VOLUME IX JULY, 1917 No. 4

OBSERVATIONS ON *BLASTOCYSTIS HOMINIS.*

BY N. H. SWELLENGREBEL, PH.D.
Zoologist of the Institute.

(*From the Section of Tropical Hygiene of the Colonial Institute in Amsterdam. Director, Prof. Dr J. J. van Loghem.*)

(With Plates XVI, XVII.)

1. *Introduction.*

In 1908 intestinal parasites of man were described by Bohne and Prowazek. The authors considered them to be cysts of *Trichomonas hominis*. The parasites consist of a highly refractile spherical body[1], staining yellow or yellowish brown with iodine and pale grey or black with iron haematoxylin; this sphere is surrounded by a narrow plasmatic layer, containing the nuclei. Bensen (1910) gave a detailed description of these bodies, with stages of autogamy, and considered them also to be cysts of *Trichomonas*. Prowazek (1911) did not subsequently change his opinion regarding the systematic position of these bodies and James (1914) confirmed Prowazek's views by stating that cats infected with the same bodies showed *Trichomonas* in their stools.

Wenyon (1910) was the first to contest Prowazek's views in holding the opinion that these bodies are degenerative stages of *Chilomastix mesnili*. Alexeieff (1911), who saw them in the gut of *Haemopis sanguisuga*, considered them to belong to a new genus and species (*Blastocystis enterocola*), showing no affinities with *Trichomonas* or any other flagellate but being related to the Blastomycetes. This opinion is founded on the following facts: The parasite has a mucous membrane; it multiplies by budding; four to eight spores are formed within its body like ascospores in an ascus, they have a germinative pore; the structure of the nucleus (peripheral chromatic granule, separated from

[1] In this paper referred to by the name of "the sphere."

the nucleoplasm by a clear space) resembles that of Blastomycetes. Subsequent authors confirm Alexeieff's views by stating that the parasite is a Blastomycete or a chytridiaceous fungus, without however describing anything like the sporulation as seen by Alexeieff. Brumpt (1912) describes the nuclei as containing a central large karyosome, differing in this respect from Alexeieff. Chatton and Lalung Bonaire (1912) observed the sphere to be eosinophilous. Mathis (1913) and Low (1916) also consider these parasites to be of a separate species. Scott Macfie (1915), although of the same opinion, thinks that it is related to intestinal amoebae. Alexeieff believes that the human *Blastocystis* and that found in the intestine of apes and rats all belong to the species *B. enterocola*, but Brumpt places these forms in different species. Alexeieff also thinks that the parasite is related to *Dermocystis pusula* Pérez (1907), a skin parasite of *Molge marmorata*, showing a refractile sphere similar to that of *Blastocystis*.

I have often observed *Blastocystis* in the stools of Europeans, having resided in the Dutch East Indies. Owing to the refractile sphere these bodies, in eosin preparations[1], are often taken for cysts, and so might cause error in diagnosis. Moreover Low points out that only after gaining a more thorough knowledge of the life cycle of this parasite will it be possible to know whether it is pathogenic or not. Therefore it seemed to me to be of importance to investigate the life history of the human *Blastocystis*.

2. *Observations on fresh material.*

In fresh material, *Blastocystis* appears as a hyaline rounded body surrounded by a less refractile fringe. The latter takes the shape of a ring or of two or three crescents. After some minutes' observation under the cover-glass, the sphere is seen to lose its refringence and to disappear, leaving only a vacuole surrounded by less refractile substance. Treated by iodine or Lugol's solution, the fringe stains yellow, the sphere remains unstained. In 5 % formalin the sphere disappears instantly and the fringe becomes highly refractile. In an eosin solution the fringe generally stains red, but sometimes it is colourless and only stains after being kept for some time under the cover-glass. I therefore conclude that this fringe constitutes a protoplasmic layer

[1] An eosin preparation consists of a suspension, under the cover-glass, of fresh fecal matter. All living elements (amoebae, cysts, etc.) remain unstained and are seen as white patches on a red background; all dead elements are stained by the eosin (cf. Kuenen and Swellengrebel, 1913).

and that it only seldom can be observed in a living condition. The sphere never stains with eosin. I cannot confirm Chatton and Lalung Bonaire's view on this subject. After being coloured by eosin, dark staining granules may be observed in the plasmatic fringe, no cyst-wall was observed to surround the cell, consequently *Blastocystis* is not a cyst.

With the object of determining whether *Blastocystis* is a normal developmental form of *Trichomonas* or *Chilomastix* the following experiments were undertaken:

Case No. 1. European, having resided in the Dutch Indies for some years, returned October 1913. The stools contain *Limax* amoebae, *Entamoeba coli* and *Blastocystis*, they were regularly observed for several months, but no other parasites were found. After the administration of four cascara tabloids, many blastocysts were present in the semi-fluid stools; after strong purgation (30 grams of sulphate of magnesia) the stools became fluid but no flagellates of any kind were found. A diet consisting of milk and some eggs only caused the blastocysts to diminish in number but not to disappear.

Case No. 2. Javanese, temporarily residing in Europe. In the stools *Limax* amoebae and *Blastocystis* were present, and observation of three months' duration did not reveal any other parasites. Purgation with sulphate of magnesia (15 grams) did not cause the appearance of flagellates.

These experiments show that *Blastocystis* may be continually present in the intestine without any traces of flagellates being found even after prolonged observation. Although in other cases I found *Blastocystis* together with *Chilomastix mesnili*, the former observations show that even if blastocysts can be formed from *Chilomastix* they also may be formed without the help of this flagellate.

3. *Observation on fixed and stained preparations.*

Wet-fixed (corrosive alcohol) preparations were stained with iron- or Delafield's haematoxylin. The results obtained from these observations will be recorded for each case separately:

Case No. 2 (Pl. XVI, Figs. 1–27).

(For particulars of this case see above.)

With iron-haematoxylin, the sphere stained yellowish-grey (Figs. 14–19). The surrounding cytoplasm stained a pale grey, it showed an alveolar structure (Figs. 14, 15, 17) or no structure at all (Fig. 16),

sometimes it consisted of nothing but a line around the sphere (Figs. 19, 20). No trace could be found of a cyst-wall, pellicula, or mucous membrane, not even in specimens showing shrinkage. The chromatic granules were generally found in the plasmatic fringe (Figs. 14, 17), sometimes within the sphere (Fig. 15). There may be one chromatic body, surrounded by a clear zone (Fig. 14), sometimes showing a granule in its centre (Fig. 16), or there may be two or more of them of equal or unequal (Fig. 23) dimensions, some of them showing an internal structure (Fig. 27). The sphere may be hyaline and without any internal structure (Figs. 14–18) or small alveolae may be present (Figs. 13, 20, 24, 27). Sometimes it stains uniformly yellowish-grey with iron-haematoxylin, or deep black (Fig. 25), sometimes the centre stains black and the periphery yellow-grey (Fig. 27).

In the stools of this case all blastocysts had their cytoplasm stained red after treatment with eosin, which shows that none were found living. Consequently all the forms mentioned here must show more or less marked necrotic changes. Which forms show the least deformity? I believe that the least altered forms are those possessing the broadest cytoplasmatic fringe because in Case No. 3, the only one in which still living blastocysts were seen, most of them showed a broad cytoplasmatic layer. In Case No. 2, most of the blastocysts only showed a very narrow cytoplasmatic layer (Figs. 19 and 20), therefore I believe that such forms should be considered as the final stages of necrosis, although some persons might be tempted to consider them as representing stages of sporulation. The problem now arises as to the origin of the stages with broad cytoplasm (Figs. 13–17).

Sometimes blastocysts were observed with a chromatic reticulum within the sphere (Fig. 12) nearly (Fig. 11) or wholly (Figs. 9, 10) filling the latter. Apparently there is a genetic relationship between these forms, but what is the first stage? Here Figs. 5–7 may suggest a solution. Fig. 5 represents an amoeba of the *Limax* type, showing within its cytoplasm a dark staining part of reticular structure surrounded by a pale staining part of alveolar structure. In Figs. 6 and 7 this differentiation is more distinctly marked and these forms are not to be distinguished from those represented by Fig. 10. In Fig. 9 there is a distinct demarcation between the two plasmatic components, also shown by differences in colour, the peripheral crescent-shaped cytoplasm being coloured grey and the alveolae between the chromatic reticulum of the centre being coloured yellow-grey. A similar differentiation is shown in Fig. 8, but here the central cytoplasm does not

show a reticular structure and the nucleus has assumed the shape of a crescent, a deformity often found in degenerating amoebae of this kind (cf. Fig. 1).

Another way in which *Blastocystis* may arise from *Limax* amoebae is represented in Figs. 1–4. The centre of the cytoplasm shows large vacuoles (Fig. 1) which become fused and produce one large vacuole (Fig. 2) filled with a grey granular or reticular substance. The nucleus breaks up in chromatic granules (Figs. 3, 4). Similar changes were observed in case No. 5 (see below).

Case No. 1 (Pls. XVI, XVII, Figs. 28–64).

(*For particulars regarding this case see p.* 453.)

The smaller forms perfectly resembled the blastocysts of the former case. A peculiarity of this case was the presence of large blastocysts, their size being due to the growth of the sphere accompanied by a reduction in the volume of the plasmatic fringe (Figs. 56, 57) and of the number of chromatic granules. Within the plasmatic fringe a peculiar differentiation was to be observed, consisting of the splitting up of this fringe in an outer filamentous often contorted part (Fig. 54) and an inner plasmatic part. Fig. 56 shows the beginning of this differentiation. The inner part disappears completely, finally leaving nothing but the sphere surrounded by the filamentous part (Fig. 58).

Divisional stages, mentioned by various authors, were often met with in this case (No. 1), showing all stages of division (Figs. 59–64). It was noticed that division was more often seen in the blastocysts showing the filamentous deformation of the cytoplasm mentioned above, which is accompanied by the complete disappearance of the cytoplasm. Consequently I think that this division is due to an unbalanced condition arising from the hypertrophy of the sphere combined with a gradual disappearance of the enclosing cytoplasm, which causes the sphere to break up into two or more parts (Figs. 62, 64), which are kept together by the surrounding cytoplasm.

As in Case 2, the blastocysts of Case 1 could be referred to *Limax* amoebae (Fig. 28) showing within their cytoplasm a more or less compact agglomeration of chromatic substance (Figs. 29–31). This agglomeration grows (Figs. 32–34) and gradually (beginning at the periphery) is converted into a structureless hyaline mass (Figs. 35–38). The nucleus is often situated within the latter mass and generally disappears, being replaced by chromatic granules at the periphery which can sometimes

be shown to arise from the nucleus. No signs of sporulation were found in this case.

Case No. 3 (Pl. XVII, Figs. 65–82).

A European suffering from amoebic dysentery.

Infection with *Blastocystis* combined with *Chilomastix mesnili* and *Entamoeba histolytica.*

In this case for the first time living blastocysts (not staining red with eosin) were encountered. The sphere was generally much smaller in relation to the surrounding cytoplasm, than in the former cases (Figs. 70–75). The blastocysts differed also from the former by the presence of a vesicular nucleus, with only small chromatic granules and a distinct reticular nucleoplasm (Figs. 71–73); in other forms only peripheral chromatic granules were to be seen (Figs. 75–77). Division was observed (Fig. 81), but only combined with the filamentous differentiation of the cytoplasm already mentioned (Case No. 1).

In Case No. 3 it was quite clear that the blastocysts were formed by *Chilomastix mesnili* (Fig. 65), which often showed near the nucleus a greyish coloured body (Figs. 66, 67) which by growing fills up the greater part of the cell (Figs. 68, 69), thus producing forms identical in appearance with the blastocysts shown in Figs. 71–73.

Case No. 4 (Pl. XVII, Figs. 83–95).

Rat infected with *Chilomastix* and *Blastocystis.* Preparations kindly furnished by Dr S. L. Brug.

The blastocysts in this case were characterised by the distinct vacuolar structure of the cytoplasmatic fringe. Divisional forms were common (Fig. 89) without however showing the peculiar changes, described in the former cases. The nucleus, when present, showed the structure described in Case No. 3 (Figs. 84, 85, 86, 93). Besides these nuclei, chromatic granules were present. Sometimes the peripheral cytoplasm was reduced to a line (Fig. 95); other forms might suggest sporulation (Fig. 92) but no other stages were found supporting this view.

As in the former case, the blastocysts were formed by the rounded forms of *Chilomastix* (Fig. 83) which accompanied the flagellate stages not figured here. These forms show a strongly vacuolated cytoplasm; between the vacuoles a grey structureless substance appears (Fig. 83) which by growing pushes the vacuoles to the periphery (Fig. 84), takes

a central position (Fig. 85) and finally assumes a distinct shape with well-marked outlines (Fig. 86).

Case No. 5 (Pl. XVII, Figs. 96–106).

European child having resided in India for several years. Infection of *Blastocystis* and *Limax* amoebae.

The blastocysts of this case can be referred to forms illustrated in Fig. 96 representing small *Limax* amoebae showing a differentiation of their cytoplasm into a dense peripheral zone and a central space of loose reticular structure. The vacuoles of this part fuse into one central vacuole (Figs. 97, 98), containing several irregular chromatic particles which probably arise from the hypertrophying nucleus (Figs. 98, 100). Divisional forms occur and also stages (such as are represented in Fig. 104) resembling sporulating stages as described by Alexeieff, but no other signs of this phenomenon were encountered.

CONCLUSIONS.

1. In two cases *Blastocystis* was found where the presence of *Trichomonas* or *Chilomastix* could be excluded with absolute certainty. Consequently *Blastocystis* cannot be a normal developmental form of either.

2. In fresh stools *Blastocystis* is but seldom found to be alive and even when encountered in this state it dies quickly. After death the central sphere soon disappears.

3. The size of blastocysts varies greatly and the larger they grow the smaller becomes the peripheral fringe of cytoplasm. Living blastocysts are relatively small and rich in cytoplasm.

4. The blastocysts of the cases mentioned here, although having some general characters in common, differed much as to details of structure. This difference was especially marked when the associated parasites were different. No blastocysts were found without an associated parasite.

5. The occurrence of blastocysts in the stools of a man fed on milk and eggs only, and the presence of living blastocysts in the man's stools, exclude the idea of their being remains of solid food.

6. It is probable from the observation recorded in this paper, 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. The resemblance seen in blastocysts

from different sources which may lead to their being regarded as belonging to one species, is easily explained by a convergence resulting from the parasites which produce the blastocysts losing their characteristics during the process of degeneration.

7. No certain stages of sporulation were seen, as described by Alexeieff, and the nuclear structure, although variable, never resembled that given in his description. It is therefore probable that Alexeieff's *Blastocystis enterocola* is different from the forms described in man under the same name.

EXPLANATION OF PLATES XVI AND XVII

Figs. 1–27. *Case No.* 2.

Figs. 1–4. Production of blastocysts from *Limax* amoebae by the formation of a central vacuole.
Figs. 5–13. The same, by the formation of a central chromatic reticulum.
Figs. 14–17, 22, 23. Blastocysts with well-developed cytoplasm.
Figs. 18–21. Linear cytoplasm.
Fig. 24. Stage suggesting sporulation.
Figs. 25, 26. Dark staining sphere.
Fig. 27. Sphere with alveolar structure.

Figs. 28–64. *Case No.* 1.

Figs. 28–39. Production of blastocysts from *Limax* amoebae by the formation of a central chromatic reticulum.
Figs. 41, 42. Well-developed cytoplasm.
Figs. 40, 43, 45, 52, 53. Linear cytoplasm.
Figs. 46, 47. Gradual disappearance of the sphere.
Figs. 48–51. Dark staining sphere.
Figs. 54–58. Large blastocysts showing disappearance of cytoplasm.
Figs. 59–64. Division.

Figs. 65–82. *Case No.* 3.

Figs. 65–69. Production of blastocysts from *Chilomastix*.
Figs. 70–73. Vesicular nucleus.
Fig. 74. Disappearance of the nucleus.
Figs. 75–77. Involution of the cytoplasm.
Fig. 78. Dark staining sphere appearing in rounded *Chilomastix*.
Figs. 79, 80, 82. Blastocysts with dark staining sphere.
Fig. 81. Division.

Figs. 83–94. *Case No.* 4.

Figs. 83–85. Production of blastocysts from rounded forms of *Chilomastix*.
Figs. 86, 87. Forms with vesicular nuclei.
Fig. 88. Only chromatic granules present.
Fig. 89. Division.
Figs. 90, 94. Linear cytoplasm.
Fig. 91. Form suggesting sporulation.
Figs. 92, 93. Dark staining sphere.

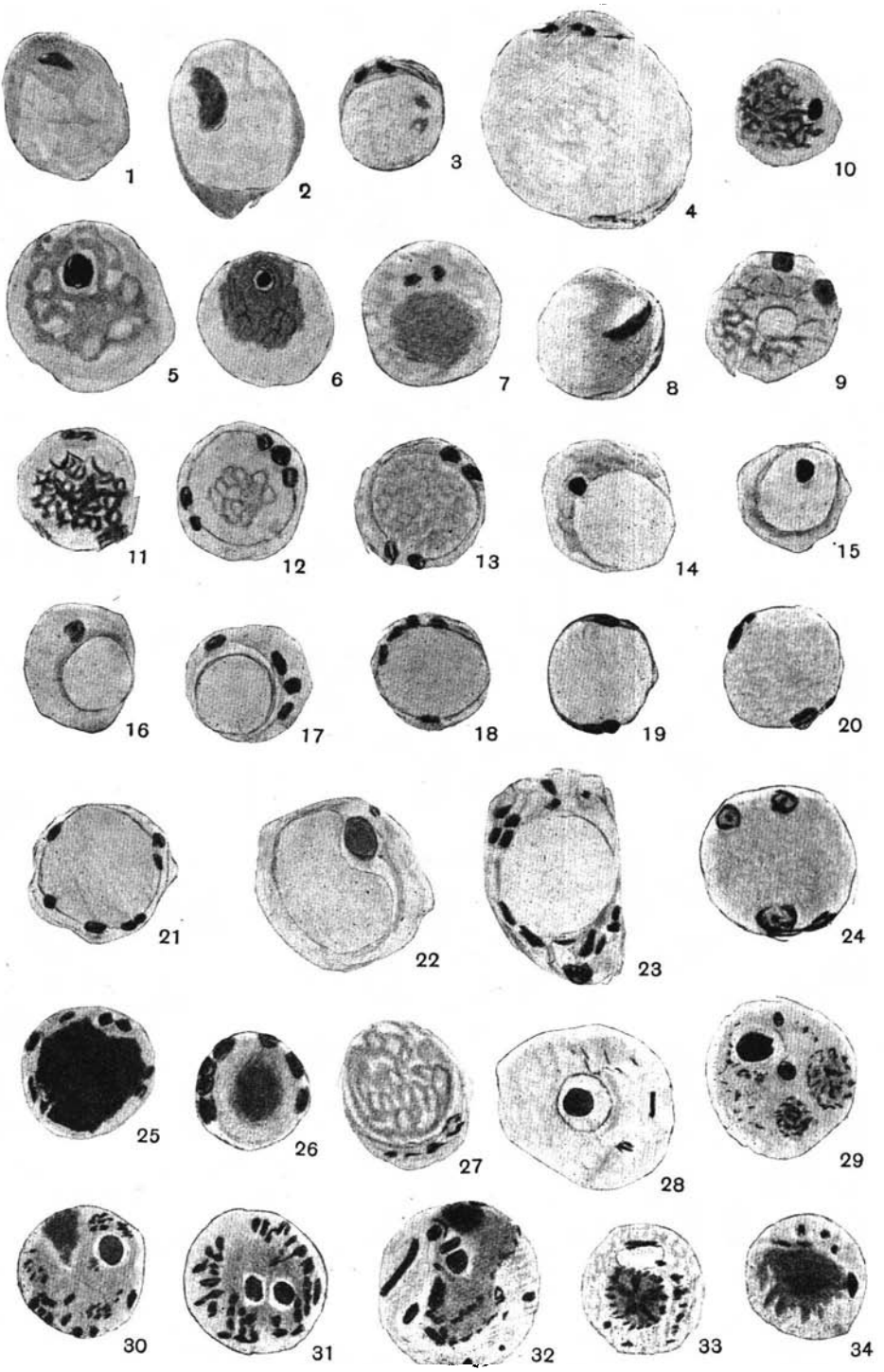
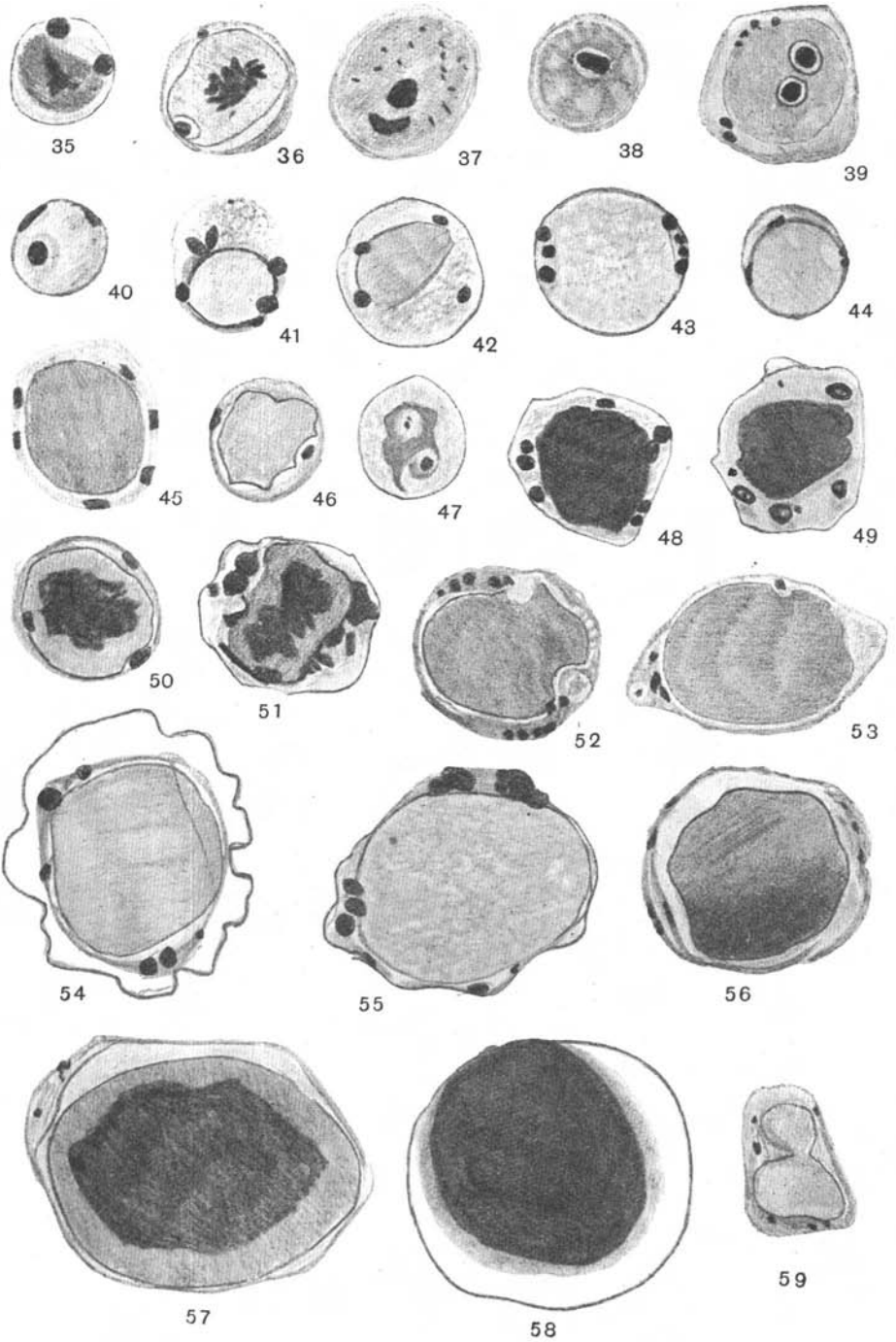

1
2
3
4
10
5
6
7
8
9
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34

PLATE XVI


35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59

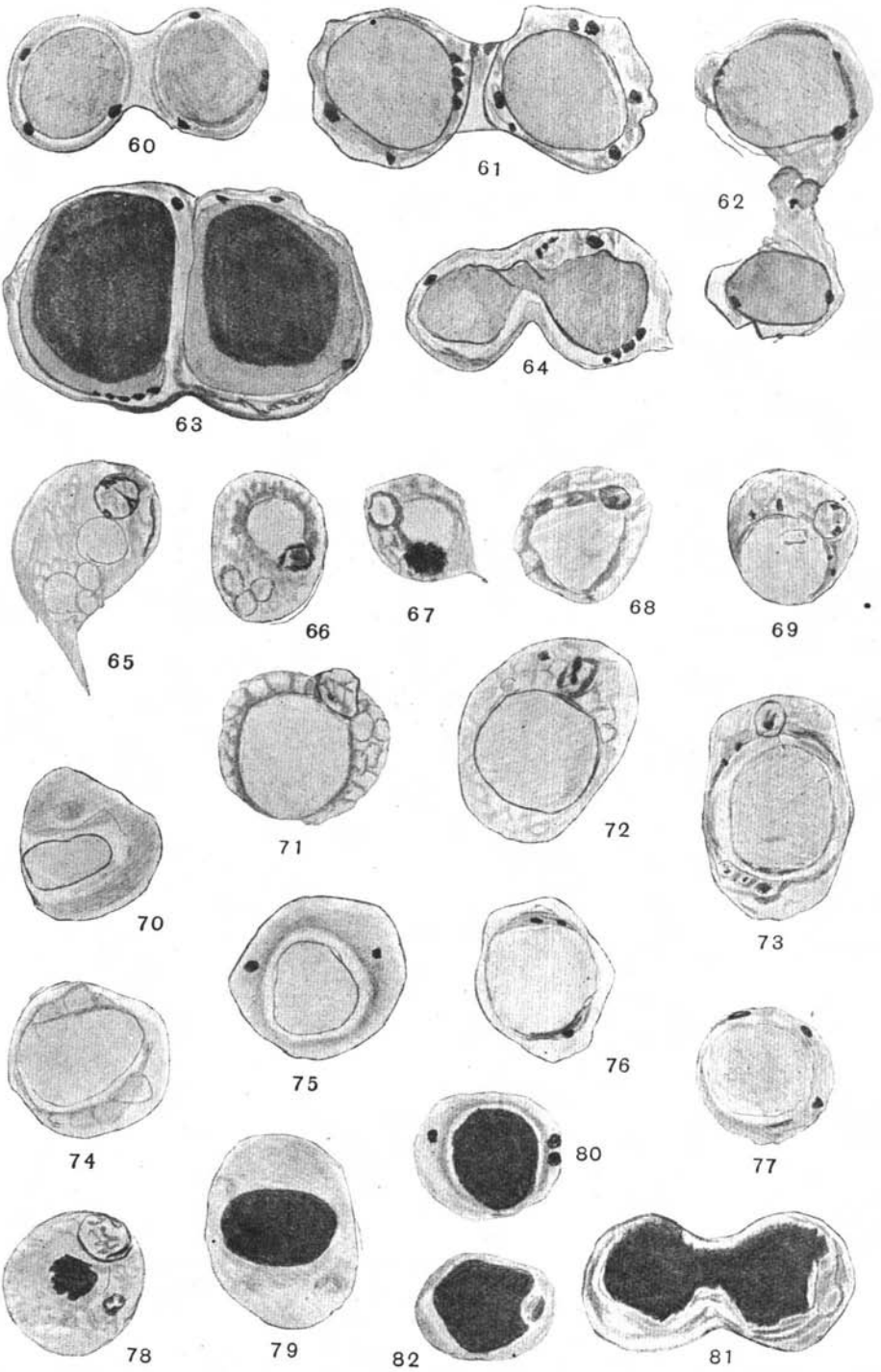
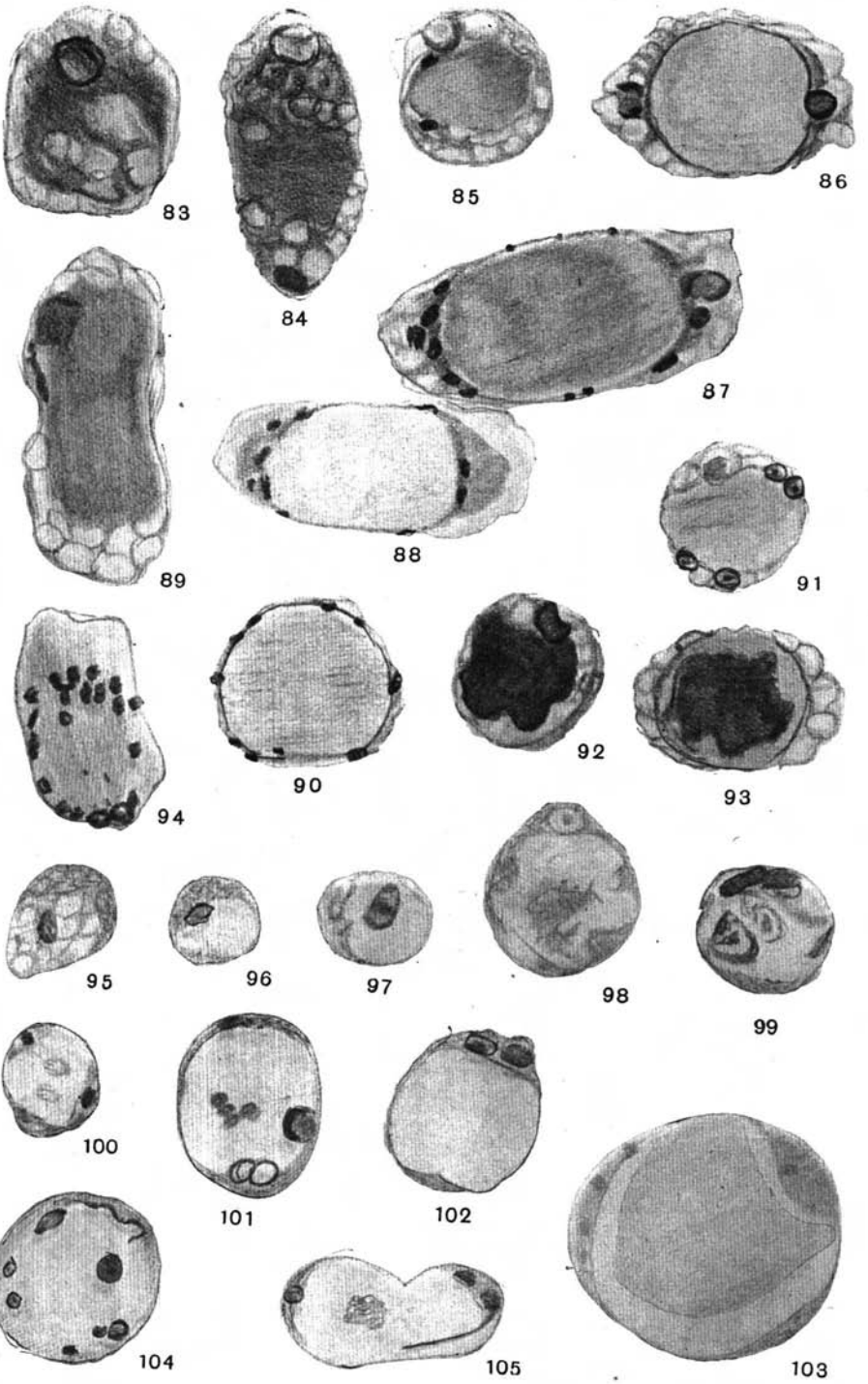

60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82

PLATE XVII


83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105

Figs. 95–105. *Case No.* 5.

Figs. 95–98. Production of blastocysts from *Limax* amoebae by formation of a central vacuole.

Figs. 99–101, 104. Dark staining patches in the sphere.

Fig. 102. Linear cytoplasm.

Fig. 103. Form resembling a stage of sporulation as described by Alexeieff.

Fig. 105. Division.

REFERENCES.

ALEXEIEFF (1911). *C. R. Soc. de biol.* LXXI. 296.
BENSEN (1910). *Arch. f. Protistenk.* XXIII. 96.
BOHNE and PROWAZEK (1908). *Ibid.* XII. 1.
BRUMPT (1912). *Bull. Soc. pathol. exot.* V. 725.
CHATTON and LALUNG BONAIRE (1912). *Ibid.* V. 135.
JAMES (1915). *Ann. Trop. Med. and Parasitol.* VIII. 133.
KUENEN and SWELLENGREBEL (1913). *Centralbl. f. Bakteriol.* Abt. I. Orig. LXXI. 278.
LOW (1916). *Journ. Trop. Med. and Hygiene,* XIX. 29.
MATHIS (8. VI. 1913). *Bull. Soc. méd. chirurg. de l'Indochine,* IV. No. 7.
PÉREZ (1907). *C. R. Soc. de biol.* LXIII. 445.
PROWAZEK (1911). *Arch. f. Protistenk.* XXIII. 96.
SCOTT MACFIE (1915). *Ann. Trop. Med. and Parasitol.* IX. 507.
WENYON (1910). *Parasitology,* III. 210.