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PART I.

Section I.

History of the discovery of the Parasite
Classification of the Parasite.

I. Benign
   (a) Quartan
   (b) Tertian.

II. Malignant or Aeetivo-Autumal.

The Parasitic origin of Malaria as a theory can be traced as far back as the times of the Roman Empire when Varro (B.C. 118-29) says "It is also to be noticed that where there are marshy places........, certain "small animalcule spring up, which cannot be seen by "the eye, but being in the air enter the body by the "mouth and nares, and cause dangerous diseases".

For centuries this was the theory most commonly accepted. No known attempt to discover the nature of these animalcule was made, until Mitchell in 1849, finding spores in usual quantities in marshy districts suggested that they might be the cause.

In 1864 Lemaire found lower organisms in larger numbers in the miasma of the marsh, than in the atmosphere of a dry healthy district, and thought that possibly they might have a causal influence.

But no theory held any general belief until

See page 11 of thesis.
Salisbury's paper was published in 1866. In turning attention to the malarial person, and away from the malarial swamp and miasma, we are taking the first step towards a correct solution of the problems which have until recently surrounded the subject of Malaria.

Binz in 1867 observed the fatal effect of quinine on protoplasm, even in weak solutions, and connecting this with the remarkably curative effect of quinine in malarial fever suggested that the pathogenic agent of malaria might be a lower organism.

Were it not for the wide-spread belief that the discovery of Klebs and Crudeli gathered round it, I should pass over their communication without mention. Like Mitchell, Lemaire and many others, they were searching for the cause in the soil, instead of in the infected person. Their belief was based on the fact that certain bacilli, which when cultivated and injected into animals, caused malarial symptoms. But, as we shall see later; malarial symptoms have other causes than the malarial parasite and to any who may still have a lurking belief in the agency of the bacilli of Klebs and Crudeli, it ought to be enough to say that the only pathognomic sign of Malaria is the presence of the malarial parasite on the stage of the microscope.

Although repeated researches in this line have
clearly demonstrated the fallacy of this theory, as recently as 1885, one so high in the profession as Surgeon-General Sir William Moore refers to their discovery as a settled fact in the causation of malaria; and Burney Yeo writing in the year 1899, whilst he warns us against belief in the Klebs-Crudeli theory, considers it sufficiently worthy of mention.

Meckel and Virchow were the first to place their feet on the right path. They observed that in the blood of persons who had died of malarial fever, masses of pigment were found enclosed in bodies of various shapes. These examinations were made when both patient and parasite were dead. They were regarded rather as the product than as the cause of Malaria.

Thirty-three years later, in 1880, whether aided by the discovery of Meckel and Virchow, or acting on an original idea, or favoured by mere chance, Laveran whilst engaged in examining fresh blood drawn from a living patient suffering from malaria, came across an exflagellated crescent. Cause and effect at once took root in his mind.

Next year he announced the observation of three distinct bodies:

I. One set of bodies were 1/6 the diameter of a red blood corpuscle and contained
specks of fine dark pigment.

II. In the second set the parasite was equal in size to a red corpuscle, sometimes even larger, and contained pigment which was coarser and more actively motile.

The above two parasites Laveran believed to be attached to the red corpuscles, and to grow at their expense.

III. He also described what is known as the crescent and observed the changes which a crescent undergoes in its transformation into an exflagellated organism.

Although Laveran had published his observations and Richard by independent work had shown full confirmation, the fallacious theory of Klebs and Crudeli still held general belief. The main reason for this delay was that fresh specimens were not examined. Even skilled investigators, like Marchiafava and Celli, believed the bodies described to be merely degenerated red corpuscles and this belief held sway until dried specimens were laid aside, and examination was made of fresh blood from a living patient.

My own experience is a repetition of this mistake. For months I prepared blood films which I fixed, dried, and carefully stained. It is true I saw beautiful examples of stained leucocytes, but to see the malarial parasite I still had to look at coloured plates.
"The beginner should work only with unstained preparation of fresh liquid blood. To the unpractised, staining is full of pitfalls. It was not until I read these words of Manson that I began to understand the appearance of the parasite.

No sooner did Marchiafava and Celli examine blood in this way than they became convinced that the actual agent in malaria was a living organism circulating with the blood. This was in 1885, and since, to quote the words of Osler, "So far as I know, not a single observer, who has had the necessary training, and the material at his command has failed to demonstrate the existence of these parasites."

As the publication of observations in different parts grew, it was found that the same description did not apply to all parasites. Golgi in Pavia found that the parasite in the blood of his patients was not the same as that described by Laveran in Algeria, and Marchiafava and Celli in Rome. He further found that in several patients with a tertian type of temperature the parasite differed from that found in those patients with a quartan rise of temperature. After this had been corroborated by further observation he was led to the conclusion that type of temperature depended upon type of organism.

Golgi further noticed that in the vast majority..."
of cases, the crescent body described by Laveran was not found. On making further comparison it was observed that not only the parasite, but the temperature chart and the severity of the fever differed considerably in Pavia, where Golgi worked, from those found in Algeria, where Laveran worked, and in Rome, from the neighbourhood of which Marchiafava and Celli obtained their cases. Gradually it became a settled fact that where the temperature was atypical and the symptoms severe, the crescent body was found; and that where the temperature was regularly intermittent, and symptoms mild, the crescent body was absent.

We thus divide malarial fevers into,

Benign forms in which the crescent body is absent.

Malignant forms in which it is present.

We can further divide the Benign forms into Tertian and Quartan.

Numerous attempts have been made to differentiate the Malignant forms; Grassi, Marchiafava, Mannaberg and Manson adopt a three-fold division, -

(a) The pigmented quotidian parasite.

(b) The unpigmented " "

(c) The malignant tertian "

Marchiafava and Bignami distinguish two varieties, -

(a) The quotidian.
(b) The malignant tertian.

Whilst the difference between a benign tertian and a benign quartan is at once apparent, in the cases of crescent infection that I have examined I have not been able to satisfy myself of any constant difference in the morphology of the malignant parasite.

As my text book I had taken Mason's "Tropical Diseases" in which he adopts the threefold classification of Mannenberg, and I was beginning to have the same sense of failure, that I had felt in trying to find the parasite in a dried stained specimen, until Major Ross stated to me that probably all forms of aestivo-autumnal or malignant malarial fevers were associated with the same parasite. I subsequently found that this is the opinion held by Hewetson and Thayer in America, Laveran in Algeria, Golgi in Italy, Hans Zieman in Germany, and James Cantlie in England in reviewing opinions in general. Where however so many competent observers have described different forms, we shall probably find that, as our methods of observation become more perfect, the differentiation of the malignant parasite will become more exact. Hence I adopt as a provisional classification of the malarial parasites:

I. Benign.

(a) Quartan.
(b) Tertian.
II. Malignant or Aestivo-autumnal.

My intention in giving this brief history of the evolution of the discovery of the parasite from the swamp and its miasma (Varro, Mitchell, Lemaire, Klebs and Crudeli); to the infected person (Meckel and Virchow); from the dead person to the living patient but with dried and stained film (Marchiafava and Celli); and from this to the fresh film of a living patient (Laveran and Richard), has been to impress what is my strong conviction that for purposes of diagnosis, clinical demonstration, and especially in the commencement of the study of Malaria, films of fresh blood should alone be used.
Section II.

Methods of Examination.

I. By fresh films.

II. By stained films.

The parasite is intracorpuscular, and thus we must attempt to so dispose the corpuscles that their surfaces, and not their edges will be exposed to view. We must further attempt to have them as little altered in their shape as possible. The finger pad or the lobe of the ear may be used - the latter is perhaps less painful, the former more convenient. Whatever part is selected should be cleansed with alcohol to remove dirt and fatty substances. The slides and cover glasses, for the same reason must be cleansed in the same way, but with more care. Having cleaned the cover slips and slides it is a convenient plan to keep them immersed in a vessel containing Methylated spirit until required. An imperfectly cleaned slide or cover will cause a film filled with bubbles. The instrument that I have found give a drop of blood most readily, and with least pain is a spear-pointed lancet. A needle will however serve the purpose. A small puncture is made, and the first few drops of blood wiped off. A minute droplet no larger than a pin's head is now expressed, and the cover glass held
by the forceps, so applied that the lower surface just touches the upper margin of the drop. The slide is then given a final wipe with a dry cloth, the cover glass applied to the slide, and the preparation is complete. The cover glasses are held by clamp forceps. By this method they are manipulated more easily, and there is no danger of moisture from the fingers condensing on the cover glass and preventing the formation of an even film. Three or four preparations are made in this way. No pressure should be exerted on the cover glass, and only the thinnest covers should be used—otherwise the corpuscles become crenated. The film, which should now be held up to the light, will have the following appearance if successfully prepared. One or more areas will be seen to contain apparently no blood, they are quite transparent. Unless one or more of these light areas be present, the preparation may be put on one side as useless, as the corpuscles when seen under the microscope will be found in rouleaux with their edges only, and not their surfaces exposed to view.

I have gone into this matter minutely because I found not only in my own experience but from watching others, that failure to readily find the parasite has been because the corpuscles have not been favourably disposed to exhibit the parasite.
Put one of these clear empty areas in the centre of the field and gain the focus with a low power (e.g. Zeiss A). The corpuscles will be seen to lie with their surfaces exposed. My own plan is then to put on the high power (e.g. Zeiss D). The benign parasites and crescents can be at once detected; and after some experience there is no difficulty in observing the malignant fever forms. The advantage, however, of using Zeiss D at this stage, is the comparative rapidity with which the field can be traversed, and a diagnosis reached. The highest objective used by Laveran was 1/6 dry lens; but in all cases when we have found the parasite we should use the 1/12 oil immersion.

If, however, we wish to go beyond the clinical demonstration and diagnosis of the malarial parasites and to study their morphology we must have recourse to methods of staining.

Again we must so dispose the corpuscles that their surfaces are exposed to view. Greater care is needed in the cleansing of the slides and covers when making a dried specimen, than when working with fresh films. My first method was to make a blood film by means of two cover glasses in the ordinary way. I found the corpuscles often crenated and otherwise deformed, no doubt by some pressure on the covers, which even with the greatest care it is impossible
to avoid. Either of the following methods will give a uniformly correct disposition of the corpuscles and without crenation or other deformity.

A droplet of blood is obtained as previously described but on a slide and not a cover. A piece of uncreinkled gutta percha tissue measuring \( \frac{1}{2} \) by \( \frac{1}{3} \) inch is applied to the drop of blood on the slide, \( \frac{1}{4} \) inch from one end of the strip of tissue. A film quickly forms and then the tissue is slowly drawn along the slide at an angle. Instead of the tissue a piece of cigarette paper of similar size, or as Dr. Neil MacLeod suggests a piece of stout note paper may be used. I personally prefer the cigarette paper.

The film as Roux first suggested may be fixed at once, by being put in a mixture of equal parts of absolute alcohol and pure ether. A better method is to allow the film to dry, and then to fix it, by allowing a little absolute alcohol to be in contact with it for five minutes. After fixing and removing the excess of absolute alcohol it is ready for the stain.

Good results may be obtained with most of the basic nuclear dyes. Loeffler's methylene blue or borax-Methylene blue (Borax 2\( \frac{1}{3} \% \) and Methylene blue
1% may be used. The former has no effect on the red corpuscles and requires about 30 to 60 seconds. The latter stains the red corpuscles a faint blue, to some extent a disadvantage, nuclei of the leucocytes a deep blue, and the parasite an intermediate tint. This stain requires a little less time than Leofflers solution, 15 to 30 seconds being quite sufficient.

Undoubtedly the stain which shows the morphology of the parasite most clearly is the one suggested by Romanovsky. We need a saturated aqueous solution of methylene blue, and a one per cent watery solution of eosin. Add two parts of eosin to one part of methylene blue, stir and pour into a beaker. The solutions may be filtered before mixing, but the combined solution, although a sediment may form must not be filtered. The older the Methylene blue solution the better. The stain must remain in contact with the fixed film for from one to three hours. It is well to prevent evaporation by covering the whole with an inverted glass beaker resting on moistened blotting paper. A point of practical importance is to have the film to be stained on the surface of the liquid, not at the bottom - otherwise the sediment which forms on mixing the two solutions settles on
the film, and is most difficult to remove without damaging the preparation. If a cover glass be used there is no difficulty in floating it film downwards.

In using a slide film, I have adopted the method of inverting the slide on a small watch glass 2/3 filled with the stain. The solution at once covers the part of the slide overhanging the watch glass.
SECTION III.

The Parasite is intracorpuscular.

Brief description of the parasites in general.

Minute: description of each variety of parasite.

Significance of various stages of the fever cycle.

Best time to examine blood for the parasite.

Before entering on a particular description of each variety, it will simplify matters if we give a description which applies more or less to all the parasites.

Commencing with earliest forms we find that spores in enormous numbers are circulating free in the liquor sanguinis. No doubt many of these spores are attacked by the leucocytes but probably the great majority escape to infect the red corpuscles. La- veran believed that the spore attached itself to the red corpuscle and was epicorpuscular. Golgi shewed that the parasite, no doubt at first epicorpuscular, soon entered into the substance of the corpuscule and became intracorpuscular.
The following three observations lead us to believe in the truth of Golgi's remark:

I. After some experience a delicate yellowish tinge can be observed lying between the eye and the parasite.

II. When the uppermost surface of the corpuscle is in focus, the parasite is out of focus, often so much so that the pigment is not even seen.

III. That though the movements of the parasite may be very active, a pseudopodium is never seen to protrude beyond the margin of the corpuscle.

In its early stage the parasite is without pigment, but as it grows the haemoglobin of the corpuscle in part is changed into the melanin of the parasite. This melanin, dark in colour as its name suggests, occurs in any form from the most minute specks to coarse clumps and rods. Distinct movement, probably Brownian, takes place in the pigment particles, active at first but slower as maturation proceeds. The parasite growing at the expense of the corpuscle, at its maturity may be from $\frac{1}{6}$ to a size equal, and sometimes larger than the corpuscle according to the
variety of the parasite. When the maximum size has been reached faint lines are seen to appear at the periphery, followed by the formation of segments and finally of spheres. During this time the pigment from being scattered is seen to collect into groups and finally forms a dense central clump. The "sporulating body" is now fully formed. The parasite has now multiplied itself and is ready to escape from its corpuscular host. The corpuscle bursts and the spores and pigment are set free in the liquor sanguinis and the cycle is recommenced.

We now pass to a more minute consideration of the parasite.

THE PARASITE of BENIGN TERTIAN FEVER.

In a fresh film the youngest stage is observed as a small round body without colour - sometimes appearing as a refractive ring with a lighter colour-ed centre. That it is not a vacuole may be determined by the fact that it is actively amoeboid - throwing out and retracting pseudopodia; has not a distinct, clearly defined edge but shades off into the surrounding protoplasm; and that its apex shews no black point on focussing above it.

As the parasite grows particles of fine pigment appear - often at the apex of a pseudopodium. They
shew incessant movement, constantly changing their position; but whether this movement is active or passive is not yet known. Towards the end they become aggregated in the centre of the parasite in the form of a ring and finally just before sporulation become fused into one or two blocks of deep black pigment.

The Parasite increases in growth until it reaches the size of the red corpuscle it occupies—sometimes both parasite and host are larger.

The movement of the parasite, active at first, becomes gradually less until when the pigment forms the central ring, it has almost ceased.

The red corpuscles become completely decolourised and in many instances, so considerably hypertrophied, that they may be twice their natural size.

On making a successful stain with Romanovsky's method the young parasite is seen as a ring of delicately stained protoplasm. At one point of the periphery is a small deeply stained spot and around this an unstained area. The former represents the nucleolus, the latter the nucleus. As growth takes place the nucleus and nucleolus become less distinct until they disappear. Just before sporulation, however, the nuclear elements again come into view and, becoming surrounded by a small mass of protoplasm form
a spore.

The parasite has now reached maturity and proceeds to the process of sporulation in the manner previously described. The segments, numbering fifteen to twenty, are arranged around the more or less centrally-placed block of black pigment. Their appearance at this stage has been compared to a bunch of black grapes.

THE PARASITE of QUARTAN INFECTION. In its earliest stages the parasite has not been differentiated from that of Tertian infection.

The Pigment, at first absent, gives the first clue to its distinction. We have no difficulty in observing that the particles are coarser, and, perhaps because of this, the colour is deeper. We note further that the pigment particles show much less activity. As the time for sporulation approaches aggregation of the pigment towards the centre of the corpuscles is taking place. It does not collect itself as a ring but assumes a radial arrangement; and finally when sporulation is complete, becomes aggregated into a central clump.

The Parasite is not only slower in growth than that of Tertian infection, taking seventy two hours to complete its cycle, but rarely reaches the same size. At its maximum while it completely occupies
the red blood corpuscle so that merely a rim is left, it never causes hypertrophy.

The movement of the parasite is sluggish and lazy from its youngest stage and onwards, until when pigment is fully formed, all movement has ceased. The red blood corpuscle is affected in a different manner — it contracts rather than expands; and its colour is deepened rather than diminished.

In its staining reactions it behaves in a similar way to the Tertian Organism.

The Sporulating body differs in that the segments number from 6 to 12, and are arranged in a perfectly regular manner round the central clump of pigment. The terms rosette, marguerite, or daisy form, have on this account been given to the Quartan Sporulating Body.

THE PARASITE of AESTIVO-AUTUMNAL FEVER.

I have already stated that many observers believe that this parasite exists not in one but in distinct varieties. I have already stated that there is only evidence of the existence of one variety of aestival parasite. No doubt much of the difficulty in the study of this parasite is due to the fact that only the earlier forms are to be found in peripheral blood. To make an accurate study of the life history,
the spleen must be punctured, an operation which I have not felt justified in performing; or immediately after death to make preparations from the viscera and bone marrow. I have had abundant opportunity of making examinations of fresh films. During the year 1899 we have treated 152 cases of malaria, but, of these, only two died, — one from malarial cachexia; and at the time of the other death I was away from hospital. My studies have therefore been limited to examination of the parasite as found in peripheral blood.

The youngest forms of the aestival parasite are very similar to those found in Quartan and Tertian infections. The only difference to be noted is that they are somewhat smaller. They are often described as ring-shaped. More careful focussing will show that the lumen of the ring is really an attenuated part of the parasite. Further, from the changes the parasite is seen to undergo we are led to believe that the "ring shaped" parasite has no lumen proper but that there is more protoplasm at the periphery than at the centre.

In some cases, the pigment is apparently absent in the early stages. But as the parasite grows, so far as my studies have shown me, it is to be found
in all cases. It is seen as particles of fine dark brown dust. Often no more than one or two particles can be observed; from this any number up to 10 or 15. Often in the earlier stages they are motionless; but as the parasites grow older, the pigment particles show active dancing movement. As the time for sporulation approaches the pigment collects into clumps, more or less centrally placed.

The parasite at its maturity reaches usually about half the size of its host. It may, however, occupy the red corpuscle almost completely.

The movement of the parasite is active, especially in its earlier forms, but becomes less active as the pigment becomes collected, previous to sporulation.

In the Tertian infection we showed how the red corpuscle became decolourised and often hypertrophied. In the Quartan the host became somewhat contracted and except immediately previous to sporulation assumed a slightly deeper tint. The greatest change however takes place in the aestival variety. The shrinkage is greater and takes place in the earliest forms. The colour is so deepened as to have the name of "Brassy body" applied to it. This change, however, does not apply to all the parasites. Many, indeed
the majority of hosts, are apparently unaltered. To staining reagents it behaves in a similar way to the Tertian Parasite.

The Sporulating Body is very rarely seen in finger blood. It is most easily observed in spenic blood obtained by aspiration; or in preparations made immediately after death from bone marrow or the deeper viscera.

The body is then seen to be composed of 6 to 8 segments arranged more or less in a heap, around the central block of black pigment.

Golgi was the first to connect these facts observed by the microscope with the facts observed at the bedside. The first step was to recognise that the sporulating body did not represent a process of dissolution but a process of reproduction. He found that where a temperature chart showed a malarial paroxysm the microscope showed the sporulating body.

Without attempting to describe the symptoms we may divide the cycle into the following stages:

1. Premonitory
2. Cold.
3. Hot
4. Sweating
5. Quiescent.

In the Premonitory stage, the parasites reach maturity. A certain number have formed sporulating
bodies - their host has ruptured and the spores have been set free. Along with the spores there is liberation of a toxin, which in small quantity causes the minor symptoms included in the premonitory stage. But, as we know, the development of the parasites is largely simultaneous, and while a few parasites anticipate their growth, the vast majority of infected corpuscles are broken up at about the same time. It is the vast amount of toxin which is thus set free that is the cause of the sudden rise of temperature, and the production of the cold stage. The latter part of the hot and sweating stage we may regard as an effort of nature to rid herself of the toxins. At the end of the sweating stage the young spores have attacked and entered their new hosts, and during the quiescent stage their growth is taking place. It is evident therefore that the time at which the parasite can be most easily demonstrated is during the earlier part of the premonitory stage.
SECTION IV.

Mobile Bodies

Description

Their nature.

I. Are they malarial?
   Observations to prove the negative

II. Are they bacilli?
   Observations to prove the negative.

III. Are they the extruded granules of Leucocytes?
   Observations by Horder, Cabot.

Their power of locomotion.

We now pass for a moment to a form of life, which, I believe, is not generally recognised. In the Liquor sanguinis are always to be noticed very numerous minute mobile bodies. In size they vary from about 0.5μ to minute specks. Unless 1/12 oil immersion lens be used they are almost sure to remain unnoticed and then careful focussing is needed to bring them into view. In shape they are sometimes round, sometimes oval. They are dark in colour and refractive on high focussing. They possess a wriggling movement in themselves and have the power of distinct locomotion.

I had only examined with 1/12 immersion the
blood of patients suffering from malaria. I had only noticed these mobile bodies in such examinations. My first impression therefore, was that they were malarial in nature; that they were either spores or particles of freed pigment. That they were not spores, I soon convinced myself by examining the blood of patients who had been free from rigors for some days, and in whom the only form of parasite present was the crescent. These bodies, however, I found in their usual numbers. Without fever and fever forms there could be few, if any, sporulating bodies, and therefore few, if any, spores. The conclusion is therefore obvious that they were not malarial spores. Against the possibility of their being particles of pigment, was not only the above fact but also the fact of their power of transference from place to place. To make the matter incontrovertible I examined the blood of 25 patients who had never been out of Great Britain, and who stated they had never suffered from fever. In every case these mobile bodies were present. I felt myself justified therefore in concluding, that they had no connection with the malarial parasite. To me these bodies were a discovery. I had not seen, and for some time could not find, any reference to them in books; and in speaking to others had difficulty in carrying conviction as to their presence.
The Hospital Pathologist spoke of them as muscae volitantes.

A wiser thought came from the Hospital Bacteriologist - that they were bacteria introduced through imperfect cleaning of fingers and material used. To test the truth of this statement I boiled for fifteen minutes in Carbolic lotion (1 to 20) both cover glasses, and slides. I then dried them over a bunsen flame, and placed a cover glass on a slide in such a position that the edge of the slide was flush with the edge of the cover glass. The instruments used in manipulating these had been boiled in solution of the same strength, and for the same time. Neither covers nor slides had been touched by the fingers. They were retained in position by clamp forceps. The finger I prepared as for the most particular operation and had a perchloride of Mercury (1 to 1000) moist dressing kept on for 12 hours. The spear-pointed lancet was heated to incandescence in a flame, immediately previous to use, and cooled in carbolic lotion (1 to 20). I felt therefore that my material might justly be considered free from bacilli. The edges of the cover and slide being applied to the droplet of blood, a film was made by capillary attraction. This experiment I have repeated, and in each case I have found these mobile bodies to be present. I felt my-
self justified therefore in concluding that if bacterial in nature at all, they were not introduced from without.

I was thus led to the conclusion that they were a normal constituent of healthy blood.

Their size and movements excluded the idea that they were blood platelets.

I had made a note on the 21st. September, that they were found in largest numbers in the neighbourhood of leucocytes; but I had come to no conclusion as to their nature. In the Lancet three weeks later I came across a note in which E.H. Horder of Pakhoi S. China describes bodies similar to those I had noticed. In his own words "The granules in the white cells began to move most vigorously and after watching them for some minutes it was noticed that they (the granules) were extruded from the cell, scattered all over the field and joined those already in the plasma". This was the first reference I had seen to bodies whose description was similar to those I had observed.

Cabot describes certain intraplastic bodies as "small round colorless granules about the size of the finest fat drops, or about ¼ to 1 in diameter. They are highly refractive and have rapid dancing molecular motion but no power of locomotion. They
are insoluble in alcohol and ether, not stained by osmic acid, and take no part in the formation of fibrin. He believes that they are the extruded granules of neutrophilic and eosinophilic leucocytes, from the fact that their staining reactions are the same.

It will be noticed that my description which was written before I had seen any printed reference, described these bodies as having a distinct power of locomotion. Subsequent observations have only led to corroboration of this opinion. That change of position takes place will be at once admitted, and that it is due to a current in the plasma seems incredible, when one body is seen passing up the field, and another passing close by on its way down.

As soon as I became convinced that these mobile bodies were not malarial, I laid aside, for the time, any further research into their nature.
PART II.

SECTION I.

The Latent Phase.

Two examples.

It is also a recognised fact that having once contracted the disease, although the patient may be amidst surroundings where malaria as a primary infection is unknown; and although years may have passed since the infected districts have been left behind, any unusual strain, bodily or mental, may cause a recurrence of the old symptoms.

The following are two interesting examples:-

R. F., age 53 was admitted on 24th August 1899 suffering from internal Haemorrhoids. The Haemorrhoids were ligatured and when on the third day after the operation his head began to ache and his temperature to rise, I thought a ligature must have slipped and septic absorption be taking place. However the following day the temperature fell to normal, and all discomfort disappeared. The eighth day after operation headache again became severe, and the temperature rose suddenly to 103.6°F, with as sudden a drop early the following morning to 99.8°F, again with the dis-
appearance of headache and other discomfort. After
an interval of a day the temperature again rose with
a rigor to 104°F. followed by a fall the same evening
to 99.6°F. This was repeated the following day the
temperature reaching 105°F.

The following is a copy of his chart:

I was struck by the fact that for four days after
the first attack of fever from the 3rd. to the 6th.
September, the patient had an absence of all discom-
fort; that the second attack was preceded by headache
commenced with a rigor, and ended, as suddenly as it
arose, by moderately profuse sweating. This clinical
picture at once led me to think of malaria. I made
enquiries and found that the patient had suffered
severely from Java fever eight years ago but since
then, had been entirely free. On the 9th., at 1.30
when the temperature was 103.4°F. I made an examina-
tion of a fresh blood film, and, to quote from notes
I made at the time found "Plasmodia about the size
of red corpuscles with fine granules of motile pig-
ment". At a later examination I found "red cells
containing small plasmodia and fine granules of pigment. I made several permanent preparations stained with Borax-Methylene blue. Unfortunately I had not then learnt the use of Romanovsky’s stain and the permanent proof of the above statements was not as obvious as I should have liked. My honorary physician, to whom I submitted a specimen of the first examination, described the sporulating form, quite a possibility considering the period of the paroxysm at which the blood was withdrawn.

G.B. age 46 landed in Liverpool in May 1897 suffering from a fractured arm, received three months previously when at sea. He had received a severe infection of malaria three and a half years ago, and had since suffered several relapses. Since the day he landed, he had practically lived in hospital. On the 11th February 1900 he had a headache followed by a sudden rise of temperature to 103.6°F., with a slight rigor. I immediately examined his blood and found an occasional aestivo-autumnal parasite. The drawing below is a film of his blood stained by Romanovsky’s method, showing the parasite.

Other well-marked instances have come under my notice, but the above two most interesting cases will serve to illustrate my point.

We must therefore admit a third phase of existence - the Latent Phase.
We know that the spores of bacilli have prolonged powers of resistance. Also with regard to the malarial parasite if a blood film preparation be allowed to dry and after some days a few drops of distilled water be added, the vitality of the parasite may be shown to have been latent only.

We shall see that the extracorporeal as much as the intracorporeal cycle, has its own distinct parasite, but whether this Latent phase can be also credited with one; or whether the parasite becomes latent in the spleen or other viscus; or whether the intracorporeal parasites, diminished in numbers and activity but still circulating with the blood, are kept in check by the leucocytes and only reappear in activity when the force of leucocytes is diverted by an attack of another enemy is still quite unknown.
SECTION II.

The Crescents:

An afebrile form

Appearance

Shape
Pigment
Male & female
Exflagellation.

Origin

Growth

Intracorpuscular nature.

Time required for exflagellation.

The Flagellum.

Motion

Appearance.

The description of Part I applies to the parasite as found at the period of fever. If the blood be examined at this time, in a case of primary infection no further forms are present. If however in a case of primary aestivo-autumnal infection, the blood be examined some days after the temperature has been normal, whilst none of the above forms are probably seen, a body is noticed which has not been seen before. This points to the fact that this body is an afebrile form. Whilst in its origin it probably has
relation to the febrogenetic parasite, its presence has no connection either with the fever, or the parasite causing the fever. In shape it is crescentic with a clump of pigment more or less collected in the middle. From tip to tip it measures 10 to 12 μ, and in breadth about 2 μ.

Some of the crescents will be noticed to be broader than others and to have their pigment scattered while the pigment of the narrower ones is collected towards the middle. In the meantime it must be taken for granted that the former is the male, and the latter the female crescent.

If one with somewhat scattered pigment be watched the shape of the parasite is seen to change. The crescent becoming ovoid, gradually assumes a circular form. At this stage the pigment particles form a ring and movement in them becomes increased in activity. The sphere begins to tremble violently, a bulging appears at one part of the periphery, and a pseudopodium is shot out which rapidly becomes lengthened into a flagellum. This is quickly followed by formation of three or four more flagella, and we have the remarkable body, which 20 years ago, led the way to the discovery of the etiology of malaria - the flagellated organism of Laveran.

Their origin is unknown. Manson holds with Man-
naberg that the crescent is the result of the conjugation of two ordinary plasmodia - a syzyium in fact - in a doubly infected corpuscle. A different view is taken by Thayer. "It seems probable that after the infection has lasted for a week or ten days certain of the full grown parasites, instead of undergoing segmentation, continue to develop in size, and to accumulate pigment". I have no suggestion to offer as to the truth of either.

Their growth, as pointed out by Bignami and Bastianelli, probably takes place within red corpuscles in the medulla of bones.

By careful use of the fine adjustment a pale ring can sometimes be made out on the convexity of the crescent, while the concavity is usually seen to be filled up by a faintly yellow bib-like membrane. From the fact that at times these can be seen to be continuous and that the staining reaction of this 'membrane' is the same as that of Haemoglobin, we are led to the belief that the membrane is in reality the remains of the red corpuscle, and that the crescent is thereof intracorpuscular in growth. After the crescent has become a sphere, and the pigment particles are beginning to show their remarkable, vigorous, dancing movement, and just before the flagella are shot out, careful observation shows that there is
movement taking place which is not an undulatory motion of the protoplasm, but suggests a flagellum moving with difficulty within the protoplasm, and as it were beating against the wall by which it is confined. I offer the suggestion that in the substance offering this resistance we still have the remains of the red corpuscle.

The time required for flagellation to take place is usually about fifteen minutes. But as the crescents become decreased in number, due either to prolonged quinine treatment or to the part that nature plays in the struggle for health, we find that exflagellation takes a longer time. I have notes of a case of a Lascar whose blood on admission on 27th November 1899 showed numerous crescents which became flagellate bodies in ten minutes. Ten days later half an hour was required. On the twentieth day after admission I commenced observation at 12.30 midday. I watched the crescent change into the oval form, and then into a sphere. The movement of the pigment particles became most active, and I was expecting the usual phenomenon of exflagellation to take place in a few minutes. However, at 3.0 p.m. movement of the pigment and protoplasm was as active, as it had been seven and a half hours previously, but no attempt at exflagellation had taken place. Although I have
not made sufficient observations to allow me to make the suggestion, it is possible in the light of our present knowledge of the function of the crescent, that a time arrives in the life of the crescent at which it is too old and feeble to undergo the changes necessary for its propagative function.

In variable time after the flagellate body is fully formed, from ten to twenty minutes, a speck of darker colored substance may be seen to shoot into the flagellum from the parent body, and almost immediately the flagellum is seen to break away, and to become free in the liquor sanguinis. By means of rapid vermicular motion it moves from place to place the speed depending apparently upon the quantity of liquor sanguinis. In the thinnest part of the film - the spot which by transmitted light is transparent and colorless, where the corpuscles are few in number, for instance, in a case of which I have a note, only ten red corpuscles were in the field of the 1/12 immersion lens - the flagellum could be followed from place to place. But as soon as it reached sufficient plasma to allow of free movement, it at once swam out of sight.

Their length varies from 15 to 20 μ.

Two swellings can usually be made out in the
course of the flagellum. The first, at one end, is apparently the head, for locomotion always takes place with this extremity in advance. This spot is refractile and sometimes diamond-shaped. The second swelling is somewhere in the course of the flagellum; sometimes in the middle, sometimes nearer the tail end. It is usually oval in shape - and at times I have thought I could make out a darker centre.

After detachment of the flagella, the parent body becomes motionless and shrunken, as if with the formation of free swimming flagella, its part in nature had now been completed.

Figs. 1, 2, 3 are drawings from my own preparations.
SECTION III.

Preparation of specimen to shew the Crescents.

Staining the Crescent.

Staining the Flagellate body.

To prepare a specimen which will shew crescent forms, Ross recommends that a drop of blood be allowed to dry on the slide, and the Haemoglobin washed away with distilled water. The crescents by this means are aggregated, and their black pigment shews up at once in the surrounding colorless area. I have never found any difficulty in determining the presence or absence of crescents in a film preparation made in the ordinary way. They are seen quite easily with 1/6 dry lens, and with this the whole preparation can be quickly examined. Ross' method is no doubt useful if crescents alone are to be sought for. There are however at least two more observations we wish to make - the presence or absence of fever forms and the length of time required for flagellation. The one film preparation serves for all these purposes.

The Stain that gives the best results is again that suggested by Romanovsky. The tips of the horns are more deeply stained, whilst towards the middle an
area remains unstained. The latter is said to be the vesicular nucleus; the function of the former is unknown. Around the margin the faint eosin-stained remains of the red corpuscle can at times be made out.

I will not detail other methods of staining. I have tried Carbol-Fuschin, Methylene blue, Borax-Methylene blue, and Melanein; with none have I got the beautiful results that can be obtained with Romanovsky's stain.

To stain the crescent is an easy matter but to obtain a stained specimen of the flagellate body is most difficult. The difficulty does not arise in finding an effectual stain so much as in obtaining a preparation of a fixed flagellate body.

Ross has pointed out that the formation of the flagellate body is hindered, if not stopped, by preventing the drop of blood from coming in contact with the air. And conversely, that exposure to the air for a minute or two encourages exflagellation. Marshall states that exflagellation is also encouraged by slight aqueous admixture. In the preparation of a film for the purpose of staining the flagellate body Manson combines these two suggestions. A sheet of thick blotting paper has several holes cut out about 1 x ¾ inch. The blotting paper is laid on a sheet of glass or other flat, smooth surface, and mois-
tened. A drop of blood is obtained on a slide and spread out with a needle in a thin layer. The slide with the film surface downwards is then placed on the blotting paper, the film being over a hole. We have thus carried out the above two suggestions and thereby obtained a perfect moist air chamber. A further use of this chamber is to prevent evaporation of the film before exflagellation has had time to take place.

After a quarter to three-quarters of an hour the slides are removed, and dried, and the film fixed with absolute alcohol in the usual manner. Manson recommends staining with weak Carbol-Fuchsine (1 to 30) for six to eight hours. I have tried this method time after time, under most favourable circumstances, and have carried out to the letter every detail mentioned by Manson, but so far have not succeeded in obtaining a single stained specimen. I have worked at several other methods that have suggested themselves to me, but all results have been negative.

I may say as indicating the difficulty that H. E. Annett, M.D., D.P.H., who has been demonstrator of the School of Tropical Diseases for the past ten months, has only once succeeded.
SECTION IV.

The Flagellate body of the Benign Infection.

The material which has been studied.

It will be noticed that the flagellate body has so far always been mentioned in connection with the crescent. It will be remembered that the crescent is only found in the so-called malignant, or aestivo-autumnal infections. The Benign Tertian and Quartan likewise have a flagellate body. Its development is not from a crescent, but from a spherical body, which appears towards the close of a cycle, when the parasite is the size of, and sometimes larger than a red corpuscle. All evidence of its host has however disappeared. The changes that take place are similar to those of the crescent-derived flagellate body. The pigment becomes tumultuous and previous to exflagellation forms a central ring; the protoplasm shews undulatory movements suggesting as Richard long ago remarked, an attempt on the part of some included body to escape; and finally three or four flagella are successively shot out.

The flagella are similar in appearance to those already described.

I should like to say here that the material
which I have had at my disposal has been largely drawn from the West Coast of Africa. As a consequence I have been largely limited to the study of the aestivo-autumnal or malignant infection. Out of nearly 400 fever cases examined at Sierra Leone by Captain Duggan and Dr. Geo. Thin, not one case of quartan and only one case of Tertian infection was observed.

I have notes of only one case of quartan and three of tertian malaria. In the former, I was fortunate in observing the flagellate body. Of aestivo-autumnal infections, I have made at least 150 examinations. I have on no occasion been fully satisfied that I have seen a sporulating body; but the other forms, from the earliest to the most mature I have observed time after time, and as often have watched the crescent and its derived forms.
PART III.

Section I.

The Function of the Flagellate Body

Four Theories.

Relation of Mosquitoes to Malaria

Brief history of supposed connection.

Two paths of investigation

(a) Trace the parasite from man to mosquito.

(b) Trace the parasite from mosquito to man.

An analogy of each.

Manson's Mosquito theory.

The function of the flagellate body has had many interpretations, which we may more or less accurately, class under four heads; three of these we shall mention and pass over.

I. That they represent degenerated, dead, or moribund plasmodia (Marchiafava - Blanchard)

II. That the crescent is a variety of parasite, distinct altogether from the ordinary intracorporeal plasmodium (Grassi - Feletti)

III. That they are sterile forms, without function, and incapable of reproduction. A theory no doubt true with regard to their life in man. (Marchiafava
IV. That they have relation to a life outside the human body, by which the species is continued. This last is the interpretation we adopt.

The likelihood of some connection existing between Malaria and mosquitoes, has occurred not only to Physicians, but to men possessed of ordinary observation. They were impressed by the fact that in those places where malaria is most rampant, mosquitoes are most numerous; that the yearly incidence of malaria is greatest at the time when mosquitoes are most abundant; by the fact that the period of the day marked 'dangerous' - the night air - is the time at which mosquitoes bite most actively; and by the fact that the parasite is a blood-contained organism, and the mosquito a blood-sucking insect.

The Roman Physician, Lancisi, a contemporary of Valsalva, hinted at the possibility of some connection in the latter half of the seventeenth century. Dr. King in 1833 wrote an article on 'Mosquitoes and Malaria'. In 1891 a note came from Laveran; and in 1892 we find Koch remarking "that the parasite might be capable of two different cycles of development, one being exogenous; and that the exogenous malarial spore might be conveyed to man, through the agency of blood-sucking insects such as mosquitoes."
1894 Manson noticed that the female filled herself with infected blood, laid her eggs and died. In December 1897 Ross observed certain cells in the stomach wall of a mosquito, which had been fed on crescents. They contained pigment which was indistinguishable from malarial pigment.

Being convinced them that the mosquito is in intimate relation with the malarial parasite, it remains for us to find its exact connection.

We may approach the subject from two sides, one being complementary to the other. We may attempt to follow the parasite from the human body into the body of the mosquito and watch its development there - and we may attempt to find the method by which the parasite returns to man.

To take an analogy of the former, we know that the filaria nocturna passes from man to mosquito; and we can follow the filaria as it escapes from its sheath in the mosquito's stomach, and as it penetrates the thoracic muscles, and there watch further metamorphosis.

To take Texas fever as an analogy of the latter we know that a parasite similar to the malarial parasite is communicated to cattle by the bite of the tick.

The former, which we shall speak of first, re-
presents the work done by Ross, and the British School; the latter the work of Bignami and the Italian school.

We take it for granted that the parasite passes from man to mosquito at the time of the bite.

Manson was the first to offer a theory of subsequent change. From the five following facts he concludes that the flagellum is a flagellated spore—that the flagellate body develops only from apparently mature forms; that it is found in every variety of malarial infection; that it only comes into existence, after removal of its parent, from the human body; that the flagellum becoming detached from its parent body is capable, for a time at least, of continuing a life of its own; and that somewhere in its course is always to be found a well-marked swelling.

In only a few instances do the crescents become transformed into flagellate bodies when watched on the stage of the microscope; but in the stomach of the mosquito Major Ross has shown, that this change takes place in the majority of cases. Manson naturally concluded that "this constancy of exflagellation cannot be accidental". Hence his theory that the "Flagellum enters some cell of the mosquito and therein like any gregarine or coccidium becomes parasitic growing, and sporulating afterwards" "I have further conjectured that the continuation and multi-
lication of the plasmodium outside the human body is secured by the passage of the mosquito-bred plasmodial spore into the larvae of the same insect. The mosquito generally dies in the water beside the eggs she has deposited. When the eggs are hatched the young larvae commonly devour the body of their parent and consequently her parasites . . . . We can easily understand from this in what way the parasites from the mosquito, may get access to the mosquito's offspring. On the infected larvae becoming mature insects, the plasmodia they have swallowed continue, I conjecture, to develop. These insects in their turn infect their larvae, and so on"
SECTION II.

MacCallum's Observation.

His theory of the function of flagellum and
crescent.

Terminology

Bignami and Bastianelli's description of intra-
 gastric changes.

Ross' description of changes in wall of stomach.

It is quite true that the flagellum enters a
 cell but it is not parasitic in this cell nor is it
 a cell of the mosquito.

MacCallum gave the first hint of the real func-
tion of the flagellum. In his study of the Haemato-
zoa of birds he observed that crows are very generally
infected with the halteridium of Labbé. He notic-
ed that there were two mature forms, identical in
outline, but differing in the appearance of their
protoplasms; one being "granular and opaque, the
other clear and hyaline". The latter, apparently
the homologue of the human crescent, underwent changes
resulting in the formation of flagella. The flagel-
la having become detached, approached and began beat-
ing their heads against the granular sphere. One
succeeded in effecting an entrance. The granular sphere, which hitherto was motionless now began to shew violent churning movement. After a period of fifteen to twenty minutes quiet, a hyaline conical process appeared at one side of the organism - the pigment collecting mainly to the opposite side. This elongation grew until it formed a vigorously motile, spindle-shaped body, or vermicule. In two instances in blood from a human subject he saw a flagellum enter an unchanged crescent with accumulated pigment, but beyond a little swelling no further change took place. This suggests that the function of the granular sphere in crow's blood and of the crescent with aggregated pigment in human blood, is that of an ovum; and the function of the flagellum that of a spermatozoon, and the process one of sexual fertilization.

A word as to terminology at this point will simplify description.

To the mature intracorpucular parasite Ross gives the name of Sporocyte. The mature extracorpucular parasite or crescent he speaks of as a gametocyte. The male crescent with scattered pigment, which proceeds to exflagellation, is named a microgametocyte - the flagellum a microgamete. The female crescent is called a macrogametocyte, resulting in
the formation of one macrogamete. The fertilized macrogamete is a zygote.

Professor Ray Lankester suggests the use of the following terms: Androspore for microgamete; gynospore for macrogamete; and gametospore for zygote. This terminology I adopt."

For the sake of continuous description I will put Bignami and Bastianelli's account of the changes of the gametospore in the stomach cavity, before Ross' account of the changes that take place in the stomach wall, and body of the mosquito. It ought to be remembered however, that it was not until after Ross had published the results of his work on the proterosoma of sparrows, in the summer of 1838, that the Italian school commenced similar investigations with the parasites of man.

A gynospore has been fertilized by an androspore resulting in the formation of a gametospore. After 10 to 12 hours (the mosquito being kept in a warm chamber at 30°F.) a small segment of the gametospore becomes hyaline, while in the remaining part the pigment becomes aggregated. The former segment, retaining its hyaline appearance, becomes elongated while the latter becomes smaller. Finally the granular portion disappears altogether, the pigment in the meantime having migrated into the elongated
portion. We now have the fully formed vermicule. It will be noticed how exactly this coincides with MacCallum's observations. The only difference being that in the one case MacCallum watched the changes taking place in actual life, while the description by Bignami is constructed from serial microscopic sections.

The vermicule now pierces the epithelium of the stomach wall. In some instances it remains here, but in most, it passes on to the outer coat. It was this stage that Ross noticed and described in December 1897.

Fig. I. Very small gametosporre attached to stomach wall of Culex fastigans x 500

Fig. II. Mature gametosporres x 60
In this situation it becomes surrounded by a delicate membrane, becomes more or less circular in outline and increases in growth.

Fig. III. A gametospore about 2/3 full size protruding into body cavity and containing blasts which are already nearly mature. $\times 500$
"Its substance divides into eight to twelve 'meres'. Each mere becomes a blastophore bearing a large number of filamentous blasts affixed to its surface by one extremity.

![Fig. IV. A full sized gametospore full of mature blasts ×500.](image)

As growth advances the blastophores disappear leaving the capsule of the zygote (or gametospore) packed with blasts.

![Fig. V. Gametospore full of blasts.](image)
The capsule now ruptures allowing the blasts to escape into the body cavity of the insect.

Fig VI.

From this they work their way into the insect's salivary gland.
Fig. VII. Four blasts lying free in the juices of the thorax. x 500

Fig. VIII. Blasts in the salivary gland of A. Costalis x 400.

Fig. IX. Individual blasts.

Passing notice must be taken of the so-called 'black spores'. The drawing shows that they are large black sausage-shaped bodies.

Fig. X. Mature gametospore to the right and capsule of another to the left containing black spores x 500.
They are found within the zygoites and disseminated through the tissues of the mosquito's body. Described first by Ross they have subsequently been noticed by investigators in Italy, Germany and West Africa. Whether they represent another life cycle, perhaps for infection of the larvae; or whether they form a product of degeneration; or have no relation at all with the malarial parasite is unknown.

Major Ross has kindly allowed me to reproduce the micro-photographs given. I have examined the actual sections of Major Ross and am only sorry that the photographs are such poor representations of his excellent preparations. Figures 5, 6, 9 are drawings by Dr. Fielding-Ould of the parasites found by him in Anopheles mosquitoes in Sierra Leone last autumn.

We have now completed the first line of investigation. We have followed the crescent from human blood into the stomach of the mosquito; we have seen it exflagellate; we have watched the conjugation of the male and female elements, and observed the various changes that take place; and the final lodgement in the salivary glands.
SECTION III.

Manson's theory
by water and air

Criticism.

Facts against infection being communicated by water

Facts against infection being communicated by air.

We now pass to the other line of investigation and attempt to shew how the parasite passed from the mosquito back again to man.

The constructive mind of Manson readily conceived a theory based on his observation, that the infected fertilized mosquito died "in the water beside the eggs she has deposited". In his own words, "Man, I conjecture, may become infected by drinking water contaminated by the mosquito; or, and much more frequently, by inhaling the dust of dried up mosquito-haunted pools; or in some similar way". We shall find immediately that although Manson was facing the right direction he was on the wrong track.

I feel confident he does not adhere to this
statement today, but it was his theory in the last edition of his book (September 1898).

We shall further see that mosquitoes bred from larvae of an infected mother are quite free from any power of infection.

We must hesitate before we accept the theory that man becomes infected by drinking malarial water. Major Ross administered water in which malarialized mosquitoes had died after depositing their eggs; but although in one case fever supervened, the greater number shewed no reaction.?

Zeri experimented as follows:—

(a) Nine people were made to drink water from malarious places in quantity and for a considerable time—results negative.

(b) Marsh water was sprayed on the mucous membrane of the respiratory passages of a number of persons—again with negative results.

(c) Five persons had injections of water from malarious places into the bowel—still were the results negative.

In view of these experiments and others which we shall speak of later, doubt cannot but exist as to the truth of this theory of infection.

I also feel doubtful whether man is infected by breathing so-called malarious air. If we divide

α: VII. p. 381.
β: XVIII p. 1179.
men into those who use mosquito nets at night time and are careful as far as possible to avoid mosquito bites and those who are careless of all precautions, we find that while both are exposed to the same influences of 'dangerous' air, the former escape while the latter catch Malaria.

I offer three examples.

Dr. Macdonald, Assistant Medical officer of Rio Tinto reports that the only Englishmen, who avoided fever, always slept under curtains, and when on the veranda at night, and therefore exposed like others to 'dangerous' air, covered their feet with towels, hands with gloves, and neck with a muffler. In another district all had fever except the 'patron' who slept under curtains. 

Again, of the recent malarial expedition to Sierra Leone 'only one member, Mr.E.Austin, Assistant in Dyptera, British Museum, became infected with Malaria, through sleeping one night without mosquito curtains'.

"Emin Pasha never failed to take a mosquito net with him on his African journeys, and attributed the fact that he was spared a malarial attack to this
precaution".

Although these examples do not prove either that mosquito-bites cause malaria, or that contact with night air does not expose the person to infection, they offer strong arguments in favour of both.

We therefore put on one side as doubtful the possibility of infection through the respiratory and digestive tracts. The only other method is by direct inoculation. The bite of the mosquito at once suggests itself as providing a suitable opportunity.
SECTION IV.

Early experiments of Bignami & Bastianelli.

Early experiments of Ross.

Ross' work with culex and sparrows.

Grassi's work with mosquitoes.

Later work of Bignami & Bastianelli

Three species of mosquitoes tested.

Points to prove that Anopheles is the infecting mosquito.

Opinions offered.

This theory of mosquito infection until eighteen months ago was rejected by most. Thayer in 1897 writes: "We are absolutely ignorant of the form in which the malarial parasite exists outside the human body and equally ignorant of the manner in which it enters". In February 1898 James Cantlie writes: "An idea scouted by most --- and at any rate not proven". Manson in September 1898 writes: "Experiment has not encouraged this idea".

Nevertheless we find Bignami and Bastianelli in 1894 performing experiments with mosquitoes and men. He brought a number of mosquitoes from a malarious station, and let them free in a chamber in which a
man had voluntarily placed himself. The man was bitten but malaria did not develop.

In August 1896 three volunteers were bitten by mosquitoes fed on malarial blood. One of these, the assistant surgeon in Major Ross' hospital was bitten by 36 malarialized mosquitoes. All results were negative.

In 1897 Major Ross "After numerous negative experiments with several species of gnats of the genus Culex succeeded in cultivating one of the Human Haemamoeobidae in two species of gnats of the genus Anopheles."

Early in 1898 experiments were resumed by Bignami and Bastianelli. Results however were still negative. Further experiments were made in August of the same year ending again in failure.

In the meantime Ross had been continuing his work. As soon as he had found that the salivary gland was the goal of the "blasts" he realized he had also found the method of re-infection. He noted that one particular species of mosquito alone allowed growth of the parasite - the "culex". It must be remembered that all Major Ross' investigations were carried on with birds, chiefly sparrows. To sum up a long series of experiments in a few sentences. He obtained one set of infected sparrows, and one
free from infection; and two sets of mosquitoes, one infected, the other healthy. First, he allowed healthy mosquitoes to bite infected sparrows. Some of the mosquitoes he then dissected, and found that they had become infected and that the usual metamorphosis of the parasite had taken place. In the second experiment he placed others of the now infected mosquitoes in a curtain cage of healthy sparrows. After the sparrows had been bitten he examined the blood of certain of them and found the parasite. In the third experiment he placed those sparrows that had been thus proved to be malarial in a cage with healthy mosquitoes. Sections were then made of mosquitoes when it was found that the usual changes had taken place. The cycle was thus completed. As further proof of negative character he allowed healthy mosquitoes to bite healthy sparrows with the negative result he expected.

To put in a diagrammatic way:

Let healthy sparrows equal \( S \)

" infected " " \( S' \)

" Healthy mosquitoes " \( M \)

" infected " " \( M' \)

" "Bit" or "bitten by" be represented by \( X \)
These remarkable results of Ross on the malaria of sparrows were made known in the summer of 1898 with a description of the mosquito used - the grey mosquito. This gave a fresh impetus to the study of the malarial parasite as found in man.

The Italian school at once saw the missing link in their chain. They had an intuitive conviction of the infective power of a mosquito bite, but the negative character of their repeated experiments could not but shake their belief. But now came the life history of the parasite in sparrows and one particular kind of mosquito of a cycle proved to be complete. Their first effort therefore was to find the human "malarious" mosquito. Thus Grassi a naturalist gave himself to the study of the difference in the species of mosquitoes to be found in malarious and non-malarious localities. He labelled as "suspected" all mosquitoes found in malarious localities and not found in healthy districts. In this way he singled out anopheles claviger, culex penicillaris,
and culex malariae. Mature insects of these three species were captured in a highly malarious district at the end of the malarial season 1898 and were let loose in a chamber in which a man had voluntarily placed himself. Bignami satisfied himself that this man had not previously suffered from fever, and that during the time of experiment he was subject to no infection by other means than that of direct inoculation. "With these species the first case in man of experimental malarial infection was obtained."

It was not yet settled, however, which of the three species of "suspected" mosquitoes was the actual infective agent. Fortunately it is a matter of ease to separate the Culex mosquito from the Anopheles.

Two points to be brief may be mentioned.

I. The wings of Anopheles are generally spotted along the anterior edge, while those of culex are generally plain.

II. The attitude adopted when seated on a wall is different. Anopheles has the axis of its body almost at right angles to the wall; Culex has its tail hanging downwards, even perhaps pointing towards the wall.
Other points are mentioned in the malarial expedition report but the above are the two outstanding differences. With a knowledge of these differences we can feel confident that in a certain experiment only the one genus is used. The Italian observers thus found that in no case did the Culex cause infection whereas in many experiments with Anopheles infective inoculation was successful. In their own words "Only by subsequent research was it demonstrated that the extracorporeal hosts of the human malarial parasite are the various species of the genus anopheles. The complete demonstration of this truth is the fruit of the work carried out in collaboration during the past year by Grassi and ourselves."

Whether their claim that they were the first to produce human experimental malaria can be absolutely accepted is somewhat doubtful. We find that Ross is reported to have read a paper before the Indian branch of the British Medical Association in which he states that he had produced human infection from mosquito bites. 
There seems, however, little doubt that while Ross' most important contribution has been the life cycle of the parasite in sparrows and culex, it is to the Italian school that we owe the main proof of the life cycle of the parasite in man and anopheles.

It was with the acostivo-autumnal parasite that these experiments were made. They then experimented with tertian and quartan parasites, and found that changes took place in the body of the mosquito similar to those of the semilunar parasite — "only in the first stages of growth is it possible to make a differential diagnosis with certainty".

We have mentioned that anopheles and not culex in India and Italy are hospitable to the human parasite. The same has been proved by the members of the Malarial Expedition to apply to at least two ports on the West African Coast, Sierra Leone, and Lagos. Proof of a negative character was obtained at Accra. No anopheles were to be found but Culex mosquitoes were present in large numbers. Here to some extent is a test case. With Culex present and Anopheles absent we should expect to find absence of malaria. "And this is precisely what we do find. All my enquiries from local medical men and colonial surgeons led me to believe that there had been no case of original infection for two and a half months before I arrived". 8
Proof is coming to hand from all quarters that where malaria exists as a primary infection mosquitoes of the anopheles genus are found, and with absence of anopheles we find absence of Malaria. Dr. Macdonald, medical officer of a district of 60 miles in Spain, divided his province into twelve regions, nine of which have endemic malaria, and three are free. In the latter not one specimen of anopheles was to be found, while in each of the former, anopheles was present.

We wish to make four more statements without attempting proof, that,

I. Only female anopheles act as hosts.

II. And apparently only female anopheles that have been fertilized. Males feed on bananas and fresh fruit but females require a meal of blood both for fertilization and the development of their ova. These two points may be mentioned however. If mosquitoes, male and female, both bred from larvae be liberated in a curtain enclosure and allowed to bite an infected person, the fertilized females are found to have become infected. But when females only, also bred from larvae, are liberated and allowed to suck malarious blood no infection will take place.

III. That a malarious mosquito does not infect
its offspring. The obvious proof of this is to be found in the dissection of mosquitoes bred artificially from an infected mother.

IV. That type of infection is maintained. A mosquito ingesting tertian gametocytes will produce the tertian parasite in man.

Although I have not omitted any essential fact in dealing with the extracorporeal life of the parasite, I have passed over the subject more rapidly than both its interest and importance demand. My reason is that the parasitology of the intracorporeal forms is the subject at which I have worked. Every statement, especially with regard to the aestivo-autumnal parasite, with very few exceptions, has been verified in the laboratory.

In conclusion, I beg to offer the following as the points on which I have ventured to lay stress,-

I. That for clinical purposes films of fresh blood should be used.

II. That there is at present evidence of only one variety of malignant or aestivo-autumnal parasite.

III. That a film should be laid aside as useless unless containing one or more clear areas.

IV. That Romanovsky's stain in all cases gives the best results.
V. That for purposes of diagnosis examination of the blood should be made during the earlier part of the premonitory stage.

VI. That the mobile intraplastic bodies have no connection with Malaria.

VII. That the parasites after years of inactivity may reproduce themselves in sufficient numbers as to cause a relapse of fever, and to allow demonstration of the parasite in peripheral blood.

VIII. That crescents and their homologues in benign fevers are afebrile forms; are male and female, and their function is the propagation of their species outside the human body.

IX. That the flagellum is the male element.

X. That the crescent with concentrated pigment in malignant fever is the female element. The homologue in benign fevers has not so far been differentiated.

XI. That conjugation of the male and female elements taking place in stomach of the mosquito commences a life history which ends by the lodgement of blasts in the salivary gland.

XII. That the bite of the insect not only affords opportunity for the commencement of the extracorporeal life cycle, but is responsible for the reinfection of man.
XIII. That only females of the genus anopheles are hospitable to the human parasite and probably only fertilized females.

XIV. That although further facts are required, each new fact adds proof to the theory that the only method of infection is by the bite of an infected mosquito.

XV. That the presence of "Black Spores" in the tissues of an infected mosquito is almost constant; but their function is unknown.
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