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ORIENTAL SORE IN BAGDAD, TOGETHER WITH OBSERVATIONS ON A GREGARINE IN *STEGOMYIA FASCIATA*, THE HAEMOGREGARINE OF DOGS AND THE FLAGELLATES OF HOUSE FLIES.

BY

C. M. WENYON, M.B., B.S., B.Sc.,
Protozoologist to the London School of Tropical Medicine.

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By C. M. WENYON, M.B., B.S., B.Sc.,
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(*The Report of the Expedition sent to Mesopotamia in* 1910 *by the London School of Tropical Medicine.*)

With Plates XII—XVI and 36 Text-figures.

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Introduction.

THE present report is an account of observations made in Bagdad during the year 1910 (March to November) on the Oriental Sore as it occurs in this part of the world. Though the sore of Bagdad may not differ very markedly from that of other places, it is so common here that all stages and types of the sore may easily be studied, and such points as the early age of infection, immunity, and duration of the disease are very clearly exemplified. Accordingly, a more or less complete account of this disease of Bagdad, known locally as the Date boil or Arabic "Uchut," will be given, though descriptions of the sore as it occurs in several parts of the world have been published by other observers. During the investigations various side issues arose, and as these suggest certain interesting points, three sections have been added dealing with (1) the life history of *Lankesteria culicis* (Ross) a gregarine of *Stegomyia fasciata*, (2) *Haemogregarina canis* and its development both in the dog and the tick *Rhipicephalus sanguineus*, and (3) *Herpetomonas muscae domesticae*, and other flagellates of the house-fly.

During the first six months in Bagdad, I derived great help from my assistant Mr Stremes, but early in September there occurred a deplorable accident which resulted in the death of Mr Stremes, and the destruction of part of the laboratory by fire, together with the bulk of the

apparatus and outfit, and a large part of my collection of flies. No sooner was work resumed after the delay caused by this accident, than an epidemic of cholera completely disorganised my plans and put a stop to certain experiments I was about to conduct with the sand fly, *Phlebotamus*. Still, the results obtained as far as they go are of interest and will be found below in the following sections.

I. Oriental Sore in Bagdad.

Plate XIV.

A. *The disease as it occurs in man.*

As it occurs in Bagdad the disease is essentially one of childhood, and just as in England all children are expected to suffer from measles, so here the sore is looked upon as the natural portion of childhood. Being a disease which is accompanied by little or no constitutional disturbance, and in consequence by no death rate, it is not feared except for its inconvenience and the possibility of its leaving a disfiguring scar upon the face. The sore first makes its appearance as a rule, between the ages of one and three, and the face of the little child with one or more sores varying in size from a sixpenny piece to half a crown, over which are constantly feeding swarms of flies, is one of the commonest pictures of the Bagdad streets. Younger children may contract the disease, and I have seen the sore on the cheek of a baby seven months old,—but apparently there is little chance of the child becoming infected till it begins to run about and assert its independence in refusing to be completely covered up as is the manner of dealing with very young children in these countries. It occasionally happens that a child escapes infection and does not get the sore till later in life. Such cases are exceedingly rare, even if the evidence on which they are based is reliable. I have seen natives of Bagdad who have lived there all their lives, who declare that they never have suffered from the Bagdad sore. However, as the sore may be nothing more than a small papule of not greater diameter than five millimetres, and as it may never exceed this measurement, its presence could easily have been ignored. Though it is possible that a native of Bagdad may completely escape infection, it still remains a fact that the vast majority of the population contract the disease when young, and between the ages of one and three.

One constantly encounters cases of the sore in adults, but, with the very few possible exceptions just mentioned, these are in people who

have come to Bagdad from other districts in which the sore does not occur. Apparently the inhabitants of the smaller villages round Bagdad and the nomad Arabs living in encampments, are not so liable to the disease as those who dwell in the town. When these people come to Bagdad to seek employment, they contract the disease and furnish a good proportion of the adult cases met with. Europeans almost invariably become infected within a year or two after their arrival though in rare cases they may escape the disease for years, if not entirely.

All classes of the population are infected alike, and the child of the rich and well-to-do seems as liable to infection as the children brought up in the dirt and squalor of the most insanitary parts of the town. The children of the Europeans living in Bagdad generally suffer from the disease, and no matter what a person's nationality, a sojourn in Bagdad of a very few months or even weeks, is liable to result in a Bagdad sore which will leave its mark in the shape of the permanent scar, known locally as the "date mark." Such scars of varying size and extent are to be seen on the faces of the greater part of the population.

Seasonal prevalence of the disease. The sore may make its appearance at any time of the year, but it is a well recognised fact that the autumn, the time of the ripening of the dates, at the close of the long spell of hot weather, is the season when the majority of sores first develop. It is this fact which has given rise to the local name of "date boil" and "date mark" and to the hypothesis which attributes the sore to eating dates or even to injuries inflicted by the thorns of the date palms. The occurrence of the disease in early childhood does away with any such hypothesis. The month of September is the first month of the "date boil" season, and during this month and the two succeeding months, the majority of new cases occur. The frequency of onset at this particular season must certainly depend on some parallel variation in the occurrence of some biting fly or infecting agent responsible for its transmission. It is quite common for the sore to make its appearance at some other time of the year, but such cases may be instances of prolonged incubation period. It is evident that infection usually takes place during the hot months of June, July and August and with an incubation period of two to three months, the sore would appear, as it does, in the autumn. Cases are known where there is a much longer period of incubation, and the sores which develop at other seasons may be of this nature. It is possible that infection always takes place in

the summer months. This of course refers to the first inoculation, as secondary inoculations or autoinoculation may occur at any time of the year by the scratching of an already existing sore and the transference of some of the organisms to an abrasion on some other part of the body.

Position of the sore on the body. The strict limitation of the sore to exposed surfaces of the body has been a long recognised fact. This is one of the chief arguments in favour of insect transmission of the disease. In very young children less than twelve months old, one rarely encounters a sore except on the face, a fact probably dependent upon the habit the people have of wrapping infants up so that the whole body is covered with the exception of the face. At an earlier age the face is usually covered also, so that the rarity of sores in children before the age of twelve months is easily explained. In older children the sore commonly occurs on the arm or leg, below the elbow or knee, though the face is still the most usual situation. In adults, almost invariably new comers, the disease appears with about equal frequency upon the face, arms, or legs. It is rare to find a sore on any other than the exposed parts of the body. I have seen a case in a boy about five years old where there was one sore on the buttock, and a second over the sternal end of the second left rib, and in another child age about three years, a sore near the umbilicus. I have been told of cases where "sores" (?) occurred in numbers all over the body, but it must be doubtful if such are true sores (due to *Leishmania*) as the disease can very readily be confused with other conditions, such as syphilis.

Upon the face the sore appears usually upon the cheek or forehead. It commonly occurs on the end of the nose, producing an unsightly condition while it lasts though the permanent scarring is not in proportion to the temporary disfigurement. The sore more rarely attacks the lips, eyelids, and ears. I have not seen a case of sore on the scalp, neck, or on any of the mucous surfaces. On the arm the disease may appear on the elbow over the olecranon process, a position in which the sore is often very painful. More usually it is below the elbow and frequently occurs upon the fingers, producing a great enlargement of these. On the leg the favourite site is on the outer side just above the ankle joint.

This practically constant appearance of the sore on the exposed surfaces of the body can hardly be explained by any other assumption than that the disease is transmitted by some biting insect or fly which

feeds upon these exposed surfaces in contradistinction to such elusive insects as body lice, fleas, and bed bugs which generally suck their blood under the protection of clothing. In this connection however one must not forget the recent experiments of Basile on the flea transmission of canine leishmaniosis.

Number of sores on each individual. It is probably safe to say that the single sore is most commonly encountered, and this one may be on the cheek, lip, nose, eyelid, forehead, arm, hand, leg or foot. Two, three, and even four sores are by no means uncommon, but cases with larger numbers are certainly rare. It often happens that during the growth of a single sore secondary sores will develop on some exposed surface, either near the primary one, or further away. Such secondary sores are most likely subinoculations from the first. A child with a sore on its face will almost certainly inoculate any wound it may have upon its hand, and any slight abrasion of skin upon the face will be infected by the swarms of flies that are constantly walking across the sore. Some of these secondary sores may however be the result of a normal infection. I have seen the case of a child about 12–18 months old, where there were eighteen sores upon the face, one on each of the limbs except the left arm which had two, making a total of twenty-three sores. I have heard of cases where the numbers have been even greater, but the character of the sores in these cases had not been established by microscopical examination. In the case of this child with twenty-three sores, it is interesting to note that they were all limited to the exposed surfaces of the body. If the disease is the local manifestation of a general infection, it is difficult to explain how the sores limit themselves to the exposed surfaces only in these cases of multiple infections. One would expect that at least some would appear upon the body. On the other hand, it is not quite clear that each sore can be a separate inoculation by some fly, though in my opinion this is the more probable explanation.

Incubation period. On the subject of the incubation period it is difficult to obtain precise information from the observation of natural infections. The youngest child I have seen with a sore was seven months old. Cases of Europeans becoming infected within two months after reaching Bagdad, are not uncommon, and I have a reliable history of a case in which two red spots appeared upon the feet a fortnight after arrival. The subject of these sores was badly bitten upon the feet while sleeping on the roof without a mosquito net. All the marks produced by the bites disappeared except the two red spots mentioned.

These persisted for upwards of a year, and in them I was able to demonstrate the typical parasite. As a rule however the incubation period is longer and the sore may make its first appearance many months after a person has left the infected district. It is probable that the time elapsing between inoculation and the appearance of a noticeable sore is very variable and may depend on several factors, such as a person's natural resistance to the disease, the state of health at the time of inoculation, or the virulence and numbers of the parasite inoculated. From the first moment of inoculation the parasities must have been multiplying in the skin at the point of infection, and it is only when their number is sufficiently great that a visible change is produced on the surface.

From experimental inoculations more precise data can be obtained, though even here one must exercise caution in drawing conclusions, as the natural manner of infection may produce a sore more slowly or more quickly than by the inoculation of parasites from man to man. I inoculated a European lady on the left forearm with the juice squeezed from a sore on the arm of her husband. By microscopical examination, the juice was shown to contain numerous parasites. The inoculation was done by placing the juice on the skin and scarifying with a sharp instrument as in vaccination against smallpox. The wound caused by the scarification completely healed, though the mark or scar persisted and no definite development took place for some time. At the end of seven weeks from the time of inoculation, there was present at this spot a typical sore in the form of a small papule of about five millimetres in diameter. Later there appeared on the other arm two small sores which were probably the result of a natural infection if we remember Dr Saati's experiments. Though in this case the mark of the wound caused by the inoculation never completely disappeared, this is not usually the case as will be seen from the following interesting information on the subject of inoculation given me by Dr Saati of Mousul, who has practised protective inoculation for some time. He has been in the habit of inoculating people with the juice of the sore as in vaccination against smallpox, on the outer side of the leg. The sore thus produced heals quickly and finally all trace of the inoculation disappears. At the end of two months, or occasionally it may be three or four, there appears at the point inoculated a minute red papule which increases in size till it assumes the form of a typical sore. Dr Saati has inoculated about three dozen cases, and in the greater number of these the incubation period has been about two months. It is important to note that the

inoculation always produces only a single sore, and that at the site of the inoculation. It then may be accepted that the incubation period is approximately two months.

Character and development of the Bagdad sore. It is generally believed by the natives of Bagdad that there exist two kinds of sore which are designated male and female. Clinically this is true, for as the types of two extremes there exist the ulcerating and the non-ulcerating sore. The ulcerating as well as the non-ulcerating form of the disease commences as a minute red papule. This has a characteristic dusky red colour, which takes on a slightly brownish red tint, as it increases in size. It is only slightly raised at this stage, and does not produce any surrounding inflammation of the skin as does the common acne spot. In accordance with its chronic nature, it produces little or no irritation. It is not tender to the touch and would probably escape observation if it did not appear on a visible surface of the body. The original papule increases slowly in size and may finally follow one of two courses, which results in it being either a male or female sore. In the female sore the central portion breaks down leaving a shallow, indolent ulcer, from which exudes a yellow fluid in which are to be found the sore parasites and the numerous bacteria with which these ulcers become secondarily infected. This ulcer gradually extends till in severe cases a large area of skin may be involved. In one such case encountered the ulcer occupied the whole of one cheek, had destroyed the lower eyelid of this side, and extended across the bridge of the nose to the other cheek. Such extensive ulceration is however uncommon, the ulcers, as a rule, not exceeding the area of a five shilling piece. When small these ulcers frequently scab over with the dried up exudation. The scab is easily removed, and if allowed to remain, only serves to retain pus and make the ulcer painful by producing a surrounding inflammation. While extending in one direction the ulcers may heal at another part, producing a condition resembling that of a lupus. The surface of the ulcer is covered with granulations varying in colour according to the extent and nature of the secondary bacterial infection. The granulations readily bleed, and a scraping from the surface reveals a mixed infection of the typical parasite, bacteria and yeasts. One never obtains the specific parasite in such great numbers in these mixed infections. I have encountered no case of spirochaete infection in these ulcerating sores.

The second type of sore known as the male sore commences in the same manner as the female sore. The papule however increases in

size and does not ulcerate. The superficial layers may form into a dry scaly covering, which breaks away leaving a thin red skin beneath. If the scaly covering is forcibly removed, the skin may be broken showing a mass of red granulation tissue from which a pure culture of the parasite can be obtained. The granulations thus exposed soon scab over, and if left undisturbed the sore shows no tendency to further ulceration. Eventually the sore shrinks and becomes less elevated, till finally there is nothing left but a dusky red patch on the skin which gradually changes to a thin, white scar, very slightly depressed. The scarring left by the non-ulcerating male sores is never so extensive as that produced by the ulcerating variety in which the secondary bacterial infection has brought about a greater destruction of tissue. It must be remembered that the two types of sore just described are connected by intermediate forms, and a sore which has been progressing as a male non-ulcerating sore may suddenly break down and ulcerate. There is nothing in the character of the parasites to be obtained from the two types of sore that would lead one to suppose that there is any specific difference between them.

It is very rarely that any deformity results from the contractions of the superficial scars or date marks. In the case mentioned above in which there was extensive ulceration on the face, the lower eyelid was completely destroyed, so that considerable deformity would be bound to result. But such a case is very exceptional.

Duration of the disease. The duration of the disease is usually about one year with a variation of three months on either side of the limit. The advent of ulceration and the infection with extraneous organisms have little effect on the life of the individual sore. The male and female sores are of about equal duration.

Immunity. It is a general rule that after the sore has healed and disappeared the person is protected completely from further attack. I have been told of persons who have had a second and even a third attack with several years intervening between each. If such cases occur they must be extremely rare. The consensus of opinion amongst the inhabitants of Bagdad is that a second attack is impossible. The fact that sores in adults who have always lived in Bagdad are never seen, is a sufficient proof that the infection of childhood has conferred a life long immunity. As mentioned above, it is frequently the case that a second sore will commence during the course of the first. Such secondary sores may be simply an auto-infection. As it is possible to inoculate the parasite by simple scarification in the presence of juice from a sore, it

must certainly happen that a child will inoculate itself at some other place by conveying the organisms on its fingers. Such infection could also very readily be produced by the house-fly, though it has not been demonstrated experimentally. These secondary sores are liable to occur only because the immunity against the sore parasite is not complete. Sometimes the secondary sore will develop towards the closing period of the first. It is difficult to explain such cases on the theory of immunity. If it is an immunity which has caused the first sore to disappear, then one would expect this to be sufficient to prevent the development of the secondary sore. However, it is possible that the virulence of the parasites of the first sore has become so attenuated that they cease to multiply, while the freshly inoculated parasites of the secondary sore are of sufficient virulence to withstand the partial immunity which dissipated the first. I have the following history of the disease in a European: A sore appeared on the right wrist. This followed the usual course and disappeared in about one year. Soon after the disappearance of the first a second sore appeared on the left elbow. This persisted for a similar period, after which no more developed and the person has remained free since. In other cases a sore will develop up to a certain stage and then commence to shrink and show signs of disappearing, and just when the patient is congratulating himself that the trouble is nearly over, it will break out again, extend and exceed its original dimensions. With these exceptions, one single attack lasting about a year is the rule, while the healing of the sore appears to be brought about by an immunity against the specific organism acquired by the person infected; such an immunity being sufficiently lasting to protect the person from further attack for the rest of life.

Symptoms. Apart from purely local symptoms due to the sore, it is impossible to trace any constitutional disturbance. The fact that nearly all sores are in very young children renders the detection of such symptoms difficult, as any slight disturbance in health can at this age be attributed to the common ailments of childhood. The onset of the disease is so insidious that the first papule is not considered to be a true sore till its persistence compels this view to be taken. Mild fever, feelings of malaise or intestinal derangement, or other slight symptoms which might be supposed to accompany such a benign infection, would most probably be looked upon as one of those slight disturbances to health so common in all hot countries. In the case of the child with twenty-three distinct sores, recorded above, there was nothing to indicate, apart from the sores, that the child was in any way abnormal.

It has been suggested that this disease is a general one, and that a sore is merely the local manifestation of a general infection as in the case of syphilis. If this is true, there is however this difference; whereas in syphilis the chancre indicates a severe general infection accompanied by various definite symptoms, and a corresponding distribution of the specific parasite throughout the body, the sore is accompanied by no such constitutional disturbances, or such as are too slight to be detected, and the sore parasite is limited to the sore and does not extend to other parts of the body.

The local symptoms are due to the presence of the sore. In the early stages the papule may cause slight irritation and itching, and be feebly tender on pressure. There is only slight surrounding inflammation of the skin, except in some of the cases, with a secondary bacterial infection. There is little pain, and the sores are not remarkably tender unless it has formed over a bone such as the tibia or olecranon process. The discharge from the ulcerating sores is often very foul and exceedingly unpleasant, not only for others, but for the patient himself, who frequently is unable to remain in a room, but has to seek refuge in the open air. Occasionally, in septic sores, there is pain and tenderness in the lymphatic glands receiving the lymphatics from the infected area of skin.

The impossibility of performing autopsies on human beings in Bagdad is a misfortune, as from this source much light might be thrown on the disease. It is very important to know whether the parasites are to be found in the internal organs as in Kala azar, or whether they occur in the lymphatic glands. Without autopsies it is impossible to decide these questions. Repeated examinations of the peripheral blood of infected persons have failed to confirm the observations of Neumann made on a single case under his care in Heidelberg, that parasites occur occasionally in the circulation. Equally unsuccessful were the attempts to obtain cultures by inoculating tubes of blood agar with blood taken from the finger or ear of persons suffering from the sore.

Treatment. Very little can be done in the way of treatment and though many medicaments have been tried, they have no appreciable effect on the duration of the malady. Excision of the sore has been attempted, and with success. The sore must be treated as a malignant growth and removed entire with a good margin of healthy flesh, great care being taken to prevent contamination of the wound by the organisms of the sore. Such a procedure is hardly practicable on the face and would probably lead to more extensive scarring and deformity than if

the sore ran its natural course. One great disadvantage of such a treatment is, that to obtain good results the sore must be excised at a very early stage of its growth, and this would be before the immunity against the organism had been acquired and would leave the patient open to further infection.

Various ointments and lotions have a certain reputation amongst the inhabitants of Bagdad. The natives plaster the sores with strong solutions of indigo, or with the green alga which they scrape from the inside of the "hubs" the native earthenware filters. This continual application of medicaments is often continued till there is formed a large, dry crust over the sore which has the effect of shutting in the exudation and bringing about extensive suppuration. Judging from the difficulty of carrying on a culture of the sore parasite in blood agar in the presence of bacteria, it might be supposed that the infection of a sore with extraneous organisms leading to suppuration might have the desired effect of killing off the parasites. Though the suppurating sore does not disappear any more quickly than the non-ulcerating type, it is a fact that the specific parasites are to be obtained in much greater numbers from the latter. Dr Saati tells me that a saturated alcoholic solution of methylene blue has sometimes an advantageous effect. It does little to cut short the disease, but tends to keep the sore dry and free from ulceration. What can be the action of the methylene blue in these cases it is difficult to say. Very possibly the dryness is merely the hardening effect of the alcohol. The French authorities in N. Africa have obtained beneficial results by the dusting of the sore with permanganate of potash.

While in Bagdad I was in the habit of giving the yellow mercury ointment as an attractive medicament. It had no effect other than the cleansing of the sores, for I insisted that a careful washing of the sore was necessary before each fresh application. When once a sore has developed, the best course to adopt is to protect it from irritation, as this may start the unpleasant process of ulceration. It must be kept clean, and thus undisturbed, it will run the ordinary course for a year producing an immunity which will protect from further attacks.

Prevention. Unless the mode of infection is definitely determined, it is difficult to lay down rules for prevention. In all probability—and this is supported by the observations that the specific parasite will develop into flagellates in the gut of both the bed bug and a mosquito—the disease is conveyed from person to person by some insect which feeds upon exposed surfaces of the body. During the hot months of

the summer, it is the custom in Bagdad to sleep at night on the flat roof tops, and in the afternoons in the Sirdarbs, which are dark cellars partially below the ground. Both in the Sirdarbs and on the roof, people are liable to be bitten by all the biting flies found in Bagdad. An ordinary mosquito net will protect against any biting insects, but the *Phlebotamus*, which is able to pass through all but the finest mesh. In order to avoid infection it is essential to protect oneself against flies which feed upon one when asleep. The common house-fly is probably at least a mechanical carrier of the disease, and every care must be taken not to sleep in the day time without some protection against these insects which are constantly swarming over the exposed sores on the faces of the children. Any open wound on the face or hands is liable to be infected, and such should be carefully protected from the flies. In the case of children care must be taken to prevent them from inoculating themselves in other places by scratching the sores.

In a town like Bagdad where the disease is so common, though one may take every precaution it will be difficult to avoid the disease if one remains there any length of time. In such cases the most reasonable course to adopt is that of preventive inoculation. Such an inoculation will enable the person to choose the site of the sore and so avoid the inconvenience and risk of contracting the disease on the face with its resulting scar. The simple procedure of inoculation on the leg or arm would prevent the unsightly scars which one so commonly sees on the faces of the Bagdad inhabitants. Of course, in some cases sores would develop at other places than that chosen for inoculation, but such would be the result of a natural infection which had taken place at about the time of the inoculation. A protective inoculation of this kind would lessen very considerably the number of people having sores on exposed surfaces of the body.

This diminution in the number of sores on the exposed surfaces of the body, would in all probability have an important bearing on the spread of the disease. A sore on an exposed surface of the body, such as the face, is always an attraction to the myriads of flies which are to be found in localities like Bagdad. A sore on an unexposed surface would be free from these attacks and would not be a possible source of infection for others. With biting flies, such as mosquitoes, it is a little more difficult. If a biting fly is the agent of transmission, does this fly take up the parasite from the circulation or from the sore itself?

Though Neumann has found parasites in the peripheral blood on rare occasions, my repeated examinations in Bagdad have failed to

reveal these in the circulation. Attempts at culture from the peripheral blood have also failed. Hence it may reasonably be assumed that it is only rarely that the parasites are to be found in the general blood stream. There is nothing in the form or nature of a sore on the face, and especially in its early or non-ulcerating form, to make it repulsive to a mosquito. As will be shown later mosquitoes readily feed on the non-ulcerating sores, often preferring the thin red skin over these, to the tougher normal skin at the side. Mosquitoes feeding in this way may take up large numbers of parasites, and often, intact, the large mononuclear cells fully infected. Hence it is probably safe to conclude that if this disease is transmitted by a biting fly, this fly becomes infected by feeding from the sore directly, though much less frequently it may become infected by ingesting the scanty parasites from the peripheral circulation. It is thus very desirable to protect the sore against the attacks of the house-fly and any biting flies, and the simplest method of achieving this end is to develop the sore on some unexposed surface of the body by inoculation before infection takes place in the natural way. Protective inoculation in the way here advocated will not only protect the person from the visible deformity of a natural sore, but it will prevent him being a source of infection to others. By following out this plan on a large scale, it is not improbable that the disease could be very greatly diminished if not entirely stamped out. Till we know exactly how the sore is acquired, naturally such a line would be experimental, but it would be an experiment well worth carrying out and with every prospect of success. The inhabitants of Bagdad are accustomed to vaccination against smallpox and inoculation with sore virus has been done in an irregular manner, but generally on an exposed part of the leg. It would of course be necessary to inoculate on an unexposed surface. The promise that this would probably protect from a sore and scar on the face would in itself be a sufficient attraction.

Great care would of course have to be exercised in obtaining the virus from a reliable source without there being any chance of the conveyance of syphilis or tubercle at the same time. Should the cultures of the parasite in blood agar produce a sore which confers immunity, this would be the best source from which to obtain a virus. Recently Nicolle and Manceaux have been successful in inoculating the sore to men by employing artificial cultures, but we do not know if such sores will result in immunity against the natural infection. As pointed out above, inoculation with juice from a sore is readily effected by proceeding as in vaccination against smallpox. The usual course is the complete

disappearance of the scarification wound and the appearance of a sore in about two months at the point of inoculation. Inoculation at one spot does not produce sores at any other.

B. *Examination of the lower animals for signs of the disease and inoculation of these from man.*

Dogs. The commonest domestic animal brought into close association with man in Bagdad is the dog, which lives in a semi-wild condition in the streets and feeds on the refuse thrown out from the houses. Since in North Africa the dogs have been shown by C. Nicolle and C. Comte to harbour the parasite of infantile Kala azar, it was thought that dogs in Bagdad might in the same manner harbour the parasite of the Bagdad sore. There is moreover a general opinion that the dogs suffer from the sore about the nose or eyes. Cases of dogs with these supposed sores on the nose I had some difficulty in finding, as they were not nearly so common as they were said to be. Several cases were discovered, but unfortunately in none of these could the parasite be found. One large dog had a condition of the legs which was said to be the sore, but repeated examination of this failed to reveal the parasite. All the cases were in pet dogs living in private houses. The lesion on the noses of most of the dogs examined did not resemble the sore in man. They were more of a papillomatous nature and much harder to the touch than the true sore. They did not show signs of ulceration. In one small dog I saw a condition of the nose reminding one much more of the true sore. This was an ulcer scabbed over. The ulcer when I saw it had been in existence nine or ten months. Unfortunately, I was unable to examine this dog, which was also a pet in the house of an English resident.

Recently the authorities of Bagdad have followed the example of those of Constantinople in taking steps to exterminate the dogs from the streets. Accordingly, all street dogs have been collected and herded together in an enclosure in the desert where they are kept in a condition even less sanitary than that in which they lived before. Amongst these dogs there was a great mortality owing to lack of food, and I obtained permission from the authorities to perform autopsies on the dogs that died. I performed autopsies on a series of one hundred and ten dogs which I examined carefully for any signs of the sore about the nose or eyes. Where any ulceration occurred smears were made from scrapings of the sore. These smears together with portions

of the liver, spleen, and bone marrow of each dog, were taken back to the laboratory where they were examined microscopically for the typical parasite of Kala azar or the sore. In no instance was I successful in finding the Leishman-Donovan bodies, so that if the dog acts as an alternative host for the sore parasite, as it apparently does for the parasite of infantile Kala azar in North Africa and elsewhere, the percentage of infected dogs must be very low indeed and quite out of proportion to the number of cases of sore occurring in man. If the dog is an alternative host, one would expect to find the number of infected dogs approaching the number of infected human beings. The fact that no typical sore was found in any of these dogs throws no light on the question as to whether the Oriental sore is a purely local skin affection or a general infection representing a mild variety of Kala azar. At any rate, we can safely say that infections of this kind if they occur are very rare amongst the street dogs of Bagdad, a fact that does not lend support to the view that the dog acts as a reservoir for the parasite which is carried from them to men by some biting insect. The number of infected human beings is so great that they themselves act as centres of infection for others, and it is not necessary, in order to account for the incidence of the disease, to assume that the dog fulfils the rôle so often attributed to it. Though the dog may not act in this manner, it is possible that it may sometimes suffer from the sore in the ordinary way. However, such a case has not come under my notice.

By banishing the dogs from the streets, the Bagdad authorities have unknowingly carried out an interesting experiment. Assuming that the dogs act as a reservoir for the sore parasite, then their removal should bring about a great diminution in the number of human beings infected. The result of this experiment can be only apparent after some time.

The examination of this series of one hundred and ten dogs has shown that nearly every one is infected with the leucocytic haemogregarine first found by Bentley in India. The reproducing forms of this parasite were to be found in the spleen and bone marrow and in the ticks taken from the dog. The development of this parasite will form the subject of a later section of this report.

Piroplasmosis is common amongst the Bagdad dogs, which are generally in a very filthy condition and covered with fleas, ticks, and flies belonging to the group of the *Hippoboscidae*.

Inoculation of dogs. All attempts at inoculating dogs with the disease have failed. Dogs of all ages were used, from very young

puppies to full grown dogs. It was impossible for the young puppies to have suffered from the disease, so that the ordinary dogs of the Bagdad streets must have a natural resistance to the disease as inoculated experimentally. The inoculations were carried out in various ways. Some of the dogs were inoculated by scarification and application of fluid from a sore; others were inoculated into the skin with the hypodermic syringe. They were inoculated both on the skin of the nose, on the inner surface of the thigh, or on a shaved patch on the back. In none was a sore produced. In two dogs, in one case on the nose and in the other on the leg, a superficial ulceration was produced at the point of inoculation quite unlike a sore and in which the parasite could not be discovered, even after repeated examination. This failure to infect dogs with the disease is strange after the successful inoculation of dogs by C. Nicolle and L. Manceaux in North Africa. These observers have found that dogs become infected about the nose after an incubation period of thirty-six days. The disease however is not of long duration, so that it is possible that my failure to inoculate means that the Bagdad dogs are naturally immune in a place where the disease is so common in man. Inoculation of dogs from artificial culture of the sore parasite was equally unsuccessful.

Cats. Cats, like the dogs in Bagdad, though less numerous live in a semi-domestic condition. Only one of these animals was examined, and that with a negative result.

Rats. Rats were exceedingly common and a great pest. The only protozoal parasite found in the blood or organs was the common *Trypanosoma lewisi* and the leucocytic haemogregarine. Inoculation of numbers of rats with material from the sores and blood agar cultures has given no result.

Rabbits. Similar experiments conducted on rabbits have given no result.

Birds. Sparrows and two small owls were likewise inoculated without success.

Ticks. An attempt was made to obtain a development of the sore parasite by inoculating the large blown-out ticks taken off dogs. The ticks survived the operation of inoculation, which was made with a fine needle into the abdomen, but no trace of any developmental forms of the parasite could be found when the contents of the ticks were examined microscopically.

Other domestic animals in Bagdad are the horse, mule, donkey, and cattle. Camels are rarely seen in the town, and then only in passing

through from one gate to another. It is unlikely that any of these animals have an influence on the disease as it occurs in Bagdad. It is popularly believed that not only dogs but other animals and even birds, such as canaries, may suffer from the disease. It has become a custom to talk of any curious and inexplicable skin lesion in any animal, as a sore. This is merely a superstition and is not supported by microscopical examinations. In fact in human beings many skin conditions are erroneously considered to be the sore. This is especially true of a semi-chronic ulcer from which white people commonly suffer. It is easily distinguished however from the fact that it is more painful and tender, is surrounded by a greater area of inflammation and that it exudes a yellow pus. The organism of Oriental sore cannot be found in the scrapings, but a diplococcus much resembling the gonococcus in appearance is constantly present.

The failure to obtain an animal suitable for inoculation experiments renders the investigation of the disease much more difficult. If transmission experiments could be carried out with biting flies as in the case of trypanosomiasis, one could more readily arrive at results. In the case of the sore all such experiments have to be carried out on man, and it is difficult to find subjects suitable and willing. In want of these experiments the following course was adopted. Flies of various kinds were fed upon the sore and these were dissected after varying intervals and search made for developmental forms of the parasite, a procedure which is much more difficult and full of pitfalls than the experiment of transmission. In the search for developmental forms one is guided by the changes in form undergone by the parasites during their evolutions in the blood agar culture.

C. *Examination and dissection of flies and ticks.*

There are of course in Bagdad a large number of arthropods any of which can be looked upon as possible transmitters of the parasite of the sore. In order to obtain some idea of the intestinal fauna of these arthropods, a dissection of such as could be caught was undertaken. This was a necessary preliminary to any feeding experiments that were to be made.

House-flies. These occur in great numbers as in all oriental towns. They appeared to diminish in numbers to some extent during the hottest part of the summer when the maximum shade temperature reached 110° F. The great heat combined with the dryness of the

atmosphere seems to have a deleterious effect upon them. When the temperature is not so high the flies abound and are constantly swarming about the faces of the children and more especially those made attractive by the sore. Such flies collected from the face of a child suffering from an ulcerating type of sore are found to have the intestine filled with the exudation of the sore in which the parasites can readily be found. This was only to be expected, as any fly feeding on the sore is bound to take up large numbers of parasites. Such a fly feeding immediately afterwards upon some fresh abrasion of the skin must certainly in a number of instances inoculate the sore parasite.

On carrying out the dissection of house-flies, one was not surprised to find that a certain percentage of these had an intestinal infection of *Herpetomonas*. In some instances these appeared to be *Herpetomonas muscae domesticae*; in others there occurred a smaller flagellate, also of the *Herpetomonas* type. In a certain number of flies the malpighian tubes were infected with flagellates which were of the trypanosome type with the kinetonucleus on the non-flagellar side of nucleus. A description of these flagellates will be found in another section. Whether these various flagellates represent different stages of one parasite of the fly is a point difficult to settle, unless one undertakes special experimental work to this end. The smaller forms of herpetomones from their resemblance to the cultural forms of the Leishman-Donovan bodies might readily be taken for similar developmental forms in the gut of the fly. Judging from experiments conducted with house-flies it is improbable that the *Herpetomonas* are related to *Leishmania*. The intestine of the fly was filled with all kinds of débris intermingled with bacteria of many kinds.

Stomoxys. From the time of my arrival in Bagdad in March till the hot weather had set in in June, these flies occurred in fair numbers, especially in the neighbourhood of the stables. They often settled upon one and were mistaken for house-flies till the pricking of the proboscis revealed their nature. They would frequently bite about the ankles, especially through dark socks. With the advent of the really hot weather these flies almost completely disappeared and could rarely be discovered even in the stables, their favourite haunt. The laboratory where these investigations were conducted was situated directly opposite a stable, and here during the cooler months it was an easy matter to capture forty or fifty of these flies in a very short time. During the hot months of July, August and September, a whole morning's search would fail to yield a single specimen.

These flies feed naturally upon the horses, donkeys, and mules, so that blood from these animals is found in their intestines. *Filaria* larvae were often encountered, and occasionally in the hind gut a flagellate of the *Herpetomonas* type. *Stomoxys* occasionally was caught feeding on the faces of children with sores, but in no instance were the parasites found in the intestine of such flies.

Hippoboscidae. Flies belonging to this group are to be found commonly on the horses. Another species is constantly present on the dogs, where it lies concealed in the fur till disturbed, when it will leave its host, or fly to hide on some other part of the body. These dog flies will bite human beings, but this is an uncommon occurrence. I was bitten only on two occasions though I lived in close association with a number of dogs, all of which had many of these flies about them. Numbers of the flies were dissected from time to time, but none of these were found to be infected with flagellates.

Tabanidae. Flies belonging to this group I have not encountered in Bagdad, but I have seen one taken a mile or two out of the town.

Fleas. Fleas are a great pest, and especially at a certain season of the year which is popularly known as the flea season. They occur in greatest numbers in the month or two before the commencement of the summer season of intense heat. They are constantly on one's person during these months and are a continual worry to anyone who is susceptible to the irritating substances they inject. Dissection of fleas has not revealed any flagellate infection. If fleas are responsible for the transmission of the sores as Basile finds they are for canine leishmaniosis then with an incubation period of two months the greatest number of sores should appear in June or July. This is not so however as the greatest number of sores appear in the autumn.

Body lice. These are found commonly on the bodies and clothes of the dirty portion of the Bagdad population, and many were dissected without result.

Ticks. Ticks are found on practically all the domestic animals. They however rarely attack man. The dogs in the streets are usually covered with them. Dissections of these ticks taken from dogs have disclosed no flagellates but on several occasions a larval *Filaria*, probably *Filaria immitis*, was found in the gut and frequently the developmental stages of the leucocytic haemogregarine of the dog, a description of which will be found below in another section.

Bed Bugs. These occur in Bagdad, but in no great numbers. They can usually be obtained fairly early in the prison where they hide in

the mats covering the floors or upon which the prisoners sleep. In only one other house have I heard of the occurrence of bed bugs. It is certain that Bagdad is not infested with bed bugs to anything like the extent of some other Eastern cities. This statement is borne out by the evidence of the European residents, who would readily detect the presence of these pests even if they passed unnoticed by the native; Europeans generally believe that the bed bug does not exist in Bagdad. It was only by instituting a careful search and enquiry that I discovered their whereabouts in the prison. It is possible that the dryness of the atmosphere combined with the intense heat of summer is unfavourable to their extensive development and spread.

Owing to the incriminating evidence brought against the bed bug by Patton in the demonstration that the parasite of Kala azar develops into *Herpetomonas* in its gut, this insect was looked upon with suspicion and was made the subject of a careful enquiry. Firstly, numbers of bed bugs were dissected as they were taken from the prison. Many of them had recently fed upon human blood, while others had not fed for varying intervals judging from the condition of the intestinal contents. In this way seventy-two bugs were dissected, smears were made of the intestinal contents and examined microscopically for flagellates. In none was I successful in finding any protozoa. This result is important in the light of the results of the experiments to be detailed below.

Sand flies. This name is used in Bagdad for any small fly that bites at night. The fly most usually described under this name is a *Phlebotamus.* It is able to pass through the meshes of the ordinary mosquito net from which it may or may not escape after feeding in the morning. To protect oneself against the bites of these insects, it is necessary to use fine meshed nets. Unfortunately, when I wished to commence experiments with these flies, very few could be obtained, so that no definite result was arrived at. This is to be deplored, for it is possible that this fly may be the transmitting agent of the disease. The delay in these experiments was caused by the unfortunate fire that took place in my laboratory, and by the outbreak of cholera which put a stop to a scheme I had planned for continuing the work. However, I hope to be able to continue these experiments at a future date.

Mosquitoes. These occur, as would be expected, in large numbers. In some years the Tigris overflows its banks and runs into the desert around Bagdad, forming pools and marshes. Under these conditions mosquitoes are said to be much more numerous than I have seen. The

mosquito fauna of Bagdad differs very much from that of Busra, which is situated near the mouth of the river. Here anophelines are very common, and in consequence there is much malaria. In Bagdad on the other hand, I did not see a single specimen of an anopheline or malaria carrier, and all the cases of malaria that I met with had come from Busra or some other town in which malaria occurred. I was told on good authority that anophelines may occur in Bagdad and that cases of malaria contracted there are sometimes encountered.

Before the hot weather had commenced from March to June, various species of *Culex* were common. Amongst these was the common *Culex fatigans*. With the advent of the hot weather the number of *Culex* apparently diminished, while another mosquito, which I had not met before, began to make its appearance. This was *Stegomyia fasciata*. As the summer advanced the numbers of this voracious insect increased till it became a constant nuisance. It lives in the houses and hides in any dark corner, especially in the Sirdarbs or semi-underground rooms into which one retires during the hottest part of the day. It is most persistent in its attacks so that one is unable to escape from its ravages unless one is protected by a mosquito net. Both the male and female are fond of alighting on the skin, but though the male apparently makes attempts to perforate the epidermis by probing about with its proboscis, it is only rarely that it gains any satisfaction in this way. It seems as if the male is attached to the human being for other motives, for repeatedly I have watched several males attempting to bite without success. The approach of a female has diverted the attention from these fruitless efforts, and the males have attacked the female on the wing and at least one and sometimes two at one time, have been successful in attaching themselves to a single female. The males seem to hover around the human being not so much to obtain a feed of blood, as because they know that before very long a female will approach.

The females, on the other hand, feed very readily, and twenty-four hours is often sufficient time for the digestion of the large quantity of blood taken up at a single feed. The female will readily feed every day.

The breeding places of the mosquitoes are generally the wells with which most houses are supplied, the large porous earthenware water filters known locally as "hubs," or the cesspools which are generally under the courtyard of each house. The cesspool communicates with the exterior through a small hole over which a round stone is rolled. Some of the wells in disused houses become very foul, and from them

enormous numbers of *Culex* come forth daily. The *Stegomyia* breeds very readily in the cleaner wells and also in the "hubs" which are filled daily with water carried from the river in skins. If left for more than a week without being emptied and cleaned, the hubs become the source of numbers of *Culex* and *Stegomyia*. During the hot weather the development is very rapid, and one week is almost sufficient for the complete development of the mosquito from the egg. During the very dry and hot season the mosquitoes rest during the day upon the moist outer surfaces of these hubs, the temperature of which is considerably below that of the surrounding objects. In the summer the air is very dry, and though a good deal of irrigation of gardens takes place, the water carried up each day from the river has dried up and evaporated before night, so that there is little chance of mosquitoes breeding in standing pools. In the town, by cleaning out the hubs regularly, covering over used or old disused wells, and by attending to the cesspools, it would be possible to rid the town of most of its mosquitoes. It is true that the river Tigris runs through the town. During the summer months the climatic conditions resemble very much those of Khartoum on the Blue Nile, and there under very similar conditions, Dr Balfour has found it possible to exterminate the mosquito. Of course, it would be impossible to carry out such anti-mosquito measures in an old town like Bagdad, for much of it is in a very insanitary condition, and the carelessness of the Eastern native would be a constant obstacle. Khartoum is a comparatively new town in which inspection and enforcement of regulations are not a very difficult matter. But Bagdad is an old town with narrow crowded streets, and many small and dirty houses in which the poorer part of the population are crowded together in conditions far from sanitary. To enforce regulations among these people is almost impossible. However, it would be possible to educate the more enlightened part of the population, and this at any rate would have the effect of diminishing the mosquitoes in the town, and thus one of the possible agents of transmission of the sore. As a matter of fact, during my stay in Bagdad the newly appointed progressive Wali moved in this direction by causing many old and disused wells to be filled in and completely covered over and by building a dyke across the bend of the river on which Bagdad stands in order to prevent the flooding of the desert around the town when the Tigris overflows its banks.

Dissection of mosquitoes as caught in and about the houses was constantly carried on. On no occasion was a flagellate found in these

wild mosquitoes. In *Stegomyia fasciata* a gregarine first noted by Ross in India, was found in the encysted condition. This gregarine was also found in the larvae and pupae, and a description of its life history will form the subject of a later section of this report.

In the larvae of *Stegomyia fasciata* a flagellate (*Herpetomonas*) was occasionally encountered both in the gut and malpighian tubes. It was never met with in the pupa nor in the adult mosquito. The presence of this flagellate in the larvae is of importance from the point of view of the experiments made by feeding *Stegomyia fasciata* on the sore. The larvae very commonly have a large spirochaete infection of the intestine.

Reduvidae. On two occasions only did I encounter members of this group. Only two examples were seen, and these were both very small forms which had alighted on my hand. One definitely bit my hand. It was captured and mounted. The presence of a member of this group is interesting, as a suggestion has been put forward by Donovan that possibly some reduvid may be responsible for the transmission of Kala azar and we know that the *Schizotrypanum* in South America is transmitted in this way. The numbers of *Reduvidae* in Bagdad are however too small to be able to account for such a common disease as the sore.

D. *Experiments undertaken with the object of infecting flies with the parasite of the sore.*

House flies. As already mentioned the house-fly is most persistent in its attempts to feed on the sore, and of all flies which might possibly feed from the sore, the house-fly certainly takes up more parasites than any other. On this account the house-fly has been looked upon with suspicion, and Dr Row has suggested that the house-fly is the natural agent of transmission of the disease. There can be no doubt that the house-fly occasionally carries the parasites to open wounds, and in this way produces sores, but it is unlikely that every sore is the inoculation of an abrasion of the skin by a house-fly, and more improbable still that the house-fly can inoculate the individual through healthy skin. I have said that, as was to be expected, house-flies collected from the faces of the infected children show numbers of the sore parasites in the gut. The same result is obtained by allowing the house-fly to feed on the open sore or on juice or scrapings from the sore. In order to determine if any development would take place in the

house-flies, they were fed on the sore one or more times and dissected at varying intervals after feeding. The possibility of the occurrence of a natural flagellate infection has to be remembered in these experiments, which were all made with flies caught about the house.

1. In every fly dissected immediately after feeding on the sore or on the exudate or scrapings from this, Leishman-Donovan bodies were found.

2. Flies fed in the same manner and dissected five hours after feeding gave negative results. The parasites had disappeared. In some of these flies natural flagellates were found, but there was no possibility of mistaking these for the sore parasites. The fact that natural flagellates occurred in a few, does not affect the result which was the disappearance of the parasites in such a short time in the large number of flies employed.

3. Batches of flies were fed on the sore daily, and a certain number dissected each day. In every case twenty-four hours had elapsed since the last feed when the dissection was made. In this way flies dissected had had one to ten feeds from the sore. Those that had had ten feeds must have taken up enormous numbers of parasites, but in spite of this no evidence could be obtained that any development had taken place. The maximum number of feeds any single fly had was ten, and the dissection was made eleven days after the first feed. The repeated occurrence of the natural flagellates in the experimental flies might tend to obscure the result, but the proportion of infected flies amongst those that had fed on the sore, was not greater than those fed in other ways. To test this latter point control flies were fed daily on human blood from an uninfected person. The different batches of flies were not all kept at the same temperature. Some were kept in the laboratory where the temperature was high and frequently attained 80°–110° F. for the twenty-four hours. Others were kept in the Sirdarbs where the temperature did not rise much above 80° F., and others were kept in small porous earthenware jars covered with mosquito netting, and standing in plates of water. The water soaked up the sides of the porous pots, and by evaporation the temperature was considerably lowered and registered inside the pot from 70° to 75° F. In all cases the results were the same and no evidence of development was discovered.

In some series of flies it was found that the number of individuals with a flagellate infection was greater amongst those that had not fed on the sore. For instance, twenty flies were fed daily for ten days on

scrapings of the sore, and another twenty daily on citrated human blood, for the same length of time. The surviving flies (twelve and fourteen respectively) were dissected twenty-four hours after the last feed. In those that had fed upon the scrapings of the sore no trace of *Herpetomonas* could be found, while in two of the control flies flagellates probably *H. muscae domesticae* occurred. In such an experiment the natural fly infections did not affect the result. In other cases the findings were reversed, but in none was there any evidence of a development of the specific parasite of the sore.

Examination of the faeces of infected flies was always negative. In carrying out these experiments the gut was removed to a slide and films made from the contents taken from the stomach and intestine. These films were stained with Giemsa's stain and examined for parasites.

The constant presence of large numbers of bacteria of various kinds in the intestine of the house-fly may account for the quick disappearance of the parasite when taken up. As in artificial cultures the sore parasites do not develop, or only to a limited extent in the presence of bacteria, so one is not surprised to find that no development takes place in the gut of the house-fly.

As will be shown below in the artificial cultures in rabbits' blood agar, the *Herpetomonas* resulting from the sore parasites may be extremely minute and merely little flagellate organisms of not more than 2 μ in diameter. Such minute forms would be extremely difficult to detect amongst the mixture of substances and bacteria one finds in the gut of such an omnivorous feeder as the house-fly. However, one would at least expect to find some larger forms as in the artificial cultures. House-flies were also allowed to feed on cultures of the sore parasites in rabbits' blood agar. Results obtained were similar to those obtained by feeding the flies on the sore. The flagellates taken up very quickly degenerated and disappeared.

Stomoxys. Numerous experiments were made with these flies which were caught for the purpose in the stable near the house. The flies occurred in greatest numbers at the early part of the summer. During the hottest season they were difficult to secure. The flies taken in the stable had in most cases already had a feed of blood from the horses, so they were starved for twenty-four hours after which time they readily fed on the sore. It was not so easy to discover the parasites of the sore in the stomachs of *Stomoxys*, even when dissected immediately after feeding as in the case of the house-fly. The difference in the method

of feeding in the two cases would account for this. It was impossible to determine what percentage of *Stomoxys* took up parasites from the sore without feeding very large numbers on the sore, and dissecting immediately. However, in some cases large numbers of parasites were taken up and even the large mononuclear cells full of parasites, and a short examination of the stomach contents sufficed to demonstrate their presence, so that it is possible that in those cases where the parasites were not found they were present in small numbers only. The experiments with *Stomoxys* were conducted on the same lines as those made with house-flies, with the difference that the *Stomoxys* were not fed on scrapings from the sore. The greatest number of feeds given a *Stomoxys* was ten, and the flies were dissected twenty-four hours after the last feed. This time generally sufficed for the complete disappearance of the blood taken up twenty-four hours before. As in the case of the house-flies the parasites very quickly disappeared from the gut and on no occasion was any trace of a development discovered. The presence of a *Herpetomonas* in the *Stomoxys* tended to obscure the result, but the flagellate was only found once in a fly fed on the sore, though several times in flies dissected immediately after capture in the stable. The *Herpetomonas* is either a flagellate peculiar to the fly, or represents some trypanosome of the horse or other animal.

Dog Flies (Hippoboscidae). I was not successful is inducing these flies to feed on the sore, and they very quickly died if kept in confinement away from a dog.

Fleas and Pediculi. Experiments with fleas and body lice were not made though numbers of these were dissected without result.

In view of the experiments on the transmission of canine leishmaniosis recently recorded by Basile, the possibility of fleas transmitting the sore must not be forgotten.

Canine leishmaniosis of this type is however a general infection, while the sore is a local skin disease. In the latter one can safely assume that the sore develops at the site of the inoculation whether this be carried out naturally or artificially. In such a case the flea can hardly be responsible for a lesion appearing exclusively upon exposed surfaces of the body.

Bed Bugs. Experiments were carried out by allowing the bed bugs obtained from the prison to feed on the sore. Only a small percentage of bugs would feed under these circumstances, even after prolonged starvation, so that it was a laborious process inducing the bugs to feed. When they did feed they became gorged with blood. Bugs which had

fed were dissected at varying intervals after feeding. On no occasion was I successful in inducing a bug to feed twice from the sore. In all, twelve adult bugs fed on the sore, and became gorged with blood. Two of these were dissected twenty-four hours after feeding, eight were dissected forty-eight hours after feeding, and the remaining two seventy-two hours after. Developmental forms of the sore parasites were found in the stomach of one which was dissected twenty-four hours after feeding, in three which had fed forty-eight hours before, and in neither of those dissected seventy-two hours after. So that in four out of the total of twelve fed on the sore were flagellates found.

Four young bugs which had hatched from eggs laid by bugs in the laboratory fed on the sore. Only three of these were dissected, all with a negative result.

It has been mentioned above that seventy-two bugs taken from the prison and dissected immediately, gave no indication of a flagellate infection, so that there seems little doubt that the flagellates found in the gut of those fed on the sore represent cultural forms of the sore parasite. It is unfortunate that it was not found possible to conduct a greater number of experiments with the bugs hatched in the laboratory, but the greatest difficulty was experienced in persuading the young bugs to feed on the sore. The great majority of the many tried either persistently walked away or became involved in the exudate and there perished.

The flagellate developmental forms of the sore parasite in the bed bugs are figured at Pl. XII, figs. 1–21. It will be seen that they very closely resemble the cultural forms obtained on blood agar. Forty-eight hours after having been taken up, all are in one flagellate condition, while in the bug dissected twenty-four hours after feeding, rounded forms and clumps of incompletely developed parasites are found in addition to completely developed flagellates. In the bugs many abnormal forms are encountered, the protoplasm is often vacuolated and the nucleus broken up or absent. It seems probable that the development in the bug is an abortive one and takes place on account of the large quantity of blood which has acted as a good culture medium.

These observations are very similar to those made by Patton on the development of the parasite of Kala azar in *Cimex rotundatum*. If the parasite of the sore can develop into a flagellate in the stomach of an insect, not its true host, then it would be expected that the very similar parasite of Kala azar would develop in the same manner. Such experiments and results only show that it is unsafe to draw the conclusion

that an insect showing such developmental stages in its gut is the true carrier of the disease, for there is no question of the possibility of the bed bug being the carrier of the sore in Bagdad. This development is then merely a partial imitation of what would actually take place in the true intermediate host.

Mosquitoes. During the earlier part of my stay in Bagdad and before the commencement of the hottest season, various species of *Culex* (including *C. fatigans*) were very common, and numbers of experiments were made with these. It was exceedingly difficult to induce them to feed upon the sore and it was only rarely that one would feed more than twice. In all thirty-one mosquitoes other than *Stegomyia fasciata* chiefly *C. fatigans*, were fed on the sore. Some of these fed twice and one three times, but the majority fed but once. These were dissected twenty-four, forty-eight and seventy-two hours after feeding. No trace of the sore parasites or developmental forms could be discovered. Five mosquitoes were induced to take up exudate from the sore but the parasites could not be traced.

Owing to the difficulty of inducing these mosquitoes to feed on the sore, the experiment was tried of turning loose mosquitoes into a net under which a boy with a sore on his face was sleeping. Those mosquitoes which had fed were collected in the morning and dissected after varying intervals. Of course in such an experiment only a small percentage of mosquitoes would have fed on the sore and taken up parasites, unless we assume that parasites are taken up from the peripheral blood in support of which assumption we have seen above there is little evidence. Close on one hundred mosquitoes were examined after feeding in this manner, but with negative results.

Much difficulty was experienced in keeping the *Culex* alive in captivity, as frequently whole batches would be found dead though they had appeared healthy a short time before. In these cases it was found that the intestine and even the other organs of the body were teeming with a bacillus which had evidently destroyed them.

With *Stegomyia fasciata* which first began to make its appearance in June, experimental work was much more readily carried out. These mosquitoes feed greedily in broad daylight, and are quite willing to feed from the sore every twenty-four hours. It was easy to make them feed on any spot on the sore by gently guiding the proboscis to this spot. So eager were they to feed that they were not unduly disturbed by this interruption. While feeding the proboscis was generally plunged in to its base, and if the blood did not flow readily, the

proboscis was partly withdrawn and again inserted till the mosquito was satisfied with the supply of blood it was drawing. These mosquitoes more often prefer to feed on the thin red skin at the margin of the sore than on the healthy skin beyond. The rapidity with which the relatively enormous quantity of blood taken up at a single feed is digested and got rid of, is remarkable. In twenty-four hours the blown out *Stegomyia* will have returned to its normal size and be ready for another feed.

Stegomyia fed upon the sore and dissected immediately afterwards were found to have taken up the parasites in about 10 % of cases. In the others the parasites may have been too few for detection. In no instance were flagellates found in *Stegomyia* which had had only one feed on the sore. In one *Stegomyia* which had fed on the sore on two successive days and was dissected twenty-four hours after the last feed, there were found rounded forms of the parasite which resembled the enlarged forms seen in the early stages of the artificial cultures.

In five other *Stegomyia* which had had a number of feeds varying from four to ten, and which had been dissected either twenty-four or forty-eight hours after the last feed, fully formed flagellates were found (Pl. XII).

In all, over eighty *Stegomyia* were fed in this way, the majority of these having had over five feeds and many of them ten. So that out of the eighty *Stegomyia* fed on the sore six were found to have evidence of a flagellate infection of the gut. This is a much smaller percentage than in the case of the bed bug, and here one can be less certain that one is dealing with developmental forms of the sore parasite and not with natural flagellates of the mosquito. In each case the hind gut was free from flagellates, a fact which is in favour of their being derived from the sore, since in natural infections the hind gut is generally the seat of the most intense infection. The experiments in nearly every instance were conducted with mosquitoes which had their first feed on the sore.

The result is complicated by the presence of a *Herpetomonas* occasionally in the gut and malpighian tubes of the larvae. On no occasion however have these flagellates been found in the gut or malpighian tubes of the pupae, nor in the mosquitoes which had not fed on the sore though very many were dissected.

As controls to the feeding experiments some six dozen *Stegomyia* were allowed to have one or more feeds on a human being, but in none of these were any *Herpetomonas* found. So that the evidence is in favour

of the flagellates found in *Stegomyia* being a cultural form of the sore parasite as in the case of the bed bug.

Attempts were made to infect mosquitoes by allowing them to feed on the artificial culture, but with no definite result. The flagellates so taken up, quickly disappeared from the gut, even though the mosquitoes were allowed to feed on a human being afterwards.

The experiments with the flies just recorded were conducted mostly on a boy of about four years of age, who had a sore of the non-ulcerating type on the cheek. Some were made with other patients, but the boy was the only one who was willing to be employed in this way regularly. One had to be very careful not to frighten the patient or his friends, who were always very suspicious of what was being done. The sore was a non-ulcerating one, and in order to allow house-flies to feed from it, it was necessary to remove some of the thin skin over it. This exposed the red granulations from which it was easy to obtain large numbers of parasites. House-flies feeding on these granulations took up numbers of the large infected mononuclears and also many free parasites. A scab formed from day to day over these granulations, and this was removed whenever it was necessary to feed house-flies or to obtain juice from the sore.

With mosquitoes and *Stomoxys* it was not necessary to have the scab removed, as these insects fed readily through the intact skin at the side of the scab. Bed bugs generally would not feed, unless the granulations were exposed, and this introduced a difficulty, for immediately the bug became in the least involved in the exudate, it refused to feed, and often died, especially in the case of the young bugs hatched in the laboratory.

The mosquitoes were kept in glass jars as recommended by Christophers and Stephens in their *Practical Study of Malaria*. These jars were kept either in the laboratory or in the Sirdarb where the temperature was lower. In order to have a still lower temperature, mosquitoes were also kept in porous earthenware pots about six inches high and about five inches across. These were covered with mosquito netting and placed in a plate of water. The water from the plate soaked up the sides and by evaporation produced a temperature of about 70° to 75° F. These pots are of the same material as that from which the large earthenware filters or hubs are made, and it is on the moist cool surface of these hubs that the mosquitoes about the house repair during the hot part of the day. The small earthenware pots covered with mosquito netting reproduced the natural conditions very exactly. In

the pots the mosquitoes would sit on the sides, and of all methods for keeping them alive this was the best. There is constant moisture, a relatively low temperature, and a good supply of air. Of course, one has to be careful to keep the plate supplied with water, and further to keep all such jars out of the reach of ants which very soon destroy the flies and mosquitoes if they can attack them. House-flies and *Stomoxys* were kept in the same manner and also in wooden boxes with mosquito netting fronts.

For actual feeding on the sore the mosquitoes or flies were liberated in a mosquito net and taken up separately in small glass tubes ($3 \times \frac{3}{4}$ inch). These were inverted open mouthed over the sore, and, after feeding, the mosquitoes or flies were returned to the respective jars. In the glass jars the mosquitoes survived much longer if a clean sterilised jar and cork were used each day.

For breeding mosquitoes in the laboratory large mosquito nets were used and in these earthenware basins of water with the larvae were kept. Larvae were collected from the wells in small nets of mosquito netting fixed to the end of long bamboos.

E. *Character of the parasite as found in the sore.*

The parasite in smears from the sore may vary very much from the classical oval body with deeply staining rod and paler nucleus. The typical parasites are found in large numbers both within the large mononuclear cells which are probably of endothelial origin and also free in the plasma of the sore. In addition to these forms others occur in which there is considerable departure from the typical oval form. Dr Row has called attention to this fact and has figured many of these forms which are often much elongated and pointed at one end. In these elongated forms the kinetonucleus is often closely applied to the nucleus so that sometimes there is the appearance of the two having fused into one nucleus. In carefully stained films it is nearly always possible to distinguish the kinetonucleus even though it may be lying over the nucleus, and the number of forms where the two are indistinguishable can easily be explained by the fact that the kinetonucleus is under the nucleus and so rendered indistinct or invisible. A reference to Pl. XII, figs. 22–24 will show these conditions. I do not think the kinetonucleus and nucleus have fused in any of these elongated forms as Dr Row thinks must be the case. Nor do I think

that they represent a higher developmental condition than the ordinary forms. I rather think that these are forms endowed with greater powers of resistance and to them may be due the fact noted by Row that the parasites of the sore are able to survive a longer time in the scrapings from the sores than do the parasites of Kala azar in the material obtained by splenic puncture.

In these pointed forms the kinetonucleus is almost invariably between the nucleus and the blunt extremity, so that the tapering of the body is not a step in the direction of flagellum formation as one is inclined to suppose. These elongated forms, so aptly described by Dr Row as torpedo or cigar-shaped parasites, often occur in great numbers in the juice from the sore, and every transition between the typical oval body and the elongated narrow forms can be found. Amongst these may be seen some that have completely lost their chromatin, and appear in the film as homogeneous pinkish bodies of varying shape. Others occur in which the nucleus is lost while the kinetonucleus remains (Pl. XII, figs. 27 and 28). Such forms are undoubtedly degenerate or involution forms. The elongated forms reproduce by division as do the more typical ones. It will be seen that the appearance in a smear from a sore differ in many ways from smears of internal organs in Kala azar. In this latter disease there is a much greater uniformity in the structure and form of the parasites, and one does not encounter the curious elongated and involution forms. The intracellular parasites, the typical oval bodies, in the two cases are indistinguishable, but the appearances in the sore smear differ very markedly in the deviation of the parasite from this type.

The typical parasite (Pl. XII, figs. 25 and 26) as is well known, is an oval or bean-shaped body with the deeply staining rod-shaped kinetonucleus and the more brightly staining nucleus usually applied to one side of the parasite. The rhizoplast, first described by Mesnil, Nicolle and Remlinger in the parasite of Oriental sore in the same year that Christophers noted it in the parasite of Kala azar, is present in most cases. In well stained films it may be detected in almost all the parasites outside the cells, whether they are of the typical oval shape or elongated (Pl. XII, figs. 22–26). It is not easily detected in the intracellular forms probably because of the obscuring effect of the protoplasm of the cell. It may thus be said to be a typical feature of the parasite. In the dried films stained with Giemsa's stain, its connection with the kinetonucleus can be made out. The kinetonucleus stains very deeply except on one side where there is a paler staining

body. This appears to be part of the kinetonucleus and not a separate structure like the blepharoplast of some trypanosomes. It is from the margin of this paler body that the rhizoplast takes origin, whence it runs to the surface of the body. The first indication of a preparation for division is the growing out from this pale body of a second rhizoplast, parallel to the first (Pl. XII, fig. 26). This new rhizoplast is at first thinner than the original one, but it gradually increases in thickness till it attains the same size. Such a connection of the flagellum to the kinetonucleus I have seen in some of the reproducing forms of *Trypanosoma lewisi*. Too much weight cannot be attached to the appearances presented in dried films, but a similar connection can sometimes be made out in films, prepared by more rational methods. The protoplasm of the parasite is usually vacuolated to a greater or less extent and finely granular. Some of the granules may stain red with Giemsa's stain. The appearance of the nucleus in these dried films is well known and calls for no remarks beyond this, that the drying process has completely destroyed the normal appearance.

In films fixed in Schaudinn's fixative and stained by the iron haematoxylin method, the normal appearance is better retained. The parasites however are so minute, that it is difficult to make out clearly such details as the relation of the rhizoplast to the kinetonucleus. This is more clearly shown in some of the larger cultural forms. However, in films from the sore fixed and stained thus, one sees clearly that the nucleus is a spherical body limited by what appears to be a delicate membrane. At the centre of the nucleus is the deeply staining karyosome which varies in size according to the extent of extraction of the stain by the iron alum solution. The rhizoplast is as a rule very difficult to detect in these preparations. The kinetonucleus stains as a black rod (Pl. XIII, figs. 9 and 15).

Examinations of the sores made to discover if any morphological change occurred in the parasite as the sore developed, showed that all the forms described above could be found in the youngest as well as in the oldest ones. In the older sores that are commencing to heal, the most noticeable feature is a diminution in the number of parasites which eventually become very difficult to find.

In the non-ulcerating sores, the parasites occur in the large mononuclear cells. The nuclei of these cells are frequently riddled with parasites and may be completely destroyed by them. Portions of these cells are often broken off in the process of film making, and one has the appearance of several parasites embedded in an enucleate

mass of protoplasm. In the suppurating and ulcerating sores one frequently finds that the parasites have been taken up by the polymorphonuclear leucocytes.

F. *Culture of the parasite.*

The parasite of Oriental sore was first cultivated by C. Nicolle and later by Row in India. I first successfully cultivated the organism on a medium made after the formula of the blood agar of Novy and MacNeal with the substitution of dog's for rabbit's blood. Subcultures were made on dog's blood agar and on rabbit's blood agar. The development was never so rapid with the former as with the latter. Eventually the cultures on dog's blood ceased to develop. Possibly this may have had something to do with the natural resistance of the Bagdad dog to this disease as the blood was taken from the ordinary dogs off the street. On rabbit's blood agar cultures were maintained for some months. The development of the parasite in the cultures follows closely that given by other observers, so that it is not necessary here to enter into a detailed description of what takes place.

Dr Row has described the first process in the culture of the parasite as a fusion of the kinetonucleus and nucleus. I have not been able to confirm this. Many appearances are seen resembling those seen in the elongated forms from the sore where the intimate association of the kinetonucleus and nucleus may have the misleading appearance of complete fusion. I do not think that either in the sore or in the culture does a fusion of the two nuclei take place.

Dr Row mentions certain differences as existing between the cultures of the sore parasites and those of Kala azar. Apparently he compares his cultures of the sore parasite made on human blood serum with the culture of the parasite of Kala azar in citrated blood. It is not safe to emphasise such distinctions unless the conditions of culture are identical in the two cases. The appearances of the flagellates in dog's blood agar, for instance, show marked differences in size, shape, and rate of multiplication from those on rabbit's blood agar. C. Nicolle says that cultures of the two parasites under identical conditions show no differences. Dr Row's cultures were made with human blood serum which is perhaps an ideal medium for these parasites, and to this is probably due the rapid development and large size of his cultural forms.

G. *Character of the developmental forms of the sore parasite in blood agar culture.*

The parasites in cultures may be studied in dried films stained by Giemsa's stain, or better in films fixed without drying and stained by Heidenhain's iron haematoxylin method. Some of the flagellate forms as seen in dried films are shown at Pl. XII, figs. 30–36. In some of these the karyosome within the nucleus is shown in stages of division, but they are most interesting from the point of view of the flagellum which shows a connection with the kinetonucleus similar to that which was seen described above for the parasites from scrapings of the sore. In these it appears as if the kinetonucleus is a structure enclosed by a delicate membrane, on one side of which lies the deeply staining chromatin mass. From the opposite side of the membrane springs the flagellum, and it is from this membrane that a new flagellum grows out when division is about to take place. Sometimes the spot at which the flagellum unites with the possible membrane appears slightly enlarged and has the appearance of a blepharoplast.

The structure of the cultural forms is very well shown in films stained with iron haematoxylin after fixation in Schaudinn's fluid (Pl. XIII). It will be seen that the resting nucleus like the nucleus of a trypanosome consists of a delicate membrane enclosing a clear space at the centre of which lies a deeply staining karyosome. The kinetonucleus is a rod-shaped body staining black. The exact connection of the flagellum with this is difficult to trace. In some it appears to unite directly with the kinetonucleus (Pl. XIII, figs. 1, 8, 10, 13). In others it cannot be traced so far, while again it may be united by a faintly staining structure (fig. 11) resembling to some extent the appearances given in the dried films. There does not seem to be a separate blepharoplast as in many trypanosomes. Such a cone-shaped structure has been described and figured (figs. 4 and 5) by Robertson and Minchin in the collar cells of *Clathrina coriacea*, and I have shown a somewhat similar cone-shaped prolongation of the nuclear membrane in the case of *Cercomonas* in which the flagellum arises from the summit of the cone.

The various division stages are very clearly shown in the wet fixed films, especially in some of the short stumpy forms. The first indication of a division is the further elongation of the already rod-shaped kinetonucleus and the formation of a second rhizoplast parallel to the first. The earliest stages of the formation of the new rhizoplast

are not easy to trace owing to the difficulty of distinguishing it till it is of some thickness. Its connection with the kinetonucleus is the same as that of the first rhizoplast. In some of the dried specimens the new rhizoplast is seen growing out from a point on the surface of the pale half of the kinetonucleus as pointed out above (Pl. XII, figs. 31 and 34). The further division of the kinetonucleus is very clearly seen in the specimens fixed without drying. The elongated kinetonucleus becomes constricted towards the middle, and the two halves separate more and more, though they remain connected by a fine filament even up to the stage when the protoplasm of the parasite is commencing to divide (Pl. XIII, figs. 7, 8, 10, 14). Meanwhile, the new rhizoplast has been gaining in thickness and increasing in length till it commences to grow out from the surface in the form of a new flagellum. This new flagellum is at first thinner than the already existing one. At first the free extremity is closely applied to the original flagellum (Pl. XIII, fig. 4). I believe this appearance is due to the fact that the flagellum is enclosed in a delicate protoplasmic sheath, a continuation of the superficial ectoplasmic layer of the body. The new flagellum grows outwards within the sheath of the first flagellum and it is only when the new flagellum is fairly long that this sheath divides longitudinally so that each flagellum has its own sheath and can exercise independent movements. The new flagellum increases in length and thickness till when the division of the flagellate is complete it may not be equal in length to the original flagellum. I think that in the majority of trypanosomes the formation of a new flagellum takes place in a similar manner and the distal end of the new flagellum is within the sheath of the original flagellum, and often closely held to it so that the end may appear to unite. Such an appearance often gives one the impression that the new flagellum is forming by longitudinal division of the old one, but a careful examination will nearly always show that a slight interval exists between the termination of the new flagellum and the side of the old one. As the flagellum grows longer the part already formed may stretch the common sheath and bring about its division, so that there are two undulating membranes in the earlier formed portion, while the distal extremity is still within the common sheath, and closely applied to the old flagellum.

Eventually the filament connecting the two halves of the kinetonucleus ruptures and the division is complete. The length and fineness of this connecting filament are remarkable. It retains the stain intensely showing up even after much extraction by the iron alum solution.

One is inclined to think that it is an indication of some intranuclear division centre though this has not actually been observed. In these flagellates the flagellum is generally traceable back to the kinetonucleus itself, and does not appear to rise from an extra nuclear blepharoplast as in many trypanosomes. It is possible that such a blepharoplast may be in some cases within the kinetonucleus and may be represented here by the pale staining portion of the kinetonucleus from which the flagellum is seen to arise. In division it would be obscured by the surrounding chromatin of the kinetonucleus though the filament connecting the two halves is visible when the chromatin has divided and retracted from around the filament. In the division of the collar cells of *Clathrina coriacea*, so clearly described by Minchin and Robertson, the blepharoplast acting as an extra nuclear division centre takes on appearances very similar to these. The position of the blepharoplast outside the kinetonucleus as in trypanosomes of the blood, may be looked upon as a higher type of development while more primitive flagellates such as those under discussion, representing as they do flagellates of the intestinal tract of insects, display a more primitive condition with the blepharoplast within the nucleus.

To return to the division of the cultural forms of the sore parasites, we find that very soon after the kinetonucleus shows signs of approaching division the karyosome of the nucleus becomes elongated and with it the nuclear membrane. The length of the karyosome increases, a constriction appears and the karyosome may be divided into two parts (Pl. XIII, fig. 6) at a comparatively early stage. More frequently however the two halves of the karyosome remain connected by a filament which may eventually stretch across the whole width of the parasite. At this stage the nuclear membrane is seen surrounding the ends of the elongated structure (Pl. XIII, figs. 8, 10, 12, 16). The appearance of the karyosome thus elongated resembles very much, though on a larger scale, the condition of the dividing kinetonucleus. The size of the chromatin mass at each end of the structure varies with the extent of the extraction of the stain. The connecting filament however remains even after prolonged extraction (Pl. XIII, fig. 2).

It would appear that in the case of the nucleus also there is within the karyosome a division centre obscured by the chromatin, and in division represented by the fine connecting filament, this filament being both in the case of the kinetonucleus and nucleus a centrodesmose corresponding to the centrodesmose described by Minchin and Robertson in the case of the division of the collar cells of *Clathrina coriacea*.

Eventually the nuclear division is complete and two daughter nuclei formed. The division of the body of the flagellate has been proceeding by a groove appearing between the rhizoplasts. The presence of the filament connecting the two halves of the kinetonucleus appears to arrest the division of the protoplasm for some time, but when this filament is ruptured the division extends to the non-flagellate end of the body and the two daughter flagellates separate.

A point of much interest in connection with the culture of these organisms is the presence of extremely minute forms. One frequently encounters examples not more than 3 μ or 4 μ in length and about 1 μ to 2 μ in breadth, while on several occasions I have seen smaller individuals barely 2 μ long and not more than 0·5 μ in thickness. It is just possible in these small specimens stained with iron haematoxylin to make out the nucleus and kinetonucleus. The flagellum is relatively large. The presence of these minute forms and the possibility of others still smaller must be borne in mind when examinations of flies is made for developmental stages. It is possible that the form transmitted to man by the insect carrier is some such minute flagellate as this. They would be extremely difficult to detect amongst the debris frequently present in an insect's gut.

H. *Character of the development forms of the sore parasite in bed bugs and Stegomyia fasciata.*

The various developmental forms in the bed bug are shown in Pl. XII, figs. 1–21. Figs. 1, 5, 8, 10–21 are taken from a bug dissected forty-eight hours after feeding on the sore. The bug was opened and a dry film made from the contents of the stomach and another from the hind gut. The stomach showed a fair infection with flagellates. In the hind gut only a single flagellate was found. It will be seen that many of the forms correspond very clearly with those met with in the artificial cultures. Others however show various abnormalities and many appear to be degenerating. Figs. 10 and 14 show two such degenerating flagellates, while figs. 3, 9, 15, are examples of abnormal forms. Apparently the development is only an abortive one and a partial picture of what would take place in the true host. Figs. 2 and 6 are from another bug and figs. 3, 4, 7 and 9 from a third. Some of these appear to be normally constituted, while others, especially the curious form at fig. 9, are evidently abnormal. Figs. 38, 39, 41 show three parasites from a *Stegomyia* which had four feeds from the sore

and was dissected twenty-four hours after the last, while fig. 37 is from one that was fed on ten successive days and dissected forty-eight hours after the last feed, and fig. 40 from one that had two feeds and was dissected twenty-four hours after. There is nothing of special interest to note about these forms except their resemblance to the cultural forms met with in the test tube cultures.

I. *Attempts to transmit the sore by the bites of Stegomyia fasciata.*

Owing to the fact that the parasite of the sore develops in the *Stegomyia fasciata* and that this mosquito is so constantly attacking man, it was regarded as a very probable transmitter of the disease. Accordingly a series of experiments were undertaken in order to test this point. Specimens of *Stegomyia fasciata* were allowed to feed on the sore for a varying number of days and in each case after a lapse of twenty-four or forty-eight hours after the last feed from the sore the mosquitoes were allowed to feed on a small area about the size of a shilling on my forearm. In this way were fed twenty-six mosquitoes. Six of these had fed from the sore on ten successive days, and forty-eight hours later on my arm, fourteen had fed from the sore on four successive days and on my arm after an interval of twenty-four hours, two had fed from the sore on five successive days, and after twenty-four hours on myself; three had six successive feeds from the sore and a feed on my arm after an interval of forty-eight hours; one had fed once from the sore and after forty-eight hours on myself.

The following table gives the details of these feeding experiments:

Number of *Stegomyia*	Number of days on which *Stegomyia* had fed on the sore	Interval elapsed between last feed from sore and feed on myself
6	10	2 days
14	4	1 day
2	5	1 day
3	6	2 days
1	1	2 days

All these mosquitoes were examined twenty-four hours after they had fed from my arm. In two of those that had fed from the sore four times, and in one that had fed five times, were flagellates found. The rest gave negative results. Nearly nine months have elapsed since this experiment was made, and there is still no sign of a sore developing at the spot where these mosquitoes fed. All of them fed from the same small area of skin. I was exposed to infection all the time I was in

Bagdad, so that unless a sore had developed at the exact spot the experiment would be valueless and even had a sore developed there, there would be the possibility of some other fly having bitten and produced the sore. Still the failure of the *Stegomyia* to produce an infection seems to give some evidence against its being the natural host of the sore parasite. It would have been better had more than twelve days elapsed between the first feed from the sore and the feeding on myself, but it is only possible to form a single experiment on oneself, and the difficulty of finding persons suitable or willing for such experiments, prevented them being extended. The experiment is interesting in so far that it has given a negative result.

J. *Origin of the Disease.*

There are many local hypotheses regarding the origin of the sore. Some people maintain that it is only those who drink the water of the Tigris or Euphrates who become infected; others that it is due to the contamination of open sores or wounds by dirt from the roads, while others think it is in some way related to syphilis. The names "date boil" and "date mark" are attributable to the view that the disease is due to the dates which become ripe at the season of maximum incidence of the sore. None of these hypotheses will explain the disease. The peculiar distribution of the sore on the exposed surfaces of the body, the character of the specific parasite and the development of this parasite into *Herpetomonas* forms in the culture tube can only be accounted for by the assumption that there is some fly responsible for the transmission of the sore. The possibility of the house-fly acting as a mechanical carrier of the disease has been mentioned above. It is almost certain that the house-fly from time to time plays the part of such a mechanical carrier and transfers some of the parasites from an open sore to a fresh and healthy wound. The parasites may be carried over in moist material adhering to the proboscis or feet of the fly. That this mechanical transmission by the house-fly is to be regarded as the normal means of infection cannot be maintained, for it is certain that sores appear on parts of the skin where there have been no wounds or abrasions. It is improbable that the mere application of juice from a sore to the healthy skin will give rise to an infection, though it would be of interest to test this point experimentally. Even if it were possible for the mobile cultural forms of the sore parasite to pass through the uninjured skin, it is difficult to imagine how the passive immobile forms

found in the sores could do so. It is possible, as Dr Row suggests, that the parasite undergoes some development in the house-fly, and is then deposited upon the skin probably in the faecal matter, and that a sore arises in this way. Dr Row is conducting experiments with the object of elucidating this matter, but the quick disappearance of the parasites in the house-fly and their failure to develop into *Herpetomonas* forms, afford a strong argument against the view advanced by Row. The fact that the parasite develops into flagellate forms in the culture tube and also in the bed bug and in *Stegomyia* is almost conclusive evidence that a similar development will take place in the true host. From this point of view the house-fly cannot be suspected. However, in the light of the experiments of Hindle on the passage of *Trypanosoma gambiense* through the normal uninjured skin of the rat, it is a possibility to be considered that the parasite of the sore is taken up by the house-fly, that it develops into flagellates in its gut, probably minute forms like those described as occurring in the culture tube and not easily to be detected, and that in this condition it is passed on to the skin and is able to penetrate and produce a sore. My experiments lend no support to such a view, and should the house-fly be the normal carrier it still has to be explained why the disease is limited in its distribution, though the house-fly occurs everywhere.

Of the biting flies in Bagdad the *Stomoxys* as the carrier of the disease, is not to be suspected, as it is not only limited in its distribution, but also fails to give any development of the parasite in its gut, even after as many as ten feeds from the sore. The *Hippoboscidae*, the ticks, fleas and body lice, will not explain the disease. The two former on account of their seldom attacking man, and the two latter because they would give rise to sores on the unexposed rather than the exposed surfaces of the body. The bed bug on account of the development of the Kala azar parasite which takes place in its gut, and its supposed responsibility for the spread of this disease, was at first looked upon with suspicion. It was soon found that its distribution in Bagdad was not wide enough for this hypothesis to be correct, and further it would not explain the occurrence of sores only upon the exposed surfaces of the body. In spite of the impossibility of the bed bug being the host of the sore parasite, it has been pointed out above that a certain development of the parasite takes place in its gut. This observation is of great importance, for it shows that the development of the Kala azar parasite, which was found by Patton to take place in the same host, may be of a similar nature. The evidence afforded by the occurrence of such a

development is not sufficient to prove the bed bug to be the intermediate host. There is just the possibility in my experiments as well as in Patton's that the flagellates seen in the bed bugs are parasites peculiar to these insects, but I consider this highly improbable since the dissection of numbers of bugs which have not fed from a sore has failed to reveal these flagellate forms. It seems therefore that the large quantity of blood taken up by these insects when they bite is able to act as a culture medium for the sore parasites, without this culture indicating in any way that the development is the true development in an intermediate host.

The only other biting flies of any note in Bagdad are the mosquitoes and the so-called sand flies (*Phlebotamus*). Of the mosquitoes the *Stegomyia fasciata* both on account of its voracity and numbers would be looked upon with greater suspicion than the various species of *Culex*. This suspicion is increased when we consider the development undergone by the sore parasite in its gut, a development which does not take place in the other mosquitoes. At one time I was inclined to regard the *Stegomyia fasciata* as the probable agent of transmission. It is essentially a house mosquito and is most persistent in its attempts to obtain a feed of human blood. When it has completed its fill it quickly digests this apparently enormous quantity and is ready on the next day for another feed. It bites as readily by day as by night, so that one is never free from its attacks. Allowing about two months as the incubation period of the disease the time of maximum incidence of the *Stegomyia* corresponds with the time of maximum incidence of the sore. The result of the experiment upon myself with *Stegomyia fasciata* detailed above, appears to me to negative such a view, so that at present it is impossible to say whether the *Stegomyia fasciata* can or cannot act as the transmitting host of the sore parasite. It is still possible that some other mosquito less numerous and not so voracious as the *Stegomyia fasciata* will eventually be incriminated, but a more probable fly has yet to be excluded, in the sand fly (*Phlebotamus*). Unfortunately, experimental work was not undertaken with these flies till later in the year. This was just commenced when the unfortunate accident to my assistant and the destruction of part of the laboratory completely put a stop to the work for some time. When this was again resumed, it was not found possible to secure *Phlebotamus* in sufficient numbers for experimental work, so that I am unable to give any experimental results in support of this fly being the agent by transmission. Its numbers and distribution both as regards place and season

are compatible with this view. It is a fly that has been suspected in other places, so that until further work is undertaken the question must remain undecided. I hope during the coming summer to resume the experiments and to obtain some further light on this interesting but difficult question.

Conclusions.

1. Oriental sore as it occurs in Bagdad does not differ essentially from that of other places. There may however be a variation in the virulence and duration of the Oriental sore in different parts of the world.

2. The Oriental sore attacks practically all natives of Bagdad generally between the ages of 1 and 3 years. Newcomers usually become infected within a year or two after arrival.

3. Occasionally individuals may escape infection though exposed to it for years.

4. There may be one, two or three sores at one time. More rarely there are more. Sometimes there are as many as twenty-three and even greater numbers are talked of in Bagdad.

5. Whether single or multiple the Oriental sore rarely appears on any but an exposed surface of the body, *e.g.* face, fore-arm, leg, hand or foot.

6. One attack confers absolute immunity for the rest of life. It is possible that the sore of Aleppo may not produce absolute immunity against the sore of Bagdad. The same may be true of other places.

7. From inoculations from man to man it is demonstrated that the incubation period is about two months.

8. Inoculation with juice from a sore as in vaccination against smallpox produces a single sore at the point of inoculation only. From this it is concluded that in cases of multiple sore each sore is a separate inoculation by the transmitting agent or a subinoculation by a house-fly or by the individual himself.

9. There is a seasonal prevalence of the disease. Though they may appear at any time of the year, sores most usually make their appearance during the months of September, October and November at the close of the hot season.

10. The duration of the disease is from twelve to eighteen months.

11. The sore commences as a small papule which increases in size. It may then ulcerate and extend (female sore of Bagdad) or it may remain dry with a scaly scab on the surface (male sore of Bagdad).

There is little pain and no demonstrable constitutional disturbance associated with the disease.

12. In all true sores the typical parasite (*Leishmania tropica*) can be found unless the sore is in the final healing stage.

13. I have not been able to demonstrate the presence of the parasite in the peripheral blood either by direct examination of the blood or by the cultural method.

14. There is a much greater variety in the shape and size of the parasites in smears from the sore than in smears from the internal organs of cases of Kala azar.

15. The parasites obtained from young sores are of the same shape, size and characters as those obtained from old healing ones.

16. I have failed to demonstrate the presence of the sore in any domestic animal nor has the examination of one hundred and ten dogs revealed the presence of canine leishmaniosis in Bagdad. Kala azar in man is not known in Bagdad.

17. House-flies collected from the faces of children suffering from the open type of sore nearly always show the sore parasites in the gut. The parasites quickly degenerate and do not develop into flagellate forms.

18. House-flies must often act as mechanical carriers of the disease to open wounds.

19. Mosquitoes, *Stomoxys*, and bed bugs fed upon the sore are found to take up parasites.

20. Only in *Stegomyia fasciata* and the bed bug do the parasites develop into *Herpetomonas* forms. This is however no evidence that these insects are the natural carriers of the disease.

The same remark applies to the development in bed bugs of the parasites of Kala azar described by Patton. Development probably takes place because of the large quantity of blood taken up acting as a culture medium.

21. The transmitting insect is probably sometimes the house-fly and more usually either one of the mosquitoes or the sand fly (*Phlebotamus*).

22. The parasites of the sore develop into *Herpetomonas* forms in rabbit's or dog's blood agar as previously demonstrated by Nicolle and Row.

23. The inoculation of dogs and other animals (not monkeys) with juice from a sore or artificial culture on blood agar has failed in my hands to infect these animals.

24. No treatment has had much effect in reducing the duration of the sore.

25. Much can be done by protective inoculation on unexposed surfaces of the body not only to avoid the disfiguring scar on the face, but to prevent ~~this~~ having access to sores and thus becoming infective to other people.

II. The Life History of *Lankesteria culicis* (Ross, 1898).

Gregarine in *Stegomyia fasciata*.

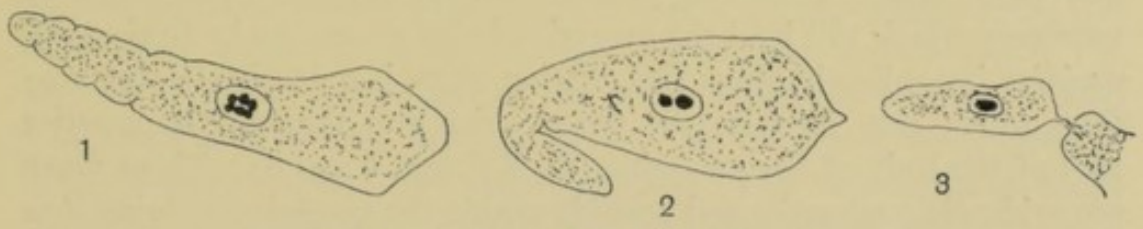
General account of the infection. Gregarines in this mosquito were first noticed and described by Ross during his classical researches into the development of malarial organisms ~~in~~ mosquitoes. The main features of the life history of this gregarine were described by him as follows.

"The youngest gregarines are found in the perivisceral cells of the youngest larvae. Growing in size, they escape from the host cell, become active and when the larva develops into a pupa, migrate into the malpighian tubes. There they become encysted with or without conjugation, and produce a large number of pseudonavicellae which are expelled with the faeces of the imago, either into water or upon the human skin." This gregarine was subsequently rediscovered by Marchoux, Salimbeni and Simond when conducting investigations on yellow fever. It is evidently the same gregarine which is to be found in many of the *Stegomyia fasciata* of Bagdad. Here the *Stegomyia* breed chiefly in the wells, and I have noticed that not all the wells are infected with gregarines to the same extent. In some wells practically every larva or pupa is infected, while in others only a small percentage and in some the *Stegomyia* appear to be free.

By a process of section cutting, somewhat laborious, of the body contents of the *Stegomyia* in all stages of its development I have been able to follow out almost the complete cycle of development of the gregarine. The body contents of larvae were dissected and placed one upon the other upon a slide kept moist under a glass cover, so as to form a small heap. When twenty to thirty had so been gathered into a heap the whole mass was fixed in Schaudinn's fixative. There was little tendency for the individual parts to separate so that it was possible to treat the collected organs as a single piece of tissue. This was washed, cleared and embedded in paraffin in the usual manner. The mass was then cut into serial sections which were stained with

haematoxylin. In this manner some beautiful preparations of the stages of development of the gregarine were obtained, and it was in this material that I have followed out the development. Unfortunately there are one or two gaps in this development owing to the fact that insufficient material had been collected, but certain stages can only be obtained by the examination of large quantities of material in the manner described above, a process which is very tedious.

In the gut the free forms are usually about 50 μ in length or smaller. I have not encountered such large forms as those described by Ross as being 200 μ long. When examined in the living condition



Lankesteria culicis of *Stegomyia fasciata*.

Figs. 1 and 2. Gregarines free in the gut of the larva of *Stegomyia fasciata*.
Fig. 3. Gregarine attached to epithelial cell.

they exhibit the typical gregariniform movements of progression, flexion, and constriction. Such free forms are figured at Text-figs. 1 and 2. In sections of the gut it is found that the gregarines remain as intracellular parasites during nearly the whole of their trophic development. Pl. XV, fig. 5 shows a gregarine completely within its cell, and fig. 8 another 50 μ long, also within the epithelial cell which shows a slight rupture at the surface. The general shape and features of the gregarine call for no special remark. They are clearly shown in the figures. The body is non-separate and the single and typical gregarine nucleus is at the centre of the body. At the anterior end of the body is a peculiar structure which reminds one of the similar body described by Siedlecki in *Lankesteria ascidiae*. It appears to be a vesicle and is possibly a suction organ connected with the endoplasm through a pore in the cuticle. It evidently functions as an organ of fixation which is used chiefly when the epithelial cell is completely ruptured and the gregarine remains attached to its degenerate remnants. The most careful examination of the point of fixation of the gregarines in sections has failed to reveal any processes or fibres passing from the fixation organ into the epithelial cell. The gregarine lies in a vacuole in the epithelial cell and the fixation organ is merely

applied to that surface of the vacuole which is nearest the basement membrane. I have used the word suction, but it must be admitted that there is no indication of a stress being produced in the protoplasm which one would expect to find if a suction force were being exerted. In the case of tape worms fixed to the gut it is easy in sections to see that such a suction is taking place, for the tissues in the neighbourhood are dragged, as it were, towards the sucker. If we can compare a gregarine with such a large organism as a tape worm we might expect to find some signs of stress though on a smaller scale. Nothing of the kind can be detected, so it is possible that the peculiar organ which has been called a sucking organ, merely secretes some adhesive substance which will hold the gregarine in its place. As in *Lankesteria ascidiae*, immediately below this fixation organ is a deeply staining area from which pass fan-wise the fibres which run through the anterior part of the body of the gregarine (Pl. XV, figs. 5 and 8). These fibres are evidently contractile and possibly enable the gregarine to bring into action the fixation organ at its anterior end.

When the gregarine is about 50 μ in length, the epithelial cell ruptures and the gregarines remain attached to the epithelial remnants for some time. They then become detached and fall into the gut cavity where they exist for a while as free gregarines. After the passage of the *Stegomyia* larva into the pupa, as pointed out by Ross, the gregarines migrate into the malpighian tubes. The stimulus acting upon the gregarines is probably the sudden cessation in the intake of food by the *Stegomyia*.

Encystment. Within the malpighian tubes the gregarines associate in pairs, becoming attached to one another by the anterior ends, the two sucking organs coming into apposition. The point of contact of the two gregarines appears in the stained sections as a deeply staining area (Pl. XV, figs. 3 and 7). A similar appearance is shown by Siedlecki in the case of *Lankesteria ascidiae*. The two gregarines become enveloped by a thin membrane forming spherical cysts. The cells of the malpighian tubes become excavated to accommodate these comparatively large gregarine cysts. Pl. XV, fig. 9 is a longitudinal section of a malpighian tube of a *Stegomyia* pupa, showing the gregarine cysts and the alteration produced in the cells. Though in a single section it may appear that a single gregarine occupies one cyst, by following the sections through the series it is seen that there are always two. I have not encountered a case of a gregarine encysted singly.

Nuclear multiplication. The newly encysted gregarines usually have nuclei with a single large centrally placed karyosome. The karyosome soon becomes vacuolated and may break up into several fragments. At the same time the nuclear membrane becomes less distinct and the karyosome eventually passes into the protoplasm. In two cases, in close proximity to the nucleus, another structure has been seen which stains more deeply than the surrounding protoplasm. In Pl. XV, figs. 31 and 32 are shown in sections of a gregarine cyst the nucleus with the karyosome in process of breaking up in one section and in the next section the elongated staining body which shows some sign of radiation at one pole. Figs. 4 and 6 are two adjacent sections of another gregarine cyst. In one is seen the nucleus with the karyosome in two parts, and in the other a definite spindle forming with radiations around the two poles. At each pole is an area of denser tissue showing a few small vacuoles. This denser tissue which takes up the stain more deeply than the surrounding protoplasm is probably of centrosomic nature. For lack of material it is impossible to follow up the stage any further, but there is evidently a preparation for the formation of the first nuclear spindle. These stages bear some resemblance to the first spindle formation and the "achromatic mass" described for *Metamera schubergi* by Duke.

Though from the material at my disposal I was able to discover some trace of the first nuclear division, I was quite unable to find a cyst showing the second division. Various stages of the third division in a gregarine cyst are shown at Pl. XV, figs. 28, 29, 33, 34. In one gregarine the division was slightly more advanced than in the other. Figs. 33 and 34 are two dividing nuclei of one gregarine. There is a centrosome at each pole of the nuclear membrane which is still intact. Around each centrosome is a definite astral system. I was not able to detect a centriole within the centrosome. Probably had I stained with the iron haematoxylin method, further details would have been revealed, but all the sections were stained with ordinary alum haematoxylin. The chromatin within the nuclei at this stage is arranged irregularly. The nuclei in this gregarine were still spherical, but in the gregarine associated with it, the spindles were slightly more advanced (figs. 28 and 29). The centrosomes have separated and the spindle has become elongated. Definite fibres can be seen running from one pole to the other and at the centre are several chromatin masses. I am unable to say if at this stage there is a definite number of chromosomes. The nuclear membrane, much attenuated, is still

present. In the former of these two gregarines the karyosome (fig. 30) with two vacuoles was lying free in the protoplasm. Though the nuclear multiplication had advanced as far as the third division, it was still intact. Attached to it at one side is a peculiar club-shaped body. A similar structure occurred in the nucleus of another gregarine (Pl. XV, figs. 1 and 2). In subsequent divisions the definite character of these spindles is lost or they are too minute to be detected readily. Pl. XV, fig. 19 shows a section of a gregarine cyst where some of the nuclei of one gregarine are already arranged on the surface preparatory to gamete formation, while others presumably in the other gregarine are in division. In this nuclear division all that can be made out is the spindle-shaped structure without any astral system round the poles, or a visible centrosome. In the minute spindles the chromatin appears at first as a single mass at the centre. It divides into two, each half supplying the chromatin of a daughter nucleus. It is possible that even at this stage the centrosome, astral system and spindle fibres exist, but that they are too small to be clearly made out. As the nuclear divisions proceed some of the nuclei cease to have any share in the multiplication process and they remain as undividing nuclei in the protoplasm (Pl. XV, fig. 19). They take no part in the formation of the gametes and ultimately degenerate with the residual protoplasm left over when the gametes are formed. During the process of nuclear multiplication the bodies of the gregarines, at first clearly distinguishable, became interlaced owing to their protoplasm becoming vacuolated and thrown into folds and ramifications. In such a cyst as that figured at Pl. XV, fig. 19, it is impossible to trace the bodies of the two gregarines. Presumably the nuclei which are still dividing belong to one gregarine, while the others which have placed themselves on the surfaces of the ramifications in preparation for the formation of gametes belong to the other.

Gamete formation and conjugation. When nuclear division is complete, the nuclei arrange themselves on the surface of the ramifications into which the bodies of the gregarines have been produced. Pointed elevations of this surface are formed and into each bud a single nucleus enters. The buds are then separated as small gametes 3·5–4 μ in diameter. The nucleus of each gamete is placed eccentrically and beyond the nucleus the protoplasm is produced into a pointed protuberance (Pl. XV, figs. 12, 14, 17). The protoplasm of the gametes shows a marked reticular structure whereas the pointed eminence beyond the nucleus is quite clear and hyaline. It is possibly a condensed portion of the thin limiting ectoplasmic layer.

Though the gametes are equal in size, this is not the case with their nuclei, which are of two kinds. There are gametes with large nuclei and others with smaller nuclei. This difference in size of nuclei evidently has to do with a differentiation into male and female gametes. A similar difference in size of nuclei has been described by Swarczewsky in *Lankesteria sp.* and Brasil has described an almost identical differentiation in the case of the gametes of *Urospora lagidis.* The difference in size of nuclei is best seen in the conjugating gametes, all stages of which can readily be followed in the sections (Pl. XV, figs. 11, 13, 15). Pl. XV, fig. 10, shows a gregarine cyst containing zygotes and several masses of residual protoplasm, in which are seen some of the incompletely divided nuclei.

Development of sporocysts. The zygotes become elongated (Pl. XV, fig. 20) and each becomes enclosed in a sporocyst which is flattened at the poles. Within the sporocyst the protoplasm divides into eight sporozoites after the nuclear divisions have taken place (Pl. XV, figs. 21 to 27). The nucleus of the zygote is a spherical body with the chromatin arranged irregularly over the surface of the membrane. The first division takes place regularly at right angles to the long axis of the sporocyst. The nucleus becomes elongated and the chromatin aggregated at the poles in the form of bands. After division the nuclei so formed migrate to the ends of the sporocyst, where they undergo a second division in a similar manner and in a line parallel to that of the first division. The final division takes place in the line of the long axis of the sporocyst and at right angles to the line of the first and second nuclear divisions. The eight nuclei then become arranged round the equator of the sporocyst.

As a rule the whole process of development from the encystment to the formation of the ripe sporocysts takes place during the pupal stage of the *Stegomyia fasciata.* In the newly hatched adult mosquito one finds the malpighian tubes containing gregarine cysts filled with ripe sporocysts. Very soon the cyst walls enclosing the sporocysts disappear and the sporocysts are found lying free in the cavity of the malpighian tubes. Pl. XV, fig. 35, is from a longitudinal section of such a malpighian tube. The sporocysts make their way into the gut and are expelled, as Ross has described, with the faeces. Evidently these sporocysts escape into the water where the mosquito lays its eggs, and are taken up by the newly hatched larvae. I have not observed the escape of the sporozoites from the sporocyst in the gut of the larvae nor the infection of the epithelial cells in the earliest

stages. There can be, however, no doubt that this is the mode of infection.

The whole course of this development bears a very close resemblance to the development of *Lankesteria ascidiae* given by Siedlecki. It differs in that there is incomplete isogamy, as distinguished from complete isogamy, where both the size of the gametes and their nuclei are equal, the unequal size of the conjugating nuclei marking a differentiation into male and female gametes. In both cases the gregarines are intracellular during the greater part of their trophic period and they both have the same peculiar fixation organ. The resemblance is so close that both forms should be included in the same genus. The name for this gregarine of *Stegomyia fasciata* will then be *Lankesteria culicis* (Ross) 1898. I did not encounter gregarines in any other mosquito though these were taken from the same well in which were breeding the *Stegomyia* showing the largest percentage of infected individuals.

III. Some Observations on the Development of the Haemogregarine of the Leucocytes of the Dog.

Occurrence of the infection. It has already been mentioned in the first section of this report that practically without exception, all the Bagdad street dogs are found to harbour this parasite. I was able to perform autopsies on one hundred and ten dogs of all ages and it was nearly always possible to find the developmental forms of this parasite in the spleen or bone marrow. Sometimes the infection was a small one and several preparations had to be searched in order to discover a single cyst.

In other cases the infection was very large, so that numbers of cysts occurred in each squash preparation of either spleen or bone marrow. The cysts can readily be detected with a low power objective in simple squash preparations of the fresh organs. It seems that when once a dog is infected with this haemogregarine it remains infected for the remainder of its life. It is probable that the duration of life of the dogs is not great owing to the fierce struggle for existence which these dogs have to endure in the Bagdad streets. The appearance of the haemogregarine as it occurs in the dried blood films stained by any Romanowsky stain calls for no remarks. It has already been fully described by several observers. In films fixed by methods more rational than those of drying (*e.g.* by Schaudinn's fixative) the haemogregarines

show a somewhat different nuclear picture. Instead of the irregular red staining mass which often is produced into strands, evidently the result of drying and partial flattening on the slide, the nucleus is made up of a group of deeply staining (Heidenhain's iron haematoxylin method) masses of chromatin. The masses appear to be bound together by a paler reticulum. The masses of chromatin are so closely packed together that it is often difficult to make out any further structure. If, however, the differentiation in the iron alum solution is carried further, it will be found that the deeply staining masses become discoloured and that some further details can be detected. The nucleus will then be seen to consist of a ring of minute granules at the centre of which is a larger granule or karyosome. This is evidently an optical section of a vesicular structure. It is difficult to say if any definite nuclear membrane is present or not, but the regular arrangement of the granules in a ring suggests a nuclear membrane. I am inclined to the view that a definite membrane is present. Within the membrane between it and the karyosome can be seen some very fine granules. The nucleus of this haemogregarine is then a vesicular nucleus limited by a nuclear membrane on the surface of which in deeply stained specimens large staining masses obscure all other structure, while in more discoloured specimens the nuclear membrane is seen to be covered with small granules. At the centre is a definite karyosome, surrounding which are still fewer granules arranged on the nuclear reticulum.

Reproduction in the spleen and bone marrow.

It has generally been supposed that this haemogregarine reproduces almost exclusively in the bone marrow, but in the case of the Bagdad dogs, at any rate, the reproducing forms are found as commonly in the spleen as in the bone marrow.

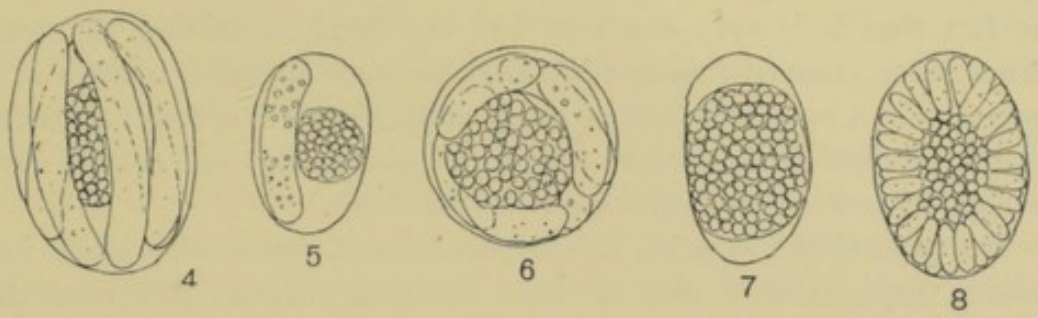
The reproduction follows two distinct lines. In one case, and this is the method which has hitherto been described, the resulting products of reproduction are numerous small bodies resembling the haemogregarines found in the leucocytes in the peripheral blood (Pl. XVI, fig. 8). In the other case, not described previously, the products of the reproduction are much less numerous (generally three) and very much larger, reaching a length of 15 μ (Pl. XVI, figs. 3 and 6). It may be stated at once that the small bodies are destined to enter the leucocytic cell in the peripheral blood, and that they are most probably gametocytes

which proceed with their development in the body of the tick *Rhipicephalus sanguineus*. The larger forms represent the asexual method of multiplication or schizogony.

A similar difference in the reproducing forms of other haemogregarines has been noted by Lutz, Prowazek, Reichenow and others, and I have described it in the case of *Haemogregarina gracilis* of the Sudan lizard, *Mabuia quinquitaeniata*. I suggested there that the small narrow forms which entered the red blood corpuscles and appeared in the peripheral blood were possibly sexual forms which would be found to pursue their further development in some intermediate host. This has been fully borne out by recent investigations. In their recent paper on the development of *Haemogregarina lutzi* Hartmann and Chagas describe a similar course of development. They mention the case of the *Haemogregarina gracilis* described by me, but they have stated that the so-called "microschizogony" products are the only form of asexual reproduction. In reality I said that the small forms are those destined to enter the red corpuscles and to appear in the peripheral blood while the large forms remain in the liver and reproduce asexually. The former are then probably sexual forms. The macroschizogony forms in the lizard as in the dog represent the asexual generation which occurs only in the internal organs, the so-called macromerozoites never appearing in the peripheral circulation.

The various stages of the development can be traced in sections of the spleen or bone marrow. In the present instance the tissue was fixed in Zenker's fixative and the sections stained with iron haematoxylin. The youngest forms are seen as small rounded bodies within mononuclear cells (Pl. XVI, figs. 4 and 13). The nucleus has a structure similar to that which has been described for the haemogregarines of the peripheral blood. The parasite increases in size and distends the host cell. At the same time the protoplasm alters in appearance, becoming filled with spheres of a refractile material. Very soon the nucleus commences to divide. The nuclear membrane is lost and the chromatin is arranged in an irregular mass of granules. With prolonged differentiation in iron alum solution it is seen that a karyosome is present in the midst of these granules. The karyosome divides after becoming elongated, and with its division the chromatin becomes collected into two masses each with its own karyosome (Pl. XVI, fig. 9). The karyosome acts in exactly the same manner as the karyosome of *Coccidium schubergi* in the dividing nuclei of the schizont as described by Schaudinn. Hartmann and Chagas have described a very similar nuclear division in the case of

the nuclear multiplication in the schizont of *Haemogregarina lutzi*. This process of division is repeated for each of the daughter nuclei or for only one of them. The nuclei resulting from the second division may again divide in a similar manner. There results a schizont filled with refractile spheres (Text-fig. 7) and having nuclei varying in number according to the exact progress of the nuclear divisions. From this are



Figs. 4–8. Reproductive cysts of *Haemogregarina canis* in the spleen of the dog, drawn from fresh material.

Figs. 4 and 6. Cyst containing merozoites and residual body drawn from fresh spleen material.

Fig. 5. Cyst containing one merozoite and residual body.

Fig. 7. Cyst containing schizont with protoplasm filled with refractile spheres.

Fig. 8. Cyst containing sexual forms with large residual body.

separated off large sausage-shaped merozoites having a fine reticular protoplasm while the refractile spheres are left behind in a large residual body. The number of merozoites correspond to the number of nuclei. In some cysts only a single merozoite is present (Text-fig. 5) with a large residual body. Here increase in size of the schizont can only have taken place without any nuclear multiplication, or only one of the nuclei resulting from the multiplication has given rise to a merozoite. Very commonly there are three merozoites but there may be four or twice this number (Text-figs. 4 and 6). The merozoites are about 15 μ in length. The nucleus varies in appearance with the extent of extraction of the stain. When fully extracted there is seen to be a nuclear membrane over which are arranged fine deeply staining granules. At the centre of the nucleus is a karyosome (Pl. XVI, figs. 3 and 6). I have not been able to decide whether the merozoites are able to again pass through a similar process of schizogony or whether each one passes on to the production of the sexual forms. I think the latter is more probably correct. If the asexual cycle could be repeated indefinitely, the infection of the dog would be much larger than it ever is. The dogs are covered with ticks and these ticks are constantly infected with the

sporozoites of the haemogregarine. Reinfection of the dogs must be constantly taking place, and sporozoites finding their way to the spleen or bone marrow. The probable course is that a sporozoite enters a mononuclear cell of the spleen or the bone marrow, increases in size, and eventually gives rise to a variable number of large merozoites which escape from their cyst and proceed to produce the small sexual forms which find their way into the peripheral blood. In favour of this view is the fact that it is very usual to find the cysts containing the small sexual forms grouped together in threes or fours in such a manner as to suggest that a sporozoite had recently produced three or four merozoites near this spot, that these have escaped from their cyst and settled down near together to produce the small sexual forms.

The process of formation of the small sexual forms takes place in a manner very similar to that of the merozoites with the difference that the nuclear divisions proceed very much further. The details are the same, and even up to the last division in suitably stained specimens the karyosome can be detected in the middle of the chromatin area (Pl. XVI, figs. 5 and 14). The number of nuclei is very large and there are produced a corresponding number of small sexual forms (Text-fig. 8). With the rupture of the cyst they escape and enter the mononuclear cells of the blood where they appear as the familiar haemogregarines in the leucocytes.

The host cell during both these processes of reproduction is reduced to a thin envelope surrounding the parasite. The nucleus of the host cell, very much flattened and altered, is seen at one side of the cyst (Pl. XVI, figs. 2, 8, 14). It is probable that in addition to this covering derived from the thinned out host cell the parasite secretes a covering of its own. These cysts are of some resistance, for in the squash preparations of the fresh organs they are not easily ruptured, and in the staining of smears which have been fixed without drying, the stain only penetrates with difficulty. The details of the contents of the cyst are only clearly made out in sections where the cysts have been opened at some point.

The fully formed cysts in the spleen and bone marrow are about 25–30 μ in diameter. The size of the cysts varies very little whether they contain the large merozoites or the numerous small sexual forms.

Further development of the leucocytic stage in the tick Rhipicephalus sanguineus.

The development of this haemogregarine in the tick was first described by Christophers. The method of investigating the development adopted by him, viz. the making of smears of the contents of the ticks, was not the best for giving clear pictures of what was taking place. By making smears it is impossible to avoid breaking up the large öocysts and scattering the sporocysts. In this way Christophers appears to have described the sporocysts as öocysts.

In the present instance the following method was adopted. Ticks were taken from infected dogs and the abdomens were opened by making a cut round the margin with fine scissors. By careful dissection with needles it was possible to remove the ventral chitinous plate intact, leaving all the organs behind. The organs were then removed in one piece and immediately fixed in Zenker's fixative. These fixed organs were brought home for examination. Serial sections were cut and these stained with haematoxylin. In these sections it was possible to follow many of the stages of development of the haemogregarine.

The first step in the development has been clearly described by Christophers and consists in the liberation of the cysts from the leucocyte and finally the escape of the haemogregarine from the cyst. The free haemogregarines can be found in numbers in the intestinal contents of the ticks.

I have also been able to trace haemogregarines within the epithelial cells of the gut and also between the epithelial cells and the basement membrane. Finally they are to be found outside the basement membrane amongst the body contents, usually, however, close to some fold of the gut.

Unfortunately, I have not yet been able to follow out the conjugation process.

For haemogregarines this has been most clearly described by Reichenow for *H. stepanowi*. The haemogregarines taken into the gut of the leech associate in pairs, and still within the gut a process of conjugation takes place which is very similar to that of the coccidian *Adelea ovata*. In one of the haemogregarines the nucleus divides into several parts, and one of these parts unites with the entire nucleus of the other haemogregarine. The remainder of the divide nuclei are discarded. After this the zygote increases in size and breaks up into sporozoites which pass through the gut wall of the leech into the

surrounding tissue. Robertson has also described a similar method of conjugation in the case of another haemogregarine *H. nicoriae.* The sexual process for the dog haemogregarine was described by Christophers, but Reichenow is of the opinion that here also the conjugation will ultimately be found to be of the *Adelea* type.

Miller, however, has described for the rat haemogregarine a complete conjugation of two unaltered haemogregarines in the gut of the mite *Lelaps echidninus.* It is possible that here the conjugation will be found also to be on the lines of that of *Adelea ovata.* Further work alone will settle the question of the exact details of the conjugation of these leucocytic haemogregarines of the dog and rat.

I have mentioned that I was able to trace the haemogregarines through the epithelium of the gut into the tissues of the body of the tick, but the forms I have seen might have been öokinetes passing through the gut wall after conjugation as do the öokinetes of the rat haemogregarine according to Miller, or they may have been even the sporozoites resulting from a sporozony undetected by me in the gut of the tick, as described by Reichenow and Robertson.

In order to follow in detail the stages which occur after the haemogregarines have reached the body tissues of the tick, one must have recourse to artificial infections. The ticks with which I conducted these investigations were taken from naturally infected dogs in which the infection was never large enough to enable me to follow in detail the next few stages. It is sometimes very difficult to distinguish the very young öocysts from the tissue cells of the ticks.

The next stage which I have been able to distinguish clearly is shown at Pl. XVI, fig. 11. Here it will be seen that a large cell has been produced, that nuclear multiplication has taken place, till there are present about thirty nuclei. I am not quite clear whether this is a growing öocyst or whether it is some stage in the production of gametes as described by Christophers. Miller has described for the rat haemogregarine an enormous increase in size of the öocyst. A similar increase in size takes place in the case of the dog haemogregarine. Eventually large öocysts of about 100 μ in greatest diameter are produced. These are found in the sections to lie outside the gut wall embedded in the surrounding tissues. These öocysts contain at first a single mass of protoplasm with thirty to fifty nuclei. By a process of budding (Pl. XVI, fig. 17) there are separated off protoplasmic masses each with a single nucleus. A great part of the protoplasm is unused in this process and is left over as a residual body. The protoplasmic

masses are really sporoblasts, and they soon secrete a covering of sporocyst. Within the sporocyst nuclear multiplication again takes place, till there are produced about sixteen nuclei in each (Pl. XVI, fig. 1). The nuclei then range themselves in groups, eight at each pole. By a process of growing out from the protoplasmic mass there are formed from each end eight sporozoites (Pl. XVI, fig. 7). A part of the protoplasm remains as a residual body. In this manner there are produced large öocysts of about 100 μ in greatest length containing from thirty to fifty sporocysts of about 15 μ–16 μ in length, and in each sporocyst sixteen sporozoites of about 14 μ in length (Pl. XVI, fig. 16). The sporozoites (Pl. XVI, fig. 12) have been described by Christophers. They are elongate bodies of 14 μ–15 μ in length and are packed tightly together within the sporocyst. The nuclei of the sporozoites resemble those of other stages of the haemogregarine. There is a mass of chromatin granules closely bound together. I have not been able to determine if a nuclear membrane or karyosome exists in this stage, but as the earliest forms found in the splenic cells have nuclei of this type, I do not doubt that the sporozoites have also.

The presence of these large öocysts was not noticed by Christophers, probably owing to his method of studying the development. In making smears of the contents of ticks these large öocysts would certainly be ruptured and the sporocysts liberated. In sections the general arrangement of the various parts is much better retained. The production of the öocysts and sporocysts corresponds in almost every detail with the development given by Miller for the very similar haemogregarine of the rat. The average number of sporozoites in each case is sixteen. The number of sporozoites in each sporocyst in the case of *Haemogregarina stepanowi* and *Haemogregarina nicoriae* is only eight.

The next stage in the development of the large öocysts in the dog tick is the dissolution of the cyst wall and the liberation of the sporocysts which wander about amongst the body contents of the ticks. Liberated sporocysts may be found in any part of the body outside the gut. In sections of ticks at this stage it is possible to find sporocysts in almost every section. The further development has not yet been traced. I have not been able to detect free sporozoites either in the body cavity or in the gut. In the case of the *Haemogregarina stepanowi* and in *Haemogregarina nicoriae* Reichenow and Robertson have described the liberation of the sporozoites from the sporocysts and their passage back into the cavity of the gut, whence undoubtedly they again enter the body of the tortoise. Christophers describes free sporozoites

in the gut contents, but in the method of smearing it is almost impossible to be sure from where the various forms seen have originally come. In the case of the rat haemogregarine Miller has not noted any escape of the sporozoites from the sporocysts while they are still within the body of the mite. He has found, however, that when treated with the intestinal juice of rats the sporocysts rupture and the sporozoites escape. This led him to feed healthy rats on crushed mites containing sporocysts. In this way, and in this way alone, was he able to produce an infection in healthy rats. It is possible that in the dog also infection takes place in a similar manner, through the intestinal wall, by the dog eating the infected ticks. The sporozoites would then be liberated in the gut, make their way into the spleen or bone marrow, enter a mononuclear cell and develop into the schizonts producing about three large merozoites. The merozoites would escape and develop into those forms which produce the well-known haemogregarines found in the peripheral blood. Whether these are really gametes or gametocytes cannot be settled till the exact process of conjugation is known. According to Miller they must be regarded as gametes which conjugate in the intestine of the tick.

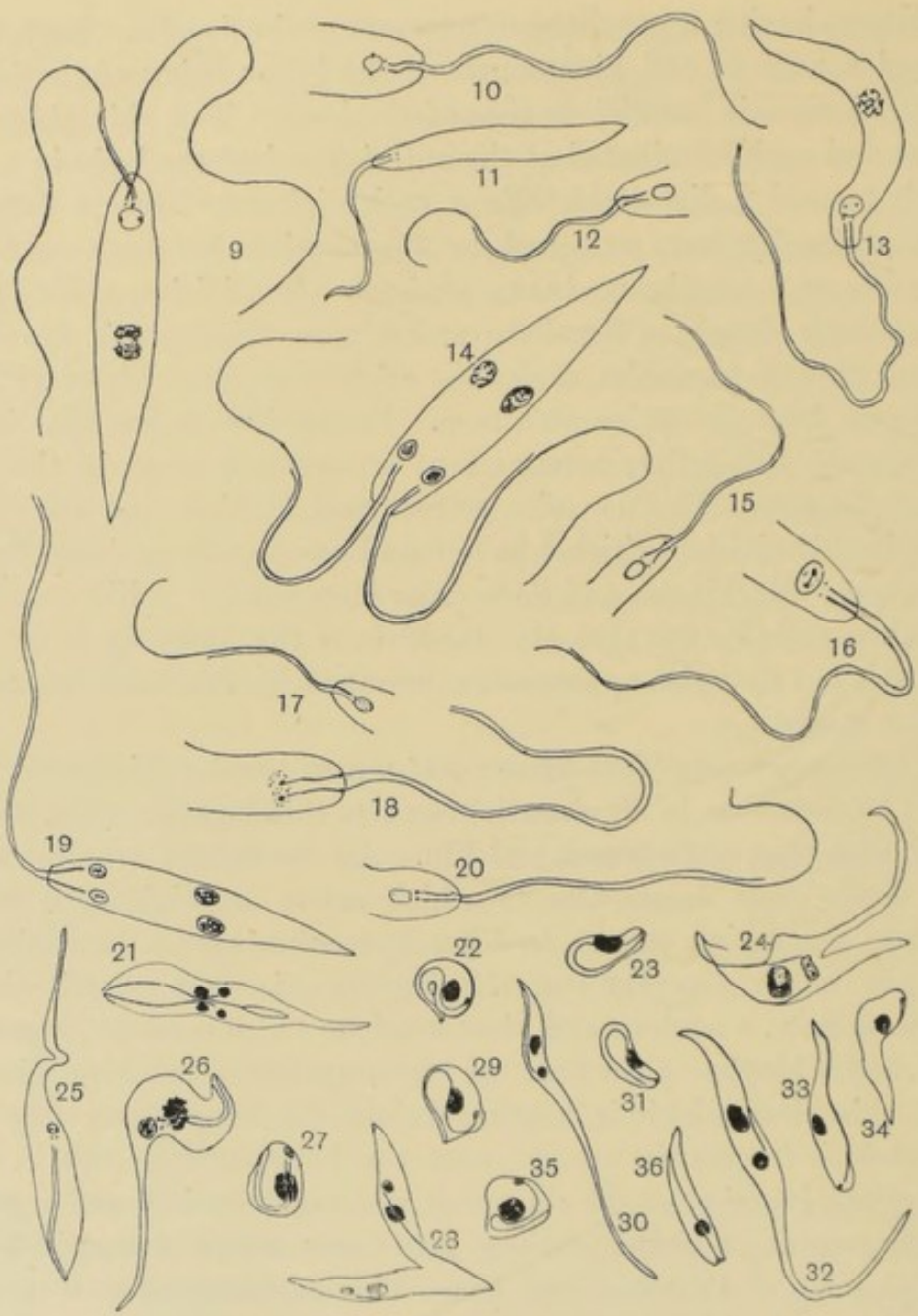
The course of the development given above for the haemogregarine of the dog, as far as it is complete, agrees in almost every detail with that of Miller, for the similar parasite of the rat. I hope at a later date to be able to fill in the gaps in this life history.

IV. Flagellates of House-flies.

As in many of the Eastern cities a large proportion of the Bagdad house-flies are found to harbour intestinal flagellates. Of these I have met with three types, but whether each type represents a distinct species of flagellate or three different stages of one and the same flagellate, I am not in a position to state. Two of these correspond with the flagellates described by Flu from the house-fly, and one is found chiefly in the malpighian tubes and is interesting in that it shows the trypanosome arrangement of nucleus and kinetonucleus. Of the two flagellates described by Flu one is the *Herpetomonas muscae domesticae* of Prowazek ; the other is spoken of by Flu as a *Leptomonas*. The chief object of the present account is to show that in the case of the former the description of Prowazek is probably based on a faulty interpretation of the appearance of the flagellate in division. Prowazek described his

flagellate as having a peculiar arrangement of the flagella. Each flagellate had a nucleus and kinetonucleus, the latter being often elongate with the long axis parallel to that of the body. Two rhizoplasts were present and each terminated at the anterior end of the body in a small granule termed a diplosome. From each diplosome arose a flagellum. The two flagella were attached to one another by some connecting substance or a membrane between them. In division one flagellum goes to each daughter flagellate and a new flagellum is formed by forward growth from the diplosome while the new rhizoplast grows backwards from the same structure. Patton has shown that in this flagellate the biflagellate arrangement is merely a stage in the longitudinal division. This is quite correct and I think that the various stages in this division which I have found completely establish Patton's contention. Mackinnon and Porter also have come to a like conclusion and agree with Patton that the flagellate of the house-fly is not a biflagellate, but that the appearances described by Prowazek are merely division stages.

A reference to the Text-figures 9 *et seq.* will make this matter clear. It will be seen that in all essential respects this flagellate (figs. 9 to 20) agrees with that of Prowazek and Flu. All the figures are drawn from films made from house-flies in which active multiplication of the flagellate was taking place. In other house-flies such a multiplication may not be in progress and the flagellate is then found with an elongated body, a nucleus and kinetonucleus which is much longer than broad, and a blepharoplast near the kinetonucleus from which arises the single rhizoplast which is continued into the single long flagellum. A vacuole is frequently present near the kinetonucleus and in many cases at the junction of the rhizoplast and flagellum is found a granule or enlargement of either of these structures which corresponds with the diplosome of Prowazek and Flu. The blepharoplast as a structure distinct from the kinetonucleus was not noted by Prowazek, and this is the only difference between my flagellate and such forms as figured by him. When house-flies in which active multiplication is in progress are examined, forms with a single flagellum may never be found and this is dependent upon a precocious flagellum formation, each flagellate being as it were in a hurry to have the new flagellum ready for a succeeding division. The result of this is that all the flagellates have either two or more flagella. A flagellate which has just resulted from longitudinal division has already two flagella arranged as shown in fig. 17. The kinetonucleus is an oval body limited apparently by a membrane. It



Text-figs. 9—36. *Flagellates of the House-fly.*

Figs. 9—12. *Herpetomonas muscae domesticae.*

Fig. 9. Dividing flagellate. The kinetonucleus shows the deeply staining granule on each side. There are two blepharoplasts and from one a new rhizoplast and flagellum are forming.

Fig. 10. Anterior end of another flagellate similar to fig. 9.

Fig. 11. Flagellate without nucleus or kinetonucleus. The two blepharoplasts and flagella are still present.

Fig. 12. Anterior end of a flagellate showing granules at the junction of the flagella and rhizoplasts. These are probably Prowazek's diplosomes.

Fig. 13. Dividing flagellate. Within the kinetonucleus are the two granules which have not yet reached the sides of the kinetonucleus which appears to have a definite membrane. The two blepharoplasts clearly shown.

Fig. 14. Dividing flagellate showing well the precocious formation of flagella. Though division is not complete the daughter flagella of the succeeding division are already forming.

Fig. 15. Anterior end of a dividing flagellate showing blepharoplasts, rhizoplasts and diplosomes.

Fig. 16. Anterior end of a dividing flagellate showing the dividing karyosome (?) within the kinetonucleus.

Figs. 17–20. Dividing flagellates. Fig. 18 from a flagellate in which the kinetonucleus has been destroyed. It shows well the union of the rhizoplast and blepharoplasts by means of a faintly staining line.

Figs. 21–36. *A flagellate of trypanosome type from the malpighian tubes of the house-fly. They possibly represent forms of* Herpetomonas muscae domesticae.

Fig. 21. Dividing form.

Figs. 22, 23, 27, 29, 31, 33–36. Various stages in the formation of the small pear-shaped forms (fig. 23) from the long forms (figs. 28 and 30).

Fig. 24. Large dividing form.

Fig. 25. Long dividing form without nucleus but still possessed of kinetonucleus and blepharoplasts.

Fig. 26. Dividing form showing two granules within the kinetonucleus and two blepharoplasts.

Fig. 28. Dividing form.

Figs. 30 and 32. Typical long forms. Note the long drawn out flagellar extremity of the body. The kinetonucleus is always on the non-flagellar side of the nucleus. There is no free flagellum as this structure terminates at the blunt end of the body.

is still single but close to its flagellar side are the products of the already divided blepharoplast. From the new blepharoplast a new rhizoplast narrower than the old one has formed, and this has grown out into the beginnings of a new flagellum. Patton believes that the new rhizoplast is divided off from the ~~fresh~~ one, but it is certainly a new formation growing out from the divided blepharoplast. The blepharoplast alone divides. Porter claims to have watched the division of the flagellum in the living *Herpetomonas muscae domesticae.* From the stained smears examined by me, I can find no evidence of such a division. The flagellum is enclosed, as I believe, in a thin sheath of ectoplasm continued from the body of the flagellate and it is within this sheath that the new flagellum grows out. As the flagellum increases in length both it and the rhizoplast increase in thickness till eventually the length and thickness equal those of the old flagellum. Such stages may be followed in figs. 17, 12, 10, 13, 16, etc. When the new flagellum has attained a certain degree of development, indications of division appear in the kinetonucleus. Within this there is apparently a structure like a karyosome.

This first divides into two equal parts (figs. 16 and 13). Each half then passes to one side of the kinetonucleus (figs. 9 and 10) and then the kinetonucleus itself is divided. Apparently the dark staining body which is within the kinetonucleus functions as a centrosome. The details of this division have been described but it must be mentioned that the films were dried ones stained by Giemsa stain. It is just possible that some of the appearances are the result of bad fixation, but they are so constant and occur in so many of the dividing flagellates that this can hardly be the case, though one has to exercise caution in drawing conclusions. After division of the kinetonucleus, division of the nucleus takes place, but it must be stated that the kinetonucleus does not divide till the new flagellum is equal or almost equal to the old one in length and thickness, so that many of the flagellates appear in the preparations with single nucleus and kinetonucleus, and two equal flagella which are bound together by some connecting material which is, according to my interpretation, the common flagellar sheath. These forms correspond exactly with Prowazek's figures and many of them show clearly the granule or diplosome at the base of each flagellum (figs. 18, 15, 10, 20). Just before or at the time of division of the kinetonucleus the common sheath which binds the two flagella together is divided longitudinally, so that the flagella may move independently of one another (fig. 9). Immediately after division of the kinetonucleus each blepharoplast divides again, a new rhizoplast is formed and a new flagellum grows out in such a way that these dividing flagellates have four flagella. This condition is very well shown in the flagellate drawn at fig. 14 and it corresponds very closely with some of Prowazek's figures of this stage.

Occasionally the formation of the new flagella may commence before the division of the kinetonucleus is completed, but this is a rare occurrence (fig. 9). The forms with four flagella are not numerous, for division of the two nuclei is quickly followed by division of the body of the flagellate. The result of this process of division is that none of the flagellates are uniflagellate, for at the time of division each half is provided with one fully formed flagellum and one partially formed one (fig. 17). It thus comes about that during the active multiplication uniflagellate forms are not to be found, and I think that this fact has undoubtedly led to Prowazek's interpretation of this flagellate as being a biflagellate. When, however, active multiplication is not in progress the flagellate is often found with a single flagellum. This is the resting form and every transition between the uniflagellate elongated forms

and the small oval forms with only a short flagellum or no flagellum whatever may be followed.

During multiplication, therefore, the biflagellate appearance is the result of a very active multiplication of flagella, a multiplication which appears to lead the way in longitudinal division of this flagellate. The protoplasmic body of the flagellate is reluctant to divide so that it is always behindhand and has not completed its division till after the daughter flagella of the succeeding division have partially developed. When the daughter flagella of the succeeding division have partially formed the flagellate, as it were, realises its backwardness, and tries to regain lost ground by very rapid division. This latter fact accounts for the comparative rarity of such forms as that shown at fig. 14.

The flagellate just described agrees with that of Prowazek in dimensions, and in most of the details of structure. The blepharoplast or achromatic structure near the kinetonucleus was not described by him, and I have failed to find the fibre which Prowazek traced through the body of the flagellate from its kinetonucleus. Flu has not figured this fibre. I think there can be no doubt that the flagellate here described is *Herpetomonas muscae domesticae*, and is the same as that described by Prowazek and Flu. According to my observations this flagellate agrees in all essential points with the other flagellates so often described as *Herpetomonas* and is not a biflagellate as maintained by Prowazek and others.

In addition to this large *Herpetomonas* a smaller one may be found in some flies. It corresponds with the flagellate described by Flu as a *Leptomonas*. In reality it should be included in the same genus as the larger flagellate of the fly. It may be a distinct flagellate as Flu claims, or it may be a stage in the life history of *Herpetomonas muscae domesticae*. In some flies the small form alone is found. In others there is a mixed infection with the large and small forms.

Still a third type of flagellate was found in some flies. These are shown in Text-figures 21–36. They occur mostly in the malpighian tubes, but may also be found in the gut. They are remarkable in showing a trypanosome arrangement of the nuclear apparatus.

There is no free flagellum, but this organ terminates at one extremity of the body, the opposite extremity of the body being drawn out into a long tapering filament (figs. 25, 26, 30, 32). The nucleus is situated in the thicker part of the body and the kinetonucleus between it and the tapering extremity. The flagellum arises from a blepharoplast lying near the kinetonucleus (figs. 25, 26, 28, 30), and is continued past the

nucleus to the blunter extremity of the body. There does not appear to be a true undulating membrane and the flagellum runs a fairly straight course over the surface of the flagellate. Dividing forms are shown at figs. 21, 24 and 26. In division the blepharoplast divides, a new flagellum is formed, after which nuclear divisions take place succeeded by division of the flagellate. Within the kinetonucleus there appears to be a karyosome which first divides as in the case of *Herpetomonas muscae domesticae* described above. A curious feature about this flagellate is the formation of small pear-shaped bodies (fig. 26) which may be stages in cyst formation. Actual cysts were not observed.

The first step in this process is the formation of small stumpy forms (figs. 33, 34, 36), some of which resemble *Trypanosoma nanum* very closely. The stumpy forms have the kinetonucleus at the blunt extremity of the body and they appear to arise by the gradual loss of the long tapering end of the long forms. The small forms then become doubled upon themselves (fig. 22), and finally the space between the two limbs is filled in and forms such as those shown at figs. 27, 29, 35 are produced, in which the flagellum follows a curious course through the body. Finally the body narrows and the small pear-shaped forms result (figs. 23, 31). It is possible that this flagellate also is some stage in the development of *Herpetomonas muscae domesticae*, but I have no facts on which to decide this point.

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EXPLANATION OF PLATES XII TO XVI.

PLATE XII.

Figs. 1–21. *Developmental forms of the sore parasite* (Leishmania tropica) *in bed bugs.*

Figs. 1, 5, 8, 10–21 from a bed bug dissected forty-eight hours after feeding from the sore.
Figs. 2 and 6 from a second bug dissected twenty-four hours after feeding from the sore.
Figs. 3, 4, 7 and 9 from a third bug dissected forty-eight hours after feeding from the sore.

Fig. 1. Dividing Herpetomonas form. Size $11 \times 2{\cdot}5\ \mu$.
Fig. 2. Developmental form with flagellum not yet showing. Size $8{\cdot}5 \times 2{\cdot}5\ \mu$.
Fig. 3. Abnormal individual.
Fig. 4. Herpetomonas form showing connection of flagellum with cone-like prolongation of kinetonucleus. Size $10 \times 2\ \mu$.
Fig. 5. Abnormal division form with double nucleus. Size $14 \times 2{\cdot}5\ \mu$.
Fig. 6. Group of partially developed parasites. One has no nuclear apparatus.
Fig. 7. Two oval flagellate forms. Length $6{\cdot}5\ \mu$.
Fig. 8. Small abnormally dividing form. Length $4{\cdot}5\ \mu$.
Fig. 9. Curious dividing form without nucleus. Length $12\ \mu$.
Fig. 10. Evidently degenerating form.
Fig. 11. Flagellate with dividing nucleus. Size $14 \times 2\ \mu$.
Fig. 12. Large clump of developing parasites.
Fig. 13. Flagellate form. Size $12 \times 2\ \mu$.
Fig. 14. Abnormal or degenerating form. Size $16 \times 2{\cdot}5\ \mu$.
Fig. 15. Flagellate with large quantity of chromatin staining material in protoplasm Size $12 \times 2\ \mu$.

Fig. 16. Elongated form without evident flagellum. Karyosome shows within the nucleus. Length 9·5 μ.

Fig. 17. Typical flagellate form. Size $7 \times 2\ \mu$.

Fig. 18. Another and larger typical flagellate form. Size $14 \times 1\ \mu$.

Fig. 19. Small curved forms. Size $5 \times 2\ \mu$.

Fig. 20. Dividing forms without flagella. Size $5 \times 2\ \mu$.

Fig. 21. Dividing flagellate form. Karyosome in division. Size $10 \times 2{\cdot}8\ \mu$.

The sore parasite (Leishmania tropica).

Figs. 22–29 *from dried smears made direct from the sore and stained by Giemsa's stain.*

Fig. 22. Torpedo-shaped parasite with the kinetonucleus closely applied to the nucleus. Such a position of the two nuclei may lead to the erroneous idea that a fusion of these has taken place. The rhizoplast running towards the blunt extremity of the body is clearly shown.

Figs. 23 and 24. Two similar parasites with the kinetonucleus not so closely applied to the nucleus. These torpedo-shaped parasites are mostly extracellular.

Fig. 25. Typical parasite from the sore. The two parts of the kinetonucleus show well, one deeply and the other lightly staining. The rhizoplast arises from the pale staining part. Length 2·5 μ.

Fig. 26. Another and larger parasite in division. The pale half of the kinetonucleus shows up clearly. The new rhizoplast is already formed though it is still smaller than the original one. Length 5 μ.

Note. These parasites (Figs. 25 and 26) which are from dried films stained by Giemsa's stain, should be compared with the similar parasites stained by Heidenhain's iron haematoxylin method in films fixed in Schaudinn's fixative without any drying. Such forms are shown at Pl. XIII, figs. 9 and 15.

Figs. 27 and 28. Two abnormal forms without nuclei. Many of these are to be found in the smears from the sore.

Fig. 29. Form with dividing nucleus.

Figs. 30–36. *From cultures of the sore parasite on rabbit's blood agar.*

Fig. 30. Cultural form showing dividing karyosome in nucleus and dividing kinetonucleus connected with which is the pale staining dome-shaped structure from the apex of which the flagellum springs. Length 11 μ.

Fig. 31. Similar form with the dome-shaped structure on the kinetonucleus. A second rhizoplast is forming. Length 10 μ.

Figs. 32 and 33. Two parasites in division. They show well the kinetonucleus with its dome-shaped structure which divides with the kinetonucleus. The second rhizoplast is a new formation. Size $8 \times 5\ \mu$.

Fig. 34. Division form showing the dividing karyosome and kinetonucleus with its dome-shaped structure and newly-formed rhizoplast. Length 7 μ.

Fig. 35. Typical cultural form. The connection of flagellum to kinetonucleus is well shown. Length 12 μ.

Fig. 36. Form showing the kinetonucleus with a deeply staining dividing structure within it.

Figs. 37–41. *Developmental forms of the sore parasites in* Stegomyia fasciata.

Fig. 37. From a *Stegomyia fasciata* which had fed from the sore on ten successive days and was dissected forty-eight hours after the last feed.

Figs. 38, 39, 41. From a *Stegomyia fasciata* which had fed from the sore on four successive days and was dissected twenty-four hours after the last feed.

Fig. 40. From a *Stegomyia fasciata* which had fed from the sore on two successive days and was dissected twenty-four hours after the last feed.
Fig. 37. Form with two rhizoplasts. Length 5 μ.
Fig. 38. Form without flagellum. Length 6 μ.
Figs. 39 and 40. Partially developed forms. Length 4 and 5 μ respectively.
Fig. 41. Typical flagellate form. Length 7 μ.

PLATE XIII.

All the figures from preparations of the rabbit's blood agar cultures of the sore parasite fixed in Schaudinn's fixative and stained by Heidenhain's iron haematoxylin method, except figs. 9 and 15 which are from preparations made in the same manner direct from the sore.

Fig. 1. Typical cultural form. Length 13 μ.
Fig. 2. Dividing form very much discoloured. The fine line connecting the separating halves of the nuclear karyosome still retains the stain. Length 6 μ.
Fig. 3. Typical form showing a clear interval between the end of the rhizoplast and the kinetonucleus. Length 9 μ.
Fig. 4. Dividing form showing a condition of the kinetonucleus comparable with that of fig. 36, Pl. I. Length 7 μ.
Fig. 5. Dividing form showing two structures in the position of the kinetonucleus. Length 7 μ.
Fig. 6. Form in which the nucleus contains two chromatin masses, possibly the divided karyosome. Length 7 μ.
Fig. 7. Dividing form with two flagella. Length 8 μ.
Fig. 8. Dividing form showing the fine line connecting the halves of the karyosome. The kinetonucleus also dividing. The protoplasm shows commencement of division in the form of a groove between the rhizoplasts. Length 7 μ.
Fig. 9. Typical parasite from the sore. Compare with figs. 25 and 26 in Pl. XII, which have been dried and stained by Giemsa's stain.
Fig. 10. Dividing culture form. The halves of the kinetonucleus connected by the fine line like that which occurs in division of the karyosome of the nucleus.
Fig. 11. Form showing the flagellum connected with a dome-shaped structure so frequently seen in the dried films (cf. Pl. XII, figs. 30–35).
Fig. 12. Form with nuclear karyosome in division.
Figs. 13 and 14. Forms with dividing kinetonucleus.
Fig. 15. Parasite from the sore. Compare with fig. 9 and Pl. XII, figs. 25 and 26.
Fig. 16. Dividing form with division of kinetonucleus completed. The halves of the karyosome connected by the long drawn out line. Length 6·5 μ.

PLATE XIV.

Figs. 1 and 2. Typical appearance of the oriental sore as it occurs on the faces of children in Bagdad.
Fig. 3. Girl age 14 with oriental sore on the face and another on the right wrist. The sore on the face is of the type of a spreading ulcer ("female sore"), that on the wrist is a non-ulcerating growth ("male sore").
Fig. 4. The same case as fig. 3, showing the spreading ulcer on the face and its tendency to heal in one part as it spreads in another.

PLATE XV.

Lankesteria culicis (*Ross* 1898) *a gregarine of* Stegomyia fasciata.

Fig. 1. Two gregarines encysted in the malpighian tubes of the pupa.

Fig. 2. Nucleus of one of the gregarines of fig. 1, showing a process budding from the karyosome.

Fig. 3. Another gregarine cyst in the malpighian tubes of a pupa. The deeply staining body between the gregarines is the remains of the fixation organs which are applied to one another when association takes place.

Fig. 4. Section through a gregarine cyst. The nucleus of one of the gregarines shown is breaking up. In the next section in the series (fig. 6) is seen the beginnings of the first nuclear spindle.

Fig. 5. A gregarine in the gut epithelium of a larva. Length of gregarine 45 μ.

Fig. 6. See fig. 4.

Fig. 7. A later section from the same cyst as fig. 1. The dark body arising from the fixation organs is shown well.

Fig. 8. A gregarine in the epithelium of the gut of a larva. Drawn on a larger scale than fig. 5. Length of gregarine 50 μ.

Fig. 9. Longitudinal section through a malpighian tube of a pupa showing many gregarine cysts. Note the excavations of the tube cells.

Fig. 10. Gregarine cyst in malpighian tube of pupa showing zygotes and residual protoplasm containing unused nuclei.

Figs. 11, 13, 15 and 16. Stages in the conjugation of the gametes and nuclear fusion. The unequal size of the nuclei of the conjugating gametes is shown well in figs. 11, 13 and 15.

Figs. 12 and 14. Gametes showing the clear hyaline pointed structure near the nucleus.

Fig. 17. Gregarine cyst from a malpighian tube of a pupa showing gametes.

Fig. 18. Formation of gametes by a process of budding.

Fig. 19. Gregarine cyst from a malpighian tube of a pupa. Last stage in nuclear multiplication. In one gregarine, very much ramified, the nuclei are ranged on the surface for gamete formation. In the other gregarine can be seen nuclear division spindles presumably of the last division. Many large unused nuclei are seen.

Fig. 20. Gregarine cyst from a malpighian tube of a pupa. The zygotes have become elongated preparatory to formation of sporocysts.

Figs. 21–27. Stages in the development of the sporocysts.

Figs. 28 and 29. Nuclear spindles of the third nuclear division of a gregarine.

Fig. 30. Karyosome free in protoplasm of the same gregarine from which figs. 28 and 29 were drawn.

Figs. 31 and 32. Two adjacent sections through a gregarine cyst from a pupa. Fig. 31 shows the breaking up karyosome and the complete disappearance of the nuclear membrane of the gregarine nucleus. Fig. 32 a structure which is probably the forming first nuclear spindle.

Figs. 33 and 34. Dividing nuclei (third division) from the gregarine which was associated with the one from which figs. 28–30 were taken.

Fig. 35. Longitudinal sections through a malpighian tube of the imago of *Stegomyia fasciata*. It is filled with liberated sporocysts.

PLATE XVI.

Development of Haemogregarina canis *in the dog and in the tick* Rhipicephalus sanguineus.

Fig. 1. Large öocyst from the tick. The nucleus of each sporocyst has multiplied till each possesses about 16.

Fig. 2. Cyst from the spleen of a dog containing schizont with protoplasm filled with refractile spheres and six nuclei.

Fig. 3. Cyst from the spleen of a dog containing three large merozoites and residual body.

Fig. 4. Young schizont in mononuclear cell in spleen. The nucleus of the parasite discoloured to show the karyosome.

Fig. 5. Schizont from spleen of dog. The nuclei show the karyosomes clearly.

Fig. 6. Cyst from spleen of dog similar to that at fig. 3 but more discoloured. The nuclei of the merozoites consist of a nuclear membrane over which fine granules are scattered. There is a central karyosome.

Fig. 7. Sporocyst from an öocyst in the tick. It shows the budding off of the sporozoites. Eight are formed at each pole. The drawing only shows four of these.

Fig. 8. Cyst from spleen of dog. The small forms are destined to enter the leucocytes and to appear in the peripheral circulation.

Fig. 9. First nuclear division of schizont in spleen of dog. The karyosome divides and functions as an intranuclear division centre.

Fig. 10. Sporocyst from an öocyst in the tick. It shows the eight nuclei arranged at one pole. Eight nuclei were similarly arranged at the other pole.

Fig. 11. Developing form in the tick. Possibly an early öocyst.

Fig. 12. A sporozoite from a sporocyst in the tick. Length 14 μ.

Fig. 13. Early schizont in spleen of dog. The nucleus shows the karyosome.

Fig. 14. Schizont from spleen of dog. Each nucleus has a karyosome.

Fig. 15. A form within an epithelium cell of the gut of the tick. Possibly a zygote.

Fig. 16. Large öocyst filled with sporocysts containing sporozoites from body of tick.

Fig. 17. Developing sporoblasts in öocyst from the tick.

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PLATE XII.

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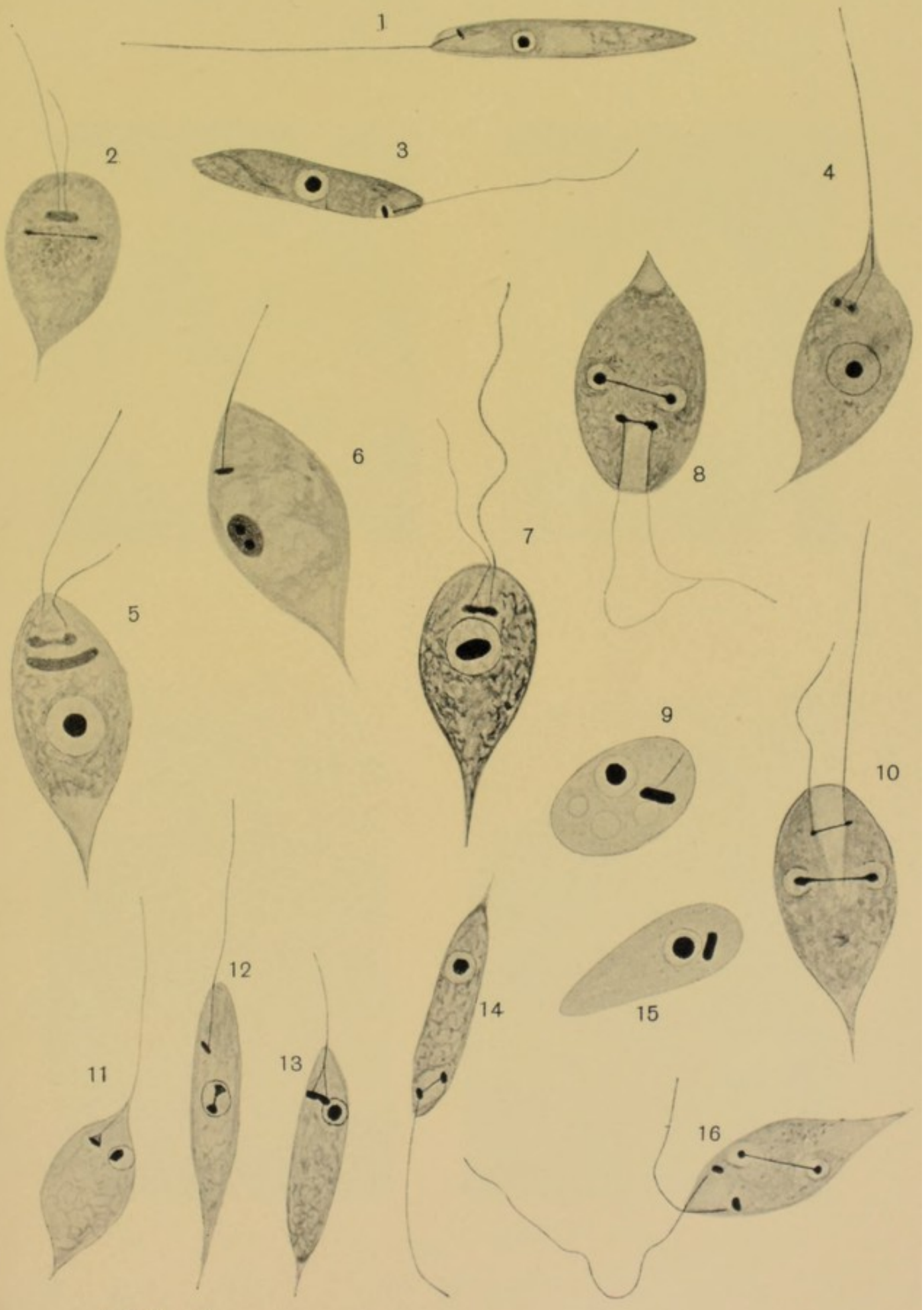

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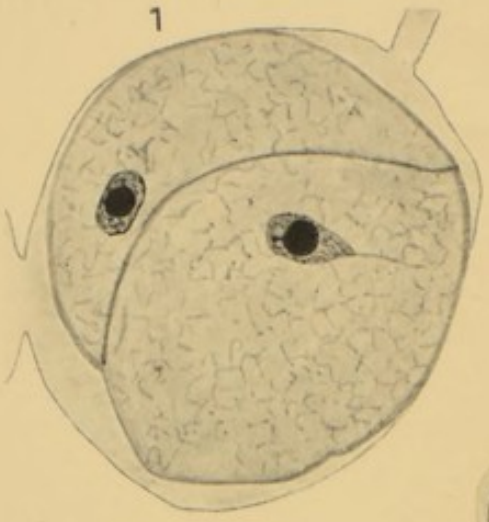

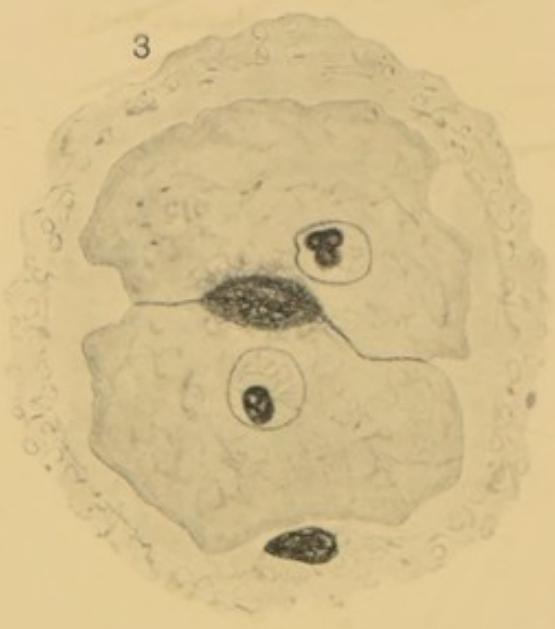

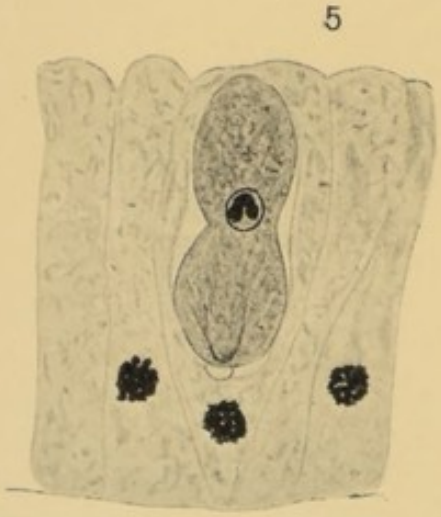

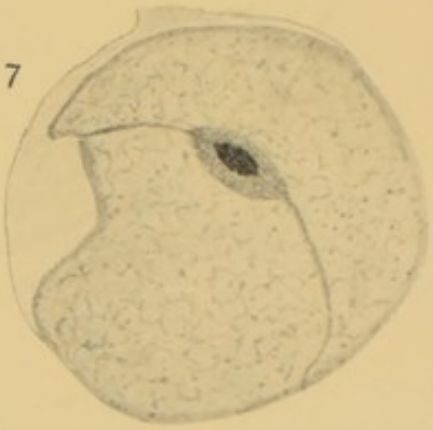

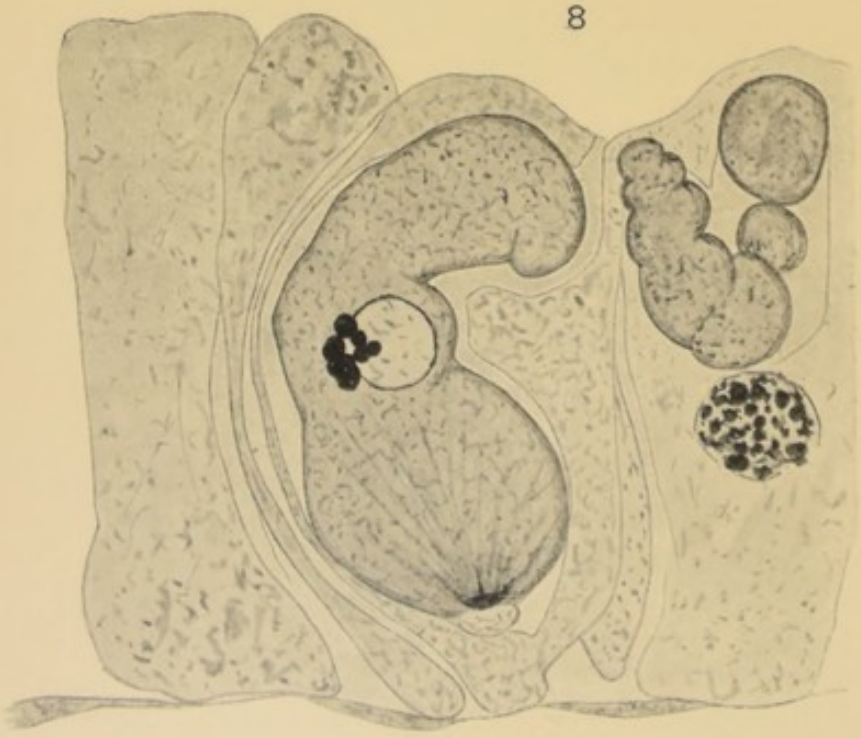

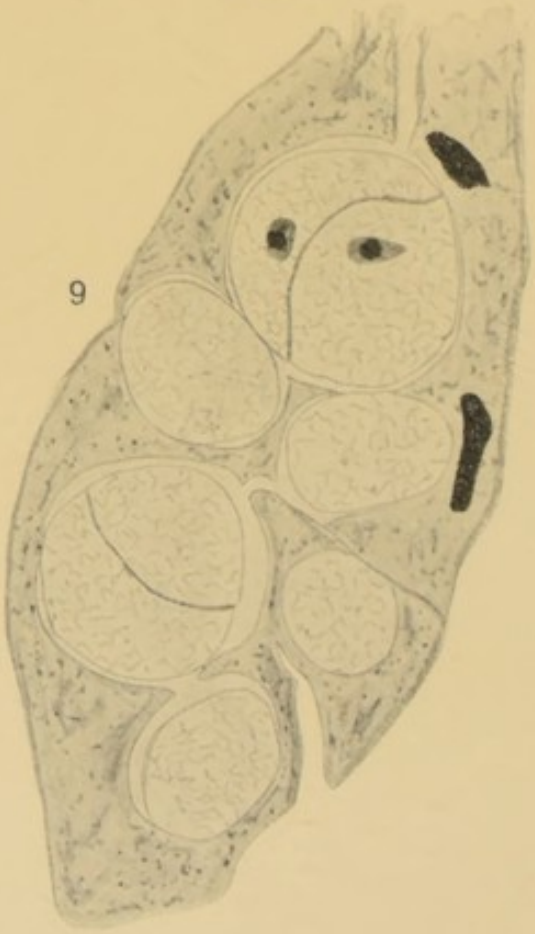

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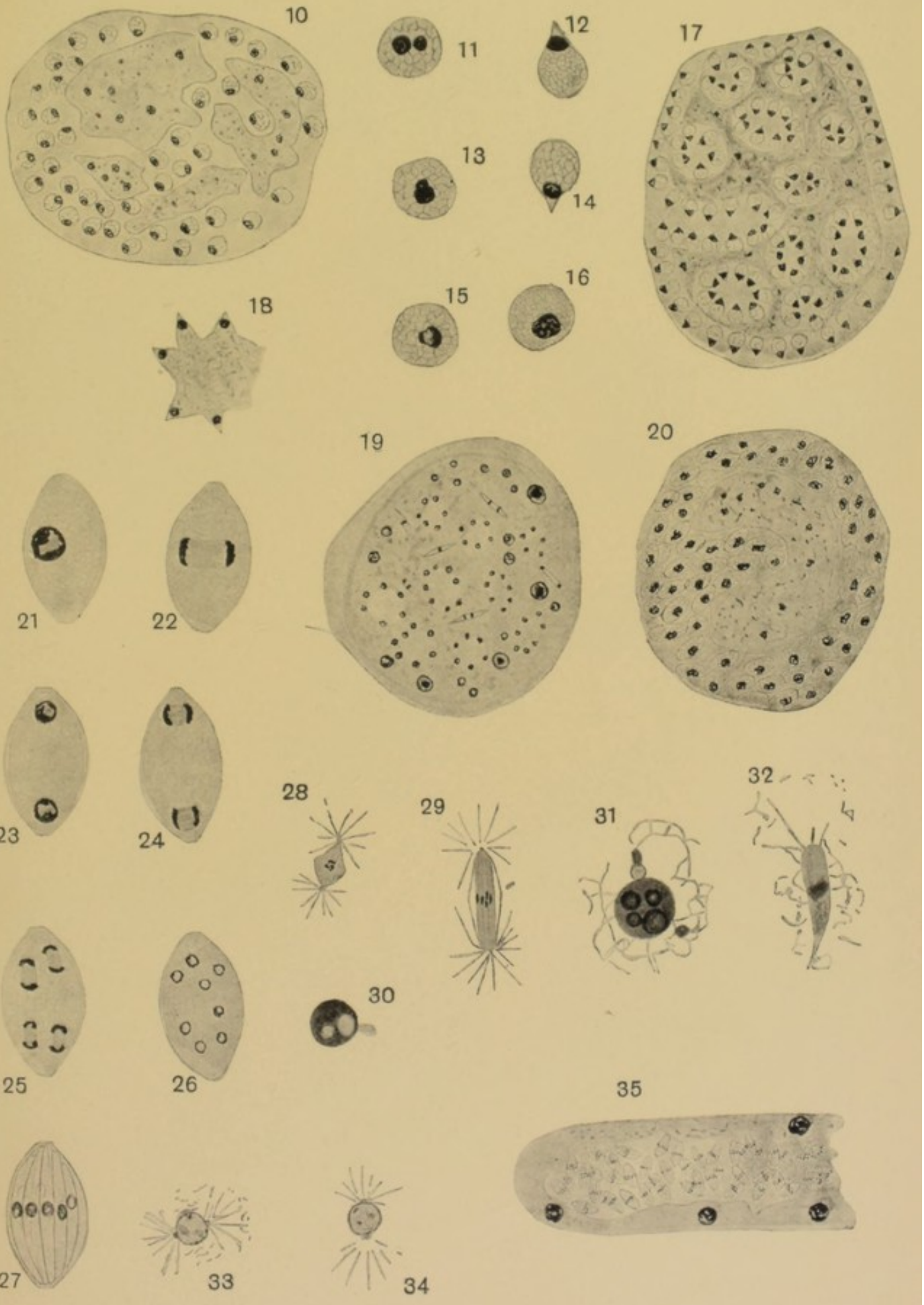
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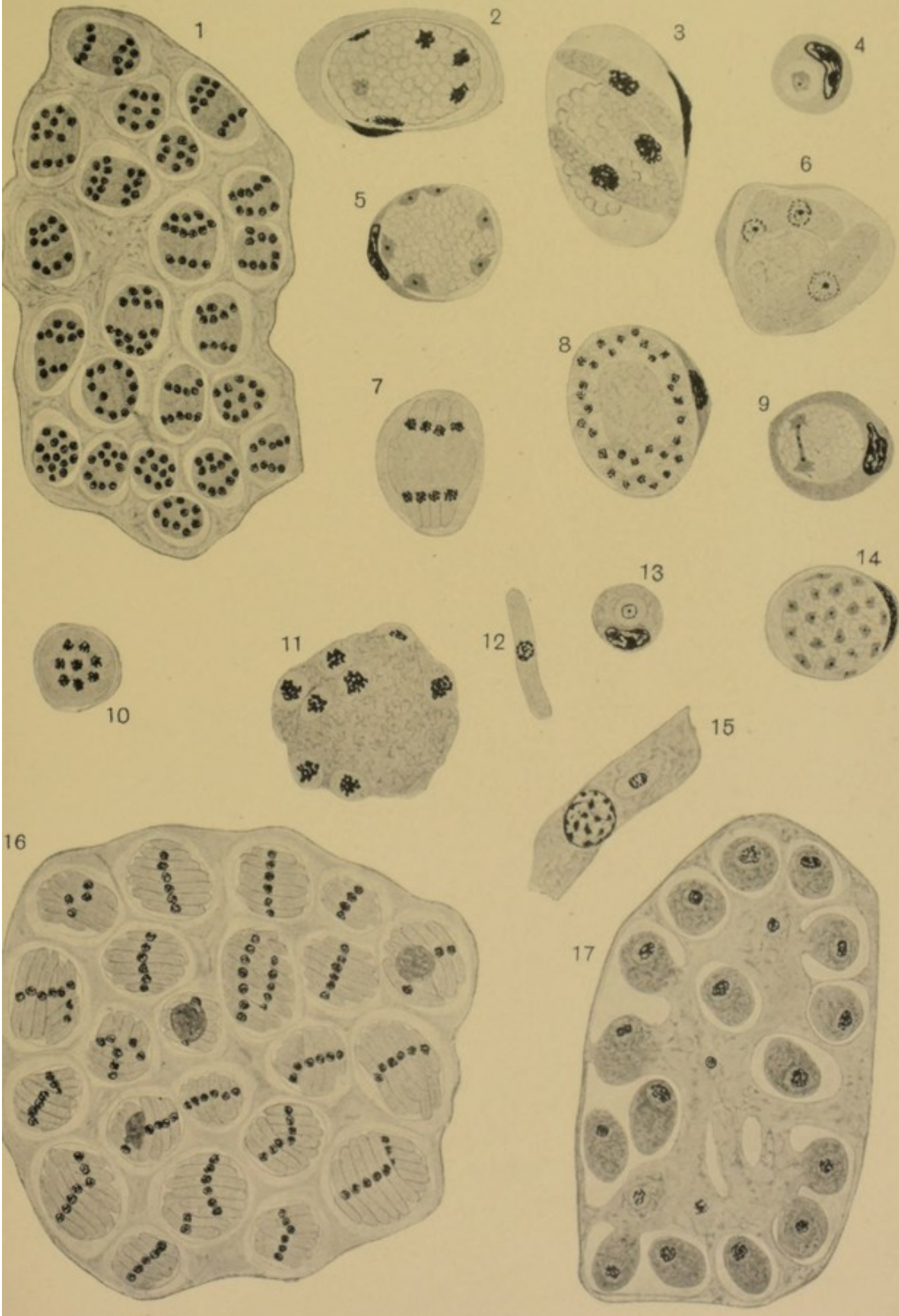

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