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On a new Coccidian, *Isospora wenyoni* n. sp., from the intestine of Indian toad, *Bufo melanostictus*.

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(With plate 8.)

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Introduction.

In July 1934, while we were examining the gut contents of frogs and toads of Calcutta, for Protozoa, we came across a coccidia belonging to the genus *Isospora* infecting the intestinal epithelium of *Bufo melanosticus*.

Only authentic account of *Isospora* from this group of animals is that of *I. lieberkühni* (Labbé) which according to Laveran and

MESNIL (1902), and NÖLLER (1913, 1923) is confined to the kindney of *Rana esculenta*. Oocysts of another form measuring 25 μ in length have been reported by WENYON (1926) from the intestine of English toad. Our species however, differs in the first place from *I. lieberkühni* in being solely confined to the intestinal epithelium of the host and, in the second place, from WENYON's species in its size and nature of the oocyst.

A close study of our material has shown that merozoites possess a pair of hyaline blades or lamina at the anterior end, a feature which has been noted by RAY (1935) in *Eimeria laminata* from *Bufo melanostictus*. Besides these there are other minor features which has warranted us to give it a new specific name *I. wenyoni*. The specific name has been given in honour of Dr. C. M. WENYON F. R. S., who first saw the intestinal type of *Isospora* from toads.

2. Material and Methods.

Infection of toads with this coccidian appears to be very rare, because out of a few hundred toads that we have examined since July 1934, only two harboured this organism. Oocysts appeared in the rectal contents, which when kept in 1 % chromic acid for three days completed their development, extra-corporally. As the examination of fresh smears of the intestinal contents did not show any particular feature we at once fixed the intestine in Bouin-Duboscq-Brasil's fluid for a closer examination of the intestinal epithelium. Sections were cut 6 μ thick and subsequently stained in HEIDENHAIN's iron-alum haematoxylin and MALLORY's triple stain (formula recommended for protozoa in Boles Lee was used). Differentiation in the case of haematoxylin stain was carried in picric acid in alcohol (50 cc. 70 % alcohol + 50 cc. saturated solution of picric acid in 90 % alcohol), and as soon as the proper degree of differentiation was obtained the slide was transferred for 5 minutes to a saturated solution of Lithium carbonate in 70 % alcohol. This method hastens the process of differentiation without injuring the brightness of the stain. Most of the drawings illustrating this paper were made from slides prepared in this way.

3. Observations on *Isospora wenyoni* n. sp.

a) Schizogony.

The smallest intracellular stage found in the epithelium of the intestine of toad measures 10 $\mu \times 3$ μ. This we take to be the young trophozoite and as we have not seen a sporozoite entering

the host cell we are not in a position to state definitely whether it has developed from sporozoite or merozoite. Prevalence of schizogony however, indicates that perhaps it developed from merozoite. As it begins to grow its nucleus begins to divide (Pl. 8 Fig. 5) and when a schizont measures 20—25 μ in diameter it has eight to twelve nuclei. Same number of spindle-shaped merozoites measuring 5×12 μ are very soon differentiated (Pl. 8 Figs. 6, 7). Each merozoite has a pair of hyaline blade or lamina situated at one end, which, in accordance with its subsequent behaviour, has been called the anterior. This feature is very characteristic of *E. laminata* which has been described in detail by RAY (1935). We have seen merozoites escaping into the lumen of the intestine and then entering fresh epithelial cells (Pl. 8 Fig. 1). We have sufficient data at our disposal to support the view that the laminar end of a merozoite is its anterior end because we have very often seen a merozoite lying against the host cell with this end directed forward. After a merozoite has got its entry into the cell it rotates and comes to lie with its anterior end directed towards the lumen of the intestine (Pl. 8 Figs. 2, 3). In between the blades there is a darkly staining area very much similar to what has been noted by RAY in *E. laminata* and constitutes of, what we may call, contractile elements. We have not seen any sucker at this end as is described by WENYON (1926) for *I. felis*. Sometimes more than one merozoite was found to enter a single epithelial cell (Pl. 8 Fig. 4) and schizogony in such cases often gave an impression as if more than twelve merozoites were formed. Such a crowding of schizonts, as was expected, gave rise to merozoitos which were slightly smaller in dimensions than the usual ones.

Occurrence of blades or lamina at the anterior end of merozoites of both *E. laminata* RAY and *I. wenyoni* n. sp., very strongly suggests that these coccidia occupy a higher place in evolution than those which are devoid of any such modification. Epithelium of the host in all probabilities, is responsible for bringing about this structural change by offering a resistance to the penetrating merozoites. It may be said that these two coccidians are more new fashioned in this respect and consequently offers an explanation for their rarity of infection.

b) Sporogony.

It was very difficult to distinguish a microgametocyte from a schizont at an early stage. With the advancement of age, however, a microgametocyte began to increase in size and at the same time

showed large number of nuclei situated towards its periphery (Pl. 8 Fig. 10). In a microgametocyte in which nuclei were actively dividing we could see four chromosomes during metaphase and two at each pole at telophase (Pl. 8 Figs 12, a, b). Soon microgametes were differentiated and when fully mature they were found to cluster round a residual mass of cytoplasm (Pl. 8 Fig. 11). A microgamete measured 2.4 μ in length and 1.5 μ in breadth.

A macrogametocyte can easily be distinguished even at an early stage from the rest by the presence of darkly staining granules in its cytoplasm (see Pl. 8 Figs. 8, 9). With growth however, the hyaline blades became invisible. A mature macrogamete measured 16—20 μ in length and 11—14 μ in breadth. In such forms the posterior end was usually turned upon itself and gave an impression as if there was short tail (see Pl. 8 Fig. 13). The nucleus at an early stage is spherical and contains a karyosome (see Pl. 8 Figs. 9, 13) but when maturity is reached the nucleus becomes elongated while the karyosome breaks up into a numbers of small granules scattered irregularly along its whole length (see Pl. 8 Fig. 15). The fertilization spindle is formed laterally as in *E. laminapta* and not along the long axis of the body which is usually the rule amongst these protozoa. Presence of hyaline blades at the anterior end prevents the formation of fertilization spindle in the usual manner (this has already been pointed out by RAY in the case of *E. laminapta*). We have often seen the fertilization spindle lying parallel to the groove formed by the turning of the posterior end of the macrogamete (see Pl. 8 Fig. 15). In few cases the spindle was even seen to open into this groove. Whether this groove ultimately serves the purpose of a micropyle for the entrance of microgamete we can not say but in Fig. 15 of Pl. 8 we just represent what we saw only once in our preparation. Here the presence of microgametes at the posterior end of the macrogamete strongly suggests that perhaps the male gamete gets entry into the latter through this groove. Fertilization evidently takes place while the organism is lodged in the epithelium of the host because in sections we have often seen specimens in which oocyst had developed (Pl. 8 Fig. 14).

Oocysts measuring 16—20 μ in length and 11—14 μ in breadth did not undergo further development in the body of the host and hence it was neccessary to keep the foecal matter in 1 % chromic acid. Within a course of three days the zygote gave rise to two sporoblasts each containing four sporozoites. There was no oocystic

residuum. Oocystic membrane has a double contour, the inner one being more prominent than the outer.

Sporocysts measure $8\,\mu \times 4\,\mu$ in dimensions with their long axis directed at right angles to the long axis of the oocysts (Pl. 8 Fig. 16).

Diagnosis:

A merozoite carries a pair of hyaline blades or lamina at its anterior end, which helps it to enter the epithelial cell: schizogony results in producing eight to twelve merozoites: Female gametocyte has a short recurved tail towards the posterior end: Oocyst subcylindrical, disporocystid tetrazoic, measure $16—20\,\mu \times 11—14\,\mu$, develop extracorporally: no oocystic residuum: Sporocysts $8\,\mu \times 4\,\mu$ have their long axis at right angles to the long axis of oocyst: sporocystic residuum present.

Habitat — Small intestine of *Bufo melanostictus.*

Locality — Calcutta, Bengal (India).

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Explanation of plate.

Plate 8.

All the figures except Fig. 16 were drawn with the aid of camera lucida and the magnification is $\times 1666$ unless otherwise stated. Figs. 1—15 were made from sections of *Isospora wenyoni* n. sp. in the intestinal epithelium of a toad. Fig. 16 is photomicrograph of an oocyst. The material was fixed in Bouin-Duboscq-Brasil's fluid and stained in Heidenhain's iron-alum haematoxylin (cilia of epithelial cells omitted).

Isospora wenyoni n. sp.

Fig. 1. A merozoite entering a cell.

Fig. 2. A merozoite rotating within a cell.

Fig. 3. Same, after complete rotation.

Fig. 4. Two merozoites in a single cell.

Fig. 5. A schizont with two nuclei.

Fig. 6. Merozoites escaping into the lumen of the intestine.

Fig. 7. Transverse section of a group of merozoites × 3500.

Fig. 8. Female gametocyte, note the granules in the cytoplasm.

Fig. 9. Slightly advanced female gametocyte showing spherical nucleus and the karyosome.

Fig. 10. Male gametocyte.

Fig. 11. Fully formed male gametes clustered round a central mass of cytoplasm.

Fig. 12. a) Dividing nucleus of male gametocyte — metaphase. × 3500. b) Sametelophase. × 3500.

Fig. 13. Female gametocyte with a recurved tail at the posterior end.

Fig. 14. Oocyst — immature.

Fig. 15. Female gametocyte-note elongated nucleus lying parallel to the groove, and two male gametes lying at the posterior end.

Fig. 16. Mature Oocyst. Photomicrograph. × 1600.


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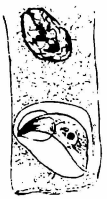
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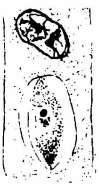
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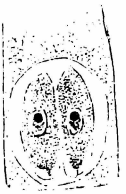
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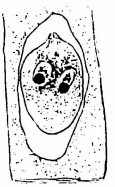
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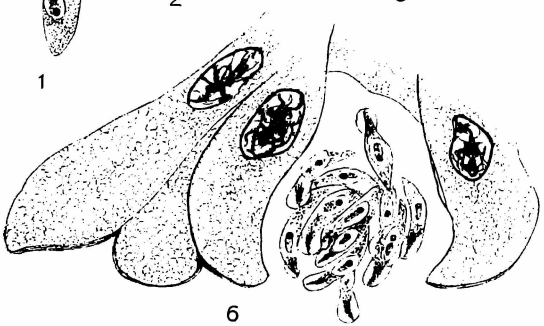
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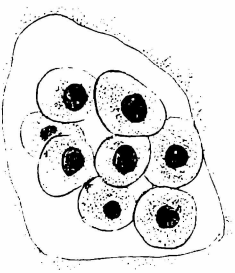
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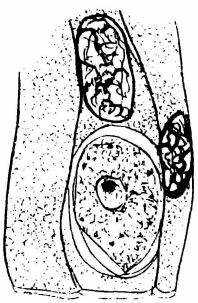
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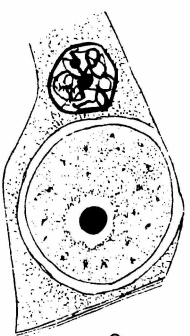
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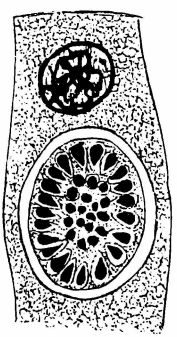
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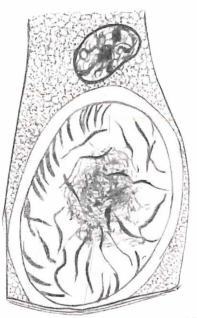
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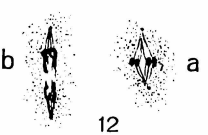

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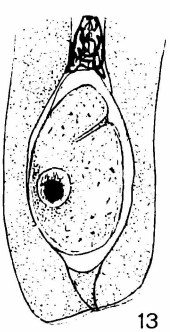
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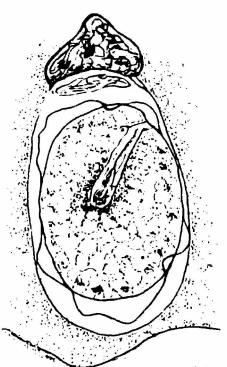
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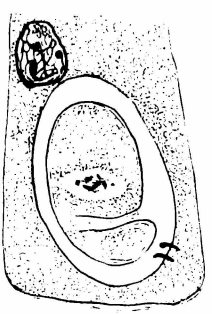
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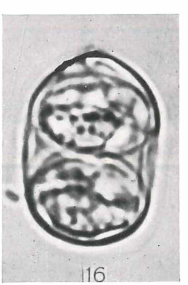
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VERLAG VON GUSTAV FISCHER IN JENA