distribution in other forms of allergy, namely individual hyper-susceptibility to allergen whatever that allergen may be. The second part of the problem is much more difficult, namely why is blackwater fever to be found only in a very few of the areas of malarial endemicity. One thing which may be relevant is that areas where blackwater fever is endemic are areas where malaria is present all the year round, e.g.compare the Bengal Dooars where blackwater fever is endemic with the Punjab where blackwater fever is not found and where transmission of malaria is seasonal. This part of the problem is again one which is linked with the question of blackwater fever

susceptibility and immunity and the part played in these by repeated infection with malaria. Until we know more of the processes at work in malarial immunity this part of the problem must also be left unanswered. As we have said of blackwater fever immunity in natives so also we may say here that this fact that blackwater fever is patchy, largely limited to areas where malaria is always endemic, cannot be necessarily put against the theory of blackwater fever being caused by antibody reaction.

Nothing further may be added to this part of the problem by quoting findings in the cases of haemoglobinuria in Razmak as no relevant experiments were made.

The first part of this work was devoted to establishing a diagnosis for six cases of haemoglobinuria in Waziristan. It was concluded that they were cases of blackwater fever.

The latter part of the work was devoted to examining the theories of causation of blackwater fever. It was suggested that the theory which explains most satisfactorily the findings in blackwater fever is that this disease is the result of antigen-antibody reaction.

This brings to a close the discussion on the possibility that immunological processes are the cause of blackwater

fever. This theory fits well several features of the disease, e.g. the escape of new-comers and the patchy distribution among individuals; there are other points in the disease which are not so well explained by this theory.

There are no features of blackwater fever which would appear definitely to rule out the possibility that the theory is correct.

It is suggested that of all the theories yet tabled as likely explanations of blackwater fever the one that most closely fits the facts is that blackwater fever is an anaphylactic phenomenon.

Summary.

Haemolysis as a cause of blackwater fever.

As a problem this was considered under three headings, namely:

1. Discussion on the general principle of circulating haemolysins in blackwater fever.
2. Discussion on lecithin and lecithin derivatives.
3. Discussion on the theory of lysin-antilysin balance.

Firstly consider the theory of circulating haemolysins.

This discussion opened with a brief review of the small amount of experimental work which has a bearing on this problem, e.g. the work of

Loutit and Mollison on acquired acholuric jaundice, which was quoted to show that the process of red cell lysis by haemolysins may not be the simple mechanism generally accepted (this had been a point of view also put forward by Browning in 1925);

and that of

Foy and Kondi who failed to produce blackwater fever in any one of 106 mental patients to whom the authors gave blood from blackwater fever cases or on whom mosquitoes infected from blackwater fever cases were allowed to bite. Fifty-eight different blackwater fever cases were used in the tests, all of which were negative;

and that of

Foy, Kondi and Moumjidis who showed that blackwater fever patients have power to destroy normal cells transfused into them. The converse experiment, namely that of transfusing blood from a blackwater fever case into a normal individual gave no results, but proved nothing because of the small amount of blood inoculated. The conclusions of Foy and Kondi were that in blackwater fever the defect is not in the red cell but in its environment; as will be seen later however these same authors did claim to have detected abnormal behaviour on the part of the red cells from blackwater fever cases, see below.

Previous to the experimental work of Foy and Kondi, other workers had attempted to transmit blackwater fever but in the light of present-day knowledge the experiments were crude, and the negative findings have no significance.

Various workers support the theory of a lytic factor circulating in blackwater fever cases although such a factor has never been proved. Various explanations have been propounded to account for this, e.g. Nocht and Mayer suggested the lysin is found only in the internal organs. Fairley and Bromfield suggested that the failure to demonstrate lysins may be because of immediate fixation of the haemolytic agent by

the corpuscles, a suggestion also supported by Gear. Low and Fairley suggested that the lysin is bound to the red cells as soon as it is produced - this suggestion would explain the finding of Brown et alia that the life of a red cell transfused from an individual with haemolytic anaemia is much shorter than the life of a normal red cell so treated. Loutit and Mollison suggested that the process of haemolysins in certain at least of haemolytic anaemias may be different from that at present generally accepted.

Secondly Lecithin and Lecithin derivatives.

It is generally accepted that certain phospholipids; especially lecithin and cephalin, play an important part in the physiology of practically every cell in the body, and also are concerned in the absorption and utilisation of fats. Certain substances of this class, e.g. lecithin and more particularly a lecithin derivative - lysolecithin - have haemolytic properties. The importance of this in blackwater fever is suggested by Krishnan and Pai and by Foy and Kondi. The first-named claim to have found a rise in blood lecithin in pre-haemoglobinuric states in the malignant malaria of monkeys, and Foy and Kondi found that red cells from cases of blackwater fever are significantly more fragile in lysolecithin solutions than are normal cells. One other finding thought by some to be of significance in blackwater fever is that the blood content of

lysolecithin is increased during passage of the blood through the spleen, particularly an enlarged spleen. Unless much more proof is forthcoming however it is not possible to accept the suggestion that increased red cell fragility to lysolecithin plays a significant part in the production of blackwater fever; the other possibility is that blackwater fever is the result of increased production of this haemolytic substance. This theory is incorporated in the next paragraph, namely:

3. Lysin-antilysin balance.

Over a period of years various authors have reported the finding of a haemolytic substance in the blood or in the tissues of normal individuals. This haemolytic substance was found to be inhibited by normal serum.

One suggestion is that the rate of haemolysis occurring in the normal individual at any one time may be a function of the balance between the tissue lytic agent and an inhibitor: a further suggestion is that blackwater fever may be due to lack of normal inhibitor rather than due to production of a special lytic substance. Certain workers have linked this theory of lysin balance with the theory of lecithin mentioned above and have claimed that the lytic substance normally present in the body is lysolecithin; this is not generally accepted.

It was felt that the evidence available does not justify the presumption that the process of haemolysin-

antihaemolysin balance is an important physiological process and it is felt to be unlikely that the cause of blackwater fever will be found to be an imbalance of a haemolytic-antihaemolytic system.

Antigen-Antibody reaction as a cause of blackwater fever.

This is the next theory to be discussed and the last to be discussed.

The consideration of this theory was opened with a short discussion on antigens. It was seen that an antigen is a substance capable of producing a specific altered reaction capacity on the part of cells with which it comes into contact. Certain of these antigens may be compound substances in which the antigenic component is specifically oriented by non-antigenic groups, "haptenes" or "partial antigens" of Landsteiner; these latter substances are incapable by themselves of causing the production of antibodies but are capable of reacting with specific antibody. The production of antibodies leads to a state of hypersensitiveness. In certain types of hypersensitiveness, i.e. allergy and idiosyncrasy it is not possible to demonstrate circulating antibodies, and the hypersensitiveness cannot be transferred, yet these are examples of true hypersensitiveness.

It is strongly stressed that in the production of hypersensitiveness in an individual there must be a hereditary

predisposition in addition to the spcific sensitising factor.

The next point noted in the general discussion was that certain of the body proteins are capable of acting as isoantigens. Specially important ones from our point of view are haemoglobin, globin and red cell stroma; only the last is a powerful antigen, the two first-mentioned being weak in this respect.

A further point of importance to us was whether or not immunity could develop against malaria. It seems generally accepted that a definite immunity to malaria may develop but that this immunity is an immunity of infection, i.e. the individual is immune because of latent low-grade disease. Furthermore, such immunity is possibly strain-specific and very labile, e.g. heat, cold, etc. all being capable of provoking exacerbations of malaria. The mechanism of immunity in malaria is not clear; at one time it was thought to be purely cellular but later work suggests that humoral factors also play a part.

Thus far it has been proved that the human red cell and certain of its constituents are capable of acting as antigens and that the malaria parasite is also capable of stimulating production of antibodies.

The significance of the above findings had not been

lost on earlier workers and from 1908 onwards workers on the subject of blackwater fever have repeatedly brought forward the theory that blackwater fever is the expression of an antigen-antibody reaction. Christophers and Bentley (1908) were among the first to suggest this; later workers favouring the theory of antigen-antibody reaction include Cleland, Cardamatis, Plehn, Thomson, Yorke, Ross, Chopra, Foy and Kondi, Nocht and Mayer, Rogers and Megaw, and Gear.

There are differences in details of the theories produced by the above authors, e.g. Thomson's theory of "brassy corpuscles" of malignant tertian malaria, but the principles are much the same, namely that blackwater fever is the result of a sensitisation following malaria, and that individual susceptibility is a very important factor. The various authors who support this theory differ only on minor points. All agree in one respect, namely that in all cases practically there is no experimental proof to support the theory. This is pointed out by Stephens and Maegraith. Among the few workers who experimentally investigated the matter are Foy and Kondi, mentioned above, p.708, and Findlay and Markson. The two last-named authors have produced a paper recently (1947) confirming that newcomers to a blackwater fever area did not begin to be affected by the disease until after some time, nine months being the time quoted. They also point out the interesting fact that the local

natives when recruited into the Army and protected against malaria also show a rising incidence of blackwater fever beginning two years after protection began, whereas the other natives living locally and not protected against malaria did not show blackwater fever. The authors consider this is an indication that in the native soldiers there had been a loss of immunity to the local strain of parasite. They certainly were able to produce haemolysis in three out of six individuals convalescing from blackwater fever by injecting blood from patients suffering from locally acquired malaria. Similar injections had no effect on normal individuals.

A review of the work so far suggests that while there is no evidence proving the theory that blackwater fever is an anaphylactic phenomena, there is also no evidence which definitely excludes this as a possibility. The failure to demonstrate circulating haemolysin does not rule out the possibility of an immunological mechanism being responsible for blackwater fever. Certain workers suggest dissolution of large numbers of parasites as a cause of the onset of haemolysis in blackwater fever but Foy and Kondi and others have shown by spleen punctures that heavy malaria infection is not common in the pre-blackwater fever phase.

This theory of anaphylaxis was then considered against the three well-known stumbling blocks which have proved to be

the undoing of so many theories, namely, firstly that new-comers escape, secondly that indigenous natives are apparently immune, and thirdly that the distribution of blackwater fever is very patchy being found only in a few of the many individuals attacked with malaria, and being found only in a few of the many places where malaria is endemic.

The first of the stumbling blocks is actually a stepping-stone to the acceptance of the anaphylaxis theory, being well explained as the time necessary for sensitisation. The second well-recognised phenomenon is not so easily explained. Suggestions are offered which, theoretically, are probable, e.g. that drug treatment or such other haptene has been administered in the case of those developing blackwater fever but is not available to the average native; however, there can be no satisfactory discussion on this point until the question of immunity to malaria is more clearly settled, for undoubtedly immunity to blackwater fever is in some way connected with the immunity to malaria. All that can be said at present is that this stumbling block while not adequately explained is not a complete bar to the acceptance of the theory of anaphylaxis as a cause of blackwater fever.

The third point is the patchy distribution of blackwater fever. Patchy distribution among individuals is well-explained by the theory and is in fact an indirect support for

it since patchiness of distribution is a feature common to all forms of hypersensitiveness, being due to variations in individual susceptibility, variations in the ability to become sensitised. The patchy distribution of areas of blackwater fever endemicity, a few of the many areas of malaria endemicity, cannot be satisfactorily explained until more is known on the question of malarial immunity. It is pointed out that such areas of blackwater fever endemicity are generally places where malaria is present all the year round, e.g. in Africa and in certain parts of India, e.g. Bengal; it is absent in places like the Punjab. As with point No. 2, this problem does not necessarily exclude the possibility of blackwater fever being an anaphylactic phenomena.

It is suggested that of all the theories yet discussed, the one that most closely fits all the facts is that blackwater fever is an anaphylactic phenomenon.

APPENDIX.

Case Report No. 1.

No. 1862. Rank. Religion.

Lascar. Mohammed Hussain. Moslem.

Unit: 27 Animal Transport Coy. R.I.A.S.C. Razmak.

Age: 35.

Service 8 years. Service in Waziristan 1½ years

Home: Punjab.

Returned with the main forces to Razmak from the Kharre Column 18.7.38.

21.7.38. Admitted to the Combined Military Hospital, Razmak, from Unit lines in Razmak, complaining of attacks of shivering and fever of two days duration. Patient said attacks were exactly the same as those experienced in 1936 during attacks of malaria. Between the attacks patient felt quite fit.

Previous History.

Army records show "Bronchitis" winter 1931, 1934, and 1935 and broncho-pneumonia February 1938.

July 1936 - attack fresh benign tertian malaria; blood film showed P.vivax asexual forms.

August 1936 - Recurrence of "malaria-like" fever; blood films negative.

Diagnosed as "clinical malaria".

Both attacks treated by the standard Army course of atebrin 0.1 g. t.i.d. 7 days - 3 days rest - plasmoquine 0.01 g. b.i.d. for 5 days.

Examination. T.102°F. P.96. R. 22 per min.

General Appearance. Below average for weight, but not seriously under-nourished. No staining of the skin or mucous membranes by malarial pigment. No obvious anaemia.

Weight 109 lbs. Height 5 ft. 1 in.

Locomotor System. Nil obvious abnormality.

Abdomen. Abdomen showed no abnormality. Spleen not palpable. Stools negative for ova cysts and amoebae.

Circulatory System. Cardiac dullness within normal limits. Heart sounds fast, regular, soft, no adventitial sounds. Blood films, thick and thin, showed ring forms and gametocytes of P.vivax.

Respiratory System. Breath sounds showed prolonged expiration with rhonchi over both sides of chest and with breath sounds somewhat distant both lungs.

Genito-Urinary System. Urine showed nil abnormal.

Nervous System. Nil abnormal detected.

Diagnosis. Benign tertian malaria (?fresh); may be relapse from 1936 but spleen is not enlarged.

Course of Illness, treatment, etc.

After admission. Place - Hospital.

To receive new course, namely quinine as required, atebrin 0.3 g. for 5 days - 1 day rest - plasmoquine 0.02 g. daily for 5 days.

Date.	Course of Illness.
2nd day, 22.7.38	Patient received grs. 10 quinine t.i.d. Temperature had settled to normal.
3rd-7th day, 23-7-38 - 27.7.38 (inclusive)	Atebrin 0.1 g. t.i.d. i.e. 5-day atebrin course.
8th day. 28.7.38	1 day rest. Transferred to Convalescent Depot.

Period in Convalescent Depot.

9th -13th day. 31.7.38 - 2.8.38 Plasmoquine 0.01 g. b.i.d. as out-patient, until treatment stopped due to patient reporting haemoglobinuria.

Total 0.09 g. plasmoquine.

After admission.	Date	Place.
13th day	2.8.38	Convalescent Depot.

At 1300 hours, one hour after ingestion of the second-last dose of plasmoquine, i.e. after a total plasmoquine dosage of 0.09 g., the patient reported that for

the previous two days he had noticed steadily increasing weakness and had noticed a "dark colour" of the urine. He also said he had vomited on several occasions during the previous two days.

Place.

Hospital. Readmitted for investigation.
T. 101.6°F. P. 110 per min. R. 22 per min.
No complaint of abdominal pain.
Liver and spleen not palpable. Blood films showed no malaria organisms. No tenderness liver or spleen. Stools showed nil abnormal. Urine passed during the night; specimen was not kept but patient reported it to be "very red".

14th day 3.8.38 Patient appeared much weaker than on first admission; physical condition worse than on first admission. Mucous membrane pale and slightly icteric; no cyanosis seen. Definite tenderness epigastrium; character of heart sounds very soft. Blood pressure 85/50.
Blood films repeatedly negative.
Serial blood-picture reports, p.747 appendix; severe anaemia with reticulocytes 30% and plasma rose coloured with oxyhaemoglobin and methaemoglobin bands. Patient passed urine deep ruby-

coloured: absorption bands oxyhaemoglobin and methaemoglobin; urine reports p. 747c appendix.

Through the day the urine continued to be ruby-coloured by reflected light. The patient said he had vomited several times. Vomiting continued until midnight when the patient slipped into a restful sleep due to paraldehyde per rectum.

Treatment throughout is summarised p 747d, appendix.

In addition to specific treatment, e.g. blood transfusion, symptomatic treatment was used, e.g. each evening paraldehyde 4 drachms given in a slow drip per rectum to give the patient rest.

Treatment given on this day included blood transfusion, Campolon, atebrin injection, antivenine injection. From the beginning of this treatment (equally applicable to treatment of later cases) it was decided NOT to try the effect of intense alkalinization but to balance the intake against the output with intake being made up largely by glucose and saline drip infusion by rectum and by as much fluid, again largely glucose saline, per oram as the patient

could take. Actual intake-output figures have not been recorded for this patient. 500 ccs. blood transfusion of fresh blood of like group given at 2000 hours, no reaction, and at 2100 hours intra-muscular injection of 0.3 g. atebrin musonate.

15th day. 4.8.38. Patient doubly incontinent during the night. Examination 6 a.m. showed marked icterus mucous membranes. It was not possible to estimate the amount of urine passed; the bed was heavily stained with an admixture of red urine and faecally-stained glucose-alkali solution which had been given per rectum. The vomiting had stopped. The blood films were still negative for malaria parasites.

The red cell count did not show any change suggesting that there had been further lysis of blood except that the count remained stationary in spite of the blood transfusion on previous day. Other blood findings showed little change. The urine could not be tested because of the patient's incontinence.

1700 hrs. Patient showed increased icteric discolouration; liver not tender or enlarged. Patient now slightly cyanosed with respirations of

32 per min.

Middle lobe right lung showed impaired percussion note with an increased vocal resonance and appearance for the first time of fine crepitations in that area. Findings suggested secondary broncho-pneumonia (patient had a history of repeated attacks chest disease).

Oxygen given continuously. Paraldehyde drs. 4 again p.m. to ensure rest.

Treatment was not changed, namely atebrin musonate injection (on the mistaken assumption that the temperature rise was due to persistent malaria) and Campolon, with roughly estimated fluid loss balanced as far as possible by rectal administration and oral administration glucose saline solution with other fluids as much as possible, e.g. soft drinks. The hot applications were continued to the kidney region.

16th day. 5.8.38. Jaundice very marked, probably obstructive, due to liver disease as direct van den Bergh reaction strongly positive. Cyanosis increasing. Patient still doubly incontinent but had slept well and condition was slightly improved.

T.103°F. P. 116. R. 30

Not all of the rectal drip saline solution had been voided; there had been absorption

of a satisfactory volume; patient taking very little by mouth; no sign of uraemia.
Blood films negative for malaria parasites.
No marked change in red cell count.
Reticulocytosis 35%. White blood count now 17000 per cu.mm. van den Bergh reaction now gives immediate direct positive reaction, almost certainly due to liver inefficiency. Plasma still rosy pink. Blood urea level rising, now 120 mgm/100 ccs. Urine not tested; stains from urine still definitely red in colour, same as the rose-red on the previous day. Lung condition still unchanged right side; few crepitations base left lung (?decubitus "pneumonia").

1800 hours. Patient said he had not passed any urine for 15 hours; vomiting had started again. Catheterisation yielded 10 ccs. mucoid urine, pink colour, much lighter than previous samples. Few red cells also present (?from catheter trauma) with many casts and masses of amorphous pigment. Treatment as previously including Campolon, atebrin, and paraldehyde per rectum.

17th day. 6.8.38 T. 102.6°F. P. 118. R. 32.
Mental condition brighter, jaundice slightly less deep; no change in cyanosis. Still doubly incontinent, vomiting still present. Blood picture:

no malaria parasites seen; marked fall in red cell count; no change in the colour of the plasma; marked rise of blood urea to 200 mgm %. Urine not tested; urine stains on bed sheet were very small in amount (probably only few ccs. passed in 12 hours) and still staining deep red.

No change in condition right lung; spread of the area showing crepitations left side.

1300 hours. 500 ccs. blood again given.

This was done in view of the low red blood count. Compolon injection; atebrin injections not to be repeated.

18th day. 7.8.38 T. 101.1°F. P. 136. R.34.

Mental condition still clear. Jaundice marked. Cyanosis present. Still doubly incontinent and the amount of urine passed in the preceding 24 hours had been sufficient only to stain the sheets, probably only a few ccs. In spite of transfusion 500 ccs. blood previous day red cell count had not risen compared with previous count. There must have been a lysis following the transfusion, or transfused cells themselves must have been destroyed; plasma lighter than on previous day, being pink; urine stain on bed sheet still deep red, no change from previous day. Chest condition unchanged.

Previous treatment continued including Campolon injection. Antivenine 10 ccs. given. The hot applications to the kidney region were being kept up.

19th day. 8.8.38. T. 99.6° F. P.144. R. 38.

General condition and chest condition much worse. Pupils small and fixed. Jaundice marked. Cyanosis marked. Only few drops urine passed in 24 hours, sufficient only to produce faint pink staining half-crown in one place. Marked fall in red cell count to 1,300,000. A severe haemolysis must have taken place in the preceding 24 hours; plasma ruby red. Patient anuric. Chest not examined, patient too weak; cyanosis increasing and respiration rapid and shallow.

1800 hours. Patient catheterised; only 2 ccs. thick slime obtained with difficulty, rose-red in colour.

Large number of casts and haemoglobin masses. Treatment as before but rectal drip infusion discontinued; fluid no longer being retained per rectum and tube was troubling patient. Intra-venous glucose-saline, 5% glucose-saline 2 pints given in 6 hours, with 10 units insulin during and after administration.

20th day. 9.8.38. Patient drowsy. T. 100.2°F. P.160 p.m.
R. 40 p.m.
No sign of any urine staining in the bed for the previous 24 hours. Blood picture unchanged. Anuria for 24 hours. Repeated catheterisation failed to yield any fluid.

Patient died 1900 hours.

Summary. This patient had obviously repeated attacks of haemolysis which destroyed not only his own cells but probably cells also from the transfusions.

Post-mortem Examination.

General Appearance. Marked jaundice skin and mucosal surfaces. Patient emaciated.

Abdomen. No ascites. Organs pale.

Kidneys. Capsules stripped readily. No irregularity of surface; paler than normal; structure of kidneys not clear-cut, pyramids difficult to detect. Friability increased.

Adrenals. Small haemorrhages but no specific change.

Spleen. Not enlarged, capsule thickened, colour deep red, consistency soft except for small firm wedge-shaped areas, probably infarcts.

Smear taken for malaria parasites, negative. Marked positive iron reaction.

Liver. Not enlarged, pale yellow-green in appearance with early nutmeg pattern and with areas of marked congestion; increased friability.

Gall Bladder. Enlarged with much thick bile in it. Iron present.

Pancreas. Nil special except small scattered haemorrhages.

Gastro-Intestinal Tract. Little abnormal except shrunken; scars old amoebiasis large bowel.

Chest.

Pleurae. General fine fibrous adhesions between the two pleural layers; small encysted effusion left side.

Lungs. Sub-pleural pin-point haemorrhages over both lungs; both lungs oedematous and congested. Small patchy areas of consolidation of all lobes both sides.

Heart. 10 ccs. straw-coloured fluid in pericardial sac; organ generally pale. Left ventricle dilated; wall thin and friable. Musculature right ventricle not markedly affected.

Brain. No abnormality meninges except congestion. The brain itself is markedly congested but shows no haemorrhages or infarcts. Smear taken for malaria parasites, negative.

Microscopical Reports.

Kidneys. Glomeruli normal. Few capsules showed some desquamation of cells with granular content in the spaces.

Convoluted Tubules and Loops of Henle. Changes widespread; much degeneration and disorganisation; in many places the lining epithelium is stripped or shows no nuclei; the degree varies considerably. Many of the tubules and loops showed plugging with a mixture of lining cells, casts, red cell debris and amorphous material showing pigment staining. The parts most affected are the terminal portions of the first convoluted tubules, two limbs of Henle's loop and the second convoluted tubules.

Interstitial Tissue. Apparent hyperaemia with oedema. The hyperaemia due largely to dilatation of veins.

Spleen. Pulp quite obscured by mass of disintegrating red cells; sinuses markedly dilated. No malarial parasites seen in the smear.

Liver. Acute central necrosis involving middle 2/3 of each lobule (this would explain the direct van den Bergh reaction). The lesion is not specific.

Lungs. Very oedematous and so filled with cells (red, white and epithelial) in the lumen of the alveoli that the condition resembles resolving lobar

pneumonia.

Heart. Extensive fatty degeneration and breaking-up of cardiae musculature.

Brain. Very congested and oedematous with blood vessels showing a high proportion of white cells. There is chromatolysis and macrophage infiltration throughout. Malaria parasites were not detected in the brain smear.

Summary. Severe toxic degeneration, affecting most severely liver and kidneys.

Blood Picture, Case No. 1.

Day after coming under obs. with lysis R.B.C.	1st 3.8.38	2nd 4.8.38	3rd 5.8.38	4th 6.8.38	5th 7.8.38	6th 8.8.38	7th 9.8.38
R.B.C. (cu.mm.)	2640000	2740000 (bl.tr. 500 ccs. 3.8.48)	2460000	1960000	2040000 (bl.tr. 500 ccs. 6.8.38)	1320000	1450000
Haemoglobin (Sahli)	48%	48%	47%	40%	41%	30%	27%
Col.Index.	0.92	0.92	0.9	1.05	1.02	1.1	1.1
Mean Corp. vol.	102	98	100	100	98	101	102
App. of red cells.							
Poikilocy.	+	+	++	++	++	++	++
Polychrom.	-	+	+	++	++	++	++
Macrocytes	+	++	++	++	++	++	++
Microcytes.	+	++	++	++	++	++	++
Immature	Few nor.	Num.nor.	Many nor.	Many nor,	Num.nor.	Nommobl.	Few nor.
Reticulocytes	30%	35%	41%	35%	21%	12%	7%
W.B.C. (cub.mm.)	10,000	18,000	18,600	17,000	16,000	13,400	17,600
D.L.C.							
Neutroph.	61%	88%	90%	88%	79%	80%	81%
Eosinoph.	3%	1%	1%	1%	1%	1.5%	1%
Basophils	2%	-	-	1%	1%	0.5%	1%
Lymphocyte	19%	7%	4%	5%	15%	13%	11%
Monocytes	15%	4%	4%	5%	4%	5%	6%
Immat. Cells.	Imm. mon.	Metamy,	Myelocy,	Few myel.	Myelo.	Myelo.	Few myel.
Sed.Rate ms. hour. (Wintrobe)	17	46	67	72	70	67	59

Blood Picture Case No. 1. (contd.)

Day after coming under obs. with lysis R.B.C.	1st 3.8.38	2nd 4.8.38	3rd 5.8.38	4th 6.8.38	5th 7.8.38	6th 8.8.38	7th 9.8.38
v.d.B. qual quant	Del. + 6	Del. ++ 5	Direct + 10	Direct +++ 9	Not done	Not done	Not done
Spectroscopic Examination. a. Plasma	methalb. bands	methalb. bands	methalb. bands	methalb. bands	methalb. bands	Not done	Not done.
b. Washed R.B.C. lysed with dist. water	HbO_2 bands	HbO_2 bands + METHAEM. BANDS	HbO_2 bands	HbO_2 bands	?mglobin nands	Not done	Not done.
Plasma colour Equivalent % R.B.C. solution	1.2%	0.8%	0.9%	1.1%	0.5%	1.8.	Not done
Wassermann reac. of Blood serum	Neg	Neg.	Not done	Not done	Not done	Not done	Not done
Kahn Reaction	Neg	Neg.	-	-	-	-	-
Donath-Landst. Test	Neg.	Neg	Neg	Neg.	Neg.	Not done	Not done
Blood group	II	II	Not done	Not done	Not done	Not done	Not done
Blood pressure	85/50	-	-	-	-	-	-
Blood urea (mgs. 100 ccs. blood)	60	80	120	200	230	280	not d.
Bleeding time, mins.	1.6		4				
Coag. time	3.4		8				
Fragility test (% sal. sol. to lyse red cells)	0.35% saline		0.3% saline				

URINE REPORT - CASE No 1.

DAY IN HOSPITAL	DATE	TIME	AMOUNT CCS.	S.G.	REACTION	COLOUR	EQUIV. % SOL. R.B.C.	PIGMENTS				PROTEIN	SEDIMENT.			
								OXY HB	MET. H.B.	UROBIL-INOGEN.	BILI-RUBIN		CASTS	R.B.C.	HB.MASS	W.B.C.
14TH	3.8.38.	a.m.	Not measured	?	Alkal.	Ruby	2.5%	++	+	++	–	+	few	nil.	nil.	–
		1500	100 ccs.	1024	"	"	"	"	"	"	"		"	"	"	
15TH	4.8.38	a.m.	oliguria			stains	stains									
		↓	incontin.	?	?	red.	red.	?	?	?	?	?			?	
16TH	5.8.38	↓ a.m.	.			stain red	? 1.5									
		1800.	Catheter 10 ccs	1030	Alkal.	Pink	0.75	+	++	++	+	+++	++	few	++	few
17TH	6.8.38		Incont. passed only few ccs.	?	?	stains Red.	? 1.5			?		?			?	
18TH	7.8.38		"	?	?	"	? 1.5			?		?			?	
19TH	8.8.38	am.	nil (anuria)													
		1800	2 ccs.	?	Alkal.	Red.	1.5	÷	+	+	+	+++	+++	few	+++	–
20TH	9.8.38	a.m.	nil													
		1900	←			D	E A T H									→

Bilirubin became positive in the urine when direct v.d.B. appeared - see p.

TREATMENT, CASE No1.

	DAY IN HOSPITAL.	DATE	TIME.	Q. dihyd. in sol. Orally.	Atebrin dihydro tablets orally.	REST.	Plasmaquine tablets orally.	Atebrin musonate i.m. inject.	Ferr. et ammon. cit. orally.	Campolon. i.m. inj.	Blood (fresh) trans.	Anti-venine serum. i.m.	Hot applic. Kidney areas.	Glucose alk. 5% Gluc. 1.5% Na Cl orally.	Rectal. Saline ¾ Na Cl + ¼ vol. Gluc.	I.V. fluids other than blood.
Hospital.	2nd.	1938 22.7.		30 grs.										oral. Fluids	rectally	i.v.
	3rd ↓ 7th (incl.)	23.7. ↓ 27.7.			0.3 g daily total, 1.5 g											
	8th	28.7.				1 day.										
Conv. Depot	9th ↓ 13th	29.7. → 2.8 (NOON)	1300 hrs.				0.02 g daily total 0.09 g									
Hospital.	14th	3.8	a.m. 2000 2100					0.3 g. i.m.		4 ccs.	500 cc	10 ccs.	continuous.	+ + + + + vomit.	1 pint. +	
	15th	4.8.	a.m.					0.3 g. i.m.		4 ccs			twice daily	±	continuous (incont.)	
	16th.	5.8.	a.m.					0.3 g. i.m.		4 ccs.			"	+ (vomit)	ditto	
	17th	6.8.	1300								500 cc.		"	sips-cont	continuous	
	18th	7.8.	a.m.							4 ccs		10 ccs.	"	"	"	
	19th	8.8.								4 ccs			"	"	"	2 pints gluc. sal
	20th.	9.8.	a.m.							4 ccs			"	"	"	
	←		1900	—	Death.	—										→

.7472

Morning and Evening Temperatures

IP 1862 Lascar MOHD HUSSAIN age 35 : service 7 yrs

Admitted 21/7/38

Disease BWF

Diet Fluids → bwl.

Result Death.

HOSPITAL | COL. DEPOT | HOSPITAL | DEATH

TEMP. (DEGREES FAHR.)

104 103 102 101 100 99 98 97

DAY IN HOSP. 1st 2 3 4 5 6 7 8 9 10 11th 12 13 14 15 16 17 18 19 20 21st

DATE 21/7/38 22 23 24 25 26 27 28 29 30 31 1/8/38 2 3 4 5 6 7 8 9 10

Case Report No. 2.

No. 13836. Rank. Religion.

Sepoy. Dost Mohammed. Moslem.

2/7 Rajput Regiment, Razmak.

Age: 22.

Service 3 years. Service in Waziristan 2½ years.

Home: Punjab.

Returned with main force to Razmak from the Kharre Column 18.7.38.

29.7.38. Admitted to Combined Military Hospital, Razmak, with complaint of headache, backache and daily attacks of fever with rigor on each of the previous three days, i.e. since 26.7.38.

Previous History.

Patient said he had only had one illness in the previous 12 years, namely malaria, July 1937. Army records confirm that the patient suffered from fresh benign tertian malaria July 1937 and that he was given the standard treatment, namely atebrin 0.3 g. daily for 7 days, 3 days rest, plasmoquine 0.02 g. daily for 5 days.

Examination. T. 102° F. p. 96 per min. R. 20 per min.

General Appearance. Below average for weight and height; general condition not satisfactory. No staining of the skin or mucous membrane by malarial

or other pigment. Conjunctivae not markedly pale. Weight 116 lbs.

Locomotor System. Nil abnormal detected.

Abdomen. Nil abnormal detected; spleen and liver not palpable; stools negative for ova and cysts.

Circulatory System. Heart sounds satisfactory; blood films, thick and thin, showed numerous ring forms P.vivax.

Respiratory System; Nil abnormal detected.

Nervous System. Nil abnormal detected.

Genito-Urinary System. Urine contained nil abnormal.

Diagnosis. Benign tertian malaria, probably fresh.

Course of Illness.

No. of Day.	Date.	
1st	29.7.38	Patient given total of 30 grs. quinine; temperature settled at once to normal Total: grs. 30 quinine.
2nd-6th.	30.7.38 - 3.8.38 (inclusive)	Atebrin 0.1 g. t.i.d., i.e. 5-day atebrin course. Total 1.5 g. atebrin.
7th	4.8.38	One day rest. Transferred to Convalescent Depot.
8th-12th	5.8.38 - 9.8.38	Given a 5-day plasmoquine course 0.01 g. b.i.d. at the out-patients

department.

Total 0.1 g. plasmoquine.

13th 10.8.38. Transferred to Unit lines where he was supposed to be given a seven-day convalescent period, free from all duties and games; this was the standard method of convalescing following malaria; By mistake patient was put on duty immediately and carried out a 24-hour stretch of duty.

14th 11.8.38 Reported to hospital and said he had been passing red-coloured urine since the previous evening.

Readmitted to Hospital.

Also complained of severe weakness, palpitations and vomiting; no complaint of abdominal pain.

On admission: T. 102° F. P. 120 p.m. R.24 p.m.

No cyanosis, but mucous membranes bleached; patient's skin was cold and clammy.

During the examination the patient fainted.

Spleen not palpable; blood films all negative for malaria parasites. Blood pressure 92/60.

Serial blood picture reports given p.755a-d

The picture on this day showed red cell count approximately 2½ millions, with the plasma a beautiful golden-red colour. Patient passed

a sample of urine on admission, burgundy in colour showing absorption bands oxy-haemoglobin and methaemoglobin. Reaction alkaline. Serial urine reports given in full, p. 755c .

In this case also it was decided to supply only enough fluid and alkalies to equal the loss of fluid from the body. Intense alkalinisation did not seem desirable or necessary. Hot applications to the loins were regarded as an important form of treatment and were kept up as long as necessary.

1900 hours. Collapsed state less marked. Patient showing marked polyuria; passed total 60 ounces urine since the morning. Urine still deep red in colour with practically no sediment. Showing absorption bands of oxyhaemoglobin and methaemoglobin. Treatment is given in detail, appendix p. 755d . Treatment included giving at least one pint alkaline solution (20 grs. per oram a.a./soda bicarb. and sod. cit. to a pint of water) every 2 hours, with flavoured drinks and other fluids to balance output.

15th day. 12.8.38. Temperature and respiration normal.
Pulse 90 p.m.
Skin still clammy but improving. Faint icteric tinge, noted in the sclerae.
Vomiting stopped; fluid intake balanced fluid output. Blood films negative for malaria parasites. Red cell count not done. Plasma lighter than previous day; van den Bergh reaction indirect plus; high value. Blood urea level satisfactory. Urine morning specimen porter coloured. 22 ozs. passed since midnight, showing epithelial casts and albumen present. Oxyhaemoglobin and methaemoglobin bands + +

2200 hours. 49 ozs. urine passed in all since morning; all specimens give the colour of port-wine, with heavy content of casts, epithelial cells and haemoglobin masses; much albumen present. No change in spectroscopic findings. No change in treatment.

16th day. 13.8.38. Pulse still fast. Temperature and respiration normal.
General condition much the same, no increase in the icterus, which is mild in degree.

?Faint cyanosis. Passed 30 ozs. urine since 2200 hours the previous day. Intake balances fluid output. Colour of plasma unchanged. Slight fall in value of the van den Bergh indirect reaction; no rise in blood urea level. Red blood count 1.9 millions per cub. mm. Haemoglobin 49%. Urine deep red in colour, lighter than previous day, otherwise no change.

2300 hours. Another 30 ozs. urine passed since a.m.; the colour much lighter than previously, being pink. Other findings as in morning. Treatment - no change.

17th day. 14.8.38. General condition much the same; pulse still 92 p.m. Icterus and cyanosis unchanged; slight in degree. Marked fall in value of van den Bergh reaction, rise in blood urea: 30 ozs. urine passed since previous night. Colour yellow tinged with pink, i.e. content of blood pigment greatly reduced and showing only faint bands oxyhaemoglobin and methaemoglobin. Heavy deposit with pink casts, many epithelial cells and masses of pigment, some of it amorphous, and some

granular and mixed with cells.

P.M. Passed 40 ozs. urine since morning, more concentrated, otherwise no change in colour; spectroscopic analysis as before.

18th day. 15.8.38 Condition greatly improved, jaundice fading. No cyanosis. 10 ozs. urine passed during night; urine normal in colour, containing urobilin and albumen in small amount, with granular casts and heavy masses of pigment.

21st day. 18.8.38 Improvement maintained, jaundice no longer obvious. Red blood count only slightly improved with reticulocytes 8%; plasma clear, blood urea level not estimated, van den Bergh test still showed 3 units indirect value with a delayed direct positive reaction. Blood pressure still low, 90/60.

Urine nil abnormal: on full diet with marmite, and iron tonic.

Patient did not appear unusually upset by large doses each quinine, atebrin, and plasmoquine given as a test on successive days.

52nd day. 18.9.38 Discharged as cured; weight 124 lbs. (8 lbs. over weight on admission) Spleen not palpable. Blood pressure 110/70. Blood picture normal with red blood count 6,000,000 per cub.mm.

Summary. This patient would appear to have had one massive intra-vascular haemolysis which took several days to clear. There was no indication of repeated haemolysis. At no time did the patient show any severe renal damage. Patient not sensitive to large dose quinine (grs. 60 in one day); also no reaction to test doses atebrin and plasmoquine.

Blood Picture, Case No. 2.

Day after coming under obs. for ?lysis R.B.C.	1st 11.8.38	2nd 12.8.38	3rd 13.8.38	7th 17.8.38	34th 13.9.38
Red Blood cells (per c.mm.)	2,800,000		1,9000,000	3220000	6000000
Haemoglobin % (Sahli)	64		49%	72	110
Colour Index	1.1			1.1	0.9
Mean corpuscular volume	114			111	91
Appearance of red cells.					
Poikilocytosis	++			+	-
Polychromatophilia	+			+	-
Macrocytes	+			+	-
Microcytes	+			few	-
Early forms	1 normobl			-	-
Reticulocytes %	6.0			8.0	0.5
White blood cells (per cub.mm.)	7,800			8,200	7,600
Differential Leucocyte count					
Neutrophile Leucocytes %	60			67	79
Eosinophile %	6			5	1
Basophile %	1			1	2
Lymphocytes %	24			19	14
Monocytes %	9			8	4
Abnormal Wh.cells	Nil			One immat. monocyte	Nil
Sedimentation Rate (Wintrobe) mms. per hour	66			46	2

URINE REPORT - CASE No 2.

DAY IN HOSPITAL	DATE	TIME	AMOUNT ozs.	S.G	REACTION	COLOUR	EQUIV. % SOL. R.B.C.	PIGMENTS.				PROTEIN	SEDIMENT.			
								OXY HB	MET. HB.	UROBIL-INOGEN.	BILI-RUBIN.		CASTS	R.B.C.	HB. MASS	W.B.C.
14TH.	11/8/38	a.m.	?	1030	Alk.	BURGUNDY	3·2%	+++	++	++	–	+	–	–	–	few +
		p.m.	60 ozs.	1008	NEUTRAL	DEEP RED	2·2%	++	+++	++	±	+	–	–	–	–
15TH	12/8/38	a.m.	22 ozs.	1008	ACID	PORT. W.	3·6%	++	+++	++	+	+	EPITH +	–	+–	–
		p.m.	49 "	1006	ACID	PORT. W.	2.8%	++	+++	++	–	++	++ many ep. cells	±	++	–
16TH	13/8/38	a.m.	30 "	1008	NEUTRAL	DEEP RED	2.4%	++	+++	++	–	+	++	–	+	–
		pm.	30 "	1012	ACID	PINK.	1.1%	+	++	+	–	+	++ + ep cells	–	++	+–
17TH	14/8/38	a.m.	30 "	1014	ALK.	YELLOW PINK	0.4%	+	+	+	–	±	++	–	+++	+
		pm.	40 "	1012	–·–	–"–										
18TH	15/8/38	a.m.	10 "	1010	ACID.	YELLOW	–	–	?	+	–	±	+	–	++	+

Appearance of bilirubin in blood was coincidental. with the appearance of the biphasic V.D.B. - see p.
Urine clear of albumen on 18.8.38.

TREATMENT. CASE No 2.

DAY IN HOSPITAL	DATE	TIME	Q. dihyd. in sal. orally	Atebrin dihydro. tablets orally	REST.	Plasma-quine tablets orally.	Atebrin musonate i.m. inject.	Ferr. et ammon. cit orally.	Marmite orally.	Blood (fresh) trans.	Anti-venene serum i.m.	Hot applic. kidney areas	20 gs. Sod. Bic. & Sod Cit. in 1 pt water	Rectal. Saline N/4 NaCl + 1/4 vol. Gluc.	I.V. fluids other than blood.
1st.	29/7/38		gr. 30												
2nd - 6th inclusive	30/7/38 - 3/8/38			0.3 g daily total 1.5 g.											
7th.	4/8/38				1 day.										
8th - 12th inclusive.	5/8/38 - 9/8/38.					0.02 g. dly. total 0.1 g									
13th	10/8/38.				1 day.										
14th	11/8/38							grs. 90 dly. until disch. 38 days later on 18/9/38.	1 oz. dly. until disch. 38 days later on 18/9/38.			+ +	1 pt. every 2 hrs. plus other fluids as patient wishes.		
18th.	15/8/38											ditto	ditto.		
52nd	18/9/38.														

UNIT CONV. DEPOT HOSPITAL

HOSPITAL

Case Report No. 3.

No. 11577. Rank.

L/NK. Gursaran Singh.

No. 7 Indian Field Ambulance, Razmak.

Age: 23.

Service 3½ years. Service in Waziristan 7 months.

Home: Punjab.

Returned with the main force to Razmak from the Kharre Column, 18.7.38.

1.8.38. Admitted to Combined Military Hospital, Razmak, complaining of fever and rigors of one day's duration.

Previous History. Hepatitis 1937.

Did not remember any other illness; definitely had never had any illness resembling the one for which he had been admitted to Hospital.

Examination. T. 103°F. P. 92 per min. R. 20 per min.

Slightly pale but otherwise fit-looking; no staining of skin or mucous membranes by malarial pigment. No icterus; mucous membranes normal in colour, no sign of anaemia.

Locomotor System. Nil abnormal detected.

Abdomen. Spleen not palpable. Stools negative for ova, cysts, and amoebae.

Circulatory System. Nil abnormal detected, except that blood films, thick and thin, showed ring forms of P.vivax present in fair numbers. Blood count not carried out.

Respiratory System.)
Genito-Urinary tract and Urine.) Nil abnormal detected.
Nervous System.)

Diagnosis. Malaria, Benign tertian, fresh.

Course of Illness.

Day after Admission.	Date.	Place.
1st	1.8.38	Hospital. Given grs. 10 quinine mixture 3 times in one day. Total grs. 30 quinine.
2nd-6th inclusive	2.8.38 - 6.8.38.	Atebrin, 0.1 g. t.i.d. for 5 days. Total 1.5 g. atebrin.
7th	7.8.38	Convalescent. One day's rest. Transferred to Convalescent Depot.
8th - 12th noon	8.8.38 - 12.8.38	Plasmoquine 0.01 g. b.i.d. as an outpatient. Total 0.09 g. plasmoquine. Stopped after the second last dose as patient reported haemoglobinuria and was found to be jaundiced.

The first six tablets plasmoquine (i.e. those given on the 8th, 9th and 10th day, total 6) were from one batch of the drug, whereas those given on the 11th and the one given on the 12th morning (i.e. three in number) were from a new batch.

12th day. 12.8.38

1800 hours. When patient reported at this time in the M.I.O. room of the hospital for his last dose of plasmoquine he was found to be markedly jaundiced; said that for the previous three hours he had been feeling weak and giddy and had been suffering from palpitation even at rest; also said he was suffering from frequency (in two hours passed urine four times) and that his urine was "red".

Readmitted at once to Hospital.

T. 101° F. P. 120. R. 24 p.m.

Markedly jaundiced; no cyanosis and no abdominal pain. Spleen one inch palpable on deep exploration. Not tender; patient is tender over hepatic area. Blood films thick and thin negative for malaria parasites; blood pressure 80/50. Blood count not done; plasma rose coloured,

equivalent to 1.2% solution red cells. Urine red in colour with absorption bands oxyhaemoglobin and methaemoglobin; albumen marked; heavy deposit urates and phosphates.

Serial urine reports p.766c appendix.

Plate No. XXI page 762 is a photograph illustrating periodicity of intra-vascular haemolytic process.

Treatment:as in previous cases, simple measures of treatment were decided on, namely: fluids ad lib per oram, mainly glucose-alkali and soda water, plus soda bicarb. - soda cit. solution sufficient to balance loss of fluid. Nil per rectum, nil intra-venously. Continued hot applications to loins.

Campolon heavy dose intra-muscularly ordered to be given next day.

13th. 13.8.38. T. 99.6° F. P. 100 p.m. R. 26 p.m. No change in general condition; still heavily jaundiced; cyanotic tinge. The patient was balancing his fluid loss by fluid intake orally; no parenteral administration. Blood films negative for malaria parasites. Serial blood pictures

given p.766 appendix.

Blood picture shows severe anaemia with red blood count 2,700,000. Plasma rich red in colour; blood urea level almost normal.

Urine total 12 ozs. passed in previous 12 hours; all specimens mixed, colour port-wine with absorption bands blood pigment. Heavy albumen content, but nil special in deposit except few epithelial cells. During the day urine showed marked fluctuation in colours. Details are given on p.766c appendix and are illustrated in plate No. XXI, p. 762 appendix. During this day urine passed on two occasions was free from any red colouration. The other specimens were ruby in colour with heavy content of methaemoglobin, and oxyhaemoglobin. The condition is one of repeated attacks of intra-vascular haemolysis. Treatment was on the same lines as previously. Also Campolon 4 ccs. intramuscularly and antivenine 10 ccs. intramuscularly.

14th day. 14.8.38. M. 98.6° F. E. 100 p.m. R. 84 p.m. General condition unchanged; jaundice not changed in degree. Slight cyanosis. Patient taking fluids satisfactorily by mouth, mainly alkaline mixtures; no vomiting. Heart rate fast, with soft sounds. Blood pressure 90/50. Blood films negative for malaria parasites. Plasma ruby red; slow rise in blood urea value and van den Bergh indirect value. Urine again heavily coloured by blood pigment; first specimens passed were port-wine in colour, then brown, then pink and by late afternoon urine showed no naked-eye indication of any blood pigment content. Spleen no longer palpable and liver tenderness reduced in degree and area.

Treatment: again given Campolon 4 ccs. and antivenine 10 ccs. Glucose-alkali solution continued by mouth; hot applications to loins.

15th day. 15.8.38 Pulse fast. Temperature and respiration normal. Jaundice less deep. Fluid intake satisfactorily balances output. Heart sounds still soft; plasma pink in colour; slight rise in blood urea level with a

PLATE XXI : PERIODICITY of INTRA-VASCULAR HAEMOLYTIC PROCESS.

fall in value van den Bergh indirect units.

Urine volume passed in 24 hours - 26 ozs. During this day the patient showed a striking example of repeated haemolyses. In all, ten specimens of urine were passed and the colour ran: brown - yellow - burgundy - orange - orange - orange - rose - yellow - yellow, i.e. apparently three haemolytic phases; details are given on page 766c appendix. Urine reaction alkaline in the majority of specimens, with casts of different types, and masses of haemoglobin pigment.

Treatment as before with 4 ccs. Campolon given, but no antivenine.

16th day. 16.8.38 General condition satisfactory. Temperature, pulse and respiration normal. Rest in bed. Jaundice slightly changed; fluid intake balances urine and other fluid loss. No abdominal pain. Blood pressure 120/70; blood count showed red cells to be unchanged, 2½ million per cub.mm., in spite of reticulocytosis 21% Plasma pink in colour; blood urea level still rising slightly with a falling van den Bergh

indirect value. One specimen urine passed during morning was coloured pink; the next specimen, 2 hours later, was yellow and the urine voided during the day remained normal in colour although still showing faint absorption bands blood pigment and with heavy deposit of granular and epithelial casts with masses oxyhaemoglobin.

Treatment: general and symptomatic; no injections given; on light diet.

17th day. 17.8.38. Nil abnormal to report; urine remained normal in colour, but still showed casts and haemoglobin masses.

Treatment: administration special alkali solution no longer necessary.

18th day. 18.8.38 Condition continued near normal until 0700 hours on this day. Jaundice present. The patient passed one specimen urine at 0615 which was normal in colour. Then after 30 minutes he passed another specimen, burgundy in colour. Obviously there had been a further intra-vascular haemolysis. Unfortunately specimen not examined.

At 1500 hours next specimen urine was

passed. The colour of the urine had returned to normal although casts and pigment masses were still present; thereafter no further haemolyses occurred, urine remained normal in colour.

27th day. 27.8.38. Nine days later; urine had remained normal in colour after the lysis of 18.8.38, and the content of casts and blood pigment masses slowly cleared. No longer detected after 27.8.38. On this day also patient gave a normal result to the concentrating test of Fishberg.

48th day. 17.9.38 The patient had no complaint after 18.8.38 other than weakness which appeared to be satisfactorily clearing up. The blood picture taken on this day showed remarkably poor regeneration of red cells. The patient had been given 4 ccs. Campolon on each of the following days: 29/8; 2/9; 6/9; 10/9; 14/9 and 17/9, i.e. a total of 36 cc. including 12 ccs. given previously yet the red cell count was only 3,000,000. This poor blood regeneration is not an uncommon finding among Indians whose haematopoeic system is so often exhausted. Another 4 ccs. given

on the following days: 18/9; 22/9; 26/9 and 30/9. This brought the total of Campolon given to 52 ccs.

68th day. 7.10.38 A marked spurt had taken place in the blood regeneration and the red cell count was normal in investigation. Patient discharged to duty. He did not develop any reaction suggestive of hypersansitiveness when he was given quinine, atebrin or plasmoquine in heavy therapeutic doses.

Summary. As shown by the records of urine, p.766 this patient suffered at least eight haemolytic crises in the space of six days. He escaped severe renal damage however and the only problem was sluggish acceleration of red cell production. The count only returned to normal after intra-muscular administration of 52 ccs. Campolon. No drug hypersensitivity noted following administration test doses of quinine, of atebrin and of plasmoquine.

Blood Picture Case No.3.

Day after coming under obs. for ?lysisR.B.C.	1st 13.8.38	2nd 14.8. 38	3rd 15.8. 38	4th 16.8. 38	5th 17.8. 38	31st 17.9. 38	51st 7.10. 38
R.B.C. (per c.mm.	2760000			2500000		3000000	5530000
Haemoglobin % (Sahli)	65%			66		90	102
Colour Index	1.2			1.2		1.5	0.93
Mean Corp. Vol.	96			104		110	98
Appearance of red cells.							
Poikilocytos.	-			-		slight	-
Polychromato.	-			-		slight	-
Macrocytes	++			++		+	
Microcytes	+			few		few	
Early forms	-			-		-	-
Reticulocytes %	14%			21%		1.5%	1.8%
Wh.blood cells (per c.mm.)	8400			6000		7200	7400
Differential Count.							
Neutrophils %	80			66		61	70
Eosinophils %	1			2		7	1
Basophils %	1			1		1	1
Lymphocytes %	14			28		23	26
Monocytes %	4			3		8	2
Abnormal Whites	Meta-myelo.			Imm.mono		-	-
Sediment Rate (Wintrobe mm/hr	45			26		25	3
v.d.Bergh Reaction. qual	Biphasic	Biphasic	Biph.	Neg.		Neg.	Neg.
quant	5	10	8	4	5		

Blood Picture Case No. 3 (contd.)

Day after coming under obs. for ?lysis R.B.C.	1st 13.8. 38	2nd 14.8 38	3rd 15.8. 38	4th 16.8. 38	5th 17.8. 38	31st 17.9. 38	51st 7.10. 38
Spectrum. exam. a. Plasma	methaem-albumen bands	mha bands	mha bands	mha bands	mha bands	oxyhaem. bands	
b. R.B.Cs, washed with saline and lysed with dist. water	oxyhaemo-globin bands and ?methaemo-globin bands.	oxyhm. bands	oxyhm. bands	oxyhm. bands	oxyhm. bands	oxyhm. bands	
Plasma colour. Equiv. % blood cells solution	1.0	2.1	2.0	0.6	0.4		
Wassermann R. blood serum	Neg.			Not done		Neg.	Not done
Kahn Reaction blood serum	Neg.			Not done		Neg.	Not done
D.Landst. T.	Neg			Neg.		Neg.	Neg.
Blood Urea	60	70	130	150	60	50	
Blood Group	IV			IV		Not done	Not done
Blood pressure	85/55			118/70		Not done	120/78
Bleeding time mins.	2.4			3		5	
Coagulation time mins.	4.6			6		5	
Fragility test (% saline sol. to lyse red cells	0.4% sal.			0.4% sal.		0.3% sal.	

URINE REPORT. CASE No 3.

DAY IN HOSPITAL	DATE	TIME	AMOUNT OZS.	S.G	REACTION	COLOUR	EQUIV. % SOL R.B.C.	PIGMENTS. OXY. H.B.	PIGMENTS. MET. H.B.	PIGMENTS. UROBILINOGEN	PIGMENTS. BILIRUBIN.	PROTEIN.	SEDIMENT. CASTS	SEDIMENT. R.B.C.	SEDIMENT. H.B.MASS	SEDIMENT. W.B.C.
12TH	12:8:38	2200	10	1014	Acid.	Red.	1.5%	++	+	+	−	++	−	−	−	Few.
13TH	13:8:38	1000	12.	1022	neutral	Port Wine	2'8%	++	++	+	−	++	Epi. cells	−	−	−
"	"	1400	9.	1010	acid.	RED.	1.5%	+	++	+	−	++	+	−	Slight	−
"	"	1600	6	1012	"	Yellow.	−	?	±	+	−	±	++	−	+	−
"	"	1900	3	1014	"	RUBY	2.6%	++	++	−	−	±	++	−	++	−
"	"	2100	2	1010	"	Yellow	−	−	?	±	−	−	+	−	+	−
14TH.	14:8:38.	0500	10	1016	alkal.	Port wine	2·8%	++	+++	+	−	+	+	−	±	−
"	"	1000	6	1018	"	Brown.	2·2%	(haematin +)	+	+	±	±	+	+	++	+
" *	"	1400	1½	1010	neutral	Pink.	1·4%	±	++	±	±	±	++	−	++	±
" *	"	1700	2	1016	"	Yellow	−	±	?	−	−	−	+	−	±	±
" *	"	2000	2	1010	acid	Orange	−	−	?	−	−	−	+	−	±	±
15TH *	15:8:38	0600	2½	1008	"	Brown	2·1%	(haematin+)	−	+	−	±	++	−	++	−
"	"	1000	2	1016	alkal.	Yellow	−	?	−	±	−	±	+	−	±	−
"	"	1200	6	1012	"	Burgundy	3·2%	++	++	++	−	++	+	−	±	+
" *	"	1300	2	1016	"	Orange	−	?	+	+	−	++	+	+	±	+
" *	"	1500	2½	"	"	"	"	"	"	+	−	++	+	+	±	+
" *	"	1600	1	1018	"	"	"	"	"	±	−	±	+	−	±	±
" *	"	1700	1	1018	"	"	"	"	"	±	−	±	+	−	±	±
"	"	1900	3	1010	"	Rose	2·0%	++	++	?	−	±	+	−	++	−
" *	"	2000	3	1016	acid.	Yellow	−	+	?	±	−	±	+	−	+	−
"	"	2100	3	"	"	"	"	−	−	±	−	±	+	−	+	−
16TH.	16:8:38	0400	2	1013	"	Pink.	0·8%	+	+	+	−	±	+	−	++	−
"	"	0600	2	1008	"	Yellow	−	±	±	±	−	±	++	few	+	−
" *	"	1000	1	"	alkal.		"	−	?	±		±	++	"	++	−
↓	↓			NORMAL												
18TH	18:8:38	0615	5	1019	acid.	Yellow	−	−	−	+	−	±	++	−	+	few.
"	"	0645	3	1020	"	Burgundy	3·1%	++	+++	+	−	+	++	−	++	−
"	"	1500	8	1010	"	Yellow	−	+	±	±	−	+	++	−	+	−
27TH	27:8:38	Normal since 18:8:38 and pigment masses and casts no longer found in the urine for the first time on this day														

concentrating test (Fishberg) normal on this day.

Urine clear of albumen.

* NOT IN PHOTOGRAPHS.

TREATMENT CASE No 3

CAN. DEPOT HOSPITAL.

NOSPITAL.

DAY IN HOSPITAL	DATE.	TIME	Q dihyd. in sol. orally.	Atebrin dihydro. tablets orally	REST	Plasma-quine. tablets orally	Atebrin musonate i.m. inject.	Ferr er ammon. cit orally.	Campolon injections	Blood (fresh) trans.	Anti-venene serum i.m.	Hot applic kidney areas	C FLUIDS orally	FLUIDS RECTALLY	FLUIDS I.V.
1ST.	1/8/38.		grs 30												
2ND - 6TH INCL.	2/8/38 → 6/8/38.			0.3g dly. total 1.5g											
7th	7/8/38				1. Day										
8th - 12TH Noon.	8/8/38 → 12/8/38					0.02g dly. total 0.09g									
12TH noon.	12/8/38														
13th	13/8/38								4.c.c.		10 ccs.	+ +	Gluc. Sal. + Soda Bic. + Citrate		
14th	14/8/38								4.c.c.		10 ccs.				
15th	15/8/38								4. cc.						
16th	16/8/38.														
17th	17/8/38														
18th	18/8/38.														
68TH	7/10/38								Total of 52 ccs. campolon given at intervals including the above 12 ccs.						

7662

Morning + Evening Temperatures

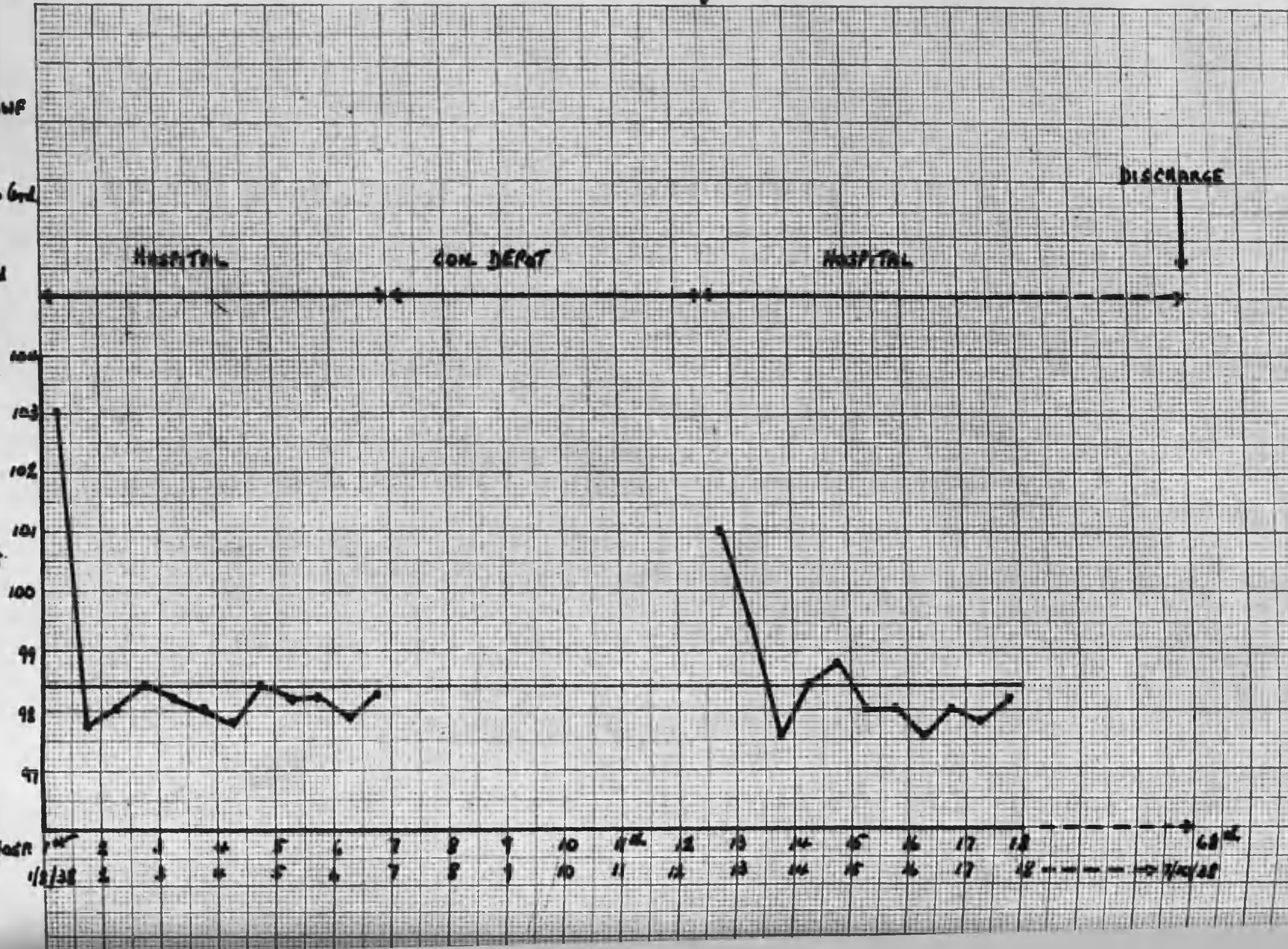
IP 11577 Lance-Naik GURSARAN SINGH aet 23: Service 3½ yrs

Admitted 1/8/38

Disease BWF

Diet. Fluid → Gen.

Result Cured



Case Report No. 4.

No. F.11. Water-Carrier Churu Ram,
3/17 Dogras, Thal Fort.
Age: 35.
Service: 12 years. Service in Waziristan 2 years.
Home: United Provinces.

This individual was on the Kharre Column with Damcol from Damdil, not with Razcol.
He therefore did not receive any prophylactic atebrin.

On return of Damcol to its camp at Damdil a Two-Company detachment of the 3/17 Dogras without stopping marched on to take over a spell of duty at Thal Fort, an isolated strong-point guarding the vulnerable part of the main supply line Bannu - Razmak. All sick and wounded from this Fort were at this time being conveyed forward to Razmak since the connection with the Base at Bannu was only weakly held. This route to Razmak was open for movement only on certain days, sometimes a week or two weeks apart.

On 28.7.38 patient developed fever and rigors; he reported sick to the Thal Fort Sick Bay where he was given such comfort and care as was available. No diagnosis could be made and quinine was withheld. The route to Razmak did not open until 2.8.38 and in the intervening four days the patient had had two severe attacks, one each on alternate

days, terminated by rigors and sweating. Clinically the condition was probably malaria.

On 2.8.38 he reached Razmak C.I.M. Hospital.

Previous History. Patient was definite that he had never had any disease resembling malaria. The medical records showed that he had not had any illness during the twelve years of Army service.

Examination: T. 99.6° F. P. 86 per min. R. 18 p.m. General condition below average, weight 99 lbs. Height 5 ft. 1 in. (Patient is a follower.) No malarial pigmentation of skin, and no jaundice; no obvious signs of anaemia.

Locomotor System. Poor physique.

Abdomen. Spleen not palpable; moderate tenderness present both in splenic and hepatic areas abdomen. No ova cysts or amoebae seen in the stools.

Circulatory System. Heart sounds soft in character. Asexual forms (ring forms) of P.vivax seen in blood films.

Respiratory System. Throat congested; rhonchi at both lung bases posteriorly (malarial bronchitis).

Genito-Urinary Tract and Urine.)
Nervous System.) Nil abnormal detected.

Diagnosis. Benign tertian malaria, fresh.

Course of Illness.

Day after admission.	Date	Place.	
1st	2.8.38	Hospital.	Given quinine grs. 10 t.d.s. for one day. Total grs. 30 quinine.
2nd-6th	3.8.38 - 7.8.38 (inclusive)	-	Atebrin 0.1 g. t.i.d. for 5 days Total 1.5 g. atebrin. One day's rest. Transferred to Convalescent Depot.
8th-9th (incl.)	9.8.38 - 10.8.38		Plasmoquine 0.01 g. b.i.d. as an outpatient.
10th	11.8.38.		i.e. after a total of 2 days plasmoquine (0.04 g. total) patient found to show jaundice of slight degree; he admitted feeling weak but denied any change in colour of urine. No cyanosis. Readmitted at once to Hospital. T. and R. normal. P. 98 p.m. Mildly jaundiced, no cyanosis. Tenderness still present over the spleen and the liver (was present at time of first admission). Stools negative for ova cysts and amoebae. Blood films negative for malaria parasites.

Blood pressure 100/60. Blood count not done; plasma not examined.

Urine showed nil abnormal (serial urine reports, p.773c

Treatment: the patient's only complaints on re-admission were jaundice, weakness and a fast pulse. Urine appeared normal on examination.

Treatment: this was thought to be a blackwater state and it was decided to treat the patient as being blackwater fever. Given the routine blackwater fever treatment, namely: Hot packs to loins, with plenty of glucose alkali by mouth, nil per rectum and nil intravenously.

11th day. 12.8.38 T. 100° F. P. 100. R. 22.

No change in general condition, jaundice slightly deeper. No cyanosis. Moderate tenderness still present over the liver and spleen. Blood films still negative for malaria parasites. Blood pressure 100/60. Urine normal in colour and content; spectroscope analysis showed no blood pigment absorption bands. Blackwater fever treatment continued.

12th 13.8.38 Temperature 98.8° F. Pulse 100 p.m. R. 22. p.m.

Patient still felt ill; jaundice showed no signs of lessening in degree.

Still moderate tenderness over liver and spleen. Heart sound soft with a marked haemic murmur; blood pressure 90/50.

Red cell count, carried out in clinical side room, showed R.B.C. 2,350,000; serial blood reports given appendix, p.773a.b

Plasma pink with absorption bands blood pigment. Urine specimen clear until 1000 hours when a red specimen was passed. At 1200 hours the urine was still red; by 1600 hours the colour had turned to pink and in the late evening an apparently normal specimen was passed.

Treatment continued as previously given. 4 ccs. Campolon injection also given.

14th. 15.8.38 No change in general condition. Lessening of the degree and area of tenderness over liver and spleen. Blood pressure 100/50. Plasma faintly pink. First specimen of urine passed was red in colour; by 1000 hours it was yellow in colour.

It remained yellow throughout 15.8.38.

Treatment, no change.

Campolon 4 ccs. intra-muscularly.

15th day. 16.8.38. Pulse fast. Temperature and respiration normal. Icterus unchanged in degree; cyanosis present for the first time. Abdominal tenderness less in degree and in area affected; stools negative for ova, cysts and amoebae. Blood films negative for malaria parasites. Blood pressure 90/50. Blood picture showed red blood count less than 3,000,000 with 24% reticulocytes. Plasma was slightly pink in colour with van den Bergh indirect value 5 units. The urine showed blood pigment colouration on three occasions during this day; the other samples voided were normal to the naked eye. From 2200 hours onwards on this day the urine did not again show any blood pigment staining. Treatment as before; no Campolon given.

16th. 17.8.38. Improvement in general condition with lessening of the degree of icterus; faint tinge of cyanosis; no abdominal tenderness. Blood pigment negative for malaria parasites; plasma not examined. All specimens

urine voided during the whole of 17.8.38 were free from any trace of blood pigment but did contain casts and haemoglobin masses.

19th. 20.8.38 The sediment in the urine no longer contained casts or haemoglobin masses; icteric tinge little changed; abdomen showed no tenderness. Fishberg's kidney function test gave satisfactory concentrating results. Hot packs and special fluids stopped. All danger of lysis seemed over. Another 4 ccs. Campolon given, also 4 ccs. Campolon given 23/8; 27/8; 31/8; 4/9; 8/9; 12/9; 16/9;
Therefore total Campolon given was 40 ccs.

48th. 18.9.38. Remarkable improvement in signs and symptoms, blood picture almost normal. Campolon stopped; Ferr. et ammon.cit begun, grs. 90 daily.

63rd. 3.10.38. Weight 114 lbs. (14 lbs. gain). Patient felt normal in every way; blood picture normal. Sent on leave following tests with quinine, atebrin and plasmoquine consecutively in an attempt to detect hypersensitiveness.

Blood Picture Case No. 4.

Day after coming under obs. for ?Lysis R.B.C.	1st 14.8.38	2nd 15.8.38	3rd 16.8.38	35th 18.9.38	50th 3.10.38
R.B. Cells (per c.mm.)	2350000		2890000	4450000	5500000
Haemoglobin % (Sahli)	38		40	87	97
Colour Index	0.8		0.7	0.98	0.88
Mean Corp.Vol.			75	89	92
Appearance of Red Cells.					
Poikilocytosis			+	-	-
Polychromat.			+	-	-
Macrocytes			+	-	-
Microcytes			+	-	-
Early forms			+/-	-	-
Reticulocytes			24	11	0.5
White Blood Cells (per cub.mm.)			7200	8600	6200
Differential Count					
Neutrophils %			73	50	39
Eosinophils %			1	4	9
Basophils %			1	2	1
Lymphocytes %			20	38	46
Monocytes %			5	6	5
Abnormal White Cells			Few metamy	One myeclo	
Sedimentation Rate (Wintrobe, mms. per hr.			78	14	7
v.d.Bergh. qual.	Indirect +	Indirect +	Indirect +	-	-
quant.	?	4	5		

Clinical side-room results.

Blood Picture Case No. 4. (contd.)

Day after coming under obs. for ?lysis R.B.C.	1st	2nd	3rd	35th	50th
	14.8.38	15.8.38	16.8.38	18.9.38	3.10.38
Spectrum exam. a. Plasma	methaem-albumen bands	m-alb bands	m-alb bands	oxyhaemo-globin bands	
b. R.B.Cs. washed with saline and lysed with dist.water.	oxyhaemo-globin bands	oxyhm. bands.	oxyhm. bands	oxyhm. bands	
Plasma colour. Equiv. % blood solution	0.6	0.8	0.2		
Blood Urea	70	60	110	45	Not done
Wassermann R. blood serum			Neg.	Neg.	Not done
Kahn Reaction blood serum			Neg.	Neg.	Not done
D.Landst.Test			Neg.	Neg.	-
Blood Group (Moss)			II	II	Not done
Blood pressure	90/50	100/50	90/50	Not done	110/75
Bleeding time mins.	1.5			4	
Coagulation time mins.	3.2				
Fragility test (% saline sol.	0.35% saline			0.3% saline	

URINE REPORT - CASE No 4.

DAY IN HOSPITAL	DATE	TIME	AMOUNT OZS.	S.G.	REACTION	COLOUR	EQUIV. % SOL. R.B.C.	PIGMENTS				PROTEIN	SEDIMENT.			
								OXY HB.	MET. HB.	UROBILINOGEN	BILIRUBIN.		CASTS	R.B.C.	HB.MASS	W.B.C.
10TH	11:8:38	am.	10	1018	Alk.	YELLOW	–	–	–	–	–	–	–	–	–	few
↓	↓		all samples normal in colour & content													
13TH	14:8:38	1000	4	1026	neutral	RED	1.6%	++	+	±	–	+	+	–	–	–
		1200	2	1022	alk.	-u-	1.6%	++	+	±	–	+	+	–	–	–
		1600	6	1016	alk.	PINK	0.8%	+	++	±	–	±	–	few	–	–
		2000	4	1010	alk.	YELLOW	–	?	–	–	–	–	–	–	–	–
14TH	15:8:48	0600	5	1012	alk	RED	1.4%	++	++	±	–	–	few	–	few	–
		1000	3	1018	acid	YELLOW	–	±	?	–	–	±	±	–	few	–
↓	↓	↓			NORMAL											
15TH	16:8:48	0800	12	1006	acid	YELLOW	–	–	–	–	–	–	few	–	–	few
		1000	6	1004	alk.	-u-										
		1400	6	1006	-u-	RED	1.4%	++	++	±	–	±	+	–	±	–
		1500	2	1004	-u-	PINK	0.6%	+	+	–	–	±	++	–	–	–
		1800	3	1008	-u-	YELLOW	–	±	–	+	–	±	++	–	++	–
		2000	6	1010	acid.	-u-	–	–	–	±	–	–	+	few	++	–
		2200	2	1016	-u-	RED	1.2%	++	++	+	–	±	++	–	+	–
		2400	4	1012	-u-	YELLOW	–	+	–	±	–	–	++	–	++	–

Thereafter normal except that it took 5 days for the casts and blood pigment masses to stop appearing in the urine.

TREATMENT. CASE N°4.

	DAY IN HOSPITAL	DATE	TIME	Q. dihyd. in sol. Orally	Atebrin dihydro tablets orally	REST	Plasmo-quine tablets orally	Atebrin musonate i.m. inject.	Ferr. et ammon. cit orally	Campo-lon. i.m. inj.	Blood (fresh) trans.	Anti-venene serum. i.m.	Hot applic. kidney areas.	Glucose alk. 5% Gluc. 1.5% NaCl. orally.	Rectal Saline N/4 Na.Cl.+ 1/4 vol Gluc.	I.V. fluids other than blood.
NAL DRT		29:7:38			NIL											
		1:8:38														
	1ST.	2:8:38		grs. 30												
HOSP.	2ND →	3:8:38			0.3 g. daily											
	6TH incl.	→ 7:8:38			Total 1.5 g											
	7TH	8:8:38				one day.										
C.D.	8TH →	9:8:38					0.02 g daily									
	9TH incl.	→ 10:8:38					Total. 0.04 g									
	10TH	11:8:38											++	Sod. Bic.		
	↓													" Cit.		
	13TH	14:8:38								4 ccs				20 gm.		
	14TH	16:8:38.								4 ccs.				in one pt. water & Gluc. Alk. ½ pt every half hour alternately with Soda water ½ pt. every half hour (Gluc. Alk. = 5% Gluc. + 1.5% NA. cit.)		
	↓	↓											↓			
	19TH	20:8:38								4 ccs						
HOSP.	22ND	23:8:38								-"-						
	26TH	27:8:38								-"-						
	30TH	31:8:38								-"-						
	34TH	4:9:38								-"-						
	38TH	8:9:38								-"-						
	42ND	12:9:38								-"-						
	46TH	16:9:38								-"-						
	48TH	18:9:38							grs 90 dly.							
	↓	↓							↓							
	63RD	10:38														

7732

Morning & Evening Temperatures

No F II Water bearer CHURU RAM age 35 service 12 years

Admitted 2/8/38

Disease B.W.F

Died

Fluids → Oral

Result Invalid

TRAL FORT | HOSPITAL | CON. DEPOT | HOSPITAL | DISCHARGE

TEMP. (DEGREES FAHR.)

103
102
101
100
99
98
97

DAY IN HOSP. 1st 2 3 4 5 6 7 8 9 10 11th 12 13 14 15 16 63rd

DATE 28/7/38 30 31 1/8/38 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 → 2/10/38

Summary. In the early stages the condition was one of blackwater fever sine blackwater. Another example of a series of haemolyses; at least four mild haemolyses in three days; little interference with kidney function, and no permanent damage. No obvious sign drug hypersensitivity.

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confirmed by his Army record sheet. The patient also said that he had never had malaria in civil life. This however is very doubtful.

Examination. T. 102° F. P. 90 p.m. R. 24 p.m.
Patient of light physique, below average weight; weight 119 lbs. height 5 ft. 4 ins. Vegetarian. No malarial pigmentation and no icteric staining seen.

Locomotor System. Slightly built, weight 119 lbs.

Abdomen. No enlargement spleen or liver; no abdominal tenderness. Stools did not show any ova, cysts or amoebae.

Circulatory System. Heart sounds nil abnormal.
Red blood count 5,000,000. Blood picture normal except for a mild hypochromia of red blood cells. Serial blood picture reports given on p.781a. Blood films thick and thin, contained ring forms P.vivax.

Respiratory System. Cough, pharyngitis and rhonchi both lung bases.

Genito-Urinary System)
) Nil abnormal detected.
Nervous System.

Diagnosis. Benign tertian malaria (Fresh).

Course of Illness.

Day.	Date.	
1st	27.9.38.	Begun daily dosage grs. Ferr. et ammon. cit. which was continued throughout the stay of the patient in Hospital. Given quinine in solution, grs. 10, t.i.d. for 4 days. Total grs. 120 quinine.
1st - 4th (incl.)	27.9.38 - 30.9.38	(Quinine given for 4 days because of the continued failure to bring the temperature under control.)
5th - 9th (incl.)	1.10.38 - 5.10.38 (incl.)	Given atebrin 0.1 g. t.i.d. for 5 days. Total 1.5 g. atebrin.
10th.	6.10.38.	One day's rest. Not thought fit to be moved to Convalescent Depot; retained to complete treatment in hospital.
11th-14th (incl)	7.10.38 - 10.10.38 (incl.)	Patient had completed 3½ days (i.e. had taken a total of 0.07 g.) of the 5 days plasmoquine course when he complained of his urine being highly-coloured; he persisted in saying that it was "deeply coloured", but denied that it was frankly red; he was not an

intelligent individual however; the patient had had rest in bed during the whole course.

14th. 10.10.38. Re-examined; pulse rate fast, temperature normal (rose to 100.5° F. at 1800 hours). Jaundice; patient's conjunctivae citrus coloured; no cyanosis and no abdominal pain; spleen one finger palpable on deep inspirations. No enlargement of liver; no tenderness abdomen; stools negative for ova, cysts and amoebae. Blood films negative for malaria parasites. Blood count not done; plasma not examined. Urine showed much bile pigment; no colouration by blood pigment and no absorption bands blood pigment; deposit urates and traces albumen. The urine continued to show much bile pigment throughout the day (serial reports p.781c). Findings strongly suggested a mild intra-vascular haemolysis, or degree insufficient to produce frank haemoglobinuria.

Treatment: plasmoquine stopped; strict bed; fluids only; (bland fluids by mouth)

15th 11.10.38. At 0600 hours patient passed red urine; fast pulse, temperature normal.

Degree of jaundice markedly increased; conjunctivae orange; tenderness over liver; definite cyanosis present now. Blood films negative for malaria parasites.

Blood pressure 100/60.

Marked fall red blood count to 2,000,000 per cub.mm. with 15% reticulocytes.

Plasma red in colour with absorption bands of oxyhaemoglobin and methaemalbumen.

Urine burgundy with heave content blood pigment and bile; albumen also present in urine with a small number of hyaline casts and red cells; obviously another haemolytic crisis had occurred at some time during the night.

During day specimens were passed suggesting a second lysis, namely: urine red - orange - red orange; amounts small.

Condition regarded as frank blackwater fever with oliguria and danger of anuria.

Treatment: treatment summarised on p. 781d

On same lines as previously, namely hot packs to kidney areaa (also tried hot retention enemas).

Fluid intake-urine output records kept.

Glucose-alkali ½ pint every hour)
) orally
Alternately with soda water 1 pint)
every hour.

Also Campolon 4 ccs. and antivenine 10 ccs.

16th. 12.10.38. Patient felt slightly better; temperature rose at 1800 hours to 99.5° F. Jaundice same in degree and mild cyanosis, liver tenderness less marked; during preceding 24 hours patient had taken 100 ozs. fluid orally and had passed only 14 ozs. urine. Plasma light red in colour; each specimen urine passed was port-wine coloured; red blood count 1.8 million, haemoglobin 43%. The specimen urine passed at 1100 hours was clear but again the specimen passed at 1900 hours showed red colouration, changing once again to yellow at 2300 hours; with granular and epithelial casts and with masses of haemoglobin in deposit.

Treatment: as before including Campolon but no antivenine.

17th. 13.10.38. Temperature normal a.m. but rising to 99.5° in evening. Jaundice little less and no cyanosis. Liver tenderness less marked. Fluid intake all per oram, 66 ozs. and urine output 20 ozs. Plasma pink in colour due to blood pigment.

Red blood count 1.7 million, haemoglobin 40%. Each specimen urine red in colour with casts and haemoglobin masses in profusion; this staining continued, although fainter, until 1600 hours when the colour became yellow; deposit still included casts and pigment masses.

Treatment continued; Campolon 4 ccs.

18th day. 14.10.38. Temperature and respiration normal. P.100 p.m. Jaundice unchanged; no liver tenderness. Fluid intake 60 ozs. output 33 ozs. Hereafter plasma and urine normal in appearance; casts and haemoglobin masses still in urine. Hot packs and special fluids stopped; repeated 4 ccs. Campolon.

19th.day, 15.10.38. Improvement continuing. Another 4 ccs. Campolon given.

21st.day. 17.10.38 Urine clear of pigment masses and casts. Begun Marmite 1 oz. daily. Jaundice fading.

35th day. 4.11.38 Marked subjective improvement; no jaundice; red blood count 3½ million with 13% reticulocytes.

27.11.38. Patient very fit. Red blood count 4.9 million.

14.12.38. Discharged cured. Weight 127 lbs. Red blood count 5.1 million. Blood pressure 120/80.

This patient had one day each on quinine, atebrin and plasmoquine and showed no ill effect from any.

Summary. This patient suffered a series of haemolytic crises while undergoing treatment for malaria. The first crisis was not sufficiently severe to cause frank haemoglobinuria. No obvious permament impairment kidney function. No drug hypersensitivity found.

Blood Picture Case No. 5.

Day after coming under obs. for ?lysis of R.B.C.		1st	2nd	3rd	24th	47th	63rd
	27.9. 38.	11.10 38	12.10 38	13.10 38	4.11. 38	27.11. 38	13.12 38
R.B.C. (per c.mm.)	5100000	2170000	1800000	1700000	3520000	4900000	5100000
Haemoglobin % (Sahli)	90	50	43	40	80	102	104
Colour Index	0.9	1.18			1.14	1.04	1.01
Mean Corp.Vol.	Not done	100			104	96	94
Appearance of Red Cells.							
Poikilocytosis		+			+	-	-
Polychrom.		++			+	-	-
Macrocytes		+			+	-	-
Microcytes		few			few	-	-
Early forms		one normobl			-	-	-
Reticulocytes %		15			13	2	1.5
Wh.Bl.cells. (per c.mm.)	6,700	6,600			7,000	6,400	6,600
Differential Count.	Before onset haemoglobinuria.						
Neutrophils %		61			64	61	63
Eosinophils %		3			2	1	2
Basophils %		1			2	1	1
Lymphocytes %		33			28	32	30
Monocytes %		2			4	5	4
Abnormal Wh. cells		1 myelocyte			-	-	-
Sedimentation Rate (Wintrobe) mms. hr.		69			9	3	2

Blood Picture Case No. 5 (contd.)

Day after coming under obs. for ?lysis of R.B.C.	1st	2nd	3rd	4th	24th	47th	63rd
	27.9.38	11.10.38	12.10.38	13.10.38	14.10.38	4.11.38	27.11.38	13.12.38
van d. Bergh Reaction. qual.	biphasic	biphasic	-	-				
quant.	9	8	2.2	3.8				
Spectrum Exam. a. Plasma	methaemalb. bands	m-alb bands	m-alb bands	m-alb bands	?	?	?	
b. R.B.Cs. washed with saline and lysed with dist. water	oxyhaemoglob. bands	oxyhaem. bands	oxyhaem. bands	oxyhaem. bands.				
Plasma colour Equiv. % blood solution	1	0.8	0.6	0.2				
Blood Urea	90	130	100	110		30		
Wassermann R. blood serum	Neg.							
Kahn R. bl.serum	Neg.							Not done
D.Landst.Test	Neg.	Neg.	Neg.	Neg				Not done
Bleeding time mins.	2				1.5	Not done		Not done
Coagulation time	5				2.6	"		"
Fragility Test (% sal. sol.)	0.35%				0.30%	"		"
Blood Gr. (Moss)	II				II	"		"
Blood Pressure	100/60				N.done	"		120/78

URINE REPORT - CASE No 5.

DAY IN HOSPITAL	DATE	TIME	AMOUNT ozs.	S.G	REACTION	COLOUR	EQUIV. % SOL. R.B.C.	PIGMENTS. OXY. HB	PIGMENTS. MET. HB.	PIGMENTS. UROBILINOGEN.	PIGMENTS. BILIRUBIN.	PROTEIN	SEDIMENT. CASTS	SEDIMENT. R.B.C.	SEDIMENT. HB.MASS	SEDIMENT. W.B.C.
14TH	10:10:38	1200	6	1024	Acid	Orange	–	–	–	++	+	±	–	–	–	–
		1600	10	1030	"	"	"	"	"	"	"	"	"	"	"	"
15TH	11:10:38	0600	6	1024	neutral	Burgundy	3·0%	+++	++	+	+	±	few hyaline.	few	–	–
		1000	2	1020	"	Red.	1.5%	++	++	+	±	±	–	–	–	–
		1800	2.	1022	Acid	Orange	–	±	?	+	+	±	casts	–	few	–
		2200	2.	1012	"	Red.	1.8%	++	++	+	+	±	"	–	"	–
		2400	2.	1014	"	Orange	–	+	–	+	±	±	+	–	+	–
16TH	12:10:38	0700	3	1022	"	Portwine	2·7%	++	++	+	+	±	granul. & epit.	few	+	–
		1100	4	1010	neutral	yellow	–	?	?	+	+	+	"	–	+	few
		1500	4	1012	"	"	–	–	–	+	+	+	"	–	++	"
		1900	3	1012	Alk.	Red.	1.2%	++	++	+	±	+	"	few	++	–
		2300	6	1008	"	yellow	–	?	?	∓	±	±	"	–	++	–
17TH	13:10:38	0800	7	1012	neutral	Red	1.6%	++	++	+	±	+	"	–	+	–
		1000	6	1010	Alk.	Pink.	0.6%	++	++	±	–	+	++	–	+	–
		1300	6	1018	"	Pink	0.6%	+	+	±	–	±	++	–	++	few
		1600	4	1014	"	Yellow	–	?	?	+	–	+	+	–	+	–
		1900	5	1014	neutral	orange	–	–	–	+	–	±	+	few	+	–
		2200	5	1010	"	Yellow	–	–	–	±	–	±	+	–	+	–

Hereafter normal except for casts and H.B. masses which finally also disappeared from the urine after four days by 17:10:38 - The urine also cleared of protein on this day.

TREATMENT, CASE No 5.

	DAY IN HOSPITAL	DATE.	TIME	Q. dihyd in sol. Orally.	Atebrin dihydro tablets orally	REST	Plasmoquine tablets orally.	MARMITE orally	Ferr et ammon. cit orally.	Campolon. i.m. inj.	Blood (fresh) trans.	Anti-venene serum. i.m.	Hot applic. kidney areas.	Glucose alk. 5% Gluc. 1.5% Na. Cl orally.	Rectal saline N/4 Na Cl. + 1/4 vol. Gluc.	i.v. fluids other than blood.
THAL FORT		25:9:38					NIL									
		→ 26:9:38														
	1ST	27:9:38		grs. 30 daily					grs. 90 daily							
	→ 4TH incl.	30:9:38		total grs 120												
	5TH	1:10:38			0.3 g. daily											
	→ 9TH incl.	5:10:38			total. 1.5 g											
	10TH	6:10:38				one day										
	11TH	7:10:38					0.2 g daily									
	→ 14TH NOON	10:10:38					total. 0.07 g									
HOSP.	15TH	11:10:38								4 ccs		10 ccs	++	gluc. alk. 1 pr. hrly alternately with Soda water 1 pr		
	16TH	12:10:38								4 ccs.		10 ccs				
	17TH	13:10:38								4 ccs.						
	18TH.	14:10:38								4 ccs						
	19TH	15:10:38								4 ccs.						
	21ST	17:10:38						1 oz. daily								
	79TH	14:12:38.														

7812

Morning & Evening Temperatures

NP 5949 Safoy DUNI aet 26 : service 4 yrs

Admitted 27/9/38

Disease BWF

Diet Fluids → Gen.

Result Cured

DISCHARGE

THEL FORT ← → HOSPITAL

TEMP. (DEGREES FAHR.)

104
103
102
101
100
99
98
97

DAY IN HOSP. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

DATE 25/9/38 26 27 28 29 30 1/10/38 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 — 31/10/38

Case Report No. 6.

No. 6579. Sepoy Tara Chand.

3/17 Dogras; Damdil Camp.

Age: 23. Service 5 years.

Home: Punjab.

Service in Waziristan 2 years.

It is pointed out that this patient was from the same Regiment as the two previous cases - Churu Ram and Duni; unlike them, however, Sepoy Tara Chand did not go forward to Thal Fort after the Kharre Column; with the remainder of his Battalion he returned from Kharre to Damdil Camp where he continued to serve until he became ill.

It has been said (p. 46) in the earlier part of this book firstly that no malarial patients from Damdil Camp were conveyed to Razmak Hospital, being treated in the Camp itself; and secondly that Damdil was thought to be mildly malarious; this is true. This particular patient, Tara Chand, was evacuated to Razmak as suffering from fever, cause unknown. Clinically it was quite unlike malaria and required investigation which was not possible in Damdil; secondly the fact that occasionally fresh malaria had developed among troops at Damdil must introduce an element of doubt whether Tara Chand contracted malaria during the Kharre Column; it cannot be proved that

he did not contract the disease in Damdil itself after the Column.

On 27.9.38, more than eight weeks after the finish of the Kharre Column this patient reported to the Field Ambulance at Damdil complaining of irregular fever and shivering and headache for the previous seven days. In the whole of his Army service he had never had malaria. He was admitted to the temporary Camp hospital; the temperature chart, p.788 shows the course of illness after admission to Damdil Camp hospital; the condition clinically did not resemble malaria, the fever being high and continued, with no obvious rigors; it was decided to evacuate the patient forward to Razmak where laboratory facilities were available. This transfer was not possible until 5.10.38.

On 5.10.38 the patient arrived in Razmak hospital; he had been ill for fourteen days previous to the transfer with a pyrexia of unknown origin, thought to be malaria probably.

This patient's Army records showed that he had not been in hospital with any disease during the five years of his service. The patient himself said that while on leave one year previously, i.e. 1937, he had had irregular fever with rigors for three weeks in his village and it had cleared up with medicine given by the local Hakim.

Examination. Temperature, pulse, respiration: Normal.
Weight 99 lbs.
Height 5 ft. 2 ins.
Anaemic in appearance; no malarial pigmentation and no jaundice.

Locomotor System. Underweight.

Abdomen. Spleen two fingers enlarged on deep inspiration; liver area normal. No ova, cysts or amoebae in stools.

Circulatory System. Heart sounds not abnormal; red blood count 5,200,000. Haemoglobin 95%.
Blood pressure 110/75.
(Serial blood pictures given p.788)
Blood films thick and thin showed gametocytes and ring forms P.vivax.

Respiratory System.)
Genito-Urinary System) Nil abnormal detected.
Nervous System.)

Diagnosis: Benign tertian malaria (?fresh).

Day after Admission.	Date.	
1st	5.10.38.	Began a course of grs. 90 of Ferr. et ammon. cit. daily which was continued until the discharge of the patient. Quinine NOT given.

1st - 5th (incl.)	5.10.38 - 9.10.38 (incl.)	Given atebrin 0.1 g. t.i.d. for 5 days. Total 1.5 g. atebrin.
6th	10.10.38	One day's rest; <u>not</u> considered fit for Convalescent Depot; <u>retained in hospital to complete treatment</u>.
7th - 9th (incl.)	11.10.38 - 13.10.38. (incl.)	Patient completed the first three days of his five-day plasmoquine course without incident, i.e. he took a total of 0.06 g. without harm.
10th	14.10.38.	On the morning of this day before the patient had taken his plasmoquine he reported a feeling of weakness; plasmoquine stopped. <u>Re-examined 1100 hours</u>. P.90 p.m. (rest in bed). Temperature and respiration normal. No jaundice, no cyanosis, no abdominal pain. Spleen <u>not</u> palpable; stools negative for ova cysts and amoebae. Heart sounds fast in rate, soft in character. Blood pressure 110/70. Blood films negative for malaria parasites. Blood count shown on p. 788a ; no significant change; red blood count 5,000,000 . haemoglobin reticulocytes 2.5%

Plasma definitely tinged with pink (% equivalent cells not estimated) with faint absorption bands oxyhaemoglobin; methaemalbumin not detected.

1200 hours. Urine red in colour with absorption bands both oxyhaemoglobin and methaemoglobin; no casts and no pigment masses (serial urine reports p.788c)

Next specimen urine normal in colour; urine continued apparently normal all during the day until

2100 hours. Again urine red in colour; marked oliguria. From then on all specimens normal in colour but pigment masses and casts were found in urine until 17.10.38.

Treatment: (Summary given p.788a)

as before, namely hot packs to loins, fluid intake and output registered.

Glucose alkali ½ pint ever hour alternately with soda water every hour orally.

Also Campolon 4 ccs. daily and antivenine 10 ccs. daily.

11th. 15.10.38 Patient felt better; temperature and respiration normal, pulse 88 p.m.; trace of jaundice; fluid intake 113 ozs.

urine output 100 ozs.

Blood films negative; plasma not examined; no biochemical test carried out. Urine normal in colour since 2100 hours previous day.

Treatment continued including Campolon and antivenine.

13th. 17.10.38. Patient felt much improved. Temperature, respiration and pulse normal. Urine free from casts and haemoglobin masses. All treatment stopped except ferr. et ammon. cit. Patient had received 16 ccs. Campolon and 40 ccs. antivenine.

21st. 25.10.38. Patient still felt weak; blood picture satisfactory. Red blood count 5,300,000. Haemoglobin 108%. Blood pressure 115/78.

38th. 11.11.38 Fit for duty; weight 110.5 (gain of 11½ lbs.) A rough test of drug hypersensitivity (oral administration heavy doses) showed no toxic reaction by patient to quinine, atebrin or plasmoquine.

Summary. This patient's urine was coloured red on two occasions only, namely 1200 hours and 2100 hours on 14.10.38.

Process was associated with marked polyuria and with minimal interference with kidney function; recovery appeared complete.

No obvious drug hypersensitivity on crude testing.

Blood Picture Case No. 6.

Day after coming under obs. for ?lysis R.B.C.		1st	11th
	5.10.38	14.10.38	25.10.38
Red Cell Count. (per cub.mm.)	5,200,000	5,000,000	5,300,000
Haemoglobin % (Sahli)	95	90	108
Colour Index	0.9	0.9	1.0
Mean Corpuscular Volume	Done in Clinical Side-room before onset of lysis.	90	96
Appearance Red Cells.			
Poikilocytosis		-	-
Polychromasia		-	-
Macrocytes		few	-
Microcytes		few	-
Early forms		nil	nil
Reticulocytes %		2.5	0.5
White Blood Count (cub.mm.)		8,000	8,600
Differential Count.			
Neutrophils %		67	63
Eosinophils %		1	2
Basophils %		1	1
Lymphocytes %		25	30
Monocytes %		6	4
Abnormal White Cells		Nil	Nil
Sedimentation Rate (Wintrobe) mms. per hr		25	4
v.d.Bergh. Direct Reaction. qual. (Quantitative exam. not done)		Indirect +	-
Spectroscopic Exam. a. Plasma		Oxyhaemoglobin bands	Not done

Blood Picture Case No. 6. (contd.)

Day after coming under obs. for ?lysis R.B.C.		1st	11th
	5.10.38	14.10.38	25.10.38
Spectroscopic Exam. b. R.B.Cs. washed with saline and lysed with distilled water		Oxyhaemoglobin bands	Not done.
Plasma Colour. Equiv. % blood solution R.B.C.		Not done.	
Blood Urea		Not estimated	
Wassermann Reaction blood serum		Neg.	Neg.
Kahn Reaction, blood serum		Neg.	Neg.
D. Landsteiner Test		Neg.	Not done
Bleeding time (mins)		2.0	3.5
Coagulation time (mins)		4.5	3.0
Fragility test (% saline solution)		0.35%	Not done
Blood Group (Moss)		III	

URINE REPORT - CASE Nº 6.

DAY IN HOSPITAL	DATE	TIME	AMOUNT OZS.	S.G	REACTION	COLOUR	EQUIV. % SOL. R.B.C.	PIGMENTS.				PROTEIN	SEDIMENT.			
								OXY HB	MET. HB.	UROBIL-INOGEN.	BILI-RUBIN		CASTS	R.B.C.	HB.MASS	W.B.C.
10TH	14:10:38	1200	6	1018	Alk.	RED	1·4 %	++	++	±	–	+	–	–	–	–
		1330	7	1018	"	ORANGE	–	?	?	±	–	±	–	–	–	–
		1500	7	1006	"	YELLOW.	–	–	–	+	–	–	–	–	–	–
		1600	4	1004	"	-"-	–	–	–	±	–	–	–	–	–	–
		1800	12	1004	"	-"-	–	–	–	±	–	–	few	few	–	–
		1900	6	1006	Neutral	-"-	–	–	–	±	–	–	"	–	–	–
		2100	10	1008	Acid.	RED.	1.6 %	+	?	±	–	+	–	–	±	–
		2200	4	1004	Neutral	YELLOW	–	–	–	±	–	+	few	–	±	–
		2400	10	1002	"	"	–	–	–	±	–	–	"	–	+	–

Thereafter normal except for casts and small amounts blood pigment masses and protein which finally dissapeared from urine a.m. 17:10:38.

TREATMENT, CASE Nº6.

	DAY IN HOSPITAL	DATE	TIME.	Q. dihyd. in sol. orally.	Atebrin dihydro tablets orally	REST	Plasma-Quine tablets orally.	Atebrin musonate i.m. inject	Ferr et ammon. cit orally.	Campo-lon. i.m. inj.	Blood (fresh) trans.	Anti venene serum i.m.	Hot applic. Kidney areas.	Glucose alk. 5% Gluc. 1.5% Na.Cl. orally.	Rectal: saline N/4 NaCl + 1/4 vol. Gluc.	i.v. fluids other than blood.
DAM DIL CAMP		27:9:38 ↓ 5:10:38			NIL											
	1ST ↓ 5TH incl.	5:10:38 ↓ 9:10:38		Nil	0.3 g daily total 1.5 g				grs 90 daily							
	6TH	10:10:38				one day										
	7TH	11:10:38					0.02 g. daily									
HOSP.	→ 9TH incl.	13:10:38					total. 0.06 g									
	10TH	14:10:38. ↓ 17:10:38								4 ccs ↓ Total. 16 ccs		10 ccs ↓ Total. 40 ccs	++ ↓	Gluc. alk ½ pr hourly alternately with Soda water ½ pr. hourly.		
	38TH	14:11:38														

7882

Morning & Evening Temperatures

No 6579 Sepoy TARA CHAND age 23 service 5 yrs

Admitted 5/x/38

Diarrhœa BWP

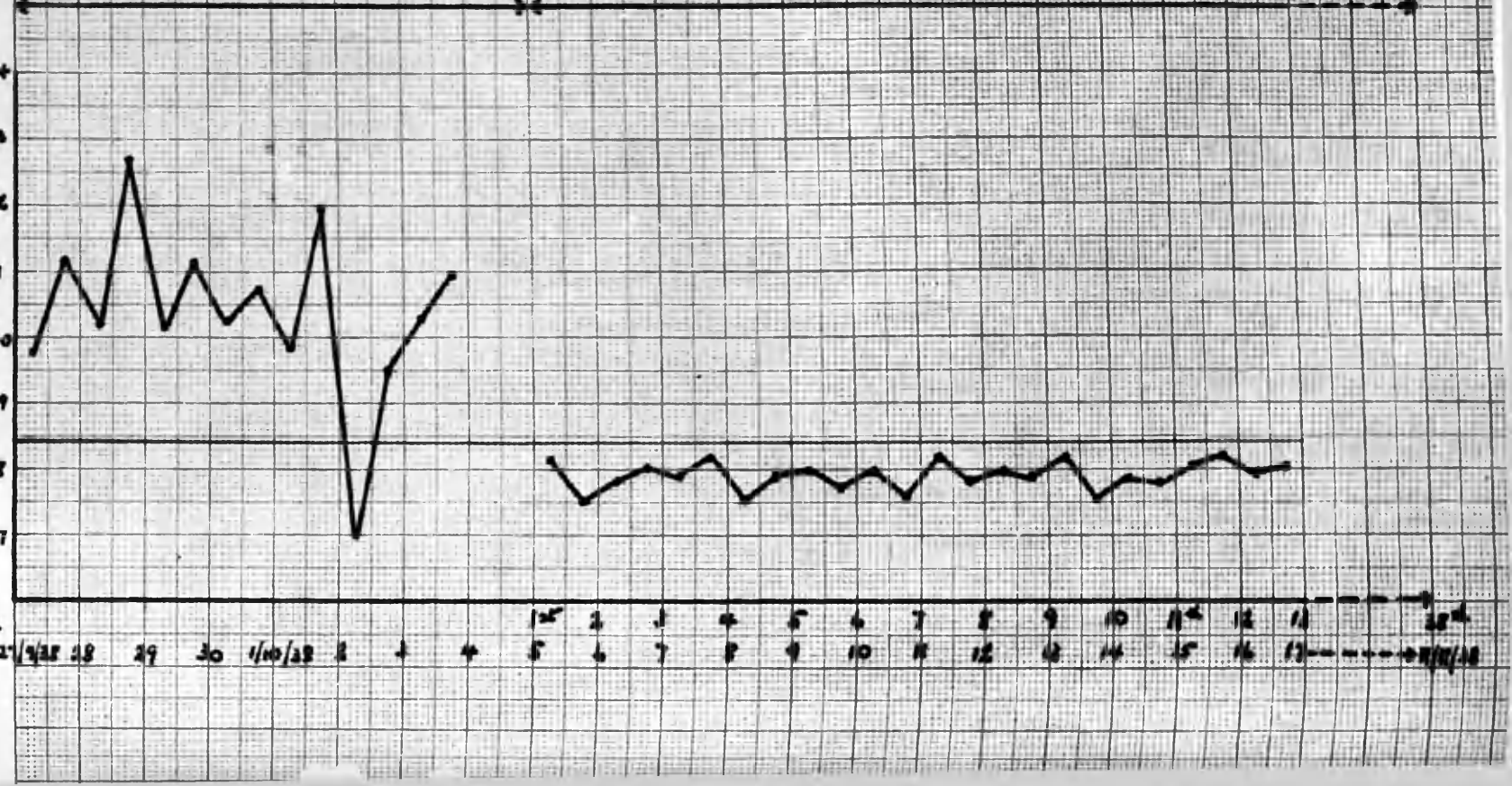
Diet Fluids → Gen

Remarks Journal

DAMDIM CAMP

HOSPITAL

DISCHARGED



INDEX.

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Browning, C.H.	1925	Immunochemical Studies, Constable, London		
Bruckmann, G. and Wertheimer, E.	1945	Brit.J.exper.Path.	26	217
Burkitt, R.W.	1943	Brit.med.J.	1	737
Burnham, R.C.	1946	U.S. Nav.Med.Bull.	46	434
Bywaters, E.G.L.	1942	Proc.Roy.Soc.Med.	35	321
ditto	1944	J.Amer.med.Ass.	124	1103
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Bywaters, E.G.L. and Beall, D.	1941	Brit.med.J.	1	427
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Calewaert, D.	1944	Rec.travaux Sci.Med. Conge, Belge	Jan. No.2	151
Cappell, D.F.	1946	Brit.med.J.	2	601.
Cardamatis, J.P.	1910	Bull.Soc.Path.Exot.	3	104
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Carreau, J.	1891	De la methemoglobinisme quinique: Point-a-Pitre.		

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Chopra,R.N.	1936	Handbook of Tropical Therapeutics. Art Press, Calcutta.		
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Chopra,R.N., and Ganguli, S.K.	1935	ditto	70	362
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Chopra,R.N.,Gupta,J.C. and Sen,B.	1936	ditto	71	309
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Christophers,S.R.	1929	Indian J.med.Res.	17	533 & 544
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Clark,H.C.	1944	Amer.J.trop.Med.	24	159

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Cohnheim, J.	1889	Lectures on Pathology. New Sydenham Society, London.		
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Coles, A.C.	1913	Lancet.	1	1230
Conant, J.B.	1923	J.biol.Chem.	57	401
Condamine, de la M.	1738	Histoire de l'Acad. Roy.des Sciences Ann.	-	226
Conley. Quoted by Field	1933	No publication (paper read at a medical meeting in Malaya).		
Corcoran, A.C. and Page, I.H.	1943	J.exper.Med.	78	205
Corre, A.	1878	Arch.de Med.Nav.	29	393
ditto	1883	Traite des fievres bilieuses, Paris		
Cort, E.C.	1929	Amer.J.trop.Med.	9	105
Craig, C.F. and Faust, E.C.	1945	Clinical Parasitology, 4th Edit. Kempton, London.		
Craige, B., Jones, R., Whorton, C.M., Pullman, T.N., Alving, A.S. and Eichelberger, L	1947	Amer.J.trop.Med.	27	309
Crosse, W.H.	1898	Brit.med.J.	2	1075
Crummings, J.C.	1859	New Orleans med.News and hosp.Gaz.	6	811
Cushny, A.R.	1926	Secretion of the Urine. 2nd Edit. London		
Custer, R.P.	1946	Amer.J.med.Sci.	212	211

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Dacie, J.V. and Richardson, N.	1943	J.Path.Bact.	55	375
Dameshek, W. and Schartz, S.O.	1938	Amer.J.med.Sci.	196	769
Das Gupta, B.M.	1932	Indian med.Gaz.	67	330
Das Gupta, C.C.	1933	ditto	68	28
Daullé. (Quoted by Stephens 1937: could not be consulted)	1857	These de Paris. original		
Davey, D.G.	1946	Trans.R.Soc.trop. Med.Hyg.	40	171
Davidson, L.S.P. and Fullerton, H.W.	1938	Quart.J.Med.	7 N.S.	43
Davidson, S. and Fullerton, H.W.	1945	Medical Annual, Wright, Bristol.		
Dawson, W.T. and Garbade, F.A.	1930	S.Amer.med.Ass.	94	704.
DE, M.N. Dem and Konar, N.R.	1940	Indian med.Gaz.	75	385
Debré, R., Gernez, C. and See, G.	1934	Bull.Soc.med.Hop.		1640
Deeks, W.E. and James, W.M.	1911	Report on Haemoglobinuria in the Canal Zone Mount Hope.		
Deeny, J., Murdock, E.T. and Rogan, J.J.	1943	Brit.med.J.	1	721
Dictionary National Biography.		Dict.Nat.Biog.	55	288
Dimson, S.B. and McMartin, R.B.	1946	Quart.J.Med.	15 N.S.	25
Director Medical Services, India.	1933	Instruction Z9295/30/DMS3 of 27/4/1933		

Authors.	Year.	Book.	Vol.	Page
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Dobbs,R.H.	1947	Lancet.	1	360
Dobreff,M.	1934	Arch.Schiffs -u- Tropenhyg.	38	288
Domagk,G.	1935	Dtsch.med.Wschr.	61	250
Donath,J. and Landsteiner, K.	1904	München.med.Wschr.	51	1590
Drew,W.R.M. and Reid,J.	1945	Lancet	2	107
Dugall,A.N. (D.A.D.P. Waziristan)	1939	Personal communication		
Dunn,J.S.	1940	Proc.R.Phil.Soc. Glasgow.	64	106
Dunn,J.S., Gillespie,M. and Niven,J.S.F.	1941	Lancet	2	549
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Eggleton, M.G., Richardson, K.C., Schild, H.O. and Winton, F.R.	1942	Brit.med.J.	2	392
Eichholtz, F.	1927	BEI. Beih.Archs.Schiffs.-u-Tropenhyg.	31	89
Eiselberg, K.P.	1927	Wien.klin.Wschr.	40	525
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Evans, C.L.	1945	Starling's "Principles of Human Physiology". 9th ed. Churchill, London.		
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Fahraeus, R.	1939	Lancet	2	630.
Fairley, N.H.	1937	Nature	139	588
ditto	1938	ditto	142	1156
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ditto	1940b	Trans.R.Soc.trop. Med.Hyg.	34	173

Authors.	Year.	Book.	Vol.	Page.
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ditto	1945	Trans.R.Soc.trop. Med.Hyg.	38	311
ditto	1946a	ditto	40	229
ditto	1946b	Brit.med.J.	2	787
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Feigl,J.	1916	Biochem.Ztschr.	76	88
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Field,J.W.	1939	Chemotherapy of Malaria. Bull.No.2,1938 from Inst.Med.Res. F.M.S.		
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Fleischer,R.	1881	Berlin klin.Wehnschr.	18	691
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Fox,C.L. and Ottenberg,R.	1941	J.clin.Invest.	20	593
Foy,H. (Quoted by Gear)				
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ditto	1937b	ditto	31	123
ditto	1938a	ditto	32	49
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Gamble, J.L.	1929	New England J. Med.	201	909
ditto	1936	International Clinics. Series 2.	46	184
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Garnham, P.C.C.	1945	Brit. med. J.	2	45
Gear, J.	1946	Trans. R. Soc. trop. Med. Hyg.	39	301
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Gibson, Q.H. and Harrison, D.C.	1947	Lancet.	2	941
Giglioli, G.	1932a	Trans. R. Soc. trop. Med. Hyg.	26	177
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Gill, C.A. Letter to Stephens, no date.				
Gilligan, D.R., Altschule, M.D. and Katersky, E.M.	1941	J. clin. Invest.	20	177
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Goodman,L. and Gilman,A.	1941	The Pharmacological Basis of Therapeutics. McMillan, London		
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Gosh,J.	1941	Personal comm.18/1/41		
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Gunther,C.E.M.	1938	Med.J.Australia.	2	1119
Gunther,H.	1924	Arch.path.Anat.	251	141
de Haan,J.	1905	Arch.Schiffs u. Tropenhyg.	9	22
Hackett, L.W.	1945	Rev.Inst.Salubridad y Enformidades Trop. Mexico.	6	239
Ham,T.H. and Castle, W.B.	1940	Proc.Amer.Philos.Soc.	82	411
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Hanschell,H.M.	1924	Trans.R.Soc.Trop.Med. Hyg.	18	42
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Harris,J.S. and Michel,H.O.	1939	J.clin.Invest.	18	507
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Hecht,G.	1933	Arch.exper.Path.Pharmak.	170	328
Heggie,J.F.	1946	Lancet.	2	436
ditto	1947	ditto	1	385
Heinemann,C.	1885	Arch.Path.Anat.u. Physiol.u.klin.Med.	102	517
Heuck,G.	1880	Berlin klin.Wschr.	17	173
Hicks,S.P. and Opie, E.L.	1942	Amer.J.Path.	18	333
Hills,A.G.	1946	Amer.J.med.Sci.	212	45
Hittmair,A.	1925	Wien klin.Wschr.	38	431
Hoops,A.L.	1934	Malaya med.J.	9	123
Hoppe-Seyler,F.	1865	Handbuch der Chem. Analyse, 2nd edit.		
Höppli and Salvioli. (Quoted by Nocht and Mayer)				
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Huntze. (Quoted by Scott)	1916			
Hynes,M.	1945	Anaemia Report, G.H.Q. India. Army Pathology Advisory Committee.		
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Innes,J.	1947	Edinburgh med.J.	54	284
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Authors.	Year.	Book.	Vol.	Page.
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James, S.P.	1924	Trans.R.Soc.trop. Med.Hyg.	18	33
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James, W.B.	1938	Personal comm. 12/8/38		
James, S.P.and Tate, P.	1937	Nature	139	545
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Josephs, H.W.	1938	Bull.Johns.Hopk.Hosp.	62	25 & 53
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ditto	1882	Gaz.Hop.Civ.Milit.	55	969
Keilin, J.	1944	Nature	154	120
Kelly, E.C.	1939	Adam's "Genuine Works of Hippocrates"		
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Kierland, R.R.	1946	Proc.Mayo Chin.	21	404
Kikuth, W.	1927	Arch.Schiffs u. Tropenhyg.	31	501
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Koch,R.	1898	Arbt.Kaiserhchen Gesundheitsamte	14	304
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Krishnan,K.V.	1933	Indian J.med.Res.	21	331
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Krishnan,K.V. and Pai, N.G.	1936	ditto	71	121
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Landsteiner,K. (Quoted by Zinsser et alia (1944) which see.	1900			
Landsteiner,K. and Wiener, A.S.	1940	Proc.Soc.exp.Biol. and Med.	43	223
Laser,H. and Friedmann,E.	1945	Nature.	156	507
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Authors.	Year	Book	Vol.	Page
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Lucherini,T.	1938	Policlinico. SEZ. PRAT.	45	1849
Lucké,B.	1946	Milit.Surgeon.	99	371
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McMichael,J.	1942	Proc.R.Soc.Med.	35	333
McNee,J.W.	1924	Brit.med.J.	2	495
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McNee,J.W. and Keefer,C.S.	1925	Brit.med.J.	2	52
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Authors.	Year.	Book	Vol.	Page
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Manifold, J.A.	1931	Jour.R.Army med.Cps.	56	321 410
Mann.W.N.	1943	Trans.R.Soc.trop.Med. Hyg.	37	151
Manson,O.F.	1886	Jour.Amer.med.Ass.	7	579
Manson-Bahr,P.H.	1927	Trans.R.Soc.trop.Med.Hyg.	20	411
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Authors.	Year.	Book.	Vol.	Page
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Mayer,M.M. and Heidelberger,M.	1946	J.Immunol.	54	89
Menk,W.	1927	United Fruit Coy.Med. Dept. 16th Annual Report.	16	78 113
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Meulengracht.E.	1920	Dtsch.Arch.klin.Med.	132	285
Mayer-Betz.F.	1910-11	ditto	101	85
Miller,D.K. and Rhoads,C.P.	1934	J.exper.Med.	59	315
Millikan,G.A.	1939	Physiol.Rev.	19	503
Minami,S.	1923	Virchow's Arch.	245	247
Mohr, W.	1944	Dtsch.Tropenmed. Ztschr.	48	177
Moir,K.T.	1933	West African med.J.	7	121
Mollison,P.L.	1943	Brit.med.J.	1	529 559
Monestier.	1873	Gaz.Hop.de Paris.	46	820
Monke,J.V. and Yuile,C.L.	1940	J.exper.Med.	72	149

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Moreschi,C.	1920	Policlin.sez.med.	27	216
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Most,H.	1940	Trans.R.Soc.trop.Med. Hyg.	34	139
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Mühlens,P.	1926	Supplem.III to Vol.30 of Arch.Schiffs.u. Tropenhyg.	30	25
Murley,R.S.	1943	Jour.R.Army Med.Cps.	80	182
Murray,A.J.	1934	West African Med.J.	8	17
Murri,A.	1896	Dtschr.med.Wschr.	22	115 136
Mushett,C.W. and Siegel,H.	1946	Blood.	1	537
Myers,W.A.	1926	Jour.Amer.med.Ass.	86	1198
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Nayudu,R.V.N.	1937	Indian med.Gaz.	72	531
Neill,J.M.	1925	J.exper.Med.	41	299
Neuwirt,K.	1922	Z.urol.Chir.	11	75
Neve,E.F.	1941	Personal comm.23/1/41		
Nicol,W.D.	1932	Trans.R.Soc.trop.Med. Hyg.	26	118
Nicol.B.M.	1940	Quart.J.Med.	9 N.S.	91
Nightingale,P.A.	1909	Transvaal Med.J.	5	253
Nocht,B.	1929	Arch.Schiffs u. Tropenhyg.	33	21
Nocht,B. and Kikuth.W.	1929	ditto	33	355
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Norris,C.H.	1939	J.cell.comp.Physiol.	14	117
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Oliver-Gonzalez,J.	1944	Proc.Soc.exp.Biol.N.Y.	57	25
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Passmore,R.	1947	Trans.R.Soc.trop.Med. Hyg.	41	189
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Paul,F.	1923-24	Wien.Arch.inn.Med.	8	531
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Pellarin,A.	1865	Arch.de Med.Nav	4	473
Peter,F.M.	1935	Trans.R.Soc.trop.Med. Hyg.	29	41
Peters,H.R.	1942	Ann.intern.Med.	16	547
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Ponfick,E.	1883	Berlin klin.Wschr.	20	389
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Salen, E.B.	1925	Acta Med. Scand. Suppl. XI	62	
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Shortt,H.E. Garnham,P.C.C. Covell,G., and Shute,P.G.	1948	Brit.med.J.	1	547
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Thonnard-Neumann,E.	1931	20th Annual Report United Fruit Coy.U.S.A.	20	57
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