The Occurrence and Diagnosis of Malaria

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SUMMARY

Malaria is readily diagnosed provided it is considered. Blood films should be examined for parasites whenever this possibility arises and the appropriate blood tests should be carried out. If a physician neglects to have them done, he will sooner or later have a stricken conscience, burdened with the memory of cases with an unhappy ending. In the Lowveld there is probably little need for this exhortation. In our large cities, unfortunately, experience almost every year tells of the need for its emphasis.

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This symposium provides an opportunity for paying tribute to some of the pioneers in the study of malaria and its mosquitoes in South Africa. Their work has led to the development of the methods of control which are still regarded as the most reliable and effective.

It may be recalled that the Portuguese settlement at Lourenço Marques was established more than a century before Jan van Riebeeck landed at the Cape on 6 April 1652. These early settlers soon became aware of the threat of malaria to their health and lives. However, one of the most heart-breaking accounts of its ravages ever written is to be found in Louis Trichardt's *Dagboek* describing the last days of the trek of his party of Voortrekkers from the vicinity of Pietersburg to Lourenço Marques. He took his party on their journey from near where Pietersburg now stands, through Chuniespoort, along the Olifants River, crossing it several times, over the Drakensberg, to the vicinity of Hoedspruit, through the bushveld now incorporated in the Kruger National Park, over the Lebombo and then on to Lourenço Marques. In his diary he described the presence of tsetse flies and noted that the indigenous inhabitants associated their bites with the fatal cattle disease known as *ngana*. Soon after his arrival in Lourenço Marques at the end of March 1838, his wife died of malaria and his description of her passing is one of the most moving ever written. Trichardt writes: 'Four days after reaching the Bay, my wife, my sons Gustav and Jeremiah and my daughter Annie, are all very sick with the fever'. Some days later 'I got up at cockcrow to see if there was any news of my dear wife, but as all was quiet at the Governor's house, I went back to bed. I could not sleep and at second cockcrow I got up again. I went at once to her to wish her good morning. She answered me so softly that I could not understand her words. "Does my dear wife know me?" I asked, and she answered: "As if I would not know you" . . . Suddenly it came over me that all I had dreaded was coming true . . . Then I lost all hold upon myself and in the depth of my sorrow I knew not what I said or did. The children wept with me and their grief made mine the harder to bear. I bade my wife farewell and told her of my hope to meet her in the home of the Heavenly Father. All our sorrow and care were in vain — my dearly beloved is taken from me for ever'. About one week later he also died of malaria, as did several others of his party. The memorial to these early pioneers is now one of South Africa's most hallowed places in Lourenço Marques.

Ever since that time, the Lowveld of the Transvaal and northern Natal has been notorious for the ravages of malaria and its associated black-water fever. It has until recently remained the most serious hazard to the health of the inhabitants of that region. The circumstances in which it occurred were studied intensively by the officers of the Union Health Department in Natal, and in particular by Dr Park Ross and Messrs Van der Wagen and Hamilton, in collaboration with the entomologists at the SA Institute for Medical Research, Drs Ingram and Botha de Meillon.

As a result of these investigations it became clear that malaria was endemic in northern Natal and the eastern Transvaal, east of the Drakensberg. When conditions were favourable, as they usually were when the rains came in summer and autumn, the infection spread up the large river valleys and then into the surrounding country to give rise to epidemics which occurred almost every autumn.

When conditions were exceptionally favourable, as they were after a prolonged drought, when there were heavy falls of rain interspersed with intervals of hot bright sunshine, malaria became intense in the endemic region and in the summer-autumn epidemic areas, and then spread widely to involve almost the whole of the Transvaal as far south as the Magaliesberg, and occasionally even as far south as the Witwatersrand. The last time that this happened was in 1938, when several locally-acquired cases were seen in the northern suburbs of Johannesburg.

Of the many species of anopheline mosquito that occur in Southern Africa, it became clear that only 2 are of importance in the transmission of malaria. These are *Anopheles gambiae* and *Anopheles funestus*. The breeding habits of these 2 mosquitoes are of prime importance in determining the incidence of malaria.

A. gambiae is a pool breeder, the larvae being found in rainwater pools, the pools left in river beds when the water has receded, and in the backwaters of rivers. Rainwater pools, fully exposed to the sun's rays and devoid of vegetation, are especially favoured as breeding places. Even small puddles which form, for example, in hoofmarks of cattle, may be found to contain larvae. *A. gambiae* spreads, and

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spreads only with rainfall, as its breeding places of choice are temporary and to a large extent dependent on rainfall. The extent of spread is also determined by the climate. Portions of the Transvaal, for instance, where the necessary rains fall, have no *A. gambiae* because the temperatures are too low.

A. funestus is essentially a stream breeder, the larvae being found at the edges of perennial streams, where the vegetation is rank, shade plentiful and the current slow. To ensure a small perennial stream a mean annual rainfall of over 760 mm is required. Other requirements for the survival of *A. funestus* are a mean monthly temperature of 16°C or over, with a range of less than 4°C.

In the Transvaal the only area in which these conditions obtain are the foothills east of the Drakensberg. In this area malaria is intense and hyperendemic, and smoulders throughout the winter. As *A. funestus* is confined to permanent streams, it does not spread with rainfall. On the contrary, heavy falls of rain may diminish the number of these mosquitoes by flushing away the larvae.

In view of the serious threat, several distinguished experts were invited by the Government to come to South Africa to take part in discussions on malaria and to advise on methods for its control. Among them was Dr Swellengrebel, an authority from Holland, who confirmed that only 2 of the more than 20 species of anopheline mosquitoes were of importance in the transmission of malaria in Southern Africa. These were *A. funestus* and *A. gambiae*. Dr Swellengrebel recommended that the antimosquito measures should be aimed primarily at these 2 mosquitoes — the principle of species sanitation. Sir Malcolm Watson, who had gained most of his experience in Malaya, suggested that antilarval measures would be most effective.

Another recognised authority on mosquito-borne diseases, especially yellow fever and malaria, was Dr Fred Soper of the Rockefeller Foundation, who attended the Pan African Health Conference in Johannesburg in 1935, where he was critical of the methods for the control of malaria developed by Dr Park Ross and his team in Natal. He had won considerable repute as the leading expert in this field by the success of the campaign he had led in controlling and eliminating yellow fever in South America. Early in World War II *A. gambiae* had been taken across the south Atlantic from Africa to Brazil apparently by French destroyers. Conditions favoured its proliferation and an extensive and severe epidemic of malignant tertian malaria resulted.

After recognising the part played by *A. gambiae* in this epidemic, Dr Soper undertook a campaign to eliminate it from Brazil. He built up a large organisation to apply the control measures and relied mainly on the spraying of huts and houses to eliminate the adults. His campaign was successful. Soon after its success had been widely proclaimed in Brazil and the USA, I met him at the Rockefeller Institute for Medical Research in New York. When he heard that I had come from South Africa, he said he was delighted to meet me since it gave him the opportunity of apologising to the malaria officers of the Union Health Department. He said he had been scornful of the value of their methods and had pointed out that for every mosquito that came inside, there would be hundreds outside and out of reach of the insecticidal sprays. He had not realised how anthropophilic *A. gambiae* were and how closely bound they were to the dwellings of man, until he saw how successful the spraying of huts and houses had been in Brazil. I was glad, and I may say proud, to convey his message of apology to my friends and colleagues in South Africa.

Before World War II the Union Health Department had established a large malaria control organisation based on the Malaria Research Laboratory built at Tzaneen and directed by Dr Siegfried Annecke. He was a forceful personality, as those of us who knew him will recall, who led his staff in an active campaign against the malarial mosquitoes, relying mainly on the spraying of huts with a pyrethrum spray, but he also included antilarval spraying of streams with Paris green. Great emphasis was placed on the protection provided by sleeping under a mosquito-net. These measures met with only limited success. Malaria remained a menace in these regions.

Soon after my return from the USA to South Africa, I attended a meeting of the Eastern Transvaal Branch of the Medical Association of South Africa, held in Machadodorp. My host on this occasion was Dr Eksteen, Member of Parliament for Middelburg. He took me by car from Middelburg to Machadodorp, and on our journey we discussed the success of the campaign undertaken by Dr Soper in Brazil and considered the possibility of undertaking a similar campaign in the northern Transvaal.

Soon after this, we learned from Colonel H. S. Gear, then Assistant Director of Hygiene, Middle East Command, of the value of DDT as shown by Professor P. A. Buxton of the London School of Hygiene and Tropical Medicine, and in field trials undertaken by the RAMC and SAMC in controlling lice and mosquitoes in the Nile Valley. This was followed by the demonstration by General Fox and Major Woodward of the US Army Medical Corps of its value in controlling lice, chiefly by applying it as a dusting powder with an air blower, and bringing the epidemic of typhus fever in Naples to a speedy end.

The formula of DDT was then a closely guarded military secret, but Major Neil Murray, then of the SAMC and Union Health Department, found it in *Popular Mechanics*. He asked Colonel Bleloch if he could make it and a method for its manufacture was worked out. Large amounts of its 2 essential ingredients, chlorine and benzene, were available and a fine plant was built at Modderfontein. Its production was able to meet the needs of South Africa and helped to meet those of the Middle East theatre of War. The first sample of this production was used to control an outbreak of typhus fever on a farm near Hendrina in Middelburg, Transvaal. It was then made freely available to the people of the Transkei, who had just experienced severe outbreaks of typhus fever, and immediately these extensive epidemics became a thing of the past.

Dr Eksteen then proposed in Parliament that financial provision should be made to support an antimalarial campaign in South Africa, relying on the spraying of huts and houses with DDT. This was readily agreed to, and the campaign was undertaken in 1945 and in subsequent years by the teams based in Natal at Durban and in the Transvaal at Tzaneen. It was directed by Dr Annecke. The

success of this campaign exceeded the anticipation of even the most sanguine health officers. Malaria was virtually eliminated from South Africa. Great agricultural development became possible along the river valleys of the eastern Transvaal and soon these were among the richest areas in South Africa. This development has continued ever since.

However, malaria had not been entirely eliminated and continued to smoulder in the border areas of the north-eastern Transvaal and northern Natal. The infection was regularly reintroduced by migrant workers from the territories to the north. The malarial mosquitoes, particularly *A. gambiae,* had not been eradicated. Indeed, recent surveys undertaken by the State Health Department have shown that *A. gambiae* is still to be found over an area as extensive as ever. However, studies initiated in the Arbovirus Research Unit in the Laboratories of the Poliomyelitis Research Foundation, have shown that the species *A. gambiae* was made up of a number of subspecies of differing habits and separated from one another by their chromosome patterns. In further investigations, 5 subspecies A - E have been identified. Two of them are of particular interest in Southern Africa.

A. gambiae A is an anthropophilic hut-frequenting mosquito and presumably was responsible for the extensive epidemics which once occurred in the northern Transvaal and northern Natal. *A. gambiae C* is a zoophilic species feeding on animals, especially cattle, and therefore not of importance in the transmission of human malaria.

Clearly the identity of the *A. gambiae* which have persisted in the once epidemic areas, must be established, and their biting habits studied. It is possible that *A. gambiae A* are now feeding on man and transmitting malaria out-of-doors.

Because of the persistence of the mosquitoes and malaria parasites, some people feared that when conditions became unusually favourable, the smouldering infection would once again burst into epidemic flame. These fears have been fully justified. In 1967 conditions became ideal for the proliferation of *A. gambiae* in the Lowveld. During the prolonged drought of the preceding 5 years, the vegetation largely withered away. This was followed by the regular heavy rainfalls interspersed with periods of bright hot sunshine in the summer and autumn of early 1967. *A. gambiae* and *A. funestus* flourished and there was a sharp outbreak of malaria.

The year 1972 will always be remembered for the bountiful and record yields of maize and other crops. The regular and heavy rainfall which ensured these good harvests also unfortunately favoured the breeding of malarial mosquitoes, and the worst epidemic since the introduction of DDT in 1945, occurred in the eastern Transvaal. The outbreak was confined to the districts east of the Drakensberg, but several thousand cases were notified and their occurrence has shaken us out of our complacency and stimulated a renewed interest in malaria and its manifestations.

PATHOGENESIS OF MALARIA

It may be recalled that 4 species of malarial parasite infect man. These 4 species of *Plasmodium* are: *P. falciparum,* (Welch, 1897), the parasite causing malignant tertian malaria; *P. vivax,* (Grassi and Feletti, 1890), the parasite causing benign tertian malaria; *P. malariae,* (Laveran, 1881), the parasite causing quartan malaria; and *P. ovale,* (Stephens, 1922), the parasite causing ovale malaria. Other species have been described but have not yet gained general recognition.

The life-cycles of these parasites resemble one another in that there are 2 cycles of development. The exogenous sexual cycle of sporogony takes place in the anopheline mosquitoes and the endogenous asexual stage of schizogony in man. Man usually acquires malaria from the bite of an infected anopheline mosquito.

Infection may also be transmitted by the transfusion of blood from an infected donor. This possibility must always be borne in mind when caring for a patient whose condition requires blood transfusion. The infection may also be acquired by a fetus from an infected mother. Theoretically it is possible for blood-sucking flies to act as mechanical transmitters, but this rarely happens.

Almost all human infections are acquired through the bites of mosquitoes. The mosquito stage infective to man is the sporozoite, which is injected, while the mosquito is feeding, in the saliva from the salivary glands. It is possible that the first development of the parasite occurs in the reticulo-endothelial cells, but this has not yet been identified in man. The first stage to be recognised in human malaria is the exo-erythrocytic hepatic stage found in the parenchymal, not reticulo-endothelial, cells of the liver. After development in the hepatocytes to a schizont, the merozoites are discharged into the blood stream to infect the red blood cells. In the red cell a vacuole appears in the centre of the cytoplasm of the parasite, pushing the chromatin to one side, giving the appearance of a ring. This ring form is the youngest stage of the trophozoite usually seen. By feeding, the trophozoite enlarges and may assume an irregular shape now known as the amoeboid form. At this stage pigment granules are to be seen. With further enlargement and division of the chromatin into two, four, eight or more masses, and subsequent division of the cytoplasm around them, a mature schizont is formed, consisting of a variable number of merozoites to some extent depending on the species of the parasite. In the course of their growth the trophozoites bring about changes in the red cell, which are of diagnostic value in differentiating the species of parasite.

After schizogony has been repeated a number of times, gametocytes appear in the peripheral blood. Their further development takes place only in an anopheline mosquito. The time taken for the completion of the cycle in the mosquito varies considerably with temperature. The optimum temperature is 24°C - 26°C, when the development may be completed in a fortnight. No development of *P. falciparum* takes place below 15°C, the parasite does not die but growth is inhibited until the temperature again rises above the critical point.

To understand the clinical symptoms of malaria it is necessary to have a clear picture of the pathogenesis of the disease. Most of the parasites in a patient in the acute stage of the infection undergo their evolution simultaneously, forming a generation. The time for this development varies in the different species, accounting for the

different periodicity of the fever attacks— 36 to 48 hours in malignant tertian malaria, 48 hours in benign tertian and ovale malaria, and 72 hours in quartan malaria.

The destruction of the red cells by the growth of the parasites results in:

1. Anaemia.
2. Malarial pigment, a form of haematin, which is phagocytosed by leucocytes, and especially by the cells of the reticulo-endothelial system, resulting in the characteristic pigmentation of the skin in patients with chronic malaria, and best seen at postmortem examination in the leaden hue of the brain.
3. Bilirubin, whose excessive production results in hyperbilirubinaemia, is responsible for the jaundice often seen clinically. It may be confirmed by finding a raised icteric index of the serum, which, because the jaundice is haemolytic in origin in uncomplicated cases, also gives an indirect reaction in the Van den Bergh test. The amount of urobilin and urobilinogen excreted in the urine is increased.
4. Reticulocytosis, indicating an increased regeneration of red blood cells, shown also by an increase in the proportion of polychromatic cells and occasionally by the presence of nucleated red cells, usually normoblasts.
5. Hypertrophy of the reticulo-endothelial system to cope with its increased work is best noted clinically in the enlargement of the spleen. In the early stages it is relatively soft and cellular. Later, due to fibrosis, it becomes firm. The liver may also be considerably enlarged.

The infected red cells, especially in infections with *P. falciparum*, become sticky, stick to one another and to the vascular endothelium, often producing a condition analogous to thrombosis or embolism, causing blockage of the capillaries. It is the blockage of the capillaries and resultant interference with the blood supply and function of vital organs, that causes the pernicious manifestations of malignant tertian malaria. The clinical picture depends on the organ which is most affected and bears the brunt of the infection: brain — cerebral malaria; liver — bilious remittent fever; suprarenal — algid malaria; gastro-intestinal tract — gastric, choleraic and dysenteric forms; lungs — malarial pneumonitis, which is relatively rare, but bacterial pneumonia may complicate an attack of malaria; bone marrow—platelets and clotting mechanisms—haemorrhagic forms often associated with disseminated intravascular coagulopathy; kidneys — uraemic form. Many cases of pernicious malaria, especially the cerebral form, show temporary improvement after treatment, only to relapse after the development of oliguria and anuria into a state of coma associated with the signs and symptoms of uraemia.

DIAGNOSIS OF MALARIA

Clinically malaria is suggested by the following signs: fever with periodicity of attacks; jaundice; splenomegaly; anaemia.

In regard to these signs it should be noted that the typical periodicity of malaria does not manifest itself until after the first week of fever. During the first week the temperature may be intermittent, showing a daily rise, or it may be remittent, with a fall between peaks, or it may be nearly continuous, especially in malignant tertian malaria.

Three stages in the evolution of a typical attack are recognised, namely cold or shivering stage; hot stage; sweating stage. The cold stage is pronounced in benign tertian and quartan malaria, but is not prominent in malignant tertian malaria. It begins with headache, joint and muscle pains, backache and lassitude followed by chilly sensations which merge into the fully developed rigor when the patient shivers violently, his teeth chatter and he looks cold with a blue pinched face and 'goose-flesh' skin. He covers himself with blankets complaining he cannot get warm. This stage lasts from 1 to 2 hours and, although the patient complains of feeling cold, his temperature is rising.

As the chill passes the hot stage begins, the patient flushes, feels warm, discards his blankets, and suffers from severe headache, nausea and vomiting. The skin is hot and dry, the temperature reaches its peak, and the patient feels very ill until the third or sweating stage begins and brings relief. The patient perspires freely and is soon bathed in his own perspiration, but he feels much better.

The whole attack lasts 4 - 6 hours in quartan malaria and 8 - 12 in benign tertian malaria. In malignant tertian malaria the cold stage is masked and the patient may experience only a slight feeling of chilliness, the hot stage is prolonged and the sweating stage is not marked. The whole attack may last as long as 36 hours, and frequently the fever does not intermit. However, a tendency to a tertian periodicity is often apparent in the temperature chart. As *P. falciparum* is responsible for over 95% of the acute attacks of malaria in the Transvaal region, it is clear that the classical textbook picture of an attack of malaria is not often seen in our hospitals. Instead the signs and symptoms may suggest typhoid fever, infectious hepatitis or meningitis. However, experienced physicians can usually recognise cases of malignant tertian malaria. Nevertheless, it cannot be too strongly emphasised that because malaria may manifest itself in atypical forms, and because many other illnesses may simulate an attack of malaria, the clinical diagnosis should always be confirmed by blood examination. Further, in all cases of fever for which no cause is apparent, malaria should be excluded.

To establish the diagnosis of malaria, both thin and thick blood films should be taken, the sooner in relation to the beginning of the attack the better, and, after staining, examined for parasites. Thin smears stained by Giemsa, Leishman or Wright stains, are of value in determining the species of parasite, but if parasites are scanty, more time should be taken for their examination. Thick smears, although reducing the time taken for their examination, may not show the characters of specific differential value clearly.

The first essential in the diagnosis of malaria is *always* to think of it. If this possibility is borne in mind and the appropriate blood examination is carried out, mistakes will rarely be made. It is because this possibility is forgotten that we still see so many fatal cases of pernicious malaria, especially in our large cities like Johannesburg and Cape Town in areas free of malaria. Most of these patients have

contracted their infection while on holiday in areas where malaria is endemic. In untreated cases of cerebral malaria and of the other pernicious forms, peripheral blood smears almost always show very heavy infections and the parasites can be detected with ease in suitably-stained blood films. However, in ordinary cases a single negative finding does not exclude malaria, especially if the film is taken later than 6 hours after the beginning of the attack, for in malignant tertian malaria, the parasites tend to disappear from the peripheral circulation after this period. If antimalarial drugs have been administered before taking the film, little reliance in excluding malaria can be placed on a negative finding, and repeated films should be examined. However, in most patients suffering from an acute attack of malaria, the parasites are readily found in blood films taken as soon after the onset as possible.

The determination of the species of *Plasmodium* is often of considerable importance in deciding treatment and in giving a prognosis. The morphology of the various species of *Plasmodia* has been described in detail. The features of especial value in differentiating the species are summarised in Table I.

The finding of parasites in the direct examination of blood films is obviously the method of choice in the diagnosis of malaria. On occasion there may be difficulty in distinguishing platelets overlying red cells from malaria parasites, and more rarely extraneous objects overlying red cells may cause confusion. It is a good rule that when there is doubt, the object in question is probably not a malarial parasite. As a rule the parasite has such typical morphological features and staining characteristics that there is certainty about its identity.

There is often doubt and considerable dispute both among experienced as well as inexperienced malariologists about the species of parasite. Over 95% of acute cases of malaria occurring in the Transvaal are due to *P. falciparum* and there is rarely any question about their identification. However, sporadic cases and relapse cases seen in this region are often caused by parasites of other species and their identification often presents difficulty and often needs careful examination and assessment of the characteristics of all the parasites and their containing red cells that can be found. The picture may also be confused by double infections.

In this regard we now have considerable doubt about many of the parasites identified as *P. vivax* in the past. Before World War II these were labelled as *P. vivax* with confidence. However, it was noted that the parasites rarely clearly showed the distinctive features of the textbook pictures of this parasite and the infected red cells. It was cheerfully accepted that this lack of clarity was due to our poor technique and the inferior quality of our stains. During World War II we saw many cases of *P. vivax* malaria in soldiers and airmen who had contracted their infections in the Middle East. The blood films from these patients stained with the same stains and methods, showed clearly, indeed beautifully, and convincingly, all the distinctive characteristics of *P. vivax* as illustrated in textbooks of tropical medicine. Since that time we have been in doubt about the identity of many of the parasites called *P. vivax* in this region.

In 1971 Dr R. Harwin, describing 12 cases of *P. ovale* infection seen in Rhodesia, noted that it is still not widely realised that *P. vivax* is a rare parasite in Africa, but that

TABLE I. FEATURES OF *PLASMODIUM* SPECIES

	P. falciparum	P. vivax	P. malariae	P. ovale
Trophozoite — stages in peripheral circulation	Rings only.	All stages.	All stages.	All stages.
Ring stage	Small hair-like rings, often with double or rod-shaped chromatin dots. Multiple infection of single cell and marginal forms common.	Relatively large rings, actively amoeboid. Multiple infection of single cell rare.	Largest ring; regular outline.	Resembles P. malariae.
Amoeboid stage	Absent, unless infection very heavy.	Irregular amoeboid forms.	Compact band forms characteristic.	Compact, tendency to oval form.
Schizont stage	Absent. If present smaller than normal red cell.	Larger than normal red cell.	Smaller than normal red cell.	Smaller than normal red cell.
Rosette or mature schizont contains:	6 - 30 merozoites.	14 - 24 merozoites.	6 - 12 merozoites.	6 - 10 merozoites.
Gametocyte stage	Crescent shape.	Round or ovoid.	Round or ovoid.	Round or ovoid.
Pigment	Coarse black granules.	Fine yellow-brown granules.	Coarse blackish brown.	Blackish.
Infected red cell	Not enlarged. Often crenated. Often 'brassy'. Shows Maurer's dots.	Enlarged, pale, shows Schüffner's dots.	No change in size on staining.	Oval (rugby football) not enlarged, pale with fimbriated margin. Shows Schüffner's dots.

P. ovale is widespread if uncommon in the Zambesi Valley, and probably occurs along the whole length of the river. Whether it is also widespread in South Africa remains to be determined. However, the last 4 films from cases of malaria not due to *P. falciparum* all showed parasites with features characteristic of *P. ovale*. It is an interesting question which should soon be answered.

SEROLOGICAL TESTS

In some cases of chronic malaria, parasites may be difficult to find. They are particularly difficult to find in cases of 'big spleen disease', presenting with marked splenomegaly, anaemia, leucopenia and thrombocytopenia, and in cases of malaria, nephritis or nephrosis. Both these conditions are most commonly associated with chronic *P. malariae* infections, which may persist for many years. The need for a reliable serological test for confirmation of the diagnosis of malaria especially in chronic cases, has been felt for a long time. At last it seems to have been met by the development of an immunofluorescent antibody test. In this test blood films containing infected red cells are exposed to the patient's serum. If it contains malaria antibody, this adheres to the infected red cell and can be shown clearly by adding antihuman antibody linked to fluorescein, which is a bright apple-green colour when examined under ultraviolet light in a fluorescent microscope.

In addition to this direct confirmation of the diagnosis of malaria, there are a number of other laboratory findings of value in assessing the condition of the patient. The full blood count shows: anaemia usually with evidence of regeneration; normal total leucocyte count or slight leucocytosis during the attack of fever; and leucopenia with a characteristic monocytosis during the apyrexial periods. Malarial pigment may be found in some mononuclear or neutrophil leucocytes. The finding of pigmented leucocytes even in the absence of parasites is highly suggestive of malaria.

The blood serum shows a raised bilirubin content and gives an indirect positive reaction in the Van den Bergh test, except in cases of the pernicious form known as 'bilious remittent fever'. In these, in addition to increased haemolysis, there is interference with the circulation of the liver, resulting in damage to the parenchymal cells, and often areas of focal necrosis. In such cases the serum gives a biphasic reaction in the Van den Bergh test.

The urine usually shows a trace of albumin, the urobilin content is increased and often the urine is more concentrated than normal, accounting for the dark colour of the urine passed during an attack. In chronic cases with malarial nephritis or malarial nephrosis, large amounts of albumin may be found in the urine.

The bone marrow obtained by aspiration or biopsy from the sternum or iliac crest may show malarial parasites or pigment, especially in chronic cases in which parasites are scanty in the peripheral blood.

Parasites may also be found in such cases in films made from the splenic juice obtained by splenic puncture. However, this operation is not without danger and should not be done.

BLACK-WATER FEVER AND OTHER HAEMOLYTIC CONDITIONS

Black-water fever is an acute illness intimately associated with malaria, most commonly malignant tertian malaria, and in most cases is apparently precipitated by the administration of quinine for an acute attack of fever. It is due to a massive intravascular haemolysis and is clinically characterised by a sudden onset, rigors, fever and backache followed by haemoglobinuria, jaundice and anaemia. This condition is now rarely seen, and its virtual disappearance followed the substitution of synthetic antimalarial drugs for quinine, both for prophylaxis and treatment during World War II.

Previous to this, cases of black-water fever were uncommon in the indigenous population but were common in recent arrivals in hyperendemic malarious areas. Those particularly liable to an attack were individuals from non-malarious areas, who had lived in a hyperendemic area for 6 months to 1 year or longer, during which time they had suffered from several acute attacks of malaria which had been inadequately treated with quinine, and who, to prevent attacks, had taken quinine irregularly. It seems that the development of a state of hypersensitivity to quinine and associated with chronic malaria, was responsible for most attacks.

The massive intravascular haemolysis resulted in the liberation of haemoglobin in the blood stream, producing haemoglobinaemia and methaemoglobinaemia detectable by biochemical tests, and in turn haemoglobinuria which, associated with red cell debris and a state of shock, resulted in kidney blockage leading to oliguria, anuria and uraemia. The death rate was about 25%, and death was most often caused by kidney failure and occasionally by anaemia and cardiac failure.

Before World War II, black-water fever was one of the most frequent causes of death in young White adults in the northern Transvaal, Rhodesia and Malawi. Dr Annecke, on one of our visits to him, pointed out a fine house overlooking the Letsitele River. Its five previous owners had each in turn died of black-water fever. At present it overlooks river banks and hillsides clothed in orchards of citrus and other tropical fruit trees and black-water fever is no longer heard of, a visible testimony to the success of the antimalaria campaign.

During World War II, it became apparent that in addition to classical black-water fever affecting patients of European descent and associated with attacks of malaria treated with quinine, there was another form of intravascular haemolysis occurring commonly in Blacks taking synthetic antimalarial drugs and especially plasmoquine. Later it also became apparent that this intravascular haemolysis was also precipitated by primaquine and a number of other drugs. This drug-induced haemolysis was shown to be associated with a genetic inherent enzyme defect of the patient's red cells known as glucose-6-phosphate dehydrogenase deficiency. Incidentally, individuals with this deficiency are less liable to severe attacks of malignant tertian malaria than are individuals with normal red cells. It may be recalled that the people with the sickle cell trait are also less susceptible to malignant tertian malaria than people

with normal haemoglobin. It is surmised that both this enzyme deficiency and the sickle cell trait have survival value in regions where malignant tertian malaria is endemic. The proportion of the population with these two characteristics is high in West and Central Africa, but the sickle cell trait is not found in the Black people of South Africa, and glucose-6-phosphate dehydrogenase deficiency is relatively uncommon. These and other genetically inherited characters of red cells and other blood constituents are at present being intensively investigated, and the results have provided clear clues to the relationships and migrations of the people of Africa. However, this is a subject entitled to a full discussion on its own merits and will not be considered further at present.