STUDIES IN MALARIA CHEMOTHERAPY.
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A THESIS SUBMITTED FOR THE

DEGREE OF DOCTOR OF MEDICINE OF

EDINBURGH UNIVERSITY

BY

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EDINBURGH,

APRIL 1946.

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pages.

ACKNOWLEDGEMENTS.

GENERAL INTRODUCTION.

This thesis is an account of the study of some aspects of experimental malaria therapy carried out in an Army Research Team during the period July 1944 to January 1946.   It covers investigations on the response to treatment of malaria cases seen under Field conditions in North Africa and Italy and deals with a continuation of similar work on anti-malarial compounds conducted at a Military Hospital in England. Until the cessation of hostilities many of the findings in this work were restricted from publication owing to their bearing on military operations, a restriction for which there is now happily no need, so that expressions of opinion on the relative merits of different drugs may be freely given and supported by factual evidence hitherto not possible to disclose.

A very great deal of research on the problem of malaria has been done in the last few years prompted by the need for the United Nations to wage war in malarious countries at a time when the main World supply of Quinine was in enemy hands.   This work, which received very high priority and official backing, was/

was carefully directed and co-ordinated in Britain
and America by combined Committees of civilian and
Service Authorities who arranged for an organised
approach to the problems presenting and for a mutual
sharing of facilities for study as well as results.
For reasons of security, little mention of this work
has appeared in recent medical literature, but there
can be few diseases which have been the subject of
more organised study than malaria and few organisms
that can have proved more wily and resistant antagon-
ists than the causal Plasmodia.   Scientists in
university laboratories, synthetic chemists in the
great drug manufacturing concerns, therapeutic trial
teams in far-flung theatres of war and field control
teams draining swamps and spraying D.D.T. from air-
craft over jungles; all have played a part and made
their contribution to the story of the united front
against malaria and to the successes that have been
achieved.   It is indeed a solemn thought for reflect-
ion that nothing less than a world-wide conflict was
required for civilisation to support, with more than
the customary financial pittance, medical research
into the prevention and treatment of the disease which
has been for long years the greatest single scourge
of the human race.

As an introduction to this thesis it will be
fitting to outline for a few pages some of the more
general/

general considerations of the way in which malaria required to be overcome in the course of the recent World War. It is hoped, by sketching the main features of the concerted attack on the disease, to place in its true perspective the part played by the work on therapeutic research which forms the subject matter of this thesis, and which will be seen to form one of many contributions to the effecting of the broad, organised plan. It will also serve to convey an idea of the encouraging progress that can result from international co-operation in medical research when applied to the relief of the commonest afflictions of mankind.

The war in the Far East and in the Mediterranean presented three main problems in connection with malaria on the solution of which the successful conduct of military operations in these theatres was largely dependent. The first of these concerned campaigns directed against the insect vector of malaria with anti-mosquito measures carried out on a scale never before attempted and covering vast areas of tropical and sub-tropical countries abounding with malaria carrying Anophelines. Through such areas large armies had to move, fight and be constantly supplied, and Malaria Control Units worked wonders in reducing the teeming mosquito population and showed how an entomologist with a bulldozer could achieve in/

in a few days results undreamt of before the powers
of modern warfare became directly interested in his
efforts. In this battle against the mosquito in its
natural habitat, chemical research produced insectici-
des and repellents of ever increasing potency, and
culminating in the introduction of D.D.T., soon recog-
nised as an agent of unparalleled effectiveness to
be used with dramatic success. The full story of
D.D.T. in the War, of its use to stamp out a typhus
epidemic in liberated Naples, and of it being fired
in mortar shells to clear mosquitoes from the path of
advancing troops in the jungles of Burma, will make
fascinating reading when written and will prove
another outstanding example of the possibilities of
co-ordinated research when the need for results is
pressing and the financial support forthcoming.

The second main problem was how to effect the
optimum personal protection against malaria for the
great numbers of men who had to keep moving through
mosquito-ridden terrain in advance of the Control
Units. These, the actual fighting men, had neither
the chance or time to carry out the anti-mosquito
measures such as could be enforced in the rear areas,
and practical experience very soon taught that in the
rapid entry or exit from a slit-trench, entanglement
with a mosquito net was the last thing desired.
Protection for these vital operational troops became
thus/

thus a question of prophylaxis by drugs and with over
ninety per cent of the World's Quinine supplies in
Japanese hands, reliance in this matter had to rest
on the employment of the synthetic anti-malarial com-
pounds.   Two such preparations were in use before the
War, both manufactured by a German firm, and their
effects in the treatment of active malaria infections
had been studied by workers in many parts of the World.
The fact that they did not enjoy a wider pre-War
popularity and their therapeutic actions had not been
more closely investigated, was due perhaps to their
formulae being among the more closely guarded secrets
of the great German chemical industry, then being
geared up for War, and also to the reason that tropical
clinicians could easily get ample quantities of Quinine,
an anti-malarial of proved value, enjoying a reputation
founded on some centuries of use in malaria treatment.

Of the two synthetic preparations, Atebrin,
later manufactured under the British name Mepacrine
was known to be effective in killing the asexual
parasites of Malignant and Benign Tertian malaria,
being of especial value in treating the former infect-
ion as it greatly reduced both the incidence of sub-
sequent relapses and of the dreaded complication,
Blackwater Fever.   It was known that it could be
used if necessary as a substitute for Quinine in the
routine therapy of active clinical malaria and as
such/

such was valuable in patients to whom the adminstrat-
ion of Quinine was contra-indicated.   It was realised
that neither Atebrin or Quinine were very effective
against the asexual forms of Quartan malaria, or in
ridding the blood-stream of the gametocytes of any
of the common Plasmodial infections.   Comparatively
little use on a wide scale, had been made, before the
War, of Atebrin for drug-prophylaxis against malaria.
Those who needed such protection had usually been
given Quinine and there was a not unnatural hesitancy
to employ, for this purpose, the prolonged adminstrat-
ion of a new synthetic compound of which it was felt
the remote pharmacological and possible toxic effects
had not been adequately investigated.   It must be
said therefore that the first real trial of Atebrin
given as a prophylactic against malaria to thousands
of otherwise unprotected subjects, was forced upon
the Armies of the United Nations by the Japanese
advances of 1942.

The second of the German synthetic anti-malarials
was Plasmoquin, later made by British firms as Pamaquin.
This drug was known mainly for its toxicity in malaria
therapy and the whole attitude of many clinicians to-
wards its use was clouded with suspicion arising from
reports of its dangerous side-effects.   It had thus
gained little place in the routine treatment of malaria,
being/

being quite overshadowed by Quinine and Atebrin.
Work of outstanding value on the action of Plasmoquin
had been carried out in the Army in India by Sinton
in the early nineteen thirties and it is more than
interesting to see how after years of argument about
the merits of this drug, the great searchlight of
War-stimulated malaria research has finally swung
right round to become focussed on its properties and
possibilities.   Much more will be said of Pamaquin
later in this thesis, but at this stage it will suffice
to say that it was never seriously considered for use
in the drug prophylaxis of malaria, being too toxic
for prolonged administration and considered at that
time to act only in destroying the sexual forms of
parasites in human infections.

An enormous amount of work was done to determine
the optimum use of Mepacrine as a prophylactic against
malaria in the Armed Forces.   Research Units were set
up in Britain, America and Australia and the drug was
given in varying doses over long periods of time to
some thousands of Service and civilian volunteers.
Investigations to show the rate of its absorption and
excretion and the concentration of it attained in
body fluids and tissues, were exhaustively conducted
and the effects of taking Mapacrine under all manner
of conditions such as physical stress and variations
in climatic temperature, were also studied.   After
many/

many forms of dosage had been tried, it was eventually
decided that 0.1 gramme of Mepacrine taken daily pro-
vided a fairly sure measure of protection against the
development of an acute attack of malaria, and was a
dosage that could be given to active troops for an
indefinite time without danger of toxic effects.
Experimental evidence had shown, and practical exper-
ience very soon proved, that in dealing with Benign
Tertian and Quartan malaria, Mepacrine was only a
suppressive drug which held an infection in check
in a sub-clinical state for as long as dosage was
maintained.   It was definitely not a true causal
prophylactic in that it could not prevent the parasites
from establishing themselves in the body following
the bite of a malaria carrying mosquito. In such
infected subjects the clinical manifestations of the
disease became apparent after the cessation of
suppressive Mepacrine dosage, presumably when the
amount of drug in the circulation fell below the
suppressive level. In dealing with Malignant Tertian
malaria, however, Mepacrine was a much more potent
safeguard; for although it did not prevent the
parasites from gaining access to the blood-stream,
it was able to suppress them and to kill them off
with such effect that the development of frank clinical
malaria subsequent to the cessation of Mepacrine ther-
apy was very much less likely to occur than with the
other/

other types of infection. Here then was a non-toxic drug which could offer unique protection against the dreaded scourge of M.T. malaria and could hold off the clinical effects of the less serious nuisance infection of B.T. malaria for as long as a steady dosage was maintained.

The immense practical importance of Mepacrine in the conduct of the War became increasingly more obvious, for it was remembered only too well from the Gallipoli campaign of the 1914-1918 War, the havoc that could be wrought by malaria in a Field Force inadequately protected against infection in hyper-endemic areas. The whole higher planning of offensive campaigns by the United Nations in the Far Eastern and Mediterranean theatres was found in 1942 to 1943 to hinge largely on the availability of sufficient supplies of Mepacrine to provide protection for the troops engaged in operations during the malaria season. The campaign in Tunisia, Sicily and Southern Italy provided the first large scale trial of the efficiency of suppressive Mepacrine and showed conclus-ively that the success of such measures depended wholly on the strict observance of the daily Mepacrine rout-ine by all personnel. A widespread incidence of acute gastro-intestinal upset followed the taking of the first few tablets of Mepacrine in North Africa in April 1943 and did a great deal to shake the faith of/

of the troops in this new much-boosted anti-malarial,
so that many of them never took it regularly there-
after.   The exact cause of this unfortunate occurr-
ence was never fully discovered but was probably
associated with the fact that the dosage first tried
was 0.2 gramme on three alternate days per week, a
routine which was at once changed to 0.1 gramme
daily.   In Sicily where malaria casualties out-number-
ed the battle casualties in the ratio of 7 to 4, most
cases of infection occurred amongst troops of the
Eighth Army, tough, hard-boiled, desert warriors who
rather scorned the new-fangled First Army idea of
being ordered to take tablets against malaria and who
realised too late that the steamy fever-ridden swamps
of the Catania plain were a very different proposition
from the hot, sandy wastes through which they had
battled previously. There was evidence indeed that
the Germans were well aware of the value of exposing the
advancing Allies to malaria in their defensive retreat
through Sicily and Italy; for apart from the purely
strategic advantages employed in defending high ground
overlooking a plain, they lost no opportunities to
encourage malaria amongst their enemy by systematic-
ally destroying the anti-mosquito measures that had
been an important feature of Italian Public Health
policy through these areas.   The flooding of the
Pontine Marshes by the Germans in 1944 was a good
example/

example in this respect, for apart from the obvious
effects of hindering land operations, the resultant
encouragement to the mosquito population in this age-
old stronghold of malaria, would have presented a
serious problem to the maintenance of the troops in
the Anzio beachhead if the final assault on Rome had
not been successful before the malaria season was
far advanced.

Apart from the Mediterranean compaigns the value
of Mepacrine, for the prophylaxis and suppression of
malaria, was shown to be of paramount importance in
the conduct of the War against Japan, both in the
Pacific Islands and in the land offensive by the Four-
teenth Army.   In India and in West Africa, the great
training areas for the Far Eastern War, this drug was
used with outstanding success for the protection of
thousands of troops, and it is indeed in a large
measure due to Mepacrine that victory in the East
was achieved in a time far shorter than most of the
World would have dared to forecast less than one
year ago.

The third great problem in dealing with malaria
in war time was concerned with the treatment of cases
who contracted the infection and where clinical mani-
festations demanded admission as casualties to medical
units.   It soon became apparent that in spite of the
most strenuous measures for mosquito control and the
most/

most emphatic directives as to the vital need for strict Mepacrine discipline on the part of all personnel, conditions at times were such that considerable numbers of malaria casualties would occur and that the medical services had ever to be ready to cope with these on a large scale. It was found, for example, that malaria could manifest itself clinically in subjects who were taking regular suppressive Mepacrine, although the number of such cases was comparatively small. This matter was considered of sufficient importance however, to merit the attention of a special research unit which was sent to Italy to determine by estimating plasma Mepacrine levels, whether these men were really taking the drug and not absorbing it or whether, as was more often the case, the tablets had not been properly issued or had been conveniently lost before being swallowed.

There were two factors which influenced the outlook on the question of the routine treatment of malaria. In the first place it was remembered how urgent was the need to conserve manpower, especially amongst the operational troops most exposed to infection. This meant that medical units should employ a standard therapy course carefully devised to produce the optimum curative effect in the minimum time and thus enabling the patient to be fit to return to duty as soon as possible. Secondly there was need for the special/

special treatment of cases who had suffered from multiple recurrences of malaria with resultant diminution in their general health and resistance.   For these men, a slowly increasing number, who spent as much time in medical units as with their own units, some real attempt was demanded to eradicate the chronic malaria infection and to restore them to their former fitness.

It was obvious that these two aspects of treatment required much careful study before standard therapy courses could be laid down for routine administration.   That such courses needed to be standardised in any one theatre of war became an accepted fact in the Services where casualties had often to be evacuated from one medical unit to another after only short stays in transit, so that only by having a uniform therapy regime could the essential continuity of treatment be obtained.   Factors such as the main types of malaria infection likely to be encountered, the sensitivity of the local strains of parasite to the action of different anti-malarial drugs, and the availability of supplies of these drugs, had all to be studied in each theatre before the important general directives on treatment could be drawn up. This work demanded the attention of whole-time research teams whose function was to investigate the relative merits of various trial therapy courses, assessing them by the clinical response of the patients, the

detailed/

detailed effects on the parasitaemia and the actions of the drugs employed. Malaria therapeutic research teams were therefore formed in India, West Africa and in the Central Mediterranean to study and to advise on the particular aspects of malaria treatment in those theatres. Each team, while remaining a separate entity primarily responsible to the local administration of the theatre, was associated with the Malaria Committee of the Medical Research Council which arranged for the central co-ordination of resources and results.

Having thus outlined, in a general way, some of the broader problems presented by malaria in the present War, it is convenient to divide this thesis into four main parts. The first part consists of a report and review of some of the studies in experimental malaria therapy carried out by the Research Team formed and commanded by the author in North Africa and Italy. It deals mainly with the use of Mepacrine in Benign and Malignant Tertian infections and shows the investigations leading up to the design of a therapy course for routine use under field conditions. It deals also with the question of treatment in cases of chronic relapsing malaria. The second part reports work carried out in England on cases of malaria returning from the Far East and contains an account of the detailed/

detailed action of Pamaquin and the new anti-malarial
compound Paludrine. A third part deals with a study
of the staining of malaria parasites in thick blood
films and a description of a new staining method is
given. The fourth and final part consists of the
main conclusions which it would appear could be
justifiably drawn from the findings recorded. Each
of the four parts is divided into appropriate sections
where necessary.

PART ONE.

A REPORT OF WORK ON MALARIA THERAPEUTIC
RESEARCH CARRIED OUT IN A MALARIA RESEARCH
TEAM IN THE MEDITERRANEAN THEATRE FROM
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SECTION 1.

Introduction, Formation of Team and Programme of Work.

The Malaria Research Team in C.M.F. was formed in June 1944 by the Consulting Physician A.F.H.Q. to carry out a Malaria Therapeutic Trial along the lines suggested by the Therapy Sub-committee of Malaria Committee of the Medical Research Council.

The number of personnel available for this Team was at first Restricted by the man-power situation in C.M.F. to two British personnel, a Specialist Physician and a senior Laboratory Assistant, but a graded Infantryman was posted as a clerk during March 1945. Two Italian Co-operators, both with civilian and military experience of laboratory work, were attached to the Team and provided reliable and valuable assistance throughout this work.

The Team was designed for attachment to a General Hospital and work was begun at No.97 (Br.) General Hospital in Bone, Algeria, early in July 1944. In September 1944 the Team was moved to Rome and attached to NO. 104 (Br.) General Hospital where it remained thereafter/

thereafter. This change in location from North Africa
to Central Italy was made necessary by the scarcity of
Malaria cases in the Bone district, owing to the rapid
clearance of Service personnel from the area and also
to the effective Malaria prevention measures that were
carried out at Bone during the Summer of 1944. The
move implied disadvantages in that it broke the con-
tinuity of the work of the Team for the month of
September, and cases met with in the Rome area differed
from the North African cases in being almost exclusively
Benign Tertian infections.

A total of 566 cases of Malaria were studied, this
number being made up as follows:

1. Active B.T. infections (a) Fresh 144 cases.
                          (b) Relapse 277 cases.

2. Active M.T. infections (a) Fresh 26 cases.
                          (b) Relapse 2 cases.

3. Active Quartan infections Fresh 1 case.

4. Cases of Malaria having)
   had multiple recurrences,) (a) B.T. 112 cases.
   admitted for investiga-)
   tion while in a quiescent) (b) M.T. 4 cases.
   stage of the infection.)

It will be seen therefore, that this report deals
mainly with active B.T. infections contracted in Italy.
The results of treatment in the series of recurrent
B.T. cases, and the findings in the very small but
interesting group of North African M.T. infections, are
however considered worthy of mention and are also
included in this report.

SECTION 2.

METHODS EMPLOYED IN THE THERAPY INVESTIGATION.

1. General Clinical Procedure.

As far as possible, the Malaria cases for treatment
by the Research Team were admitted to one ward of the
General Hospital.   Changes in the nursing personnel
took place during the investigation, but precautions
were taken to see that Nursing Officers and orderlies
looking after the cases were fully aware of the nature
of the work being done, of the importance of taking
accurate clinical records, and the need for ensuring
that patients took the exact treatment prescribed.

Detailed histories were taken in each case and
particular attention was paid to each patient's frank
description of the suppressive measures he had taken.
Once the personal interest and co-operation of the
patient had been obtained, it was found that details
such as the average weekly suppressive Mepacrine dosage,
use of mosquito nets etc., were quite freely given.

The history included details of any previous
attacks of Malaria and the patient's full account of
the onset of the current attack. The question of any
recent antecedent illness which could have played a
part in precipitating the attack was enquired into and
recorded.   Only slide-positive, uncomplicated cases
of Malaria were accepted for the purposes of this
therapeutic/

therapeutic trial. A physical examination was made of
every patient and the presence or absence of splenic
pain or enlargement was noted·

New cases were seen on admission and brief clinical
details were taken. The course of therapy to be given
was then determined and cases were started on treatment
in strict rotation of courses and according to whether
they were Fresh or Relapse infections. A treatment
chart showing the drugs and exact dosage times was
made out for each patient and was attached to the
temperature chart at the bedside. Each dose was ad-
ministered by a Nursing Officer and the co-operation of
the patient was sought to ensure that the dosage regime
was carried out as specified. Patients who had been
treated on several occasions previously, with the
standard treatment in use in the theatre, became quite
interested when they found themselves on different
forms of therapy and when the purpose of the investi-
gation was explained to them.

As far as possible all therapy courses were started
at the same time of day. It became necessary half way
through the investigation to change this starting time
from morning to afternoon. This change was found to
facilitate the existing ward and laboratory arrangements
and it was felt that it in no way altered the interpre-
tation of results.

2./

2.   Laboratory Investigations.

Parasite counts. The original intention was to
record parasite counts done both in the morning and
evening of each day of parasitaemia. This was done on
the first 87 cases studied, but thereafter counts were
done once daily in view of there being only one person
to do the tedious work involved and also that it was
apparent that the rate of diminution of parasites was
such that adequate data could be obtained from counts
done at intervals of 24 hours.

Some time was spent in determining the best method
of counting the parasites in the thick blood films
taken before and during treatment. Several methods
were tried and the results compared. It was finally
decided to count the parasites seen in proportion to
the number of leucocytes in thick evenly-spread films
and to express the result in parasites per cubic milli-
metre after estimation of the white-blood-cell count.
It was found that by using films of more than average
thickness, an even distribution of white cells was
routinely obtained and with the staining method employed
there was little or no distortion of the leucocytes
which could be easily counted against the parasites on
a dehaemoglobinised background. In such thick films
the distribution of parasites is much more even than
in thin films where the parasitised cells tend to
collect mostly at the ends and edges. In order to
obtain/

obtain an even distribution in thick films it is im-
portant to prevent auto-agglutination of the red cells.
The latter may be avoided by drying the freshly taken
films in a warm oven or incubator.

The staining method employed was a modification of
Field's stain with a dilute Leishman counterstain.
This method was elaborated after an investigation of
the staining of malaria parasites in thick blood films
and these experiments are fully described in Part Three
of this thesis.   The main advantage of the stain in
this work was that by dehaemoglobinising very thick
blood films, it was possible to count parasites as
they diminished during treatment, even though they
were present in very small numbers.

Among other methods of counting parasites that
were used to determine the accuracy of the above
technique, was a modification of Sinton's chicken
blood method which may be mentioned here.   Sinton
recommended that the parasitised blood be mixed with
a known suspension of the nucleated chicken red blood
corpuscles, against which the asexual parasites and
gametocytes could be counted.   This method is best
suited for work under field conditions, enabling one
worker to collect a great many prepared blood slides
for counting at a later time.   Apart from the dif-
ficulties of obtaining supplies of chicken blood, the
leucocyte/

leucocyte method was preferred as repeated white cell counts were readily available under laboratory conditions. It was found that Sinton's method could be carried out using a known suspension of fixed and stained human red corpuscles prepared in the following manner. Red blood cells from oxalated blood were washed with normal saline and then transferred to Methyl Alcohol containing Methyl Blue, which both fixed and stained them a dark blue. As this dye is insoluble in watery solutions, it was possible to continue washing the stained cells in saline until all clumps had been eliminated and then to prepare a known dilution of a suspension of the blue cells for admixture with the parasited blood.

The stage of the parasite asexual cycle at which treatment was started, was noted in every case and therefore the daily parasite count recorded the numbers per cubic millimetre of both asexual forms and gametocytes. On an average, about 300 leucocytes were counted for each parasite count and the routine thick blood films employed were such that about 10 to 20 leucocytes were present in each oil immersion field. It will be described in a later section of this report how it was found of great interest to record the various stages of the parasite asexual cycle seen at intervals during treatment, thus making a qualitative or differential parasite count as well as an actual quantitative enumeration.

White/

White blood cell counts were done on all patients
before, during and at the end of treatment courses, so
that details of the leucocytic reaction in the active
Malaria infections studied are available in the general
results. Haemoglobin estimations were made in every
case at the start and finish of therapy, although the
initial level was sometimes unduly high owing to the
state of dehydration that was present in pyrexial cases
on admission during hot weather. Red blood cell counts
were done at the same times as the Haemoglobin estima-
tions in the first 87 cases, but were subsequently
discontinued owing to shortage of laboratory personnel.

3. Follow-up of cases.

An effort was made to secure a follow-up of cases
over an adequate time as it was felt that in this way,
more than in any other, was it possible to arrive at a
true assessment of the value of the various treatment
courses on trial.

On discharge from hospital each patient was given
a form which he was asked to retain and to produce it
to a Medical Officer if he should be admitted to a
medical unit with a relapse of Malaria. The form asked
that if the relapse occurred in the area where the
Research Team was working, the case should be readmitted
to them for treatment and where the patient was seen
and treated elsewhere, it was asked that details of
the recurrence should be recorded and the form returned.
The/

The co-operation of each patient was sought in ensuring this follow-up.

It was considered that many of the above follow-up forms would be lost or go astray and accordingly a further form was posted to each patient 6 months from the end of his treatment. The patient was asked to supply details of any subsequent attacks during this period and to take the completed form to his Medical Officer or Orderly Room for posting back to the Research Team through the Official Mail. This method of follow-up proved most successful and it was felt that the time-interval of 6 months was of sufficient length to be of some value in assessing the curative value of the treatment courses employed. These forms were returned by many patients who had in the meantime left the theatre, but when there were no replies, an effort was made to trace the men by addressing a further form to them through the Records Office of their particular branch of the Service. By such means it was possible to obtain finally 6 month follow-up details in 78% of the total number of cases treated.

4. The Therapy Courses.

The Therapy Courses used on the active cases of Malaria were those laid down by the M.R.C. Malaria Committee, with the addition in 1944 of the standard treatment course in use in C.M.F. and B.N.A.F. during that season. The courses, which were employed in rotation were as follows:-

A.   For Fresh infections:

Course 1   Quinine gr 10 T.I.D. for 3 days.
            Mepacrine 0.1 gm. T.I.D. for 5 days.
            Interval of 2 days (this was omitted in all
                                but 2 cases).
            Pamaquin 0.01 gm. T.I.D. for 3 days.

            Totals Quinine 90 gr. Mepacrine 1.5 gm.
                    Pamaquin 0.09 gm.

Course 2.   Mepacrine 0.2 gm. Q.I.D. for 2 days.
            Mepacrine 0.1 gm. T.I.D. for 5 days.

            Total   Mepacrine 3.1 gm.

Course 3.   Mepacrine 0.2 gm. T.I.D. for 1 day.
            Mepacrine 0.1 gm. T.I.D. for 6 days.

            Total   Mepacrine 2.4 gm.

Course 4.   Mepacrine 0.2 gm. T.I.D. for 1 day.
            Mepacrine 0.1 gm. T.I.D. for 6 days.
            Pamaquin 0.01 gm. T.I.D. for 3 days.

            Totals Mepacrine 2.4 gm. Pamaquin 0.09 gm.

Course 5.   (Standard C.M.F. course 1944).

            Quinine gr. 10 T.I.D. for 3 days.
            Mepacrine 0,2 gm. Q.I.D. for 2 days.
            Mepacrine 0.1 gm. T.I.D. for 3 days.

            Totals Quinine 90 gr. Mepacrine 2.5 gm.

B.   For Relapse infections.

Course R1 Mepacrine 0.2 gm. Q.I.D. for 2 days.
          Mepacrine 0.1 gm. T.I.D. for 10 days.

          Total   Mepacrine 4.6 gm.

Course R2 Quinine gr. 10 + Pamaquin 0.01 gm. T.I.D.
                                      for 10 days.
          Totals   Quinine 300 gr. Pamaquin 0.3 gm.

C.   For cases that relapsed after treatment with either
      Course R1 or R2,

Course R3   Quinine Gr 10 + Pamaquin 0.01 gm. T.I.D.
                                      for 14 days.
            Quinine gr 10 + Pamaquin 0.01 gm. B.I.D.
            with Pamaquin 0.01 gm. 1 dose for 7 days.

            Totals Quinine 560 gr. Pamaquin 0.63 gms.

SECTION 3.

RESULTS OBTAINED IN THERAPEUTIC TRIAL.

A. Numbers of Fresh Benign Tertian cases treated
on each course.

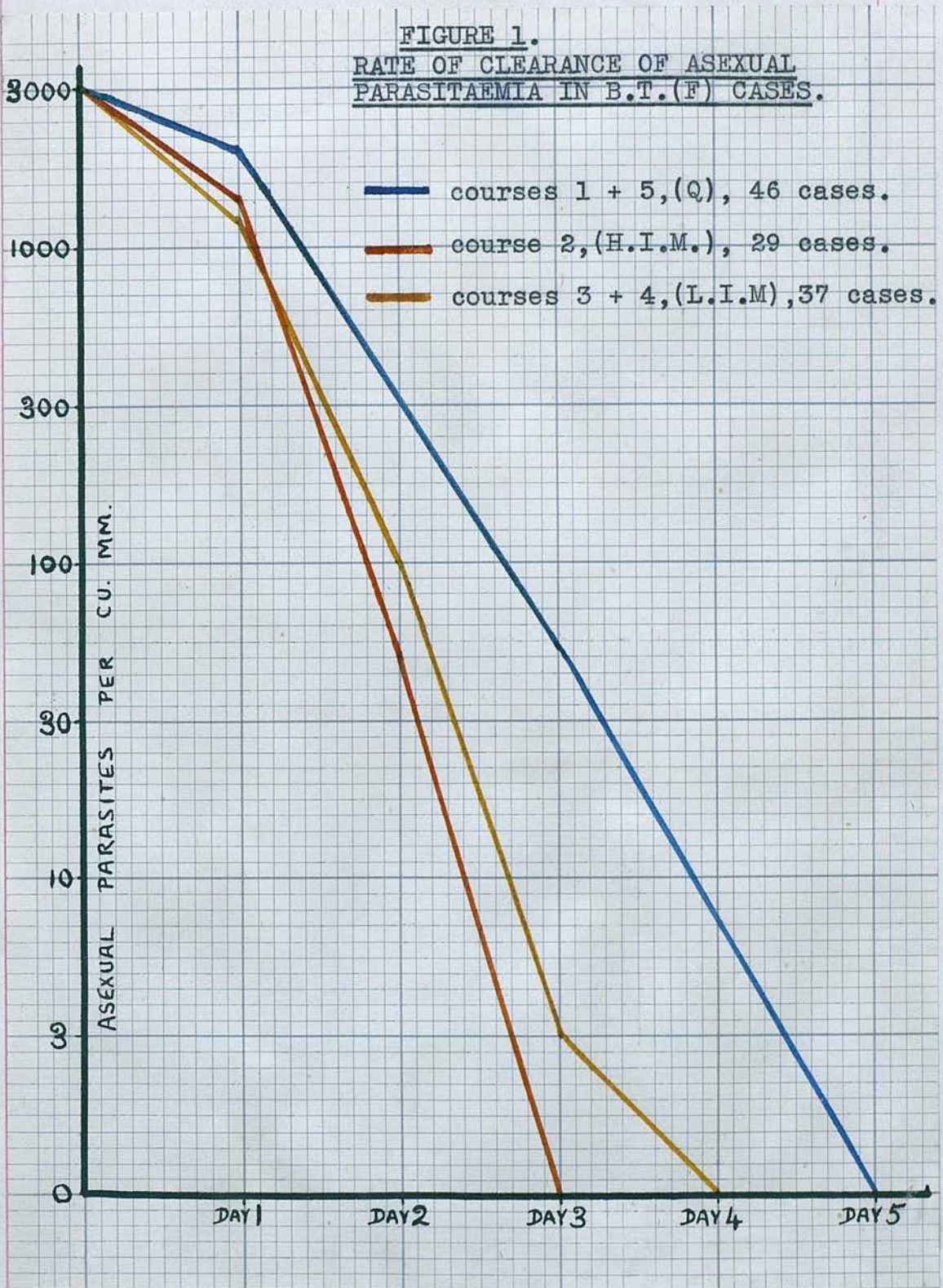|	
Course 1.	29 cases
Course 2.	30 cases
Course 3.	29 cases
Course 4.	29 cases
Course 5.	21 cases.

1. Rate of disappearance of Asexual Parasites.

Daily parasite counts enable the rate of fall-off
of asexual parasites to be studied on a mathematical
basis during treatment.

Figure 1 shows the asexual parasite count plotted
against the time in days while on therapy. In the
construction of this graph, some adjustment of the
initial and daily parasite figures was necessary to
start all the cases at the same average initial
parasite count. The differences thereby made to the
average daily counts were so slight that this purely
mathematical correction was felt justifiable in view
of the greatly increased ease with which the resultant
graphs could be compared.

Owing to the extremes of parasite count figures
involved, a logarithmic scale has been employed in
many of such graphs.

As Figure 1 shows only the average rate of parasite
disappearance on the different dosage regimes, the
following examples of actual parasite counts from cases

are/

FIGURE 1.(page 27)

FIGURE 1.
RATE OF CLEARANCE OF ASEXUAL
PARASITAEMIA IN B.T.(F) CASES.

courses 1 + 5,(Q), 46 cases.
course 2,(H.I.M.), 29 cases.
courses 3 + 4,(L.I.M),37 cases.

FIGURE 1.(page 27)

are quoted to show the variations that can occur around these averages.

Asexual parasites are expressed in numbers per cu.mm.

Course 1.- Quinine.

Case No.	Initial Asex.Count.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
102.	4000	Nil	Nil	Nil	Nil
317.	4200	330	110	Nil	Nil
441.	1100	1000	160	3 seen	Nil

Course 3.- Mepacrine - low initial dosage.

359.	4000	2500	Nil	Nil	Nil
171.	2300	1260	Nil	Nil	Nil
294.	1200	1300	220	2 seen	Nil

Course 2.- Mepacrine - high initial dosage.

103.	600	Nil	Nil	Nil	Nil
293.	2500	200	Nil	Nil	Nil
170.	3000	1600	2 seen	Nil	Nil

2. Duration of Asexual Parasitaemia.

Figure 2 shows the percentage number of cases who showed asexual parasites during the first few days of treatment and conveys better the finding that in the majority of cases the asexual parasites disappear from the peripheral blood in from 3 to 4 days from the commencement of treatment.

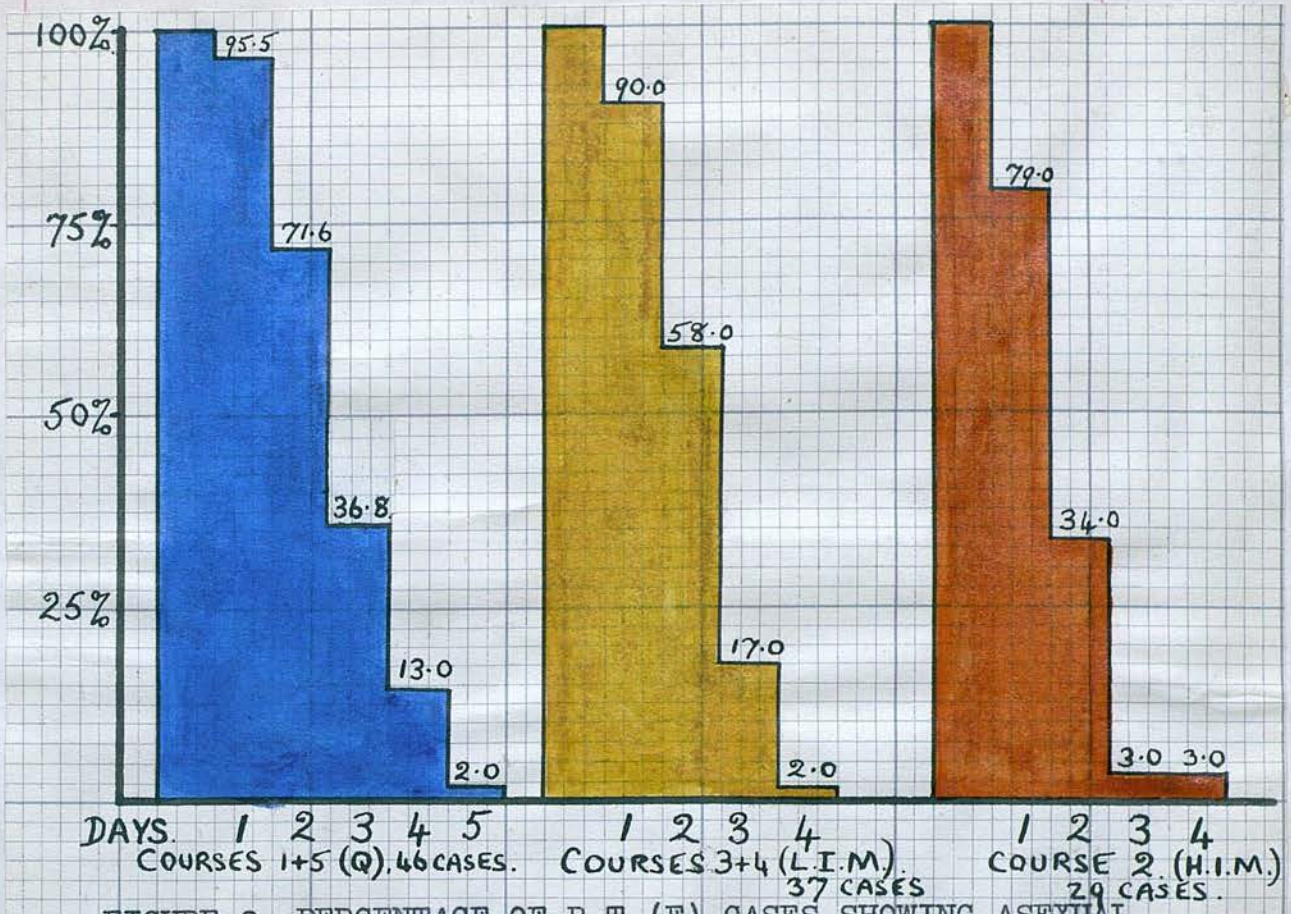Figure 3 shows this in the average duration of asexual parasitaemia for the same group of cases.

FIGURES 2,3,4. (pages 28,29).

FIGURE 2. PERCENTAGE OF B.T.(F) CASES SHOWING ASEXUAL
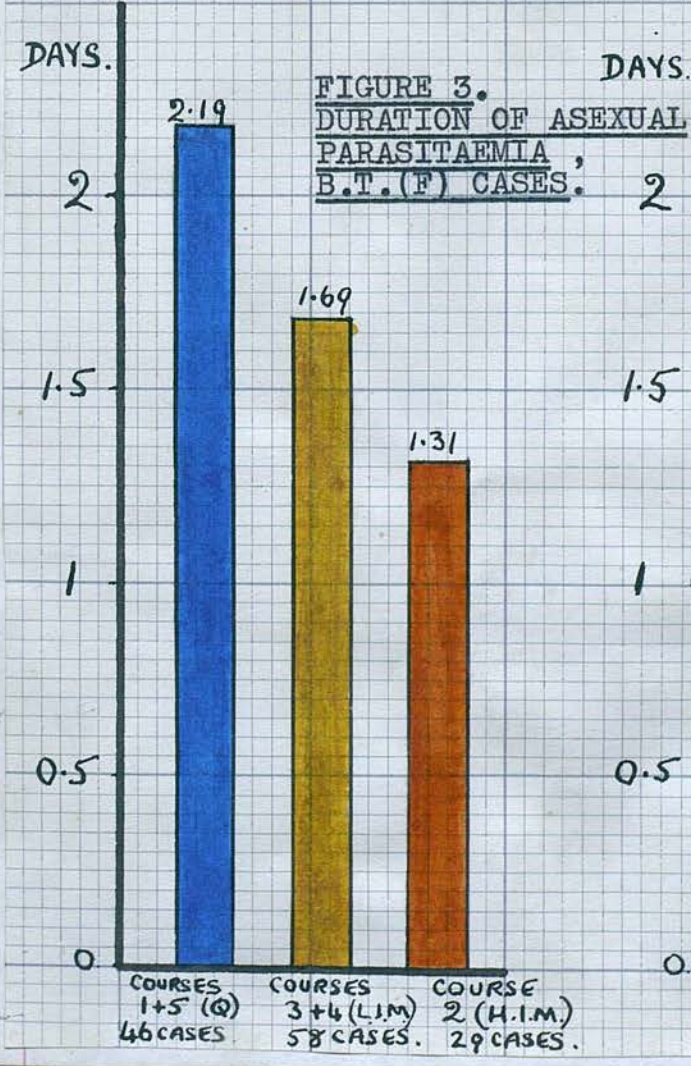PARASITES DURING FIRST FEW DAYS OF TREATMENT.

FIGURE 3.
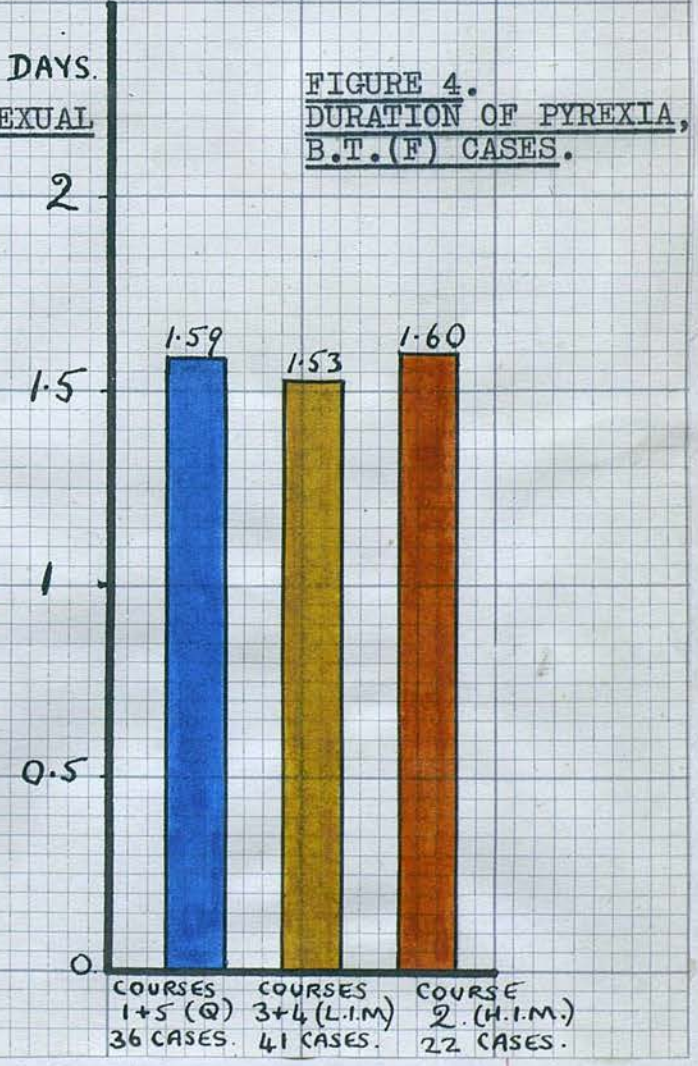DURATION OF ASEXUAL
PARASITAEMIA,
B.T.(F) CASES.

FIGURE 4.
DURATION OF PYREXIA,
B.T.(F) CASES.

3.   Duration of Pyrexia.

Figure 4 shows the average duration of pyrexia from the start of treatment in the Fresh B.T. infections treated on the therapy courses described. It will be seen that on the whole, the cases became afebrile after about 36 hours of treatment which was before the asexual parasites had disappeared from the peripheral blood.

4.   Rate of disappearance of Gametocytes.

Figure 5 shows the percentage number of cases who showed gametocytes during the first few days of treatment and Figure 6 shows the average time required for their disappearance on the various therapy courses

It will be seen that on the whole the gametocytes are very much slower in disappearing than the asexual forms and in many cases continue to appear in fresh showers after the asexual parasites have been cleared from the peripheral blood.

Individual counts — of which three are quoted below as examples — also show considerable variations from day to day. It was noticed that the large B.T. female gametocytes were more subject to daily variations, sometimes continuing to appear until late in therapy.

Examples/

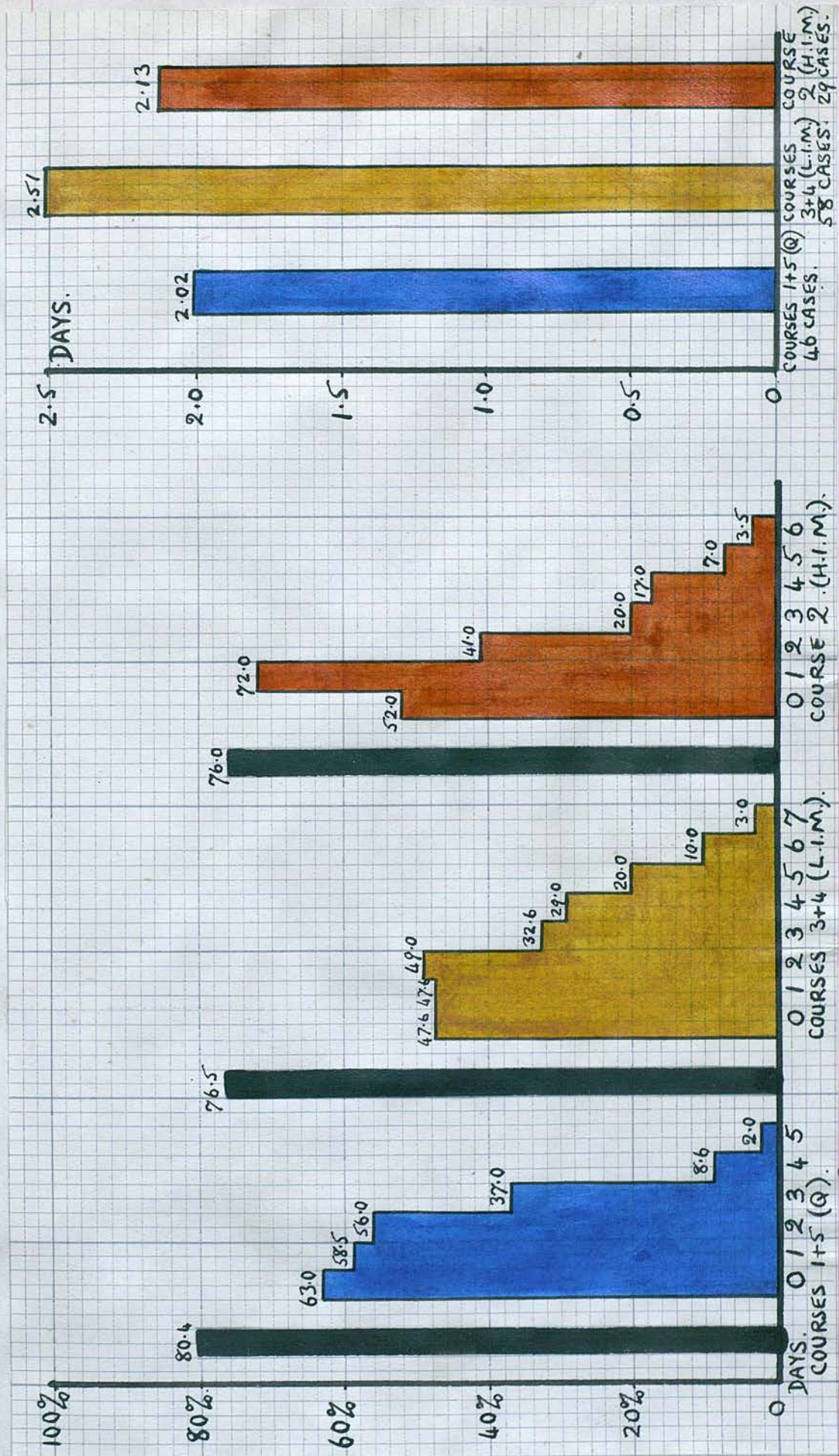FIGURE 6. AVERAGE DURATION OF GAMETOCYTES, B.T.(F) CASES.

FIGURE 5. PERCENTAGE NUMBER OF B.T.(F) CASES WITH GAMETOCYTES DURING TREATMENT. FIRST BLOCK SHOWS PERCENTAGE OF TOTAL CASES IN WHICH GAMETOCYTES APPEARED.

FIGURE 7.(page 30).

FIGURE 7. CLEARANCE RATE OF GAMETOCYTES IN B.T.(F) CASES.
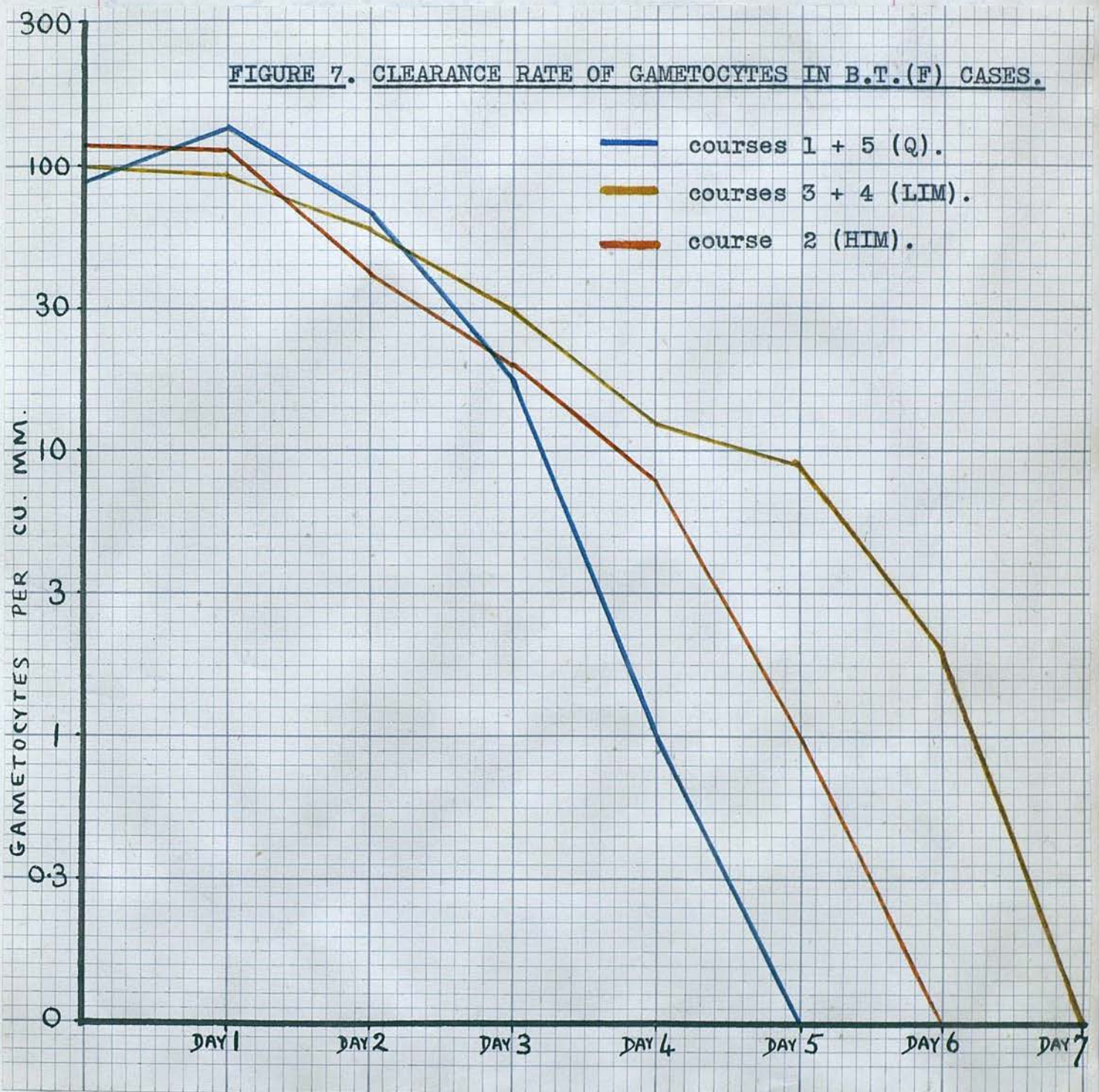
TABLE 1.   AVERAGE INITIAL AND FINAL WHITE BLOOD CELL
           COUNTS OF B.T. FRESH CASES TREATED ON
           COURSES 1 - 5.

Course and No. of cases.	Length of course-days.	W.B.C. per cu.mm. before treatment.	W.B.C.per cu.mm. at end of treatment.
1 (29) (Cases)	11.	6,100.	7,800.
2 (30) (Cases)	7.	5,400.	7,300.
3 (29) (Cases)	7.	4,800	6,000.
4 (29) (Cases)	10.	4,500	7,100.
5 (21) (Cases)	8.	5,550	8,000.

It will be seen that the average initial white
count on the 138 B.T. Fresh cases studied showed a
slight leucopenia, which had returned to a normal
average count at the end of each therapy course.
The intermediate white counts during treatment are not
shown, but it was found that the duration of the
initial leucopenia was rarely more than four days
irrespective of the treatment course employed.

Examination of over 30 sternal marrow smears from
these cases showed that there was a slight maturation
arrest at the late metamyelocyte and early non-segmented
polymorph stage of the granular leucocyte precursors.
This would account for the peripheral blood picture in
the stage of active parasitaemia.

6./

6.   Haemoglobin percentage before and after treatment.

Table 2 shows the average Haemoglobin percentages before and at the end of treatment in the 138 B.T. Fresh cases and shows also the length of the courses concerned.

TABLE 2.   AVERAGE INITIAL AND FINAL HAEMOGLOBIN PERCENTAGES OF B.T. FRESH CASES TREATED ON COURSES 1 - 5.

Course and No. of cases.	Length of course—days.	H.B.Percentage before treatment	H.B.Percentage at end of treatment.
1 (29) (Cases)	11.	97.	94.
2 (30) (Cases)	7.	97.	95.
3 (29) (Cases)	7.	100.	97.
4 (29) (Cases)	10.	100.	97.
5 (21) (Cases)	8.	99.	95.

Ferrous Sulphate gr.6 in tablet form was given T.I.D. to all cases as a routine on the fourth day of treatment and thereafter. Cases on short therapy courses of 7 or 8 days had thus only 3 or 4 days of Iron before the final Haemoglobin level was estimated whereas other cases on courses 4, R_2 and R_1 had 7 or 9 days of Iron.

It/

It will be seen that on the whole there is little significant change in the Haemoglobin levels between the beginning and end of treatment in the 138 Fresh cases studied.

Many of the cases in North Africa showed a fall in Haemoglobin during the first few days of treatment, coincident with their recovery from a state of dehydration and haemoconcentration present on admission during the very hot weather.

B.   Numbers of Relapse Benign Tertian Cases Treated.

$$Course \quad R_1 \quad - \quad 87 \text{ cases}$$
$$Course \quad R_2 \quad - \quad 94 \text{ cases}$$
$$Course \quad R_3 \quad - \quad 8 \text{ cases}$$

As Course R_2 and R_3 both start with the same dosage of Quinine plus Pamaquin for the first ten days, it is possible to consider cases treated on both courses as one group when studying the rate of disappearance of parasites and pyrexia, thus making 102 cases treated in this way.

1.   Rate of disappearance of Asexual Parasites.

Figure 8 shows the rate of disappearance of asexual parasites on courses R_1 and R_2 + R_3 by plotting the parasite counts done at twenty-four hour intervals from the start of treatment.
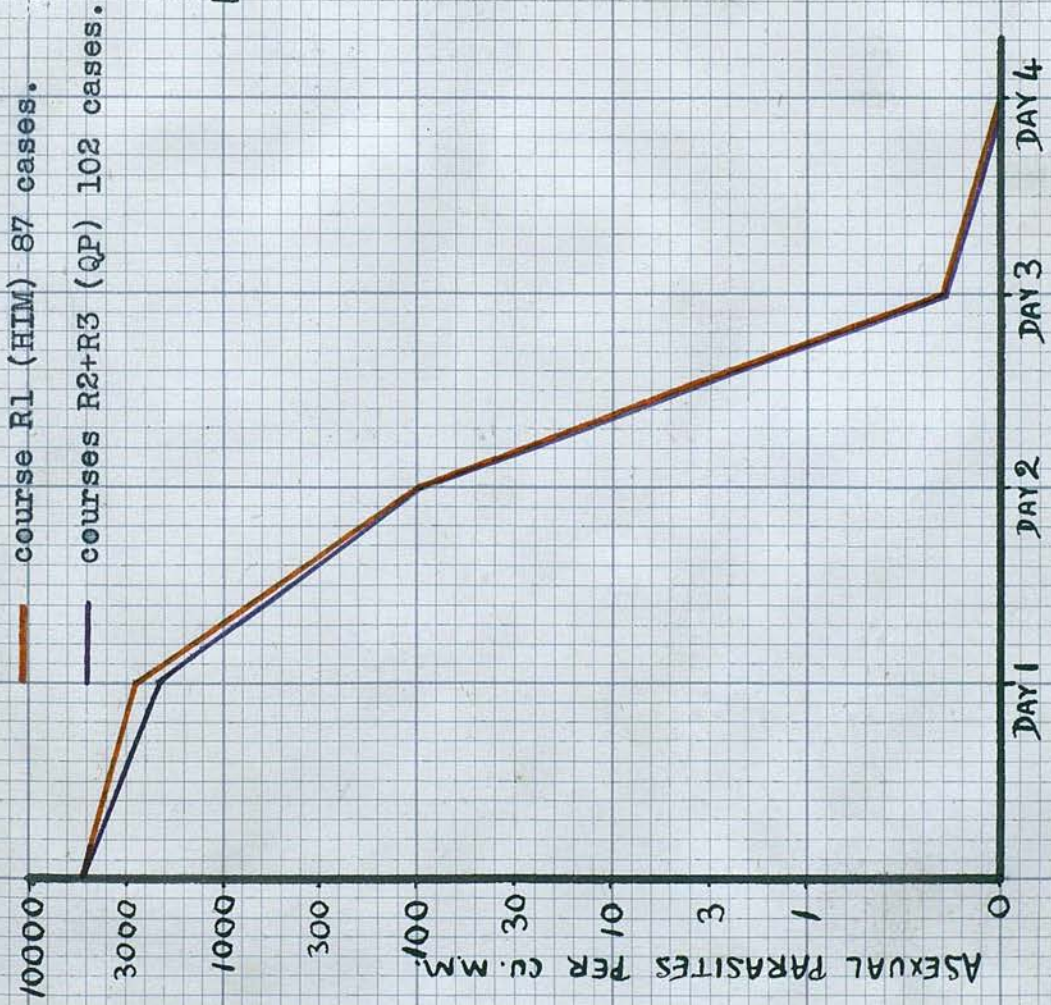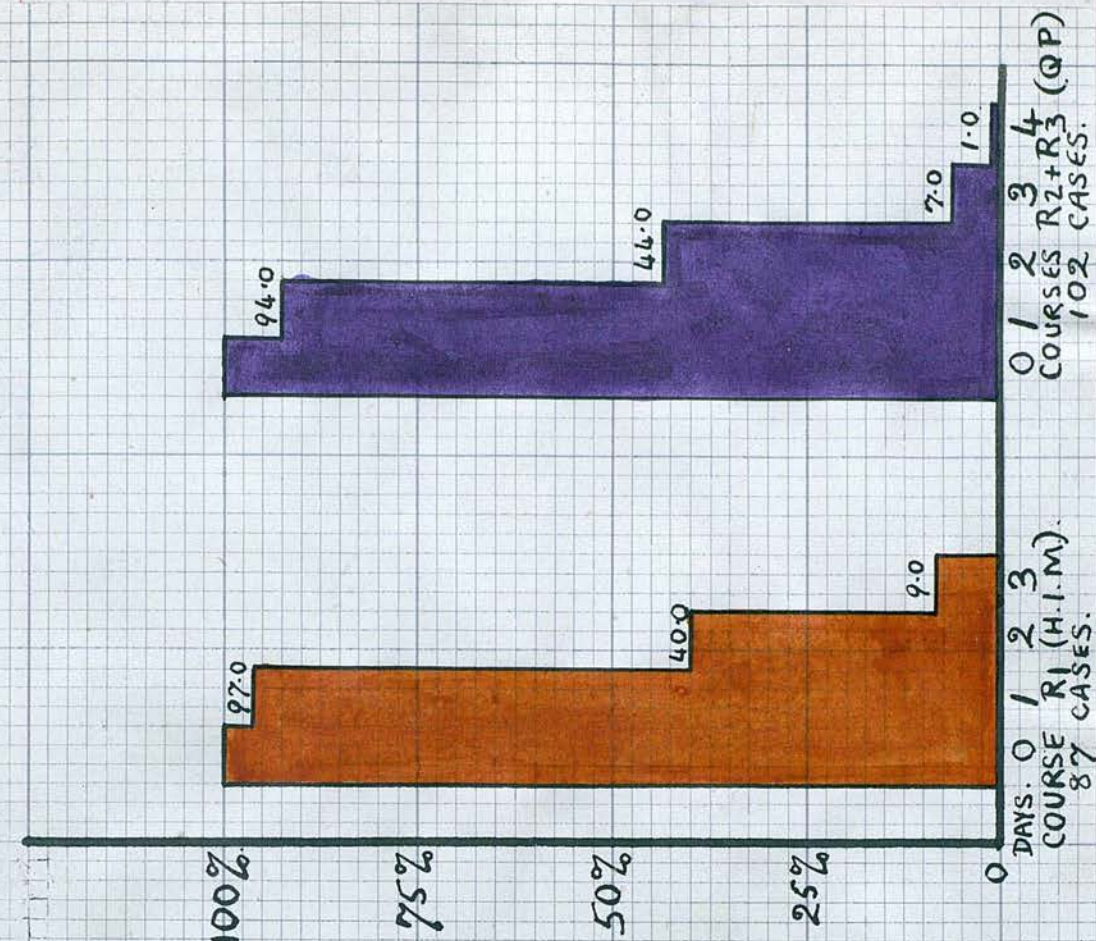
The/

FIGURE 9. PERCENTAGE NUMBER OF B.T.(R) CASES
WITH ASEXUAL PARASITES PRESENT DURING FIRST
FEW DAYS OF TREATMENT.

FIGURE 8. RATE OF CLEARANCE OF ASEXUAL PARASITES
IN B.T.(R) CASES DURING TREATMENT.

The following are examples of actual parasite
counts and illustrate the rapidity of clearance of the
initial parasitaemia that occurs in some cases on
treatment.

Asexual parasites are expressed in numbers per cu.mm.

Course R1. - Mepacrine.

Case No.	Initial Asex.Count.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
259.	17000	480	Nil	Nil	Nil
297.	8200	1700	Nil	Nil	Nil
214.	3700	2500	210	Nil	Nil
Course R2. - Quinine plus Pamaquin.					
443.	17000	Nil	Nil	Nil	Nil
369.	5700	220	Nil	Nil	Nil
392.	3600	2700	250	Nil	Nil

2.   Duration of Asexual Parasites.

Figure 9 shows the persistence of asexual parasites
in terms of the percentage number of cases in which
they were present on each day of treatment: Figure 10
shows the average duration of asexual parasitaemia in
the same 2 groups of cases.

3.   Duration of Pyrexia.

Figure 11 shows the average duration of pyrexia
from the start of treatment in the two groups of
Relapse B.T. infections.
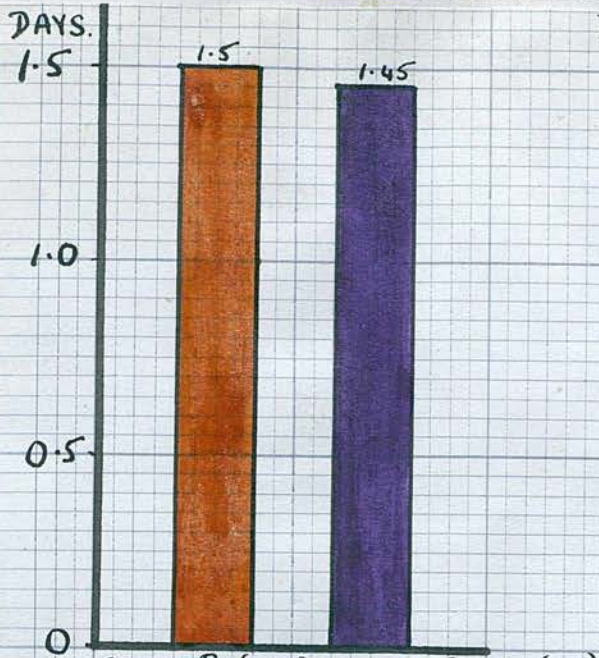
4./

FIGURES 10,11,12. (pages 34,35).

FIGURE 10. B.T. (R) CASES
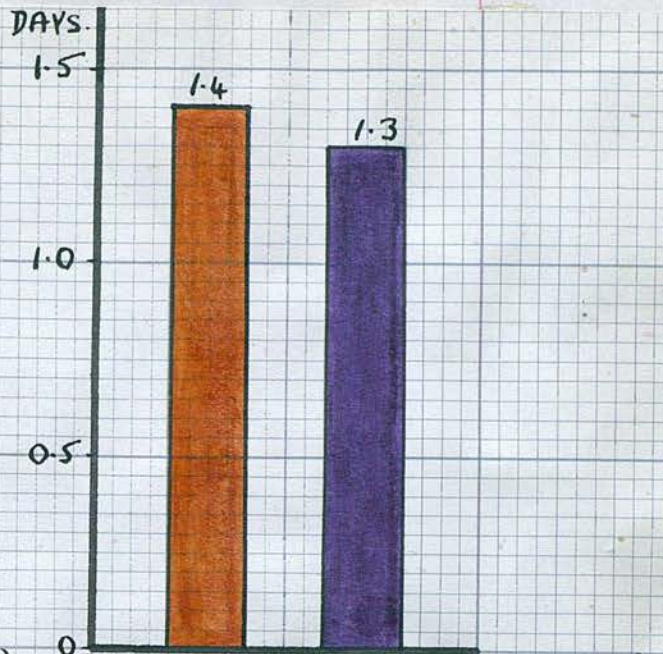DURATION OF ASEXUAL
PARASITAEMIA.

FIGURE 11. B.T. (R) CASES
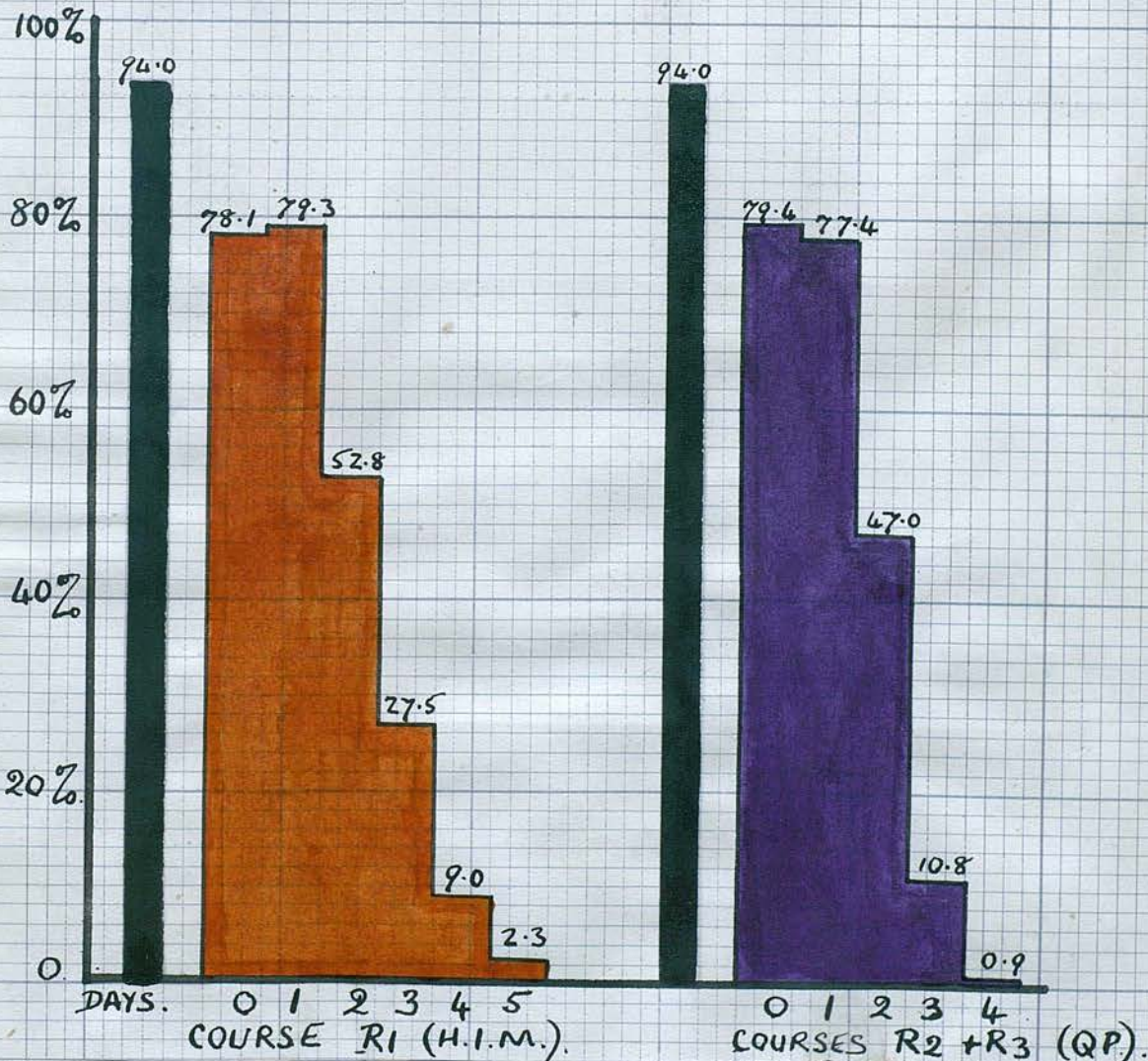DURATION OF PYREXIA.

FIGURE 12. PERCENTAGE NUMBER OF B.T. (R) CASES WITH GAMETOCYTES DURING TREATMENT. FIRST BLOCK SHOWS PERCENTAGE OF TOTAL CASES IN WHICH GAMETOCYTES APPEARED.

4.   Rate of disappearance of Gametocytes.

Figure 12 indicates the persistence of gametocytes on Courses R_1 and R_2 + R_3 as shown by the percentage number of cases with these forms present on the first few days of treatment. It will be seen that as in the Fresh infections, the gametocytes persist longer during treatment than the asexual forms and the same tendency for them to appear in showers during the first few days of therapy was observed. It is also evident from the results that the Quinine and Pamaquin combined treatment is more effective in clearing the gametocytes, than is the course with Mepacrine alone.

Examples of Asexual parasite and Gametocyte counts in B.T.(Relapse) cases during treatment. Counts in numbers/cu.mm.

(1) Therapy Course R1 - Mepacrine. Cases 374 and 324.

	Initial count.	24 hrs.	48 hrs.	72 hrs	96 hrs.	120 hrs.	144 hrs.	168 hrs.
Asex.	6700	2000	Nil	Nil	Nil	Nil	Nil	Nil
Gamet.	490	150	70	2 seen	Nil	Nil	Nil	Nil
Asex.	4000	4200	480	Nil	Nil	Nil	Nil	Nil
Gamet.	400	400	220	280	160	2 seen	Nil	Nil

(2) Therapy Course R2 - Quinine + Pamaquin. Cases 211 and 347.

	Initial count.	24 hrs.	48 hrs.	72 hrs	96 hrs.	120 hrs.	144 hrs.	168 hrs.
Asex.	7500	920	Nil	Nil	Nil	Nil	Nil	Nil
Gamet.	420	240	70	1 seen	Nil	Nil	Nil	Nil
Asex.	1960	120	Nil	Nil	Nil	Nil	Nil	Nil
Gamet.	240	200	3 seen	1 seen	Nil	Nil	Nil	Nil

Figure/

FIGURE 14.

B.T.(R) CASES, AVERAGE
DURATION OF GAMETOCYTES.
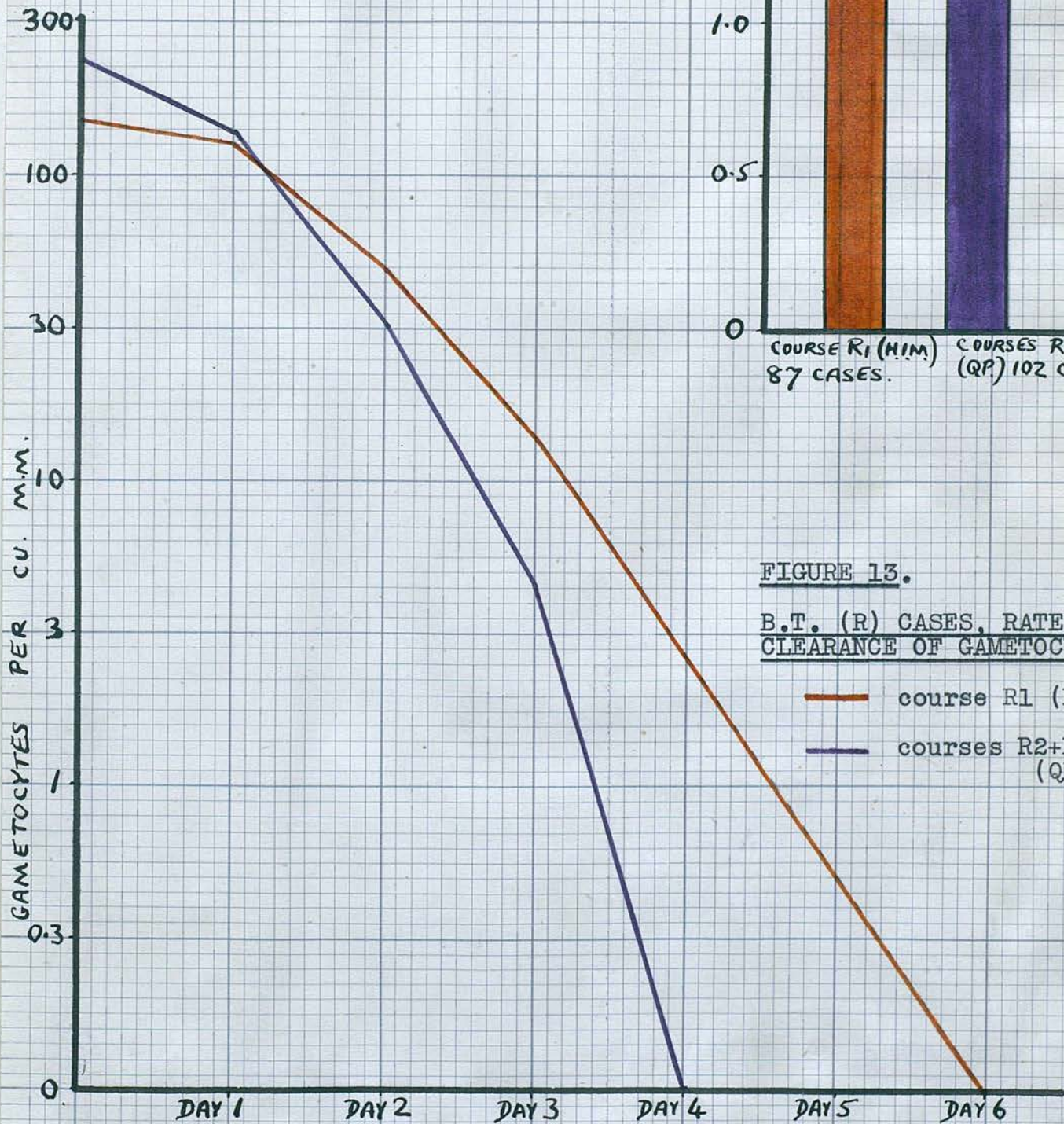
FIGURE 13.

B.T. (R) CASES, RATE OF
CLEARANCE OF GAMETOCYTES.

course R1 (HIM)

courses R2+R3
(QP)

COURSE R1 (HIM)
87 CASES.

COURSES R2+R3
(QP) 102 CASES.

Figure 13 shows the rate of disappearance of gametocytes on Courses R_1 and $R_2 + R_3$ in graphic form with the average daily gametocyte count for the first six days of treatment.

Figure 14 shows of average duration in days of gametocytes in the same groups of cases.

5.　The average white blood-cell counts at the start and finish of Courses R_1 and R_2 are shown in Table 3. As in the Fresh infections, a very slight initial leucopenia was present in the 181 Relapse cases studied but at the end of the 10 or 12 day courses the white count had returned to within normal limits, the change taking place during the first 3 or 4 days of treatment.

TABLE 3.　AVERAGE INITIAL AND FINAL WHITE BLOOD CELL COUNTS OF B.T. RELAPSE CASES TREATED ON COURSE R1 and R2.

Course and No.of Cases.	Length of Course-days.	W.B.C. per cu.mm. before treatment.	W.B.C. per cu.mm. at end of treatment.
R1 (87) (Cases)	12.	5,800	7,800.
R2 (94) (Cases)	10.	6,100	7,800.

6.　Haemoglobin percentages in Relapse cases treated on Courses R_1 and R_2.

The average initial and final Haemoglobin percentages in cases treated on these courses are shown in Table/

Table 4. As in the Fresh infections, there would appear
to be little significant change in these levels and the
average figures for the whole group of 181 Relapse
cases show no evidence of anaemia consequent upon the
recurrent nature of the infection. It would not seem
that in this series, the administration of Pamaquin
0.01 gm T.I.D. for ten days had any adverse effect on
the Haemoglobin level.

TABLE 4. AVERAGE INITIAL AND FINAL HAEMOGLOBIN
PERCENTAGES OF B.T. RELAPSE CASES TREATED
ON COURSES R1 and R2.

Course and No.of Cases.	Length of Course-days	HB.Percentage before treatment	HB.Percentage at end of treatment.
R1 (87) (Cases)	12	93.	98.
R2 (94) (Cases)	10	95.	96.

Follow-up of Fresh and Relapse B.T. Cases after Treat-
ment in the Active Phase.

1. Fresh B.T. infections.

It was possible to follow-up 120 cases who were
treated on therapy courses 1 to 5. Under the follow-up
system/

system adopted, cases who relapsed within a relatively short time of leaving hospital were notified by other medical units or were readmitted for treatment with Course R_1 or R_2. All cases were sent follow-up forms six months after completing treatment for their last known attack and the nil returns of this type constitute a record of six months freedom from active Malaria following the therapy course on trial. The results of this follow-up of B.T. Fresh cases are shown in Table 5.

TABLE 5.

Course.	1.	2.	3.	4.	5
No. treated.	29	30	29	29	21
No. followed for 6 months.	26	23	27	25	19
RELAPSE.	5	9	7	3	5
NO RELAPSE.	21	14	20	22	14

The numbers of cases treated and followed-up are too small to allow any conclusions to be drawn regarding the respective values of the 5 therapy courses concerned. Application of the Chi-square Test to these figures shows that according to the generally accepted level of significance of 1 in 20, there are no really significant differences between the follow-up results obtained on any of these courses. This negative/

negative finding is in itself of some interest, although
the same courses tried on much larger series of cases
might well have shown significant differences in re-
lapse rates. It seems permissible however, to draw
attention to the two following points which are shown
in the actual figures of the limited results given in
Table 5.

Courses 2, 3 and 5, which contain no Pamaquin,
show a higher relapse rate than Courses 1 and 4 which
included three days of Pamaquin at the end of the
treatment.

The heavy Mepacrine course No.2, giving 3.1 gm
in 7 days, would appear to be no more effective in
preventing relapses than Course 3 which gave only 2.4
gm. in the same time.

2.   Relapse B.T. infections.

Follow-up details were obtained in 139 cases of
B.T. Relapse infections treated on Courses R1(Mepacrine)
and R2 (Quinine + Pamaquin).   These results are shown
in Table 6.

TABLE 6.

Course.	R1.	R2.
No. treated.	87	94
No. followed for 6 months.	67	72
RELAPSE.	24	10
NO RELAPSE.	43	62

In this follow-up the numbers of cases treated are much larger than in Table 5 and statistical analysis of the results by the Chi-square Test shows a significant difference between the two courses, favouring the 10 day Quinine + Pamaquin treatment as being more effective than the 12 day Mepacrine course in the prevention of further malaria relapses. (Chi squared = 7.88. Probability = less than 0.01. Yates' correction for Continuity applied).

In the patients who relapsed after Course R2, it was found that the recurrences occurred within an average of less than one month from the completion of treatment. Following the Mepacrine course R1, the average time before recurrence in the 24 patients who relapsed was nearly three months. This comparison suggests that an erroneous impression as to the efficacy of a treatment course might arise if too short a follow-up period were adopted. It also raises the interesting possibility of there being an essential difference in the mode of action of Quinine + Pamaquin and Mepacrine in attacking the more resistant parasite stages of B.T. malaria in cases who have already had more than one clinical attack.

SECTION 4.

PART A. RESULTS OF TREATMENT IN MALIGNANT TERTIAN CASES.

As previously stated, the number of cases of M.T.
Malaria available for study, was disappointingly small,
being less than a total of 30 cases. Of this number,
the majority were Fresh infections contracted in the
Bone area in North Africa and they were treated on
the same therapy courses 1 to 5 in rotation as were
the Fresh B.T. infections. Parasite counts done at
12 hourly intervals, morning and evening were obtained
for most of these cases, and whereas the small number
makes them of no certain statistical value, they are
included in this report as a matter of interest and
as they constitute a record of M.T. malaria parasite
counts for cubic millimetre under the influence of
therapeutic drugs.

As in the case of the Fresh B.T. infections, it
is convenient to group together cases on Courses 1 and
5 as being treated with Quinine for the first 3 days,
and Courses 3 and 4 which started treatment with low
initial mepacrine dosage, as distinct from Course 2
where heavier mepacrine dosage was given. Only the
asexual parasite counts are given here, as gametocyte
counts on so few cases could have little value. The
results are shown in Table 7, and the following are
the/

TABLE 7.(page 41).

TABLE 7.

SHOWS ASEXUAL PARASITES PER CUBIC MM AT 12 HOURLY INTERVALS IN M.T. FRESH CASES DURING TREATMENT.

COURSES 1 and 5 Q. (9 CASES).

INITIAL COUNT.	12 HRS.	24 HRS.	36 HRS.	48 HRS.	60 HRS.	72 HRS.	84 HRS.	96 HRS.	108 HRS.	120 HRS.	132 HRS.	144 HRS.
300	300	120	–	–	–	–	–	–	–	–	–	–
18000	18000	560	–	–	–	–	–	–	–	–	–	–
8000	11000	2200	–	–	–	–	–	–	–	–	–	–
90000	30000	11000	5000	680	1 seen	–	–	–	–	–	–	–
42000	51000	25000	1100	60	–	–	–	–	–	–	–	–
420	480	300	–	–	–	–	–	–	–	–	–	–
1400	90	–	120	–	–	–	–	–	–	–	–	–
10	14400	11500	1900	130	–	–	–	–	–	–	–	–
300	200	–	–	–	–	–	–	–	–	–	–	–

*

COURSES 3 and 4 L.I.M. (9 CASES).

INITIAL COUNT.	12 HRS.	24 HRS.	36 HRS.	48 HRS.	60 HRS.	72 HRS.	84 HRS.	96 HRS.	108 HRS.	120 HRS.	132 HRS.	144 HRS.
600	–	20	30	–	–	–	–	–	–	–	–	–
80	30	–	180	–	–	–	–	–	–	–	–	–
30000	14000	400	–	25	–	–	–	–	–	–	–	–
11400	9800	400	420	120	–	–	–	–	–	–	–	–
20000	18000	80	90	–	–	–	–	–	–	–	–	–
150	180	70	40	–	–	–	–	–	–	–	–	–
600	14000	45000	70000	15000	280	11200	14000	–	200	–	120	–
5000	800	200	1 seen	1 seen	–	–	–	–	–	–	–	–
7600	800	300	18000	2000	420	–	–	–	–	–	–	–

* *

COURSE 2 H.I.M. (6 CASES).

INITIAL COUNT.	12 HRS.	24 HRS.	36 HRS.	48 HRS.	60 HRS.	72 HRS.	84 HRS.	96 HRS.	108 HRS.	120 HRS.	132 HRS.	144 HRS.
1500	540	–	14000	8400	2000	600	–	–	–	–	–	–
500000	300000	80000	40000	17000	2200	–	–	–	–	–	–	–
70000	30000	30000	–	–	–	–	–	–	–	–	–	–
260	1600	400	–	–	–	–	–	–	–	–	–	–
8800	300	–	–	–	–	–	–	–	–	–	–	–
220	–	4700	–	2 seen	–	200	–	–	–	–	–	–

* = Cases showing great variation in Parasite Count, with periodicity.

the main points that it would appear justifiable to
make from the study of this small series:-

1. There tends to be a great variation in the asexual
    parasite count with high and low counts following
    one another in a rough periodic formation. This is
    shown especially in the cases marked with an asterisk
    which demonstrate also how a low initial count may
    suddenly increase with great rapidity.

    In several cases it was noticed that the highest
    parasite counts corresponded exactly with the
    highest peak of temperature. Thus in cases having
    a continuous temperature, the rise of one or two
    extra degrees every 48 hours was associated with a
    striking soaring up of the numbers of trophozoites
    in the blood stream and such high counts subsided
    as quickly once the peak of temperature was passed.
    From this it would seem that the best time for the
    diagnostic demonstration of the parasites in blood
    films would be at the extreme peak of the pyrexia,
    when their number may be disproportionately high
    in relation to the temperature.

    These observations might also suggest that the
    clinical peak of pyrexia would be the 'optimum time
    for the start of treatment with intravenous Quinine
    or intramuscular mepacrine. At that time the
    infection is represented by a great number of
    circulating/

circulating young ring forms and there would seem
to be much less risk of causing a sudden liberation
of toxic products, than when powerful drugs are
injected to act on the schizonts which in M.T.
Malaria lurk in the capillaries of the visceral
organs.

2. The Mepacrine courses (2, 3 and 4) allowed the
asexual parasitaemia to persist for 72 hours in
3 out of 15 cases, and 1 case on low Mepacrine
dosage continued to show trophozoites for 132
hours from the start of treatment. In the 9 cases
treated with Quinine only 1 case showed a very
scanty parasitaemia 60 hours from the start of
treatment. The clinical impression was formed
that the Quinine was more effective than Mepacrine
in the initial control of pyrexia and general
symptoms.

Follow-up of M.T. Fresh infections treated on Courses
1 to 5.

Of the 24 cases treated, 16 were followed up for
six months.

Of this number only 2 had relapsed and both had
been readmitted for treatment by the Team.

The 2 cases that relapsed had both been treated
originally on Course 1, which will be noted, contained
less Mepacrine (Total 1.5 gm) than any of the other
courses.

PART B.   STERNAL MARROW EXAMINATIONS ON CASES OF B.T.
          AND M.T. MALARIA.

A study was made of smears of sternal marrow
obtained from 52 cases of B.T. and M.T. infections
treated in North Africa and Italy. The observations
made in this small but interesting series may be
summarised briefly as follows:-

1. It was not found possible to demonstrate asexual
    parasites of either B.T. or M.T. Malaria in the
    sternal marrow when they were not demonstrable in
    thick films of the peripheral blood. When present
    in the marrow blood, the estimated parasite count
    was not found to exceed that in the peripheral
    blood.

2. The gametocytes of M.T. Malaria were found amongst
    sheets of marrow cells in several Fresh cases on
    the third day of treatment; that is about 3 days
    before they appeared in the peripheral blood.
    Many of these marrow forms were rounded and im-
    mature, and it seems possible that in M.T. Malaria
    the gametocytes may be formed among the cells of
    the reticulo-endothelial system, of which the
    sternal marrow is a representative sample.

3. The initial leucopenia so commonly present in
    active Malaria infections is shown in sternal
    marrow/

marrow smears to result from a maturation arrest
at the metamyelocyte stage of the granular white
cell precursors. It would appear to be a temporary
toxic suppression of polymorph formation and dis-
appears quickly when specific anti-malarial therapy
is instituted.

4. A most careful search was made in all the marrow
smears for any non-pigmented form of parasite that
might represent an extra-erythrocytic phase, but
nothing convincing was observed. Several cases
showed small rounded extracellular bodies with
red granules set in a pale blue cytoplasm and
which at first appeared rather like parasites.
For want of a more apt description they were called
"curious bodies" and their presence or absence was
recorded in all the cases studied. It seemed from
this work that these structures bore no relation
to the stages of the infection in the peripheral
blood and they were observed also in marrow smears
from patients who had no history of Malaria. It
was concluded that the "curious bodies" represented
only rounded granular cytoplasmic debris from
damaged myelocytes and were thus artefacts. It
seemed that the finding of similar bodies was being
studied by some Italian civilian workers, and photo-
micrographs/

photomicrographs of such structures might appear to
have many of the characteristics that may be looked
for in the long-sought human extra-erythrocytic
parasite phase.

SECTION 5.

PART A.

A STUDY OF THE EFFECTS OF THE
ANTI-MALARIAL DRUGS ON THE ASEXUAL
PARASITES OF BENIGN TERTIAN MALARIA.

During the daily examination of blood films
from patients under treatment for B.T. infections,
it was noticed that in some cases, disappearance of
the asexual parasites occurred more rapidly than in
others.   It was thought worth while to pursue this
study and to see what evidence could be found as to
the more detailed action of the anti-malarial drugs
employed. With this object in view, it was decided
to record not only the actual number of asexual
parasites per cubic millimetre in the films, but to
note also the stages of development of the parasite
asexual cycle represented by each count.

Owing to the somewhat cumbersome terminology
involved in describing the various stages of the
parasite/

parasite asexual cycle, it was found convenient to
introduce an abbreviated notation to indicate 10
distinct stages of the cycle which could be recognis-
ed microscopically.   This notation sub-divided the
48 hour cycle into 6 stages of trophozoites, (T 1 to
T 6), 3 stages of schizonts, (S 1 to S 3), and a
merocyte, (M).

In order to make quite clear the microscopical
appearances implied by each symbol in this notation,
the following few pages are devoted to a series of
diagrams and descriptions of the various parasite
stages.   The sketches were made from Leishman-
stained thin blood film preparations of the common
strain of Italian Benign Tertian parasite and the
approximate time of appearance and duration of each
stage in the 48 hour cycle is indicated.   To
complete this series of parasite diagrams, drawings
and descriptions of gametocytes are also included.
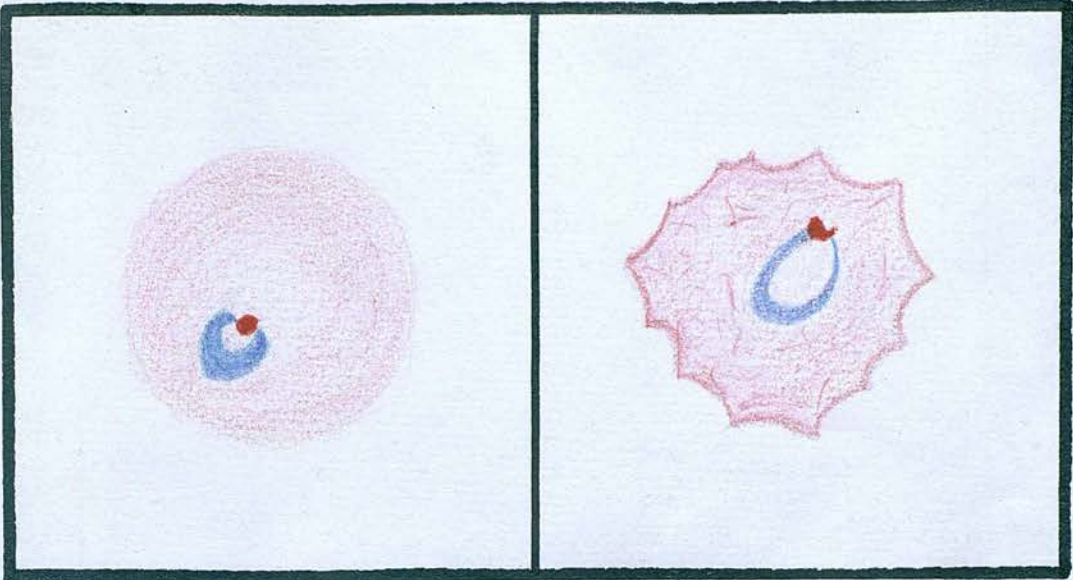
T 1.   Very young trophozoites found just after
the clinical paroxysm and often accompanied by a few
late schizonts.   Two forms are shown, (a) a young
parasite that has just entered a fresh red cell after
sporulation, and (b), the parasite seen a few hours
later as an early ring trophozoite.   At this stage,
the red cell has contracted with a well-marked fim-
briated outline and the first signs of Schuffner's
dots have formed from changes in the cell envelope,
The dots are seen as faint, short, curved lines in
the parasitised red cell.

    Approximate duration of T 1,   0 to 6 hours.
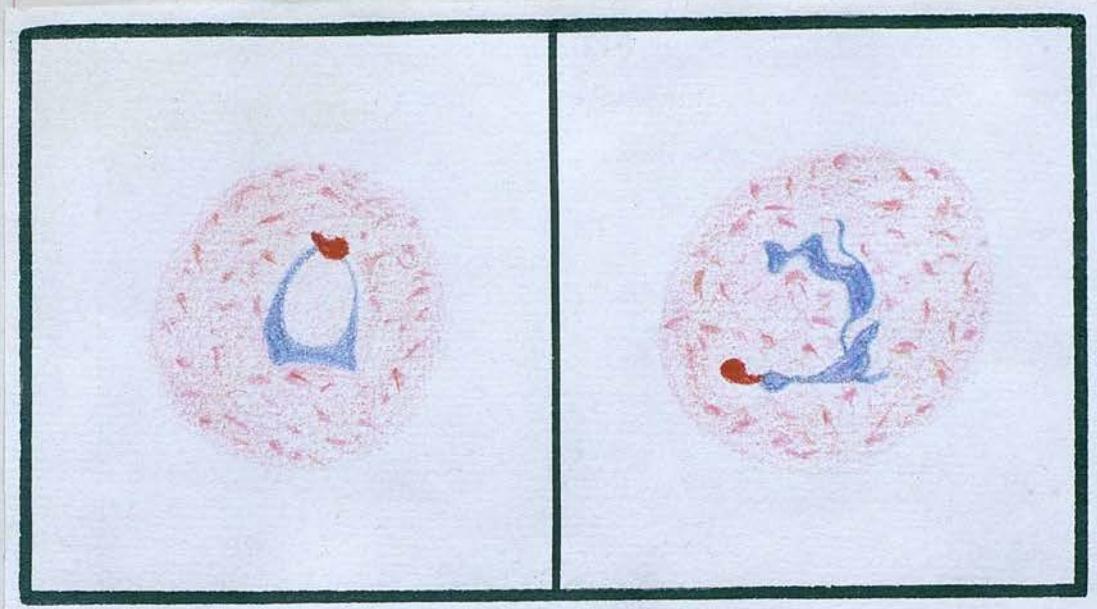
T 2.   Ring-form trophozoites, morphologically
similar to stage T 1 but growing slightly larger and
beginning to show early amoeboid activity.   Two forms
are shown, (a) a young T 2 seen as a definite ring,
and (b), a later T 2 in which the parasite has grown,
has rougher cytoplasm, and has wriggled so that the
appearance of the vacuole is lost.   The red cell has
a smooth outline and begins to enlarge during the T 2
stage as a result of parasite activity.   The Schuff-
ner's dots become well-marked as small, rod-like flecks
in the cell envelope.

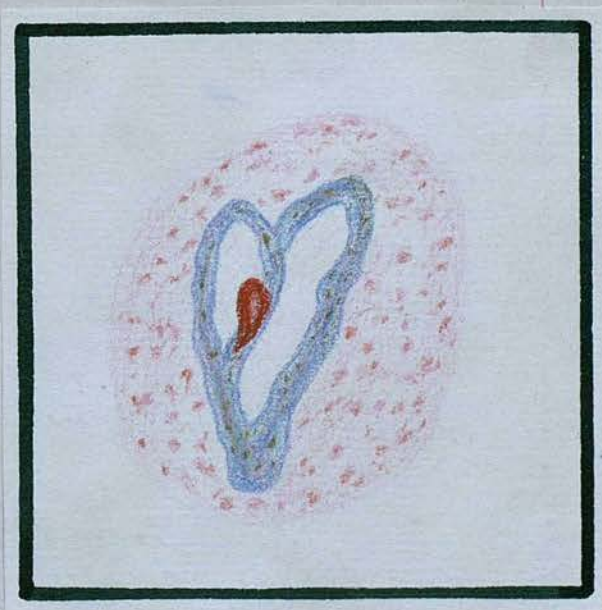Approximate duration of T 2, 6 to 10 hours.

T 3. A growing amoe-
boid trophozoite. The
parasite has become
actively amoeboid and
its coarse cytoplasm
is often spread about
its red cell. Pigment
has begun to collect
at one end of the
parasite. The red cell
is by now much enlarged and Schuffner's dots are numer-
ous, prominent and less rod-like than previously.

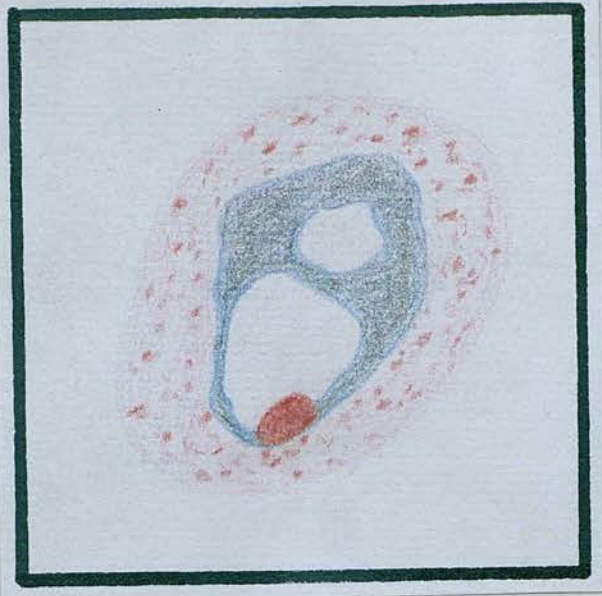Approximate duration of T 3.   10 to 20 hours.

T 4.   An almost fully-
grown amoeboid trophozoite
in which the amoeboid
activity has begun to
slow down so that the
cytoplasm becomes con-
tinuous again and vac-
uolation- often multiple-
reappears.   Pigment is
seen scattered through-
out the cytoplasm.
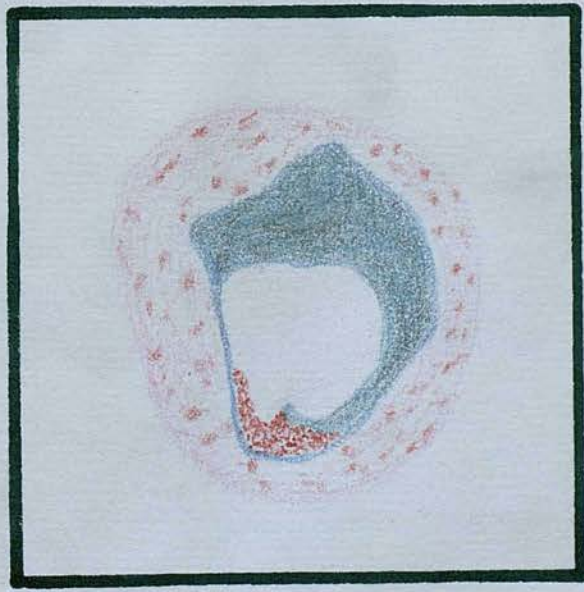

Approximate duration of T 4,   20 to 26 hours.

T 5. A fully grown
amoeboid trophoizoite,
now only sluggishly
amoeboid. Pigment is
accumulating through-
out the cytoplasm,
making it a greyish-
blue colour. The
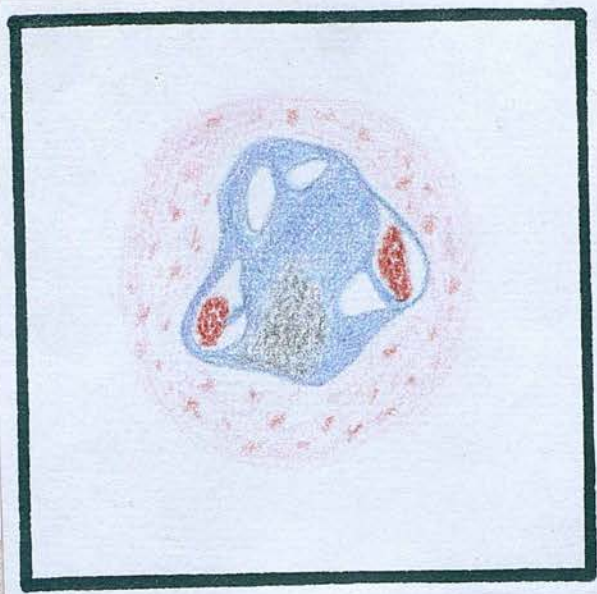chromatin nucleus be-
comes larger and less
dense.

Approximate duration of T 5, 26 to 30 hours.

T 6. A dividing
trophozoite. The chro-
matin nucleus splits
into many fine partic-
les preparatory to
segmentation. The
cytoplasm is vacuolated
and is a grey-brown
blue colour, due to
diffuse pigment. The

whole parasite is more rounded, as the amoeboid activ-
ity has ceased. Schuffner's dots can still be seen
in the envelope of the red cell.

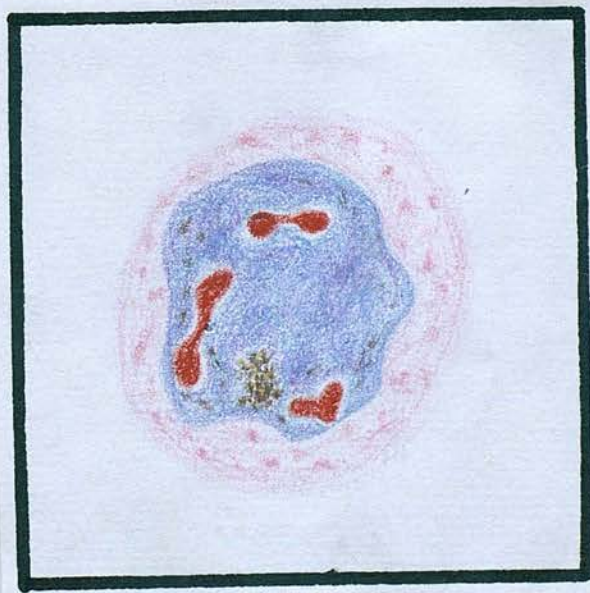Approximate duration of T 6, 30 to 36 hours.

S 1.   A young
schizont.   Two to
four distinct chromat-
in nuclei can be seen,
and there are usually
several small vacuoles
in the cytoplasm which
stains a clear blue as
the brownish pigment
is collecting together
at one spot.   The
parasite and red cell have become slightly smaller.
Approximate duration of S 1,   36 to 42 hours.

S 2.   A mid-stage
schizont with four to
eight chromatin nuclei,
and in which further
nuclear sub-division is
occurring.   The vacuol-
es in the blue cyto-
plasm have disappeared
at this stage and a
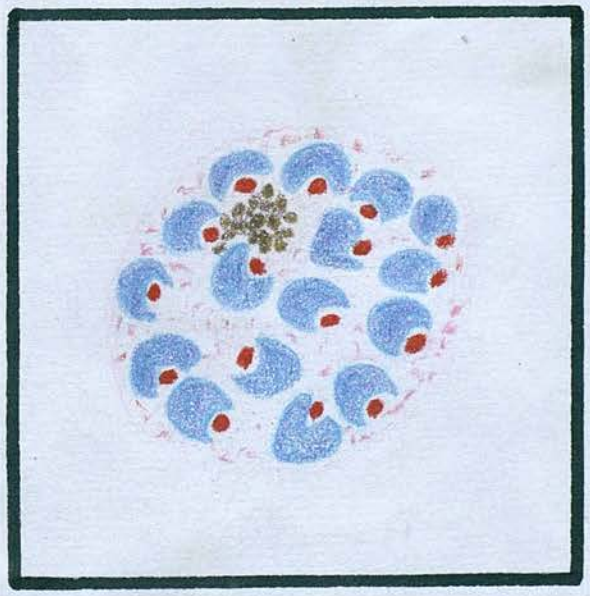collection of dark-

brown pigment can be seen.
Approximate duration of S 2, 42 to 46 hours.
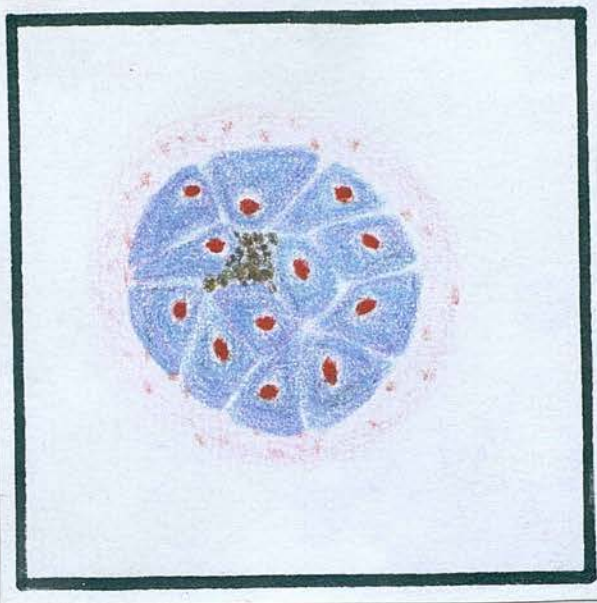
S 3. A late
schizont with eight
to twelve chromatin
nuclei and with the
cytoplasm starting
to divide up around
each nucleus.

Approximate duration of S 3,   46 to 47¾ hours.

M. A complete
merocyte with sixteen
chromatin nuclei and
attached cytoplasmic
fragments(merozoites).
A dark brown mass of
malarial pigment can
be seen amongst the
merozoites and the
shadowy outline of
the red cell with faint Schuffner's dots is just vis-
ible.   This stage is very fragile and lasts only a
matter of minutes before bursting to liberate a new
generation of young trophozoites.
     Approximate duration of M,   47¾ to 48 hours.
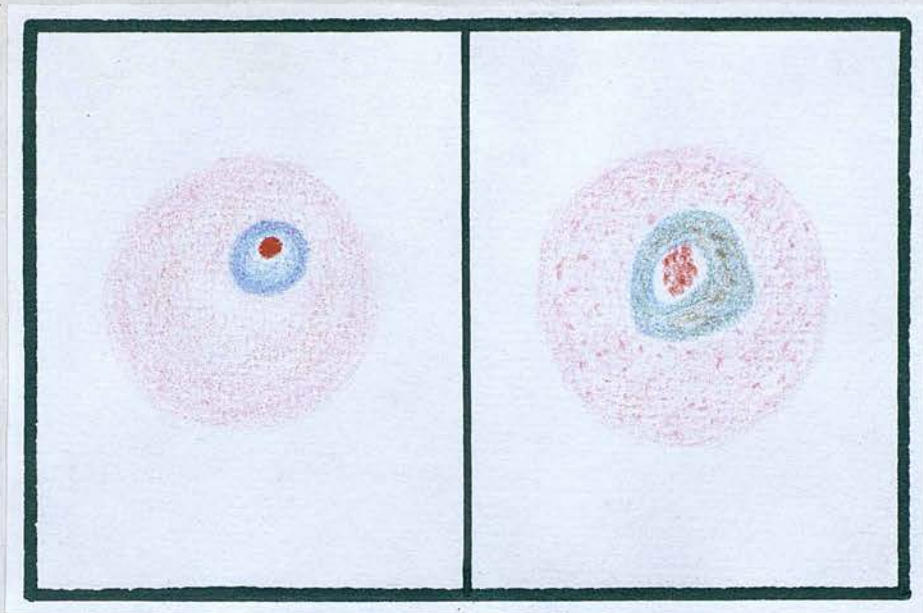
Young and Growing Male Gametocytes.
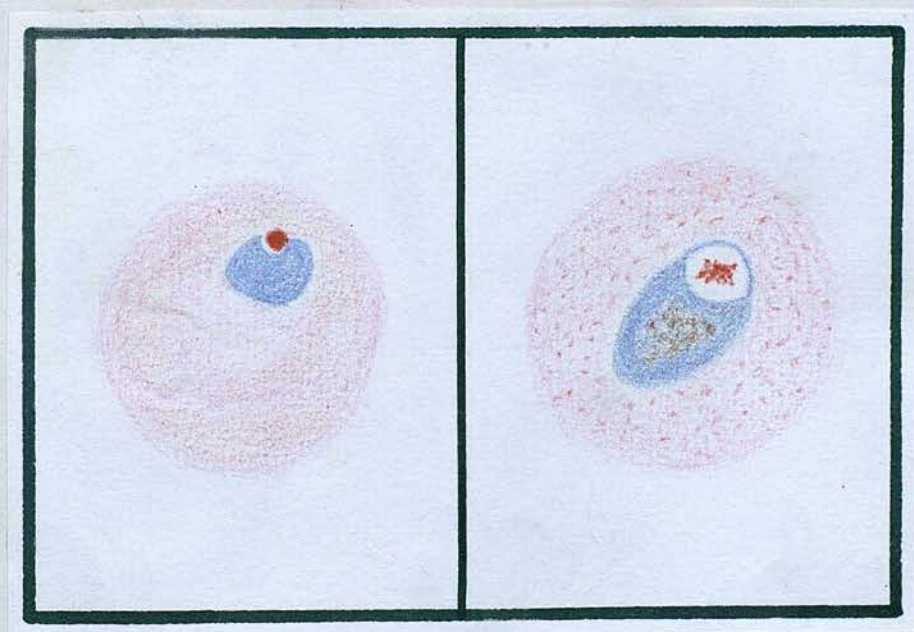
Young and Growing Female Gametocytes.

Very young male and female gametocytes are
presumed to be formed from merozoites.   Two forms
are seen, one with a central chromatin nucleus and
which/

which probably developes into a male gametocyte, and
the other with an eccentric nucleus which is probably
the early female gametocyte.

The growing gametocytes are quite clearly dis-
tinguishable from one another.   The male form is
rounded in outline, has greenish-brown cytoplasm with
golden-brown pigment scattered in it and the parti-
culate chromatin nucleus is central and surrounded by
a clear space.   The developing female gametocyte is
more oval in outline, has deep blue cytoplasm with
scattered dark-brown pigment and the chromatin of the
nucleus is situated in a clear space at one end of
the parasite.   The developing gametocytes do not
cause so much enlargement of the red cell as the
asexual forms, as they show little amoeboid activity.
Schuffner's dots occur, but are pale in colour.

Mature   male and female

gametocytes:-   see next page.
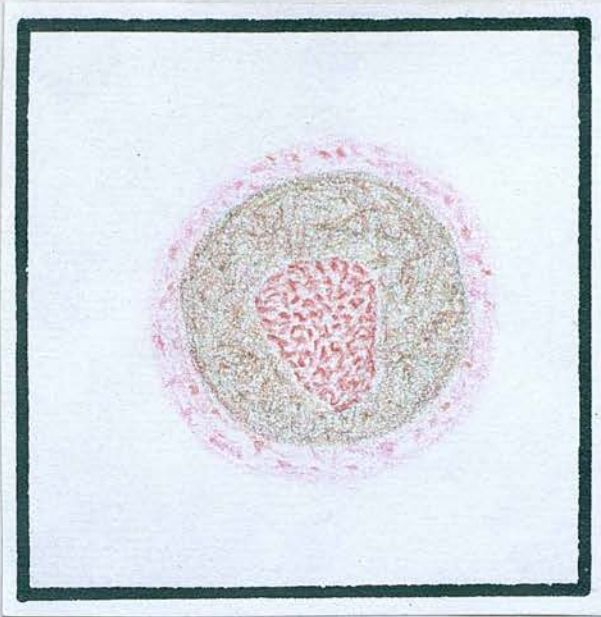
The mature male gametocyte ,(micro-gamete), has pale greenish-blue cyto-plasm with golden-brown, rod-like pig-ment scattered through-out it. The nucleus is large and central and has an open, reticular, chromatin structure.

The mature female gametocyte, (macro-gamete), is larger than the male gameto-cyte and is the larg-est form of malaria parasite found on blood examination, being bigger than any of the asexual stages.
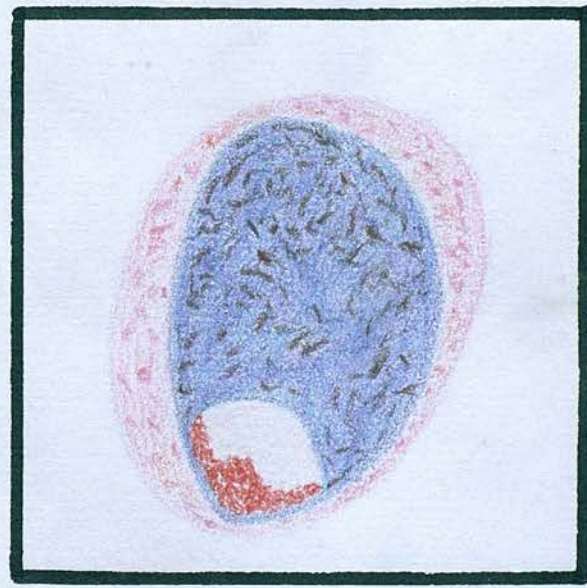
It is an oval parasite with purple-blue cytoplasm containing scattered dark-brown granular pigment. The chromatin nucleus is more concentrated than that of the male gametocyte and is situated at the periphery of the cell in a clear halo. Schuffner's dots are present in the envelope of the distended red cell.

THE MAIN MICROSCOPICAL APPEARANCES SEEN IN THE B.T.
ASEXUAL PARASITES DURING TREATMENT WITH MEPACRINE,
PAMAQUIN AND QUININE, WERE RECORDED AS FOLLOWS.

A.   Mepacrine.

Three effects have been consistently noted due to
Mepacrine. The first and most striking of these is
the bunching up and rounding off of the trophozoite
resulting from the disappearance of the vacuole and
a collapse of the amoeboid ring form.

The second effect noted is an alteration in the
pigment normally found in the asexual parasites. This
would seem to result from the collecting together and
extrusion of the pigment and under these circumstances
the parasite cytoplasm stains a clear blue colour in
contrast to the usual pale grey blue staining.

Once the above two effects begin to show themselves
in the developing trophozoites, the latter tend to be
retarded in their developmental process.

The third abnormal appearance seen with Mepacrine
action is usually found comparatively late in treatment
when the parasites appear to be ragged and bedraggled
in outline and seem to be on the point of cytoplasmic
disruption.

B.   Pamaquin.

This drug was found to have an action on asexual
parasites/

parasites very similar to that of Mepacrine, although
it was much slower in producing the first visible
microscopic changes in the parasite cell. This finding
when reported, caused some surprise amongst authorities
(P.G. Shute et al.) who understood that Pamaquin was a
powerful gametocide but produced little effect on the
asexual forms of B.T. malaria. It was therefore
decided to study this action of Pamaquin in greater
detail at a later date and some of the findings in this
investigation are reported in Part Two of this thesis
which deals with work done on cases of B.T. malaria
treated in Britain in the Autumn of 1945.

C.   Quinine.

No visible changes were noted in the morphology
of the asexual parasites during the action of Quinine,
though those found late in treatment often presented
a pale appearance and had a ragged cell outline as with
Mepacrine.   Quinine also was observed to inhibit the
development of the parasites.

In addition to the general slowing effect of all
the three drugs on the whole asexual developmental
cycle, it was noticed that when cases were started on
treatment during the middle trophozoite stage, there
were occasions when the parasites ceased to develop
beyond the stage of chromatin division though on other
occasions/

occasions development continued with the production of some young trophozoites. The retarded development forms corresponded to the T6 stage on the notation adopted, and were found to persist for 12 to 24 hours after other parasites had undergone schizogomy and had become young trophozoites. While there is no direct evidence to show that the T6 stage is more specifically arrested than other stages of the cycle, it is interesting to note that it represents the largest form of the trophozoite series and by virtue of its size alone, may be less susceptible to phagocytosis than the smaller, younger forms. It was thought that it would be a matter of great interest to reproduce the conditions governing this delay by giving drugs in such a manner as to encourage the maximum formation of these retarded T6 parasites. This and the allied investigations described below, were carried out only with Mepacrine.

In the first experiment, 2 cases having a moderately heavy infection with tertian fever, were left untreated until the parasites had developed to the T6 stage. They were then given an intramuscular in-jection of Mepacrine 0.2 gm. (Atebrin Dihydrochloride – Winthrop.) and no further treatment was given during the following 24 hours. Blood films were examined 24 hours after the injection when it was found that instead of the expected "hold-up", a striking disappearance of the/

the asexual parasites had occurred. In one case (No. 246) the initial count of 14,000 per cubic mm. had fallen to 50 per cubic mm. in 24 hours, and in the second case (No.249) all the parasites which were present initially — 4,200 per cubic mm. had disappeared within the 24 hours. Such a rapid clearance of para- sites from the peripheral blood had no comparison with the effects of any of the ordinary treatment courses observed. For the time being, attempts to cause a block or delay at the stage of division were abandoned in favour of this more interesting effect which had come to light. When Mepacrine is given intramuscularly at the T6 stage, there is a delay of 2 to 4 hours before any of the morphological changes due to the drug are seen in the parasites. The first appearance is the collection together of the malarial pigment, but the parasite continues to develop without apparent arrest and there is little or no change in the parasite count for 5 to 8 hours. During this period the parasites have nearly all become young schizonts and at the same time many of them have begun to extrude the malarial pigment into the circulation.

Up to this time, 5 to 8 hours, the patient usually feels comparatively well, but corresponding with the extrusion of the pigment by the parasite, (still a young schizont S1), the patient begins to feel the symptoms/
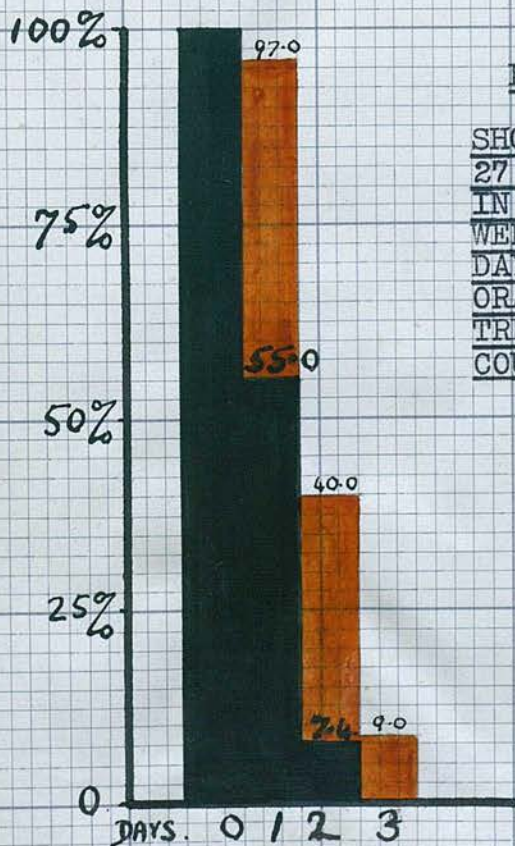
symptoms that are normally associated with a malarial
paroxysm at sporulation time. These symptoms however,
become gradually more severe with a rise of temperature
and vomiting may follow. The latter may be such as to
interfere with the subsequent administration of oral
Mepacrine. Many patients complain also of muscular
pains and cramps. These clinical effects are of
interest in that they constitute a malarial paroxysm
due to the pigment being set free before the time of
schizogony. The temperature chart shows a premature
peak in what has hitherto been a regular tertian fever.

The great fall in the parasite count occurs be-
tween 4 and 12 hours after the injection. These are
extremes of time and many cases have shown no change
at 6 hours after the injection, yet have had no para-
sites in films taken at 12 hours. The rapid clearance
occurs at a time when the parasites have developed into
middle and late schizonts (S2 and S3) and it appears
that the young schizonts (S1) are only slightly
affected. Cases with a heavy initial parasitaemia
(6,000 - 14,000 per cubic mm) can be cleared in a
matter of approximately 6 hours by this means.

27 Fresh and Relapse B.T. cases were injected at
the T6 stage as described above. In the great majority
of them, especially the Relapse cases, there occurred
a rapid disappearance of asexual parasites with a
premature/

FIGURE 15.

RATE OF CLEARANCE OF
ASEXUAL PARASITAEMIA IN 27
CASES OF B.T. MALARIA GIVEN
0.2gm MEPACRINE BY INJECTION
AT THE T6 STAGE OF THE
PARASITES.
ORANGE GRAPH = CONTROL CASES
TREATED ON ORAL MEPACRINE
COURSE R1 (H.I.M.).

Y-axis: ASEXUAL PARASITES PER CU.M.M. — 10000, 3000, 1000, 300, 100, 30, 10, 3, 1, 0

X-axis: DAY 1. DAY 2. DAY 3. DAY 4.

FIGURE 16.

SHOWS PERCENTAGE NUMBER OF
27 CASES TREATED BY MEPACRINE
IN WHICH ASEXUAL PARASITES
WERE FOUND DURING INITIAL
DAYS OF TREATMENT.
ORANGE BLOCKS = CONTROL CASES
TREATED ON ORAL MEPACRINE
COURSE R1 (H.I.M.).

Y-axis: 100%, 75%, 50%, 25%, 0

Bar values: 97.0, 55.0, 40.0, 7.4, 9.0

X-axis: DAYS. 0 1 2 3

premature clinical paroxysm. The results of the whole
series are given in Figures 15 and 16 and in which the
rapid rate of clearance can be compared with that
obtained on the oral therapy courses. (Figs 1, 2, 8
and 9).

Some cases, however, showed a variation in the
type of response both as regards clearance of parasites
and clinical reaction. Thus a few experienced no
clinical upset yet had a very rapid parasite disappear-
ance, while others suffered a severe reaction and after
24 hours still showed scanty parasites. These observa-
tions are difficult to explain but the following further
experiments may throw some light on the problem.

After injection of intramuscular Mepacrine had
failed to cause the expected delay in the development
of the dividing trophozoites, it was attempted to
produce the hold-up by the administration of small
oral doses of Mepacrine given before the parasites had
reached the T6 stage. It was then thought that possibly
too much Mepacrine destroyed the parasites, but the
action of the drug and the delay before it takes effect
were not fully realised. No hold-up effect was
observed after these small oral doses and further ex-
periments to produce this arrest were temporarily dis-
continued.

On the findings of the injection results, it was
hoped that an even more rapid clearance of parasites
could/

could be effected by giving heavy oral Mepacrine dosage
in the first few hours following the intramuscular in-
jection. The result of this attempt was again contrary
to expectations as a true block of the parasites at
the T6 stage was observed for the first time. This
hold-up was produced in a number of cases but was found
difficult to maintain for more than 24 hours as the
frequent occurrence of severe clinical symptoms with
vomiting usually prevented the maintenance of heavy
oral dosage. It was also found that a single dose of
0.8 gm. of Mepacrine given orally at the T4 stage of
the parasite may likewise produce a temporary block at
T6. The following case (No. 402) is a good example:

Initial Count and stage	12 hrs.	24 hrs.	36 hrs.	48 hrs.
14,800	9,000	4,000	50	Nil
(T4) T5 + few T6 S1	(T6) S1 S2 S3 M + Scanty T1	(T6) S1 + Scanty T1 T2	(T3) Scanty T6	

Predominant Stage is shown in brackets.

Up to the present it has not been possible to in-
vestigate as fully as desirable the plasma Mepacrine
levels attained during the above injection and blocking
experiments as such estimations could only be carried
out from the end of March 1945 till the end of May
1945. The results obtained to date in 8 injection
cases are recorded in Section 8 of this report.

A/

A further experiment was carried out to determine
the oral dosage of Mepacrine required to produce the
same rapid clearance effect on the asexual parasites
as the injection described above. It was found that a
dose of 0.2 gm. was insufficient but that a dose of
0.8 gm. could effect the desired result. To allow for
the delay in absorption, the oral dosage must be given
about 5 hours earlier than the injection, thus corres-
ponding to the T4 to T5 stage of the parasite.

The effect of giving an intramuscular injection
of 0.2 gm. of Mepacrine to a case with a tertian cycle
of parasites at other stages of their development than
T6 was also investigated.   5 suitable cases were in-
jected when the parasites were either younger forms of
trophozoites or schizonts and in none of these was
there a rapid reduction in the parasite count nor did
any premature clinical paroxysm follow the injection.
From these 5 cases and from other observations, it was
concluded that in order to produce the maximum rapid
clearance of asexual parasites, it was necessary to
inject the Mepacrine exactly at the T6 preschizont
stage of the parasite cycle.

An interesting experiment in connection with the
above findings was to observe the effect of a Mepacrine
injection in a case where two distinct B.T. asexual
cycles were running concurrently (i.e. quotidian fever),
giving/

giving the injection when only one of the cycles was at
the T6 stage. It was found that in such cases, the
cycle injected at the T6 stage could be eliminated
within 24 hours, whereas the second cycle being at a
different stage continued in its development until a
day later when it also succumbed to the Mepacrine.

Follow-up of cases given initial Mepacrine injections.

A 6 month follow-up was obtained in 22 of the
cases in which treatment was started by the above
injection method. Of this number 12 suffered from a
recurrence of malaria during this time, most of the
relapses occurring within 2 months from the completion
of treatment. Whereas this appears at first to be an
unduly high relapse incidence, the figures are too
small to draw definite conclusions, but would seem to
indicate that further work along these lines might
prove of interest.

SECTION 5.

PART B. OBSERVATIONS ON SOME FACTORS WHICH INFLUENCE
THE RATE OF CLEARANCE OF ASEXUAL PARASITES
FROM THE PERIPHERAL BLOOD DURING THERAPY.

The following observations are incidental to the
main programme of work but are included in this report
in the hope that they will be considered of sufficient
interest and as they constitute a record of the re-
sponse to therapy of the B.T. parasites encountered in
the cases treated by this Team.

1.   An analysis of parasite counts in 189 Relapse
cases treated on courses R1, (Mepacrine) and R2 + R3
(Quinine plus Pamaquin), shows that a high initial
count is reduced to nil almost as quickly as a low
count by the same course of therapy.   In order to
illustrate this point, the cases have been classified
into those with high, medium and low initial counts,
and the average clearance rates for each of these
groups on Courses R1 and R2 + R3, are shown in Figure
17.   It will be seen that after 48 hours of therapy,
the average parasite counts of all groups fall within
a close range of one another and all have disappeared
at the end of 72 hours.

This implies that in Relapse cases on treatment,
the rate of clearance of asexual parasites is irres-
pective/

FIGURE 17.(page 67).

FIGURE 17. CLEARANCE RATES OF HIGH, MEDIUM AND LOW INITIAL ASEXUAL PARASITE COUNTS IN 189 B.T.(R) CASES.

course R1 (HIM)
courses R2 + R3 (QP)
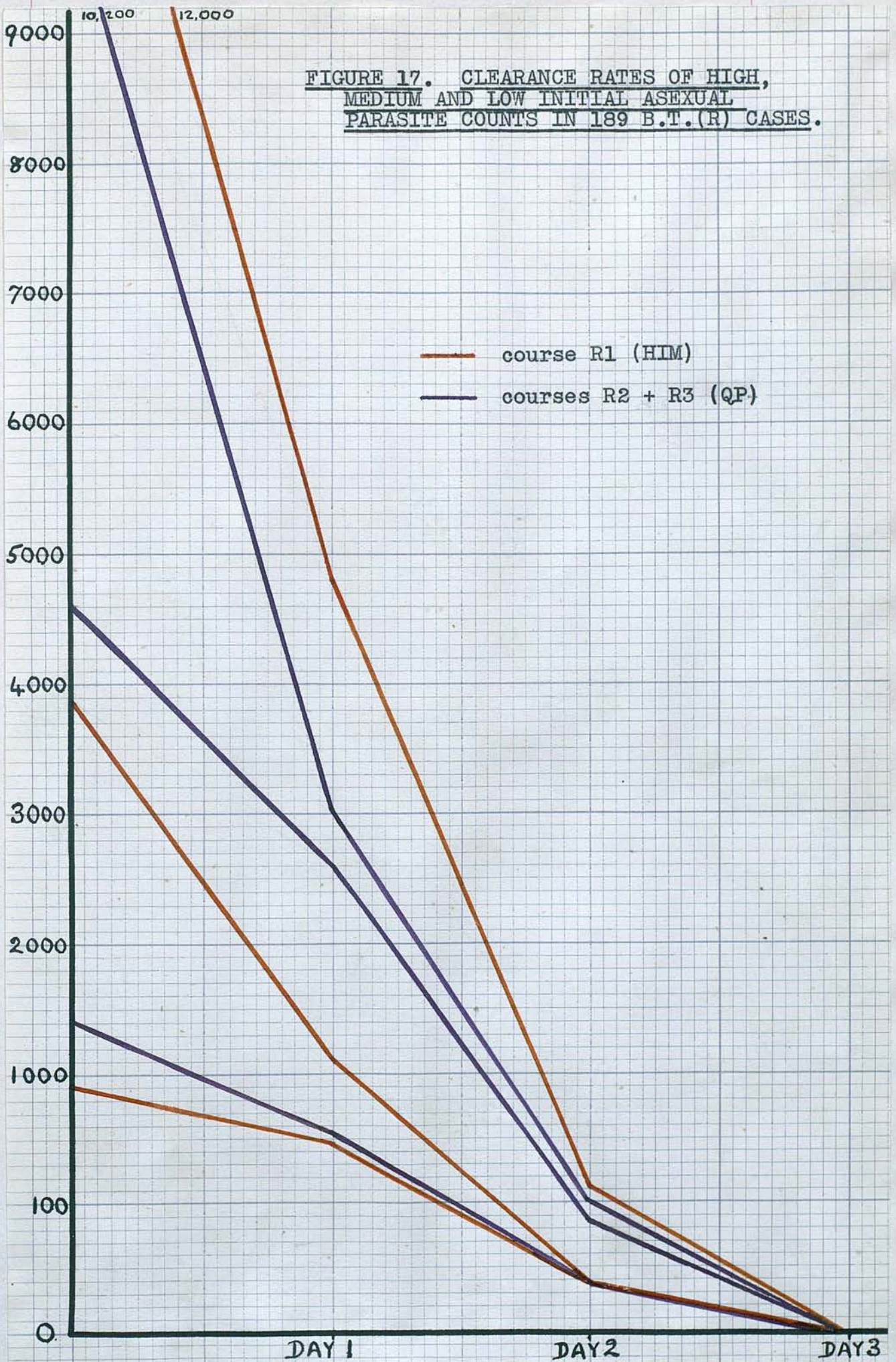
irrespective of the degree of initial parasitaemia.

2.   Among military cases in B.N.A.F. and C.M.F. it was
uncommon to find B.T. cases with initial asexual para-
site counts higher than 20,000 per cu mm. and such
figures did not tend to increase even if no treatment
was given. When it is considered that each B.T.
merocyte usually gives rise to 16 merozoites (Italian
strain), each of which is capable of starting a fresh
generation in a red cell, it follows theoretically
that the parasite count should multiply by 16 times
each 48 hours. The fact that the count remains more
or less constant, implies that the immunity mechanism
of the host is able to cope with 15/16 of the parasites
without the aid of drugs. In actual practice however,
it is usual to find that there is a slight rise in the
parasite count after sporulation, but this raised
count falls rapidly for the first few hours and there-
after decreases gradually as the parasites grow and
become larger until the stage of schizogony is again
reached. Figure 18 shows, in graphic form, an
approximation to the general effects described above
in an untreated case of B.T. Malaria.

It would thus seem that the young trophozoite
stage is a most critical period in the existence of
the B.T. parasite. This may mean that the body defences
are/

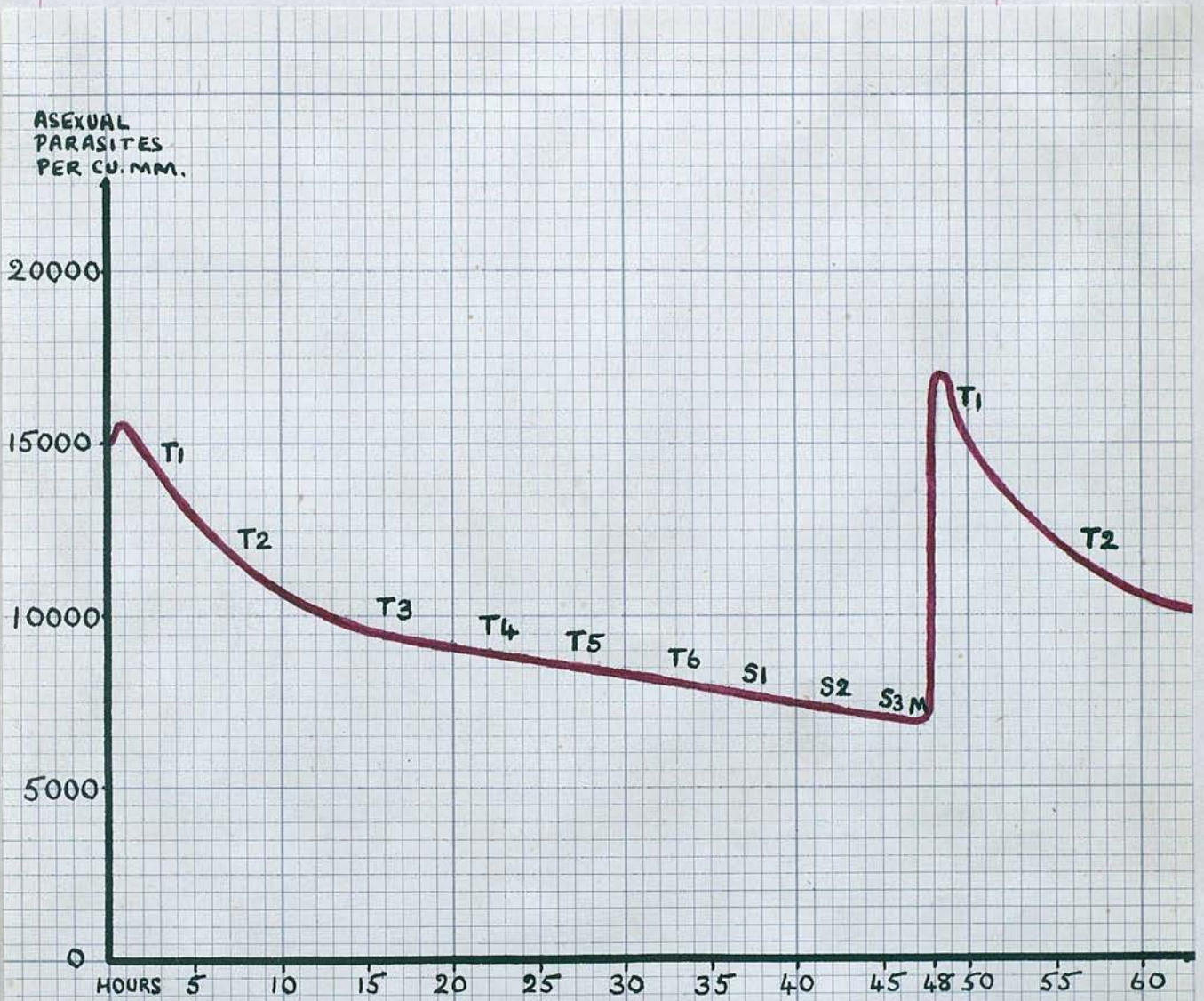
FIGURE 18.(page 68).

FIGURE 18. AN ILLUSTRATION OF THE VARIATION IN THE ASEXUAL PARASITE COUNT IN A MODERATELY HEAVY, TERTIAN, B.T. INFECTION WITHOUT SPECIFIC TREATMENT.

are best able to cope with the parasite at this stage.
Another possibility is that the young trophozoites
disappear from the peripheral circulation to continue
their development elsewhere, but in B.T. Malaria there
seems no reason to suppose that this occurs.

Assuming that the young trophozoite is a most
susceptible stage, it follows that any drug, which did
nothing more than slow the parasite developmental cycle,
would result in a progressive diminution in the parasite
count, since the parasite would not be able to make up
its numbers within the 48 hour period in sufficient
strength to replace those removed by the immunity
mechanism. If the action of the drug were to take
effect at the young trophozoite stage and so produce a
retardation in growth, a much greater fall in the
parasite count could be expected since the young
parasite would be held in check at a time when it is
most susceptible to phagocytosis. An example of this
is seen in Case No.443 where an initial count of
17,000 per cu mm. at stage T1 was reduced to nil
within 24 hours by routine oral Quinine plus Pamaquin
dosage.

3.   Although the young trophozoites (T1) can be shown
as above to be a comparatively susceptible stage of the
parasite, the following observations tend to show that
the slightly older trophozoites, T2, are relatively
resistant/

resistant to therapy.

Quotidian fevers, having two separate cycles of approximately the same number of parasites in each cycle, are useful to show the effect of a given concentration of a drug acting under the same conditions against two different stages of parasites. For example:-

Case No. 310 Course R1.

	Initial	Day 1	Day 2
Parasites/cu mm.	7,200	240	1 seen
Stage	T2 & T4	T3	T4

Case No. 397 Course R1

	Initial	Day 1	Day 2
Parasites/cu mm.	5,000	3000	Nil
Stage	T2 & T4	T3	

The counts given are those taken at the time that treatment was begun. When it is remembered that there is usually a delay before Mepacrine begins to take effect on the parasites, it will be seen that the parasites would have been slightly more advanced than the stages quoted before the drug acted. In the above two cases the late amoeboid trophozoites, present in the initial count, are presumed to have developed into schizonts which were subsequently destroyed, while the more firmly established young trophozoites though somewhat retarded, developed into young amoeboid forms which were found 24 hours later. In Case 310, one trophozoite, still in the amoeboid stage, was found after 48 hours.

Other/

Other examples are 2 cases with semi- continuous fever showing a wide variety of parasites in different stages of development when treatment was started.

Case No. 309   Course R1

	Initial	Day1	Day 2
Parasites/cu mm.	8,900	240	Nil
Stage	T1 T2 T3 T4 S3	T3	

Although 5 different stages were present in the initial count, only young amoeboid trophozoites (T3) were found after 24 hours. This shows the comparative resistance of the growing young trophozoite.

Case No. 240   Course R1

	Initial	Day 1	Day 2	Day 3	Day 4
Parasites/cu mm.	10,500	4,800	500	30	Nil
Stage	T1 T2 T3 T4 T5	T3	T4	T6	

This case shows not only the resistance of the growing trophozoite compared with the other forms, but also the inhibition of development due to Mepacrine.

The above observations help to explain why treat-ment commenced either at the stages of very young or very late trophozoites, often brings about a rapid clearance of parasitaemia, whereas such rapid dis-appearances are seldom seen when treatment is started at the stage of growing trophozoites.

4./

TABLE 8.(page 72).

COURSE R1 (MEPACRINE)				COURSES R2 + R3 (QUININE AND PAMAQUIN)			
CASE No.	INIT-IAL COUNT	DAY 1.	DAY 2.	CASE No.	INIT-IAL COUNT	DAY 1.	DAY 2.
374	6,700 T_4	2,000 $S_2S_3T_1T_2$	Nil.	362	4,100 T_4	Nil.	Nil.
376	1,300 T_4	80 S_2T_2	Nil.	369	5,700 T_4	220 T_5T_6	Nil.
389	3,600 T_4	120 $T_6S_1S_2T_1$	Nil.	382	4,000 T_4	2,220 T_6S_1	3 Seen
437	4,000 T_4	2 Seen T_1 T_2	Nil.	393	5,200 T_4	2,400 T_6	Nil.
228	120 T_4	Nil.	Nil.	371	2,300 T_4	1,500 T_6	1 Seen
263	7,100 T_4	20 T_1T_2	Nil.	406	4,000 T_4	2,000 T_6	Nil.
313	800 T_4	180 $T_6S_1T_1$	Nil.	407	3,500 T_4	600 $T_6S_1S_2T_1$	Nil.
360	3,700 T_4	360 T_2	Nil.	410	2,600 T_4	640 T_6S_1	Nil.
259	17,000 T_4	480 $T_6T_1T_2$	Nil.	161	11,000 T_4	5,200 $T_5T_6S_1S_2$	Nil.
221	160 T_4	50 T_5T_6	Nil.	168	2,800 T_4	580 T_5	Nil.

NOTES.	NOTES.
8 cases show young trophozoites on Day 1.	1 case only shows young trophozoites on Day 1.
Only very small delay in the division and young schizont stage	Many parasites delayed at the division and young schizont stage.
Great general fall-off on Day 1.	Small general fall-off on Day 1.

TABLE 8. A comparison between the effects of Mepacrine and Quinine plus Pamaquin when treatment is started at the T_4 stage of the asexual parasite cycle. (20 cases of B.T. Relapse Malaria.)

4.   A comparison of the effects of Mepacrine alone and
Quinine plus Pamaquin when given to a group of cases
in which the stage of parasite at the start of treatment
was the same, is recorded in Table 8. The principle
differences observed after 24 hours are noted at the
foot of the Table. This shows that in the 10 cases
treated on Mepacrine, schizogony was not prevented so
that young trophozoites were found 24 hours later,
whereas in the 10 cases treated on Quinine plus Pamaquin,
parasite development was inhibited before schizogony
in all but one case.

It would seem from the above comparison that the
differences noted are not so much related to a variance
between the mode of action of Mepacrine and Quinine
plus Pamaquin, but rather that the latter therapy course
is more rapid in its initial action on the parasite.
It is necessary to administer Mepacrine at an earlier
stage of the parasite development to produce a similar
effect to that seen in the Quinine plus Pamaquin group
noted above.

SECTION 6.

THE "RELAPSE FACTOR" IN BENIGN TERTIAN MALARIA.

It has been consistently observed that in B.T.
Relapse infections a comparatively high initial asexual
parasite count following a short period of premonitory
symptoms, is a characteristic finding. In contrast with
this, B.T. Fresh infections have been noted to have a
low initial asexual parasitaemia with a much longer
period of antecedent clinical manifestations.

In this respect it was considered interesting to
correlate the initial parasite count with the days of
symptoms in the form of a simple mathematical expression.
This was termed the "Relapse Factor", "R", and is
obtained as follows:-

$$R = \frac{\text{Initial asexual parasite count per cu.mm.}}{\text{Days of symptoms x 100}}$$

Examples.

Fresh Case.　　Initial count = 800 per cu.mm.
　　　　　　　　Days of symptoms = 4.

$$R = \frac{800}{4 \times 100} = 2.0$$

Relapse Case.　Initial count = 7,000 per cu.mm.
　　　　　　　　Days of symptoms = 2.

$$R = \frac{7,000}{2 \times 100} = 35.0$$

In effect the Relapse Factor is a measure of the
degree of peripheral asexual parasitaemia that the
individual/

individual is able to withstand before feeling acutely ill, and thus possibly represents the tolerance to the malarial toxin.

It was hoped that a correlation would be found between this factor and some other feature of the disease and that the factor would also provide additional information in cases where there was doubt as to whether the infection was Fresh or Relapse. On taking the Relapse Factors in 156 Fresh cases and in 254 Relapse cases of B.T. Malaria, it is seen that cases having a Factor of less than 15 are mostly Fresh infections, while nearly all the Relapse infections have a Factor higher than 25. Apart from this observation, no other application has so far been found between the Relapse Factor and other characters of Malaria infections.

The Relapse Factor is not uniformly high in all Relapse cases, or invariably low in Fresh infections, and while the average figures are consistent, some inexplicable deviations from the general rules are found. It should be noted however that the criterion of Fresh or Relapse is based upon a clinical history and hence no absolute certainty is possible under these circumstances.

Figure 19 shows graphs of the average Relapse Factor of all Fresh and Relapse cases for each month from/

FIGURE 19.(page 74).

FIGURE 19.   MONTHLY RELAPSE FACTORS
JULY 1944 - MAY 1945.
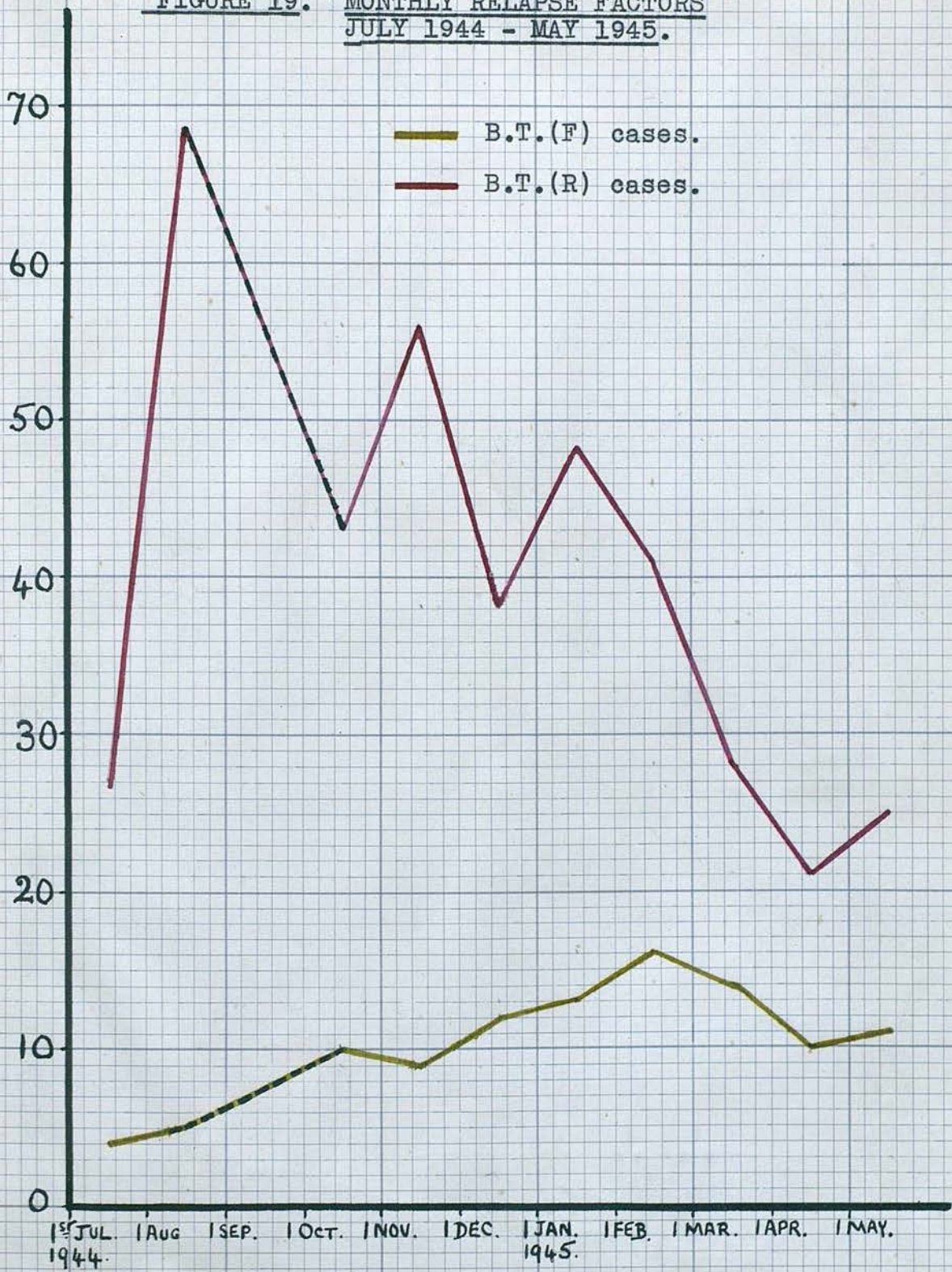
Dotted line represents period during Sept. 1944
when no cases were investigated.

from July 1944 to May 1945.   (The figure for September,
1944, was not obtained as the Research Team was moving
from North Africa to Italy during that month).   It will
be seen that the very high Factors in the Relapse
cases from August to November, fell gradually through-
out the Winter and Spring until April when they were
but little higher than the Factors for the cases who
gave no previous history of Malaria.   The Factors for
the latter cases had in contrast tended to increase
gradually over the same period.   It would have been
interesting to see whether the two groups would have
again diverged during the Summer months.

Until 15th November, 1944, the troops in the areas
in which this work was carried out were issued with
0.1 gm. suppressive Mepacrine daily.   The highest
Factors in the Relapse cases thus corresponded not
only with the height of the Malaria season and hot
weather but also with the suppressive Mepacrine.   If
the high Factors do bear any relationship to the latter,
then this would imply that relapses occurring while
under the influence of suppressive Mepacrine manifest
themselves with great suddenness and with a heavy
parasitaemic infection.   So few patients openly admit
that they have not taken any suppressive Mepacrine,
that an insufficient number of such cases are available
for comparison with the above.   It can be stated, how-
   ever/

however, that most of the cases with very heavy B.T.
infections that have been studied by this Team, have
had some suppressive Mepacrine, and that few heavy
parasitaemias have been encountered amongst those who
have had little or no suppressive Mepacrine.

SECTION 7.

CHRONIC RELAPSING CASES.

Cases of Malaria with multiple recurrences occurring in C.M.F. were sent to one of two centres for investigation and treatment. Cases from Sicily and Southern Italy went to No. 92 (Br) General Hospital in Naples and cases from Central and Northern Italy were directed to No.2 Malaria Research Team in Rome. A total of 116 cases (comprising 112 B.T. infections and 4 M.T. infections) of this category were dealt with by this Team between October, 1944, and May, 1945. It is felt that the observations and results in the group of B.T. cases are worth recording briefly under the headings below.

1.   Number and frequency of attacks.

Of the 112 recurrent B.T. cases, 105 had had 5 or more attacks, 14 had had 10 or more attacks and 4 had had 14 or more attacks. The average number of attacks for all cases was 7.

In the majority of the cases the primary attack and the recurrences had occurred over a period of 12 - 15 months, and many patients had contracted their first infection during the Summer campaigns of 1943 in Sicily and Southern Italy.

It was interesting to note how infections contracted/

contracted in certain areas about the same time had a particular tendency to repeated relapses and this may probably be explained as being a characteristic of the strain of the local infecting parasite. One Company of specialised troops, who for operational reasons spent two nights near a river in the Barletta area in September, 1943, had an especially high incidence of cases with multiple recurrences, and several of their men are included in the group reported here as having had more than 10 attacks. Another strain of parasite with a tendency to several relapses was encountered in cases who contracted their primary infection in the Anzio area during August and September of 1944.

2.   Clinical Notes.

Nearly all the cases of this series were referred for investigation by other medical units who had given them the standard C.M.F. Malaria treatment (same as Course 5). They thus arrived in a quiescent phase of their infection and had usually already been 7 to 10 days in hospital.

The clinical findings in these cases show that in general the recurrent B.T. infection was much more of a "nuisance disease" than a cause of chronic ill-health or cachexia. Many of the cases were sent to the Research Team for special treatment on account of their repeated travels in and out of hospitals which rendered/

rendered them unsuitable to be counted against the
effective strength of their units. Cases of this
nature had few symptoms between attacks and their
general health appeared to have suffered little. Other
cases complained of gradual loss of weight, easy
fatigue, headache, and inability to stand cold. A mild
degree of hypochromic anaemia was present in many such
cases and responded slowly to iron therapy.

Splenic enlargement could be detected on physical
examination in about 50% of all these cases, but the
finding of a really large and hardened spleen was
present in only 3 or 4 cases of the whole group.
In some cases with large spleens, radiographs taken
before, and at intervals after the subcutaneous in-
jection of 0.5 cc Adrenalin showed that the spleens
were capable of contracting down to about one third
of their size within 10 minutes of the injection.

3.   Treatment.

It was decided to use these cases for a trial of
Mepacrine versus Quinine plus Pamaquin in the treatment
of chronic B.T. Malaria. Two lines of therapy were
therefore adopted and patients were treated on these
in rotation.

The 55 cases treated on Mepacrine were first
given a repeat of the Mepacrine dosage of the standard
C.M.F./

C.M.F. course and the plasma levels attained during
this were estimated. Cases in whom these levels reached
a satisfactory value, were given no further Mepacrine
for 7 days and were sent to a Convalescent Depot nearby.
While there, they were given two further courses of
oral Mepacrine comprising 0.2 gm T.I.D. for 5 days with
an interval of 7 days between these courses. They
thus received a total of 8.5 gm of oral Mepacrine over
a period of 29 days. Cases who failed to show satis-
factory plasma Mepacrine levels, were given intra-
muscular injections of Mepacrine and further levels
estimated as described in Section 8 of this report.
These cases were also sent to the Convalescent Depot
for the same oral dosage as given to the previous
group and thus all of them received a minimum of 8.9
gm. of Mepacrine of which at least 0.4 gm. had been
given intramuscularly.

The other group of 50 cases were given an initial
course of Quinine 10 grains plus Pamaquin 0.01 gm T.I.D.
for 7 days. They then received no treatment for 7 days
and were sent to the Convalescent Depot. There they
were given two further courses of Quinine 10 grains
plus Pamaquin 0.01 gm T.I.D. for 5 days with an interval
of 7 days between these courses. They thus received
a total dosage of Quinine 510 grains and Pamaquin
0.51 gm given over a period of 31 days. None of these
cases was given any additional treatment.

It/

It will be noticed that an intermittent dosage
regime was adopted in these chronic cases. This method
was chosen for three main reasons. First, it was felt
that a trial should be made of the value of intermittent
Mepacrine as against intermittent Quinine plus Pamaquin
in contrast to prolonged continuous courses with these
drugs. Secondly, any undesirable effects likely to be
produced by prolonged administration would be minimised
by this method. Thirdly, it was considered that all
these patients would benefit greatly from a period of
3 to 4 weeks supervised convalescence, and method of
dosage allowed treatment to be spread over this time.

No toxic manifestations were encountered in any
of the cases during treatment with Mepacrine or Quinine
and Pamaquin and the patients were allowed to remain
ambulant throughout the courses. It can be recorded
here that many patients receiving the Quinine plus
Pamaquin therapy stated, after about 10 days treatment,
that they had not felt so well for a long time.

4.   Follow-up of Chronic Cases.

The same follow-up system was adopted for these
recurrent cases as was used for the active infections
treated on the routine therapy courses. The follow-up
took place mainly during the Winter and Spring months
when the chances of re-infection were minimal.

It/

It was possible to obtain 6-month follow-up particulars from 72 of the 105 patients who were treated on the intermittent therapy courses of Mepacrine or Quinine plus Pamaquin. The results are shown in Table 9.

TABLE 9.

Therapy Course.	Intermittent Mepacrine.	Intermittent Quinine-Pamaquin.
Number treated	55	50
No. followed for 6 months.	38	34
RELAPSE.	17	NIL. *
NO RELAPSE.	21	34

* One man wrote that he had felt shivering and had treated himself with a few doses of an Italian patent medicine. Another patient was subsequently admitted to hospital as a suspected case of malaria but no parasites were found on blood examination.

In the 17 patients who relapsed after the Mepacrine course, the recurrence took place after an average period of 7.5 weeks following the completion of treatment.

The above figures are strikingly in favour of a 30 day course of intermittent Quinine plus Pamaquin therapy as being a remarkably effective treatment for recurrent B.T. Malaria. Further reference to the success/

success of this course will be made in PART FOUR of
this thesis, when discussing the general conclusions
as to the value of Pamaquin in the prevention of B.T.
relapses.

SECTION 8.

ESTIMATIONS OF PLASMA MEPACRINE LEVELS.

From October, 1944, until January, 1945, the
Malaria Research Team was co-operating with No.1 Field
Section of the Malaria Research Unit who went to
Italy from Oxford to study plasma Mepacrine levels in
cases of Malaria and particularly in those cases where
the infection had appeared during the taking of sup-
pressive Mepacrine. It was arranged that the Field
Section should also carry out plasma Mepacrine estima-
tions on the cases being treated by the Research Team
in their therapeutic trial and that patients being
treated on account of multiple recurrences of Malaria
should have their Mepacrine absorption investigated.

When No.1 Field Section returned to U.K. in January,
1945, the special equipment required for Mepacrine
estimations was taken over by the Research Team, but
as it was necessary to wait for a new Ultra-violet lamp
for the Hilger fluorimeter to be sent from U.K., it
was not possible to begin Mepacrine estimations until
22nd March, 1945. These were done from 22nd March
until 23rd May when the Team ceased work in Italy.
The small total number of 330 estimations done during
this period was determined by the fact that no further
personnel were available for the extra laboratory work
involved/

involved. The results obtained in this somewhat limited study may be briefly discussed under 3 headings. The plasma Mepacrine levels were estimated by a modification of Mason's method (J. Biol. Chem. 148. 1943) and a Hilger Spekker photo-electric fluorimeter was used.

1. Plasma levels during therapy courses.

Estimations were made of the plasma Mepacrine levels attained at intervals throughout the therapy courses Nos. 1, 2, 3, 4, 5 and R.1. The results were similar to those obtained earlier by the Field Section and showed that an average plasma Mepacrine level of 40 to 45 micrograms per litre was reached after 48 hours administration of Mepacrine in all the treatment courses, and that such a level was maintained fairly steadily thereafter. Differences in initial dosage, high or low, affected the rate at which the steady plasma level was attained, but did not materially alter the equilibrium figure ultimately established. Considerable individual variations occurred from the average levels quoted but in general it was not found possible to detect any obvious correlation between this evidence of Mepacrine absorption and other findings such as the rate of clearance of asexual parasitaemia or subsequent tendency to relapse. It is of interest to record in passing that a plasma Mepacrine level of 25 to 30 micrograms per litre was found by the Field Section/

Section and other workers to be the average level
achieved on a routine daily suppressive dosage of 0.1
gm. Mepacrine. Here again, however, there is apt to be
a wide margin of individual variation, which may be an
important factor in those cases who develop an acute
attack of B.T. malaria while adhering strictly to the
regular suppressive dosage.

2.   Plasma levels in cases injected with Mepacrine at
     the T6 stage of the parasite.

A preliminary investigation was made of the plasma
levels attained in 8 cases who received 0.2 gm. Mepacrine
by intramuscular injection at the T6 stage of the B.T.
parasites and who were given no oral Mepacrine during
the subsequent 16 hours. Levels were taken at $3\frac{1}{2}$ hours
and 16 hours after the injection but in the 1 case
done by the Field Section, levels were obtained at
$\frac{1}{2}$, 1, 2, 4, 8 and 12 hours after injection.

These results, though few in number, are interest-
ing because they can be compared with the fall-off in
asexual parasite count which occurs so rapidly when
Mepacrine is injected at the T6 stage. Figure 20 shows
graphs of the plasma levels attained and rate of clear-
ance of asexual parasites that occurred in these 8 cases.
These findings suggest that a high concentration of
Mepacrine in the plasma is reached half an hour after
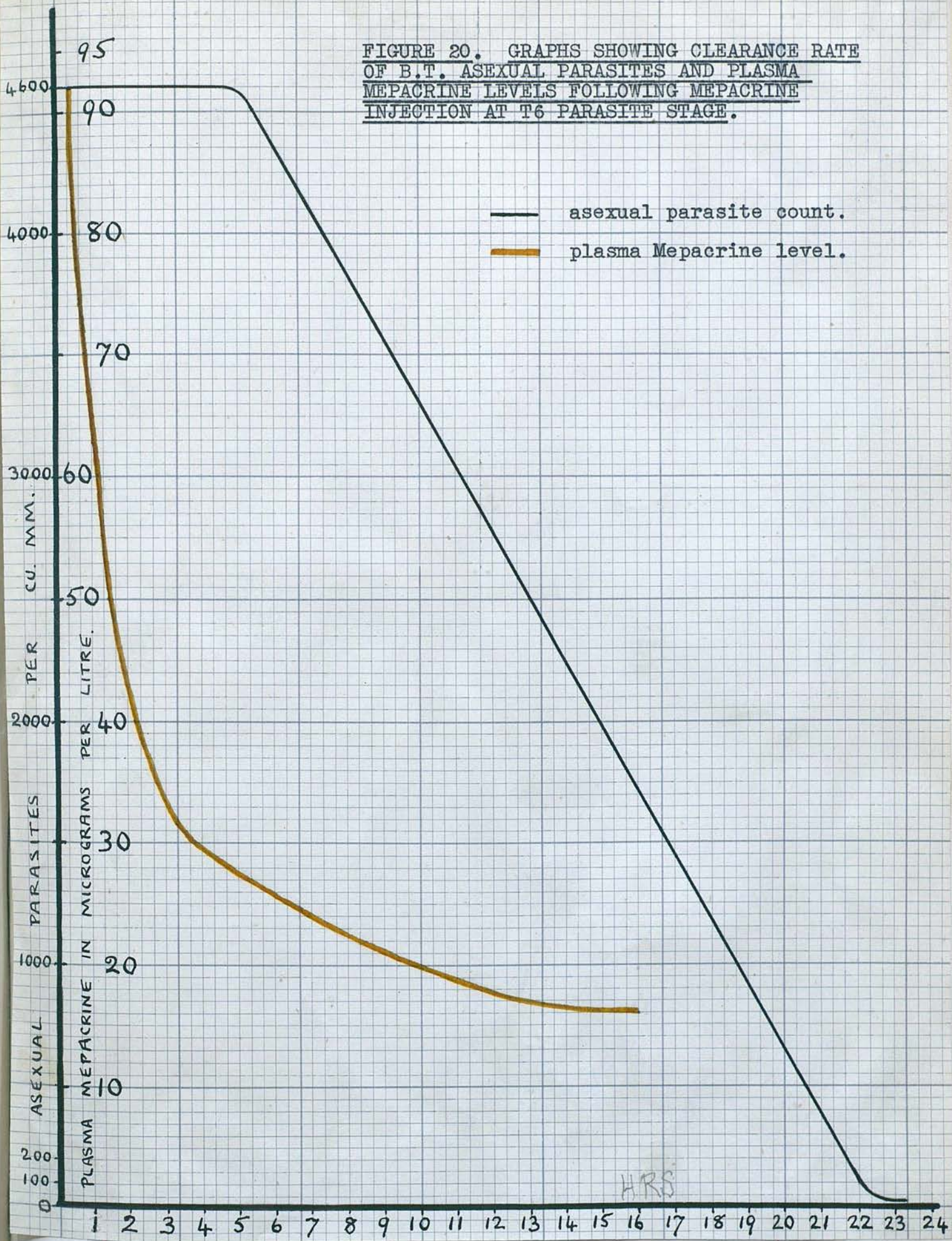injection but falls within $3\frac{1}{2}$ hours and continues to
fall/

FIGURE 20.(page 86).

FIGURE 20. GRAPHS SHOWING CLEARANCE RATE OF B.T. ASEXUAL PARASITES AND PLASMA MEPACRINE LEVELS FOLLOWING MEPACRINE INJECTION AT T6 PARASITE STAGE.

fall until 16 hours which is the last recorded level. The maximal parasite clearance effect occurs during the period from 5 to 12 hours after the injection. The patients studied remained afebrile during this time.

Studies by Shannon and other workers on the distribution of Mepacrine in the blood, show that the fall in the initially high plasma level is brought about by the transference of the drug to the circulating red and white blood corpuscles and also to the tissues. Whereas insufficient cases have been studied by the Research Team to draw any definite conclusions, it would appear likely that the time of parasite clearance observed, corresponds with this transfer of the drug from the plasma to the parasitised red cells. Further work along this line might prove of interest in the study of the action of Mepacrine or other anti-malarial compounds.

3. Plasma Mepacrine levels in cases with multiple recurrences of B.T. Malaria.

Plasma Mepacrine levels were estimated in a group of patients who had suffered from multiple recurrences of B.T. malaria and who were treated with further Mepacrine courses to investigate whether failure of absorption of the drug might be a factor in allowing their infection to relapse so frequently. A number of such cases was studied by the Field Section who found that these patients did in fact attain plasma levels which/

which were lower than average while being treated with
the standard therapy dosage. Similar results were
obtained in a smaller group of cases investigated by
the Research Team and the following example is worthy
of special comment.

Case No. 116.C. B.T. Malaria 14 attacks between
September 1943 and March 1945. He was given oral
Mepacrine 0.2 gm Q.I.D. for 2 days at the end of which
his plasma Mepacrine level was found to be nil. The
fact that he took the tablets was established. After
3 further days on oral Mepacrine 0.1 gm T.I.D. the plasma
level was 4 micrograms per litre. In each of the 2
following days he was given an intramuscular injection
of 0.2 gm Mepacrine and the levels taken 18 hours after
each injection were 5 and 18 micrograms per litre
respectively. This case is quoted as an example of
how Mepacrine given in the usual therapeutic dosage
may be present in the plasma in very low concentration
in cases of recurrent Malaria.

8 of the cases of recurrent B.T. Malaria in whom
the plasma Mepacrine levels were estimated by No.1
Field Section during therapy are known to have relapsed
subsequently. The average plasma levels attained by
this group while on the standard course of oral
Mepacrine were 36.0 and 36.5 micrograms per litre as
compared with levels of 44.8 and 40.2 micrograms per
litre/

litre which were attained after the same dosage of a
similar course by a group of cases undergoing routine
treatment. Of these 8 cases, 6 had received two intra-
muscular injections of 0.2 gm Mepacrine at the end of
the 5 days oral course and the average plasma levels
on the days following these injections had been 41.0
and 46.0 micrograms per litre respectively. In this
small group who relapsed, the additional raising of
the plasma Mepacrine level by injection did not appear
to have been efficacious in preventing a recurrence.

PART TWO.

AN INVESTIGATION OF THE ACTION OF
PAMAQUIN AND PALUDRINE (M.4888) IN THE
THERAPY OF BENIGN TERTIAN MALARIA.

SECTION 1.   Introduction.

In the Summer of 1945 the Malaria Research Team
returned to Britain from Italy and was re-equipped to
proceed to India for the study of problems of interest
in malaria therapy in the Far East.   The sudden end of
hostilities against Japan altered this programme as it
became apparent that with the cessation of active
military operations in the East, the local problems of
malaria would become little more than those of string-
ent mosquito control and personal protection amongst
relatively static troops in selected locations.
Following the institution of rigid Mepacrine discip-
line among such troops, the incidence of malaria,
especially M.T. infections, had declined to a minimal
level.   It was felt therefore that little was to be
gained by sending a Research Team to work on the
prevention and treatment of fresh infections, particul-
arly when the more acute operational demands for
conserving manpower no longer existed.   On the
other hand, the Services would at once begin to return
to Britain in their thousands, troops who had lived in
malaria-ridden lands and who would be returning home as
reservoirs/

reservoirs of Mepacrine-suppressed Plasmodia from which much recurrent sickness could be expected to arise. There were also large numbers of those liberated from Japanese prison camps to be cared for, who had received little or no treatment for malaria during over three years of imprisonment in hyper-endemic areas of infection.   It was therefore decided by the War Office, in consultation with the Medical Research Council, that the Research Team could be most usefully employed in the treatment of cases of malaria amongst personnel returned from overseas and that such work would provide an opportunity for the further study of anti-malarial compounds with particular reference to their effect in producing a radical cure of the infection.

In September 1945 the Team was accordingly attached to the Royal Herbert Military Hospital at Woolwich where excellent clinical and laboratory facilities were available and which served a Garrison area in which large numbers of troops were stationed on their return from service abroad.   It was decided to work only on cases of Benign Tertian malaria as this type was the one most likely to prove a recurrent "nuisance" disease and the chances of continued activity of Malignant Tertian infections were remote after prolonged suppressive Mepacrine dosage, which had been continued during the voyage home and for four weeks after disembarkation for all personnel.   The Team started work at Woolwich early/

early in October 1945 and it was found that the number
of cases admitted and suitable for therapeutic research
was actually greater than the number available for
investigation in Rome during the corresponding months
of 1944.

The programme of work was devoted mainly to the
study of the action of two drugs, Pamaquin and the new
synthetic compound Paludrine (M.4888).   Pamaquin was
selected for special attention as of all the many anti-
malarials tried out in various parts of the World, it
alone had been found to lower consistently the relapse
rate in Benign Tertian malaria.   Much work also had
been started in America on the pharmacology of Pamaquin,
and preliminary reports showed that a great deal of
promising information was likely to be learned from a
study of the blood concentration and urinary break-down
products of this drug.   Finally it was desired to con-
tinue and confirm the observations made by the Team in
Italy on the action of Pamaquin on asexual B.T. para-
sites during an active infection.

It is possible to report in this thesis the
results of the therapeutic trial of Pamaquin when used
as the only anti-malarial drug in the treatment of a
series of cases of B.T. malaria.   As this work was
only started in the Autumn of 1945, follow-up figures
over a period of six months are not available and up
to the time in January 1946 when the author left the
Team/

Team on being released from the Army, only the initial
stages of the biochemical investigation of Pamaquin
had been planned and started.   A brief mention of the
latter work will be given however, as it will indicate
the present trend of thought in regard to Pamaquin and
show how this previously much-maligned drug is at last
taking a place in the limelight of malaria therapeutic
research.

The other drug with which the Team was working at
Woolwich, was the compound M.4888, later designated
"Paludrine",which had been produced in 1945 by Imperial
Chemical Industries (Pharmaceuticals) Ltd. at their
research department in Manchester.   This drug, one of
a very large series of compounds tested for anti-malar-
ial activity, was synthesised by Rose & Curd and was
tried out in the treatment of chicken malaria by Davey,
working in the same laboratories.   It was found to be
quite outstanding from other drugs in being able, in
small non-toxic doses, to arrest not only the blood
forms of the parasites, but also the extra-erythrocytic
tissue phases of the infection that are the usual cause
of death in chicken malaria.   Although the latter forms
re-appeared when dosage with M.4888 was discontinued
and proved fatal through blockage of cerebral capill-
aries, the mere fact that the drug was capable of acting
on the tissue forms when given in therapeutic doses,at
once attracted attention and suggested that it might
prove/

prove worthy of trial in human infections.   While the
existence of extra-erythrocytic parasites in human
malaria has never been demonstrated,there are strong
reasons for believing that they must be present, on
the grounds of analogy with avian malaria, on the fact
that there is still a missing link between the mosquito-
injected Sporozoite stage and the young Trophozoites
in the blood, and as the resistant forms responsible
for relapses long after all traces of parasitaemia
have been cleared by treatment.   Any drug therefore
which might act on these hypothetical tissue parasites
in addition to killing the asexual stages in the blood,
would therefore be of great interest and possible value
in the treatment of human malaria, and especially the
types of infection most prone to relapse.

M. 4888 was first tried on cases of human malaria
at the Liverpool School of Tropical Medicine and was
found to be very successful in the treatment of the
clinical attack in both B.T. and M.T. infections.   The
drug, administered orally in the form of tasteless,
white tablets was effective in clearing parasitaemia in
dosage as low as 10 Mgm. given twice daily.   It pro-
duced no toxic manifestations, other than occasional
nausea and epigastric discomfort within a few minutes
of swallowing, in doses as high as 500-700 Mgm. twice
daily and so appeared to be a drug with a very con-
siderable margin of safety between the effective
therapeutic/

therapeutic dose and the toxic dose.   In the control

of malaria fever and termination of the clinical attack,

Paludrine was at least equal to Quinine and Mepacrine

and with the additional advantage of low toxicity,

promised to be a real advance on other anti-malarials.

In one respect only, were the early hopes of it not

fulfilled, for B.T. cases treated with full courses of

the new drug continued to show a relapse rate at

least as high as with Quinine or Mepacrine.

Following the initial trial at Liverpool,

supplies of Paludrine were made available for more

extensive investigation by Army Research Teams.   One

series of cases was treated at Colchester where the

relapse incidence in B.T. infections was shown to com-

pare unfavourably with a control series treated on

Quinine plus Pamaquin, although the drug worked well

in the control of the active malaria attack.   Another

batch of the compound was sent to Brigadier Hamilton

Fairley working in Australia and was used in experiments

to evaluate its action as a prophylactic against

mosquito-borne malaria infections.   Fairley's work

showed that Paludrine, in doses as low as 25 Mgm. a

day, was a true causal prophylactic against Malignant

Tertian malaria, acting apparently on the primary

tissue stages of the parasite so that blood stream

infection did not develop.   That in itself was a

tremendous advance over other drugs and ensured for

Paludrine/

Paludrine a lasting place of the greatest value in
the prevention of malaria.   It meant, in short, that
by taking one tablet daily (Fairley's recommended
dose = 100 Mgm.), immunity from M.T. malaria was
ensured.   It is doubtful whether the implications
of these facts have yet been widely realised, as the
results are still unpublished and the supplies of the
drug still limited to experimental use;   but it is
indeed the dawn of a new era in tropical medicine
when what is perhaps the most widespread fatal infect-
ion of all, can be prevented by a single daily tablet.

In B.T. infections, Fairley found that Paludrine
was not a complete causal prophylactic, in that it
did not prevent mosquito-borne Sporozoites from estab-
lishing asexual parasites in the blood-stream, although
the latter were suppressed for as long as the drug
was administered, in the same manner of suppression as
with Mepacrine.   His results in the therapy of both
M.T. and B.T. acute cases with M. 4888, were in agree-
ment with the Liverpool work and he observed that in
both infections the drug exerted its action by being
a powerful schizonticide.   The above findings by
Fairley's Team were recorded in a Progress Report of
that unit in February 1946 and work with Paludrine
is being continued.

The third therapeutic trial with Paludrine was
allocated to the Malaria Research Team at Woolwich
and/

and it was decided to adopt two lines of work.   The
first of these was to study on a relatively small
series of cases, the precise mode of action of the
drug on the parasites of active B.T. infections, using
methods of parasite counting and observation as had
been employed in Italy to study the effects of Mepacrine.
The results of this investigation, the first of its
kind, were reported to the Medical Research Council
in December 1945, and will be recorded in this thesis.
The second line of work was to examine the possibility
of combined therapy with Paludrine and Pamaquin and
to see whether the two drugs might have an additive
effect in reducing the relapse rate in B.T. malaria
cases.   The early stages of this work are also
mentioned in this thesis.

It was realised that since most of the cases
seen at Woolwich had contracted malaria in India or
Burma, it might not be possible to draw conclusions
as to the comparative efficacy of drugs on the basis
of previous studies made in dealing with Italian and
North African strains of parasite.   A short pre-
liminary study was therefore made to determine the
essential features of the parasites encountered in
the cases at Woolwich and the effects of Mepacrine
therapy in producing microscopic changes and clearance
of/

of parasitaemia, were noted in a short series of cases.
Thereafter the ten-day Quinine-Pamaquin course (R2)
was adopted as a control treatment for the subsequent
work.

Another point to which attention was paid in
the investigation at Woolwich, was to select for the
purposes of trial therapy, only those cases in which
the degree of parasitaemia and other features indicat-
ed that spontaneous recovery was unlikely to result
within a few days in the absence of specific treat-
ment.   This was important as many of the patients
had acquired a considerable immunity from previous
attacks and those who were having their first
Mepacrine-delayed attacks, might still have retained
sufficient of the drug to modify the clinical course
of the acute phase.   Evidence of a mounting or
sustained asexual parasitaemia over a period of 48
hours, was therefore adopted as the main criterion for
the acceptance of a case as being suitable for study.

SECTION 2.   The Action of Pamaquin in

the treatment of B.T. Malaria.

28 cases of active Benign Tertian malaria were
studied during treatment with Pamaquin, given in a
dosage of 0.01 gramme three times daily for 10 days.
No other anti-malarial drug was given to these patients.
The response to therapy was noted clinically and by
observations on thick blood films examined at 24 hour
intervals, recording the changes in the parasites as
well as the asexual and sexual parasite count per
cubic millimetre.   The results obtained may be con-
sidered conveniently under the following headings.

1.   Clinical Response.

The drug was well tolerated during the acute,
febrile stages and caused no vomiting or other
disturbances.   The average duration of pyrexia after
the start of treatment with Pamaquin was 3.0 days, as
compared with 2.0 days in the control cases treated
with Quinine plus Pamaquin.   The patients were allow-
ed up after the temperature had remained normal for
48 hours and in no case did the drug need to be stopp-
ed on account of toxic manifestations.   In 2 cases
there was slight cyanosis evident during the last 3
days of the 10-day course, and 3 patients complained
of mild abdominal discomfort which appeared to be
associated with fairly marked splenic enlargement.
Leucocyte counts, done repeatedly during treatment in
connection/

connection with the parasite counts and Haemoglobin
estimations at the start and end of therapy, showed
no evidence of toxic suppression of haemopoietic activ-
ity and no sign of increased blood destruction.
There were no abnormal urinary findings.

The general clinical impression formed was that
Pamaquin alone was quite capable of controlling the
malaria fever, although it was slower in producing
this effect than Quinine, Mepacrine, or Quinine and
Pamaquin given together.

2.   Clearance of Asexual Parasitaemia.

The average duration of treatment required to
clear asexual parasites from the peripheral blood
was 3.1 days, as compared with 1.5 days for the con-
trol Quinine-Pamaquin cases.

Figure 21 shows the rate of disappearance of
asexual parasites on Pamaquin therapy by plotting the
average of parasite counts done at 24 hour intervals
from the start of treatment.   A logarithmic scale
has been used in this graph.

Figure 22 indicates the percentage number of
cases in which asexual parasites were present on each
day of treatment and shows that, in the majority of
cases, clearance of parasitaemia was complete after
48 hours.

3/

FIGURES 21,22.(page 100).

FIGURE 21. CLEARANCE RATE OF ASEXUAL
PARASITAEMIA ON PAMAQUIN THERAPY(28 B.T.CASES)

——— Pamaquin cases.

——— control QP cases.
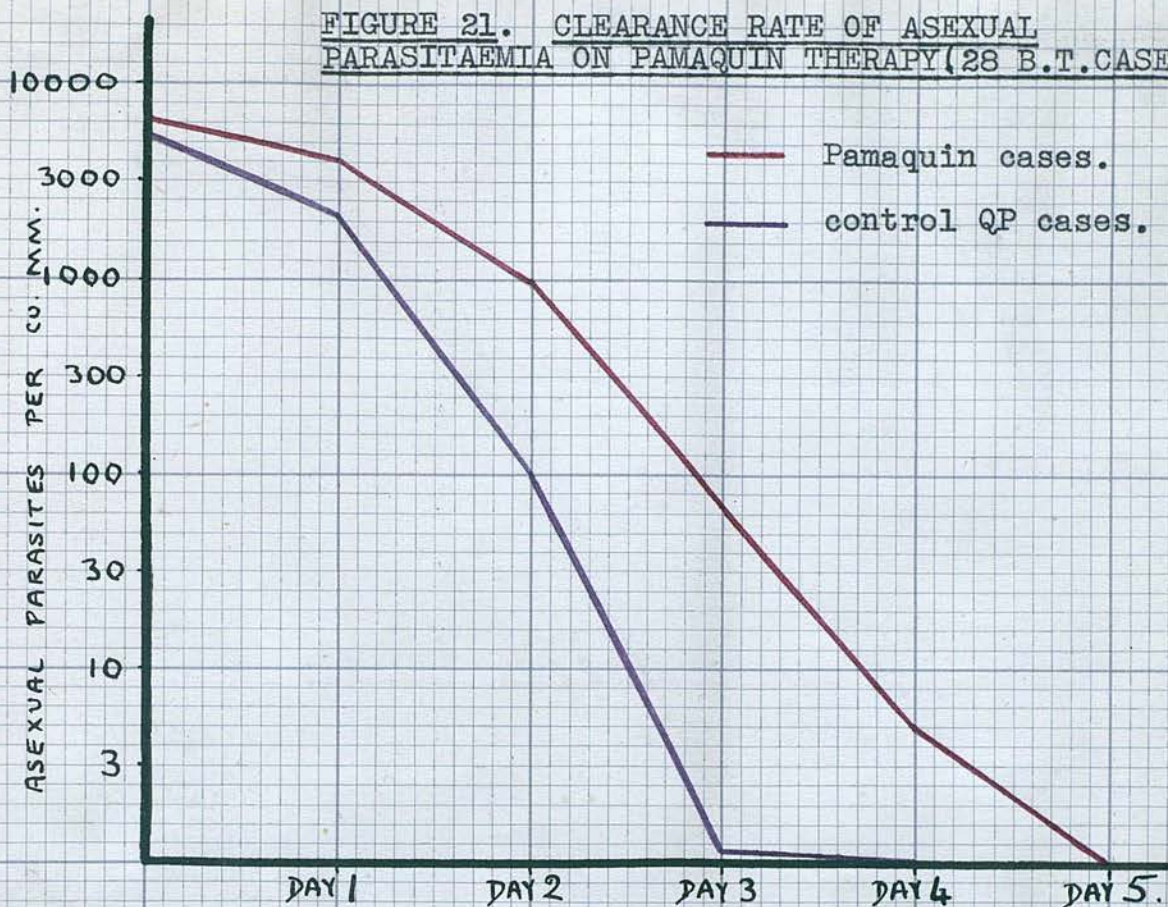
FIGURE 22.
PERCENTAGE NUMBER
OF CASES SHOWING
ASEXUAL PARASITES
DURING INITIAL
DAYS OF PAMAQUIN
TREATMENT.

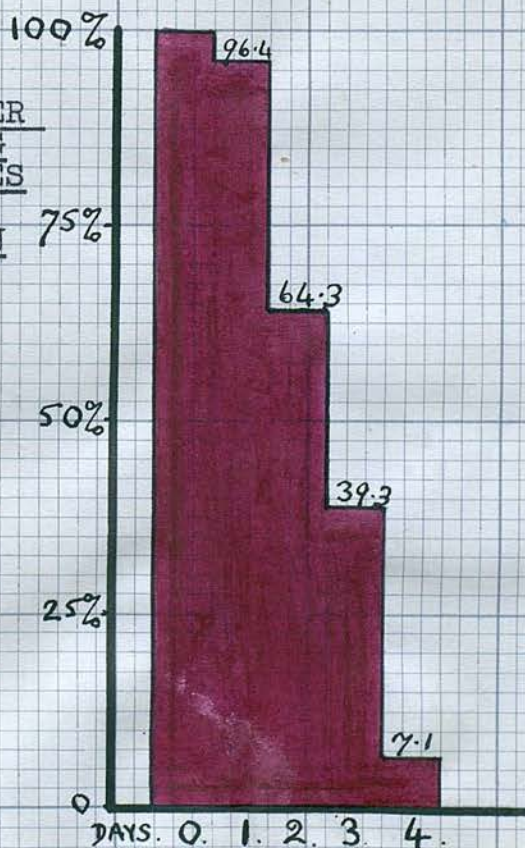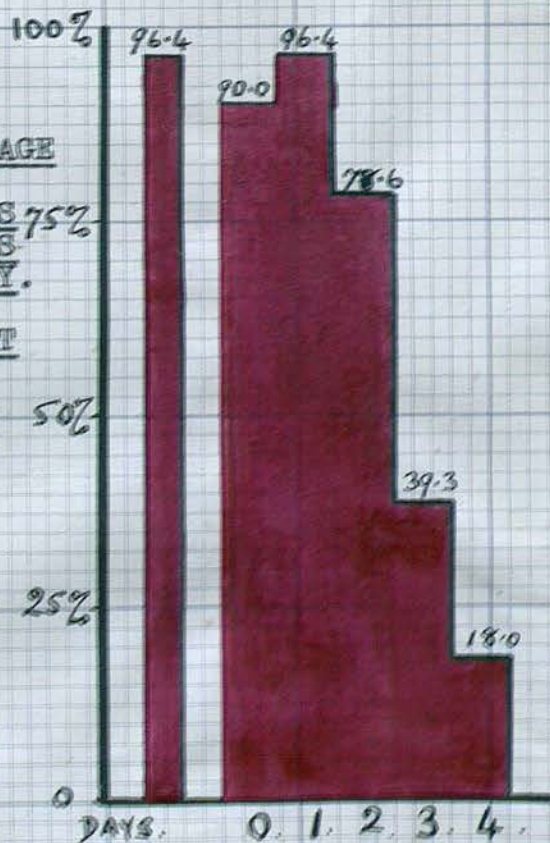# FIGURE 23. CLEARANCE RATE OF GAMETOCYTES ON PAMAQUIN THERAPY (28 B.T. CASES).

Pamaquin cases.

control QP cases.

FIGURE 24. PERCENTAGE NUMBER OF CASES SHOWING GAMETOCYTES DURING INITIAL DAYS OF PAMAQUIN THERAPY. 96.4% CASES SHOWED SEXUAL PARASITES AT SOME STAGE OF TREATMENT.

3.　Clearance of Gametocytes.

Figure 23 shows in graphic form (logarthmic scale), the average daily gametocyte counts from the start of therapy, and Figure 24 records the percentage number of cases in which sexual parasites were present initially and on each of the first 6 days of the course.

The average duration of Pamaquin treatment required to clear the peripheral blood of gametocytes was 2.5 days.　Whereas this time compares unfavourably with the gametocyte clearance time of Quinine alone (2.02 days in 46 B.T. Fresh infections in C.M.F.) or Quinine plus Pamaquin, (1.5 days average in 130 cases in C.M.F. and at Woolwich), it will be noted that Pamaquin by itself is able to clear the blood of gametocytes in a time as short, or less, than it requires to get rid of the asexual parasites.　This is in direct contrast to the findings in therapy with Mepacrine alone, which, in both high and low initial dosage, produced clearance of the asexual forms in less time than disappearance of gametocytes.

4.　Effects on the development of the B.T. asexual cycle.

As might be expected from the time taken by Pamaquin to check both the clinical fever and asexual parasitaemia in active B.T. infections, there is a considerable delay before its action on the asexual developmental/

developmental cycle becomes evident on microscopical
study. Compared with Quinine and Mepacrine, which
produce demonstrable retardation of parasite develop-
ment within 12 to 18 hours, this effect usually occurs
much later, - after 24 hours -, with Pamaquin, a fact
which has no doubt contributed previously to the view
that this drug has little direct action on asexual
parasites.

When the Pamaquin-effect does begin, however,
there is a marked slowing in the maturation of the
growing trophozoites as occurs with Quinine and
Mepacrine, though it differs from the latter drugs
in causing very little tendency to an arrest of
parasite development at the pre-schizont stage (T6 in
the parasite notation scheme described in Part One).
The trophozoites therefore are subjected to a general
inhibition in their growth, but appear to undergo
nuclear segmentation relatively unimpeded and reach
the later stages of schizonts before they are destroy-
ed. The maximum destructive effect of Pamaquin would
seem to be exerted on late schizonts and merocytes
(S 2, S 3 & M), and those parasites that survive this
phase and escape from the bursting merocyte to become
young trophozoites again, may struggle on until the
late schizont stage of the next cycle before they are
overcome.

5/

5.   Morphological effects on the
      asexual parasites.

Collapse of the amoeboid forms of trophozoite
occurs with Pamaquin action,though the change is later
than with Mepacrine therapy and not so intense.

Conglomeration of malarial pigment in late
amoeboid trophozoites is often seen with Pamaquin,
as with Mepacrine, and the bunched up pigment may be
extruded from the parasite cell.   If this liberation
of pigment should occur "en masse" from large numbers
of late trophozoites, at approximately the same time,
it may result in a typical attack of rigor and malaria
fever.   Such a clinical paroxysm may be caused in
this manner at a time when the asexual parasites are
only at the stage of young de-pigmented schizonts,
so that the fever occurs prematurely according to the
usual timing of the parasite cycle.   A good example
of this effect, produced by oral Pamaquin therapy,
is depicted in Figure 25 (Case No. 452) which shows
the occurrence of a peak of temperature coinciding
with the extrusion of pigment from late amoeboid
trophozoites.   This case shows also the slowing effect
of the drug on the maturation of the late trophozoites
and it will be noted that the maximum fall in the
asexual parasite count occurred within 6 hours during
which the developing schizonts were destroyed.

FIGURE 25. (page 103).

FIGURE 25. CLEARANCE OF ASEXUAL PARASITAEMIA AND
TEMPERATURE CHART IN A CASE OF B.T. (R) MALARIA
TREATED WITH PAMAQUIN. PARASITE STAGES ARE SHOWN
WITH THE COUNT FIGURES.
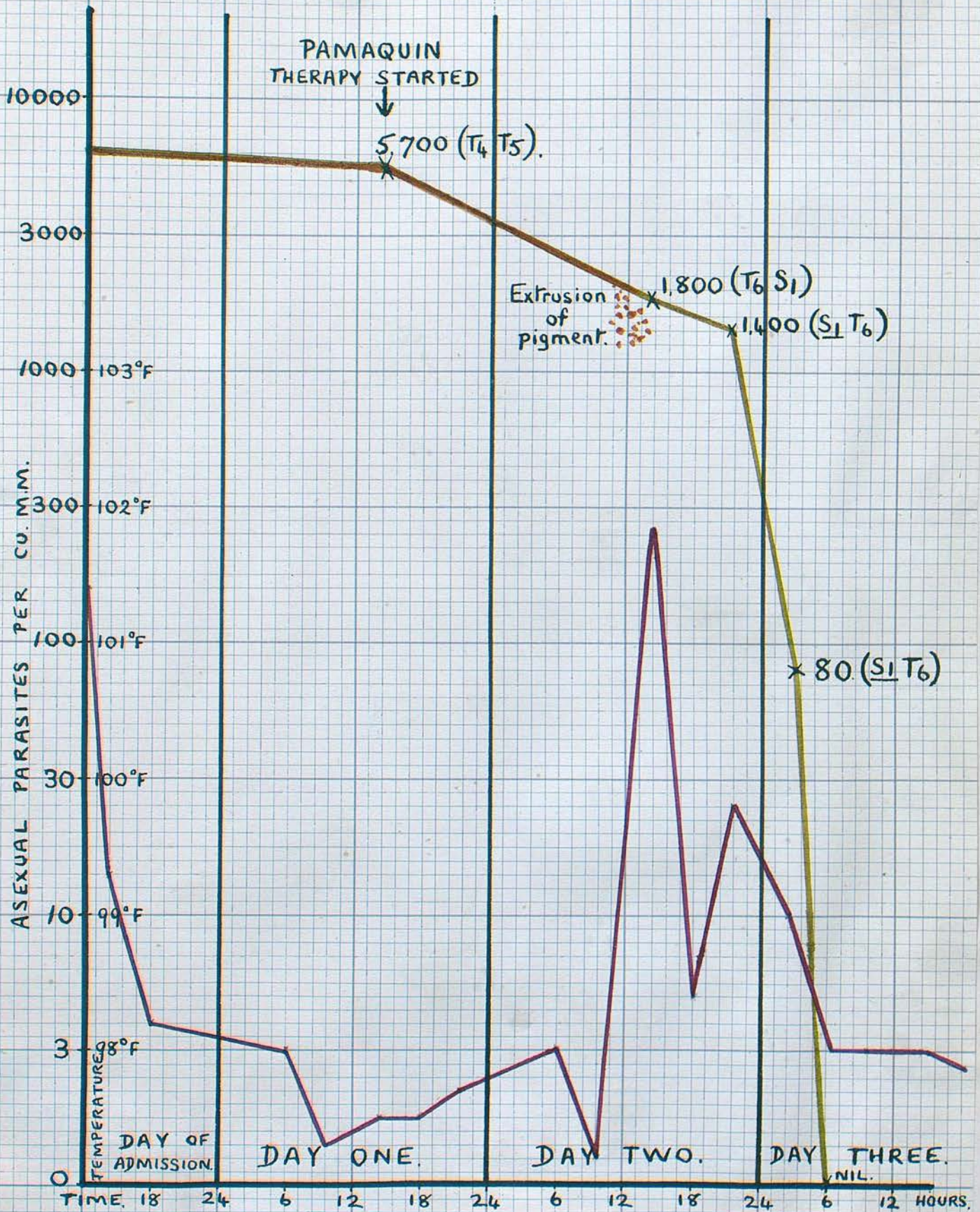
OBSERVATIONS ON THE PHARMACOLOGY OF PAMAQUIN.

The results reported above of the effects of
Pamaquin therapy on the asexual parasite of B.T.
malaria, indicate that, although generally unrecog-
nised, it is actually a powerful schizonticide. This
property becomes all the more impressive when it is
considered that the daily dosage of the drug, (30 Mgm),
required to produce this effect, is 20 to 30 times
smaller than the Mepacrine dosage necessary to bring
about an equivalent destruction of schizonts. When,
in addition to these findings, it is realised that the
gametocidal action of Pamaquin is widely accepted,
and that it has as yet proved the most effective
measure in the reduction of the relapse rate in B.T.
infections, it becomes more and more apparent that
this drug is indeed a potent anti-malarial and one
well worthy of further investigation into its action.

The great limitation to the extensive use of
Pamaquin is that it may produce very undesirable toxic
effects when dosage of more than 40 Mgm is given daily.
These effects, abdominal colic, methaemoglobinaemia,
and more serious degrees of intravascular haemolysis,
are well known, and the risk of producing them has
usually not seemed worth while to clinicians who have
regarded Pamaquin as being merely a gametocidal agent.
In recent years, however, the value of this drug in
preventing recurrences of B.T. and Quartan infections,
has/

has lead to its employment on a much wider scale and
has prompted closer study of its pharmacological pro-
perties.   Most of the latter work has been done with-
in the past year (1945-46) by Shannon and his colleag-
ues at the Goldwater Memorial Hospital, New York on
behalf of the National Research Council of America.
Their results in this investigation, which is still
continuing, have so far appeared only in restricted
publication, but it is possible to refer here to some
of the more interesting findings and to indicate some
of the lines of promise which might arise from them.

A chemical technique for the estimation of Pama-
quin in whole blood, plasma and urine was evolved
and permitted studies to be made of the absorption,
utilisation and excretion of this drug.   It was found
by these means that Pamaquin is absorbed rapidly into
the blood stream and is removed almost as quickly to
be stored in the tissues or broken down into degrad-
ion products.   This disposal was suggested by the
finding that little or no unchanged Pamaquin appears
in the urine.   There seems to be no steady balance
established between the amounts of the drug present
in the circulation and the tissues, as is the case
with Mepacrine, for Pamaquin plasma levels were found
to vary greatly in relation to times of dosage and
other factors, and as such were considered to be of
little value for study as an assessment of the efficacy
of therapy.   Shannon noted that Pamaquin plasma levels
were/

were consistently higher in subjects who were taking
Mepacrine as well, and he concluded that the latter
drug can displace Pamaquin from storage in the body
tissues, thereby raising its blood level to some
6 or 8 times that usually found. He suggested that
this fact may provide the explanation for the apparent
increase in Pamaquin toxicity when it is given concurr-
ently with Mepacrine;- a point long recognised by
clinicians who have had experience of the undesirable
effects produced in patients by this peculiar form of
drug incompatability.

Shannon next investigated the isolation of the
degradation products of Pamaquin as it seemed possible
that the anti-malarial activity of the drug was brought
about by one or more of these, rather than by the com-
pound itself. He was successful in isolating two
fractions from the urine of subjects taking Pamaquin.
Fraction A was fluorescent, stable and did not appear
to be toxic, whereas Fraction B was non-fluorescent,
unstable and highly toxic, causing rapid haemolysis of
red blood corpuscles. Considerable variations were
found to occur in the quantities of these two products
excreted by different patients.

Information regarding Shannon's findings was
received in Britain in September 1945 and it was decided
that it might prove of interest for the Malaria Re-
search Team to undertake a similar line of investigation
while/

while studying the action of Pamaquin on recurrent
B.T. cases at Woolwich.   As a preliminary observation
it was found possible to demonstrate a fluorescent
substance in the urine of patients receiving Pamaquin,
by using the same extraction technique as for urinary
Mepacrine and by selecting only those patients who had
taken no Mepacrine for at least 2 months previously.
The quantities of this substance varied greatly from
day to day and in different patients and bore little
apparent relation to the dosage or clearance of para-
sitaemia.   It was, however, absent from the urine of
control cases being treated on Paludrine.   A consid-
erable delay was occasioned in obtaining the necessary
reagents and in working out the complicated technique
for plasma Pamaquin estimations, and by January 1946
when the author left the Team, this work was only just
getting under way.   It is possible, however, to record
here some of the plans made for lines of work to be
followed, which will give an idea of the aims and hopes
in the field of investigation.

Interest was centred mainly on the fluorescent
substance present in the urine of patients taking
Pamaquin and which was possibly the same as Shannon's
Fraction A.   An attempt was to be made, by passing
the whole daily urinary output through a chromatographic,
absorptive column in ultra-violet light, to isolate
this fluorescent fraction and to identify its chemical
nature/

nature.   It was hoped that this fraction might prove
to have anti-malarial properties, and might actually
represent the non-toxic active principle arising from
the break-down of Pamaquin.   It was also hoped to
record quantitatively the urinary output of this
fraction for comparison with the parasite clearance
from the peripheral blood, and the subsequent follow-
up record in each case treated with Pamaquin alone,
or in combination with Paludrine. (Paludrine is stable
and non-fluorescent and is excreted unchanged in the
urine).   It would be of great interest to know wheth-
er the 10 to 15 per cent of patients who relapse after
Pamaquin therapy, have shown any difference from the
other cases in their urinary output of this fluorescent
factor.

The toxic urinary product, Shannon's Fraction B,
is obviously of little interest even though it may
possess anti-malarial action.   Its toxicity and in-
stability would preclude its use as a drug.   If, how-
ever, the stable, non-toxic, fluorescent Fraction A
could be shown to have anti-malarial activity, espec-
ially in regard to prevention of relapses in B.T.
malaria, methods might be found to increase the for-
mation of this degradation product from Pamaquin given
in its present dosage.   It seems not too much to
hope that before long, the isolation, analysis and
eventual synthesis of the active principle derived
from/

from Pamaquin, may make available in a simple non-
toxic form an anti-malarial of unique potency.

SECTION 3.

THE ACTION OF PALUDRINE (M.4888) IN THE
THERAPY OF BENIGN TERTIAN MALARIA.

The initial trials of Paludrine at Liverpool
and the subsequent work by Hamilton Fairley in
Australia, soon established this new drug as being
an anti-malarial of outstanding importance.   In M.T.
malaria it fulfilled nearly all the criteria of the
ideal drug, being a true causal prophylactic for the
prevention of infection and a potent therapeutic
agent for the cure of the acute attack.   In B.T.
malaria it proved a good suppressant of infection and
a safe, effective measure for the clinical cure of
the active phase of the disease.   There remained, how-
ever, the problem of relapsing B.T. malaria in which
Paludrine did not appear to be as valuable as earlier
work in chicken seemed to suggest;   and also the
question of its action in resistant Quartan infections,
about which no controlled therapy trial has yet been
published.

The following report deals with the detailed
action of the drug on the parasites of B.T. malaria
as studied by the methods of parasite counts and
microscopic observation previously described.   The
findings are based on 24 active cases treated with
250 Mgm tablets of Paludrine given twice daily for
10 days.

1.   CLINICAL RESPONSE/

1. CLINICAL RESPONSE.

All patients tolerated the drug very well in
the acute, febrile stages and the average duration of
pyrexia after the start of treatment was 2.0 days,
being the same as in the control series treated with
Quinine plus Pamaquin.   Patients were allowed up
after they had been afebrile for 48 hours.   In 5
cases there were mild complaints of nausea and slight
epigastric discomfort within a few minutes of swallow-
ing the early morning tablet from the 5th to 7th days
of the course.   These symptoms were easily prevented
by giving the tablet after food, or with a full half
pint of water, or with a small dose of an alkaline
mixture.   Patients who experienced these slight
manifestations of direct gastric irritation in the
middle of the course, had no complaints during the
later days of dosage.   In no case did the drug have
to be stopped owing to intolerance, and daily blood
and urine examinations revealed no indication of
toxic effects.

The general impression formed was that Paludrine
was clinically in every way satisfactory in the treat-
ment of the acute malarial attack.

2. CLEARANCE OF ASEXUAL PARASITAEMIA.

The average duration of treatment required to
clear asexual parasites from the peripheral blood
was 1.6 days.   This compared with 1.5 days for the
control/

FIGURE 26. CLEARANCE RATE OF ASEXUAL
PARASITAEMIA ON PALUDRINE THERAPY (24 BT CASES)

Paludrine cases.

control QP cases.
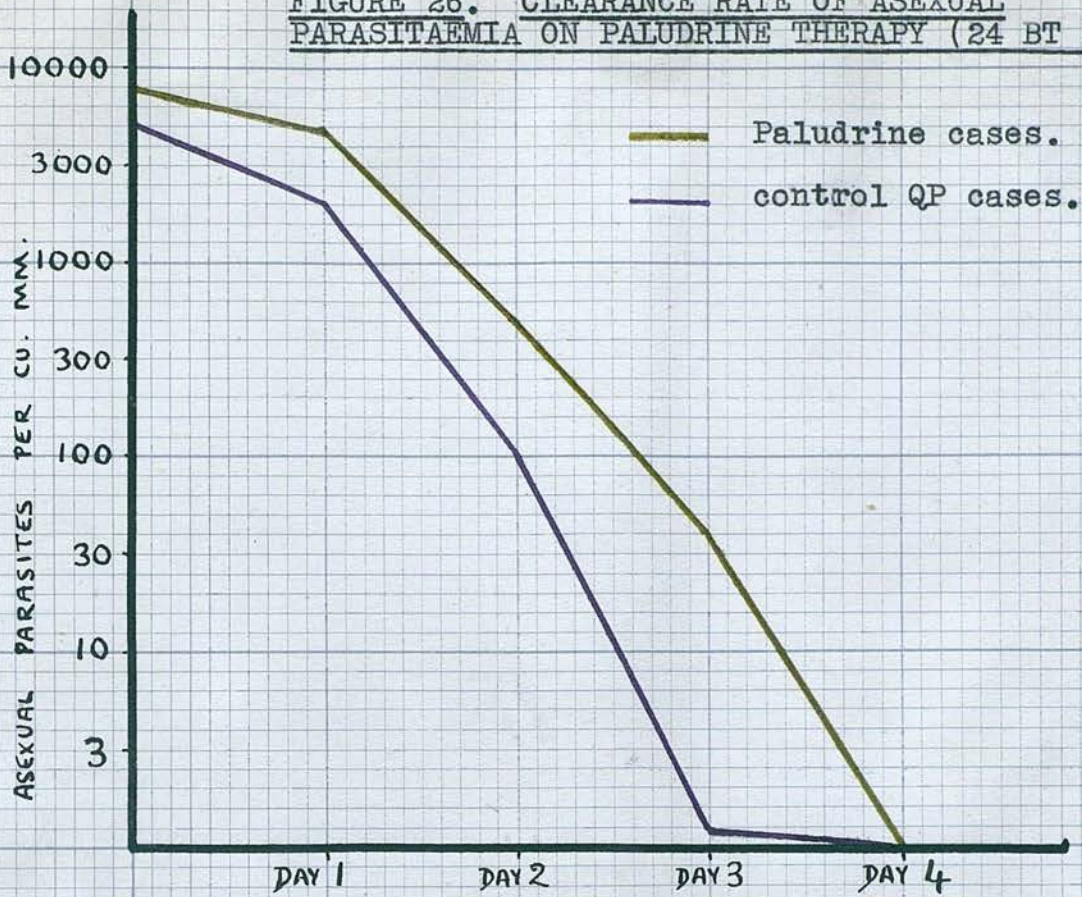

FIGURE 27.
PERCENTAGE NUMBER
OF CASES SHOWING
ASEXUAL PARASITES
DURING INITIAL
DAYS OF PALUDRINE
TREATMENT.
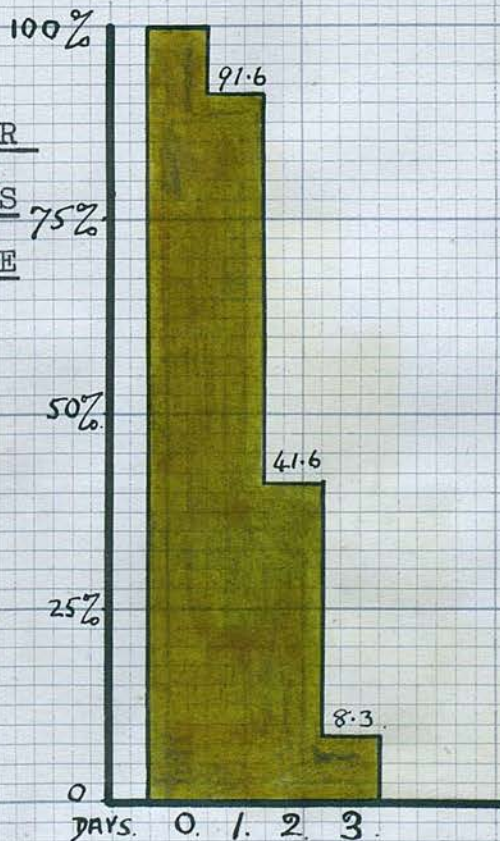
FIGURE 28. CLEARANCE RATE OF GAMETOCYTES ON PALUDRINE THERAPY (24 CASES B.T.).
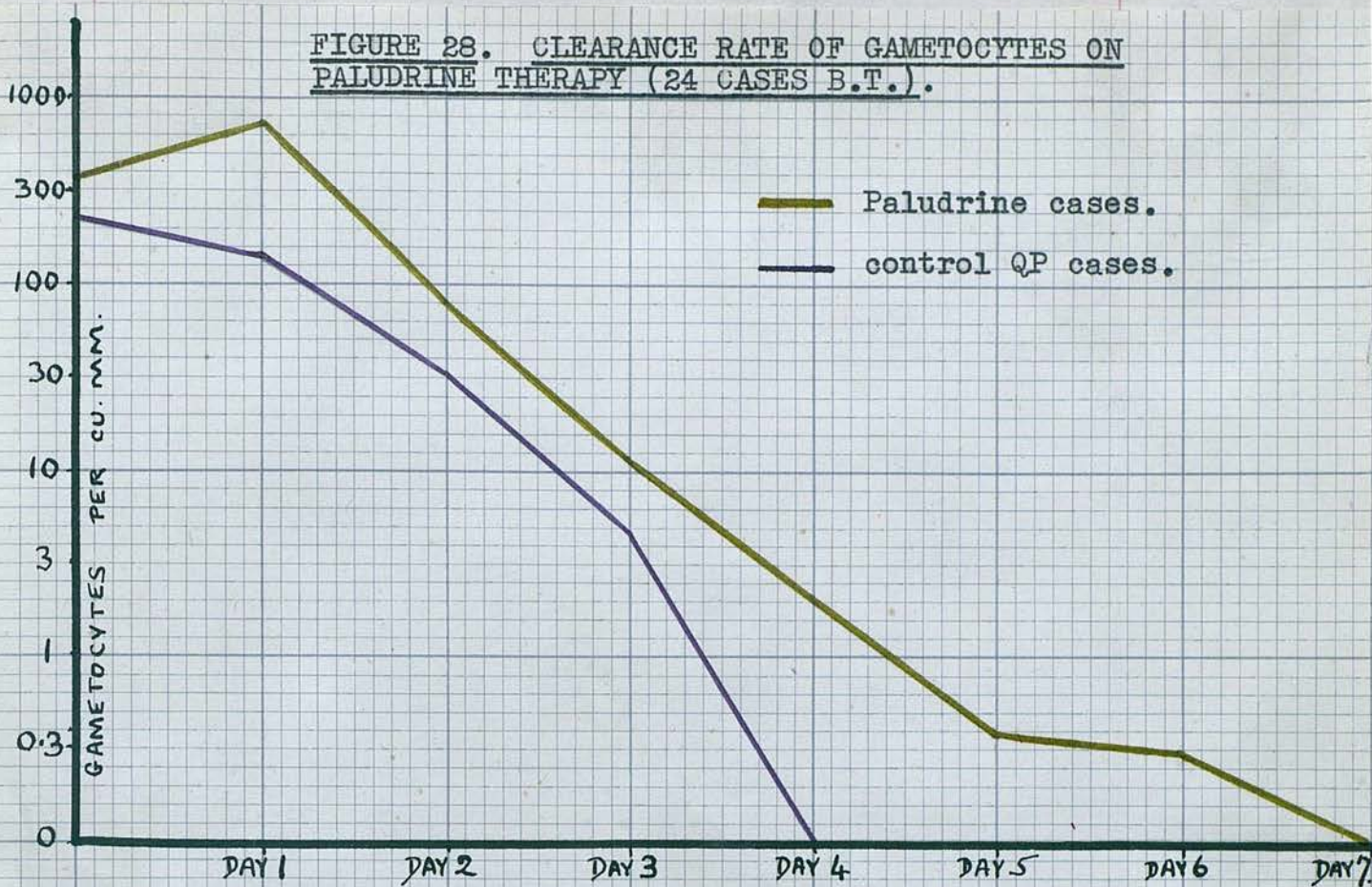
— Paludrine cases.
— control QP cases.

FIGURE 29.
PERCENTAGE NUMBER OF
CASES SHOWING
GAMETOCYTES DURING
INITIAL DAYS OF
PALUDRINE THERAPY.
100% CASES SHOWED
SEXUAL PARASITES
AT SOME STAGE OF
TREATMENT.

control Quinine-Pamaquin cases.

Figure **26** shows in graphic form the fall-off in
the daily asexual parasites, and Figure **27** indicates
the percentage number of cases in which asexual para-
sites were present on each day of treatment.

3. CLEARANCE OF GAMETOCYTES.

Figure **28** shows the average gametocyte counts
plotted at 24 hour intervals from the start of ther-
apy and in Figure **29**, the percentage number of cases
is recorded in which sexual parasites were present
initially and on each of the first 8 days of the
course.

It will be seen that B.T. gametocytes may per-
sist in blood films up to one week after the start
of Paludrine, a time which compares unfavourably
with the gametocyte clearance-time of any of the
other anti-malarials studied.   The persistent forms
of gametocyte were all observed to be females.   It
is of interest to recall, in this connection, that
the large fully-grown female gametocyte probably
represents a non-feeding stage of the parasite and
as such would be unlikely to be much affected by a
drug which had to be ingested to produce its action.

4. EFFECTS ON THE DEVELOPMENT OF THE
    B.T. ASEXUAL CYCLE.

Inhibition of trophozoite maturation is seen
with Paludrine, but occurs later than when Mepacrine,
Quinine, or Pamaquin is used for treatment.   There
is/

is thus a slightly longer latent period with Paludrine
than than with these other drugs between the time of
administration and the first visible effects on
parasite development.

Patients who start treatment with young tropho-
zoites do not as a rule eradicate their asexual
parasites until the stage of segmenting trophozoites
is reached.   The biggest disappearance occurs mainly
after the latter stage.   If inhibition of develop-
ment takes place before the parasites can reach this
stage, then much longer persistence results.   This
finding is not peculiar to Paludrine alone, but has
been observed with all the other anti-malarials
studied.

Patients who start treatment with late tropho-
zoites in the blood stream, show a much more rapid
parasite clearance, as segmentation of the nucleus
has occurred before the inhibitive effect on develop-
ment has had time to prevent the parasites from reach-
ing the highly susceptible schizont stages.   The
drug appears to have a destructive effect on earlier
forms of schizonts, (S 1 S 2), than any of the other
anti-malarials, which attack later schizonts, mero-
cytes, and in the case of Quinine, the merozoite-
young trophozoite transition stage.

5.   MORPHOLOGICAL EFFECTS ON THE ASEXUAL PARASITE.

Collapse of the amoeboid forms of trophozoites
occurs/

occurs with Paludrine, though this effect is later
and less complete than that produced by Mepacrine.
The action resembles that of Pamaquin in this
respect.

The malarial pigment present in late tropho-
zoites becomes coarsely granular with Paludrine
therapy.   It is not bunched together or extruded as
with Mepacrine and Pamaquin.

There appears to be a powerful interference with
the dividing chromatin nucleus during Paludrine ad-
ministration, and this occurs in a more striking
manner than has been seen with any of the other drugs
studied.   The differentiation of the chromatin of
the segmenting trophozoite into numerous rod-like
structures, occurs in the normal manner, but instead
of these re-grouping to form two distinct nuclei, the
chromatin remains in this differentiated phase appar-
ently inhibited from further development.   Parasite
forms showing this unusual state of nuclear chromatin
arrest are produced, sometimes in large numbers, and
are seen to persist for long past the time when they
should normally have undergone complete schizogomy.
These are not uncommonly the last forms of asexual
parasites to be found in the peripheral blood during
Paludrine therapy.   It proved only possible to
distinguish these damaged nuclear types in thin blood
smears, as in thick films they appear closely similar
to/

to female B.T. gametocytes which as noted, are them-
selves unduly persistent during Paludrine treatment.
This point of differentiation is one likely to cause
considerable difficulty in enumerating B.T. asexual
parasites in the blood of cases receiving this drug.

A general effect, seen late in Paludrine therapy,
is that B.T. asexual parasites in various stages of
degeneration are found, with fading cytoplasmic stain-
ing and indistinct chromatin.   These degenerate forms
may occur at any phase in the developmental cycle and
are more commonly seen with this drug than with Quin-
ine, Mepacrine or Pamaquin.   Parasites thus affected,
may fade quietly out of the blood picture without
showing any gross morphological changes.

COMBINED THERAPY WITH PALUDRINE
AND PAMAQUIN.

The idea of combining Paludrine and Pamaquin
in a therapy course arose when it was found that a
disappointing high proportion of B.T. cases treated
with Paludrine alone had further recurrences.   In
preliminary follow-up estimates of such cases, the
relapse rate was in the region of 30 per cent, being
as high as with Mepacrine therapy.   This finding
again pointed to the inadvisability of drawing too
close/

close an analogy between human and chicken malaria infections, as in the latter Paludrine in non-toxic doses, had proved much more effective in suppressing the tissue phases of the parasites than had heavy toxic dosage with Pamaquin.

It was thought that it might be of interest and value to try the effect of Paludrine and Pamaquin together in human malaria, as the new drug was a stable product, quite unrelated to Pamaquin in chemical structure, and might possibly produce an additive effect in the form of a combined assault on the resistant tissue forms of parasite that are believed responsible for recurrences of the infection.

Remembering Shannon's finding that Mepacrine could displace Pamaquin from the tissues and so produce toxic levels in the circulation, it was realised that a similar displacement might result when giving Paludrine and Pamaquin together. Accordingly preliminary trials of the combined dosage were made in chicken and animals by Dr. D.G. Davey of Imperial Chemical (Pharmaceuticals) Ltd., and only when these showed no obvious effects, was similar work begun by the Malaria Research Team on patients at Woolwich.

The first cases to receive this treatment were given simultaneous doses of Pamaquin, 10 Mgm and Paludrine 50 Mgm twice daily. Both drugs were given in tablet form and administration was continued for

10/

10 days. No ill-effects were noted and after 6 patients had completed the course without toxic manifestations, the dosage was increased to thrice daily. It was decided not to give more than 30 Mgm of Pamaquin each day and so succeeding groups of cases were given gradually increasing doses of Paludrine added to the constant amounts of Pamaquin. By these means it was found possible to work up the combined dosage to 30 Mgm Pamaquin plus 750 Mgm Paludrine per diem, for a course of 10 days. Careful clinical and laboratory observation revealed no signs of drug intolerance other than the occasional 5th to 7th day slight gastric irritation which occurs with Paludrine alone, and mild cyanosis noted in one patient from the 5th day of treatment.

It was agreed to treat a series of 100 or more cases on this therapy regime and to compare the 6 month follow-up results with those of the control series receiving Quinine and Pamaquin. By January 1946, insufficient cases had been treated to allow time for such a follow-up, but it is of interest to record that while cases were being re-admitted to hospital with recurrences following treatment with Paludrine alone, there had then been no relapses reported amongst the patients who had been treated in the various trial groups receiving increasing doses of the combined therapy.

As regards the effects of Paludrine and Pamaquin together/

together on the parasites of B.T. malaria, no especial-
ly rapid clearance of parasitaemia was found to re-
sult.   The average duration of asexual parasitaemia
after the start of treatment was 1.7 days in the
first 26 cases receiving the highest dosage course.
It was noted that with Pamaquin being given, the
gametocytes were cleared much more rapidly than with
Paludrine alone.

The investigation of this combined course also
included estimations of the plasma concentrations of
Pamaquin and Paludrine, but the findings in this
work are at present incomplete.   It is sufficient
here however, to have drawn attention to the proof
that it is a practicable measure to combine Paludrine
with Pamaquin in the treatment of B.T. malaria and
to record that such therapy would appear to constitute
a rational approach to the problem of minimising
recurrences of this "nuisance" infection.

PART THREE.

A STUDY OF THE STAINING OF MALARIA
PARASITES IN THICK BLOOD FILMS WITH A
DESCRIPTION OF A MODIFICATION OF FIELD'S
STAINING METHOD.

At the outset of the work on malaria therapeutic
research, it was realised that the staining method
employed for the demonstration of malaria parasites
in thick blood films used for parasite counts would
be of great importance in attaining reliable results.
It appeared also that with such a large number of
films to be examined, a rapid staining technique
such as that introduced by J.W. Field in 1941 (Trans.
Roy. Soc. Trop. Med. Hyg., 35, 1, 35-42), would save
a great deal of time as compared with other methods.
Accordingly a study was made of the merits of Field's
and other staining techniques and from observations
and experiments made in the course of this investigat-
ion, it was found possible to produce a modification
of Field's method which ensured that optimal staining
was more regularly achieved.   It is intended to de-
scribe here some of the main points of this work and
to give details for the preparation and use of the new
stain.   A short description of the latter was publish-
ed in September 1945; "A Staining Method for Malaria
Parasites in Thick Blood Films" by J.C.B. Fenton and
James Innes, Trans. Roy. Soc. Trop. Med. Hyg., 39, 1
87-90).   In the following report, a knowledge of
Field's and other methods of staining thick blood
films/

films will be assumed.

Dilute Romanowsky stains, such as Giemsa stain have been used for many years for the demonstration of malaria parasites in thick blood films. Providing that they are not excessively thick, the films do not need to conform to any definite specifications and lose most of their haemoglobin whilst staining. The malaria parasites and leucocytes are thus seen against a clear white background and on one plane of focus. Many workers prefer such a dehaemoglobinised background to the darker pink background that is seen with Field's staining method.

The main disadvantages of the dilute Romanowsky stains are that they tend to cause distortion of the leucocytes and their staining qualities are very sensitive to small changes in the pH values of the water diluent. Thus variable results are sometimes recorded, particularly with Leishman stain which varies in its staining power from batch to batch, and under field conditions the necessary staining corrections to adjust these variations are not always easy to apply.

Field's isotonic Methylene-Blue-Azure and Eosin stain has a great advantage over thick blood film stains in the speed and ease of its method of use. Its early widespread adoption by a great many pathologists indicated the real need that existed for a reliable rapid staining method for malaria diagnosis.

Its/

Its introduction implied a great saving of time in busy laboratories with many films to examine and the relatively minor disadvantages in the method were outweighed by the advantage gained in the time factor.

One disadvantage of Field's method is that the blood films stained must be somewhat thinner than those used with the dilute Romanowsky stains and the films must be made to conform with certain specificat- ions of size. Furthermore, although the staining properties of Field's stain solutions are fairly con- stant, very careful attention to the correct staining times are necessary to produce consistent results. While this presents no problems when dealing with thick films properly made and intended for staining by the rapid method, experience shows that blood films are frequently received for examination that are impossible to stain correctly on account of being far too thick or too small and thin. Occasionally even with properly made films, one finds that only certain parts of the film, lying between the extremes of red and blue, are correctly stained.

A common mistake in staining a badly made thick film by Field's method, is to overstain in the Eosin solution. Once this has been done, the resultant faulty staining cannot be corrected by returning the slide to the Methylene-Blue-Azure solution. It was found that in such cases of overstaining in the Eosin, the/

the fault could be easily corrected by a gentle rinsing of the slide in a very dilute Leishman stain. Furthermore, the result of staining a slide in this manner appeared much superior to that obtained by staining with the usual Field's technique.   It was also found that by allowing the slide to stand in the dilute Leishman stain until it becomes transparent, complete dehaemoglobinisation of the film took place with no loss of the stain from the leucocytes or malaria parasites if present.

It would seem that, used as above described, dilute Leishman stain can be made to act as a corrective and differentiating agent of Field's stain, thereby greatly enhancing the staining qualities of this method.   In the dilution employed, the Leishman stain itself is too weak to take part in the direct staining of the film, but acts in conjunction with the Methylene-Blue-Azure and Eosin solutions both of which form necessary constituents of the stain.   Blood films immersed in either of the latter solutions alone and then washed in the dilute Leishman do not give satisfactory results.

Having recognised the superior staining properties of this combined Field-Leishman stain, an investigation was made to determine the optimum conditions for the staining of malaria parasites in thick films using this method.   This involved mainly a study of the staining/

staining effects produced by varying the pH value of
the solutions of Methylene-Blue-Eosin, Azure 1 and the
dilute Leishman stain.   This work, conducted on a
"trial and error" basis with a large number of variat-
ions in the formula of the combined stain, brought to
light the many complicating difficulties liable to
offset the desired result, and served to emphasise the
extreme importance of the correct pH values in the
various stain solutions.   For instance, it was found
that the clearest differential staining of the parasite
with Methylebe-Blue and Eosin occurred at a pH of 7.4,
but at this same pH, the other component of the stain,
Azure 1, stained and mordanted the fibrin threads and
red cell envelopes comprising the basis of the film
and so gave rise to a highly coloured red background.

The results obtained, in this study of the stain-
ing properties of the individual stains in acid and
alkaline solution, may be briefly summarised as
follows:

1.<u>Methylene Blue</u>.

(a)   In acid solution (pH approximately 6.0)
         No affinity for red cell envelopes, slight
affinity for parasite chromatin and moderate
affinity for parasite cytoplasm.

(b)   In alkaline solution (pH approximately 7.6)
         Great affinity for red cell envelopes and
parasite cytoplasm, but very little affinity for
parasite chromatin.

2/

2.Eosin.

    (a)   In acid solution (pH approximately 6.0)

           Very great affinity for red cell envelopes
and parasite chromatin. Slight affinity for
parasite cytoplasm stained with Methylene-Blue.
This results in a partial decolourisation which
tends to destroy the sharp contrast between the
red chromatin and blue cytoplasm.

    (b)   In alkaline solution (pH approximately 7.6)

           Marked affinity for haemoglobin and
parasite chromatin.

3.Azure 1.

    (a)   In acid solution (pH approximately 6.0)

           Slight affinity for red cell envelopes
and blood platelets, with moderate affinity for
Schuffner's dots and parasite chromatin.

    (b)   In alkaline solution (pH approximately 7.6)

           Very great affinity for red cell envelopes,
fibrin threads, parasite chromatin, Schuffner's
dots and blood platelets.

           The staining activity of Azure solutions
appears to be affected by changes in temperature,
being much increased in warm weather.

From the above experiments it was concluded that
the optimum pH value for the staining of malaria
parasites in thick films lay between pH 6.6 and pH 7.0.
At this range the more common background debris such
as/

as fibrin threads and red cell envelopes are least conspicuous. It was also apparent that the exact optimum pH was dependent upon the degree of dehaemoglobinisation of the film and that this factor was of great importance in determining the final appearance of the stained film.

A film which contains free haemoglobin after staining has a tendency to turn blue during drying. This may be due to a change in pH towards alkalinity which occurs by reason of the buffering power of the haemoglobin protein. Under such conditions, Eosin would be absorbed by the residual haemoglobin and a blue colouration of the film would result. This effect can be avoided by one or two procedures, namely staining with Eosin to an excess, or by staining at a pH slightly more acid than the optimum. If on the other hand the blood film contains no free haemoglobin, the optimum pH for staining was found to be pH 6.8 to 6.9.

In the new stain described here, the pH optimum of 6.8 has been chosen in order to allow for a very small amount of residual haemoglobin such as is usually left in the blood film after staining. Furthermore, the final staining and dehaemoglobinising solution contains a small amount of phosphate buffer, and since the slide is taken out of this solution and allowed to dry without further washing, the buffer that is left on the film acts as an additional control to mimimise changes in pH.

The/

The following are the directions for the preparation and use of the modified stain:

PREPARATION.

There are three staining solutions-A, B and C: A being the Methlylene-Blue Azure solution, B the Eosin stain, and C a corrective Leishman solution.

Stain A.   Is made from a mixture of two solutions which should be prepared separately.

Solution (1) Methylene-Blue ... . 1 gramme,

Disodium hydrogen
phosphate (12 H_2O) .. ... 5 grammes,

Distilled water .. ..250 c.c.

Place this solution in a 500 c.c. conical flask and immerse in a bath of boiling water for 2 hours 15 minutes.   The neck of the flask should not be plugged, but allowed to have free access to the air, as the formation of azure depends on the oxidization of alkaline Methylene-Blue by the atmospheric oxygen. If the azure is prepared under these conditions, the right proportion of azure in relation to the Methylene Blue will be formed.   Azure made by this polychromizing method has been found more satisfactory for staining than pure Azure 1 added to Methyleneblue in the preparation of this stain.   The polychromizing should not be carried on beyond the stated time or too much azure will be formed, and too much is almost as bad as too little.   If the azure is in excess, then fibrin threads will stain in the background/

ground of the films, the parasite cytoplasm will not stain a good blue colour and the parasite chromatin may appear black.

A heavy deposit may separate out when the solution is heated and the flask should be set aside, say overnight, to allow this to re-dissolve.   When cold, the solution should be made up to its original volume and should appear a purple blue colour when viewed in artificial light.   Too much azure production results in a more reddish purple colour.

Solution (11) Methylene-Blue ... ... 1 gramme.

Potassium dihydrogen
phosphate . ... 2 grammes.

Distilled water .. ..500 c.c.

To prepare Stain A, add two parts of Solution (11) to one part of Solution (1).   This method of preparation ensures that there is always some unchanged Methylene-Blue present in Stain A rather than a mixture of poly-chromed by-products.

Stain B.   Yellow water soluble cosin 1 gramme.

Disodium hydrogen
phosphate (12 H_2O ....   3.3 grammes.

Potassium dihydrogen
phosphate ... ...   1.3 grammes.

Distilled water .. ...   500 c.c.

The pH of the above stains A and B should be approximately 6.8.

Stain C/

Stain C.   A stock buffer solution is required to
prepare this stain, and consists of:-

> Disodium hydrogen phosphate
>   (12 H_2O) ... ...12.5 grammes
>
> Potassium dihydrogen
>   phosphate... ... ...5 grammes.
>
> Distilled water... ... ... 250 c.c.

Ether, 0.5 to 1.0 c.c., may be added to preserve
this solution from bacterial contamination.

The stock buffer solution should have a pH value
of approximately 6.8.   This should be checked with an
indicator if there is any reason to suspect that the
sodium phosphate has lost some of its water of crystall-
isation.

To prepare the corrective Leishman stain, make a
1 in 50 dilution in distilled water of ordinary 0.15
per cent. Leishman stain, and add the stock buffer
solution at the rate of three parts in 100 of the
total mixture.

METHOD OF USE.

The staining times for a thick blood film are
recommended as follows, although these can be varied
a great deal without producing an adverse result:

1.   Dip in Stain A for 1 to 2 seconds.

2.   Wash in distilled water for 1 second.

3.   Dip in stain B for 2 to 3 seconds.

4.   Wash in distilled water for 1 second.

5.   Place in stain C for 10 to 15 minutes in
     a small staining dish.

The/

The slide should be taken out of the Leishman stain and stood upright to dry without further washing in water.

NOTES ON THICK FILMS STAINED AS ABOVE.

1. No definite specifications as to size or thickness of the blood film need be laid down for staining by this method.   The films can be much thicker than those recommended for use with Field's technique as dehaemoglobinisation occurs as in the dilute Romanowsky staining method.

2. The naked eye appearance of a successfully stained film is that it should be completely transparent and without any residual haemoglobin.   The colour should be a very faint purple blue.

3. After staining, the film may be dried in an incubator if desired, but like other Romanowsky stains, if dried by direct heat it will turn blue.

4. No harm is done by leaving films for much longer than the specified 10 to 15 minutes in the dilute Leishman stain.   In very thick films such longer time will produce better results by ensuring complete dehaemoglobinisation.

5. Films that turn blue on drying have either

   (a) not been completely freed of haemoglobin and should have been left longer in the Leishman stain; or

   (b) have been differentiated in a Leishman stain more alkaline than pH 6.8.

6./

6.　　Films that have an excessively red appear-
　　　　have been differentiated in a Leishman
　　　　stain more acid than pH 6.8.

THE MICROSCOPICAL APPEARANCES OF A
SUCCESSFULLY STAINED THICK FILM.

The stained leucocytes, platelets and malaria

parasites are contrasted against a clear white back-

ground, thus resembling in general a film well stained

by dilute Giemsa.　Since the Methylene-Blue-Azure

solution A has a mild fixative action, there is little

or no distortion of the leucocytes.　The granules of

the cytoplasm of the polymorphonuclear cells are often

powerfully stained.　In Plasmodium vivax infections

the Schuffner's dots usually stain intensely in the

thinner parts of the film.　This gives rise to the

"ghost cell" effect that is seen also with Giemsa

stain and when present forms a diagnostic indication

of the benign tertian parasite that mey be most valuab-

le evidence in a case where young ring forms are seen

only in the film.

The parasite cytoplasm is stained a blue or grey-

blue colour and fine details in its structure can often

be seen.　The parasite chromatin stains a deep red.

In experiments to determine the maximum thickness

of film stainable by this method, films up to 1 milli-

metre in thickness were satisfactorily stained.　The

only modification in technique required for staining

these, was a slightly longer immersion of the slide

in/

in Stain C. than the time of 10 to 15 minutes recommen-
ded for routine films.   In such ultra-thick blood
films, between 60 and 80 leucocytes are seen per oil
immersion microscope field in blood with a normal total
white-cell count; and whereas preparations of this
nature are of experimental interest only, they do
indicate the very high diagnostic capability of the
thick-film method when a suitable dehaemoglobinising
stain is employed.   Since the rate at which dehaemo-
globinization occurs is increased by a rise in temper-
ature, it is usually possible to stain thicker blood
films more easily during warm weather.

The above staining method was used on several
thousand thick films taken for routine diagnostic and
research purposes and was found to give consistently
successful results.   It proved of especial value in
the estimation of parasite counts, as the thickness
of the films made it possible to count small numbers
of parasites as they diminished following the start
of treatment; and also in the many, tedious hours
of microscope work involved in this investigation, it
was a great advantage to be looking at parasites and
leucocytes in dehaemoglobinised films rather than
against the pink background of Field's stain preparat-
ions.   The method was adopted by the laboratories of
several of the military hospitals working in Italy
and/

and the pathologists who tried it reported favourably
upon the results obtained which, they agreed, were
such as to increase considerably the chances of find-
ing malaria parasites in cases with only a scanty
parasitaemia.   It is hoped that this modification
of Field's stain may prove of some value to those for
whom malaria diagnosis is an important part of their
routine laboratory work.

PART FOUR.

GENERAL   CONCLUSIONS.

PART FOUR.

GENERAL CONCLUSIONS.

It is fitting that the last part of this thesis should draw attention to the main points of interest which it would appear reasonable to extract from the mass of results and theoretical argument making up the subject matter of the foregoing pages.   It is intended to present these conclusions in a general way with reference to their supporting evidence, practical application and scientific interest, and it is hoped that in this manner it will be possible to set forth in a few pages, some angles on malaria chemotherapy that may be of value to both the clinician and the laboratory worker.   The conclusions are considered under numbered headings as they deal with different aspects of the work reported, and as such do not form an entirely continuous sequence.

1.   THE THERAPEUTIC TRIAL OF ANTI-MALARIAL DRUGS.

The assessment of the therapeutic value of an anti-malarial compound should be based on a wide series of observations covering the following investigations. These are:- (a) its toxicity in both therapeutic and prolonged suppressive doses and its compatibility with other drugs, (b) its speed of action in clearing asexual parasitaemia as estimated by parasite counts recorded/

recorded at regular intervals from the start of treatment, (c) its detailed mode of action on the asexual cycle of different varieties and strains of human malaria infections, (d) its possible action on tissue phases of the parasites both in regard to causal prophylaxis of primary mosquito infections, and in its effectiveness in influencing the relapse rate of established infections, (e) its fate in the body, absorption, utilisation, storage and excretion, and (f) its effect on gametocytes.

2. PARASITE COUNTS AND STAINING.

Parasite counts form an important part of a controlled therapeutic trial, but require a skilled and highly efficient technique if their interpretation is to be of real value. They are best done in thick blood films in which the parasites are counted in proportion to the leucocytes and their number per cubic millimetre calculated from the white blood cell counts. Very thick blood films are needed to detect the scanty parasitaemia seen as treatment progresses, and optimal staining of such films is an essential, with dehaemoglobinisation a great advantage.

An investigation into the staining characteristics of malaria parasites in thick blood films showed the pH of the staining solutions to be the determining factor in the production of good results. It was possible from these experiments to introduce a modification/

modification of Field's staining technique which has been used with success in the parasite counts reported throughout this thesis.

3. FINDINGS IN M.T. INFECTIONS AND STERNAL MARROW EXAMINATION.

Only a very limited number of cases with M.T. malaria were available for study, but it was possible to make certain observations from the results of parasite counts done at 12 hour intervals during the treatment of this small series.   Such counts suggested that the clinical peak of pyrexia would be the best time for the diagnostic demonstration of parasites in the peripheral blood and would be also the optimum time for the start of parenteral therapy with Quinine or Mepacrine.

Examination of sternal marrow smears in 52 cases of B.T. and M.T. malaria showed that parasites were found no more frequently in this site than in thick films of the peripheral blood.   It was possible, however, to demonstrate M.T. gametocytes in the marrow about 3 days before they were detected in blood films, suggesting, that these sexual forms are developed from visceral rather than from circulating parasites. No evidence was found in the marrow of any extra-erythrocytic phase of parasites.

4. COMMENTS ON THE TREATMENT OF B.T. FRESH AND RELAPSE CASES IN THERAPEUTIC TRIAL IN C.M.F.

(a)   The clearance of asexual parasitaemia in Fresh/

with heavy oral

Fresh cases was found to occur more rapidly than with low oral Mepacrine dosage, and both of these were quicker than dosage with Quinine.

(b)   In Relapse cases there was no significant difference between the rate of clearance of asexual parasites with heavy oral Mepacrine and with Quinine plus Pamaquin.

(c)   Quinine and Quinine plus Pamaquin were both more effective than Mepacrine dosage in clearing gametocytes from the peripheral blood in both Fresh and Relapse infections.   With low Mepacrine dosage, in some Fresh cases, the female gametocytes were able to persist beyond the last day of the treatment course.

(d)   On a 6-month follow-up, relapses were fewer after all the courses which had contained Pamaquin.   Although the small numbers of Fresh cases treated on each therapy course meant that the follow-up figures showed little significance when analysed statistically, on the larger series of Relapse cases, the advantage of Pamaquin in preventing B.T. recurrences was significantly demonstrated.

The above findings show that in the routine treatment of B.T. infections, dosage with Mepacrine should be initiated as early as possible, as this drug produces the most rapid effect in the reduction of the asexual parasitaemia.   It was therefore recommended/

recommended that a standard therapy course for malaria cases in C.M.F. in 1945 should contain Mepacrine dosage earlier than had been hitherto employed.   It was considered necessary to leave Quinine as the treatment on the first day of the course to allow for M.T. injections, in which it was felt that Quinine is a better drug in the control of initial pyrexia and general symptoms.   Three days of Quinine and Pamaquin dosage were also recommended at the end of the course, both for the value of Pamaquin in reducing the tendency to B.T. relapses, and for its gametocidal action.

The following are particulars of the dosage suggested for an 8-day standard treatment suitable for all fresh and relapse cases of B.T. and M.T. malaria.

Day	Drugs & Dosage.
1.	Quinine 10 grains, 3 doses, 8 hourly.
2.	Mepacrine 0.2 gm, 4 doses, 6 hourly.
3.	Mepacrine 0.2 gm, 4 doses, 6 hourly.
4.	Mepacrine 0.2 gm, 3 doses, 8 hourly.
5.	Mepacrine 0.2 gm, 3 doses, 8 hourly.
6.	Quinine 10 gr & Pamaquin 0.01gm, 3 doses, 8 hourly.
7.	Quinine 10 gr & Pamaquin 0.01gm, 3 doses, 8 hourly.
8.	Quinine 10 gr & Pamaquin 0.01gm, 3 doses, 8 hourly.

<u>Totals</u>. Quinine   120 grains.

Mepacrine 2.8 gm.

Pamaquin   0.09 gm.

In/

In patients with B.T. infections who had had
multiple recurrences, it was suggested that a further
4 days of Quinine and Pamaquin dosage could be added
at the end of the course.

5. THE ANTI-MALARIAL EFFECTS OF PAMAQUIN.

The action of Pamaquin in the treatment of B.T.
malaria had been studied in detail, as it appears that
future work on the pharmacology of this drug may lead
to important advances in malaria chemotherapy.

Contrary to general opinion, Pamaquin has been
shown to exert a delayed but powerful schizonticidal
action in the clearance of asexual parasitaemia in
B.T. infections. This action is quite apart from its
gametocidal effects which are recognised to be more
active than those of other drugs. It seems likely
that after absorption, Pamaquin is broken down in the
body yielding degradation products, some of which are
toxic substances, but others being potent anti-malarial
factors with an action against resistant, tissue phases
of parasites as well as against the demonstrable
blood forms. Work is proceeding in an attempt to
isolate the active, non-toxic products which might
prove of great value in the treatment of recurrent
malaria.

This thesis contains 6-month follow-up records
of 424 cases of B.T. malaria, which includes cases
treated for Fresh, Relapse and Multiple Relapse
infections/

infections.   Of this number, 202 received treatment
containing Pamaquin dosage of 3 to 20 days duration
as part of various therapy courses which also con-
tained Quinine and Mepacrine.   The other 222 cases
received from 7 to 20 days treatment with Quinine and/
or Mepacrine, but were given no Pamaquin.   The sub-
sequent relapse rate in these two comparable groups
of cases is shown in Table 10.

TABLE 10.

TREATMENT	Quinine and Mep. Courses.	Pamaquin Courses.
RELAPSE	57	18
NO RELAPSE	165	184

The above results are statistically highly
significant in showing the influence of Pamaquin on
the relapse rate in B.T. infections, (Chi-square
19.28 with Probability less than 0.001.)

Another follow-up of interest was in the group
of 50 cases, treated for multiple recurrences of
B.T. malaria, where no further relapses were recorded
after Quinine plus Pamaquin therapy given intermitt-
ently over a period of 30 days.   The reasons for the
unique success of this treatment regime are not easy
to postulate, but it is known that early recrudescenc-
es are the common form of relapse after Quinine and
Pamaquin therapy, and it is therefore possible that
an intermittent course would be more successful than
a/

a continuous course in dealing with an infection which tended to remain inhibited in some latent and resistant form during the administration of anti-malarial drugs. It should be noted in this connection, that both Quinine and Pamaquin are fairly quickly excreted from the body and any such inhibition exerted by them would thus be soon removed during the intervals in the treatment course, whereas with a slowly excreted drug like Mepacrine, any action on latent parasite forms would tend to be much more sustained.

It must be mentioned that other recent workers have not always reported similar favourable results with Quinine-Pamaquin therapy.   A therapeutic trial conducted on 384 British cases of chronic B.T. malaria in Southern India Command in 1945, yielded a 34.2% relapse rate after a 10-day Quinine plus Pamaquin course, within a follow-up period of 3 months.   The peak incidence of relapses occurred in the third week after treatment, which is in keeping with the observation to this effect made above, but the difference in the results is not easy to explain.   Much of the original work which drew attention to the anti-relapse value of Pamaquin was carried out in India by Sinton, and in the recurrent B.T. cases from India treated by the Research Team at Woolwich during the Autumn of 1945, the 10-day Quinine-Pamaquin course appeared to be producing results comparable with those in the earlier/

earlier C.M.F. cases with a relapse rate much lower than 30%. One point which may have influenced the different findings, is that the patients in C.M.F. and at Woolwich were mostly treated during the winter months and were not taking suppressive Mepacrine just before the clinical attack. In view of the property of Mepacrine to displace Pamaquin from the body tissues, it is possible that the question of previous suppressive Mepacrine is an important factor which must be taken into consideration when assessing the relapse incidence of B.T. infections treated on Pamaquin therapy.

6. THE ACTION OF PALUDRINE (M.4888) IN B.T. MALARIA.

Therapeutic trials with this new drug are still in progress, but from the results so far obtained, it promises to be the greatest advance in malaria chemotherapy yet made. In M.T. malaria, Hamilton Fairley has found it to be a true causal prophylactic against natural infections and therefore ideal for the prevention of this disease. It is also highly successful in the treatment of clinical attacks of M.T. malaria. In B.T. malaria, while not a causal prophylactic, it is a good suppressive drug, being easy and safe to administer over long periods. It is also effective in the treatment of acute B.T. attacks, although it does not prevent the tendency to relapse. Its action in Quartan malaria has not yet been reported.

It/

It seems likely that in the near future, Paludrine
will entirely supersede Mepacrine for the routine pro-
phylaxis, suppression and treatment of M.T. and B.T.
malaria.

This thesis reports the first trial of Paludrine
in which detailed observations were made of its action
on B.T. parasites during treatment.   It has been shown
that it brings about clearance of asexual parasitaemia
in approximately the same time as taken by Quinine
or Mepacrine, though there is a rather longer latent
period before its initial effects than with these
drugs.   It produces an inhibition in the development
of growing trophozoites similar to that seen with
other anti-malarials, but it causes an arrest of the
parasites at the stage of their first nuclear division.
Schizonts of an earlier form are destroyed by this
drug than is the case with Quinine, Mepacrine and
Pamaquin and the optimum rate of clearance of asexual
parasites occurs when the administration of the drug
is started on the day following the clinical paroxysm.
Paludrine does not appear to have a powerful action
on B.T. gametocytes.

It has been shown that Paludrine in adequate
therapeutic doses can be safely given at the same time
as Pamaquin.   In this respect it has advantages over
Mepacrine which is pharmacologically incompatible with
Pamaquin, and it is hoped that combined Paludrine-
Pamaquin/

TABLE 11.(page 143).

THERAPEUTIC PROPERTY.		DRUGS.		
	QUIN.	MEP.	PAM.	PALUD.
Usual daily dosage.	2.0 gm.	0.8 gm.	0.03 gm.	0.75 gm.
Toxicity.	++	+	++++	+
Speed of initial action.	++++	+++	+	++
Reduction of pyrexia.	++++	+++	+	+++
Inhibition of parasite cycle.	++++	+++	+++ (late)	++
Parasite stages destroyed.	M - T 1	S2S3MT1	S3 M	T6S1S2
Rate of Asexual clearance.	+++	++++	++	+++
Collapse of amoeboid forms.	-	++++	++	+
Bunching of pigment.	-	++++	++	+
Action on Gametocytes.	+++	++	++++	+
Reduction of relapses.	+	++	++++	++

TABLE 11.   A COMPARISON OF SOME PROPERTIES AND

EFFECTS OF QUININE, MEPACRINE, PAMAQUIN AND

PALUDRINE IN THE TREATMENT OF B.T. MALARIA.

Pamaquin dosage may prove an effective measure in
reducing the relapse rate in B.T. malaria and possibly
in the treatment of Quartan infections.

7.   A COMPARISON OF THE ACTION OF
     ANTI-MALARIAL DRUGS IN B.T. MALARIA.

It is felt that a summary of the action of
Quinine, Mepacrine, Pamaquin and Paludrine in B.T.
malaria, may usefully be given in tabular form and
based on the findings recorded in this thesis.   This
is shown in Table 11 in which symbols are used to
represent the relative intensity of effect under the
various headings dealing with the main properties of
anti-malarial drugs.

8.   SOME PRACTICAL CONSIDERATIONS
     IN B.T. MALARIA THERAPY.

The rate of clearance of asexual parasites is
a measure only of the rate of clinical cure.   With
the present-day drugs, this cure appears to be effect-
ed in a time so short that little advantage would be
gained by attempts to shorten it further.   For
instance, it has been shown that an extra rapid clear-
ance of parasites occurs when Mepacrine is injected
intramuscularly at a time when the parasites are most
susceptible.   This procedure, however, often causes
so much clinical upset, that it is more of academic
interest than of practical importance.   Such measures
would be justifiable only if they could be shown to
alter the subsequent relapse rate.

It/

It has been seen that a rapid clinical cure of
B.T. malaria can be brought about by the use of heavy
oral Mepacrine or Paludrine.   Accordingly it would
seem logical to suggest that in areas where the chances
of reinfection are high, treatment of this nature
should be employed during the infective season, rather
than a therapy course designed to produce a high pro-
portion of radical cures.   The Mepacrine and Paludrine
courses would have the additional advantages of being
of short duration and of not interfering with the
routine suppressive use of these drugs.

On the other hand, when there is little risk
of further infection, such as during the Winter months
and when suppressive drugs are not in use, there is
need for a more drastic attempt to eradicate those
infections which have become recurrent. A Quinine plus
Pamaquin course is much more effective than Mepacrine
or Paludrine in achieving this result and an inter-
mittent dosage regime, such as reported in this thesis,
would appear to be worthy of consideration. It seems
likely that combined Paludrine and Pamaquin therapy
may soon be used rather than Quinine plus Pamaquin
in the treatment of recurrent B.T. malaria and may
produce still better results in the eradication of
this "nuisance" condition.

9./

9. THE VALUE OF PARASITOLOGICAL STUDIES IN THE TRIAL OF ANTI-MALARIAL COMPOUNDS.

Much of the work in this thesis is based on microscopical studies of malaria parasites during treatment with anti-malarial drugs, and attempts have been made to assess the therapeutic value and use of these compounds from the observations made. There are probably those who would consider that this microscopical approach has been given undue prominence in this work and that practical considerations, such as rate of reduction of pyrexia, should have received more attention as being of greater importance than the determination of the exact stage in the parasite asexual cycle on which the drug is most active. Such critics might also hold that studies such as the "Relapse Factor" can have little bearing upon the problems of malaria chemotherapy; whereas in fact they shed valuable evidence on the influence of acquired immunity on the clinical manifestations and response to treatment in B.T. infections.

While it is agreed that the ultimate criteria on which the adoption of a new anti-malarial drug must rest, are its properties for benefitting the patient in the clinical sense, it is maintained that a detailed study of its effects on the causal parasite provides an essential spotlight on its mode of action with which most of its clinical effects can be correlated.

As/

As an example of how it is possible to forecast the
value of such a drug from its parasitological action,
it could be reasonably expected that Paludrine would
produce no striking new cure of B.T. malaria as soon
as it was evident that its main effect on the parasite
cycle was to attack the schizont stages in much the
same manner as Quinine, Mepacrine and Pamaquin.   No
compound yet tried has been found to destroy specific-
ally the growing trophozoites of B.T. malaria and it
would indeed be interesting to observe the curative
value of such a drug which might well be expected to
produce a new form of therapy response in this condit-
ion.

In conclusion, therefore, a plea is entered
for the parasitologist and biochemist to join forces
with the clinician so that together they may rational-
ly demand from the synthetic chemist, new, calculated
therapeutic compounds that will one day bring final
victory over human malaria and write a new chapter
in the history of chemotherapy.