

# A Contribution to the Study of parasitic Ciliata

by

DR. CESAR FERREIRA PINTO.

(With Plate 76)

## Introduction.

While examining the intestine of some frogs (*Leptodactylus ocellatus*) from Mangui-nhos and other localities of Rio de Janeiro, I found, among other Ciliata and Flagellata, an apparently new species of *Opalina*.

In the blood of the same frogs I found *Trypanosoma rotatorium* GRUBY, which was studied by ASTROGILDO MACHADO and a hemogregarine which I intend to describe in a future paper.

## *Opalina brasiliensis* PINTO, 1918.

(Pl. 76 figs 3a, 10 & 11).

LÉGER and DUBOSQ (Arch. de Zool. Expér. sér. 4, tome 2, p. 343, 1913) divide the genus *Opalina* in two groups, according to the number of nuclei: a) *Opalinae* with many nuclei and b) *Opalinae* with from one to five. *O. brasiliensis* PINTO belongs to the latter, since it has from one to four nuclei.

## Fresh material.

Unstained specimens of this species have a yellowish colour. The nuclei are visible and the endoplasm contains a great many rounded or rod-shaped granulations. The

body moves rapidly and displaces itself forwards or sideways with a certain amount of agility. Cilia are visible on the whole outline.

## Morphology and Size.

*Opalina brasiliensis* is pear-shaped and has a slight elevation to one side of its anterior end, while its posterior extremity ends in a blunt point, which is sometimes also deflected to one side. The specimens I observed were 46  $\mu$  long and 22  $\mu$  wide. Larger forms may attain a length of 115  $\mu$  and a width of from 10 to 12  $\mu$ .

## Stained Material.

### Methods.

The specimens were fixed in their natural medium by an alcohol-sublimate solution (Schaudinn) and stained with iron-hematoxylin (Heidenhain).

## Structure of the Protoplasm.

The cytoplasm is divided in ectoplasm and endoplasm. The ectoplasm is more or less homogenous and of yellowish colour; many cilia issue from its edge. The endoplasm, which is brown, contains many

rounded or rod-shaped granules which stain deeply when treated with iron-hematoxylin (chromatoid bodies of LÉGER and DUBOSQ).

The lines of cilia are spiral (fig. 3a) and go from one side of the body to the other; they are not smooth as in *O. longa* BEZ-ZENBERGER, but composed of minute granules which stain very well with iron-hematoxylin. They are more or less equidistant and I never saw them fork. In a specimen measuring  $22\ \mu$  (width) by  $48\ \mu$  (length), there were twenty four rows. The nuclei, of which there are mostly two, are generally in the middle. (Fig. 3a).

### Nuclear Structure.

(Pl. 76 figs. 1, 8, 9, & 9a.)

Quiescent state. The nuclei are more or less rounded; when there is only one, it occupies the middle of the cell; when there are two, they are also near the middle, one behind the other and somewhat obliquely to the longitudinal body-axis. (fig. 3a). The nuclear membrane is clearly visible and sometimes shows slight elevations and depressions.

Like in other species of the same genus, the nucleus of *O. brasiliensis* contains rounded or elongate masses of chromatin, which are generally near the surface of the nuclear membrane. (fig. 1). To the inside of these, there are sometimes other very small achromatic granules (fig. 3 & 3a), or a mass of smooth achromatic substance. (fig. 9 & 9a).

### Division of the Nucleus.

The nuclear division of *O. brasiliensis* corresponds more or less to the *paratenomitos* of ALEXEIEFF (no centrioles).

The first stage is characterised by the

stretching of the nucleus, the disappearance of the nuclear membrane and the elongation of the chromatin masses, which either go to the edge or remain in irregular groups in the middle (fig. 2).

After that (fig. 3 and lower nucleus of 3a), there appears a pseudo-centrodesmosis which binds one of the granules on one side to another granule on the opposite side, while the nucleus becomes longer and narrows at its equator (fig. 4); this enables one to perceive the pseudo-centrodesmosis quite clearly.

At a later stage (fig. 5), the equatorial stricture becomes more marked and the pseudo-centrodesmosis is still seen, connecting the chromatin mass on one side to a small granule on the opposite side. Then (fig. 6) half the pseudo-centrodesmosis disappears and the rest of it remains attached to a chromatin mass on one side of the nucleus only. After that, the stricture gets more and more accentuated (fig. 7) and the pseudo-centrodesmosis can be seen no longer, but only rounded or elongated chromatin masses are visible; they may be isolated or arranged in loose groups in the vicinity of the membrane.

When the rounded daughter-nuclei are formed, they are still connected by a slender filament (fig. 8). Later on the filament tears and frees them (fig. 9 & 9a.).

I have observed stages of the division of binucleate specimens of *O. brasiliensis* and have seen specimens with four resting nuclei (fig. 11).

Specimens with only one nucleus are generally much smaller than the plurinucleate forms, a fact which leads me to believe that they must be younger individuals.

### Explanation of Plate 76.

All the figures of plate 76 were drawn from cover-glass preparations, fixed by sublimate - alcohol (Schaudinn) while wet, and stained with iron-hematoxylin (Heidenhain).

Fig. 5 was drawn with compensating eye-piece 6 and immersion lens 1/12; fig. 3 with compensating eye-piece 4 and immersion lens 1/12; the others with eye-piece 2 and lens 1/12. Drawings at the level of the table, with Zeiss microscope and Abbé camera-lucida.

Fig. 1 Resting nucleus. Chromatin masses of different sizes and shapes at the edge of the nuclear membrane. A large oval chromatin granule with a clear halo near the center.

Fig. 2 Elongated nucleus; beginning of mitosis; disappearance of nuclear membrane. Rounded and elongated chromatin masses at the edge of the membrane and inside.

Fig. 3. *Opalina brasiliensis* showing the spiral lines of cilia; they are composed of very small and deeply stained granules. The cilia and the granulations of the endoplasm are not drawn. Lower

nucleus and that of fig. 3 show the formation of the pseudo-centrodesmosis between two granules; fig. 3 is more enlarged.

Fig. 4. Later stage of mitosis. Pseudo-centrodesmosis present, going from an oval mass on one side, to another equal one on the opposite side.

Fig. 5 Advanced stage of division. Pseudo-centrodesmosis going from a chromatin granule to a chromatin mass on the opposite side. Deeply stained (Iron-hematoxylin) and irregularly disposed chromatin masses.

Fig. 6. Involution of half the pseudo-centrodesmosis.

Fig. 7. Final stage of mitosis. Very marked stricture. Irregularly and differently shaped chromatin masses.

Fig. 8. Daughter-nuclei, still attached by slender filament.

Fig. 9 & 9a. Free daughter-nuclei at rest. Crescent-shaped or rounded chromatin masses in the vicinity of the membrane.

Fig. 10. *Opalina brasiliensis* with two dividing nuclei.

(The details are not drawn as the specimen was not well differentiated).

Fig. 11. Specimen with 4 nuclei.

## Literature

- 1 ALEXEIEFF, A. 1913 Systématisation de la mitose dite "primitive". Sur la question du centriole. Archiv fuer Protistenkunde. Iena Vol. 29, pp. 344-363.
- 2 ARAGÃO, Dr. H. de B. Noticia sobre o *Nyctoterus cordiformes* Stein. Mem. do Instituto Oswaldo Cruz. Rio de Janeiro. Vol. IV, n. I, p. 126.
- 3 BEZZENBERGER, ERNEST 1904 Uber Infusorien aus asiatischen Anuren. Arch. fuer Protistenkunde. Iena. Vol. 3, pp. 138-174, P. XI.
- CEPEDE, CASIMIRO 1904 Recherches sur les infusoires astomates. Arch. Zool. exp., vol. 3, pp. 341-609, Pl. IX-XVIII.
- 4 CHAGAS, Dr. CARLOS 1911 Sobre as variações ciclicas do cariozoma em duas especies de ciliados parasitos. Memorias do Inst. Oswaldo Cruz. Rio de Janeiro, Vol. III, n. 1, pp. 136-144, Pl. 9 and 10.
- 5 DOFLEIN, Dr. F. 1911 Lehrbuch der Protozoenkunde, pp. 956-58.
- 6 FONSECA Dr, O. DA 1915 Estudos sobre os flajelados parasitos. Trabalho do Instituto Oswaldo Cruz. Rio.
- 7 FAURE FREMIET, EM. 1905 La structure intime du protoplasme chez les protozoaires. C. R. Soc. Biol. Paris, Vol. 2, pp. 612-14 and 697-699.
- 8 HARTMANN, MAX. e CHAGAS, Dr. C. 1910 Estudo sobre flajelados. Mem. do Instituto Oswaldo Cruz, Rio de Janeiro. Vol. II, n. 1. pp. 64-124, Pl. 4-9.
- 9 KENT, W. S. 1881 A Manual of the Infusoria. Vol. III, p. 558.
- 10 KUNSTLER, J. e GINESTE, Ch. 1905 Les spherules trophoplasmiques des infusoires ciliés C. R. Acad. des Sc., Vol. 141, pp. 907-8.
- 11 " 1906 L'orientation du corps des Opalines. C. R. Soc. Biol. Vol. 61, p. 113.
- 12 LEGER ET DUBOSCQ 1904 Notes sur les infusoires endoparasites. Arch. de Zool. Expér. V. II, pp. 337-356, pl. IV.
- 13 LOEWENTHAL, Dr. W. Notizen uber *Opalina ranarum* Purkinje et Valentin; Archiv fuer Protistenkunde, Iena, Vol. 113 p. 115-120.
- 14 MACHADO, ASTROGILDO 1911 Pesquisas citologicas sobre o *Trypanosoma rotatorium* Gruby. Mem. do Instituto Oswaldo Cruz Rio de Janeiro. Vol III, n. 1, pp. 108-132, Pl. 7 and 8.
- 15 METCALF, MAINARD M. 1909 *Opalina*. Archiv fuer Protistenkunde. Iena. Vol. 13, pp. 195-375, pl. XIV-XXVIII.
- 16 " " " 1914 Notes on *Opalina*. Zoologischer Anzeiger. Vol. XLIV, n. 12. July the 21st, 1914, pp. 533-541.
- 17 NERESHEIMER, Dr. EUGEN Die Fortpflanzung der Opalinen. Archiv fuer Protistenkunde, Iena, Suppl. 1 pp 1-43, Pl. 1-3.

18 PINTO, CESAR FERREIRA 1918

Contribuição para o conhecimento dos ciliados pa-  
razitos. Brazil - Medico, n. 37, July the 6<sup>th</sup>,  
1918.

