THE RELATION OF PROTOZOA TO PREVENTABLE DISEASE.

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IN the whole range of modern scientific medicine there is no subject of deeper interest to the physician than the relation of the parasitic protozoa to disease, but the complexity of the subject, and the rapidity with which important results have been published by observers of established reputation, render the presentation of an adequate and up-to-date summary of even a small portion of the accepted facts of recent medical protozoology a task of considerable difficulty.

Firstly, it is essential to briefly define what we mean by protozoa.

In the words of Sir Ray Lankester, "the protozoon individual is a single corpuscle of protoplasm varying in size when adult from less than one-thousandth part of an inch in diameter (some sporozoa and flagellata) up to the diameter of an inch (nummulites), and even much larger size in the plasmodia of mycetozoa."

The term protozoa originated as a translation of the German word *Urthiere*, and the members of the group are definitely distinguished from all the rest of the animal kingdom, being "essentially unicellular animals."

Minchin points out that growth and reproduction by fission may in these little creatures continue for many generations, but sooner or later their true animal character is demonstrated by the introduction of a process of sexual union into their life-cycle. The biological import of this process is not clear, but in some cases it is certain that it frequently produces a rejuvenescence of organisms which have become effete and feeble by a prolonged vegetative existence.

The sexual process may intervene at any period of the life-cycle, and may either precede or follow rapid *reproduction by fission*; and conjugation, though generally associated with reproduction, may occur independently of it. True conjugation in protozoa consists of the fusion of the nuclear substance from two individuals.¹⁵

¹⁵ A list of references is given on page 408.

The individuals who go through this process are called *gametes*, and if the union extends to the fusion of the whole bodies of the organism the resulting individual is called a *zygote*.

The gametes may be similar in all respects, or may be differentiated into *microgametes*, regarded as males, which are smaller and more motile, and *macrogametes*, regarded as females, which are more bulky, often full of reserve material, and less, or not at all, motile.¹⁵

In some cases not only are the conjugating individuals different from one another, but the individuals of the generation from which the gametes arise may be different from ordinary individuals of the same species.

The mother-cells of the gametes are then distinguished as gametocytes, and styled macrogametocytes or microgametocytes according to the variety of gamete to which they are the precursor.

I must beg you to remember these terms if they are not already familiar to you, as they recur in describing the development of different families of the organisms.

Ray Lankester²⁰ gives an elaborate classification of protozoa, but the arrangement best suited to the purpose of this paper is that of Butschli, who divides them into the following four classes, arranged in the ascending scale of animal development:—

1. Sarcodinia (or Rhizopodia), comprising those forms having the general characteristics of Amœbæ;
2. Mastigophora or Flagellata, *i.e.*, forms having a new flagella;
3. Infusoria or cilitia, being those organisms having an elaborate arrangement of cilia or flagella;
4. Sporozoa, *viz.*, those forms of protozoa which are rarely amœboid and multiply by a form of sporulation.²⁰

As it is obviously impossible, in the space at my disposal, to consider, however briefly, *all* the protozoa concerned in the production of disease, I propose to confine my remarks to six diseases of special interest to the medical officer of health: (1) amœbic dysentery; (2) trypanosomiasis; (3) syphilis; (4) kala azar; (5) malaria; (6) piroplasmosis.

It may be objected by some that these diseases with one exception are maladies peculiar to tropical countries, and that they are therefore of comparatively small interest to practitioners in this country.

I would meet this objection by pointing out that “so widespread is our empire, so intimately connected are we with countries of different, and for the most part warmer, climates than our own, that there is scarcely a family in these islands that has not some relative who occasionally returns to his native place from abroad. In many instances the tropical resident

is sent home on sick leave or to recoup after a long spell in a trying climate. He may be suffering from actual illness on landing, but it is not uncommon for a tropical resident whilst at home to develop some latent illness, such as malaria, liver ailments, or dysentery."⁴

It comes about, therefore, that medical men in the most remote corner of Dartmoor, or some obscure village on the rocky coast of Cornwall may find themselves face to face with tropical ailments with whose very name they may be unfamiliar.⁴

A short account of some of the more common protozoal diseases may therefore be not only interesting, but useful to medical men, especially those in practice in one of the most important ports of Great Britain.

The first disease to claim our attention is dysentery, a condition which recent research has taught us is not one malady, but at least two distinct diseases. One variety due to bacilli of very varying characters, and, therefore, of no present interest to us, and another caused by a protozoon and called—

AMŒBIC DYSENTERY.

The organism which induces amœbic dysentery belongs to the rhizopodia or lowest class of protozoon, and proceeding on zoological lines from the lowest form of development to the highest, it comes first on our list of diseases.

In examining the fauna of the intestine of man it must first be clearly understood that a great variety of amœboid organisms flourish in this human jungle, some of which are true amœbæ, whereas others are merely amœboid stages in the development of various *Infusoria*, such as *Trichomonas* and *Lambliæ*, hereafter referred to.

True amœbæ are divided into two groups: (*a*) thecamœbæ, or those provided with a hard envelope or shell; and (*b*) gymnamœbæ, or those consisting merely of homogeneous protoplasm. The members of the first group are not concerned in disease, but two individuals of the second group are of great interest to the physician.

One of these, the *Amœba coli*, which has been renamed by Schaudinn the *Entamœba coli*, is a harmless parasite, whereas the second, which he styles the *Entamœba histolytica*, is one of the most lethal of pathogenic protozoa.

The life-history of these simple forms of animal life is very complicated, and we owe our present knowledge of its intricate details to the industry and genius of Schaudinn, whose untimely death all interested in the subject of protozoology must deplore.

Schaudinn's work has been carefully studied by McWeeney, who has

conferred a boon on all interested in the subject by summarising the abstruse scientific German of Schaudinn's articles in a brilliant paper read before the Epidemiological Society in January, 1905.¹⁷

Schaudinn describes the *Entamœba* as a shapeless mass of protoplasm, with a large nucleus, but not showing any of the differentiation into clear ecto- and granular endoplasm which marks the pathogenic form.

He found the parasite in the excreta of about half the persons which he examined, and Schuberg afterwards demonstrated the fact that as the organism dwells in the upper regions of the colon, by hurrying the onward passage of the fæces the protozoon could nearly always be found in the dejecta of healthy individuals.

The *Entamœba* has two well-marked methods of reproduction, one merely vegetative and the other clearly sexual in type.

The asexual form consists in simple segmentation of the nucleus into eight parts, which surround themselves with protoplasm and separate into "a characteristic brood" of young amœbæ.

In the sexual cycle of reproduction the organism comes to rest, contracts, and "surrounds itself with a gelatinous coat which becomes the cyst wall."¹⁷

The nuclei, after undergoing reconstruction, divide, copulate, and then divide again, so that the parent nuclei eventually form eight young amœbæ which are enclosed within the cyst-wall, and like the cysticercus of the tape-worm cannot start in life on their own account until the cyst has been taken into the stomach of a new host and the gelatinous cyst-wall dissolved by the intestinal juices.

The *Entamœba histolytica*, as the *Amœba dysenteriae* is called by Schaudinn, differs widely in appearance and mode of production from the non-pathogenic type of organism.

It has a clear tough ectoplasm and an ill-defined nucleus, and Schaudinn considers that this tough outer layer enables the parasite to force its way between the epithelial cells of the intestinal mucous membrane into the deeper layers and submucosa, where it undergoes development, destroys the tissue, and thus forms the ulcers characteristic of dysentery.

In support of this view he states that he has actually observed the protozoa forcing their way between the cells in the freshly-excised bowel of a cat which had died of amœbic dysentery.

Entamœba histolytica in its asexual form multiplies by simple fission or forms new amœbæ by budding; "the characteristic brood formation" of the non-pathogenic organism does not occur.¹⁷

The cystic stage is also produced in a different way, and it is of special

interest to note that it is not formed till the patient is beginning to recover from his attack and the stools are becoming solid, a fact which is of the utmost importance in framing preventive measures against the disease.

The protozoon throws out from its surface a series of little knobs, about three microns in diameter, each containing a particle of chromatin. These knobs break off and, having developed a firm capsule, ultimately become hard, opaque little bodies called, for want of a better name, "spores."

These bodies are expelled in the fæces, and on being taken into the intestinal tract infect a new host.

An experiment of Schaudinn's seems to prove this conclusively. He allowed some of the fæces from a case of undoubted tropical dysentery to dry in air, and assured himself by careful microscopy that it contained nothing resembling the cysts of *Entamoeba coli*, but only the "spores" of *Entamoeba histolytica*.

He took the material from the slides actually examined, and having made it into an emulsion with water gave it in food to a healthy young cat, whose fæces had been proved to be free from dysentery organisms. In three days the cat passed stools containing typical *Entamoeba histolytica*, and it died of dysentery on the fourth day.

The autopsy showed typical dysenteric ulceration of the colon with entamœbæ "in all stages of penetration of the intestinal wall."

Schaudinn gave large quantities of fæcal matter from this case to another cat but the animal remained healthy, showing that the amœboid form of the organism is innocuous.

He then administered a little of the dried-up fæces containing some of the spore-like bodies from the tropical dysentery case, and the animal, which was larger and stronger than the first cat, developed dysentery in due course and died in a fortnight.

It is, therefore, to the spores of *Entamoeba histolytica* contained in dust and water that we must look for the cause of the propagation of amœbic dysentery, and this discovery of Schaudinn's is of enormous practical value to the physician, as it emphasises the necessity for complete segregation of the sick and convalescent from the healthy, and the adoption of every possible precaution to prevent the contamination of water and food by these long-lived and lethal cysts of the protozoon of dysentery.

Before leaving the subject of dysentery I would especially ask you to never make a diagnosis of dysentery from the mere macroscopic appearance of the stools.

In an article on tropical dysentery published in the *Lancet* two years ago I said that "the stools of all patients suffering from intestinal flux should be subjected to a microscopical examination," and declared that my chief object in writing the paper was to call attention to the fact that the character of the stools alone is not to be considered as a test of the absence, or presence of dysentery.

This view has been supported by Cantlie who writes: "Because a patient has resided in the tropics, one is apt to forget that there are other causes of loose motions, and mucus in the fæces with, or without, blood."

"Of these the more common are intestinal parasites, which are so frequently met with in the fæces of tropical residents that medical men in Britain would do well to submit the stools of all cases of patients coming under their care who present symptoms of intestinal flux, to a pathologist."⁴

In women the pelvic organs ought to be examined in all cases of intestinal flux. Cantlie says he "'cured' instantly several cases of so-called chronic dysentery and sprue, and the passing of intestinal mucous casts by replacing a retroverted or retroflected uterus and inserting a ring-support. In other cases left pelvic adhesions of the uterus and left ovarian trouble (from the fact that they involve the rectum or sigmoid flexure) set up intestinal troubles, which when the cause is removed the so-called dysentery or chronic diarrhœa disappears."

"What is called chronic dysentery, but in reality is simple diarrhœa, is a common feature of hepatic disease in patients returned from the tropics. All too frequently the simple morning diarrhœa of the tropics is due to hepatic derangements set up by alcohol, a fact that is apt to be overlooked because the patient is home from the tropics, and the ailment is looked upon as "climatic" when the cause is frequently whisky."⁴

In long-standing cases of this nature the condition of the liver will help in the diagnosis, and if, in addition to morning diarrhœa and hepatic atrophy or hyperplasia, there is albumen in the urine, associated with hepatic tenderness on palpation, the part played by alcohol should not be difficult to arrive at.⁴

Tropical dysentery as met with in Britain is, I consider, invariably of the amœbic type, and, as I have pointed out elsewhere, I regard *ippecacuanha* as a specific in its treatment.

As a point of practical interest in the laboratory I may remind you that amœbæ can only be found in the stools within twelve hours after being passed, as after that interval they begin to break up. They are

found most readily in the smaller pieces of mucus. The organisms disintegrate with heat so the films cannot be fixed in the Bunsen flame. They should be dried in air and then placed for five minutes in Gulland's fixing solution, which is made up as follows:—

Absolute alcohol	...	...	...	25 cc.
Pure ether	...	...	...	25 cc.
Solution of corrosive sublimate in				
Alcohol (strength 2 grammes in 10 cc.)				5 cc.

The films should then be immersed in carbol fuchsin for three minutes, washed vigorously in water for thirty seconds, and dried in air.*

TRYPANOSOMIASIS.

The next class of protozoa on the upward scale is the Mastigophora containing the sub-class Flagellata, which includes the great family of Trypanosomata, a group of organisms which has claimed the undivided attention of many observers in various parts of the globe for the past five or six years.

This variety of parasite has been known since 1876, when Surgeon-Major Lewis described it as a harmless parasite existing in the blood of healthy rats. Since that year different observers have noted the presence of varieties of the organism in different animals, but the present access of interest in Trypanosomata dates from 1901, when Dutton recognised as a member of this group the "vermicule" which Ford had discovered in the blood of an European at Bathurst.

Dutton named the organism the *Trypanosoma gambiense*, and Castellani showed in 1903 that this protozoon was present in the cerebro-spinal fluid of patients suffering from sleeping sickness, a discovery which led the way for the investigations of Bruce, Greig, Nabarro, and Todd, and our present exact knowledge that sleeping sickness is merely a *late* manifestation of human trypanosomiasis.¹

This malady has a sorrowful interest for members of the R.A.M.C., as the first recorded case in a British officer was Lieut. Forbes Tulloch, who succumbed to his zeal in the interests of protozoology only a year or so ago.

The trypanosome of all varieties is a somewhat fish-like structure with a fin-like projection on one side called a flagellum. In the body are two basic-staining granules and two chromatin masses, one in the middle called the macronucleus, and the other in the tail or posterior called the micronucleus.

The pathogenetic properties of the organism depend on their power of adapting themselves to various hosts when transferred to individuals of a new species, from animals in which they naturally occur, by a variety of biting flies. They flourish exceedingly in their new hosts, and thus work their destruction, *e.g.*, the Nagana trypanosome multiplies so exceedingly in the blood of a rat inoculated with the protozoon that just before death there are almost as many trypanosomes as red corpuscles in the blood.¹⁵

The features of development common to all forms of trypanosomata are somewhat as follows:—

The individual organisms are differentiated into male, female, and indifferent forms. All three varieties may multiply by splitting longitudinally, a process which takes place in the following way: (1) the macronucleus becomes dumb-bell shaped and splits in two; (2) the micronucleus behaves in a similar way; (3) a second flagellum is produced; (4) fission begins at the tail-end; but (5) actual separation commences at the head and goes on till two fully formed trypanosomes are produced.

This process may fall into abeyance when sexual forms become fully developed. The three types may be observed in the vertebrate host, but are usually not clearly differentiated except in the invertebrate host.

The males are much more slender than the females, and are so delicate in constitution that they soon die off if they do not conjugate.

The indifferent types are hardier than the males, but the females are the most resistant of the three, as they have a quantity of reserve material stored up in their interior.

The male and female forms undergo a process of maturation in the gut of the biting fly, which acts as intermediate host, and it is in this position that the act of union can alone take place.

The sexual forms undergo complete fusion in the process of conjugation, and the resulting zygote is an individual similar to the oökinet, or vermicule stage of the malarial parasite. This stage, however, gradually develops an undulating membrane and flagellum, and becomes an ordinary trypanosome which takes on a vegetative cycle of existence.

Under adverse circumstances the female trypanosome can undergo a species of self-fertilization and repopulate its vertebrate host; its descendants being gradually differentiated into male and female.¹⁵

Such, very briefly is the ordinary life-cycle of these curious little animals, but of course many modifications exist, and certain phases and forms of trypanosomata cannot be attributed to these comparatively simple data.

Closely allied to the trypanosomata is the group of organisms known

as spirochætæ, which were long confused with the class of bacteria called spirilla.

Schaudinn has, however, traced the development of the organism through somewhat similar stages to those which are shortly to be described in the protozoon of malaria until male and female individuals capable of exercising sexual functions are formed. This brilliant and industrious observer worked out the development of the spirochæta in a plasmodial parasite of the barn-door owl.²²

He considered that owl-malaria was propagated by a mosquito called the *Culex pipiens*, and found that as a result of fertilization an oökinet, or locomotive zygote is formed, which becomes converted into male, female, and indifferent trypanosome-like bodies in the front of the mosquito's stomach. The process of development is extremely interesting, but space compels us to proceed at once to follow the organism into the blood of the owl, whither it is conveyed by the bite of the mosquito in the same manner as the sporozoite of malaria.

On entering the blood the spirochætæ adhere by their flagella to the red cells, bore their way into them, and there develop into a plasmodial form. They do not proceed to form rosettes or crescents as in human malaria, but at first simply reside in the cells of the spleen and bone marrow as a snail lives in its shell. They leave their host cells at night and swim about freely in the blood, but re-enter the corpuscles in the day-time. They reach their full stage of development in about six days, and begin to multiply by longitudinal fission. The minute trypanosome-like bodies so formed proceed to attack fresh cells until the blood becomes inundated with parasites.

At this stage the protozoa gradually die down until only a small number of indifferent forms survive, which become gradually converted into micro and macrogametocytes, which can only complete their development in the body of the *Culex pipiens*.

This observation, for a time, raised the question whether all spirochætæ or spirilla might not be merely features in the development of trypanosomata, and McWeeney stated that "should Schaudinn's work be confirmed by other observers then it follows that trypanosomata and spirochætæ are really different names for the same thing."¹⁷

SYPHILIS.

This disease has peculiar interest for the protozoologist, as on April 10th, 1905, appeared a paper by Schaudinn, in which he published an account of his discovery in various syphilitic affections of a small

actively motile spirochæte which he looked on as the probable cause of syphilis.

This organism he, at first, named the *Spirochæta pallida*, but as this title was not distinctive of the zoological class he altered it to *Treponema pallidum*. In October, 1905, he announced that his discovery had been confirmed by more than one hundred independent authorities, and since that date it has come to be a generally accepted fact that syphilis is a chronic disease due to *Treponema pallidum*.

This spirochæte is only one of many similar organisms found in various pathological conditions, but it has the following well-marked characteristics which serve to distinguish it:—(1) It generally measures about seven microns in length and consists of several slender spirals which have a peculiar rigid appearance suggesting that the organism has been “turned on a lathe.” (2) Its coils are more numerous than those of other spirochætæ. (3) It never stains deeply. (4) By Giemsa’s stain, which is a modification of Leishman’s in which azure is used instead of methylene-blue, it is coloured a delicate red, whereas other spirochætæ take on a bluish colour. (5) It has sharp tapering extremities, whereas the “pseudo-pallida,” which is found in ulcerating carcinoma for example, has blunt rounded extremities.

Schaudinn looked upon spirochætæ as modified trypanosomes, and, in his work on Ziemann’s parasite, describes an oökinet which becomes coiled up and then divides into a large number of small trypanosomes which get spirally twisted round their long axis so as to constitute what have hitherto been known as spirilla. His idea that *all* spirochætæ are probably members of the protozoa class, which quite recently claimed the support of many eminent bacteriologists, must now be abandoned, as Novy and Knapp have shown that the best known member of the group, the *Obermeiri Spirillum*, is a true bacterium.¹⁵

Two Polish investigators, Krzysztalowicz and Siedlecki, describe forms which are considered to be sexual individuals, and suggest that the zygote passes into a resting stage and become transformed into some sort of spore, which, having been carried to a remote portion of the body, forms new spirochætæ and gives rise to the secondary symptoms of syphilis.¹⁵

Minchin says: “The transmission of *treponema pallidum* from one host to another by the contagion of coitus without an intermediate host is analogous to dourine, in which infection occurs in a similar way.”¹⁵

The prevention of disease caused by this interesting protozoon is a subject of the widest importance to all sections of the community. It is

the fashion to pretend that syphilis is a malady chiefly of interest to the military surgeon, but everyone with any considerable experience of out-patient work in a large hospital knows that the malady is probably as common amongst the civilian as the military population.

There is this difference: that, nowadays, the soldier rarely, if ever, conceals his disease, and has the advantage of a year or two of compulsory medical treatment and supervision on the very latest scientific principles, whereas only too frequently the civilian is ashamed to consult a reputable practitioner, and falls into the hands of quacks and charlatans with the most disastrous effects.

This being so, the work of Roux and Metchnikoff on the prevention of syphilis is of the highest importance. These distinguished investigators have been working for some years to discover an antisyphilitic serum, but their prolonged investigations have as yet resulted in failure.

They have, however, reported to the French Academy of Medicine that an ointment consisting of one part of calomel to two parts of lanoline has proved to be an absolute prophylactic if rubbed into the genitalia within twenty hours after exposure to infection.

In support of their contention they give details of many experiments, not only on monkeys, but also on a young medical student, who, in the interests of humanity, permitted himself to be inoculated with syphilis.

This discovery is, I think, one of the highest value, and one which cannot be too widely known, both in and out of the profession, as the sanitarian aims at preventing disease, and must consider all legitimate means justifiable in attaining his laudable end.

Closely related to the spirochætæ is the genus of *Flagellata*, known as *Herpetomonas*. This sub-order is of special interest to us, as zoologists now refer to it as THE LEISHMAN-DONOVAN BODIES.

These peculiar bodies were discovered in 1900 by Leishman, and independently in 1903 by Donovan in smears of spleen pulp from cases of kala azar, and are invariably found in the spleen pulp and bone marrow of patients suffering from kala azar, a peculiar type of fever found in India, China, and tropical Africa.

The Leishman bodies "have been the subject of much controversy as regards both questions of hypothesis and matters of fact."¹⁵

Even the very situation of their occurrence has been disputed, for whereas Donovan described and figured them in unaltered red blood corpuscles, both in the spleen and general circulation, Christophers found them only in the leucocytes peripheral blood, and doubts the specific

nature of the bodies in the red blood corpuscles, whether in the peripheral circulation or the spleen or blood marrow.

The Leishman bodies are ovoid or pear-shaped, about three microns in the longest and two microns in the shortest diameter. They contain two nuclei or chromatin masses, situated in the short axis of the body.

One of these masses is much larger than the other, more or less spherical in shape and stains faintly, whereas the other is generally rod-shaped and stains very deeply. Sometimes a bridge of protoplasm unites the two nuclei. The parasites multiply by binary or multiple fission.

The reason for including these curious little bodies in this group is the remarkable fact, discovered by Rogers, that they can be grown in citrated blood into distinct flagellate organisms.¹⁴

No conjugation occurs, however, in the laboratory grown organisms, which simply degenerate and die off without further development.

The artificial development of the organism has been carefully studied, but none of the stages of natural development have as yet been observed.

The agency by which the parasite is disseminated has not yet been clearly ascertained, but Rogers has produced considerable evidence to show that the bed-bug is the invertebrate host.¹⁵

This brings the prevention of kala azar within the sphere of practical sanitation, as the extensive destruction of body-parasites in affected areas, although presenting many difficulties, is not impossible, and must lead to marked diminution in the spread of the disease.

We now arrive at the third class of protozoa, and find that the *Infusoria* have little interest for us, as, of the known members of the group, *Trichomonas vaginalis* is a harmless parasite frequently found in the vagina, and familiar to medical jurists on account of a superficial resemblance to the spermatozoon, whereas *Trichomonas hominis* and *Lambli intestinalis* are rarely associated with pathological conditions, and only here referred to on account of an amœboid stage in their development which may be confused with true entamœbæ.

MALARIA.

Passing to the last class in the zoological arrangement adopted, we find the most interesting organisms yet considered, namely, the parasites concerned in the production and propagation of malaria.

The *Sporozoa* are so called because at some time in their life-history the individual sporozoön breaks up into a number of small bodies resembling spores.

The order of the zoological class to which the malarial parasites belong is called the *Hemosporidia*, because they live in, and at the expense of, the blood-corpuscles.

There are many varieties of organisms found in the blood of various animals, and it is generally accepted that at least three varieties are found in man, although M. Laveran, the discoverer, still maintains that there is but one form of human malaria.

The *Hemosporidia* found in man are included in the Genus *Plasmodium*, viz.:—(1) The *Plasmodium malarie*, producing quartan ague; (2) the *Plasmodium vivax*, producing benign tertian; and (3) the *Laverania malarie Grassi*, the cause of malignant tertian ague. All three have a double cycle of development, one sexual and the other asexual.

The asexual cycle can take place entirely in the blood of man, but the sexual required a change of host-species as distinguished from host-individual, and cannot occur without the intervention of the mosquito.

The process of development starts from the small spore-like bodies, from which the sporozoa class derived its name, finding their way into the blood, fixing themselves on to the red corpuscles and boring their way to the interior of these cells.

In the second stage of development we have, therefore, the plasmodium inside the red blood corpuscles forming the ring shape, with the chromatin at one spot, as shown in Major Morgan's beautiful plates which are given in the *Journal of the Royal Army Medical Corps*.*

From the ring shape the parasite may take on the sexual or asexual form. Let us follow the asexual cycle first. We find that the parasite grows at the expense of the corpuscle, absorbs its fluids and rapidly increases in size. It abstracts the hæmoglobin from the stroma of the cell, and alters it into a black or brown pigment called melanin.

In the interior of the corpuscle the parasite is motile and throws out processes which alter in shape, but when it comes to occupy the greater part of its host cell, the chromatin of which it is largely constituted begins to split up into two, four, eight, twelve or more segments.

On the completion of this process of segmentation the protozoon has divided into several fragments of chromatin, each surrounded with protoplasm, and arranged in the form of a daisy or rosette with melanin granules in the centre, forming the stamens of the daisy or centre of the rosette.

The petals of the daisy or loops of the rosette constitute the small

* Vol. VI., No. 1, Jan., 1906, pp. 50 and 52.

bodies originally referred to as spores, and when this curious malarial blossom is full-blown, the remains of the red cell rupture and its baleful contents are set free to attack fresh red blood corpuscles and start the cycle anew.

The melanin passes into the blood stream, and is carried by the leucocytes to the tissues producing the darkening of the skin and organs which is so familiar in subjects of malaria.

The process detailed constitutes an asexual developmental cycle of the parasite, and in quotidian ague takes only twenty-four hours from spore to spore.

If quinine is not given this goes on until "owing to the production of specific anti-bodies," or "the exhaustion of the reproductive power of the parasite under the strain of a repeated asexual division this process gradually ceases, and specially differentiated male and female forms" appear in the following way:—

Starting from the ring shape the sexual cycle proceeds at first in much the same way as in the asexual. Melanin and chromatin are formed, but when the parasite is one-third grown the latter can be seen in a clear unstained zone called the vacuole, which, Leishman teaches, is characteristic of this form of development. Another special point in this form of development is that the chromatin mass shows no tendency to segment.

When the protozoon nearly fills the red cell it is called a gamete or gametocyte, and if the patient is not bitten by a mosquito at this particular period the parasite dies, but if prophylaxis has been lax, and the organism passes into the stomach of a mosquito, its true sexual life begins.

The male gametes are called, as we have seen, microgametocytes, and the female macrogametocytes.

Ten minutes to half an hour after the blood has been sucked in by the mosquito the microgametocyte, which is readily recognized by its hyaline plasma, large nucleus, and coarsely granular pigment, exflagellates four to eight filaments about twenty microns long and one micron thick.

These flagella are actively motile and are soon set free, being given the name of microgamete when in the free state. Meanwhile the macrogamete has been preparing itself for fertilization by ejecting part of its nucleus, and McWeeney thinks that this extruded matter may act chemotactically in attracting the microgametes.

On the approach of the male the macrogamete protrudes a "receptive eminence" or microphyle, and as soon as a male adheres to this the eminence is at once withdrawn, dragging the struggling microgamete into the body of the female. This completes the act of copulation, and the

macrogamete now secretes a coat of mucus which keeps away all other males, its name changes, according to the nomenclature of Schaudinn and Lühe, to zygote or fertilized macrogamete.

The zygote changes rapidly into an oökinet, or motile body, which reaches the wall of the mosquito's stomach, worms its way through the epithelium, and comes to rest between the layers of the muscular wall of the stomach.

In this situation it is called an oöcyst, and having originally been only 7.5 microns (*i.e.*, the diameter of a red blood corpuscle) it hextuples its size and becomes 50 microns, so that it can be seen with an ordinary lens.

A tough resistant membrane develops round the oöcyst which soon begins to segment like a yolk into a mulberry-shaped mass. This mass finally splits up into a number of secondary cysts, or sporoblasts, which are full of elongated thread-like bodies, each containing chromatin, called sporozoites. The sporoblasts eventually burst, and the sporozoites are accordingly set free in the general blood-stream of the mosquito. They are carried everywhere including the neighbourhood of the salivary glands from whence they enter the salivary cells and ducts, so that when a mosquito bites an animal he injects, with the venom which he always instils, several of these fully developed sporozoites which proceed to attack the red blood corpuscles and start the malarial cycle.

Some of the sporozoites are said by Schaudinn to enter the ovary of the mosquito and there infect the ova, so that the young brood of next spring harbours the parasite, and these young mosquitoes can confer malaria without having sucked the blood of an infected individual, a condition of things which, if confirmed by other observers, will explain many epidemiological problems which have puzzled medical officers of health in every part of the tropics.

This sketch of the life history of the malarial parasite is true of all varieties, but I have given in the attached table the special characteristics of each parasite, and will here merely summarize the points in structure common to all three.

1. All live in and at the expense of the red cells.
2. All are amœboid, *i.e.*, are endowed with movement said to be due to (*a*) melanin bodies moving round and (*b*) pseudopodia being formed.
3. All manufacture melanin.
4. Nearly all produce various degenerate changes in the red blood corpuscles.
5. In the fresh state all are colourless, but when dead the protoplasm stains blue and the chromatin red by Romanowsky's method of staining, or Leishman's modification.
6. Each variety has a definite period of growth, 72 hours in Quartan, 48 in Tertian, etc.
7. All possess

sexual and asexual forms, *i.e.*, are capable of forming gametes and rosettes or daisies. (Leishman).

The complex and elaborate details of our knowledge of the connection of the hæmosporidia with malaria we owe to the labours of Laveran,¹³ who discovered the organisms; Golgi, who classified them zoologically; Ross²¹ and Manson,¹⁶ who ascertained the alternative host; McCallum, who recognised the sexual method of development; Grassi,⁹ who identified the variety of mosquito; and last, but not least, the illustrious Schaudinn, who observed the entrance of the sporozoite into his own red blood corpuscles, and thus completed the developmental cycle.

PIROPLASMOSIS.

In view of the fact that Koch,¹² in a recent address, says "our knowledge of protozoal diseases is based on three great discoveries: that of the malarial parasite by Laveran; of the *P. bigminium*, the cause of Texas fever or red water in cattle, by Smith; and the discovery of trypanosome in tsetse-fly disease," it is necessary to say a few words about tick-fever, especially as Bowhill has found both trypanosome and spirochæte forms in the blood of oxen and dogs affected with this malady.

Briefly stated, there is a group of parasites conveyed by ticks which produce a condition called piroplasmosis, which is found in man, oxen, horses, dogs, etc. These parasites are pear-shaped bodies which are found in the red cells, and affect the infected animal with all the symptoms of progressive hæmolytic, viz., asthenia, anorexia, debility, jaundice, etc., ending in death. They constitute a special genus in the same order and class as the malarial parasite.

The ticks which convey the disease lay millions of eggs, which are hatched out on the ground into larvæ, which crawl "to a summit of a blade of grass or grass-stem, and there await the passage of some animal," to which they cling.

They live on this animal for a few days and then fall off, developing on the ground, first into nymphæ and later (with or without the intervention of a second host) into full-grown insects of both sexes, which get on the body of an animal and mate. The female then falls off and lays eggs, which in due course develop into larvæ and start the cycle anew.

The tick may convey disease in any of its stages of development, and some varieties lay eggs which produce larvæ which are infective.²¹ (See Dr. Theiler's excellent plate, *Journal of the Royal Army Medical Corps*.*)

* Vol. III., No. 5, Nov. 4, 1904, p. 476.

This discovery of propagation of disease by body-insects is of the greatest interest to the physician, as it serves to explain obscure epidemics of relapsing and other fevers, which have frequently been carried from point to point in a, hitherto, quite inexplicable way.

The ticks, it must be remembered, are very hardy, and in the intermediate stages can resist starvation for very long periods; so that a larva, nymph, or adult tick may remain perched at the end of a blade of grass or other structure for some months thoroughly capable of infecting a suitable animal.

Diagram showing Life Cycle of Tick with three Hosts.

(From Col. Sir David Bruce's Plate, Journal of the R.A.M.C., Vol. VII., p. 26.)

Rhipicephalus appendiculatus is the type of this variety. Other ticks, such as *Rhipicephalus evertsi*, only require two hosts. The nymphs develop in the latter variety into full-grown insects, on the grass, in twenty-four days.

The blood of an affected animal does not give rise to Piroplasmosis when directly transferred under the skin of a healthy one. It is only after it has passed through the body of a tick that the piroplasma can give rise to disease.

With these remarks I must bring to a close my necessarily brief account of the association of protozoa with a few diseases of special importance in Preventive Medicine, in the hope that I have at least been

able to bring out the supreme importance of the subject, not merely to specialists, but to every medical officer of health.

The splendid discoveries of Bruce and Leishman of my own corps should interest every earnest physician in blood-diseases and blood-parasites, for in this direction appear to lie discoveries yet undreamed of, as protozoology seems to begin where bacteriology leaves off, and experts consider it more than probable that the long-looked-for *causa causans* of smallpox and yellow fever will be found in the protozoal class of the animal kingdom. In the words of Cantlie,

“During the last quarter of the nineteenth century bacteriology usurped the attention of medical observers, but with the advent of the twentieth century the protozoologists would appear to be about to oust the bacteriologists from the foremost place in our search after the origin and the epidemiology of disease.”³

I think it is clear that some knowledge of the development of the organisms referred to in this paper is desirable in every Medical Officer of Health, as without such knowledge he cannot intelligently adopt methods of prevention, which, rather than mere means of cure, is the aim of those on whom the responsibility for the health of the nation depends, not only in this country but in “that Greater Britain beyond the seas.”

To accompany "Protozoa and Disease," by Major ROBERT J. BLACKHAM, R.A.M.C.

Name of Parasite and Type of Fever it produces.	Effect on the Red Blood Corpuscles.	Character of Melanin produced.	Number of Segments in "Daisy" or Rosette.	Degenerative or other Special Changes produced in Red Cell.	Special Characters of Ring Shape.	Effect of Quinine.	Effect of Parasite on the Spleen.
Plasmodium Vivax produces Benign Tertian Fever.	The corpuscles are enlarged to two or three times their normal size, and paler.	Coarse light brown granules.	15 to 25.	A number of red staining spots called Shüffner's dots can be demonstrated with Leishman's stain. They increase only in size and not in number.	Ring larger than in Malignant Tertian.	Quinine, <i>per os</i> or hypodermically, destroys the parasite and cures the fever.	Enlargement is common but not invariable.
Plasmodium Malaria produces Quartan Fever.	The corpuscles are diminished in size, and do not become paler. The parasite fills the whole cell.	The granules are coarser and darker in colour. It is in this variety you get the true daisy shape.	8 to 12. Number of rosettes in peripheral blood is greater than in B. Tertian.	No degenerative changes can be seen, and if your technique is correct this enables you to settle type of fever.	Do.	Do.	This is the variety of fever at one time common in England which produced the "Ague cake" of the Pers.
Laverania Malaria Grassi produces Malignant Tertian or Estivo-Autumnal Fever.	The corpuscles are not enlarged. They do not become paler, but on the contrary some grow darker. The parasite does not fill the cells, which frequently become shrunk and cremated.	"The Melanin is arranged as in Quartan parasite." — Leishman.	There is much difference of opinion, and Leishman teaches that it is best to say "uncertain."	Following out rings which are going to become rosettes, Maza found certain markings which appear to be marks of scars made in stroma of cell by parasite. Increase in number but not in size.	Rings are smaller and more delicate in shape than in other varieties. Present in peripheral blood in larger numbers than in other varieties, and if many rings observed, in blood film, suspect Malignant Tertian.	Very resistant to Quinine.	Splenic enlargement is rare.

NOTE 1.—Malignant Tertian Malaria has been called: (1) Pernicious Malaria, (2) Estivo-Autumnal Fever, (3) Tropical Malaria, (4) Remittent Malaria, (5) Sub-Tertian Fever (Manson). It may be defined as a form of Malaria in which alone the gametes have a crescent form, and might well be called "Crescent Tertian Fever," a synonym now recognised in the official Nomenclature of Diseases. Chromatin is difficult to stain, but exflagellation is easily demonstrated in crescent.

NOTE 2.—The names applied by different authors to the Malarial parasites are confusing. The terminology followed above is that of the Nomenclature of the Royal College of Physicians. The official title of the parasite of Malignant Tertian has not yet been generally adopted. The parasite has been called Plasmodium immutatum by Schaudinn, P. Falciparum by Welch, P. Precox by Grassi and Felletti, P. Falciparum by Blanchard, and Hemozonas Precox by Ross. *Quid auctores tot nomina!*

NOTE 3.—It was hoped to reproduce Major Morgan's plates with this paper, but it has been found impossible to do so. The author invites the special attention of all medical officers of health interested in the subject to these plates, as he considers them the best hitherto published.

July, 1908.

REFERENCES.

1. Bruce. "Sleeping Sickness in Uganda." *Journal of R.A.M.C.*, Vol. III., page 17.
2. Bruce. "South African Stock Diseases." *Journal of R.A.M.C.*, Vol. VII., page 22.
3. Cantlie. President's Introductory Address, Section of Tropical Disease, Annual Meeting B.M.A., 1907.
4. Cantlie. "Tropical Ailments as they are met with in Britain." *B.M.J.*, June 22nd, 1907.
5. Castellani. *Journal of Hygiene*, Vol. IV., p. 508.
6. Clarke, J. Jackson. "Protozoa and Disease." London, 1904.
7. Daniels. Addendum on Malaria in Scheube's "Diseases of Warm Countries." John Bale, Daniellson, and Sons. 1903.
8. French, H. "Medical Laboratory Methods." London, 1903.
9. Grassi. "Die Malaria." Jena, 1901.
10. Harvey. "Note on *Spir. pallida*." *Journal of R.A.M.C.*, Vol. V., p. 409.
11. Koch. "La Malaria." *Rif. Med.* 1901, I. p. 505.
12. Koch. "African Tick Fever." *Mun. Woch.*, Aug. 22, 1905, p. 1620.
13. Laveran. "Paludisme." Rep. to 13 Int. Cong. of Med. Paris, 1901.
14. Leishman and Statham. "Development of L. Body in cultivation." *Journal of R.A.M.C.*, Vol. IV., p. 321. Also (Leishman alone) *ibid.*, Vol. III., p. 287.
15. Minchin. In Allbutt and Rolleston's "System of Medicine," Vol. II., Part II.
16. Manson. "Tropical Diseases." London, 1907.
17. McWeeney. "Protozoa and Disease." *Trans.*, Epid. Soc. Lond. N.S. Vol. XXIV., 1904-1905.
18. Novy and Knapp. "Relapsing Fever and Spirochaetes." *Ibid.*, Dec. 1, 1906.
19. Novy and Knapp. "Studies on *S. Obermieri*." *Journal of Inf. Dis.* Chicago, May, 1906.
20. Ray Lankester. "Treatise on Zoology (Protozoa)." London, 1902. And article, "Protozoa," *Encyclopæd. Britannica*.
21. Ross, Ronald. "Malarial Fever." Liverpool School of Trop. Med., 1902.
22. Schaudinn. *Arch. a. d. Kaiserl. Gesundheit*, Bd. XIX., 1903.
23. " " " Bd. XX., p. 169.
24. " " " Bd. XXII., p. 527, dated 10 April, 1905.
25. " *Deutsch Med. Woch.*, Oct. 19, 1905.
26. " "Selected Essays." New Sydenham Society, Oct., 1906.
27. " and Hoffman. *Berliner Klin. Woch.*, Vol. XLII., 1905.
28. Scheube's "Diseases of Warm Countries." London, 1903.
29. Stengel. "Text-book of Pathology." London, 1903.

Dr. T. M. CLAYTON (M.O.H., Gateshead) said that he agreed with the suggestion of Major Blackham that the subject of tropical diseases was one which should interest medical officers of health generally. He had studied particularly Trypanosomiasis, but generally he found a knowledge of tropical diseases useful even in the town of Gateshead, coming across such cases as malaria, leprosy, elephantiasis, etc. If, therefore, it was useful in Gateshead, it must almost amount to a necessity amongst port medical officers.

PROFESSOR SPOTTISWOODE CAMERON also took part in the discussion.

[The Proceedings of the Congress will be continued on page 413.]