

OBSERVATIONS ON THE INTESTINAL PROTOZOA OF THREE EGYPTIAN LIZARDS, WITH A NOTE ON A CELL-INVADING FUNGUS.

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(With Plates XIX and XX, and 2 Text-figs.)

WHILE I was in Egypt conducting investigations into the etiology of dysentery with Captain F. W. O'Connor at Alexandria in 1916 I was able to make some observations on the intestinal protozoa of three species of lizard. The occurrence of an *Entamoeba* and a *Chilomastix* (*Tetramitus*) in one of these, *Agama stellio*, was mentioned in our publication on the *Human Intestinal Protozoa in the Near East*, p. 147 (1917). The other lizards investigated were *Chamaeleon vulgaris* and *Lacerta agilis*. I will describe the observations on the protozoa under their respective hosts.

1. *CHAMAELEON VULGARIS*.

The only protozoon found in the gut of the chamaeleon was a flagellate of the leptomonas type and it was confined almost entirely to the cloaca, though sometimes it extended in small numbers into the rectum. The only previous record of such a flagellate in the cloaca of the chamaeleon is in a paper by Bayon (1915), who discovered it in *Chamaeleon pumilus* in Robben Island in 1914. I had the opportunity of examining Dr Bayon's preparations and there can be no doubt that the measurements he quotes are erroneous. The length of the flagellate bodies is given as 25–75 μ ; breadth 2–10 μ ; diameter of trophonucleus 2.5–4 μ . In none of his preparations did I see flagellates of this size, nor have I found them in the parasite of the Egyptian *Chamaeleon vulgaris*. Evidently some mistake in calculation was made and the measurements are at least three times what they should be.

Structure of the flagellate.

The organism (Pl. XX, Fig. 1) as it occurs in the Egyptian lizard has the usual leptomonas structure—an elongate, flattened and pointed body, a central nucleus and a terminal and anterior kinetoplast with a long flagellum directed forwards, in which direction the flagellate progresses. The kinetoplast, which in many individuals is laterally placed (Fig. 1, *o*), as in other organisms of this

group, consists of a dark-staining granule, the parabasal, in front of which is a paler and smaller granule, the blepharoplast or centrosome from which the rhizoplast, continued into the flagellum, actually arises. Between these two granules, and also frequently behind them, is a clear area. Whether the whole structure represents a true nucleus of which the dark granule is the karyosome and the limits of the clear area a nuclear membrane on which the centrosome lies, as in the nucleus of *Cercomonas*, is still a matter requiring elucidation. I have brought forward some evidence in favour of the true nuclear nature of this structure in a paper entitled "Observations on *Herpetomonas muscae domesticae* and some Allied Flagellates" (1913). Most observers, however, seem to regard the structure as not being a nucleus and I quite agree there are many arguments against this view. Kofoid (1915) has suggested the abandonment of the name kinetonucleus and with it the binucleate conception of this group of flagellates. According to him the darkly staining granule is a parabasal body, a term first used by Janicki (1911), and homologous with similar structures which are associated with the flagellar origin in many other flagellates (*Trichomonas*, *Chilomastix*, *Giardia*, etc.) and which occurs in the flagellate *Prowazekella lacertae* considered below. The blepharoplast is the centrosome and leads the way in division. It must be admitted, however, that in division the behaviour of the centrosome and the subsequent division of the darkly staining mass by elongation and constriction, evidently under the influence of the centrosome, bears a striking resemblance to the division of the trophonucleus of these flagellates or of the nucleus of *Cercomonas*, as I have shown elsewhere (1913). Whether there is a nuclear membrane or not surrounding the clear area in which the darkly staining granule lies is largely a matter of interpretation, for one is dealing with such tiny objects that it is exceedingly difficult to determine this point with accuracy. In such a decision one is naturally influenced by what one has observed in larger flagellates. On account of the doubt surrounding the true nature of this structure it is better to avoid any term implying a nuclear nature so I have adopted the name kinetoplast, first suggested, I believe, by Alexeieff. The use of this word avoids the necessity of such ponderous names as kinetonucleus and trophonucleus. The kinetoplast includes both the parabasal and the blepharoplast. In degenerate trypanosomes in which the cytoplasm has disappeared the kinetoplast may remain as a compact body showing the parabasal surrounded by what appears to be a membrane on which the blepharoplast lies. The flagellum may or may not remain still attached to the blepharoplast.

As a rule the cytoplasm of the organisms is quite clear. In some, however, darkly staining granules are present, but these are probably dependent upon the state of nutrition.

The length of the body of the largest individuals (Pl. XX, Fig. 1, *f* and *m*) is about 15μ and the flagellum is slightly longer than this. The width of the body of these long forms is under 3μ . From these there may be traced a series of gradually diminishing individuals of a great variety of shape and size, as shown

in Fig. 1. Finally, very minute forms (Fig. 1, *a* and *b*) more or less spherical or circular in outline are seen, having a diameter of about 2μ . These minute flagellates have relatively long flagella. Some of them are devoid of flagella (Fig. 1, *b* and *e*) and from them small ovoid bodies with a more definite outline appear to arise (Fig. 1, *d*). These are probably encysted forms such as occur in the similar flagellate of the flea, *Pulex irritans*. I have shown in this case that complete drying of the spread-out flea faeces for 24 hours does not prevent a culture being obtained when introduced into N.N.N. medium (1912). Between these tiny parasites and the elongated form every intermediate stage can be easily traced (Fig. 1, *g*, *i*, *j*, *k*). In the small forms the nucleus and kinetoplast lie close together.

Multiplication takes place in the usual manner by longitudinal fission after division of the nucleus and kinetoplast. The new flagellum is formed by an outgrowth from the daughter blepharoplast (Fig. 1, *h*, *l*, *n*).

I have been quite unable to make out any connexion between the kinetoplast and the nucleus, nor have I seen any indication of an axostyle.

Distribution of the flagellate.

The flagellate was found in all the chamaeleons examined—about six. As already stated, it was confined almost entirely to the cloaca into which the rectum and ureters open. It was encountered in small numbers in the rectum, but not in the ureters, while in the cloaca itself it was limited almost entirely to the surface of the mucosa where it occurred in the mucus in enormous numbers. When the lizard defecates it passes a cylindrical mass of faeces or solid white urine which is covered with this mucus in which swarms of flagellates can be found. In the actual urine itself they do not occur and in the faeces only in small numbers. It is probable that the latter are forms which have passed down from the rectum. The mucus from the cloaca is a clear transparent substance and is remarkable in that there is practically no contamination with bacteria or with faecal material. In stained smears no bacteria were seen and the only organism met with apart from the flagellate was the fungus described below, which was present on two occasions.

Careful examinations of the various organs of the body both by smear and cultural methods failed to reveal any tissue infection. This is perhaps surprising in the light of the work of the Sergeants, Lemaire and Senevet (1914) and the later work of Chatton and Blanc (1918). These observers have shown that cultures of leptomonas can be obtained by inoculating N.N.N. medium with the blood of the gecko, *Tarentola mauritanica*. Examinations of smears of the blood and organs failed to reveal any leishmaniform parasites, the flagellates only being demonstrated by the culture method. It seems probable that the leishmaniform bodies seen by Chatton and Blanc (1914) within the red blood corpuscles of the gecko on an earlier occasion have no connexion with the leptomonas obtained by them later in cultures. In the light of these successes it would be interesting to make further attempts to isolate a lepto-

monas from the blood and tissues of the chamaeleon, for it would seem that in this animal the intestinal infection may indicate the path by which the blood infection of the gecko was acquired.

The exact relation of the flagellates of the chamaeleon to the mucosa is best studied in sections. It is found that the infection is a very large one and that all the glands of the cloacal mucosa have their ducts packed with the organism (Pl. XIX, Fig. 1). No trace of invasion of the cells could be found, so it would appear that the flagellate is limited to the ducts of the glands and the surface of the mucosa. Fig. 2 shows a single gland more highly magnified and though many goblet cells are present there is no tendency for the flagellates to make their way into these. There was no evidence that the flagellate in any way inconvenienced its host.

Source of the infection.

As was suggested by Bayon (1915), the most probable source of infection would appear to be some insect which the chamaeleon has eaten. Flies could very readily infect themselves by feeding on the mucus covering the faeces passed by the chamaeleon, which in its turn could be infected by eating an already infected fly. Such a hypothetical cycle would seem to be the probable one.

The contents of the stomachs of chamaeleons were examined with a view to identifying the flies on which they had fed, but little information was obtained, chiefly through lack of time to follow the observations properly. However, some experiments were conducted with the ordinary house-fly, *Musca domestica*. Pupae were collected and it was found that the flies hatching from these were free from flagellate infections. A batch of these hatched flies was fed on the infected mucus from the chamaeleon's cloaca and kept alive by feeding on sugar and water. Another batch was used as control and kept alive in a similar manner.

24. VI. 16. Flies fed on infected mucus.

25. VI. 16. Several flies examined.

1. Stomach with large infection of very active flagellates.
2. Ditto.
3. Active flagellates in stomach and rounded forms in intestine.
4. Active flagellates in both stomach and intestine.
5. Doubtful forms seen.
6. Ditto.
7. Rounded forms in intestine.
8. Active flagellates in stomach and round form in intestine.
9. Ditto.
10. Active forms in stomach and intestine.

26. VI. 16. One fly had resting forms in intestine.

30. VI. 16. Four flies all negative.

1. VII. 16. Thirteen flies all negative.

None of the flies in the control experiment showed any infection.

It is evident therefore that the flagellates taken up by the flies can survive for at least two days, and the character of the infections in the flies resembled a natural fly infection, but the fact that the flagellates eventually disappeared would seem to indicate that the house-fly is not the true host of the chamaeleon flagellate.

Unfortunately time did not permit of the investigations being carried any further.

Nomenclature.

The flagellate of the chamaeleon is undoubtedly of the leptomonas type and it is of especial interest as being the only known leptomonas which is parasitic in the gut of a vertebrate. In addition to the flagellates of the gecko described by the French observers others have been recorded from vertebrates, apart of course from the well-known natural leishmania infections of man, dog and cat. The Sergents (1907) recorded their observation of a leptomonas in the blood of the pigeon. Fantham and Porter (1915) say they saw a leptomonas in mice but Mesnil (1915) is doubtful of the correctness of this observation. Balfour (1916) also states that he and Archibald saw such a flagellate in the gerbil in the Sudan. Dutton and Todd (1902) claimed to have seen this flagellate in Gambian house mice (sp. ?), but in a subsequent examination of the films Todd (1914) found the flagellate to be in reality a trypanosome which he identified with *Trypanosoma acomys* (Wenyon). *Trypanosoma lewisi* is a very active flagellate and occurs in such a variety of forms that when seen only in the fresh unstained blood it is quite easily mistaken for a leptomonas and it seems probable that the forms described by the various observers noted above were in reality *Trypanosoma lewisi*. In any case the descriptions are so meagre that it is impossible to identify the flagellates. In 1919 Marcel Leger described a flagellate (a leptomonas) which he named *L. Henrici* from the blood of two out of thirty lizards (Genus *Anolis*, Fam. Iguanidae) examined in Martinique. The organisms were typical leptomonas forms measuring 15–16 μ in length and 3–4 μ in breadth. The flagellum was longer than the body. Rarely were rounded leishmania forms seen. He subsequently found that over half the lizards examined harboured what was apparently the same leptomonas in the rectum. It would appear, therefore, that the only cases of leptomonas infections of the blood and tissues of animals are those of the N. African gecko and the S. American *Anolis*. In 1909 Knuth recorded the finding of a leptomonas in the heart blood of a roebuck but, as the animal was partly devoured and had fly larvae in its lungs, the infection may have been of extraneous origin.

On the other hand, it has been demonstrated, chiefly by Laveran and Franchini and Fantham and Porter, that many insect flagellates of the leptomonas and crithidia types are inoculable into mice and other animals and even bring about their death. These inoculation experiments are of the greatest interest from the point of view of the spread of leishmaniasis, and the French

observers (1914) have suggested that the flagellate of the gecko may in reality be *Leishmania tropica*, and that it is transmitted to man by the *Phlebotomus* which feeds on the lizards. On account of their importance it would be well if the inoculation experiments were repeated by other observers.

In the present state of our knowledge it is difficult to name many of these flagellates. Some time ago (1913) I outlined a scheme for the classification of these flagellates in the following manner. There is a group, confined entirely to the insect or invertebrate host, which in its most highly developed stage has the leptomonas structure. Small round leishmaniform bodies, protected by what must be a cyst wall, are developed from the leptomonas forms and escape in the insect's faeces. They are ingested either by the larvae or adults of the insect and lead to their infection. There is thus only a single invertebrate host and such forms can be distinguished, at any rate at present, by the generic name *Leptomonas*. This name was first employed by Kent (1881) for a flagellate seen by Bütschli in 1878 in a nematode worm *Trilobus gracilis*. Kent named it *Leptomonas bütschlii*, but whether it is a leptomonas as we now understand it cannot be determined till this organism is re-examined in the light of present knowledge. Meanwhile we shall employ the name *Leptomonas* as above defined.

Another group of flagellates, in which the highest stage of development is again the leptomonas form, includes the parasites of leishmaniasis. Here, however, there is a vertebrate host in which the leishmaniform parasite is most usually seen, but also occasionally the leptomonas, as I have shown (1915). The latter is generally encountered only in cultures in the test-tube or in certain invertebrates which have ingested the leishmania forms along with a quantity of blood. There is undoubtedly an invertebrate host of this flagellate and in it the infection would be expected to resemble a true leptomonas of an invertebrate. In order to distinguish these flagellates which have a vertebrate as well as an invertebrate host from the purely insect form—the true *Leptomonas*—the generic name *Leishmania* can be employed.

A third group of flagellates attains a still higher stage of development and the individual flagellate is known as a crithidia. In it the kinetoplast is close to, but still in front of, the nucleus, and there is a short undulating membrane running from just in front of the kinetoplast to the anterior end of the flagellate. Along the edge of this runs the flagellum, to become free at the anterior extremity of the flagellate. The crithidia live in the intestine of invertebrates and in the posterior part of the intestine there are produced small leishmania forms, as in the leptomonas, and they are responsible for the spread of infection from host to host. There is again only a single host—an invertebrate—and such forms may be considered under the generic name *Crithidia*. We know of no flagellate of this type which has both a vertebrate and an invertebrate host corresponding to the *Leishmania*.

A fourth group shows a still higher development in that the true trypanosome structure is attained. The kinetoplast is further back at the posterior

end of the body and there is a long undulating membrane along which the flagellum runs, to become free at the anterior extremity of the flagellate. In the insect's intestine these forms show not only the trypanosome type but also intermediate ones—crithidia and leptomonas—and finally the leishmania forms. In flagellates of this group there is again only a single invertebrate host and the infection is spread from insect to insect by the leishmania forms as in *Leptomonas* and *Crithidia*. A flagellate of this group is *Herpetomonas muscae domesticae* of the common house-fly and it is convenient to distinguish these forms by the generic name *Herpetomonas*.

In a fifth group there are the same forms as in the preceding group, but in place of the single invertebrate host there is a vertebrate one as well. This is the group of true trypanosomes which have the generic name *Trypanosoma*.

This scheme of classification may have many points against it, but at any rate it is convenient and is as far as we can go in our present state of knowledge. To subdivide these groups into different genera simply leads to further confusion. All we can do is to wait for further data, and such an attempt as that made by the late Dr Albert Chalmers (1918) to split up the genus *Trypanosoma* into his extraordinary series of new genera only tends to bring confusion into an already difficult subject.

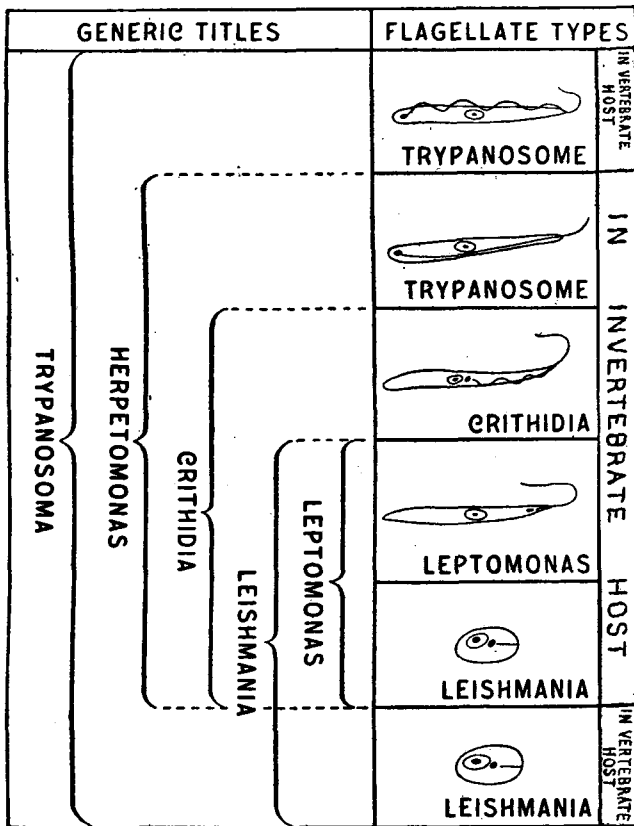
The scheme I have outlined above has the merit of simplicity and it can be arranged in tabular form in the following manner. The names in the right-hand column (Text-fig. 1) are merely descriptive and should be used in an adjectival sense. They may be employed for any particular stage which may appear in the development. Those in the left-hand column are generic titles and, as such, should be written in italics and with a capital. We can speak, for instance, of the leptomonas stage of *Leishmania tropica*, the crithidia stage of *Trypanosoma lewisi*, or the leishmania stage of *Herpetomonas muscae domesticae*.

There is one group of flagellates which do not enter into the above scheme. I refer to the curious leptomonas form first described by Lafont from the latex of Euphorbias as *Leptomonas davidi*. The work of Lafont, Bouet and Roubaud, and more recently of França (1919), has demonstrated that this flagellate undergoes a cycle in a hemipteron and is transmitted by it to healthy plants. Thus we have a flagellate of the leptomonas type which has both an insect and a plant host. Evidently it cannot be included in any of the genera defined above. For these flagellates we may employ the name *Phytomonas*, first suggested by Donovan (*Lancet*, 1909), the type species being *Phytomonas davidi* (Lafont, 1909).

If the flagellates of this most confusing group were named according to the scheme outlined above we should at any rate understand from their names something of their life history and structure.

The question now arises into what group we are to place the flagellates of the gecko, the anolis and the chamaeleon. I think we are safe in assuming that there must be invertebrate hosts. The two former would undoubtedly

fall into the group leishmania, according to the definition given above, and would receive the provisional names of *Leishmania tarantolae* and *Leishmania Henrici*. If it should subsequently be found that the flagellate of the gecko is in reality identical with that causing Oriental sore then it would of course become *Leishmania tropica*, but proof of this is at present wanting. The flagellate of the chamaeleon occupies an intermediate position. It undoubtedly has an insect host but, unlike the parasite of the gecko, it is not a tissue parasite and as far as my investigations go it appears to be confined to the



Text-fig. 1. Diagram of classification of the trypanosomes and allied flagellates.

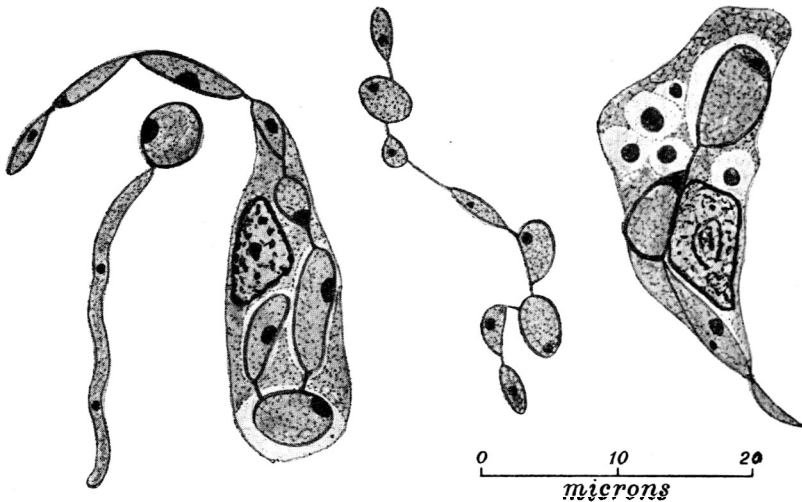
intestine and cloaca. However, it comes within the definition as having both hosts and it will therefore become *Leishmania chamaeleonis*. It seems better to place it in the genus *Leishmania* than in *Leptomonas*, for the latter would imply that it was a purely insect, or at least invertebrate, flagellate.

The intestinal habitat is of interest as it is an infection probably acquired from some insect and indicates a stage by which such an insect flagellate may eventually become a tissue parasite. It occupies an intermediate position between the purely insect flagellate and the vertebrate ones such as that of

the gecko, and of Oriental sore and kala-azar. Moreover it suggests the possibility of an intestinal mode of infection in the latter disease. In the case of *L. Henrici* it appears as if the intestinal infection, still persisting in the lizard, has already given rise to a blood and tissue infection.

Note on a cell-invading fungus.

In some of the chamaeleons examined it was noted that a fungus (Text-fig. 2) was present, and in smears and sections it was found that there was a definite invasion of the cells of the cloaca. The fungus occurs in the form of ovoid bodies embedded in the cytoplasm of the cells. Reproduction takes place by budding till the whole cell is invaded. In this process the nucleus becomes pushed aside and distorted and the cell eventually degenerates. Buds are



Text-fig. 2.* The fungus which was found invading the cells of the cloaca of *Chamaeleon vulgaris*. C. M. W. del.

also formed, protruding into the cavity of the cloaca and when this has occurred growth into the lumen takes place with the formation of more elongate elements and finally filaments. No culture of this organism was attempted and nothing more of its life-history is known. The general characters are depicted in the drawing shown in Text-fig. 2.

2. *LACERTA AGILIS* AND *AGAMA STELLIO*.

The protozoal organisms seen in the gut of these two lizards resembled one another and I have no reason to suppose that the forms common to these two lizards belong to different species. Those common to the two are *Bodo lacertae*, Grassi, or, as Alexeieff (1912) has renamed it, *Prowazekella lacertae*, *Chilomastix* sp., and *Entamoeba* sp. In addition to these three organisms, in

Agama stellio was also found a trichomonas and a trichomastix. These are probably identical with *Trichomonas lacertae*, Prowazek, and *Trichomastix lacertae*, Bütschli. It is possible that if a number of *Lacerta agilis* had been examined they would have been found in them also. The Entamoeba may possibly be identical with one seen by Dobell (1914) in the gut of *Lacerta muralis*.

Prowazekella lacertae Grassi.

This flagellate has been the subject of some controversy on account of its association in the gut of the lizard with a blastocystis, or structures which have a striking resemblance to this. Prowazek (1904) claimed to have demonstrated that the blastocystis was in reality the autogamy cyst of the flagellate. It is very improbable that any autogamy takes place in the life-history of the flagellate, but nevertheless it is undoubtedly true that the flagellate encysts and that the cysts produced have frequently been spoken of as blastocystis. Chatton (1917) describes the blastocystis as a stage in the developmental cycle of the flagellate and finds that under certain conditions not well understood the large blastocystis resulting from growth of the smaller forms produces large numbers of flagellates. He supposes, and probably correctly, that a conjugation between the flagellates takes place in association with the encystment.

Description of flagellate.

The flagellate (Pl. XX, Fig. 3) is in its adult stage an elongate, flattened organism with two tapering flagella, one of which is directed forwards and is several times the length of the body, and the other, a thinner and shorter one, directed backwards. There is a single anterior nucleus, on the anterior surface of the membrane of which lies a granule from which the flagella arise.

The body of the flagellate, as already mentioned, is flattened like a blade of grass, but not perhaps to the same extent, and sometimes, as noted by Prowazek (1904), the edges are folded or the body may be twisted on itself, but as usually seen it is simple in structure with the posterior end pointed and tapering to a varying extent. The anterior end is also pointed but less acutely than the posterior end. The cytoplasm is vacuolated and may contain granules. Near the anterior end lies the spherical nucleus, which is closely surrounded by a number of deeply staining bodies. In iron haematoxylin preparations these tend to obscure the nucleus unless the differentiation is carried far enough. The nucleus itself is a spherical body consisting of a nuclear membrane and a centrally placed karyosome. Fine granules may also be seen within the membrane, either on its inner surface or between it and the karyosome. On the anterior surface of the membrane is the granule from which the rhizoplast arises. This granule does not retain the stain as intensely as the karyosome of the nucleus nor the bodies which surround the nucleus. In some cases it appears that the granule may be double but it is difficult to make out whether this is merely an early stage of division or not. From the

granule there runs forward a fine rhizoplast. The deeply staining bodies which surround the nucleus vary considerably in number and arrangement. Sometimes they are closely applied to the nuclear membrane while at others they are separated from it by a distinct interval. There may be many fine granules or a few larger masses. They may surround the nucleus or be limited to one side only or be entirely behind it. There may be only two bodies, one on each side of the nucleus, and of such a shape that they are swollen posteriorly and tapering anteriorly, with their anterior extremities nearer one another than the posterior ones. Prowazek (1904) notes these bodies and regards them as composed of chromatin material derived from the nucleus. I can find no evidence that these bodies arise from the nucleus. In those cases where they appear to be within the membrane careful observation shows that they are merely overlying it. Prowazek (1904) figures forms in which these bodies are on the inner surface of the membrane but in all the forms I have seen they are outside it even if lying against it. Prowazek's idea that they are chromidial in nature was first contested by Dobell (1908), who wrote: "I think sufficient has been said to show that autogamy and chromidia are as yet unproven in the case of *Bodo*." They can best be spoken of as parabasal bodies similar to those which are associated with the origin of flagella in other flagellates.

The rhizoplast arising from the blepharoplast or centrosome on the nuclear membrane passes forwards and is continued into the two flagella. At the extreme anterior end of the organism at the point where the flagella arise from the rhizoplast is occasionally seen a granule or thickening but I have been unable to make out any structure in this (Pl. XX, Fig. 3, *a, c, g*). Prowazek (1904) figures the rhizoplast as terminating near the anterior end of the body in a sort of cone, beyond which is a second granule from which the flagella actually arise. If this structure is really present and there is a break in the rhizoplast then the flagellar origin is of a very specialized type. Is it not possible that this appearance is due to some peculiar plasticity of the anterior end of the body, which by retraction at the point of exit of the flagella gives rise to the cone-like appearance? At any rate in my preparations many of the flagellates seem to have a rhizoplast continued directly from the blepharoplast into the flagella. I have been unable to trace any connexion between the blepharoplast and a granule within the karyosome of the nucleus such as Prowazek describes.

The anteriorly directed flagellum is often at least five times the length of the body of the elongate flagellates and in some of the smaller and ovoid forms it is even longer in proportion. The posteriorly directed flagellum reaches as much as three times the length of the body in the long forms. It is very much finer than the anteriorly directed flagellum and in some individuals it appears to be attached to the body for a short distance (Pl. XX, Fig. 3, *a, c, e*), an attachment which may have to do with maintaining its direction. In many of the flagellates, however, there is no such attachment.

Multiplication.

The flagellate multiplies in two ways, either by simple division or cyst formation. I have not been able to trace the division in much detail, as dividing flagellates were not numerous in my preparations. However, evident dividing forms were seen in which two nuclei were present, each with its blepharoplast and rhizoplast passing into two flagella. The parabasal bodies were divided between the two nuclei so that in nuclear division those of the original nucleus are evidently divided into two more or less equal groups, as occurs in nuclear division within the cyst.

Encystment takes place in the hinder part of the gut of the lizard and is preceded by a change in shape of the organism. It becomes an ovoid body and apparently two of these become encysted together (Pl. XX, Fig. 3, *n*). A feature of the encystment is that clumps occur, the individuals of which are all in approximately the same stage of development. The clump appears to be held together by an adhesive material in which various bacteria and other intestinal debris are included.

The first stage is the formation of the ovoid body, which loses its flagella. Two of these come together and a cyst wall is formed round the pair (Fig. 3, *j* and *o*). The cyst wall is evidently of a gelatinous nature, for bacteria and other debris adhere to it. Within the cyst the two organisms fuse, as noted by Prowazek (1904), and this, I believe, is followed by fusion of the nuclei (Fig. 3, *p-s*). I can see no evidence of an autogamy as described by him.

The next stage is the appearance of a vacuole in the cytoplasm of the zygote and this gradually increases in size, evidently by absorption of fluid through the cyst wall, till the cyst becomes many times its original bulk. Concurrently with this vacuolation nuclear division takes place (Fig. 3, *t-y*). The original nucleus of the zygote has the same structure as that of the flagellates, being spherical with a nuclear membrane and central karyosome (Fig. 3, *r* and *s*). The parabasal bodies lie around the nucleus. The exact similarity between the nuclei of the cyst and the flagellates makes it practically certain that they are in reality flagellate cysts, quite apart from the stages of encystment where every step can be followed. In division the karyosome divides and the two parts separate, while they remain connected by a fine fibre (Fig. 3, *u-w*). The nuclear membrane elongates at the same time, and finally two nuclei are formed by constriction at the middle. The parabasal bodies remain at the equator of the elongating nucleus for some time and then they are divided into two groups which pass to the daughter nuclei. These bodies are of various sizes and they do not appear to form anything in the nature of chromosomes. I cannot say if any actual division of each separate mass takes place but each of the resulting groups of daughter parabasal bodies contains more or less an equal amount of material. The nuclei continue to divide in the same manner till as many as 32 are formed within the now very enlarged cyst (Fig. 3, *m, l, i*). The majority of the cysts are spherical but some are

elongate or even dumb-bell shaped. The growth of the cyst is remarkable but a similar growth takes place during the development of the oöcyst of the malarial parasite in the stomach of the mosquito. It is evident that the cysts are not very resistant bodies, but that they are destined to pass out of the body is supported by their occurrence in greatest number at the hinder end of the intestine.

The fully formed cyst contains comparatively little cytoplasm, which is grouped around the nuclei on the inner surface of the cyst wall. The bulk of the cyst contains liquid, through which strands of a coarse network of more refractile material run and which connect with the nuclear areas. Even though not possessed of very tough or impermeable walls it is possible that these cysts would take a considerable time to dry completely on account of their enormous fluid content. The development of the cyst beyond the 32 nuclear stage I have not been able to follow but Prowazek (1904) and later Chatton (1917) have seen these large cysts give rise to numbers of flagellates.

The description I have given depends entirely on stained films as prolonged observations on the living cysts were not made. That two individuals encyst together seems undoubted but if one wished to be hypercritical one could suppose that the two associated individuals either before or after encystment were the results of division and do not represent conjugation. My interpretation of the appearances seems the more probable one and is in conformity with Chatton's own observations. It must, however, be admitted that an absolute proof of the process has not been obtained by me. Further observations on this parasite might yield some interesting facts in connection with the conjugation process of flagellates and the origin of the parabasal bodies and centrosome.

Blastocystis.

As already pointed out, many of these flagellate cysts bear a striking resemblance to blastocystis and as a matter of fact I have been accustomed to regard them as such. In the lizards I have examined, all the so-called blastocystis have the very characteristic nucleus with the surrounding parabasal bodies, so there can be no doubt that they are stages in the development of the flagellate cysts. Whether side by side with the true flagellate cysts there exists a "Blastocystis" of vegetable nature I cannot say at present, but it cannot be doubted that *Prowazekella lacertae* encysts in the gut of the lizard and that the cysts have been frequently styled "blastocystis."

What then is the blastocystis which occurs so commonly in the human intestine and that of other animals? Prowazek (1911) maintained that they were cysts of *Trichomonas* but there is no evidence to support this view. Swellengrebel (1917) has suggested that they are degenerate forms of various intestinal protozoa, while Jepps and Dobell (1918) have noted that certain degenerate forms of *Dientamoeba fragilis* resemble dead blastocystis. I myself have, for want of evidence to the contrary, always regarded them as of a vegetable nature and this may be the case in spite of the resemblance to

the cysts of *Prowazekella lacertae*. For a similar reason I regarded the I-cysts as being probably vegetable organisms, but they are now known to be cysts of *Iodamoeba williamsi*. I had also noted (1910) that degenerating *Chilomastix mesnili* could assume appearances closely resembling blastocystis. Swellengrebel's (1917) conclusion is that blastocystis "is not the name of a zoological genus but of a peculiar form of degeneration to which representatives of different genera of intestinal protozoa may be liable." On the other hand future investigations may show that blastocystis is derived from amoebae and it must be admitted that the large binucleate cysts of *Entamoeba coli* with the large vacuole occupying almost the entire cyst bears some resemblance to binucleate forms of "blastocystis." Frequently in stained preparations containing small entamoebae, such as *Endolimax nana*, and blastocystis, it is possible to trace what might be regarded as a complete series of connecting links between a typical amoeba and a typical blastocystis and one is constantly tempted to adopt the view that the series traceable is a real one. Macfie (1915) regarded certain blastocystis associated with an entamoeba in the monkey *Cercopithecus petaurista* as cysts of the entamoeba. His proof of this, however, appears to be wanting and furthermore we know that the entamoebae of the monkey produce the typical entamoebic cysts with four or eight nuclei.

Alexeieff (1911) says that at one time he regarded the blastocystis of the lizard as derived from the lizard flagellate but that later observations have altered his opinion and that he has come to look upon blastocystis as a purely vegetable organism. He was largely influenced in this by the character of the development of a yeast (*Schizosaccharomyces octosporus*), and has suggested the name *Blastocystis enterocola* for the intestinal blastocystis.

Dobell (1908) in criticizing Prowazek's work on the autogamy cysts of *Bodo* compares them with very similar cysts he had seen in the gut of the frog and which he proved by germination to be of a vegetable nature. It appears to me that Prowazek's cysts, though not autogamy cysts as he describes them, are at any rate true cysts of the flagellate. At least this can be stated of some of those he figures.

It is evident therefore that there is a difference of opinion as to the true nature of blastocystis and we must await further information. It seems possible that under the name blastocystis three distinct structures have been confused: the true protozoal cysts like those of the lizard flagellates, vegetable organisms like the cysts Dobell studied in the frog or which Alexeieff saw in the case of his yeast *Schizosaccharomyces octosporus*, and thirdly, degenerate intestinal protozoa or even tissue cells, some of those in the human intestine belonging to the second group and others to the third.

Trichomonas lacertae Prowazek, and *Trichomastix lacertae* Bütschli.

I have nothing to add to the description of these two organisms as given by Prowazek (1904). I have seen none of the conjugation forms described by him. The flagellates are shown in Pl. XX, Fig. 2, *h* and *i*.

Chilomastix sp.

This is a small organism of the usual structure and in size corresponds with the two flagellates just mentioned. Its general appearance is shown in Pl. XX, Fig. 2, *g*.

Entamoeba sp.

The entamoeba is a large organism in its fully grown form (Pl. XX, Fig. 2, *a*). Smaller forms also occur and the general character of the organism is shown in Fig. 2, *a-f*. It will be noted that it bears a striking resemblance to *Entamoeba coli* and, like it, it feeds upon most of the contents of the lizard's intestine, frequently ingesting the flagellates or their cysts (Fig. 2, *b*). It produces an eight-nuclear cyst which again is not distinguishable from that of the human *Entamoeba coli* either in size or characters.

Of the lizard hosts mentioned above, judging from the contents of their intestines *Chamaeleon vulgaris* is entirely an insect feeder. This is true to a large extent of *Lacerta agilis*, though in it some vegetable matter is also present. *Agama stellio* feeds on insects but also largely on vegetable matter, as large pieces of leaves of plants and grass are to be found in the stomach. It is not surprising therefore that the two last named can infect themselves from vegetable matter contaminated by other infected lizards, while the chamaeleon only harboured the flagellate which it had probably acquired from some insect, which had in its turn taken up the flagellate from the faeces or rather cloacal mucus of the animal.

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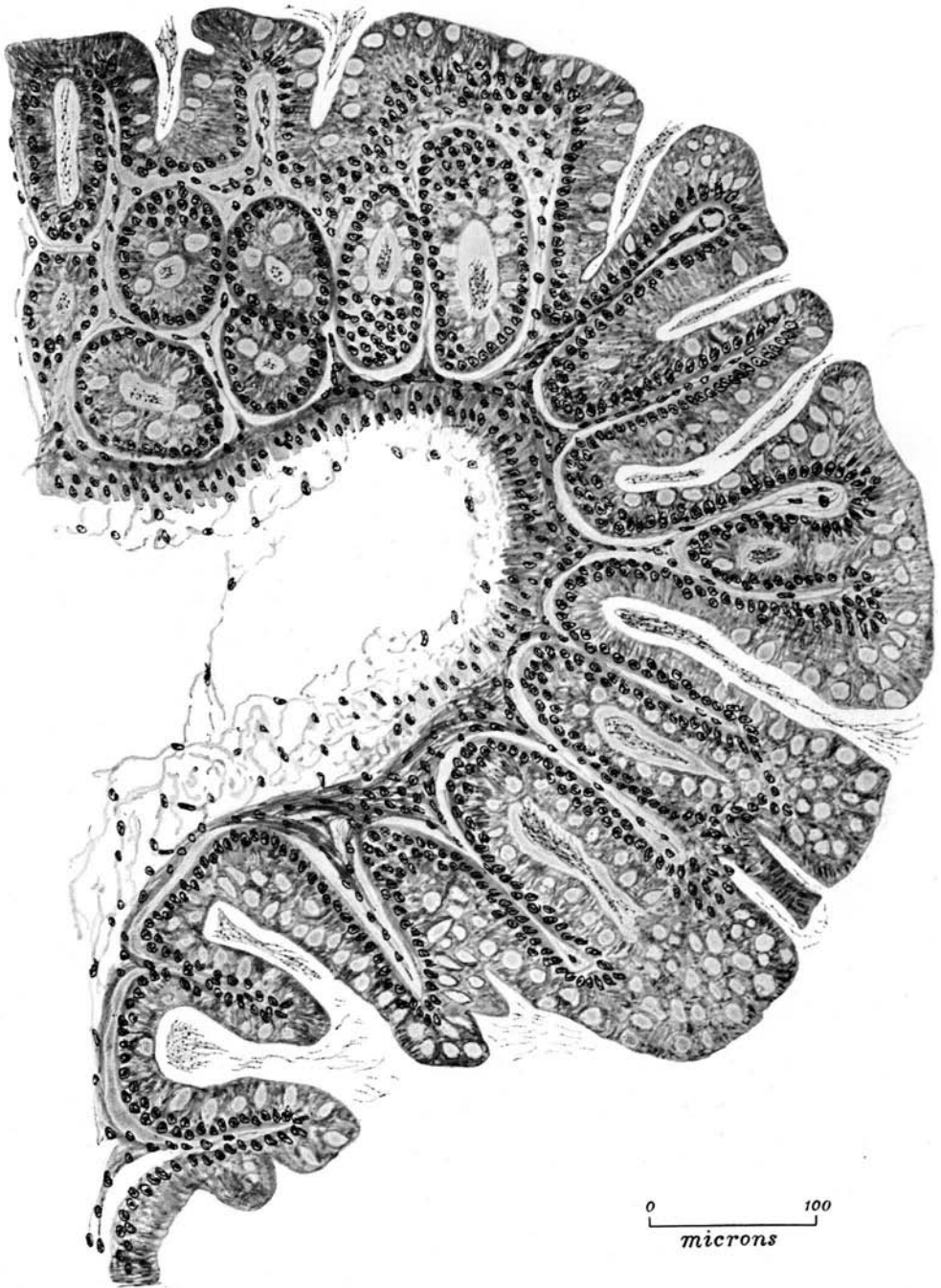


Fig. 1. Section through part of the wall of the cloaca of *Chamaeleon vulgaris* to show the lumen of the glands filled with clusters of *Leishmania chamaeleonis*. (C. M. W. del.)

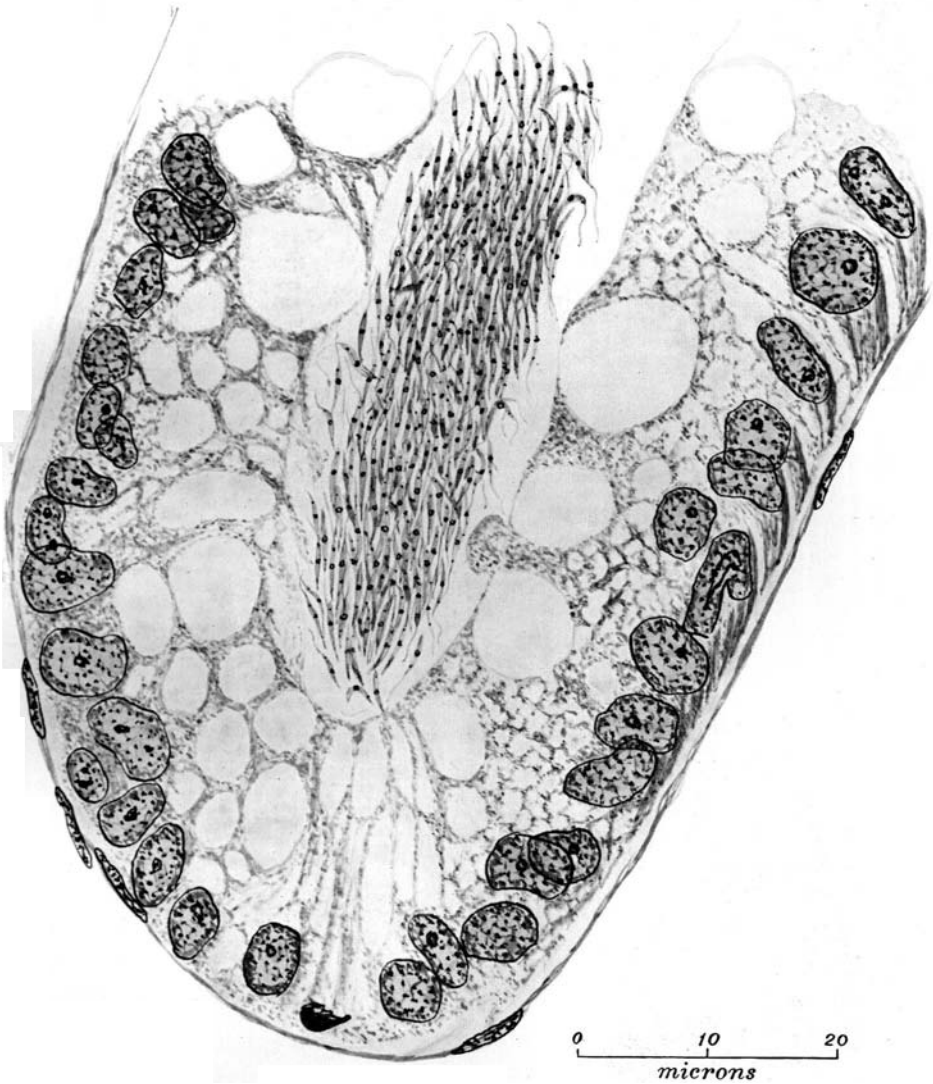


Fig. 2. A single gland in the same section as shown in Fig. 1 drawn to a larger scale. There appeared to be no invasion of the cells by the flagellates. (C. M. W. del.)

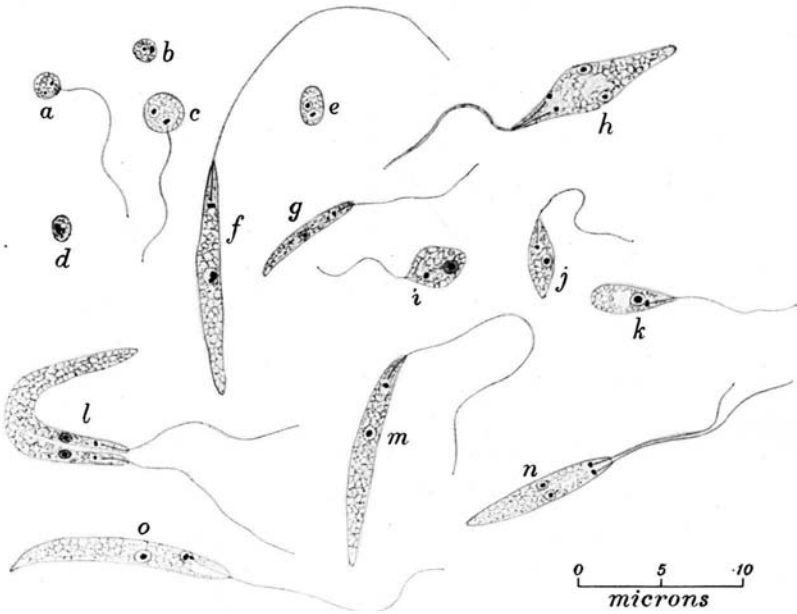


Fig. 1. Various types of *Leishmania chamaeleonis* found in *Chamaeleon vulgaris*. Every intermediate shape and size between the long forms (*f, l, m, n, o*) and the minute ones (*a, b, c*) are to be found. A possibly encysted form is shown at *d*. (C. M. W. del.)

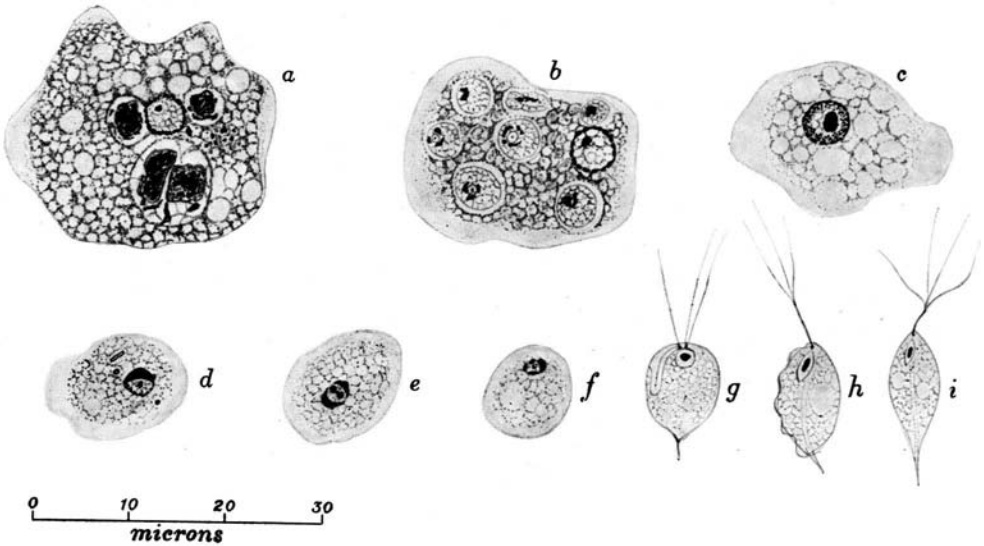


Fig. 2. Protozoa found in *Lacerta agilis* and *Agama stellio*. The entamoeba which produces cysts exactly like those of *Entamoeba coli* is shown at *a-f*; *Chilomastix* sp. at *g*; *Trichomonas lacertae* at *h*; *Trichomastix lacertae* at *i*. (C. M. W. del.)

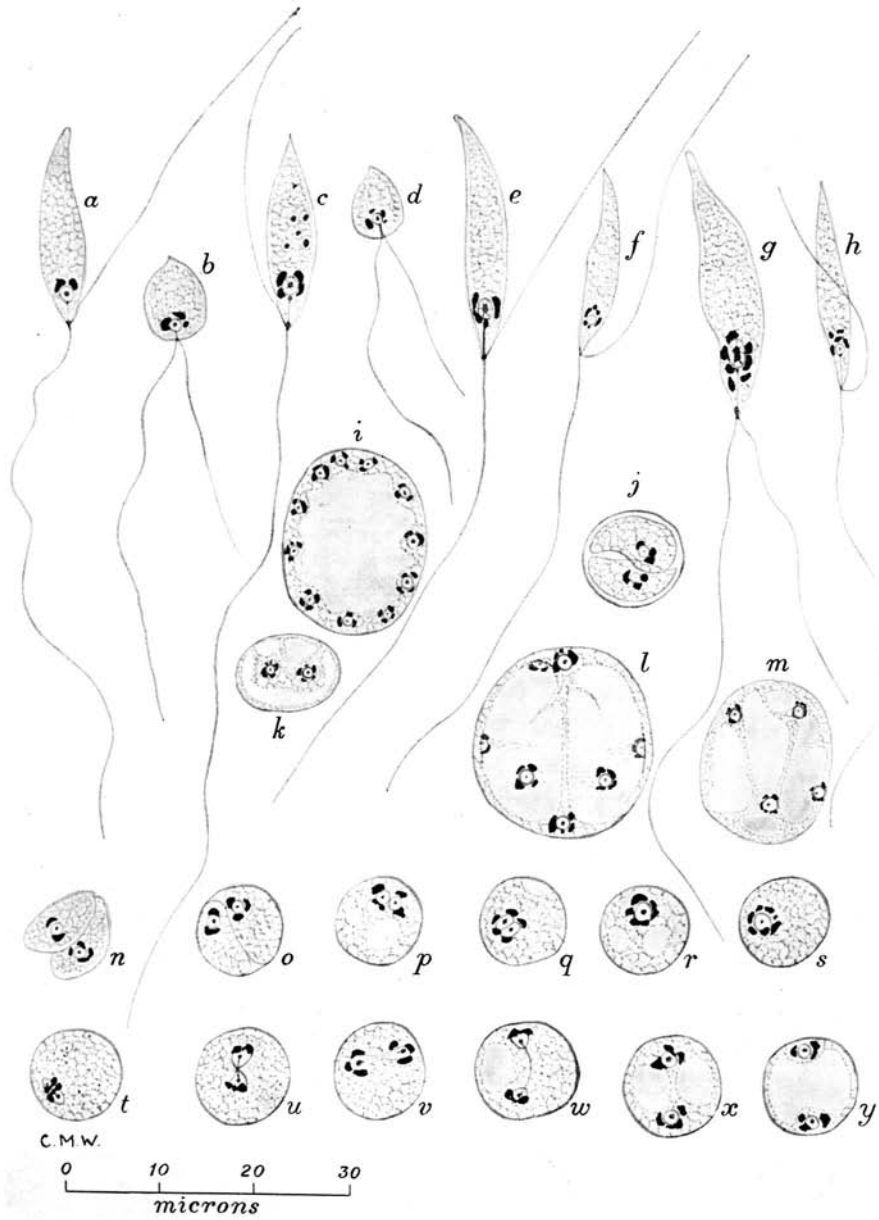


Fig. 3. *Prowazekella lacertae*. The forms figured at *a*, *c* and *e* show the appearance of the attachment of the "trailing" flagellum. In others both flagella appear to be quite free. The series *n* to *s* shows what is probably the conjugation and encystment, while *t* to *y* show the first nuclear division of the zygote and the formation of the vacuole. At *j* and *k* are two cysts corresponding to that at *o*. Later stages of the cyst development are shown at *m*, *l* and *i* in which nuclear multiplication to the 32 nuclear stage is reached. At all stages the parabasal bodies are seen surrounding the nucleus and they are roughly divided into two groups at nuclear division both of the free flagellates and the encysted forms. (*C. M. W. del.*)

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