On Entamoeba serpentis

by

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(With plate 19).

The present paper is a supplement to earlier researches, published in a preliminary communication (see Brazil – Medico, Vol. 31, Nº 34). It deals with an *Entamoeba*, described by us, from the intestine of a brazilian snake, *Drymobius bifossatus*, which was examined for parasitic protozoa.

We have little to add to the general morphology, and only wish to give a few more particulars about the changes in the appearance of the nucleus. They are like those observed by HARTMANN in *Entamoeba testudinis* HARTMANN, and may be considered in both cases as the result of cyclic variations of the caryosome.

Though we did not give much time to the study of fresh material, we noticed some interesting facts. Live entamoeba of this species are generally nearly spheric, except when pseudopodia are formed. They move rather quickly. Their protoplasm is neatly divided in two layers: the external (ectoplasm) and the internal (endoplasm) containing inclusions, mostly of bacteria. No inclusion of red bloodcells was observed.

After fixing by sublimate-alcool (SCHAUDINN) and staining with iron-hematoxylin (HEIDENHAIN), the entamoeba shows a well marked dimorphism; the two extremes are very different, but all intermediary stages between them are found.

The first of these forms (plate 19, figs. 1, 7 and 8), is larger and its outline changeable on account of active movement. The difference between the broad external zone of hyaline ectoplasm and the alveolated layer of endoplasm is well marked. The nucleus, surrounded by the endoplasm, is vesicular, large and rounded, the central caryosome large and, as a rule, formed of several granules of chromatin, suspended in a less chromophilous stroma. The caryosome is surrounded by a large liquid zone, which, though generally structureless, may contain a few small chromatin granules, forming a continuous layer; this layer may be more developed on one side than on the other (Plate 19 fig. 1).

The other form (Pl. 19, figs. 9, 11 and 12) of the entamoeba is generally smaller, nearly always spheric with circular outline. The protoplasm stains more intensely than in the large form. There is no clear demarcation between endo and ectoplasm; the latter is limited to a narrow periplastic layer which forms the contour of the organism. The nucleus is also spheric, with regular outline; like the rest of the cell it is reduced and smaller than in the other form. The nucleus shows a relatively large central caryosome, containing a compact mass of chromatin, generally without distinct granulations; sometimes it is attached to the nuclear membrane by fine linin threads. The caryosome is surrounded by a liquid zone, which may be structureless, or contain achromatic filaments, or chromatin granules mostly in small number. Without, at the edge of the nucleus, sometimes even adhering to the membrane, are the masses of chromatin which constitute the outer nucleus. They are large and compact, either isolated or in groups; sometimes they are grown together and form a thick chromatic ring, parallel with the nuclear membrane (Pl. 19, fig 2).

The changes in the nucleus, most of which may be interpreted as cyclic variations of the caryosome, are, as we already mentioned, one of the most striking features of this species. The following seems to us the order in which the different phases probably occur:

In the larger form there are many fine; granules in the periphery of the nucleus sometimes they occupy only the part nearest to the membrane (Pl. 19, fig. 1), at others they are also found in the liquid zone (Pl. 19, fig. 7). At the edge of the nucleus these fine granules gradually melt into each other, thus forming larger ones; they are symmetrically and regularly arranged (Pl. 19 figs. 3 and 6). A fact, which seems in favour of our interpretation, is the existence of forms similar to that of fig. 7, already refered to. The greater part of the nucleus shows large granules at the very edge, while in the remaining part the granules are fine and have not yet reached the periphery. When at the edge, the granules unite and form at first larger granulations, then compact masses (Pl. 19 figs 4 and 5); at its maximum, the agglomeration looks like that shown in fig. 12.

While the granules are fusing, the caryosome, which at first always looks small, also grows larger (fig. 11). In this drawing, the large size of the caryosome seems to coincide with a beginning of desaggregation in the external chromatin. This may be previous to its expulsion, as the stages, represented in figs. 9 and 10, seem to prove.

Occasionly, we noticed a chromatin granule near the caryosome (pl. 19, figs. 3 and specially 7, 8 and 10); its meaning is uncertain, but we find that HARTMANN also noticed one in *Entamoeba testudinis.* This species has more points in common with ours, as may be seen by comparing our illustrations with his.

Sometimes there are netlike formations in the liquid nuclear zone (Pl. 19, figs. 2, 4, 6, 7 and 8). They seem more developed in the period eliminating fine chromatin granules (fig. 6). This drawing shows the achromatic network only in that part of the nucleus, in which the granules are migrating to the periphery.

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The species which comes nearest to *Entamoeba serpentis,* is the one described by HARTMANN (Memorias do Instituto Oswaldo Cruz, vol. 2, fasc. 1, pp. 3–10 Pl. 1) under the name of *Entamoeba testudinis;* but no dimorphism is seen in this species and the forms, described by HARTMANN, resemble only the larger form of our species.

Another species, found in reptilia, is *Entamoeba lacertae* (HARTMANN & PROWAZEK 1907 in Archiv f. Protistenkunde, Vol. X, p. 314, fig. 42). The authors only mention its small size and a peculiar stage in the division of the nucleus. Later on, (1914) this species was studied and fully described by DOBELL (Arch. f. Prot. Vol. 34 pp. 146–159, Pl. 8).

Explanation of Plate 19.

Fig. 1 – Large form of *Entamoeba serpentis* with an annular zone of fine chromatin granules at the edge of the nucleus.

Fig. 2 – Intermediary form between the larger and smaller ones, showing a chromatin ring, due to fusion of expelled granules.

Fig. 3 – Nucleus with granulations, due to fusion.

Fig. 4 & 5 – Formation of masses, due to the fusion of chromatin granules.

Fig. 6, 7 & 8 – Nuclei of large forms showing a network in the liquid zone.

Fig. 9 & 10 – Nuclei with desaggregating chromatic granulations.

Fig. 11 & 12 – Small forms with big caryosomes and very large chromatic masses at the periphery.