NORMAL AND ABNORMAL MORPHOLOGY OF *TRYPANOSOMA LEWISI*, IN THE BLOOD OF THE RAT.

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(With 15 Diagrams.)

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WHEN comparing the results of the recent morphological study of *T. lewisi*, we note a general tendency to bring these observations into accordance with the views expressed in Schaudinn's well-known publication (1904). In this paper I propose to discuss the morphology of this interesting flagellate under normal and morbid conditions, and to consider whether the present tendency to adopt Schaudinn's views is justified. Since I shall take occasion to compare my observations with those of other authors, it will not be necessary to give a historical review of the subject. A full list of references will be found in the papers of Rabinowitsch and Kempner (1899), Wasielewski and Senn (1900), Laveran and Mesnil (1901), and Prowazek (1905). I have discussed matters of technique in a previous paper (1910) to which the reader may refer.

1. *Forms of the chronic infection.*

As is well known these forms appear 8—13 days after infection, they no longer divide. It is not necessary to insist on the general aspect, this being well-known from the description of the authors already

mentioned. In the first period of chronic infection curious forms may be seen, with a very elongated hind end (Diagram VI, fig. 2). Prowazek mentions them and considers that they have no supporting fibrillae and are consequently very metabolic.

(a) *The protoplasm.* Between the nucleus and the blepharoplast a filament may frequently be observed, staining blue with Giemsa. I have already referred to this structure in an earlier paper (1910) (Diagram I, fig. 1). This filament is probably to be homologised with similar structures found in *T. gambiense* and *T. brucei* (Robertson, 1906). Moore, Breinl and Hindle (1908) do not mention it as occurring in *T. lewisi*, but I have observed it so frequently and under so many conditions of fixation and staining that a mistake can be excluded. Generally the filament is quite continuous, but sometimes it breaks up in a row of irregular granules, which are, however, different from the chromidia, which stain red with Giemsa (Diagram I, figs. 1, 2).

Diagram I.

Fig. 1. Continuous axial filament.

Fig. 2. Axial filament broken up.

The structure of the protoplasm was considered in my previous paper.

(b) *The blepharoplast* is a rod-shaped organelle, and can only be studied in preparations carefully stained with Giemsa. If it is overstained the achromatic part cannot be distinguished from the chromatic part. In many cases the blepharoplast is composed of two chromatic granules, united by a substance staining pink with Giemsa (achromatic substance) (Diagram I). The presence of these two granules does not indicate a division of the blepharoplast, it is a premature division of the centriolic apparatus such as is often met with (Prowazek, 1909). Moore, Breinl and Hindle (1908) have described a similar structure, which

Minchin seems not to have noticed (1909). It is not always to be seen, sometimes only one chromatic granule is present.

Degenerated flagellates may sometimes be observed, in which the blepharoplast has lost its compact structure. In these cases the presence of an achromatic and chromatic substance becomes particularly clear (Diagram II). The blepharoplast is swollen and the two chromatic granules wide apart (fig. 1). Subsequently the chromatic part is broken up into many granules, still united by the swollen achromatic substance (figs. 2, 3). We shall have occasion to refer again to this degenerative hypertrophy of the blepharoplast.

Diagram II.

Fig. 1. Swollen blepharoplast. Protoplasm showing "periplast line."

Fig. 2. Isolated flagellum with corresponding blepharoplast.

Figs. 3—4. Hypertrophied blepharoplasts.

(c) *The flagellum* seems often to be free from the blepharoplast. At its base the basal granule is frequently though not always to be seen. Sometimes an achromatic (pink) filament is seen uniting the base of the flagellum to the blepharoplast. This filament was mentioned by Rosenbusch (1909) and by Breinl, Moore and Hindle. In degenerated cells it may be seen that the flagellum and the blepharoplast are really connected: the blepharoplast has lost its definite structure, the protoplasm does not stain well; the achromatic part becomes particularly clear and is always united to the base of the flagellum (Diagram II). In normal cells where the protoplasm is well stained, this filament is

often invisible. In isolated flagella, which are sometimes to be found, the blepharoplast is nearly always present, the filament uniting flagellum and blepharoplast is very distinct in such cases. Minchin thinks that shrinkage, due to defective fixation, may separate the flagellum from the blepharoplast. If this connection were so delicate, one would never be able to find free flagella with their blepharoplasts, so I think this separation is due to some other cause.

(d) *The nucleus.* The nuclear structure has already been described in my previous paper. Giemsa (1910) holds that the old method of fixation and staining does not show the minute nuclear structure. Diagram III, figs. 1, 20, 21 show the same structure as Giemsa described. It is very common in preparations made according to the old method.

Diagram III.

Changes in the structure of the non-dividing nucleus.

The structure of the nucleus is not so simple as that described by Moore, Breinl and Hindle. The simplest form shows a central karyosomatic granule, united with the periphery of the nucleus by some achromatic filaments (figs. 1, 21). This central chromatic mass can completely disappear, so that only a central achromatic structure remains (fig. 20). The karyosome may also be divided (figs. 2, 4, 6—8) and the two parts may divide again (figs. 15, 16) or only one of them may divide (figs. 10, 14). If no division takes place curious deformation of these granules is observable, due probably to the fact that the chromatin is spread over the achromatic reticulum, so that X- or H-shaped structures are produced (figs. 9, 11—13); at the end the chromatin is dispersed more or less completely over the achromatic filaments of the nucleus, so that these filaments become very distinct and the chromatin seems to be present in the form of rodlets or of a zigzag line (figs. 22, 23). A third

change of structure is the production of a central clear space in the nucleus (fig. 3), the karyosome breaking up and forming a circle of chromatic granules round it. The granules may be equal or unequal (figs. 17—19).

Prowazek (1905) has interpreted some of these stages (such as figs. 6—8, 10, 13—16, 18) as "autosynthesis" of the nucleus, by which the karyosome divides in four parts, two of them degenerating and two combining to form a new karyosome (cf. Prowazek's figs. 5—8 with figs. 7, 14, 15, 18 of Diagram III). I think however that these forms selected by him out of a great variety of forms, have no more significance than any other of the nuclei of Diagram III so that I do not see that it is proved in the least that this autosynthesis really occurs. Minchin, who holds the same opinion, thinks that Prowazek's views are based on an incorrect observation due to a defective technique, but I think his observations (not the interpretation) are quite right.

Diagram IV.

Figs. 1 and 2. Flagellates of the chronic stage of infection with two nuclei, one of them pycnotic.

Fig. 3. The same, but both nuclei pycnotic, one of them fragmented.

The peripheral granules of nuclei such as are represented in Diagram III, figs. 1, 20, 21, are generally regarded as chromosomes and their number is said to be constant. This is not the case in the nuclei shown in Diagram IV. I do not think them to be real chromosomes, because they do not play any part in the nuclear division.

Very large karyosomes nearly occupying the whole nucleus were never observed. I have already stated elsewhere the probability that they are due to shrinkage, the shrunken nucleus being taken for the karyosome (1910).

In the period of chronic infection, two nuclei may be observed in one cell (Diagram IV) sometimes united by a thin chromatic filament (fig. 1). One of the two nuclei is normal in structure, the other is always pycnotic and looks degenerated. This nuclear division is never followed by that of the cell, but it might be a reduction previous to the formation of gametes. Apart from these forms, we find others, where also a nuclear division takes place but where now two of them are obviously degenerated and fragmented (fig. 3), consequently I believe that these nuclear divisions are abnormal and to be regarded as pathological fragmentations.

(e) *Chromidia*. The study of the formation of chromidia is easier under abnormal than under normal conditions, consequently the subject will be more fully dealt with when treating of the abnormal morphology of *T. lewisi*. Flagellates containing chromatic granules may be frequently observed in rats' blood (Diagram V). Fig. 1 seems to indicate that

Diagram V.

Figs. 1—5. Different stages of the formation of chromidia.

the chromidia may be formed under the influence of the nucleus and the blepharoplast. In fig. 4 the two forms of chromidia are yet to be separated, although they have become more diffuse; in fig. 5 they have intermixed. Figs. 2 and 3 show an agglomeration of the chromatic substance, leading to the formation of one or few compact chromatic masses, sometimes resembling a nucleus (fig. 3). These figures are interesting because similar stages have been described as an autogamy by Moore, Breinl and Hindle, the blepharoplast emitting chromatin

which wanders towards the nucleus, the latter being previously reduced. Fig. 1 might represent the expulsion of the chromatin from the blepharoplast, figs. 2 and 3 the wandering towards the nucleus and fig. 4 the arrival of the chromatin near the nucleus. I believe, however, that fig. 5 seems to indicate that sometimes the chromatin may not reach its destination and may be dispersed on the way. The observations of the authors I have mentioned indicate only that the chromidia may be produced near the nucleus and the blepharoplast; but that this phenomenon has anything to do with an autogamy is not proved. Hindle (1909) seems to hold the same opinion regarding *T. dimorphon*.

Prowazek's "nuclear reduction" occurring in the blood of the rat must be regarded as a stage in the formation of chromidia.

As to the origin of these chromatic granules, I have no actual proof that they are really formed by the nucleus or the blepharoplast, although this seems to be probable judging from the phenomena observed in degenerating cells (see section III: abnormal morphology). Their chemical constitution could not be studied because they were not frequent enough to be found in normal cells.

Diagram VI.

Fig. 1. Isolated periplast showing longitudinal striae.

Fig. 2. Trypanosome with long hindend and two striae.

(f) *The periplast* covers the protoplasm as a thin layer, which sometimes (especially in young cells) may be demonstrated as a delicate red line surrounding the cell. The periplast is more resistant than the rest of the trypanosome and is sometimes (with the flagellum and the blepharoplast) the only thing that remains of the flagellate (Diagram VI, fig. 1).

In such isolated periplasts longitudinal striae are sometimes to be seen, but whether they are myonemes or folds of the periplast I am not able to say, although the latter supposition seems the more probable. In normal cells I sometimes observed two longitudinal striae such as have been described by Prowazek (fig. 2) but this is all I saw of the complicated system of "myonemes" and "anchoring fibrillae" described by this author. Minchin doubts the existence of all these structures and I am inclined to share this doubt.

2. *Stages of division.*

(a) *Division of the cell.* (Diagram VII.) The normal mode of division, by formation of rosettes, has been described so carefully that it is not necessary to repeat this here (fig. 1). The products of this

Diagram VII.

- Fig. 1. Rosette.
- Fig. 2. Dividing "leptomonas."
- Fig. 3. Equal binary division.
- Fig. 4. Equal multiple division.
- Fig. 5. Unequal binary division.

division ("leptomonas-forms") may divide again before recovering the normal shape of a trypanosome (fig. 2). These forms have been called "latent bodies" by Moore, Breinl and Hindle, who seem to think that they are resistant bodies. Why they are called "latent" I cannot imagine, for they divide and move about, and are as active as ever they can be¹.

There may also occur (though not very often) an equal multiple division without the production of a rosette (fig. 4). Many nuclei and flagella are formed and the probable result is the production of flagellates of the normal shape and size, although I cannot say this with certainty because I only met stages as drawn in fig. 4. So far as I know this mode of division of *T. lewisi* has not yet been described. This multiple equal division leads to the binary equal division (fig. 3) already described by Swingle (1907). I never saw any stage but that shown of fig. 3, so I do not know whether division actually takes place at this stage or not. Perhaps fig. 3 represents only a stage previous to the one shown in fig. 4, so that both of them belong to the same mode of division. Fig. 5 shows unequal binary division, very seldom to be observed; I saw it only once.

(b) *Nuclear division.* (Diagram VIII.) Two types may be distinguished: (a) a division in which a karyosome seems to take a leading part; (b) a division where no central chromatic mass is to be seen.

Type (a) (figs. 1—6). The nucleus becomes elongated and the karyosome takes the shape of a dumb-bell (fig. 1); often, though not always, the two poles of the dumb-bell are united to the periphery of the dividing nucleus by radiate fibrillae. The karyosome divides (fig. 2), the nucleus becoming more and more elongated. The two karyosomes may divide again before the nuclear division is complete (fig. 3). A clear space now appears in the equatorial region of the nucleus (figs. 4, 5) which becomes more distinct and indicates the line of demarcation of the two nuclei; they become rounded at the edges and the two daughter-nuclei are constituted (fig. 6). Sometimes the division of the karyosome is delayed, so that the nucleus is practically divided at a time that the two daughter-karyosomes are yet united by a filament (fig. 4). I did not often observe these stages, but sometimes they may be frequent and then the two nuclei may be separated by a long distance, the karyosomes still holding together by a very elongated filament as is shown in the figures of Moore, Breinl and Hindle and as I myself observed in Prof. Minchin's preparations. It is worth men-

¹ Fig. 4 (Diagram X) shows a curious spur-like appendix to such a "leptomonas form." Its significance is unknown to me.

tioning that in stages like figs. 3—5 it is not uncommon to see the karyosome very distinctly in one half of the nucleus, whereas it is invisible in the other half.

Type (b) (figs. 7, 8). Here the nucleus becomes equally elongated (fig. 7) and in the centre a structure may be seen resembling the karyosome of fig. 1, only it is achromatic. Whether this structure must be considered as a karyosome which has lost its chromatin, or simply as

Diagram VIII.

Figs. 1—6. Nuclear division with karyosome.

Figs. 7—8. Nuclear division without karyosome.

Fig. 9. Pseudomitosis.

a part of the achromatic reticulum I do not know. In fig. 8 (nucleus on the left hand) the last stage of division is represented. The two daughter nuclei are already separated but still linked together by a filament uniting the two central achromatic structures. All the chromatin is situated at the periphery.

If this central achromatic structure is only a part of the achromatic reticulum supporting the chromatin, I seriously doubt whether a karyosome does really exist in the nucleus of *T. lewisi*. The figures of Diagrams III and VIII seem to indicate that the chromatin may wander

from the centre to the periphery (and *vice versa*). If it is assembled in the centre, a "karyosome" seems to be present (not only stained but also in the "standard preparations"), but if the chromatin goes to the periphery the "karyosome" disappears. Hartmann would perhaps interpret this phenomenon as an evidence of the cyclic properties of the karyosome, but I doubt whether this view is quite correct.

In preparations fixed with Flemming's fluid and stained with iron-hematoxylin, nuclei may be seen of the shape drawn in fig. 9. The nucleus has undergone considerable shrinkage, two chromatic granules fill up the equatorial region and two others are situated at the poles of the spindle-shaped nucleus. Similar structures were sometimes interpreted as mitotic division. These nuclei are only to be found after wet fixation (especially Flemming's), so I think these figures are artefacts produced by the considerable shrinkage sometimes following upon this mode of fixation.

It may be noted that in studying the structure of the nucleus and the blepharoplast with the help of Giemsa's stain it is necessary not to stain too long. In many cases the staining is just suited for the nucleus, at a moment when the flagellum is not yet stained.

(c) *Division of the blepharoplast.* (Diagram IX.) The division begins with the augmentation of volume of the achromatic substance (fig. 1 *a*). Then the whole blepharoplast is elongated (fig. 1 *b*), the

Diagram IX.

Figs. 1—3. Stages of the division of the blepharoplast.

chromatic substance becomes dumb-bell shaped (fig. 1 *e*) and presently is separated into two parts (fig. 1 *d*). The achromatic substance now becomes very much elongated (fig. 2) forming a regular "centrodesmosis." At last it is broken and the two small daughter-blepharoplasts are formed each with their chromatic and achromatic components. Sometimes the chromatic portion is divided in three parts (Diagram VIII, fig. 8) but

I do not know whether or not this leads to a triple division of the blepharoplast. In blepharoplasts where the chromatic portion is already divided (such forms occurring already in the chronic stage of infection) the two parts are only separated, but of course these forms cannot be distinguished from those resulting from a division of a blepharoplast with a single chromatic granule.

Prowazek considers the chromatic part of the blepharoplast as the karyosome. Fig. 1 seems to indicate that this part of the blepharoplast governs the division of the whole organelum.

I never saw any sign of mitotic division of the blepharoplast as was described by Rosenbusch (1909), but my description corresponds perfectly with that of Moore, Breinl and Hindle.

(d) *Formation of the new flagellum.* (Diagram X.) Moore, Breinl and Hindle affirm that the new flagellum is formed out of a granule arising from the blepharoplast. Laveran and Mesnil (1901) long ago stated that the new flagellum was formed by the separation of a part of the base of the old flagellum, this part growing out. This view, contradicted afterwards, is the correct one in my opinion.

Diagram X.

Figs. 1—7. Production of a new flagellum.

This process is best studied in the "leptomonas-forms" and small trypanosomes arising from the division of larger forms. In these cells the flagellum continues to divide when still very small. In fig. 1 a new flagellum has been formed and is dividing again. There are two basal granules present, out of the left-hand granule arises a tiny flagellum,

the right-hand granule is still aflagellar. In this case the filament uniting the basal granule to the blepharoplast is very distinct, it shows only the beginning of a longitudinal division, its fission follows that of the basal granule. This figure shows that the flagellum is divided before the blepharoplast. Fig. 2, a "leptomonas," shows the same as fig. 1, only here no connection is to be seen between basal granule and blepharoplast. Here the right-hand granule produces a flagellum which as yet is very short. It shows that there is no sharp differentiation between the flagellum and the basal granule, but that the latter is only the thickened basal part of the first. Fig. 3 is similar to fig. 2 only here the right-hand granule is more developed. Flagella in different stages of growth may be seen also in figs. 1 and 2 (Diagram VII). Fig. 4 shows how the undulating membrane is produced, the flagellum not piercing the blepharoplast, but pushing it forward, when growing out (fig. 4).

Figs. 5—7 show the same phenomena in small trypanosomes, fig. 5 shows the division of the basal granule, figs. 6 and 7 the growing out of the new flagellum.

The thickened base of the flagellum (basal granule) is consequently the active part of this organellum; it divides and by growing out produces a new flagellum. The blepharoplast has nothing directly to do with it, except that the basal granule is united with the latter and so in a certain sense forms a part of it. The basal granule was never seen to be formed by the blepharoplast, nor did it produce the flagellum by a division (as is the case in *T. noctuae*).

It seems that the basal granule, which is very distinct during the division of the flagellum, disappears after division is over. This seems to indicate that it is not an independent organellum as are the blepharoplast or the nucleus but only a part of the flagellum itself, endowed with a special function.

(e) *Different forms of the trypanosomes during the period of division.* (Diagram XI.) During this period there is a marked polymorphism among the full-grown cells, affecting the length and breadth, the undulating membrane, the free flagellum and the way in which the protoplasm is stained by Giemsa.

Figs. 1 and 7 are two extreme types: fig. 1 short and broad, with a short free flagellum, the protoplasm staining dark blue; fig. 7 slender, spirochaetiform, with a long free flagellum and protoplasm staining violet. The first trypanosome is of the "female" type, the second of the "male" type. Between these two are all sorts of intermediate forms (figs. 2—6), so I do not think that in this case the dimorphism has

anything to do with sexual differentiation, but may be explained by the fact that such forms as figs. 1 to 3 are about to divide¹.

In this period the curious long-tailed forms, mentioned already, are also to be found (Diagram VI, fig. 1).

Diagram XI.

Polymorphism of full-grown cells during the period of division.

(f) *Autogamy*. In a recent article Schilling (1910) describes some changes in the relative position of the nucleus and the blepharoplast in dividing forms of *T. lewisi*. He holds that these changes indicate an autogamy. The blepharoplast comes near to the nucleus and seems to come in contact with the karyosome for a short time.

Diagram XII.

"Autogamy."

¹ This explanation of the dimorphism does not apply to *T. gambiense* and *dimorphon*. Here we have perhaps a real sexual differentiation.

I have seen similar changes, only the nuclei of my preparations do not possess such large karyosomes as Schilling has drawn. Minchin (1910) and I (1910) have shown elsewhere that these karyosomes are artefacts; Diagram X, fig. 4 shows the beginning of the autogamy, Diagram VII, fig. 2, Diagram X, fig. 1 and Diagram XII the subsequent stages. They can all of them be explained more naturally by superposition. So it is in Schilling's figures, except fig. 2, but there it is by no means proved that the intranuclear granule near the karyosome has anything to do with the blepharoplast. I think that the existence of autogamy is not demonstrated by Schilling's observation.

3. *Abnormal morphology.*

It is known that by preserving blood containing *T. lewisi* in the refrigerator, the trypanosomes may survive for a considerable time (up to 20 days). During this time they become agglomerated, the protoplasm becomes granulated and at last they degenerate completely. No attempt has been made to study systematically the different morbid changes occurring in trypanosomes under these circumstances, although this study is important so as to be able to interpret correctly the different forms found in the gut of invertebrate hosts (lice, fleas, bugs, ticks). In the paper by Strickland and myself (1910) we showed how many abnormal forms are found in the flea's gut, etc. shortly after the infecting meal, before the real development begins. These forms have been interpreted by some authors (*e.g.* Prowazek) as sexual forms. I will try to show that similar forms are to be found in the blood preserved in the refrigerator, so that probably the corresponding forms of the invertebrate hosts must be regarded as degenerative.

The blood was kept for 7—11 days in the refrigerator in capillary tubes (mixed with a 0.5 % solution of citrate of sodium in 0.5 % saline). After 11 days the changes to be described here were well marked, after that time all the trypanosomes died and were dissolved except the flagella, so my description refers mainly to forms preserved for 11 days in the refrigerator. The different changes mentioned here occur all ante-mortem.

(a) *Changes of the blepharoplast.* (Diagram XIII.) The blepharoplast becomes hypertrophied, sometimes the achromatic substance seems to free itself from the chromatic part (fig. 3), the latter may be divided in several portions (fig. 2). Also the achromatic part can become much elongated and curved, the chromatin distributed over it

as minute granules (fig. 4). Sometimes the whole blepharoplast is divided in two unequal parts (fig. 7), the flagellum being sometimes connected with the smaller one (Diagram XIV, fig. 6). Equal division may also take place and the two parts may divide again (fig. 5). All these equal or unequal divisions are continued (figs. 6, 8—10) so that at the end it is difficult to say where the true blepharoplast is situated,

Diagram XIII.

Figs. 1—4. Hypertrophy of the blepharoplast.

Figs. 5—8. Division of the blepharoplast.

Fig. 9. The blepharoplast wanders towards the nucleus.

Fig. 10. Pseudomitosis of the blepharoplast.

Fig. 11. Reticular chromidia near the blepharoplast.

Figs. 12—13. Formation of large chromatic masses.

Figs. 14—16. The blepharoplast emits chromatic substance which wanders towards the nucleus.

especially so because the base of the flagellum becomes often very indistinct. Many dividing granules are to be observed which often look very regular and suggest a "reduction" of the blepharoplast (figs. 5, 6, 8, 9) or even mitotic spindles are simulated (fig. 10). Sometimes the blepharoplast, and with it the flagellum, wanders in the direction of the nucleus (fig. 9), but a real crithidia facies is never produced. In other cases the products of hypertrophy and division of the blepharoplast are less regular and take the shape of achromatic reticula with chromatic granules distributed over it (fig. 11). All these changes may be produced on a much larger scale, large masses of chromatic and achromatic substance being formed (figs. 12, 13) or long chromatic strings being loosened from the degenerating blepharoplast (figs. 14, 15). Some of these chromidia, as they may correctly be named, wander towards the nucleus, so repeating on a large scale Moore, Breinl and Hindle's "autogamy."

Many of the changes of the blepharoplast described here bear a marked resemblance to the reduction of the blepharoplast during the parthenogenesis seen by Prowazek in cultures, and previous to copulation in the gut of *Haematopinus*. I think all these phenomena do not belong to the normal life cycle of *T. lewisi*. As in the blepharoplasts of fig. 3 (Diagram II) the distinction of chromatic and achromatic substance is well marked in these degenerating blepharoplasts.

(b) *Changes of the nucleus.* (Diagram XIV.) The nucleus becomes first enlarged and the karyosome becomes often elongated as during a division (fig. 1); then it becomes larger and seems to absorb all the chromatin, the rest of the nucleus becomes pale and indistinct (fig. 2). The karyosome becomes longer and takes the shape of a twisted filament, the rest of the nucleus becoming indistinct (fig. 7) and disappears at last (fig. 8), so that instead of a nucleus there appears a filament, wound in the form of a spiral or zigzag line (figs. 9, 14) closely resembling Prowazek's "male" form.

In other cases the chromatin may be expelled from the nucleus (fig. 3) in the form of one or more granules or irregularly shaped masses (figs. 4—6). These forms correspond to the stages of nuclear reduction in the rat's blood and in the louse's gut as described by Prowazek. If these were the only stages to be found, this supposition might be possible, but the formation of chromidia continues (fig. 10), the nucleus becoming pycnotic at the same time. At last only some dumb-bell shaped rodlets are to be found and when the blepharoplast is broken up at the same time (which is not always the case), the cell is filled with

scattered remains of the nucleus and the blepharoplast (figs. 11, 12, 14)¹.

Diagram XIV.

Figs. 1—3. Karyolysis.

Figs. 4—6. Formation of chromidia.

Figs. 7—9, 14. Formation of a chromatic filament.

Figs. 10—12. Complete destruction of nucleus and blepharoplast.

Fig. 13. Round cell with normal nucleus and blepharoplast and short flagellum.

¹ These chromidia are not composed of "volutin" as might be expected, after what is known in other Trypanosomes (e.g. *T. brucei* and *gambiense*).

It would take too long to compare in this paper all the stages of degeneration with Prowazek's figures of nuclear reduction, gametogenesis, etc., but if the figures are compared, it will be seen that they are remarkably alike and that their resemblance cannot be explained as merely due to coincidence.

(c) *Changes of the shape of the cell.* (Diagram XIV, fig. 13.) Little is to be said on this point. Cells as shown in fig. 13 were not commonly seen. Perhaps these forms are to be compared with Moore and Breinl's "latent bodies," but this cannot be affirmed with certainty.

It might be argued that these degeneration forms and the stages of development found in the gut of *Haematopinus* are not to be compared, although bearing some superficial resemblance to each other. This, however, is not the case. As Strickland and I have shown and as may be seen also in Diagram XV, both the same phenomena, viz. hypertrophy

Diagram XV.

Degenerating *T. lewisi* in the gut of *Haematopinus spinulosus*.

and fragmentation of the blepharoplast (figs. 1—3) and formation of chromidia and fragmentation of the nucleus (figs. 4, 5), are also to be found in trypanosomes in the gut of the rat-louse, if no further development takes place.

4. Summary.

The development of *T. lewisi* in the blood of the rat is accompanied by a division of the nucleus which is more like an amitosis than a mitosis.

The division of the blepharoplast, always preceded by the division of a karyosome-like granule, might be called a promitosis (*sensu*: Nägler, 1909).

It is not proved that autogamy or parthenogenesis takes place in rats' blood or in cultures. The evidence brought forward by Prowazek

and by Moore, Breinl and Hindle proves only the existence of chromidia; neither are Schilling's recent proofs suggestive.

Under abnormal conditions hypertrophy takes place affecting the nucleus and the blepharoplast, with subsequent formation of chromidia and final fragmentation. Similar stages found in the rat-louse have been described as sexual cells.

Considering these results in connection with previous observations of Strickland and myself, we may conclude that as yet nothing whatever indicates the occurrence of any sexual process in the life-cycle of *T. lewisi*, both in the vertebrate and invertebrate hosts.

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