The Life-Cycle of *Entamoeba Ranarum*, Grassi (1879) [1].

By

Elizabeth P. Sanders,
Department of Tropical Medicine Harvard University Medical School
Boston, Massachusetts.

(With Plates 9—10.)

Entamoeba ranarum, an intestinal amoeba of the frog, has long interested protozoologists because it is morphologically indistinguishable from *Entamoeba histolytica*. It was first observed by Lieberkühn in 1854, but Dobell (1908—1909) was the first one to adequately describe the organism from the trophozoite through its development to the cyst. He compared it minutely with *Entamoeba histolytica*, but in 1918 he convinced himself experimentally that the two organisms were not the same. This was done by feeding cysts of *Entamoeba histolytica* to laboratory raised tadpoles, free of infection with *Entamoeba ranarum*. He was not able to produce in this manner infections in the animals.

For a long time the exact process of development following excystation, metacystic development, was not known. A rather complicated process of metacystic development for *Entamoeba histolytica* was described by Dobell (1928) and confirmed by Cleveland and Sanders (1930). The discovery of this part of the life-cycle of *Entamoeba histolytica* was not possible until a culture medium was devised which was suitable for a complete and continuous development of the amoeba. Dobell and Laidlaw (1926) made a tremendous step forward when they added rice starch to the media, but

[1]) This work was aided by a grant from the De Lamar Mobile Research Fund.

with their media it was necessary to grow and then wash the mature cysts, planting them in fresh media to secure excystation, while in the liver infusion agar medium devised in this laboratory (CLEVELAND and COLLIER, 1930) the life cycle is a continuous process, and all stages can be readily found in one test tube.

It seemed worth while to investigate the complete life-cycle of the other histolytica-like amoebae. *Entamoeba terrapinae* was the first amoeba to be described with this in mind (SANDERS and CLEVELAND, 1930), and it was found to have a different and simpler metacystic development. Now the development of *Entamoeba ranarum* has been followed in culture, and it is possible to describe its whole life-cycle, show further similarities to *Entamoeba histolytica* and to indicate a few differences.

Cultivation.

Entamoeba ranarum was first cultivated by BARRET and SMITH (1926) in a very simple medium — one part of inactivated human serum and nine parts of 0,5 per cent sodium chloride solution. In their cultures the amoebae grew well and encysted. The morphology of these culture organisms as described by TALIAFERRO and FISHER (1926) was identical with that described by DOBELL (1909). Their amoebae were obtained from tadpoles. DOBELL obtained amoebae from both frogs and tadpoles of several species.

The amoebae described here were obtained from tadpoles collected in Virginia. *Entamoeba ranarum* was found to be far from common in frogs and tadpoles of this locality (Boston) and in frogs and tadpoles from Louisiana. In the few cases in which it was found after examination of hundreds of animals, cultivation was prevented by the presence of other organisms, particularly *Blastocystis* and *Trichomonas*. The original cultures of our strain were obtained in a medium composed of one part of horse serum and nineteen parts of 0,5 per cent saline with rice flour added. The cultures were kept for six weeks at room temperature before any amoebae were observed. In this medium both cysts and trophozoites of *Entamoeba ranarum* and *Chlamydophrys stercocea* were found. Transfers were made to liver infusion agar and LOEFFLER'S slants covered with 1:6 horse serum and saline with sterile rice flour added. TANABE'S asparagin medium was also used (TANABE and CHIBA, 1928). For RINGER'S solution we substituted with good results 1:6 serum saline. In all three of these media *Entamoeba*

ranarum grew very well, but *Chlamydophrys stercorea* was soon lost. *Entamoeba ranarum* has been in culture for six months now on these media, and will apparently grow indefinitely if transferred with sterile precautions. It is necessary to subculture about every ten days to keep the culture in good condition, but transfers have been made successfully from fourteen and sixteen day old cultures. When a transfer is made at the end of ten days there are usually many cysts and a few trophozoites. Two days after transfer of culture no cysts are present. In a five-day culture many large active trophozoites, engorged with starch (Fig. 2) are present, and a few uninucleate cysts. In six and seven day cultures cysts are fairly abundant. At ten days there are still some trophozoites present but practically all of the cysts are quadrinucleate with many chromatoidal bodies and large glycogen masses.

The cultures have been maintained at room temperature. At incubator temperature, 37° C they persisted for two weeks but during this time gradually decreased, finally disappearing entirely. An attempt was made to grow them in the cold room at a temperature of from 0° to 4° C. It was thought that they might encyst more rapidly there, since Dobell (1909) had found encysting forms most frequently in frogs during the winter months. The amoebae stopped multiplying at this low temperature and further encystation did not take place. Cultures made from cold room material at intervals varying up to two weeks grew well when placed at room temperature. Cysts will probably live much longer.

Description of Organism

Size.

Trophozoites of *Entamoeba ranarum* vary from 10,4 to 18,8 microns with an average of 13 microns. Five hundred measured cysts ranged in size from 8,8 to 13,6 microns with an average of 11 microns. The race of *Entamoeba ranarum* which Dobell studied was larger than this one, the trophozoites having a range of 20 to 30 microns and sometimes 60 microns, and cysts of 10 to 16 microns. Taliaferro and Fisher (1926) described a race of *Entamoeba ranarum* nearer the size of Dobell's race — the trophozoites ranging from 12 to 38 microns, the cysts from 6 to 20 microns. The race of *Entamoeba ranarum* described here is practically the same size as *Entamoeba terrapinae*, which had an average cyst size of 10 microns. Race variation in size would be expected in *Entamoeba ranarum* as well as in *Entamoeba histolytica*.

Morphology.

Trophozoite: The trophozoite in the living state was a typical *histolytica*-like organism, moving about actively with characteristic explosive pseudopodia, and occasional streaming *limax*-like movement. In some organisms the nucleus could be seen as the animal moved about. In the cultures the animals ingested large quantities of starch grains, but they also ate bacteria and debris. No evidence of cannibalism was found. Specimens stained with HEIDENHAIN'S haematoxylin revealed the characteristic *histolytica*-like nucleus (Fig. 1—2).

Cysts: As in cultures of *Entamoeba histolytica* and *Entamoeba terrapinae*, the cysts of *Entamoeba ranarum* were found in large clumps apparently stuck together. In *Entamoeba ranarum* this clumping and adhesion was more readily explained than in the cultures of the other amoebae. Every cyst examined was found to have a jelly-like, irregular outer wall (Fig. 3—9). This was first noticed in an iodine preparation, where it stood out very clearly. It was then searched for and found in both living and stained preparations. Its exact nature could not be determined, but in every preparation it resembled a clear hyaline-like capsule of irregular outline rather than part of a thickened cyst wall and was only visible because of the irregular line beyond the outline of the cyst. All cyst measurements were based on the inner, even outline and not on the uneven, outer limits. Iodine preparations of *Entamoeba histolytica* and *Entamoeba terrapinae* have been observed many times, but such a capsule has never been seen. The irregular cyst of *Chlamydophrys stercorea* is very different from this capsule. It is obviously a wrinkled cyst wall and in the stained preparations takes on a bluish tinge. The capsule of *Entamoeba ranarum* remains clear when the organism is stained. The cysts of this race of *Entamoeba ranarum* vary considerably in shape, many being oval instead of round.

The uninucleate cyst contains a large glycogen mass, which often pushes the large nucleus to one side (Fig. 3). They usually contain many chromatoidal bodies, large and small. The development from a uninucleate to a quadrinucleate cyst occurs in exactly the same manner that has been described so often for *Entamoeba histolytica* and *histolytica*-like amoebae (Fig. 6—8). The glycogen mass is more persistent with *Entamoeba ranarum* than in other amoebae however. In ten day cultures, when practically all of the organisms are in the quadrinucleate stage, the cysts will still contain glycogen

masses pushing the four nuclei close to the periphery of the cysts (Fig. 8).

Evidence was found that the cysts occasionally contain eight nuclei. One cyst showed six nuclei, four small and two large ones (Fig. 9). The two large ones would give rise to four others, producing a typical cyst with eight nuclei and a chromatoidal body.

Excystation and Metacystic Development.

To produce metacystic amoebae it was necessary to wash and concentrate the cysts, store them in the cold room for a period of seven to ten days and then incubate the cysts at 37° C for from four to ten hours. Cysts of *Entamoeba ranarum* in culture are provided with a large amount of glycogen and many chromatoidal bodies and until these have disappeared form the cyst they will not excyst (Fig. 10). It is believed that this is the reason they are slow to excyst. Washed uninucleate cysts, as in *Entamoeba histolytica*, never developed further after washing and storage, but quadrinucleate cysts would excyst if stored for a sufficient length of time, that is from ten days to two weeks or longer and then incubated. The amoeba excysted in exactly the same manner as *Entamoeba histolytica*, namely as a small quadrinucleate amoeba with clumped nuclei (Fig. 11).

The most interesting thing about this strain of *Entamoeba ranarum* is that its metacystic development is identical with that of *Entamoeba histolytica*. The encysted amoeba excysts as a four-nucleate organism. After excystation each nucleus divides once, thus giving rise after cytoplasmic division to eight amoebae, just as in the metacystic development of *Entamoeba histolytica* (Dobell, 1928; Cleveland and Sanders, 1930). Only certain stages of the process have been figured (Fig. 11—24). A cystic nucleus is referred to as N and metacystic as n. The stages most commonly found were Nn and Nnn, which probably means that cytoplasmic division is more rapid than in *Entamoeba histolytica*, where amoebae with eight metacystic nuclei (nnnnnnnn) are abundant. Metacystic development occasionally took place in the cultures (Fig. 16 and 20).

Mercier and Mathis (1918) have described cysts of *Entamoeba ranarum* with from four to thirty nuclei, the thirty-nucleate cysts they believed to be schizonts, and those with four nuclei, gamonts, but there is no evidence to sustain this view. They figure a trophozoite with four nuclei that is identical with the excysted amoebae here described.

COLLIN (1913) described trophozoites with as many as thirty nuclei. Some of the stages that he figured might be metacystic amoebae with nuclear combinations, NNNN, NNn, NNnn, but he believed that the small nuclei in these amoebae originated by budding from the large nucleus. Budding nuclei were not observed, nor have trophozoites been found with more than eight nuclei.

References cited.

BARETT, H. P. and SMITH, N. M. (1926): The cultivation of Entamoeba ranarum. Ann. Trop. Med. and Parasit. Vol. 20 p. 85—89.

CLEVELAND, L. R. and COLLIER, JANE (1930): Various improvements in the cultivation of Entamoeba histolytica. Am. Journ. Hyg. Vol. 12 p. 606—613.

CLEVELAND, L. R. and SANDERS, E. P. (1930): Encystation, multiple fission without encystment, excystation, metacystic development, and variation in a pure line and nine strains of Entamoeba histolytica. Arch. f. Protistenk. Bd. 70 p. 223—266.

COLLIN, B. (1913): Sur un ensemble de protistes parasites des batraciens. Arch. Zool. Exp. and Gen. Vol. 51 p. 59.

DOBELL, C. (1908): On the intestinal protozoan parasites of frogs and toads. Cam. Phil. Soc. Proc. Vol. 14 p. 428—433.

— (1909): Researches on the intestinal protozoa of frogs and toads. Quart. Journ. Micr. Sci. Vol. 53 p. 201.

— (1918): Are Entamoeba histolytica and Entamoeba ranarum the same species? Parasit. Vol. 10 p. 294—310.

— (1919): The amoebae living in man: A zoological monograph. London.

— (1928): Researches on the intestinal protozoa of monkeys and man. I. General Description. II. Description of the whole life-history of Entamoeba histolytica in cultures. Parasit. Vol. 20 p. 357—412.

DOBELL, C. and LAIDLAW, P. P. (1926): On the cultivation of Entamoeba histolytica and some other entozoic amoebae. Parasit. Vol. 18 p. 283.

MERCIER, L. and MATHIS, C. (1918): Kystes gamogoniques et schizogoniques chez Entamoeba ranarum. Bull. Soc. Path. Exot. Vol. 11 p. 47.

SANDERS, E. P. and CLEVELAND, L. R. (1930): The morphology and life cycle of Entamoeba terrapinae spec. nov., from the terrapin Chrysemys elegans. Arch. f. Protistenk. Bd. 70 p. 267—272.

TALIAFERRO, W. H. and FISHER, ANNA (1926): The morphology of motile and encysted Endamoeba ranarum in culture. Ann. Trop. Med. and Parasit. Vol. 20 p. 89—95.

TANABE, M. and CHIBA, E. (1928): A new culture medium for Entamoeba hystolytica. Acta medicinualia in Keijo Vol. 2 p. 1—4.

Description of Plates.

Plates 9—10.

These figures were all drawn with the aid of a camera ludica, from culture material fixed in Schaudinns's fluid at room temperature (22—23° C) and stained in Heidenhain's haematoxylin. All figures have a magnification of 2500 diameters.

Plate 9.

Fig. 1. Active uninucleate trophozoite.

Fig. 2. Large uninucleate trophozoite engorged with starch grains. Age of culture four days, with no cysts.

Fig. 3. Uninucleate cyst, stage found in culture after about five days. Note large glycogen mass pushing nucleus to the wall of cyst as in *E. histolytica*. The outside irregular line indicates the jelly like mass of material surrounding all the cysts of this strain of *E. ranarum*.

Fig. 4. Large irregular-shaped uninucleate cyst. Many cysts were not spherical.

Fig. 5. Uninucleate cyst showing large nucleus, large glycogen mass and heavy chromatoidal bodies.

Fig. 6. Binucleate cyst.

Fig. 7. Trinucleate cyst.

Fig. 8. Quadrinucleate cyst.

Fig. 9. Unusual cyst with six nuclei, four small and two large. This indicates that eight nucleated cysts may be occasionally found, since the two large nuclei will divide.

Fig. 10. Mature quadrinucleate cyst, without glycogen and chromatoids.

Fig. 11. Metacystic amoeba with four cystic nuclei, NNNN, a recently excysted amoeba.

Fig. 12. Metacystic amoeba with four cystic nuclei but with nuclei in pairs, indicating perhaps that cytoplasmic division may precede nuclear division.

Plate 10.

Fig. 13. Metacystic amoeba with two cystic and two metacystic nuclei (NNnn).

Fig. 14. Metacystic amoeba with two cystic and three metacystic nuclei (NNnnn)

Fig. 15. Metacystic amoeba with one cystic and five metacystic nuclei (Nnnnnn).

Fig. 16. Metacystic amoeba with one cystic and four metacystic nuclei (Nnnnn). This and Fig. 20 were found in eight day cultures, but the other metacystic stages were from cysts, excysted after incubation for three and four hours at 37° C.

Fig. 17. Metacystic amoeba with two cystic and one metacystic nuclei (NNn).

Fig. 18. Metacystic amoeba with one cystic and three metacystic nuclei (Nnnn).

Fig. 19. Metacystic amoeba with two cystic nuclei. This would follow cytoplasmic division of a stage such as Fig. 12.

Figs. 20—21. Metacystic amoebae. Each with one cystic and two metacystic nuclei.

Figs. 22—23. Metacystic amoebae. Each with one cystic and one metacystic nucleus.

Fig. 24. Dividing amoeba with two metacystic nuclei.

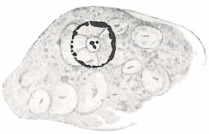

Fig. 1

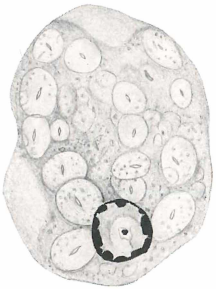

Fig. 2

Fig. 3

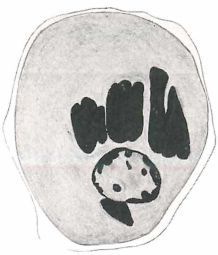

Fig. 4

Fig. 5

Fig. 6

Fig. 7

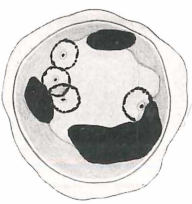

Fig. 8

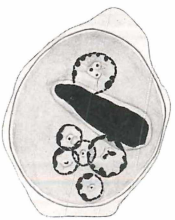

Fig. 9

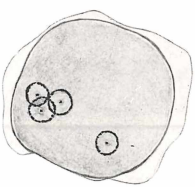

Fig. 10

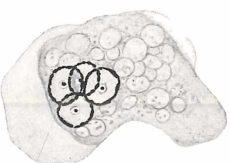

Fig. 11

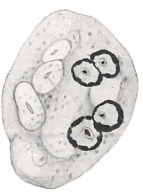

Fig. 12

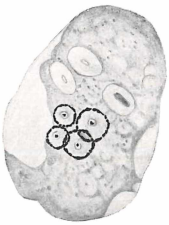

Fig. 13

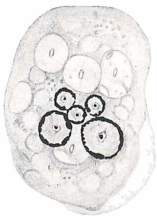

Fig. 14

Fig. 15

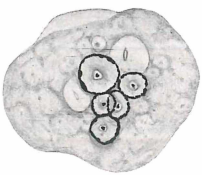

Fig. 16

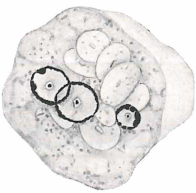

Fig. 17

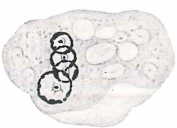

Fig. 18

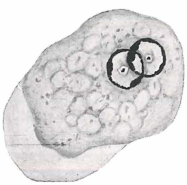

Fig. 19

Fig. 20

Fig. 21

Fig. 22

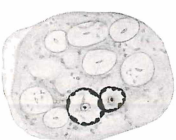

Fig. 23

Fig. 24