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To Surgeon General Sir Joseph Fayrer with the compliments of Surgeon Captain P. Hehir M.D. 11 Novbr 1896 Hyderabad

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THE HÆMATOZOON OF MALARIA

AND

ITS DISCOVERY IN WATER AND SOIL,

BY

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In the year 1892, I published a brochure entitled *Observations on the Hæmatozoon of Malaria* which contained many statements opposed to the malarial pathology of recent times. The opinions on the work as expounded in the reviews that followed its appearance were expressed with caution and reserve, but with that open-mindedness that has always been one of the laudable characteristics of the scientific medical press.

2. Since that time I have worked uninterruptedly at the subject in one of the most malarious parts of Southern India. In my original monograph I endeavoured to give the life-history of the various parasitic forms met within malarial blood; and I referred to having met with certain sporozoa in water, which presented features somewhat similar to the malarial parasite as met with in human blood. In the present paper I propose to give a resume of the conclusions arrived at as the result of three series of investigations in the same connection. This will include in the first place some remarks on the materials employed, and the nature of the cases, and the way in which these new phases in the life-history of the parasite were ascertained. This is followed by a description of the parasitic forms met with in malarial blood, and those met with in water, incidentally remarking on the peculiarities and functions of the parasite, and the question of the unity or plurality of species. As there are several forms included which have not been described or even mentioned by other writers, and other forms which, although described by previous workers, are here differently interpreted even as to their origin, these have been specially dealt with.

3. My practical acquaintance with the *plasmodium malariæ* was made in Burma in the year 1887, when I observed certain peculiar moving bodies in the blood of sepoys returning from Wonthoo to Mandalay, after an expedition to that portion of Upper Burma. At that time whilst on field service, having nothing but dry objective lenses with me, I was unable to advance much in these investigations, but late in the same year, on arrival in Hyderabad, finding a practically unlimited field for these observations, I re-commenced the work with a certain degree of enthusiasm, which grew progressively, as the proof to my mind, regarding the etiological relationship between the *hæmatomonas malariæ* and malarial diseases, became established.

4. The special object in view, however, was to endeavour to verify or refute the observations made by different observers—Laveran, Marchiafava, Celli, Councilman, Sternberg, Osler, Vandyke-Carter, and others. For six years, I worked on the same lines as these distinguished pathologists, but in 1892 I formulated a new line of investigation, which has, I believe, yielded more satisfactory results.

5. Method of Investigation adopted:—The following was the method of preparing the blood for examination in the majority of cases, and its adoption can be recommended as giving good results:—

6. The finger from which the blood is to be removed is to be thoroughly washed with soap and warm water. This removes dirt and loose squamous epithelial scales, and dilates the cutaneous capillaries. Next, the finger is washed in methylated spirit and thoroughly dried. No string round the finger is necessary. The finger (middle one preferred) is directed downwards, held pretty firmly, the blood being pressed towards the end of the flexor aspect by the investigator's thumb, and a smart prick made with a clean needle; the sharper the needle the less pain felt by the patient. A straight surgical needle is very good in this respect. The washing process should be carried out in one's presence. Every precaution should be taken to conduct this stage with perfect cleanliness.

7. In the examination of fresh blood, after the puncture has been made, the drop of blood is allowed to ooze freely without any squeezing or pressing of the finger. A bacteriological (specially thin) slide is brought into contact with the centre of the drop; the slide should barely touch the drop of oozing blood. A cover-slip is allowed to fall gently on the slide. Too much pressure of the cover-slip against the slide causes the red blood cells to run together rapidly. What is necessary is that the layer be thin and uniform, and the red blood corpuscles, if possible, seen singly. The blood rapidly diffuses as a thin film and as a rule should be at once examined in the fresh state, that is, whilst the blood is still living. Occasionally, however, especially when there are many rouleaux of red blood corpuscles, it is advantageous to wait for five or ten minutes, so as to allow the component red-blood corpuscles of the rouleaux to separate. As a rule after a period of half an hour, unless special precautions are taken to exclude air and prevent evaporation by properly cementing the preparation, the corpuscles run together, thus rendering accurate observation unsatisfactory, although in most preparations some portions of the slide may be watched for several hours and observations distinctly made.

8. If any characteristic or peculiar forms of the parasite are found in the slide-preparation so obtained, a few stained cover-slip specimens should be sealed with zinc white or Hollis' glue. If we have obtained only a few slide-preparations, and cannot get further samples, and the preparations turn out to be specially good ones, it may be advantageous to know that the fresh specimen may be fixed and stained after raising the cover-slip from the slide with the sharp blade of a penknife. This is much better than sliding the cover-slip apart from the slide, which latter of course, cannot be done if any marginal cement has been used.

9. Precautions necessary in the Examination of the Blood:—The slides and cover-slips should be thoroughly cleaned and kept in methylated spirit or clove oil till about to be used. All fluids, particles of dust and dirt, and the lines, particles, and debris left by dirty cloths used in wiping the glasses, add their quota

to complicate matters. Scales of the surface epithelium from the fingers are often present, and are not very dissimilar to the large pigmented endothelium-like cells sometimes found in malarial blood. The least amount of gentle pressure on the cover-slip is advantageous by forcing some of the blood towards the periphery, where a layer of fibrin accumulates and coagulates and thus prevents speedy evaporation, thereby facilitating prolonged examination without the aid of any cementing material. In this layer of plasma the organism sometimes lives for several hours, and in a few instances I have seen the small free amœboid bodies moving in it as long as 24 hours after withdrawal. Neither too small nor too large a drop is to be taken; if the drop is too small the film dries up too rapidly, and thus causes cessation of movement. About a quarter of an ordinary drop is required for each cover-slip.

10. The highly elastic character of the red-blood corpuscles permits of their assuming various forms under varying amounts of pressure, and as each change in shape is associated with a different degree of refrangibility in the cell, every conceivable form may be visible under these conditions. This precaution would not have been expressed were it not for the confusion created by the multiform character of the corpuscles under this condition.

11. Nature of cases Investigated :—The registered cases were chiefly those of sepoys and bandsmen in His Highness the Nizam's Regular Troops admitted into the Staff Hospital, members of the staff of the Chadarghat Municipality, and cases from the civil population. Regarding the sepoys and bandsmen, it may be interesting to note that they live in the Staff Lines—a notoriously unhealthy locality, and one always spoken of as "very malarious." Further, the sepoys were chiefly "Pioneers," constantly employed in road making and in earth excavations. From the nature of their work, the municipal servants were constantly employed in the most insalubrious parts of the municipal area.

12. As each case presented itself, a brief history of his illness, together with notes as to his present condition, was recorded. All the cases, with the exception of those specially alluded to hereafter, were taking quinine at the time, or had been taking it up to the period they were brought under observation.

13. During the nine years these investigations continued, no fewer than 7,250 slide-preparations of malarial blood were examined—4,770 fresh and 2,480 stained. The illustrations which are the combined results of the three groups of investigations, from 94 cases systematically and daily examined from the date of admission into hospital to that of discharge "cured." Those depicting chlamydospores and illustrating the various forms of engulfing and division were drawn from the last series of observations. As a rule, four slide and two cover-slip preparations (for staining) we made from each case at the time of the observation. The amount of labour represented in these investigations may thus be gauged. Notes, which were dictated to my assistant regarding each observation, we made at the time and drawings when necessary were at once made, usually with the aid of Zeiss' "Zeichenprisma."

14. During the course of these investigations, the blood of 619 cases was examined. In 94 cases the blood was examined daily and systematically from a minimum period of 3 days, to a maximum period of 31 days. The average duration of each of these 94 cases under observation was 9·5 days. Of the 94 cases, 85 were instances of quotidian ague, 7 of tertian, and 2 of

quartan. In the other 525 cases, from 1 to 19 observations only were made. All the 94 were selected cases. Of the 525 cases, 102 were chosen because they presented the typical clinical characteristics of the different forms of malarial fever. Of these 89 were quotidian, 9 tertian, and 3 quartan. The rest were cases that came under observations accidentally, or such as presented themselves for examination every now and then, and of which no regular record could be kept. In every case much care was taken in the first examination of the blood, to note all departures from the normal. Of the 223 cases 8 were typical examples of the malarial cachexia. On 162 occasions intermittent fever in one or other stage was present at the time of making the examination. Although these cases are not registered as they usually only attended casually for treatment, I am indebted to them for much valuable information. In the 94 systematic cases, observations were made both during the pyrexial period and during the apyrexial intervals. In only 7 of these was hypertrophy of the spleen absent. Of the 525 cases, enlargement of the spleen was present in 396, enlargement of the liver in 37, and enlargement of both liver and spleen in 22 cases. Anæmia was well-marked in 81 cases, slightly in 40 others.

15. Eight cases proved fatal, 3 from intercurrent maladies, 2 from diarrhœa, 1 from abscess of the liver, 1 from hæmaturia, and 1 from intestinal hæmorrhage, 3 from general exhaustion supervening on malarial cachexia, and the last succumbed to a supervening attack of acute pneumonia. In 45 cases the results as regards the presence of the more mature forms of the malarial organism were negative. Cases were examined just before, during and after the paroxysm and for several succeeding days, whilst 18 cases were examined hourly for six hours before and the first six hours, during the attack of fever.

16. The morphology and developmental processes in connection with Sporozoa invading the blood of man and the lower animals can only be successfully investigated by prolonged, patient, and repeated observation of the parasite during the various phases of its existence, supplemented by the careful and systematic use of appropriate stains. It is almost unnecessary to state that most of the sporozoa are for the greater part of their existence so transparent that the bright light needed for the combination of lenses of the immersion system, passes through them without defining the objects we are in search of. Some, of course, are not so difficult of observation, such, for instance, is the case with the *trypanosomata* in the blood of birds, the hæmatomonads of rats, etc.

17. Indeed, it may be said that the difficulty which even the trained observer experiences in following out the life-history of these parasites, depends to a considerable extent upon this characteristic of transparency. It may therefore be taken for granted, that it is impossible to trace out all the peculiarities of the malarial parasite in fresh specimens, a statement the truth of which is borne out by the present meagreness of our knowledge in connection with that parasite. The same may be said with regard to the hæmatozoon of cholera, the very existence of which is almost unknown to the profession.

18. Until recently we were particularly wanting in the means of defining in fresh specimens the various fine flagellated processes; the presence or absence of cyst-walls and the hyaline and transparent contents of various cysts which are also difficult to see in nuclei and nucleoli; the early stage of sporulation; recognition

of the forms and characters of minute bodies discharged from certain globular cells in combination with a more or less homogeneous fluid, which does not mix with the liquor sanguinis ; these and other particulars in the absence of which, it would be impossible to arrive at even an approximately correct conception of the nature of the sporozoon under consideration. These circumstances demand from the microscopist both a knowledge of the use of stains, as well as the methods of their employment, throughout researches of this description. More than half the work of these investigations has to be carried out with stains, and without them it is absolutely impossible to complete such researches. The enormous aid which they afford us is admirably shown by the employment of such stains as do not interfere with the structure, constitution or functions of these parasites in any way, while they enable us to avail ourselves of all the properties of the pigment as a staining material.

19. I was particularly impressed with the value of staining fluids for this purpose when investigating the malarial organism in marsh waters, and I believe it is a method capable of being more widely employed than at present with advantage.

20. I would incidently remark, that I have received much help from the use of weak solutions of warmed Loeffler's alkaline staining fluid, added to fresh moist preparations of malaria and cholera blood. In combination with osmic acid vapour it is invaluable. Any one who has not already carried out examinations of malarial blood with these, will be both surprised and gratified at the results obtained.

21. Another important supplementary part of these examinations consists in the use of fixing agents on fresh preparations, especially osmic acid.

22. I would now enter upon a brief description of the various methods of staining adopted. Before doing so, I might for the benefit of the tyro in blood examinations, state that to acquire anything beyond a mere theoretical knowledge of the subject, he will have to spend many days in familiarising himself with the different fixing and staining solutions that are employed in examination of malarial blood. The time spent in this way, however, is not wasted, for when he has acquired a certain degree of facility in the use of stains, and learnt their effects produced on the blood cells and these hæmatozoon, he may consider that his path has been smoothed. After employing the various ordinary solutions recommended, he may endeavour to seek out some new combinations. Assuming that these experiments with new stains are diligently conducted, the young worker will be amply repaid by the beautiful effects he will find himself producing. The most valuable preliminary aid is the recorded experience of the best workers in this special field of research. He will find most valuable assistance in consulting such works as LEE's *Microtomist's Handibook* ; STRICKER's *Pathological Histology*, HALLIBURTON's *Pathological and Physiological Chemistry* ; HEITZMANN's *Histology*, BIZZIZERO's *Pathologie du Sang* ; LOEFFLER's *Parasitic Protozoa of the Blood* ; amongst works specially devoted to malarial organisms he will find the following very valuable, MANNABERG's *Parasites of Malaria* (New Sydenham Society's Translation, 1894), LAVERAN's *Paludism*, THAYER and HEWETSON's *Researches on the Malarial Parasite* (*John Hopkin's Hospital Reports*, Baltimore, 1895). Of all these perhaps the best two guides are the first and the penultimate, although the monograph by THAYER and HEWETSON is perhaps second to none in the subject and contains much valuable information.

23. Those who have worked conscientiously and earnestly in search of accurate and definite knowledge in connection with such questions as the unity or plurality of the malarial parasite, their general and specific characters, modes of infection and reproduction, together with the relations of the different forms of the parasite to one another, and to the effects they produce on the human economy, will at once on the perusal of this brochure, come to the conclusion that it is next to impossible to follow out these various questions in the way that has been described herein, by any ordinary means of observation carried on exclusively on human malarial blood. I must confess that I would acknowledge the justification of such a statement. The results recorded are obtained by observations of the most diverse nature. At the present moment I find it difficult to recall the numerous sources from which this knowledge has been obtained; indeed, I might state that I obtained many valuable hints in this connection from sources far and wide from the subject of malaria itself; I made the first step in material progress from observations on the hæmatozoa of lower animals. In this way having obtained a clue for instance in regard to the life-history, it was put to the test in human malarial blood. There are many of the hæmatozoa of lower animals, which it is possible to follow out with a certain amount of facility, such as the tripanasomata of birds, tricomonads of rats and fishes and in a blood-organism recently discovered by myself of the cobra (*Naja tripudiens*). Thus, at one time a hint would be obtained from the general characters of an apparently irrelevant organism, or the changes it passed through in a particular period of its life-history. Often, however, the source of information was by no means so significant—such apparently trifling incidents as the diameter of a cell, the presence or absence of a cyst-wall, an extra flagella, the position of the nucleus, the number of spores produced, the size or shape of the spore, the method of division, the time taken in sporulation of a parasite, its relation to the red cell, the form or forms of conjugation or engulfing, the quantity, character or position of granules or plaques of pigment, the degree of transparency or visibility of one or other forms of a parasite, the method of forming chlamydospores, and the effect of different kinds of stains on the different forms of the parasite—each point had its meaning when tested by comparative observation on human malarial blood. Sometimes a verified observation of this kind was sufficient to fill up a broad hiatus. Such was the case, for instance, in the formulation of the theory of parthenogenesis; finding that there were two distinct forms of multiplication in the malarial parasite, simple division and endogenous multiplication, or rather sporogenesis within chlamydospores in the former; it having been observed that in one variety of spherical cell, the actual protoplasmic substance of the cell itself was split up into a certain number of parts, whilst in several other large cystic cells, formed by the union of two or more ovoid or irregular cells, the process of division began in the nucleus, which underwent karyokinetic segmentation after a very definite method.

24. In this case the encysted fluid protoplasm yielded the necessary nutritive pabulum for the process itself, as well as for the preparation of the spores to lead an independent life, affording them nutrition until such time as, having been set free, they were able to utilize the required constituents in the *liquor sanguinis*, or they invaded a red blood cell, where they could find an excellent medium for further development. I will not weary the reader, however, with any further illustrations of this kind. It is, however, necessary to enter upon these few details to indicate, that the statements made have been arrived at only after many years of direct and indirect investigation of the subject of malarial infection in the

human being, supplemented by systematic collateral observations on the hæmatozoa of lower animals.

25. The foregoing remarks may likewise exclude argument to the effect that the views herein expressed have been arrived at by a series of guesses, and the origin of the impression that the writer having discovered a few main facts, the lacunæ between these were filled up by suppositional data or hypothetical suggestions.

26 One of the most useful combinations which I have found was osmic acid vapour and methylene blue. This gave a faithful representation of the blood as it is in the natural state when used as follows: the finger is picked in the ordinary way and a cover-slip applied to the drop, whilst the hand is dependent and the drop lowermost. This drop is then spread out by means of a needle and rubbed over the surface. The fresh moist blood is then held over the vapour of osmic acid with blood side downwards, and allowed to remain there for from 15 to 30 seconds according to thickness of film of blood. To the moist blood is now added a drop of half saturated aqueous solution of eosin, and another of weak aqueous solution of methylene blue. The preparation is allowed to dry. It is then washed in distilled water and alcohol, until only a faint blue colour is left. It is then allowed to dry, and mounted in that state or in canada balsam.

27. Another excellent stain is the combination of chromic and osmic acids—a ·05 per cent. solution of the former, containing ·5 per cent. of the latter.

28. By fixing the fresh preparations with osmic acid, we cause all structures not actually contained within the red cell to stand out prominently in relief, and occasionally we may in an infected red cell in profile, actually see the line of junction between the parasitic embryo and the red cell itself. The first effect of this preparation is to fix all animate objects instantaneously in the position they occupied in the preparation the moment it touches it, and presumably we get a result which at least simulates the relations of the parasite in the circulating blood. Again, with certain stains such as Loeffler's alkaline solution used full strength, or Ziehl-Neelson stain used quarter strength, a few of the parasites may be seen to be resting on the surface, especially if the staining has been preceded by fixing with osmic acid.

29. By fixing fresh blood preparations hourly with osmic acid, we may follow out the life-history of the parasite with a certain degree of precision.

30. Loeffler's stain to which may be added ·05 per cent. of chronic acid, is by far the most satisfactory that I have used. It has numerous advantages over practically all other combinations. Personally I think its particular virtues dwell in the following qualities :—

1. It gives excellent definition.

2. Stains red cell as well as the plasmodium.

3. Shows pigment in its different forms.

4. Shows difference between the hyaline body and the red cell, and demonstrates any particle of protoplasm in or on the capsule, besides exhibiting the rim of hæmoglobin outside contained spherical bodies.

5. Stains all flagellate bodies perfectly, whether (*a*) attached, (*b*) free, or, (*c*) intra-cystic.

6. Stains small amœboid bodies attacking red cells from margin.

7. Defines flagellated spherical bodies.

8. Defines cystic bodies.

9. Shows the structures of the different forms from the small nucleated spherical flagellated spores, or the exceedingly small spores of free flagella to the largest chlamydospores.

10. Stains remnants of hæmoglobin of red cells, bright red; although it might be here incidentally remarked that such posinated remnants are very rarely to be seen.

11. Stains contained plasmodia blue and the containing red cell pink.

12. Shows the flagella of small spherical bodies well.

31. We can colour the red cell with eosin watery solution and other parts unaffected, and then can follow its action very easily. By these means I was able to distinguish them being granules.

32. Solutions of osmic acid and alcohol have the important property of preserving the shape and appearance of the red blood cells. Their use in this respect cannot be exaggerated.

33. For rapid observation it is useful to apply a drop of the staining fluid on the tip of the cleaned finger, and through this drop make the puncture. For this purpose Lœffler's stain is best. The *slide* is then to be brought into contact with the drop, and a cover-slip laid on it gently, and the preparation being observed at once, or better after ten minutes.

34. Much has been stated against the use of stains in malarial blood preparations, and it has been specially objected that they cause abnormal appearances not present in the blood. Whilst this argument may apply to the coarser stains and those containing large percentages of alcohol, it does not hold good under the use of non-alcoholic stains and sterilised staining fluids of low alcholic strength.

35. Often all the hæmoglobin is dissolved in the serum in an hour or so after abstraction of the blood, and then acts as a natural pigment on the parasitic forms present in the blood, so that we can watch all the phases of development or disintegration at will, if we keep the stage warm. This is only an exaggeration of what is constantly taking place in the blood of malarial patients—solution of the hæmoglobin of red cells, as a result of the active properties of the toxines formed by the multiplication of the parasitic bodies.

36. As the quantity of hæmoglobin dissolved in the *liquor sanguinis* is incapable of staining to any great intensity the parasite forms that have been set free, as they have usually a semi-transparent or whitish appearance, such of them as are ordinarily invisible, now stand out in contrast with the more deeply colored fluid in which they move about. In the case of the encysted and nucleated parasites as the intracystic bodies and nuclei stain the pigment more than the other parts, this serves to map them out distinctly.

37. The practical worker can make use of this natural staining in various ways; thus he may now irrigate the field and wash out the hæmoglobin whilst many of the parasites from their greater adhersiveness remain. In this there is a natural isolatation and staining without extraneous addition in the form of artificial pigments to the blood, to which the opponents of the parasitic theory take such exception.

38. It may be remarked, that when there is an unusual quantity of hæmoglobin dissolved in the blood, all cellular structures contained possess the same color, and it is often difficult to distinguish red cells from spherical bodies of different kinds, including the spherules which have developed from the unfolding of crescents. The experienced observer, however, can usually recognise some peculiarity in each cell under such circumstances which enables him to distinguish its nature.

39. This fact I observed some years ago, and in a note on the subject I wrote :— "It must not be forgotten that when the slide blood preparation has remained for sometime, much of the hæmoglobin naturally contained within the red blood cells is dissolved in the serum and sometimes the quantity thus dissolved out suffices to give the *liquor sanguinis* a distinctly reddish hue, but this effect is much more manifest in the perepheral part of the preparation. This solubility of hæmoglobin in malarial blood has one point of practical importance and that is, when the *liquor sanguinis* is rich in hæmoglobin, it may impart a yellowish or even a reddish tint to those forms of the parasite which, as a rule, are either white or quite transparent."

40. Perhaps the best method of demonstrating the different varieties of "invisible" structures—especially the highly transparent sporocysts and their contents —is that of staining the blood directly by one or other of the various preparations of methylene blue, the most satisfactory being Loeffler's alkaline solution and plain methylene blue itself.

41. THE METHOD IS CARRIED OUT AS FOLLOWS :—A small drop of blood is caught up on the slide in the usual position, and to this is added an equal quantity of the fresh Loeffler's solution. The cover-slip is now carefully laid on and very gently compressed so that the combined liquids make a uniformly thin layer which spreads to the edge of the cover-slip. The preparation is then sealed with Hollis' glue on a turn-table and allowed to stand for 15 or 20 minutes, by which time the various contained bodies including the red blood corpuscles, will be sufficiently stained

to commence the observation, remembering of course, that the intensity of the stain increases as time progresses for about 6 hours, and that no matter how carefully we endeavour to prevent evaporation, the alcohol in the stain blood will volatilize. This latter, however, occupies some hours, so that if observations are carried out during the first hour and a half the complicating effect of pseudo-vacuoles is excluded.

42. Many of the forms referred to in this paper are quite invisible without stains, but staining can only demonstrate one phase of the parasite's life-history,* periodically throughout the course of observation of a single parasite. This can be easily arranged by the use of a moist, warm chamber in which a dozen preparations of the same parasitic form as that under observation are kept; as each phase is followed a step further, one of these specimens from the moist chamber is stained, registered, and numbered, the time of removal from the chamber being noted to avoid error and confusion later on, and the condition of the blood found described at once.

43. The greatest amount of patience is demanded. In my original observations on the hæmatozoon of cholera, on one occasion, I was obliged to watch the changes in the various parasitic forms for 17 consecutive hours, during which time I did not leave the microscopes under which the observations were being carried on. As the malarial parasite is a sporozoon closely allied to the organism, I discovered in cholera blood, the same kind of close observation is a *sine quâ non.*

44. It was by such close observation that it was ascertained that the process of reproduction is more than one of simple division of the different plasmodia into two parts; it is in most forms a definite physiological process precisely identical with the early period of reproduction in the higher forms of the Animal Kingdom; that is, there is a phase in which two or more elements unite and commence the process of germination. In every form of the parasite the stage of segmentation of nuclei is preceded by an amœboid change.

45. Having met with many difficulties and disappointments in this work, I would advise those who are anxious to follow out the life-history of any one of the more complicated of the smaller protozoa, to recognise that patient and attentive search is absolutely indispensable. It is, of course, essential to have reliable optical appliances to work with, and in this respect it should be observed that in following some of the details of structure and modes of development of the smaller parasitic forms, a magnification of three thousand diameters is indispensable.

46. Such constant and attentive observation is the more necessary when we recognise that some of the changes occur quite abruptly, and in a way that is opposed to all expectation, and if these observations are not carried over an adequate length of time, it is probable that some important change in the developmental process will be lost. As interpretations of what takes place must be strictly in accordance with what we see, and as we cannot see all the changes without prolonged watching, it is obvious that such interpretations must be at most to a certain extent hypothetical without uninterrupted attention. In short, granting that the observer is careful to accept nothing from inference, but verifies every fact

* It is possible with certain precautions to keep the parasite alive and watch its developmental changes in the stained state, but this requires most careful regulation of the constitution of the staining fluid and calls for extensive experience before success can be looked for.

by direct observation, that he is not influenced by any preconceived ideas, it should be possible to obtain the same results at will, and thus test, one's own observations. Indeed, I believe the non-observance of these rules has been the cause of much error in previous work.

47. There is no series of experimental observations in which the danger of inference drawn from incomplete observation, or in which the error of conclusions based on probability, is more forcibly demonstrated than in these on malaria. Every phase in the developmental process has its important significance and as each phase varies considerably in duration, it is possible by a short absence from the microscope to miss one or more important events, without which the whole fabric of this theory dwindles to an incomprehensible and chaotic jumble of unconnected data.

48. Hitherto the teaching in connection with the life-history of the malarial parasite has had simplicity as one of its salient features; but those who took the trouble to peruse my original brochure on the subject, must have recognised that several years ago the extreme complexity of the life of the parasite was to some extent anticipated.

49. I might here state that although future years may elicit certain additional details or modifications of the statements made herein, they at present seem to me to possess a tolerable degree of certainty, and if the methods advised are followed there should not be great difficulty in testing these conclusions.

50. But this is another subject and one which is urgently in need of a great deal of attention, and from which, I would take this opportunity of predicting a vast amount of information of an overwhelmingly interesting nature will be forthcoming. I have made a series of observations in this connection, and hope later on to publish them, but they are at most of a perfunctory kind, although they will serve to indicate the chief forms which we are likely to meet under different circumstances.

51. Another important fact with which I became familiar was, that the parasite may retain vitality for an indefinite time in the dried state. This, I became acquainted with on one occasion by accident in the case of a patient whose blood on abstraction seemed to be 'alive' with organisms. The patient left the station within two days, and I was unable to get any further specimens of his blood, the dried ones being useless for defecting the forms they presented when moist. The original unstained slide-preparations were moistened with distilled water, and the activity of the various forms was restored within 20 minutes.

52. It is now exactly 9 years, since the investigations which form the subject of this communication, were commenced, and during that period the changes that have occurred in the views on the subject of malaria have been most marked. Indeed up to the year 1886 the theory of Klebs and Tommasi-Crudeli still formed the basis of the recognised pathology of malarial disease, and was referred to in all our leading text-books. From 1886 onwards, however, Laveran's views came more and more into prominence, but it may be stated that at no previous period has the question of the parasitic origin of malaria received more attention than it does at the present day. In almost every medical journal we take up there is something about the nature of malaria. It is well that there is discussion on

the subject, for it is only by the honest argument of earnest searchers after the truth, that we can arrive at the real solution of the question.

53. It is outside the purpose of this paper to deal with the history of the literature of the pathology of malaria, which is now to be found in every good text-book on medicine and pathology, but it is necessary to recite the history of the present paper. Even several years ago there was a considerable disparity in the views I propounded from those ordinarily accepted, and as the present *resume* of my work is an advancement on my original researches, it is necessary to recite, in general terms, the nature of these differences, and what I consider to be the causes of our having remained at a stand-still, as regards our knowledge for the last ten years.

54. Perhaps the most suitable way of dealing with the subject is that of describing the individual forms met with, remarking upon their inter-relation, and subsequently dealing with their morphological and developmental relations.

55. INSTRUMENTS EMPLOYED.—The instruments used in the first series were Zeiss' No. 1ª stand, No. 3 eye-piece, DD and E dry objective lenses, his $\frac{1}{12}$th and $\frac{1}{16}$th inch spochromatic oil immersions, with Abbe's achromatic condenser. For coarse observation, I found Zeiss's DD objective satisfactory, and with it one was also able to watch the effects of irrigation of the field with solutions of quinine and other drugs. As each slide was brought under observation, a preliminary general examination was made with the DD objective lens, and subsequently, as a rule, a detailed examination with the $\frac{1}{12}$ th inch oil immersion was carried out. In the second and third series from start to finish apochromatic oil immersion lenses were employed, (from $\frac{1}{12}$ th to $\frac{1}{20}$th inch), in conjunction with compensating oculars, Nos. 4 to 12. Whenever attention was attracted to something new or peculiar, the $\frac{1}{20}$th inch was used to analyse it. Until the year 1893, I did not find the powers beyond $\frac{1}{12}$th inch of any special use, except in occasionally enabling me to follow in fuller detail the structure of the more minute organisms. In the third series of observations I first used the $\frac{1}{25}$th inch homogeneous immersion lens, (REICHERT's) with from Nos. 6 to 12 compensating eye-pieces, and it was during the period of their employment that I found the extraordinary forms and the processes about to be described. Now that one has become familiar with these peculiar structured, the very high powers are found to be less urgently necessary. In the first series I found that the most satisfactory staining fluids were saturated alcoholic solutions of (*a*) methyene blue; (*b*) fuchsine; (*c*) gentian-violet; and (*d*) methyl-violet, ; which have been given in order of merit. Methylene blue is a highly satisfactory staining fluid it gives accurate definition to the outline of the cells, demonstrates the stroma, and stains all pigmented cells deeply, and, as in an investigation of this description, it is necessary to adhere to a uniform system of staining of some kind for comparison this was the dye used for that purpose. In the second series, methylene blue and eosin with or without a previous film of osmic acid vapour were almost exclusively used. The stains used in the third series are given in detail later on. Nothing, can equal the beauty of preparations and the success of the results obtained by Lœffler's alkaline solution of eosin and methylene blue; especially, when combined with a weak mixed solution of osmic acid and chronic acid.

56. It was impossible for Laveran working with a magnification of 460 to follow out the life-history of this organism, indeed we might state that it is scarcely possible to discern the gross characters of the parasite with this power. Even with a $\frac{1}{12}$th inch apochromatic objective and moderately high oculars, the different forms of the parasite can only be made out with difficulty, but we cannot distinguish the extremely minute points observed in the ooze or fluid discharged by some of the chlamydospores, nor can we follow out the transparent flagella, or clearly see the flagella of the smaller spore forms, except in an unusually good light. To carry out this research with facility, we require unusual magnification which is now-a-days, under the excellent definition given with homogeneous or apochromatic objectives, attainable with a $\frac{1}{16}$th or $\frac{1}{18}$th inch lens, when combined with a No. 8 or 10 compensating eye-piece (Zeiss' formula).

57. It is a curious circumstance that in most cases, except those of characteristic malarial infection, we note the same stage occurring simultaneously in all parts of the slide. This, however, may not always be the case, and it is markedly influenced by the temperature of the preparation at the time. If we keep one-half the slide more or less cool by the constant irrigation of the surface with cold water, all further development ceases, but only temporarily, for if the temperature be again raised, assuming that we have rendered evaporation impossible by an impermeable border of cement, the parasite resumes the developmental processes.

58. In every instance the more developed the parasite, the less distinct and the more transparent and invisible it is, and this invisibility advances, till the parasitic forms disappear from the field of view, or can only be seen with difficulty. There is one exception to this and it is chiefly met with in the quartan form—where the hæmoglobin is largely dissolved in the plasmatic fluid, it gives the appearance of the parasite having been stained.

59. One of the most remarkable facts in connection with this aspect of the subject is the facility with which each form of the parasite is capable of finding a suitable partner for itself, as if some pschyic intuitive or instinctive influence were in operation. This is no doubt one of the important features of the flagella, and it is possible that the pair of globules in the anterior part of the major parasite has some influence in this direction. This feature is seen in every form of the parasite and in one sense it is no more wonderful than the elective affinity they have for the red blood cells as a source of oxygen and suitable pabulum as well as an appropriate temporary residence.

60. I now entertain no doubt as to the existence of two distinct and easily differentiated species of the hæmatozoon of malaria and were we to investigate the condition of the blood in three consecutive cases of quotidian, tertian and quartan ague, all doubt would be dispelled.

61. There are some extraordinary features about the quartan parasite in many instances—such as is the enormous numbers of spores developed from some of the parental structures, the much larger number of the asexual ovoid bodies than in the other form, etc.

62. I have no doubt but that had such enlargements as these been employed by other observers in combination with suitable pigments, the new forms hereinafter described, would have been discovered earlier. Granting the possession of good immersion lenses of the newer formulæ and compensating oculars, some of the larger micro-organisms described become so conspicuous that they at least strike us as being curious. Indeed when making my first observations in this connection, it was for the moment thought that perfectly new parasitic forms were being investigated, but the finding of the same micro-organisms in the blood of several consecutive cases of malarial disease dispelled this notion. I was particularly misled by the presence of cilia in all the larger and most of the smaller forms, a general idea of which may be obtained by reference to (Plates III, V, VII and VII); of large vesiculated bodies such as are shown in (Plates V, VI and VII); the phenomena of engulfing, as represented in several places on (Plates III, V, VI and VII); the finding of large chlamydospores (Plates I, V, VI, and VII); with their contained embryonic forms (Plate I, figs. 1 and 6); the existence of cyst-walls to all the major parasites; the occurrence of two distinct groups of spherical cells in pairs, one irregular and granular, the other even and homogeneous, (see references to Plate III), both these bodies looking very much like red blood corpuscles, one of the two being 'absorbed' into the other (several such instances are given on Plate III, and few on Plate VI); the multiplicity of infinitesimally small moving bodies (Plate VI); the occurrence of a structureless substance discharged into the field from certain of the larger sporocysts cells (Plate VI); the comparatively numerous ovoid, sub-ovate, semi-circular, spindle-shaped bodies (all flagellated) met with (Plates V to VII); the simple division or fission of a certain number of spherical cells, and the division of certain others into 4 or 6 cyst-like structures as shown on in several places on (Plates IV and V); the great activity of a large number of certain small bodies of varying shape, but generally of ovoid or oblong form, in most instances with one acuminate end which has an exceedingly transparent flagellum of varying length, but usually from one and a half to three times longer than the body, in active motion; and lastly, the occurrence of a number of transparent globular (homogeneous) bodies, some of which seem to make their appearance in places where nothing was previously visible. All these seemingly new, or at least unfamiliar forms, were undoubtedly calculated to detract one's attention from what had been previously learnt and observed regarding the malarial parasite, and to engender the impression that a perfectly new protozoon, or several protozoid forms, were the objects of investigation. A few days' work soon dispelled this notion, especially when the various bodies were traced back by employing gradually decreasing magnifying powers, a line of observation which showed the different relations between these various bodies, and those I was already familiar with. It was this method which also taught one how utterly hopeless it was to acquire any degree of certainty as regards our knowledge of the real nature of this sporozoon, with the ordinary magnifying powers used, especially in the absence of proper staining.

General Remarks on the Malarial Parasite.

63. When, a few years ago, I first discovered the real nature of this parasite and its power of penetrating the tissues and gaining access to the blood vessels, I expressed the opinion that the poison might reach the system either through the alimentary tract or be inhaled. I have now, I believe, enough evidence to make this statement in positive terms.

64. At one time I thought that the hæmatozöon of malaria was similar to that of cholera, but I have now fully satisfied myself that they are distinct species of the same genus. The differences between the two groups of parasites is not imaginary—they are absolute and unequivocal, but their behaviour as regards destruction of the red corpuscles, their method of division both by sporulation or endogenous multiplication after union of nuclei, and by simple division, the growth and development of spores, are all similar. But there are conspicuous differences in the details of these processes. Amongst the gross differences we might state that the cholera organism stains much more readily and more deeply than the malarial parasite. In short, both these hæmatozoa are monads, and as such the laws which regulate their life-histories are at least similar.

65. Each polymorphic form of the parasite has mostly its own form of sporocyst, which is described in the following remarks; but this is by no means uniformly so, because the chlamydospore producing a particular form may vary considerably in shape, size and appearance ; thus the rosette of Golgi is a true sporocyst, but the same form of spore may be produced in a large multilocular structure producing from 40 to 60 spores in one brood.

66. This I first ascertained by finding that in many instances they contained one or two embryos in their meshes, the spore for some reason or another not making its exit in the natural way.

67. In many preparations made with hardening or fixing agents, we find a number of ragged looking and elongated fibres of protoplasm. Some of these are of considerable length and may show branchlets here and there. I had for years mistaken many of these for actual flagellate structures. During the last two years, however, I have satisfied myself that they are really the disintegrated frame-work of the larger chlamydospores. In other instances, similar pseudo-flagella are the straightened out cell-wall of circular discoid parental bodies, which remain after rupture of the cell-wall and setting free of their embryonic contents. Or they may be the condensed and hardened part of the margin of the ooze or discharge which is secreted by one form of parent cell. They are, nevertheless confusing, and were a source of considerable trouble throughout these investigations, as I had never been able to trace their origin from a cyst, nor from any one kind of crescentic body.

68. The question of the unity or plurality of the poison of malaria is one which has for many years roused a great deal of interest. The fact that notwithstanding

the enormous amount of attention it has attracted, and the patient labour employed to solve it, while there is still so much difference of opinion, leads one to consider the matter still *sub judice*.

69. Personally I have devoted no small share of time in endeavouring to arrive at a definite conclusion on the subject, but it is only recently that I found myself in a position to be able to express an opinion regarding it. The great complexity in the life-history of malarial parasites is to a certain extent accountable for the difficulty met with, but by far the most potent cause of failure has been the fact that in many cases we have to deal with multiple infection; in other words, the different forms of malarial organisms are co-existent in the blood, and going through their life-history. On one occasion I met with no fewer than five generations of organisms in one case, that of double quotidian ague. To add to this complexity, we have the fact that in the blood the malarial organism is rarely stationary, at least as far as the peripheral circulation is concerned. Indeed it is seldom that we get precisely identical characters in the field, even if we examine the blood at intervals of an hour.

70. When I originally began these researches, I accepted the views propounded by LAVERAN that the malarial virus consisted of only one organism which was polymorphic in its nature, and I may here confess that I wasted a great deal of time in endeavouring to satisfy myself on this point. I have now arrived at the conclusion that there are only two forms of malarial parasite, the tertian and the quartan, and this opinion I have held for the last 15 months. I do not mean by this, however, that in every case of malarial fever we get either the tertian or the quartan parasite alone. In a number, many cases met with, we have the two poisons circulating in the blood at the same time —a mixed infection. It is invariable, however, for one or other species of the parasites to predominate, and most frequently that form is the quartan parasite. We get it in the most peculiar forms of periodicity—thus it alone is frequently responsible for the large and important group of malarial fevers which we speak of as "irregular." Again it is most frequently the cause of malarial cachexia, as it is also the condition popularly spoken of as "low malarial fever." It is likewise the cause of simple quartan ague, of irregular quotidian, and often of pure quotidian. In cases where there are two generations of the quartan parasite present we may have one attack of ague to-day, another to-morrow, the third day being missed. Or we may have three generations of the parasite with a regular quotidian, or, double quartan with two generations of the parasite sporulating on the same day, separated by from 8 to 12 hours.

71. There are two definite varieties of the parasite, the quartan and the tertian, the former alone occurring in human blood about 15 times more frequently than the latter alone, whilst mixed infection occurs in about 14 per cent. of cases. The quartan parasite gives rise to by far the largest majority of the malarial fevers met with.

72. As previously stated the researches of which this is a summary have extended over 9 years, in a locality where malarial fever occurs throughout the year with epidemic exacerbations.

73. Amongst the more common varieties of fever caused by the quartan parasite are—

(*a*) Quotidian (by far the most frequent).

(*b*) True quartan.

(*c*) Irregular, which often begins as a quotidian, but in which one of the broods ceases to exist every now and then.

(*d*) A fever coming on every 8 or 10 days, every fortnight or every month.

(*e*) Chronic malarial fever, with a temperature not exceeding 100° F., but with enlargement of the spleen and liver.

(*f*) Malarial cachexia.

(*g*) Primary enlargement of the spleen with or without subsequent attacks of pyrexia.

(*h*) Mixed tertian and quartan infection, causing an atypial form of intermission.

(*i*) Lastly, the quartan parasite is responsible for many of the complications arising from malarial infection. The *tertain* parasite is most frequently met with in the blood of patients suffering from one or two forms of malarial fever—true tertian, or quotidian; but it may be found in cases where the quartan exists, the latter speedily effacing it. As the tertian species has less tenacious vitality it is often crushed out of existence by the quartan—"the survival of the fittest." The tertian parasite most frequently leads to either—

(*a*) true tertian ague, or

(*b*) ague quotidian with double infection of two generations of the parasite carrying out their life-history in the blood at the same time;

(*c*) irregular intermittent fever, when it co-exists in the blood with the quartan parasite, it gives rise to simple tertian ague when only one generation of the tertian parasite is carrying out its life-history in the blood.

74. In the Deccan the major varieties of fever produced by malarial parasites may be at once recognised by stating that in one hundred cases of malarial fever, the average distribution will be approximately as follows :—

(*a*) *Produced by the Quartan species* :—

Quotidian fever	...	...	73
Quartan fever	...	...	3

(*b*) *Produced by the Tertian species* :—

True tertian fever	...	...	15
Quotidian fever	...	...	7

(*c*) *Produced by mixed infection* :—

Alternating quotidian and tertain	...	2

75. Many of the regular types of fever may become irregular, the intermission varying ; *per contra*, some of the irregular forms may become regular intermittents. It is no uncommon circumstance to find that a quotidian that has recurred daily for say a week ceases for one or two days, and then comes on as a quartan, or even as a tertian. In the former case the infection was due to three generations of the quartan parasite, of which two ceases to multiply, in the latter to two broods, if the tertian of which one ceases to multiply.

76. Both species are most complicated in their developmental processes, and perhaps the tertian is the more so. As in the case of the quartan the tertian parasite produces spherical spores from red corpuscles, but there are certain differences. In the tertian the spores are developed in the red blood cells, but (*a*) red cells are smaller ; (*b*) lighter in colour ; (*c*) more uniform in outline ; (*d*) spores are smaller and more numerous, and the residue of pigment is less in quantity and occurs as small particles. I do not attach much importance to the area of the red cell occupied by the spores.

77. Small sized free flagellated structures develope in spherical cysts which become oblong, and from which they are set free ; or such flagella may be produced within the red cells themselves. There are also enormously large chlamydospores within which free flagella of varying size develop, but without any special wall. They form large egg-shaped sacs, which fit into corresponding excavations in the chlamydospores. In forming flagella these ovoid bodies enlarge, especially in the longitudinal direction ; some of the flagella are long and broad and curled up at the ends which are pointed ; others are long and narrow, and all split up longitudinally into their ultimate flagella ; and later may even split across into minute rod-shaped spores. These are peculiar in staining deep blue and not red with Loeffler's solution, whilst the surrounding protoplasm of the chlamydospores stains red.

78. There are also considerable numbers of ciliated spherical bodies, the cilia being long, firm, and pass out at right angles abruptly, leaving spaces between each other, in which spaces arise a considerable number of varying sized cyst-like

bodies which are set free, and become the hyaline spherical body mentioned above. As a rule this latter occurs either when the fever is present or during the intermediate stage. These spherules stain light purple with Loeffler. In the blood, preceding the fever stage, we have the small spherical spores from red cells, and the groups of spores in the chlamydospores set free.

79. Simultaneously with the production of the small spherical spores there are formed—(*a*) small ovoid bodies ; (*b*) minute ciliated and nucleated spherical spores ; (*c*) minute crescent ; and (*d*) minute amœboid bodies. At the same time, we notice that the red cells are to a large extent being infected with these embryonic structures, which in a few cases lie on the surface, but in most, make use of it as an encasing cyst.

80. During the intervals between the attacks of ague, if we examine the blood, we find that the chief parasitic structure of the tertian species is the chlamydospore, of which there are several kinds :—

1 Enormously large and coarse with large ovoid loculi, and a moderately thick frame-work, but the ovoid-cystic cells are never in contact with one another. These are the special sporocysts for the tertian form of spores ;

2 Spherical bodies ;

3 Large triangular cystic-cells. The mesh-work in the first named is much finer than the last, looking like that of ordinary adenoid tissue, or a small round-celled sarcoma, whereas in the last it looks like the stroma of a schirrhus cancer, with these differences—the alocoli do not communicate, and they only contain one cell each. They are best seen when the process of disintegration is half advanced (*vide* Plate VIII, which gives a general view of these chlamydospores).

4 Chlamydospores which are smaller and with a finer mesh-work ; the meshes contain ovoid nucleated cells, the whole staining red with Loeffler. These are the sporocysts of quartan spherical embroynic spores and are sometimes very numerous.

5 Chlamydospores within which cystic bodies are produced, and ;

6 those forming the group of small spherical spores in the red cells.

81. Associated with the tertian parasite we also have star-shaped bodies of medium size, with moderately long cilia, having also two contractile vesicles, the whole structure developing spherical cystic bodies. These stellate structures are developed from another variety of chlamydospore to be described later on. If we keep the blood from coagulating, by careful observation it is possible to keep these bodies in view till they rupture. Either methylene blue or Lœffler's alkaline solution stain them well, especially the latter, the eosin of which maps out the outline of the contained bodies, whilst the contractile vesicles, nucleus and flagellum are well brought out ; these effects are intensified if osmic acid vapour is used before the stains.

It is then seen to stand out in relief, because of shrinking due to the vapour of osmic acid to which the fresh moist blood was exposed. All the forms illustrated on Plate VIII were fixed and stained in this manner. The use of osmic acid in causing the stained preparation to retain the appearance presented at the moment of abstraction of the blood, no matter what form of aniline dye be used subsequently, is I believe one of the more useful of recent improvements in the colouring of blood preparations. It is also of special value in causing the red cells to "take" any one of the aniline dyes, whereas without this film of osmic acid the hœmoglobin of red cells stains perfectly only with eosin. Personally, I am not aware that this idea in regard to the enhanced usefulness of osmic acid preparations has been advocated previously. Anyhow it is a most important addition to the means at our disposal in blood examinations.

82. One variety (the largest) is formed in fevers produced by the quartan parasite and the other in those of tertian origin.

83. Some excellent descriptions of the process of formation of these forms have been given, especially by Golgi and Mannaberg, and there is little to add to them. It is necessary to state, however, that I do not consider the spores thus produced to be formed by direct division, but that they simulate in a diminutive form, the *plasmodium* of the *Myxomycetes*; that is, they are true sporocysts, formed by the previous conjugation and fusion of two embryonic spores in which the nucleoli disappear, the nuclei fuse and undergo division, and by a true karyokinesis or physiological sporocystic envelope being early formed.

84. It is only exceptionally that we get a case in which the developmental processes are regular and uniform. In almost all, there are several phases of the parasite undergoing changes simultaneously. Most frequently, however, we find that there is a quotidian from infection with the quartan parasite.

85. There is a whole class of bodies to be met with which does not appear to have been mentioned by other authors, *viz.*, free flagella. These do not refer to the free flagella developed from elongated crescents, but to ones that are developed in special cysts. There are three varieties, large, medium and small. The large ones for a time look like silk fibre in the field, but soon become granular or variously striated and eventually disintegrate to give origin to different forms of spores. Whilst the long and narrow kind are produced either singly within special cysts, or in larger cysts of the same kind, and set free.

86. They are met with specially in tertian ague, and the more continued forms of malarial fever, whilst the crescentic varieties are met with chiefly in quotidian fever. Besides these there are other forms of flagella which I have termed pseudo-flagella. They and the foregoing will be dealt with in detail later on.

87. No part of the malarial parasite undergoes disintegration; there is no waste of tissue or developmental force anywhere—even parts of the frame-work of the sporocysts are reproductive. This is well seen in the case of the tertian parasite, in which the flagellate forms after full development subsequently undergo sporulation by transverse division.

88. In every severe case of quotidian fever, after the blood has been under observation for a time, what appear to be the red cells are seen to change their

characters so as to develop into two kinds of cells, one hæmogeneous, the other granular. Close observation and proper staining show that both kinds are flagellated. Later on it will be noted that these pair off so that one granular is engulfed by the hyaline and the combined body so formed develop into one large chlamydospore.

89. Having fused their nuclei. sporulation begins, the combined body breaks up into a number of spherical oval or oblong bodies which grow to the size of their parts.

90. In some cases two bodies of the same kind, either hæmogeneous, or granular, unite to produce spherical sporocysts, but this is rare.

91. In other instances we find one of these spherical cells enlarge, become globular and finally split up directly; that is, the protoplasm of the cell itself breaks up into, elongated flagellate spores (Plate V fig.). In all instances, except the last named, the process of reproduction appears to be one of sporulation through the neuclei—a true karyokrosis.

92. Besides the two groups of large oval cells mentioned, there is a third which also results from the fusion of an organism of a similar kind, and develops within it transparent or slightly opalescent fluid which goes on increasing till it bursts its envelope and is poured out in the hæmoglobin of the ruptured cells, but does not mix with the *liquor sanguinis*. The quantity of this fluid is seemingly very great indeed. The outer limit of the area is well-defined. In the area itself, after a varying number of hours, many yellowish spots develop, which ultimately grow into large flagellated ovoid bodies like the parent cell.

93. In the quartan parasite there is developed one large elongated mass which forms a sporocyst within which spherical cells develop which look very much like red blood cells. A similar group of cells forms in the large irregularly oblong chlamydospores, but they have a closer resemblance to the flagellated spherical body which develops within the red cell.

94. As in the case of quartan ague one variety of the large oblong or irregularly spherical cystic-cells produce a peculiar fluid which is discharged from the ruptured part of the sac, and in which we see minute points of a yellowish color which increase in size and become flagellated. The area occupied by this fluid is very rarely occupied by other forms of the parasite.

95. In tertian ague we get two, and sometimes even three, forms of flagellated structure; (1) a long and broad variety; (2) long and narrow variety; (3) medium-size stunted form.

96. The long and broad variety breaks up into small spherical cyst-like bodies or into excessively minute refractive spore-like bodies, which soon separate and move free in the field and develop into oval flagellated bodies. Sometimes these flagella develop small crescentic rods, the concavity of which is soon filled with protoplasm; or fine rod-like, processes may be thrown off which give off branchlets at acute angles the intervals being filled by protoplasm, or they

throw of minute rapidly moving small irregular bodies which develop into amœboid bodies or actually enter any cystic bodies found in the field, and develop into oval flagellated bodies.

97. It is in tertian ague that we especially get the large granular "swarming" cell (Plates VIII, *a*, VI *n*), which by many observations have been considered to be large white cells, or macrophajes. This, however, is an interpretation of their nature which these researches do not appear to justify. I consider them to be as much a form of sporocysts as are large bodies shown in (Plate I and Plate VIII). I arrived at this opinion on ascertaining that they are provided at an early stage with a cyst-wall, that they are flagellated, provided with vesicles, that they contain large nuclei, after that they are formed by engulfing of one body into another, subsequent to which, the so-called granules are developed. Close observation of these granules, however, shows that each one has a limiting wall of its own as well as a nucleus and a nucluolus, develop flagella free themselves and wander about the field. The longer these large white bodies wander about, the fewer become the granules which are, in short, nucleated spores. In some cases of tertian the spores are excessively small. There is no structure in the malarial parasite which more accurately answers to the description of a "plasmodium," using the term in its zoological sense, than the one just referred to. It is so important that it will be necessary to revert to it more in detail when describing the individual forms of this parasite.

98. The largest form of chlamydospores is that within which is developed medium-sized granular and ciliated spherical bodies, which latter on being set free, closely simulate crenated red blood cells. Some of these spherules commence sporulating immediately after making their exist, but others undergo binary division and subsequent union with other bodies before doing so.

99. Another large sporocyst develops a clear cyst-like structure which, if set free grows rapidly; these seem to correspond with the hæmogeneous variety of round cells (Plate I, fig. 1, *d*,). A fifth form of sporocyst also seems to develop an exceedingly small spherical spore rapidly acquiring long flagella (Plate I, fig. 5, *a*). The parent has three long flagella.

100. With a considerable experience in blood examination, I have no hesitation in stating that no investigation should be considered complete until the process of staining and fixing herein described have been carried out. By the exclusive adoption of other methods it is possible to fail to discover even some of the larger forms of the parasite, especially is this so, with regard to some of the chlamydospores, transparent spherules, as well as liquid protoplasmic discharge. This method must however, be carried out with care for overstaining renders a preparation useless, even obscuring the details of structure, while deficient fixing or staining fails to bring out such forms as are present. An observer who has not carried out this process before, will be astounded with the first results of an observation on a well-marked case of malarial fever.

101. Therefore microscopical investigation of malarial blood can only be reckoned complete when it has embraced the following:—

(1) Examination of the fresh blood at least every hour from the commencement of an attack to that of the next;

(2) Examination of the fresh blood that has been fixed while in the moist state; that is immediately after abstraction;

(3) Examination of the fresh blood that has been stained in the moist state, by adding a drop of the weak watery hyaline stain selected to the still liquid blood or applying a drop of the latter to the finger and pricking through the staining fluid and examining it at once.

(4) Examination of the fresh blood bottom stained and fixed, according to the plan laid down on a previous page.

(5) Examination of the blood carried out in the same way as for bacteria—drying, fixing by passing through a flannel three times, staining according to rules for the stain or stains used, washing, drying and mounting in zylol balsam.

Examination of the fresh blood should be carried on half hourly in connection with each species of the parasite, and each one of the polymorphic forms of such species, from the commencement of one attack to the commencement of the next, a feat, the achievement of which, involves an amount of labour only those who have carried it out, can duly comprehend. There are some cases in which at the commencement of an investigation it is absolutely necessary to watch the changes in the parasite forms continuously.

102. Fixing is usually best carried out by means of osmic acid vapour which when properly done has the effect of causing the cessation of all movement in the parasite, as if by an electric shock, each form being made to permanently retain the shape it possessed the moment the vapour penetrates it. If this is done, we obtain a series of permanent preparations which should be numbered, recorded, and described at once, and kept for reference. Staining is best done by one of the combinations of methylene blue and eosin. Loeffler's being I think the best. It has the advantage over fixing that the latter does not touch the more attenuated or delicate forms of the parasite, especially those in water, a fact which points to be high value of the combination of their methods.

103. The combination of fixing and staining yield by far the best results. We should recollect that the stain continues to heighten in colour for a few hours. This process has the effect of mapping out practically every part of the plasmodium on the slide, including the intra-corpuscular and the intra-cystic. The special virtue however resides in the prominence it gives to structures, which have hitherto been so transparent in fresh blood as to be invisible to the most experienced and trained eye. It has the further advantage of enabling us to see all forms of the organism with an ordinary $\frac{1}{12}$th objective, and a moderately high compensating ocular. It also enables us to see one form of the spherical cell dividing into two parts, the division being clearly commenced in the nucleus, the flagella of all forms which were previously highly transparent now coming into view; the engulfing of the granular by the hyaline spherical cell; and of the three forms of ovoid cells, etc.

104. In concluding of this preliminary account of the malarial parasite, I might state that notwithstanding the constancy of the different species of the malarial parasite, and of the characteristics of the polymorphic forms, some of them present differences which have sometimes tempted one to believe that new forms of the parasite were under consideration. If we take this circumstance together with the fact that these different forms are in some instances of momentary duration, and again that the predominating forms vary with the severity of the attack, we have an adequate explanation as to the different descriptions of the various forms of the parasite given by different writers as well as the different interpretations propounded although, I am compelled to adhere to the behalf that no previous worker in this field has seen, or described what I have termed the invisible forms of the parasite.

105. Some of the micro-organisms to be watched and followed in malarial blood, are amongst the smallest bodies found under the microscope. We have an instance of this in the minute micrococcus like-spore produced by the disintegration of the flagellum of one form of this parasite, another in the infinitesimally small bodies produced in the fluid formed by one special variety of chlamydospore; and in one form of the extremely small cystic body produced in one large chlamydospore.

106. In most of the chlamydospores small ovoid bodies are formed and separated; thus all the spores shown on Plate VIII, become ovoid and acquire flagella, the most conspicuous flagella being those at the narrow end. In some instances these changes occur before they are separated from the parent cell. The mesh-work likewise disappears, breaking up into an irregular flagellated structure.

107. We may now find many fine flagella appearing in the field in places where nothing had previously been seen. They are usually in syzygies. Lastly, there is a most curious arrangement due to infolding of the fine flagella, the intervals between the folds being filled up with thin protoplasmic material, and in the meshes, at a later stage, we see the commencement of sporulation.

108. There are now also numerous small nucleated cystic bodies in the field which have been given off by large chlamydospores formed in pigment derived directly from the red cells.

109. When only one flagellum is present, frequently the size and general proportions of the flagellum is out of all ratio to that of the spherical body, in which case it looks like a true flagellum. Sometimes under the same circumstances one long and one short flagellum is attached to the body. Frequently we see one or two particles of pigment on the flagellate process, but there is no certainly about this, nor is there any regularity in regard to the position of the pigment patches.

110. As the quantity of hæmoglobin dissolved in the *liquor sanguinis* is incapable of staining to any great intensity the parasitic forms that have been set free, as they have usually a semi-transparent or white appearance, such of them as are invisible now stand out in contrast with the more deeply coloured fluid in which they move about. In the case of the encysted and nucleated parasites, the intra-cystic bodies and nuclei " take " the pigment more than the other parts and maps them out distinctly.

111. If we gently irrigate the field now, we can wash out the hæmoglobin whilst the parasites from their greater adhesiveness remain. In this way we get what we may term a natural staining, without adding anything extraneous in the form of aniline pigment to the blood, to which the opponents of the parasitic theory take such exception.

112. It may be remarked incidentally that when there is an unusual quantity of hæmoglobin dissolved in the blood, all cellular structures contained in it, possess the same colour, and it is often difficult to distinguish red-cells from spherical bodies or spherules which have developed from the infolding of crescents. There is always, however, one or other modification in each cell which enables us to distinguish its nature. This does not apply to the ciliated spherical cell which looks immensely like a crenated red-cell.

113. In the tertian species the developmental processes are carried out with less capriciousness, than is the case with the quartan parasite or the parasite in cases of mixed ague. Free flagellate forms are on the whole much more common in tertian than in quotidian ague.

114. We often see the large free flagella double up and produce an incomplete circle, the central part of which is filled up by thin protoplasm in which are developed a number of small nucleated spherical spores which eventually burst through the wall and are set free; in other words it forms a true plasmodium before rupturing as do most of the other forms.

115. The special directions in which we have hitherto fallen into error are:— In recognising that the process of sporulation is one of direct occurrence in the red blood cell, and we shall see that this is a fundamental mistake; considering that the ciliated spherical bodies, with irregular margins, are crenate corpuscles, and the hyaline spherical cells (also flagellated) are "shells" or spherical bodies from which the "spherical body" has been set free; neglecting to follow out the future history of the very large spherical cells, which having become indistinct, ultimately disappear in the plasma for a time, varying from 8 to 24 hours, and at the end of that time re-appearing; neglecting to ascertain the nature of the fluid discharged by some of the large chlamydospores; neglecting to attach any importance to the infinitely small yellowish-white particles that appear in this fluid in progressively increasing numbers, and to inquire into the nature and origin of the oval or crescentic bodies which are developed from these; believing that the whole of the spherical bodies make their exit from the red blood cells— an unavoidable error, unless we watch the process in several fields for several consecutive days, which would teach us that the two cells, from being at first merely in contact with one another, the larger ultimately absorbs the smaller.

116. Referring to the nature of the fluid discharged from certain cells referred to above, the absence of any conspicuous contents in the discharged fluid, or of any distinct peculiarity in the nature of the fluid itself, are no doubt accountable for the fact that its very presence has hitherto been neglected or unnoticed. When preparations of human blood containing it are stained with methylene blue, it may be noted that the fluid acquires a faint blue color, and that it is fairly well-defined from the plasma. Even in the freshly abstracted blood it may be well marked, especially in cases of fever due to the quartan parasite. Allowing that this be left unnoticed on a slide containing a large quantity of it, there is a some difficulty in

recognising why the small yellowish bodies which gradually appear in it should have hitherto escaped attention. They must have been seen by scores of observers, and, no doubt, the temptation to consider them pigment particles is very great, especially when we take into consideration the fact that there are in the plasma larger and more conspicuous particles of pigment almost all over the slide. Lastly, we arrive as the time when these small bodies begin to move about, first by a sort of lateral oscillation simulating "Brownian" movement without change of position, but soon they do actually alter their position, and are seen to move about with great rapidity, appearing as minute nucleated spore-like bodies, and when there are a large number of these bodies in motion simultaneously, we get a most peculiar and characteristic appearance, which on close observation presents some of the characters of "bubbling" or "swarming," the extent of their perigrinations being still limited to the area occupied by the fluid from which they are developed. At this time we have a highly transparent granular body which is still of globular form. It now however rapidly acquires an ovoid or sickle-shape, its flagellum lengthens, and it wanders throughout the field.

117. Again, the process of "swarming" has hitherto been mistaken to be an agitation of granules of pigment in the containing body, and supposed to be due to a vibration of the contained fluid, but in reality it is an active movement of a large number of small nucleated organisms, which are seen to move about in the plasmodium with great speed. The parent cell now assumes a somewhat amœbic character, and moves very rapidly for about 15 minutes. It now assumes a spherical form and it becomes stationary; the flagella continue to move for a few minutes, and then curiously disappear. At this time a most curious phase may be noticed which will be reverted to later on.

118. One of the commonest errors is that of believing that all the spherical bodies develop within red-cells. This undoubtedly does occur, but the majority of the spherical cells are developed in independent chlamydospores. Another serious error is that of mistaking the granular spherical cell of malaria, for crenated red blood cells. When the blood is stained with eosin and methylene blue we can recognise the difference at once as by the effects differs in the case of each of these structures, eosin staining the crenated red-cell rose pink, but it has no effect on the malarial parasite, whilst methylene blue, which does not affect the red-cell, shows the granules of the spherical cell to be of a light blue colour. Further, in fresh preparations, if we watch the spherical cell we note that it undergoes one of two changes—when of the crescentic series it usually become quite smooth and spherical again, and then break up into spores; or more frequently it unites with one of the hyaline spherical cells to form a large body in which are developed sickle-shaped spores.

119. Again, we may from the shape and appearance of the red-cell, recognise that it is a discoid body which has been blown out as it were, whilst the malarial body is uniformly spherical, that the former stains well with eosin, whilst the latter does not, but that methylene, which has no effect on the red-cell, stains the malarial spherical body light blue colour; the red-cell has no contents except the serous fluid which swells it, whilst the malarial cell has some visible modifications as regards contents.

120. Early in 1891, I learnt that the summer heat of Hyderabad during the day, and the heat of the artificial illumination required for night work, were sufficient to keep the parasite living in the blood-plasma, and at the same time to some extent prevent coagulation of the blood. The facts stated in my first brochure were dependent on this discovery, to which I owe much of whatever success has attended my efforts in their researches; and I believe a great deal may still be learnt by this procedure. This point was altogether overlooked by my critics. It was at once apparent, however, that the success under these circumstances must necessarily be limited, from the small quantity of fluid in any one slide-preparation.

121. In a large number of cases of chronic malarial infection, one of the most frequent changes in the blood is that of finding that certain parasitic forms pass through a considerable part of their life-history within the red blood cell. In some instances the red cells remain more or less transparent throughout, whilst in others they become quite opaque. In the former case the developmental phenomena may be easily pursued, but in the latter all the essential changes, are hidden from view, so that much at least of what is going on in the red blood cell has to be arrived at confecturally. In some we are almost able to follow the principal phases by the fact that we see certain parts of peculiar forms contained in outline, together with elevations, and depressions, etc. In that case we have to await further opportunity to verify the conclusions arrived at. This latter method, however, is one to be condemned rather than followed, unless from exceptional circumstances, we are able to fill in the details, and this is less desirable since there is often a bias, which once created, is often difficult of eradication, a condition compatible with the most unimpeachable integrity of purpose, for unfortunately the human mind is, in its operations, neither mathematically accurate, nor is it cast in a mould, nor worked on the basis of a machine. The small degree of elasticity under which the mental operations of the honest labourer after the truth in scientific research are brought into force, is perhaps the great cause of our advance in knowledge, by reason of its originating that " difference of opinion " out of which so much has sprung.

122. I have not hitherto alluded to the intrinsic nature of this parasitic organism beyond general statements. I hope, however, to show that it is a true monad, having its natural habitat in water, contaminated by organic matter, probably animal matter resulting from the disintegration of the myriads of the different forms of aquatic monads, infusoria, crustacea, fish, amphibia, etc., and resulting in the almost illimitable destruction of the red blood cells. Indeed, it is possible by the inoculation of healthy blood by malarial water to actually follow the history of this parasite, at least, in the earlier stages of its development, and were it not for the great solubility of hæmoglobin in water, I believe it would be possible to carry on a complete investigation in this way. My investigations seem to point to the fact that either the fully formed spore containing chlamydospores may be the medium of communication or the spores themselves, the latter probably being the case when infection occurs from malarial air. Let us suppose that we are commencing an observation on the parasite after its consumption :—The following seem to me to be the interpretation of the ordinary course The ova are drunk, reaching the stomach part are set free, and begin to multiply, whilst the rest enter the blood vessels in which the process of enlarging and multiplying goes on uninterruptedly.

123. The hæmatozoa of malaria and cholera, the *amœba coli* of dysentery (which I have proved is a true flagellated sporozöon, acting on the columnar

epithelian of the large bowel, just as the former two act on red blood cells), and other hæmatozoa, which I propose to describe later on, all pass through an encysted embryonic stage, a flagellate stage, an amœboid stage, a stationary stage, and lastly a stage of disintegration, during which the spores are set free. We see this specially well marked in the sporozöon to be found in the blood of syphilitic patients, during the period between the second and third stage of that disease. I might in passing state that this latter sporozöon has specially well defined characteristics, and that the so-called bacillus of LUSTENGARTEN, is only one of the embryonic flagella of this sporozöon, syzygies of which from large chlamydospores which give origin to ovoid flagellated spores. Before paring in this way they often unite at one end with a small spherical spore producing a drum-stick like body and, in a few instances, this alone develops into a parental structure. The chief body however is a well defined plasmodium or sporozöon, which has characteristics very similar to those of the sporozöon depicted and described as occurring in malignant tumours. There are also in syphilitic blood two forms of free flagella. These I have described and illustrated in another special monograph.

124. It is sometimes difficult to distinguish certain of the other small forms from the amœboid bodies, especially is this so when they are moving about, for the small spherical spores, shortly after detachment, sometimes assume forms very similar to small amœboid bodies. As a rule, however, we ought to be able to distinguish the spores because their main body remains more or less homogeneous, and they do not throw out irregular pseudopodia as the smaller amœboid bodies do.

125. When we consider that several forms of the malarial parasite pass through an amœboid stage, as one of the natural phases of their development, we should be cautious in regard to the structure to which we give this term. Amœboid movement is a phase of the general developmental processes of all sporozoa.

126. The flagella of both spores and parent cells are sometimes thrown out with great rapidity—one cilium or flagellum after another appearing in the field, until in some the whole surface seems to have discharged a number of processes, like an angry porcupine shooting its quills. This is specially well seen in some of the forms met with in marsh-water. In malaria however it is never seen so characteristically as it is in cases of amœboid dysentery.

127. There are certain general characteristics in-regard to the malarial parasite which may be here mentioned.

128. All forms are changed into a larger form by the combination of two similar or dissimilar bodies. After this incorporation important changes occur, the chief of which are :—

(1) The occurrence of an amœboid stage ;

(2) change to a spherical form ; and,

(3) to segmentation into two parts, then four, eight, and so on, till sporulation having attained its maximum as regards numbers of embryos, the latter move about with great rapidity ; throughout this long period the parasite is invisible.

129. With regard to the larger forms of this organism it is necessary to indicate that they have certain special characters :—

(1) They are all flagellated, usually possessing from one to three flagella.

(2) They pass through an amœboid stage as well as through a stage of great activity, and finally a stationary stage.

(3) They all incorporate another form or body usually of the same shape as themselves, but smaller and having visible differences in the details of structure, and all undergo one or other form of sporulation.

(4) The larger spherical bodies produce a highly granular body with a cyst-wall, containing a large, highly granular nucleus.

(5) Many of the ordinary ciliated spherical bodies split into two parts by binary division, producing two flagellated structures, which eventually become the parents of one form of spore-body.

130. As a rule, we find that the parasites aggregate near an air bubble, or near the edge of the slide ; especially when all the red blood cells have been used up, bearing out the statement that the malarial parasite is aërobic, and seeks the source of oxygen.

131. The whole process arises from the decomposition of organic matter in an organic infusion, or in a liquid containing animal organic matter. Outside the body this is met with in water, in the circulation the red blood cells bear the brunt of the destructive process. The process is therefore one connected with the decay, decomposition or putrefaction of organic matter. The infecting agents are doubtless, the extremely minute spore-like bodies generated in the sexually mature parasitic cells—the spores bodies, reaching the moist surfaces of the respiratory tract when inhaled or the digestive tract by imbitition when taken with water.

132. I think it would be well for us to consider that this parasite is highly complex in the various phases of its development. In the table enumerating the different parasitic forms in malarial blood given latter on, it may be seen that there are many different structures to be found in the blood of malarial patients, and although it has not been possible to connect all forms in the developmental process, I have no doubt but that such connection does exist.

133. The following enumeration indicates that there are several main structures with their sub-divisions. There are also two special species of the parasite, quartan and tertian. We might consider these *seriatim*, or deal with them in the order of their development or of their importance.

134. The most visible form of sporocyst is that which occurs in the red cells, although it is doubtful if more than a small percentage of the embryonic structures occurring in malarial blood is thus produced. It is, however, the one with which writers and workers are most familiar. There are two chief varieties producing two forms of small spherical spores in single red-cells, but we shall see

that the spores thus formed are few in comparison with those produced in the larger chlamydospores. Numerous red-cells infected with the embryos of this form of the parasite may however fuse, and this is one of the ways in which the largest aggregation of spherical spores (such as that seem in Plate I, fig. 6,) is produced.

Description of the Polymophic forms of Malarial Parasites.

135. We have next to consider the nature, peculiarities, and structure of the various parasitic forms met with in malarial blood, and would begin by enumerating the polymorphic varieties of this hæmatozöon, dealing specially, however, with those met with in my last series of observations and embraced in the forms I have ventured to designate "invisible plasmodia."

136. For convenience, we might in this enumeration, consider the malarial organism as consisting of several varieties of parasitic forms, related to one another in their life-cycle.

These different forms as—

(*1*) *Sporocyst or chlamydospores;*

(*2*) *Spores;*

(*3*) *Crescents;*

(*4*) *Spherical bodies;*

(*5*) *Amœboid bodies;*

(*6*) *Stellate bodies;*

(*7*) *Plasmodia;*

(*8*) *Flagellate bodies;*

(*9*) *Cystic bodies;*

(*10*) *Phagocytes;*

and in connection with these we may allude to the pigment, free and contained which gives rise to malarial melanœnia which is so characteristic of malarial infection.

137. Like other amœbæ, the *plasmodium malariæ* seemingly has a tendency to settle and colonize during the reproductive period of its life-cycle, and this they do by selecting certain internal organs for their temporary abode. We may also note that they do not adhere to places where there is a full and powerful current of blood. They do not remain in the more active parts of the general circulation, but in places where it is sluggish, such as the parenchyma of the spleen, liver, the red marrow

of bones, the capillaries of the *pia mater*. We see in this one of the earliest indications of an instinctive faculty, in which there is manifested a sort of pschycical or elective affinity—the rich oxygen-carrying cell being chosen by the malarial organism.

SPOROCYSTS, SPORES, AND SPORULATION.

138. The foregoing list includes the polymorphic forms met with in the two species of the malarial hæmatozöon. We commence our description of the various forms of the malarial parasite by a consideration of the sporocysts, which are the most important structures because they generate the embryonic forms, the "birth" of which may determine an outburst of pyrexia.

139. The sporocyst or ovum presents several forms, but the following table includes most of those met with in cases of malarial fever occurring in the Deccan.

VARIETIES OF SPOROCYSTS.

I.—The Sporocysts occurring in red blood cells are :—

(*i*) *The sporocysts containing the group of small spherical spores of the tertian parasite.*

Do. do. quartan parasite.

(*ii*) *Sporocysts developing groups of cystic bodies—there are two kinds, one developing four to six cysts, the other developing only two.*

(*iii*) *Sporocysts giving origin to small free spherical bodies from masses of red cells.*

(*iv*) *Of flagella, from either single red cells or masses of red cells met in the tertian only.*

(*v*) *Sporocysts developing groups of two or more forms of crescentic, sickle-shaped and other forms.*

II.—The Sporocysts met with in liquor sanguinis are :—

(*i*) *Sporocysts in which are generated small irregular spherical bodies.*

(*ii*) *Do. do. regular spherical bodies of quartan parasite.*

(*iii*) *Do. do. do. tertian do.*

(*iv*) *Do. oval or angular cysts of tertian parasite, the sporocyst having a coarse frame-work.*

(*v*) *Do. oval spores, with and without a fine reticulated framework in the tertian and quartan parasite respectively.*

(*vi*) *Sporocysts oval spores without any intercellular network between the spores—found only in the quartan parasite.*

(*vii*) *Sporocysts in which the body protoplasm of itself is split up into numerous elongated cylindrical bodies or with rounded ends.*

140. It is difficult to separate an account of the sporocysts from either the process of spore-formation, or a description of the spores themselves. For this reason, as well as for convenience, I have to some extent incorporated the facts brought out in connection with the spores with those of sporocysts and sporogenesis.

141. It will be convenient, therefore, to enumerate here the different varieties of spores.

VARIETIES OF SPORES.

The varieties of spores occurring in the blood are :—

(*a*) Small *spherical spores* of *tertian* species—

(*i*) develop in red corpuscles.

(*ii*) do. sporocysts.

(*b*) Small *spherical* spores of quartan species—

(*i*) developed in red corpuscles.

(*ii*) do. sporocysts.

(*c*) Small and medium-sized spherical cyst-like bodies, developed in red blood cells in both tertian and quartan species.

(*d*) Small spherical spores occasionally developed in crescents.

(*e*) *Invisible* spherical spores developed in the discharge or ooze, from one of the large varieties of sporocysts, which is similar in both tertian and quartan parasite.

(*f*) Oblong flagellum developed from sporocysts.

(*g*) Oval, oblong, or triangular cysts, developed in tertain sporocysts.

(*h*) Large ovoid spores of quartan sporocysts.

(*k*) Spores of *flagella* developed from—

(*i*) crescentic flagella,

(*ii*) large and large medium-sized flagella;

(*iii*) from frame-work of sporocysts.

(*iv*) the discharge of one variety of sporocyst, and invisible under ordinary circumstances.

(*l*) Elongated cystic structures giving origin to "flying-bird" forms.

(*m*) Spores of different forms appearing in the field in places where no such bodies were seen previously, and probably from the fluid contents of one of the "invisible" mature parasite forms consequently of unknown origin.

142. We may now make a few remarks in connection with the special varieties of sporocysts formed in both species of the malarial parasite.

143. In Plate 1, figs. 1 to 6, we see several different forms of mature parasites as well as the stages in the formation, of the different varieties of sporocysts. They are even better seen in Plate V; but in Plates VI and VII the whole process of engulfing, sporulation, etc., as well as the flagella, vacuoles, and "eye-spots" are distinctly visible. In addition to the foregoing the formation of the large ovoid or triangular bodies (Plate VIII, c^{ii}), produced in tertian sporocysts, the engulfing carried out by two forms of ovoid bodies, and the production of spores in these, are seen in Plate VI.

144. The parasite shown on Plate I *a*, is one of the commonest forms met with in marsh-water, in which, however, it retains its flagella to the end. In water it is often seen to possess a greyish-green, or even a green color, indicating its origin from a vegetable cell, which had been infected by one of the embryonic forms of the malarial parasite. When it has split up, it leaves, as a rule, an unusually large quantity of pigment. In fresh blood, during the early stage of its formation, it is quite distinct; it has not hitherto been noticed, probably on account of its semblance to a swollen or degenerated red cell; it is seen only during the intermediate period (amœboid stage) of its development, and even then it is by no means well-defined; lastly in the globular stage it is invisible. Even when it has split up, the embryonic forms produced are less defined than the parent. If, however, we see it early and keep it in focus, we may observe it gradually vanishing from the field, but we may, by retaining the field in position observe its re-appearance, in a new guise.

145. In the stained preparation the next most frequent and by far the most conspicuous body, is a large sporocyst containing a varying number of spores of large size, and of spherical or ovoid shape; these are seen to distend the sac, which gives away at one part and sets these spores free in a stream, one by one or in single file, until the sac is comparatively empty and may actually collapse; but, as a rule the sac remains distended, the place of the freed cells being taken by the plasma this fluid being quite transparent or only faintly coloured by the dissolved hæmoglobin.

146. The next most frequent body is a multilocular sporocyst, developed by the union of two ovoid bodies, within which medium-sized spherical bodies are formed, eventually set free, and look extremely like red blood cells. This

body in some instances splits up into two similar parts, each part possesses a cyst-wall, and is provided with flagella. Most of them unite with another hyaline spherical body and become amœboid, wandering about for a time, then pass through a stage in which it is stationary, develops spores, disintegrates and sets the spores sickle-shaped free.

147. Another important body is the sporocyst produced by the union of two similar spherules, which after passing through remarkable changes, ends in producing a liquid protoplasm which it sets free, but which does not mix with the plasmatic fluid of the blood. In this fluid minute spots of a yellowish colour develop, and these eventually, grow into large ovoid bodies which assume the same characters as the parent cell.

148. If we follow one of the large ovoid bodies developing this liquid sarcode from the beginning, we note that it becomes spherical, produces a small clear space within which is developed a nucleus, that it elongates, acquires a flagellum, sets itself free, and develops into a large ovoid body, this body soon acquires another flagellum moves about, and develops a contractile vesicle. It now assumes the globular form, loses its flagella, splits up into two parts, then into four, and eventually forms a multiplicity of embryos similar to itself, by the breaking up of its own body-protoplasm; finally, it bursts and sets these free. These free bodies may subsequently be seen to undergo inclusion in other large bodies of the same kind, which, however, are somewhat granular; the line of junction is lost, the united body bursts, and sets free a large quantity of the discharge or mentioned above.

149. In another class of the large ovoid cells we notice a segmentation into two parts, forming spherical bodies which detach themselves each one, subsequently undergoing a transformation, by combining with other bodies of the same, or of a different kind.

150. There is a fourth kind of large ovoid body, the lower half of which is highly granular (Plate V, 71[a]), which unites with a smaller smooth ovoid body (seen to the right of the granular cells), and subsequently changes to a globular form (Plate V, 72), after which it commences segmenting. It first divides into two and then into four, and finally filling out the sac, bursts the sac-wall and sets free the ovoid spores. It is curious to notice these embryos always uniting with embryos of the same kind (Plate V, 78).

151. One of the most extraordinary forms of conjugation and fusion met with is that in which two moderately large ovoid cells combine. As soon as by their long anterior flagella, they ascertain their adjacency to one another, the one begins to move in the direction of the other, and if they are running parallel, one of them advancing takes a half turn, and by a nice adjustment of its body brings its narrow or narrow end into contact with the fine end of the other (Plate V, 48). During this and the succeeding stage, the flagella are in active lateral vibration, and the two bodies fuse to form one large creature of oblong shape, which ultimately becomes round, loses its flagella, and finally splits up into a number of embryonic forms similar to the parent structures.

152. Another noteworthy fact in connection with the life-cycle of malarial parasites is the facility which, in the process of pairing, each form of the parasite is capable of finding a suitable partner for itself, as if some psychical, or instinctive influence, was in operation. This is probably one of the important functions of the flagella, and it is possible that the pair of flagella connected with a pair of vesicles in the anterior part of the major parasite have some influence in this direction.

153. At a certain stage in the life-cycle of the parasite there is a pairing off of the two kinds of medium-sized spherical cells, both varieties of which appear like red cells, and which, I believe, are parasitic forms occupying the red blood cells; whether this is really so or not regarding the *granular* cell, there can be no doubt but that a large number of the hyaline form of spherical cell are developed from the latter. By further magnification (to 3,000), and adequate fixing with a combination of osmic acid and tannic acid, to which a few drops of glacial acetic acid have been added, we may observe that these two forms of spherical body are always flagellated, and that most of them have been developed from special sporocysts. In fresh blood preparations we notice that after a few hours both varieties are quite transparent and look like cysts. The sporocysts producing the spherical hyaline cells are shown in (Plate I, fig. 2), this is the variety which the red cells produce, but in comparatively small numbers. The sporocyts producing the granular cell is shown in Plate VIII *d*. In this process a curious phenomenon may be observed. The general rule is for cells of a dissimilar kind to unite—the granular variety of spherical cell is included in the smooth one (Plate V, 54, 57, 62), and this takes place in the vast majority. Here and there, however, we note that two smooth cells conjugate, and after a momentary contact, one is absorbed completely within the other (Plate V, 52, 63). In the same way two cells of a granular character may combine to form one large cell, but this is even rarer than the union of two hyaline forms.

154. In a well-stained preparation it is possible to see the earlier stage of nuclear division in spore formation; thus, we may see the nucleus sub-divide into two, four, eight, and so on, in the centre of a large clear space, which space is ultimately filled with these bodies; this is partly shown in Plate I, fig. 3, *a*, *d*. Some of the sporocysts develop bodies which are already ovoid in shape, and which by a slight transformation become crescentic. In many instances two of the round spores in ordinary sporulation combine to form a syzygy. This is very common in the spores of crescents and of flagella.

155. When two embryos unite to form a sexually mature structure, we may observe a series of highly interesting phenomena ensue. As soon as the pair come into contact to form a syzygy, or to undergo engulfing, we at once notice that protoplasmic envelope enclosing both is thrown out. This envelope however affords only a temporary covering, for like all the other forms of sporozoa the parasite soon develops a cyst-wall for itself. In only one instance do these spores arise from a single embryo. That instance occurs as one of the stages in the remarkable phenomenon of parthenogenesis in relation to the latent vitality of the malarial parasite under unfavourable circumstances.

156. In almost all cases two separate bodies combine to form a pair or syzygy. This coalescence occurs either before the separate bodies enter, a red cell or other

cystic structure, or before they throw out a cell-covering of their own manifacture. But it may not take place till after they have entered. With the higher powers of the microscope, 3,000 and upwards, it will be found that the two differ from one another in some particulars—usually, in one being more transparent than the other, or in containing excessively small granules of pigment, whilst the other does not. Both are nucleated and nucleolated, but after their union, the nucleoli disappear, whilst the nuclei fuse. The united body goes on enlarging, assuming the most peculiar and diverse shapes, finally, going through an amœboid stage before presenting any actual divisions into spores. This division begins in the united nuclei, and is a true *karyokinesis*; and I would take this opportunity that in every instance, with one exception, where spores are produced, the process begins in the nucleus, all other parts of the cell protecting, nourishing, and in other ways subserving the wants of nucleus, which is the all important, essential and vital part of the cell. The exception referred to is that of partheno-genetic division of the body protoplasm in the absence of a nucleus (see paragraph 162). By the time this division commences, the cell-wall has formed, and with proper stair ing and close observation, we may discern both the wall and the divisions. In sum such as the endo-globular sporocyst of spherical spores of the quartan parasite the divisions are large but almost invisible and are only brought out by the use of suitable dyes; but in the case of the tertian, these divisions are better formed and of more definite shape. When forming in a red cell, between the limiting wall of the sporocyst (for such it is) and that of the red cell, we may often see that there is a distinct interval. When available, red cells are preferably chosen by spores, although there is often a strong tendency for the infested spores to amalgamate to form one large sporocyst, within which a vast brood may be developed.

157. As already stated, in every case of severe malarial infection, a large quantity of the normal hæmoglobin has been dissolved in the *liquor sanguinis* by the toxines formed by the parasite—the quartan parasite possessing this property of dissolving hæmoglobin much more than the tertian one.

158. All varieties of the parasite are capable of reproducing themselves in the *liquor sanguinis,* in doing which, they appropriate the dissolved hæmoglobin.

159. On slide preparations, long before all the red cells have been utilized, many of them are invaded by one embryonic form, which multiplies to produce from four to six medium-sized cystic-cells; these unite with the ciliated granular spherical cell (developed from either red cells or large sporocysts), the combined body forming a large sporulating structure, the ovoid flagellated spores of the latter being the most numerous body produced in the blood in malarial infection. These bodies *are seen in the freshly abstracted blood*, showing that they are present in the blood during its circulation in the blood vessels.

160. In most cases, if we keep the unstained blood alive for 10 or 12 hours, we find that all the red cells have disappeared, and that there are vast numbers of these cysts produced. These unite with granular ciliated spherical bodies, to produce large embryo-containing sporocysts.

161. In *marsh water* the green colouring matter of vegetable cells, takes the place of the hæmoglobin in the blood. It is a curious fact that the embryonic forms

appear to choose either the young cells or the old and partially decayed ones, in which to carry out the intermediate stage of their life-history.

162. The foregoing facts are often proved by the embryonic, and even the mature forms, of the parasite, which in the ordinary condition are quite transparent and colourless, occasionally becoming deeply pigmented in human blood. This is especially the case with the second generation produced in the *liquor sanguinis*, and in the absence of the red cells; and in water, we occasionally see the embryos possess a greenish hue—a corroborative proof of the importance of this observation. In blood where all the hæmoglobin has been appropriated, we often see the parasite crowd around the margin of the cover-slip, or around an air-bubble. The same also is the case in water, only more conspicuously so, from the beginning, which may be interpreted as showing the äerobic nature of this parasite.

163. Hitherto, we have considered that the process of spore-formation went on in the red cells only, and that in them it was always direct; that is, without the occurrence of conjugation, production of syzygies, or engulfing, of any form. In no case, however, with one exception, have we met with direct division. This exception is the one referred to in connection with the parthenogenetic process, one phase of which is represented by a large spherical structure breaking up into a number of elongated bodies with rounded ends; there being no nucleus visible in either parental cell, or the brood of embryos. In this case the whole of the body protoplasm is split up into from 6 to 12 divisions, not even the remnants of the cell wall being left.

164. As the sporocystic bodies form the source of the different kinds of spores or embryonic structures found in malarial blood and malarial water, it may perhaps be well to commence by reciting one's experience regarding the nature of these structures. While the descriptions are taken chiefly from the preparations of fresh blood, I might state that the illustrations are from nature, and well represent what we see.

165. We should note that their extreme transparency in the fresh blood renders investigation difficult, and progress in substantial knowledge in regard to their relations, origin and destination, is extremely slow. In the series of researches under consideration, this tardy progress was emphasised from the circumstance that in no single instance was the result of staining exclusively relied on; and although numerous points were suggested by stained preparations, not one was accepted or theorised upon, until verified by observation in fresh preparations—some of these observations were spread over several months, whilst some still remain unsettled.

166. There was much in the results of my previous observations which it was found impossible to adjust with other facts until a few years ago. This was specially the case in regard to the very small moving forms that appear in the field without any seeming origin, developing in places where absolutely nothing was to be seen previously; and numerous observations rendered it impossible to ascertain the manner in which certain spore forms infected the large structures. The different varieties of spores enumerated, and their manner of development has been given under the heading of each form of sporocyst in which they are generated. We might here, make a few general remarks in regard to spores.

167. In many of the red blood cells true sporulation appears to occur, but it appears to do so in a somewhat irregular manner. In red blood cells infected by small, oval, or spherical spore bodies, the infecting spore develops into an irregular hyaline mass; but only after fusing with another spore already in the red cell, although sometimes this union occurs before the two spores have entered the red cell. Eventually we observe two bright granules, and finally from four to six of these arranged around a central body. Shortly afterward we observe that they are surrounded by soft protoplasm and that the whole mass has well-defined limits. At this stage they are stationary and gradually enlarge, the nucleolus seems to disappear until eventually they fill the whole cell with spherical bodies. As the process of sporulation continues, the whole cell increases in size, becoming more and more regular and uniform in outline; it likewise becomes much paler and more transparent; towards the close of the process of sporulation all indications of the original red cell have entirely disappeared, and in its place are left the now well-defined spores, granular debris irregularly distributed particles of pigment, and here and there the dim outline of the red blood cells, and in some cases, there may still be small irregular bits of hæmoglobin left.

168. That the corpuscular pigment, as well as the homoglobin dissolved in the plasma, helps in the formation of the cystic envelope, and is utilised in the nutrition of the embroyos, is shown by the fact that in many cases the spores produced within the sporocysts, whether they be spherical falciform, flagellate or crescentic, preserve the same colour as the encysting envelope. Thus, when the whole of the envelope is of a dark brown colour, the limiting wall of the hæmatozoa has the same colour, and retains the colour until such time as its pigment is utilised in the nutrition and growth of the body.

169. As a rule, however, the shade of colour of the embroyos produced is a little lighter than that of the walls of the sporocyst. The embryos enclosed and set free from this large sac, have their outline perfectly defined by the colouring matter just as if they had been artifically stained by some pigment.

170. Indeed, it was in this way that I was able to trace the living embryonic spherical spores, crescentic, spindle, and falciform spores, into red cells, and to watch the earlier period of their development. I was further able to watch the development of those small bodies into adults, while free in the *liquor sanguinis.*

171. Another method in which masses of round spores are produced is that of partial fusion or union of the edges of infected red cells. In the case of the quartan parasite, the spherical or oval spores, when contained in sprocysts, are arranged irregularly in large groups, but whereas when this form of sporulation occurs in a red cells, the spores occur in a single layer of eight; whereas in the tertain parasite these spores occur in a group of from twelve to fifteen.

172. In a large number of these sporocysts we can observe that almost every variety of spore is developed, that embryonic amœboid bodies, flagella, cysts, and even small spherical cells, are placed side by side, and that they do not appear to interfere in any way with each other's development at this stage, although such impartiality is absent at a later period. These large sporocysts are formed by the amalgamation or fusion of several red cells which have been infected individually by several embryonic forms of the parasite.

173. Whilst the distinction between *disintegration* of and *sporulation* in red blood cells is easily made, it is necessary that we should know how to make it. In *sporulation* we have a vital process, which is but little affected by accidental circumstances, and in which under proper conditions, we can follow the process of development of the spores, nothing remaining of the original red cell, but the pigment residue; the spores stain light blue with Loeffler's alkaline solution; *disintegration* is a mechanical process due to one of several causes, but we never have any indications of vitality, no developmental process, no movement or enlargement of the individual particles, whilst they stain rose-pink with Loeffler's solution.

174. It is likewise necessary to distinguish crenated red cells from those in which spores have formed, as well as from the medium-sized granular spherical bodies produced in certain sporocysts, and the enlarged cyst-like spores arising from either the red cells, or from one large variety of sporocyst. That this is not always an easy matter is seen from the fact that such cells as these last have hitherto not been recognised at all. The distinction sometimes needs great attention to detail, but by closely watching the successive changes, and the effects of stains these difficulties should disappear.

175. It is necessary also to distinguish disintegrating red cells from spores, especially when the particles of the former break up into several spherical or globular fragments. On the fresh spore we note that in the spores rapidly acquire from three to nine small flagella which enable it to move about, that it rapidly increases in size, and that it stains blue.

176. There is great disparity in size in the engulfing bodies. This diversity in size is chiefly witnessed in the ovoid forms. It is noteworthy that, as a rule, both bodies are egg-shaped or at least, much longer than broad, with one narrow end in almost all instances. Occasionally, by a sort of accident, a *spherical unites* with an ovoid form.

177. It is a curious circumstance that in most cases, except those of irregular malarial infection, we note that after the blood is abstracted, the same stage of development is occurring simultaneously in all parts of the slide. This, however, is not always the case, and it seems to be in some way influenced by the temperature of the preparation at the time. If one half of the slide under observation is kept more or less cool by placing a small piece of ice on the upper surface of that half of the slide (as far as possible from the cover-slip), the different forms present, *after a time* cease to develop, but only temporarily, for if the temperature be again raised, assuming that we have rendered evaporation impossible by an impermeable ring of some varnish, the parasite resumes its developmental activities. If, however, we at the same time, keep the other half warm, we find the parasite very active in the warmed part.

178. In every instance the more developed the parasite, the less distinct and the more transparent it is; and this goes on till it eventually disappears from the field of view or can only be seen with difficulty. There is one exception to this, and it is chiefly met with in the quartan form.

179. The fact that in the majority of cases, only a few parasites are met with in any one field at the beginning, is due to the larger number of red cells; but this

is not always the case, for I have examined at least seven cases of chronic malarial infection in which all the corpuscles were infected, and in which the whole field presented an appearence which would lead an observer to deny its being human blood—the vast number of parasites present, giving the aspect of what may be termed "universal swarming."

180. In the seven cases alluded to, in addition to every red blood cell being infected, there were crowds of various forms of the parasite, at the moment of abstraction of the blood. But in every simple case of serious malarial infection, if we keep the blood warm, prevent coagulation, and keep the parasite alive, we will find within six hours, that whilst the red blood cell are rapidly decreasing in number, the parasitic forms are very rapidly increasing, and that in ten or twelve hours, there is not a single red blood cell to be found, all having been invaded by one or other form of the parasite and undergone absorption. Indeed, I have examined at least twenty cases in which even at the end of six hours all signs of red cells had disappeared, whilst the whole preparation quivered with life, and every field presented myriads of spore forms, which being produced vastly in excess of the cystic bodies, were unable to infect anything, and very soon brought about their own dissolution by their very numbers. I have repeatedly transferred the different embryonic forms from these preparations *en masse*, and with them inoculated preparations of healthy blood and kept both living on a warm stage, with the result that healthy red blood cells were speedily infected, especially in the quotidian species. The decrease of red cells above mentioned is of course, easily accounted for, when we consider the rapidity with which the spores are created whilst there is no such increase in red cells.

181. The data accumulated tend towards the conculsion, that an attack of ague occurs simultaneously with the development of a large number of embryonic forms, and on the other hand, that defervescence of the fever is connected with the disappearance of these spore bodies; the real cause of reduction of temperature probably being the cessation of the manufacture of further quantities of the pyrogenetic toxine, and the exhaustion of the effects of that already produced, or its elumination, destruction or disentegration in some hitherto unknown way.

182. It is in cases of chronic malarial infection, associated with re-current ague of the irregular form, that we get the most marked modifications in the blood. Thus, during an accession of fever in such cases, we may get every red cell in the body infected by one or other form of the parasite. Indeed, it is no unusual circumstance to find sporulation of both varieties of small spherical cells going on in the red blood cells, together with the development of sporocysts of the large kind, and the formation of medium and large-sized spherical bodies with flagella, and lastly, flagellate forms of different kinds in the blood without any pyrexial symptoms, the possessor of these being to all appearance in fairly good health.

183. I have in the Staff Hospital of His Highness the Nizam's Regular Troops the present time, two cases presenting these phenomena in a remarkable degree; in neither is there fever, but all the blood cells are diseased, and the *liquor sanguinis* teems with life, no matter what hour of the day or night we examine the blood.

184. It is necessary to state that in rare cases of even severe forms of malarial infection, we may not get any parasite forms on the first or second examination, a fact which may throw us off our guard and lead us to consider the case to be one of

some other disease. When we consider, however, that malarial infection in many parts of India is universal, amongst the inhabitants, that it complicates most morbid condition, we find that the value of this organism as a diagrostic agent is reduced. I shall have to revert to this subject later on.

185. There can be no doubt in regard to the fact that in ordinary simple cases of ague, a period of exacerbation corresponds with the occurrence of sporulation. Indeed, any one who has abstracted a drop of blood in a well-marked case of ague during the hour preceding the attack, or during the cold stage itself, or when there is no cold stage at the time of accession of the pyrexial phenomena, must be convinced that the extraordinary changes which he then observes in the blood cells, could not take place throughout the whole human economy without some very remarkable pathological changes arising. Indeed, it is often most phenomenal that such little re-action is produced in the system with these remarkable changes. But more curious still is the fact that cases are met with locally, in which all the blood changes occur without any real fever, the person harbouring these parasite complaining only of those peculiar general sensations that are so well known to precede an attack of ague.

186. In the human being the malarial parasite causes a form of modified putrefaction in the blood, in which it effects a true necrobiosis of the red blood cells; that is to say, the brunt of the effects of the parasite is borne by the red blood cells, the hæmoglobin of which it appropriates—that the parasite is äerobic, many of the facts herein recited tend to prove. Apart from these latter facts, the toxines when in sufficient quantity are capable of dissolving out the hæmoglobin from the red blood cells—a fact shown in every slide preparation of fresh malarial blood if the parasite has been kept alive for a few hours. I have found large quantities of hæmoglobin in the urine during the pyrexial stage of malarial fevers, an in chronic malarial infection without pyrexia, there is always a moderate quantity of hæmoglobin dissolved in the urine, from which I have precipitated it. The details of the observations on this subject were recorded in a series articles which appeared in the *Indian Lancet* in 1894 under the heading of *Malarial Melasma*, a disease which I believe occurs in malarial infection. In conducting the series of observation by which I was enabled to prove this I employed one of Zeis's Micro-spectroscopes, and I would take this of opportunity of recommending it to any one desirous of verifying the facts just referred to.

187. The term *sporulation* has hitherto been limited to the form of spore production which occurs in the red cells only, and it has been described as a process of direct division of the plasmodium contained in the red cells. I believe it is possible to show, that both statements are not in accordance with what may be observed in fresh preparations. The red cell, although a favourable position for the embryo to develop in, affords only a temporary abode; like all Sporozoa, each polymorphic form of both species of the malarial parasite, is able to generate a cyst-wall for itself as one of the phenomena of its development, and therefore the form of the parasite under description, soon develops a cyst-wall for itself. Further, in no instance do these spores arise from a single embryo. In all cases a pair have united to form a syzygy, either before entering the red blood cell, or they have entered separately and united subsequently, and with the higher powers of the microscope, it will be found that the uniting couple differ from one another in some particular; usually in one being more transparent than the other, or less frequently, in one containing

excessively small granules of pigment, whilst the other does not, or in one staining well, and the remaining clear, or in other particulars. Both are nucleated and nucleolated, but after union, the nucleoli disappear. This united body goes on enlarging, forming the most peculiar and diverse shaped structure, but finally going through an amœboid stage before presenting any actual division into spores. By the time this division commences, the cell-wall has formed, and with proper staining and close observation under the higher powers, we may discern both the wall and the divisions. In the case of the quartan parasite the divisions are large and for the most part invisible, but in the case of the tertian, these divisions are better formed and of more defective shape. Between the limiting wall of the sporocyst (for such it is), and that of the red cell, we may often see that there is a distinct interval.

188. The 'spore' is the true infecting medium, and is to be found in the water, air, and soil of 'malarial' districts, and it is peculiar in being one of the few forms of the parasite met in malarial air. We have frequently given utterance to this view, and further observation leads us to confirm our opinion in this regard.

189. It may here be noted that the individual parasites of the same generation do not form spores at the same moment, but one after another, at short intervals.

190. Another remarkable phenomenon is that on the slide and in fresh preparations of blood, the developmental processes in each succeeding generation become easier to recognise. This fact is explained by the larger quantity of pigment contained in the plasma in consequence of the solution of the hæmoglobin and the partial evaporation of the fluid portion of the blood. In this way one has observed the development of this body upon single slides until by their very numbers they had crushed one another out of existence. Further, as development advances in the human serum outside the body, and on the slide, each generation is smaller than the other, as if the quantity of nutritive material were insufficient to produce the large and robust bodies like their predecessors.

191. The parasite is endowed with latent vitality. I have proved this by simply allowing a small quantity of warm distilled water to irrigate old slide preparations of dried blood. Within a minute of doing so the organism which had been dried for months were resuscitated. In the case of lungs even the spores would have a very good chance of sustaining and getting into blood vessels. We have here, therefore, at once a perfect interpretation of the danger of using from dried of swamps, marshes, or other places in which organic matter had been retained for any length of time.

192. For the acquisition of malarial infection in the case of *air*, all that is necessary is that the dried spores or sporocyst of the different varieties of parasite should be inhaled ; for these reaching the moist surfaces of the mucous membrane of the respiratory passages, once more light into activity, the delicate capsule bursts, and the embryos find their way to the blood cells. In the penultimate section I have shown that the spores and spherical forms of malarial parasite, are to be found in the air over drying marshes and dried up irrigation chanels. With these facts before us, the process by which infection occurs in the human being, is one of great simplicity, and it indicates the truth of the statements that have already been made in regard to the infectivity with air and water in malarial places.

193. In the section on THE RELATION OF THE MALARIAL PARASITE TO MARSH WATER, it is shown that there are several parasitic forms similar to those of human blood to be found in malarial water.

194. In the case of water all that is necessary is that the sporulating cystic structures, or even the spores of the various forms of embryos, should be drunk; if the sporulating cyst are consumed the delicate envelope is dissolved by the gastric juice.

195. It is a remarkable fact that in every form of this polymorphic parasite a nucleus is present, and in most parts, a nucleolus exists, at one time or another. Indeed, it would seem that this form is the most essential part of the organism, and it is probable that it has a vital influence on the selective process in pairing. It seems also to play an important part in the chemical composition of the protoplasm, and its integrity of form; in short, it seems to be the essential and most indispensable constituent in the carrying out the functions of re-production and assimilation.

196. Here, as in other instances, the nucleus of each of these spores stains more deeply than the surrounding protoplasm, from their affinity for aniline dyes.

197. During the intra-corpuscular life of the parasite the red blood-corpuscles change in size, shape, and colour. In most instances they lose their colour, but this is chiefly the case during the development of those bodies which produce pigment in their growth. Sometimes the corpuscle is distorted, often it enlarges to twice or three times its normal superficies. In tertian ague the infected blood cells are frequently two or three times the natural size, but in quartan they may be even larger.

198. When infected red cells form rouleaux, the process of development appears to go on uninterruptedly within the cell. The length of time occupied in the development of the different forms varies very considerably; some completing the whole cycle of development of the main parasitic forms present, in from 18 to 24 hours; others occupying two or three days. The different forms of the parasite may be seen at different stages, and it is rare to meet with the same stage in the developmental process in two cases except that of chronic malarial infection, and in which crescents are chiefly forming.

199. The small spherical spores of the tertian parasite developed in red cells are almost always arranged in a double circle, concentrically around a small mass of pigment, and the cells are not only smaller, but they are more numerous, and occupy a smaller part of the red cells than do the quartan.

200. *The worker in this field cannot err in regard to spores if he adopts the rule that the spores of malarial parasites are only to be recognised as such when a nucleus and a nucleolus, with a protoplasmic envelope, is present.* Products of segmentation which do not possess this structure, are therefore *not spores, but residual bodies;* they are often to be observed under various conditions (in fever, owing to quinine, spontaneous cure), and in consequence of non-attention to the details of structure, they have often been erroneously taken to be spores.

201. We may look upon those red cells in which spherical spores are generated, as one form of ovum, within which one phase of this Sporozöon's life-cycle is carried out. In the circulating blood, of course, there are fresh uninfected red cells ready to hand at every moment, so that their infection is progressive and uninterrupted, so long as there are spores generated in sufficient numbers.

202. Many of the small spherical spores develop in the red blood cells, and as a rule, do so in the regular way described so well by various writers. They are so well known as to need no further description here. But from my observations it is necessary to state, that the selection of the red blood cell as a sort of temporary host is only the accident of position, from its being the most suitable and available cystic structure in which this form of sporulation could take place. Outside the human body where there are no red corpuscles, the malarial organism has either to form a cell-wall for itself or to develop in vegetable cells.

203. In a well-marked case of malarial infection we see every stage of the development the moment the blood is abstracted. Besides this, we note that there are myriads of minute moving bodies which require the closest attention to detect. But once they have been seen if they are kept in view, we note their rapid increase in size.

Crescents.

204. There is no form of the malarial parasite which has given rise to so much controversy as the crescent. This is probably the natural outcome of its complexity, the important part it plays in the life-history of the parasite, the different phases through which it passes, and the great variation in shape which it assumes under different conditions. Personally, I believe it to be by far the most difficult form of the *plasmodium malariæ* to investigate, and although one has arrived at certain conclusions after nine years' work in an area in which all forms of the malarial parasite are both endemically and epidemically met with, it is found impossible to write about the crescent with the same confidence that one does in dealing with some of the other polymorphic forms of this sporozöon. In making the following remarks, regarding the malarial crescent, one does so under the apprehension that it will be necessary to make some modifications at some future time. Hitherto, writers have described the crescent as a body with very definite morphological characters, and as one that is specially connected with the *quartan* species of the parasite and the more chronic forms of malarial fever. Only a few writers have considered it to be an encysted structure, most writers describe it as a product of degeneration of either other forms of the parasite, or even of normal histological elements, few have believed it to be connected with any form of sporulation, and only one authority has made any reference to the existence of its flagella. Some have thought it to be a degenerate condition of the white blood corpuscle, and have entered upon more or less complicated theories in explanation of the mechanism by which such a change of shape should occur in a circulating body. In short, it may be stated that almost every worker in connection with the malarial parasite has given his own interpretation as to the nature of the crescent; a few agree in regard to some of the main facts, none do so in connection with all the details here advanced.

205. Every form of the crescent is nucleated, and the nucleus can sometimes be brought out by suitable stains—the best seems to be a film of osmic acid vapour succeeded by a short immersion in Lœffler's alkaline solution.

206. In many cases the first stage in the development of the crescent is that of corpuscular infection. This is seen in two tiny particles of protoplasm appearing in a red cell, which are really two small spores. At first separate with separate nuclei and nucleoli, and in two distinct protoplasmic envelopes, they fuse to become one body in which both nucleoli disappear, and the nuclei blend, both bodies being now enclosed in a common envelope, around which a well-defined cell-wall is formed; it now forms a fully developed spherical body which being set free from the red cell, soon changes its shape, becoming oblong and eventually crescentic.

207. On its way to develop into a crescent it has to pass through a variety of forms, and on reaching any one of these forms no further change may take place, hemispherical, oblong, ovoid, trichomonad-like, falciform, etc, but the crescent so-called having attained a certain shape, retains it.

208. In a disintegrating sporocystic ova of the crescentic kind, it may be observed that while one form of embryonic crescentic spore is almost invariably produced, there are, as a rule, one or more other crescentic spore forms present, some being almost straight, others irregularly triangular, some ovoid, and other pear-shaped or even oblong, these forms being to some extent due to the pressure to which in their growth they were subjected. This is not always so, as I have seen the same varieties in water, under circumstances where the pressure was not excessive, and I have traced one of the semi-crescentic forms through red blood cells to its development into a flagellate form of the long and narrow variety, and similarly I have traced another kind till it formed one of the medium-sized flagellate bodies.

209. We occasionally meet with a small body which seems to have a limiting membrane and looking like a double crescent, united back to back and moving about in a gyratory manner. This is merely an encysted flagella of the crescentic form undergoing changes, and producing embryonic falciform bodies. In by far the largest majority of cases, such altered forms seem to be spherical bodies of the crescent series in an early stage of karyokinesis.

210. Many of the crescents are sickle-shaped bodies some of which are within and others *outside* the red corpuscles. In the red cell they become semilunar in shape, but more pointed at one extremity; if we fix our attention, we see that it recedes and advances in an intermittent manner. At the same time we observe that the whole body of the red blood cell is suddenly moved in an egg-like case, as if something within were endeavouring at that point to make its exit. If we look at the body of the red blood cell, we will see that it also undergoes an intermittent agitation. Eventually we note that a corresponding change occurs at the other end, and now we find that in some instances the contained parasite throws out a flagellum from either end, which begin to lash about and shortly after its appearance the contained form is set free. There is still, however, a double contour in the corporeal part, showing that it has not as yet left the red blood cell.

211. The enumeration which follows shows the many forms the crescent may assume, and the extent to which it may remain single, or unite with another similar body to form a syzygy; in the latter case the parasite rapidly acquires a common cyst-wall; fusion of the two forms takes place, the result being the produc-

tion of a large spherical body within which spores are developed. It is, however, by no means uncommon for two red cells containing separate crescents to join together, in which case the fusion alluded to occurs within the united red cells, the embryos resulting being eventually set free by the disintegration of the envelopes within which they are formed, at times the latter may even reach a certain size before being set free. In stained preparation we are occasionally able to trace the different stages of these processes.

212. There are other sources of crescent-like bodies which remain to be considered—one is from large, oval, pyriform, or falciform bodies, which develop within the meshes of the sporocysts. In this case they soon go on developing to form crescents or demilunes, and then sometimes to elongated flagellate bodies which are set free and finally doubling inwards undergo sporulation. Another is from the large spherical cells with long flagella. It may also arise from the transverse divisions into which the flagella of different forms occasionally develop. In this case one part seems a mere concavo-convex double-contoured line. In the centre of the concave part a small mass appears, which goes on developing to fill up the interval between the ends of the crescent. In some cases crescentic bodies of excessively small size develop in certain parts of the field, from what was before an invisible body; in the same way as the large ovoid cells are developed from the ooze or discharge of certain large sporocysts.

213. The great activity of the crescent shaped-bodies during one stage of their existence, then reproductive vigour, the "swarming" which may sometime be seen in the contained pigment particles, its active developmental changes, union with other bodies, the vital process of sporulation, all of which have been seen as positive events in the life-history of this phase of the parasite, are quite opposed to the theory of a degenerative metamorphosis of either the crescent or any other form of the malarial parasite or of the normal constituents of the blood. We at times observe that the crescent about this stage may become spindle-shaped, or even pass on to form a more or less cylindrical flagellate body of moderate size.

214. Up to this stage all forms are encysted, have a separate and independent cell-wall, within which the parasite moves about freely, until the stage of rest.

215. As soon as the crescent is set free, and sometimes even before that, the flagellum, or flagella, which it had acquired on the surface during the amœboid stage, are gradually lost, become more or less undefined or actually disappear; the body itself ultimately acquiring a smooth and uniformly spherical outline.

216. At this stage one of two further changes may occur—

(*a*) It may, and most frequently does, unite with another body of the same kind, but as a rule such fusion occurs before it reaches the spherical shape. and the pair develop into a large sporocyst in which a number of small curved or oval, or falciform spores develop and are set free.

(*b*) It may divide into two by simple division, one of these two uniting with another body of much the same kind, but in reality different, the two fusing and after varying period developing embryos. Sometimes, perhaps in most instances this division into two occurs after the crescent has developed into a spherical body.

(*c*) It may split up into a number of embryos, on each of which a flagellum from one or both either ends are developed, which enables them to move about with moderate freedom.

217. During the active stage of its existence the crescent seldom remains more than a few hours as such. It then seems to develop into an amœboid body, and wander about for a time, but during these peregrinations it is gradually changing its shape to that of a spherical body. The irregularities are formed by the splitting up of the protoplasm itself into a number of separate individuals. Two may unite end to end whilst they are crescents become fused, and then divide into these spore bodies. Very often there is seemingly no such union of two separate individuals at this stage, and yet the one body proved to proceed a brood of embryos. In this case close observation will usually show that this body was a syzygy in which the point of union was badly defined. These syzygies sometimes form very early.

218. Although, the spherical body developed within the red cell, is the most conspicuous origin of the crescent, and the one from which the development of the crescent can be most directly traced, it may be remarked that, with two or possibly three exceptions, in every phase of its parasitic life, the malarial organism passes through what may be termed an imitation of the crescentic form, which for purposes of reference, we may describe as the *true crescent*.

219. We can trace the true crescent in sporocysts, several forms of which produce several kinds of spores as already described :—

(*a*) Small spherical or oval spores, from red cells (Plate IX, *l*.).

(*b*) Medium-sized spherical bodies (Plate II, fig. V, *b*.).

(*c*) Small spherical cystic bodies (Plate I, fig. II, *b*.).

(*d*) Oval, oblong or pear-shaped uniflagellate spores (Plate I, fig. III, *c*.).

(*e*) Crescentic spores—

(i) ordinary (Plate IX, fig. *h*.);

(ii) flagellate (Plate IX, fig.);

(*f*) Large oval cystic bodies (Plate VII, fig. *c*, III.).

(*g*) Large, irregular, and broad bodies, producing medium-sized spherical, ciliated, cystic bodies (Plate I, fig. II, *a*.).

(*h*) Small spherical spores from the breaking up of crescentic syzygies (Plate IX, fig. *e*.).

(*i*) Oval or kidney-shaped bodies from united crescents (Plate IX, fig. *g*.).

(*j*) Nucleated and pigmented small spherical and irregular or angular spores, from the large amœboid granular "swarming" cells (Plate VIII, fig. *a.*).

(*k*) Direct fusion of certain spherical cells into oval or oblong bodies, which look like red cells from which pigment had been removed producing falciform spores (Plate III, 57.).

220. It might be thought that it is an unnecessary refinement to include such a large number of crescentic forms in the enumeration. But I would justify this enumeration by stating, that it seems from the literature of the subject, to be really these differences in shape, size, and grouping, that has hitherto led to such confusions, and given origin to such wide range of opinion as regards their nature. The different forms of crescent met with at different times are represented in Plate IX. They were drawn from the last 42 cases that came under observation.

221. There are at least two forms of what appears to be *simple division* in the crescent, but both are extremely seldom met with. One is a division of the whole body protoplasm of the crescent, whilst it is in the crescentic form, into, from four to six parts, by transverse lines running at right angles to its long axis, the individual parts forming spherical bodies without any visible nucleus or nucleous. The other form, which is a process of simple gemmation, occurs when the organism is first becoming spherical, the small spherical bodies which occasionally surround the inner half of the circle, being cast off as separate bodies in which a nucleus is not visible. In this case the small spherical seem to unite with other small spherules, and form syzygies producing oval or falciform bodies.

222. The former of these two, has been seen only five times out of many thousands of slides, the latter only twice in the same number; both forms are extremely rare, and apparently not an indispensable phase in the developmental processes. In this, we have another instance of what appears to be a process of parthenogenesis one of the most interesting phenomena in the life-history of this parasite. It will thus strike the reader, that in some at least of the developmental phases of this parasite, the process of biogenesis is occasionally represented. The great importance of this phenomenon rests in fact that it imparts a latent vitality to the malarial virus, a circumstance which has been referred to already, and to which we shall revert in our concluding remarks.

Spherical Bodies.

223. There are several forms of spherical bodies, some merely temporary and passing through a phase of development, such as the spherical body of the crescent series; others are permanent, such as the medium-sized hyaline and flagellated as well as the granular forms mentioned as uniting by engulfing. The former variety we need not deal with here, beyond stating that it is sometimes almost impossible to ascertain by a single observation of the blood, whether a spherical body met with is one of the temporary or permanent class—the only means of deciding being that

of using suitable stains, or watching the fresh blood. The following list embraces the more important of the—

SPHERICAL BODIES :—

1 Ciliated spherical body of medium-size (Plate II, fig. V, *e.*).

2 The granular spherical body (Plate II, fig. V, *e.*).

3 The homogeneous, provided with flagella (Plate II, fig. *c.*).

4 The large irregular spherical body from which are developed the spherical cysts (Plate I, fig. II.).

5 The small spherical spore from the red cells (Plate I, fig. VI, *b.* and Plate IV, fig. IV, *d.*).

6 Large spherical bodies resulting from the union, engulfing or conjugation of two of the large ovoid bodies (Plate VII, fig. 7) ultimately give rise to—

(*a*) the spherical, oval, oblong, or bean-shaped, flagellated spores, which are met with in such swarms in the blood during the first few hours of, and for several hours preceding an attack of ague (Plate VII, fig. *h.*);

(*b*) spores similar to the above, but more uniformly bean-shaped or ovoid, and resulting from the direct splitting up of the protoplasm of the large globular cell (Plate VI, *c*, fig.); or

(*c*) giving rise to a peculiar liquid protoplasm in which subsequently round ovoid, ciliated bodies develop (Plate VI, figs. 8, *a*, *c.*).

7 Intracorpuscular spherical cells, which on being set free develop into one of three structures—

(*a*) crescent,

(*b*) flagellated bodies of medium-size ;

(*c*) small flagellated spores (*vide* several figs. Plate IX.).

224. The foregoing enumeration of the *spherical bodies*, suffices to indicate the marked difference between all previous opinions expressed regarding the nature of this body, and those propounded in the present series of observations.

225. With regard to the first variety named above it may be remarked that the medium sized cell enlarges further, spots appear within it, which are later on seen to be part of a network, and still later the long flagellated processes, may be seen coiled up within. In others the broad and short forms a circumferen-

tial boundary. Its disintegration begins by the occurrence of little fissures in the circumferential flagella, which increased in number and breadth until the whole of the circumference is broken up into a number of small bright bodies. It is then found that a disintegration of the rest rapidly takes place, and when the surface covering is removed, it is noted that the deeper structures consist of small ovoid crescentic, oblong, irregularly quadrilateral, and minute rod-shaped flagellated structures, pigmented at both ends.

226. Ciliated spherical bodies in some instances change to crescentic bodies, but a very large number go on developing and reach a considerable size, retaining their rounded form for a time, as well as their granular condition, they then throw out flagella of considerable size in various directions, and in their interior are seen a number of nuclear structures; they alter their shape very considerably and their flagella are exceedingly active.

227. The cilated spherical bodies undoubtedly join hyaline and seemingly non-ciliated cells make their exit singly from the red blood cells, They are seen developing from active amœboid bodies in the red blood cells, and are subsequently set free, or set themselves free by their own activity. Whilst in the red blood cells, and for a short time afterwards, it appears to have only two cilia, and to be quite homogeneous, but it has the capacity to change its shape, its movements and growth are rapid.

Large "Swarming" and Irregular Bodies.

228. Hitherto the descriptions given seem to indicate that there is only one form of spherical body or one only form is described under this appellation. It is likewise stated that this body invariably develops in red cells. We may, however, find them arise from at least two sources, red cells, and large sporocysts, and present the varieties referred to in the above enumeration.

229. This large "swarming" structure has given great difficulty in the elucidation of its origin, growth, development and its general and specific relations. It is far more frequently met with in the tertian than the quartan species, but even in tertian it varies very considerably in number, in some cases there being only a few on each slide, whilst in others there may be as many as from 5 to 15 per cent. of the red cells affected.

230. The earliest stage of development is in the infection of the red blood cells, appearing as an amœboid body; ultimately the pigmented protoplasmic envelope having burst, the contained organism is set free. For the second time it now begins a sort of amœboid action, but it is soon provided with flagella which are sometimes both long and wide; it then passes on to develop a globular structure which is seen to contain from six to eight large spherical spore-like bodies, which are set free, and within which other forms of the organism are developed or unite with another spherical body to produce a large sporocyst VIII, *a*, *e*.

231. Sometimes they contain cystic bodies which may form a syzygy contained in a common envelope, at others they seem to contain flagellate bodies. It has always got a separate envelope of its own, and its movements are active. It is a curious circumstance that this is one of the special forms that undergo development in the apyrexial period, the crescent being another. It has not hitherto been found possible to ascertain its exact position in regard to the different other

forms. Or they develop into large "swarming" cells of which several may be in one field. When in doubt as to the nature of these large cells, we may clear the way by resorting to one of the many isolating, or differentiating stains, but perhaps the following method is as good as any that can be adopted.

232. Dry the film of blood on the cover-slip in air; fix for half an hour in equal parts of alcohol and ether; stain for ten minutes in a solution of three ounces of saturated aqueous solution of methylene blue, adding constantly a few drops of alcohol, and then add 7½ grains of eosin previously dissolved in water. This cover-glass preparation is now washed in water, dried and mounted either dry, or in FARRANT's medium. The result with malarial blood is typical. The red blood cells are rose coloured, the plasmodia light blue, but the nuclei of the white cells are deep blue. The granules of macrophages are also stained pink, the special structure under consideration is stained sky blue.

233. Their movements are essentially amœboid and inconspicuous. Some are seen to force their way through the intervals between groups of corpuscles. They circumvent all red cells one after another, but curiously in most instances they not only avoid visiting the same cell twice, but when a cell had been circumvented by another body previously, it is subsequently not visited by other large cells. Their *natural shape* is spherical, but when not in contact with any corpuscle or other structure, they throw out long processes in various directions, usually only two, but sometimes as many as four.

234. They usually go round the red cells and then flow away from them, but sometimes they completely envelope them so that their granules seem to pass through the red blood cell. I believe the small spherical spores which are carried by these large bodies around the red cells infect the latter in their rapid movements, for they seem to leave spore-like bodies behind, which rapidly become amœboid.

535. As the activity of these large cells lessens the number of granules decrease considerably, and the cell appears to contain only a few of the larger forms, with various processes. These processes appear to be the frame-work or skeleton of this body which remains after the granules have left. When only a few granules are to be seen the whole body appears to be homogeneous. It sometimes at this stage, has the appearance of a large cell in the rosette stage of karyokinesis.

236. These large granular cells do not appear to take up the stray particles of pigment or foreign matter in the field, for these are left after the body passes them. Indeed, the only apparent reason for their wanderings is that of going round the red cells and infecting them. They push many of the red cells out of the way; but they can attenuate the pseudo-podia so as to insinuate them between corpuscles in contact with one another, separate them, the rest of the body flowing in the direction of the fine processes afterwards. It thus appears that the structure under consideration is merely a developmental stage of the malarial parasite, and that this stage is one which is common to the developmental processes of many other organisms which infest the blood, and results apparently from the inclusion of the spores which are undergoing development within the boundary of the cell membrane.

237. In every case if we abstract the blood at a special time, we can find the large cells with their spores already formed. The process of " bubbling " or

"boiling" is sometimes distinctly manifest in large irregular granular cells, whilst several of the so-called "granules," are seen to be setting themselves free and moving about in the field as small amœboid bodies or small spherical or oval spores.

238. We may trace definitely the manner in which some of the large spherical bodies develop into enormous granular plasmodia from the red cell, together with the way in which they infect the red cell during their peregrinations and finally undergo disintegration.

239. Sometime many of the red cells unite to form a large irregular body with undefined outline, this loses hæmoglobin as time goes on, and leaves this large irregular body as a mass of homogeneous matter, containing one or other form of the malarial parasite in the cell the spores of which may be "swarming" prior to making their exit. When these large granular cells are not advancing the "swarming" movements are well seen.

240. In some of these large bodies we find cystic structures. These cysts are often of large size, but mostly kidney-shaped or oval. Their centres are at first clear and hyaline, then we note that in the centre certain undefined bodies appear, which enlarge and multiply till they fill the cyst, and lie parallel to each other, but as growth and development goes on they coil round one another in a seemingly inextricable way, forming skeins; and when the large flagellum is formed, several of the fibrillæ are intertwined in a most complicated way. Whilst the parent grows the fibrillæ are seen to create a commotion in the body, and this accounts for the agitation seen in it throughout. Eventually the large cell bursts at one end, or the capsule disintegrates and sets free these complicated skeins of free flagella.

241. From the various bodies met with in different large "swarming" cells, I have been driven to conclude that they are different forms of sporocysts, which instead of remaining invisible as they mostly do, are more or less distinctly visible. I might in conclusion state that the bodies under consideration are quite different to the several forms of large multinucleated leucocytes and macrophages, which are also in many cases to be seen in the field simultaneously.

242. These large "swarming" structures exist during life in the blood stream; for one has found them in the blood the moment it is abstracted, as well as in preparations fixed with osmic acid solution at the time the blood was oozing from the finger, by abstracting the blood with a prick through the drop.

The Amœboid Bodies.

243. From an early date in the history of the malarial hæmatomonad, it became customary to speak of "an amœboid body," under which heading one of the polymorphic forms of this parasite has been described in every detailed account of malarial organisms. The term thus used seems to indicate that there is but one form of amœboid body, and by a large number of authorities it is considered to be entirely endo-or epi-corpuscular, that it is never met with free in the *liquor sanguinis*. With all due deference to the able workers who have dealt with this part of the life-history of the malarial parasite, I am of opinion that this term has been applied in too limited a sense, this restricted signification arising from the fact that the exact nature of the whole parasite has not according to the views herein propounded hitherto been recognised.

244. In the first place I would ask the reader to assume that the following statements regarding this special body are correct, and then put them to the test for himself. I ask this because these bodies are specially prone to be taken for white blood cells, and once that view is accepted, one may be unconsciously engendering an opinion in opposition to the description given, leading to what may become, an unconquerable prejudice.

245. I would also make a few propositions, which will form a basis of argument:—

1 Like all monads the malarial parasite goes through an amœboid stage of existence.

2 Every phase of its parasitic life and every variety of the parasite is at one period or another amœboid.

3. Whether we deal with the spherical body, the crescent, the small flagella, the flagellated small or medium spherical body, the large ovoid bodies before conjugation or the same after engulfing, the large globular bodies during the sporulating stage, etc., we find that they all go through an amœboid stage of existence. In some, this stage lasts for only a few minutes and may be entirely lost, but in others such as the large granular "swarming" cells may continue for several hours. This latter fact probably accounts from the difference of opinion in regard to the description as to the nature of the bodies. The amœboid stage in most, but not in all, precedes that of rest, and would seem to be essential to the maturation of the parasite, with subsequent sporulation.

246. In malarial fevers of all types, amœboid bodies are found occupying a certain number of the red blood cells, adhering to their margins, or resting on the surface; this is well seen in ordinary stained slide preparations; but they are much better seen in fresh blood fixed with osmic acid vapour, and subsequently stained in the moist state with a mixture of eosin and methylene blue. With Lœffler's solutions the amœboid bodies are stained of a light blue, the red are pink, and sometimes they are so faintly stained and are so transparent that we make out their presence by contrast with the deep blue coloured white corpuscle. In the fresh state they derive pigment from the hæmoglobin of the red cell, develop and increase in size at the expense of the red cell, the pigment being contained in their substance as distinct granules. I have not been able to find the "rods" of pigment described by some writers. They vary much in size, and some seem to become half or even two-thirds as large as the red blood cells. They are colourless and transparent. I have never been able to satisfy myself that those bodies undergo segmentation in the red corpuscles, although many form the irregular hyaline structures found in the red corpuscles, and some seem to develop into the small variety of simple spherical cell, which when set free, goes through a plasmodic stage, elongate, double up and form a spherical body, with a smooth surface, and finally break up into a number of small spherical spores.

247. The nucleated condition of small amœboid bodies is sometimes well shown especially in stained preparation. Many of the small amœboid bodies, when actively moving have the appearance of large micrococci or diplococci, but if we

watch one whilst we irrigate the field with a one per cent. solution of quinine, we shall see that they gradually become stationary, and are then seen to be one-sixth to one-eighth the size of a white blood cell.

248. Whilst the amœboid are among the most constant forms present in the blood in malarial fevers, they are chiefly present from a period several hours antecedent to the commencement of the attack, up to its acme. Yet in some cases they may be absent during one attack and during the next be found in hundreds. This occurred in about 18 per cent. of the cases. They may be entirely absent in one field, and numerous in the adjacent one. This was very common, but in all unequivocal cases of quotidian paroxysmal malarial fever, a daily search for three consecutive days never failed to bring them into view.

249. In many others it would seem that the embryonic flagellate is the infecting agent, it rapidly enlarges, producing a large amœboid-looking body from which division radiate to the circumference, these bodies produce large cells in multiplication. The stationary stage of the large spindle cells is preceded by an amœboid stage; but it is during the latter that the early rosette phases of karyokinesis goes on preparatory to sporulation during rest. The sporocystic sac is highly refractive and splits into irregular division setting free its embryonic structures.

250. On several occasions we have observed the small ovoid or falciform spores with a single flagellate at the narrow end, as well as amœboid bodies, and small ciliated spores, attacking red blood corpuscles. In some cases their movements cease when they had advanced about half their bodies through the wall of the corpuscle; in others they gain entire access; the process taking from 40 to 50 minutes to complete, during the whole of which time their movements appear to be moderately vigorous. At intervals of ten minutes, I tried the effects of pressure on the cover-slip, to expedite the entrance of the spore, but it neither reduced its activity nor helped its progress, showing that it was a vital rather than a mechanical action.

251. In every case we see that the small amœboid body is provided with a nucleus. Its movements are progressive and essentially amœboid, *i.e.*, it shoots out pseudopodia in every conceivable direction, the rapidity of its movements absolutely preclude the possibility of acquiring a knowledge of the details of its structure while it continues to move, but when it becomes quiescent it is seen as a small shapeless mass of protoplasm.

252. Many of them enter into a red cell before they can undergo any further changes, thus, the small oblong or ovoid bodies after entering the red cell become spindle bodies, whilst an amœboid body will probably develop into spherical bodies which disintegrate into amœboid bodies.

253. For a time after acquiring attachment to the surface of, or actually entering, the red blood cell, the small amœboid body continues to throw out pseudopodia, but it soon becomes stationary and slowly undergoes increase in size, some acquiring pigment granules, others not. Even when most active, however, there does not appear to be any special local change of position with the amœboid movement; it is chiefly to be recognised as a general alteration in shape; naturally, in the close quarters of the red blood corpuscle, there is not much room for change of place.

Flagella.

254. We might enumerate the different forms of Flagella under the following headings :—

Varieties of Flagella :—

A.—*Attached.*

(*i*) Ordinary spores (Plate VIII, *b.*).

(*ii*) Oval spores (Plate VIII, *c. iii*).

(*iii*) Spherical bodies (Plate II, fig. V, *b*, *c*, *e.*).

(*iv*) Crescents (Plate IX.).

(*v*) Sporocysts (Plate VI, and VII.)

B.—*Free.*

(*i*) Long and narrow (Plate VIII, *f.*).

(*ii*) Medium sized, encysted (Plate IX, *d.*).

(*iii*) Of small and medium-size naked probably crescents (Plate IX, *e.*).

Pseudo-Flagella :—

(*i*) From frame-work of sporocysts (Plate VIII, *g.*).

(*ii*) Condensed ring of red cells (Plate VIII, *h.*).

(*iii*) Circumferential part of various cystic and cellular bodies, which after setting free their contents, straighten out (Plate VIII, *i.*).

(*iv*) Stretched hæmoglobin and remains of red cells (Plate VIII, *k.*).

(*v*) Condensed thickened, and coagulated marginal part of large patches of plasma, which from manipulation, or even pressure on the cover-slip, chips off and remains isolated. If separated during staining they attract more pigment and thus are a shade or so deeper than the general background of the plasma, which may still further confuses the observer. Sometimes this margin instead of breaking, off folds over the edge of the hardened plasma, and again, seen in double fold, has a deeper hue ; in this way I have seen the most remarkable likenesses to flagellate and other forms produced. In passing, one might refer to similar chipping from the central parts of hardened fields of plasma, and as the edges of the openings thus made attract more pigment or interfere with its removal by washing, we have the appearance of a cyst with transparent fluid contents, with pigmented wall.

255. In some cases of tertian infection the formation of the flagellate body can be witnessed from start to finish in the red blood corpuscles, from which it is ultimately set free. Sometimes however it is developed in a homogeneous spherical cell body. From the high transparency of the liquid sarcode of the cyst, and the more opaque condition of the flagellum, the latter can be watched with considerable certainty throughout the intra-cystic stage of its development. Further, in a number of cases it is possible to see the embryonic flagellum entering one of the cellular structures, usually as an elongated spore, covered with a loose soft protoplasm envelope.

256. After having been set free it wanders about, and having united with another free flagellum possessing characters similar to itself, goes through a sporulating stage once more. The united flagella double inwards and become oval or globular in shape, and finally create numerous small spherical spores.

257. Occasionally we see elongated cylindrical bodies like large flagella, in the field. These cylindrical cells are sometimes of very great size but are never seen to move about. They soon split up into numerous small flagella. When I first described these bodies, it was stated that I had depicted and described cotton fibre as malarial parasites.

258. These develop from large cystic bodies of oval or oblong shape; the latter forming in the meshes of large spherical flagellated cell. The flagellum elongates the cyst-wall till it ruptures; but it is often coiled up within the cyst, sometimes taking three turns on itself.

259. Sometimes similar flagella but long and narrow in shape, are set free from the containing cell before the latter are set free from the large parental sporocysts, and when eventually the latter disintegrates, we find that a number of the flagella are endeavouring to get away from the sporocysts. This has been seen only twice but I have seen them move within the chlamydospore on several occasions, the whole presenting the movement of swarming flagella.

260. In two instances I have also seen the smaller variety of flagellum follow one another, eventually meet, join end to end, one becoming incorporated into the body of the other by the process of engulfing; finally a number of oval ciliated half-moon, or pyriform embryos, were produced which soon acquired the size and shape of the parasites.

261. There is no doubt that some, at least, of these flagella are formed from the invisible structure developed in the ooze. It may be at once seen that this variety of flagella has little to do with the crescentic body.

262. The long and narrow flagellum is seldom met with; I have only notes of it in nineteen cases (fourteen of them chronic malarial infection, the other five cases of quartan infection), but when found, it is a body of very definite structure, having a well-defined double contour, a whip-cord like fine end, and a broad end, it varies from $\frac{1}{1,200}$th to $\frac{1}{300}$th of an inch in length. Usually met with singly and developed in a cyst, it may be formed in groups in which the individual flagella are closely coiled round one another.

263. The minute pigmented spores (Plate VII, fig. *k.*), described by the Italian writers as connected with pernicious malarial fever, were only found twice, both

were cases of chronic malarial infection , and all possible means were adopted to discover a nucleolus without success, a nucleus however, may always be found; they occur in groups of from 12 to 36, and are readily seen when present.

264. A small *cylindrical* flagella (Plate II, fig. II, *c.*), was found in eleven cases, but there was no constancy about the nature of the cases in which they occurred except that six of the eleven were cases of chronic infection with large spleens. This body stains deep blue, with methylene blue and thus differs from the ordinary flagella.

265. It is the opinion of some, that we should be able to see the parasite at once on examination, but these are usually workers who have no familiarity with sporozoa, or who have been accustomed, work with stained bacterial organisms. I have no hesitation whatever in expressing the opinion that the subject of researches in connection with sporozoa in general, especially that of tracing out their life history is one of the most difficult in the whole range of original microscopical investigation.

266. Indeed, sporozoa, especially the varieties met with as hæmatozoa, are infinitely more difficulty to carry out, much less satisfactory in results, and require much more patient observation than do the investigations in regard to schizomycete. This is because the sporocysts particularly in their resting stages are quite transparent and invisible, are much more complicated in their developmental processes, they cannot be cultivated, inoculation is for the present at least infractions in results whilst the developmental processes are comparatively slow.

267. Sometimes in fresh preparations one has to spend a whole hour in examining the blood before we find a single parasite, and probably go over several slides, and yet one knows that they are present in practically every field but in most cases the practised eye seldom fails to detect them in almost every field.

268. A great deal has been written as to whether the smaller amœboid forms of the parasite gain access to the interior of the red blood cell, or merely remain on the surface. It is extremely difficult to decide this question from simple observations of fresh specimens; a statement borne out by the wide difference of opinion expressed on the subject. I must confess that my own opinion varied from time to time till eighteen months ago. I had subjected this point to a special series of observations, in one series I used fresh blood, in another moist preparations of blood held over osmic acid vapour till they had dried, and then examined them in the dried state, and lastly preparations stained with various solutions, with and without previous fixation with osmic acid vapour.

The Malarial Plasmodium in Water.

269. I have found most of the forms of the malarial parasite in the water from surface wells, stagnant ponds, marshes, tanks, etc., several of its forms in the silt forming the bed of tanks and irrigation channels, from surface well and stagnant ponds, marshes, tanks, etc. A few of its forms, especially spores, of several kinds were seen in the air over drying marshes and irrigation channels.

270. So far back as June, 1891, I wrote.—" Concurrently with the statements herein made, it would appear that the only condition incident to the production of malarial infection in the human being, is the introduction of the plasmodium into

the alimentary tract or the lungs, to multiply in the system, and after a time to multiply and give rise to one or other forms of ague." I had not up to the time the above was written (May, 1891), recognised the quality of species; indeed, on the contrary, I supported the unity theory.

271. At the same time in continuation of the above I wrote—"It is noteworthy in this connection, that I have found several of the spore-forms of the malarial parasite in the waters of certain local wells, but chiefly in marsh-ponds, tanks, and irrigation channels."

272. I now propose to enter upon a brief account of the method in which the investigations in connection with waters containing the parasitic forms of malaria were conducted.

273. To examine water for the malarial parasite, it is only necessary for the microscopist to carry the cleaned collecting vessel along the plastic ooze found on the surface of most stagnant ponds, or to gently scrape the surface scum of any unused surface well, or hold a clean bottle below the surface of any water suspected to contain this organism; or to dive the vessel between the different water-weeds and bring it out full.

274. The water having been collected in *thoroughly clean* glass stoppered bottles, should be divided into three parts, kept in three tall transparent glass vessels, which have also previously been thoroughly cleaned with sulphuric acid, boiling water, and finally distilled water, these vessels should also be provided with glass stoppers or rubber caps. The contents of one vessel should be examined without further preparation as soon as possible. To the second, we should add Lœffler's alkaline staining fluid to the extent of 1 to 250, and to the third, simple methylene blue solution, 1 to 100. In examining the first fluid, it is as a rule not necessary to do more than decant the upper part after the water has stood for an hour, for it usually contains an abundance of the parasites we are in search of. But as evaporation is active we should be careful that the field is irrigated with a drop of distilled water from time to time, to enable us to continue our observation. The examination of, say half a dozen slides, will give us a fair average of the specimen of water. If any particular forms of interest are seen, they may be fixed over osmic acid vapour by raising the cover-slip from the slide, and having allowed this fixed preparation to dry, mount it in FARRANT's fluid, and surround the edges with HOLLIS' glue, or zinc white.

275. The second specimen when allowed to stand for six hours, has all the forms of the parasite thoroughly coloured, but still active, and this will be found of great convenience to the worker. As in the above case, slides of this group may be readily made into permanent preparations when necessary. Many of the flagellate forms, and those contained in sporocyst which have just ruptured, will be stained with the eosin, and excellent contrasts with those coloured by methylene blue. In the third set of preparations the real parasitic forms stained are of a much lighter hue than the other forms met with.

276. The observer of the waters of Indian tanks and channels will soon find, after the examination of say a dozen waters from different probable sources of the malarial organism, that he has discovered one or two sources in which the malarial forms are the chief parasite in the whole specimen. The waters alluded to in the

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Part III which includes the description of the nine Plates illustrating the parasites of Malaria, will appear in a few weeks in The Lancet.

following remarks, and those from which the drawings were made, are from three sources only—a large Irrigation Tank known as the Seetarampett Tank the marshes below a large tank known as the Hoosain Saugor Tank, the latter being the source of our local public water-works and the tanks in the local Botanical (Public) Gardens.

277. The observer should at the beginning compare his specimens generally with those of carefully stained blood specimens of well marked cases of malarial fever, and from this go on to the varieties of malarial parasitic forms, beginning with the quartan parasite (the most visible). In the mixed forms of infection he will be surprised to find the large variety of forms of the parasite met with. So much is this the case that he will be tempted to consider that he is dealing with new varieties of the parasite; but a little experience, especially with fresh-preparations, will prove that he is only dealing with different stages of the same parasite in which some of the specimens are of diseased, deformed, disintegrated, parasites; whilst others are affected by the influence of quinine. In all he will find that in general terms they have the same structure, the same methods of reproduction, the same means of locomotion, and in general terms the same habits, characters and peculiarities.

278. In water as in blood, we find that the spore-forms always predominate, even the partial structures, and in almost every slide of well-marked specimens of malarial water, we may find each and every form of spore represented—small spherical, crescentic, kidney-shaped or falciform; rod-shaped, embryonic flagella, embryonic cysts, and small amœboid bodies. As in the case of the blood we notice that as each form is set free, it soon seeks out an unoccupied cyst or vegetable cell, with which it comes into contact. In short in their general morphological characters they are identical with the malarial organism as met with in blood.

279. Spherical cystic bodies are also always present, sometimes forming the predominating element. They are formed from the large variety of sporocysts, and also from infected vegetable cells. In the former case they vary much in size, but in the latter like those produced in red cells of the blood, they are usually six in number and of uniform size.

280. With regard to the spherical body I have not been able to trace out the same definite relation between its different varieties and those met with in the blood, nor is it met with in such considerable numbers as in the blood, if we except the large, and the small flagellate varieties. Whilst the free flagellated spherical body is one of the commonest and most characteristic forms met with in water, often moving in gyratory circles, or revolving constantly in its progress. The uniflagellate form also, which is so common in the blood of early tertian and in early irregular agues, is less frequently met with, and this is a noteworthy difference when we consider the very important part it plays in malarial blood. On the other hand, we often see the free flagellum formed from the large oval cystic body, and at once recognise it by its having one comparatively large round, and one elongated tail-like end. The spherical bodies, also developed in special cysts, are well-represented in every specimen of water. Before being set free we can recognise the movements of the confined flagella, which are more defined than in the case of the blood.

281. As regards the *crescent*, they are similar to the blood parasite of that shape. Thus, we in almost all specimens see the large, small and medium-size varieties produced within cystic bodies, vegetable cells, or the large sporocysts.

282. In water, in the absence of red cells, and in the uncertain presence of vegetable cells, by far the largest number of spores are created in sporocysts, and there is a remarkable similarity between the bursting sporocyst of water and that of malarial-blood, whilst their intracystic nature is even thus early well shown, especially in stained specimens. In a few instances I have seen the spores, (which are all ciliated or flagellated), in the interior of larger cell forms, but I have not seen them develop to the larger variety of crescent.

283. By far the most common form found in the *silt of irrigation channels* and the *bed of tanks* was the large sporulating cystic structures, together with varieties of sporocysts; these latter were, as a rule, shapeless masses of protoplasm, containing pigmented spherical, oval, oblong spore-like and short rod-like bodies, all of the forms possessing a nucleus. These small spores were pigmented; the mass itself contained embryonic forms of various kinds, was always flagellated, its flagella (usually three or four in number) sometimes reaching a greater size and enabling it to move about with rapidity. Even while thus progressing, it may often be noticed that an agitation is taking place in the protoplasmic substance, later on, the spores begin to alter their position. Finally, rupture occurs, and the embryos are set free; but this rupture and escape of spores is never so conspicuously accomplished as in the blood. The whole process takes place almost passively, the wall gradually thinning till it is here and there invisible.

284. Similarly the air over marshes was systematically investigated, and once in eighteen observations or more correctly in two out of thirty-six observations found to contain spherical, oval, flagellate, and sickle-shaped spores. In the first series of observation, carried out with POUCHET's äeroscope, not a single form could be found in twelve observations. In the second series of twenty observations, made with HESSE's apparatus, in two, I found the spores only, but none of the larger forms of the parasite. In the third series of four observations over a dried-up paddy-field, also with HESSE's apparatus, the results were purely negative. I am not acquainted with any previous experiments which satisfactorily demonstrate that such observations were made in the light of recent acquisitions in knowledge regarding malarial parasites. The observations were conducted during and just before the ordinary periods of epidemic malarial fever. The area chosen was below the Hoosain Saugor Tank, in the swamps to the west of the bund or dam. This is a charactically malarious place, full of swamps and marshes, and for many years a rice-producing locality, having a rich organic soil, in which the sub-soil water is always close at hand. The medium we employed for collecting the parasites in the first twelve experiments was sterilized glycerine, that in the remaining twenty-four observations sterilized glycerine jelly. Although, I am forced to pronounce these investigations on malarial air failures, I am disposed to recommend their continuance. The labour involved in such observations is great, and the results so far, altogether incommensurate.

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