

29. A New Ophryoscolecoid Ciliate, *Entodinium insolitum*, sp. n., from the Indian Rhinoceros. By JOHN M. WATSON, A.R.C.S., F.Z.S. (Lecturer in Zoology in the Northern Polytechnic). From the Wellcome Laboratories of Tropical Medicine, London.

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(Text-figures 1-4.)

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INTRODUCTION.

The new species of *Entodinium* which forms the subject of this paper was first discovered in a sample of a stool obtained from an Indian rhinoceros (*Rhinoceros unicornis*) on exhibition in the Gardens of the Zoological Society of London. The animal in question was suffering from severe diarrhoea and wasting, and in August 1933 a sample of its faeces was sent by the Society's pathologist, Colonel A. E. Hamerton, to Dr. C. A. Hoare of these Laboratories. Dr. Hoare found that two kinds of ciliates were present, one a new genus of Cycloposthiid ciliate, *Triplumaria hamertoni*, which has already formed the subject of a special paper (Hoare, 1937), the other a typical *Entodinium* which has not hitherto been described.

According to Hoare (1937), parasitic ciliates had never been recorded from the Indian rhinoceros prior to his own work, and a diligent search of the literature since that date has revealed no further records.

The particular interest of the ciliate hereinafter to be described is that it is the first species of *Entodinium* to be recorded from any member of the Perissodactyla (horse, tapir, rhinoceros), although numerous species of this genus have been recorded from various members of the Artiodactyla (cattle, sheep and goats, deer, antelopes, pigs, camel, hippopotamus) in comprehensive works by Dogiel (1927), Kofoid and MacLennan (1930), Das Gupta (1935) and others. Several ciliates have, however, been recorded from the African rhinoceros (*B. bicornis*), but these are all members of the family Cycloposthiidæ, namely, *Tricaudatia brumpti*, *Prototapirella clypeata*, *P. cristata* and *Bozasella rhinocerotis* (Buisson, 1923 a, 1923 b).

I take this opportunity to acknowledge my indebtedness to Dr. Hoare for placing the material at my disposal, and for guiding and criticizing the work recorded below. I am also deeply indebted to Dr. C. M. Wenyon, F.R.S., formerly Director-in-Chief, and the authorities of the Wellcome Foundation for so generously placing their admirable facilities at my disposal, and to Prof. E. Hindle, F.R.S., for much helpful advice.

METHODS OF INVESTIGATION.

The material handed to me was preserved in formalin. Specimens were examined unstained or lightly tinted with Weigert's iodine or Noland's fluid

in order to determine the general shape and structure. The details of the nuclei, the oral and digestive apparatus, and the contractile vacuole were studied in specimens stained with permanganate-ripened hæmatoxylin (Watson, 1943). The neuromotor apparatus was also examined in silver preparations, but it was found that, on the whole, it was better displayed in the hæmatoxylin preparations.

For statistical purposes one hundred specimens, chosen at random from several smears, were carefully drawn by means of Abbé's camera lucida and measured by means of a stage micrometer.

In the cutting of sections Peterfi's double embedding method was used (Peterfi, 1921), as recommended by Strelkow (1929), the sections being subsequently stained with Mayer's acid hæmalum and counterstained with eosin. The ciliates were first of all separated from the coarser particles of fæces by filtration through fine gauze and were then concentrated by centrifugation or by sedimentation in agglutination tubes, in the course of which most of the finer fæcal particles were pipetted off. The resulting concentrate of ciliates, which was, of course, a mixture of *Triplumaria* and *Entodinium*, was embedded, sectioned, stained and mounted (see Hoare, 1937). By courtesy of Dr. Hoare, I was enabled to make use of serial sections prepared by him in connection with the description of *Triplumaria hamertoni*.

The system of axes and ratios used in making the measurements of size and proportions was adopted from Kofoid and MacLennan (1930).

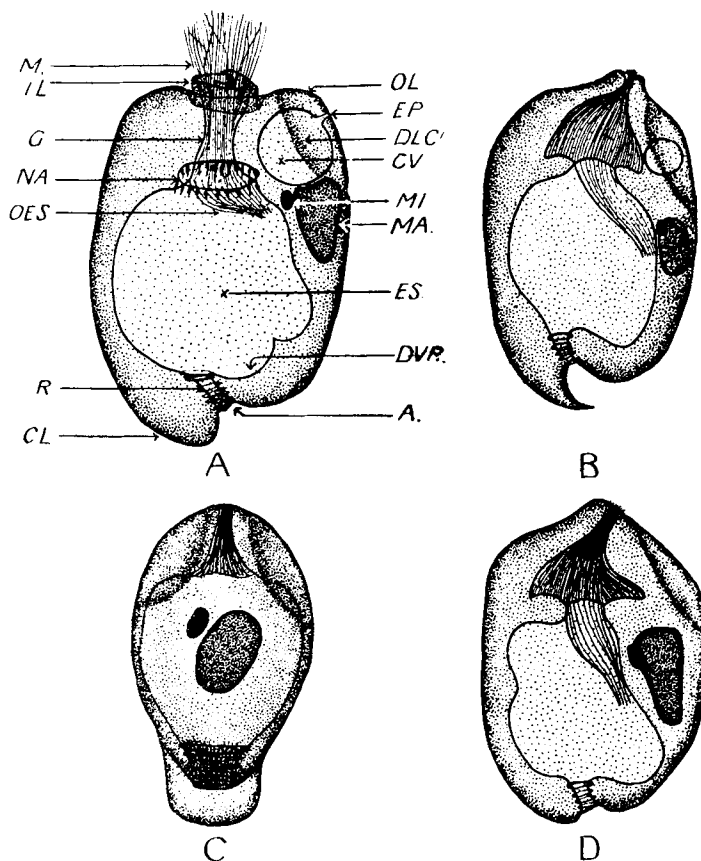
DESCRIPTION.

The new ciliate, which is described in detail below, was considered by Dr. Hoare to belong to the genus *Entodinium* (of the family Ophryoscolecidae), a provisional identification which I was soon able to confirm. As is well known, the members of this genus have a single zone of membranellæ, forming a spiral surrounding the mouth at the truncate anterior end of the body. A specialized "digestive apparatus" has been evolved, consisting of a pharynx and gullet leading posteriad from the mouth into the capacious "endoplasmic sac," where the food is digested, fæcal residues being voided from the body through a short rectal tube and anus at the posterior end of the body. There is a conspicuous macronucleus situated in the ectoplasm on what is generally accepted as the dorsal side of the body, with a small micronucleus anterior to the middle and on the left ventral side of the macronucleus. The contractile vacuole lies on the left, close to the anterior end of the macronucleus, discharging dorsally. There are no skeletal plates, but in many species the posterior surface of the body is produced into one or more lobes or spines (see text-fig. 1 A, B).

Form and Proportions.

The body of the new ciliate is almost rectangular in outline, 1.21 to 1.81 dorsoventral diameters in length, and is laterally compressed to 0.69 to 0.85 dorsoventral diameters. For the greater part of its length the dorsal surface is slightly convex, increasing to a more pronounced curvature at the ends. The ventral surface for the greater part of its length is almost straight or even slightly concave, but, like the dorsal surface, increases suddenly to a more pronounced curvature at the ends. Thus the outlines of the dorsal and ventral surfaces are almost parallel to one another. This tendency, however, is more pronounced in contracted than in fully expanded specimens, in which the curvature of both surfaces shows a greater degree of convexity (see text-fig. 1 A & D). The lateral surfaces are smoothly convex in outline throughout their length, and the greatest transdiameter (*i. e.* the greatest distance between the lateral surface in a line perpendicular to the main axis) being in the anterior half of the body, they approach more closely posteriorly, so that, viewed from above, the body is somewhat ovoid (see text-fig. 1 C). The anterior end of the body is quite sharply truncated at right angles to the main axis to form

Text-figure 1.

*Entodinium insolitum*, from whole mounts.

- A. *E. insolitum* forma *insolitum*. Fully expanded specimen seen from the right side. ($\times c. 1000.$)

A. Anus.	M. Membranellae (broken up into constituent cilia by action of fixative).
CL. Caudal lobe.	MA. Macronucleus.
CV. Contractile vacuole.	MI. Micronucleus.
DLC'. Dorsolateral concavity.	NA. Neuromotor apparatus.
DVP. Diverticulum posterius.	OES. Oesophagus.
EP. Excretory pore.	OL. Outer adoral lip.
ES. Endoplasmic sac.	R. Rectum.
G. Gullet.	
IL. Inner adoral lip.	

- B. *E. insolitum* forma *uncinatum*. Contracted specimen seen from the right side. ($\times c. 1000.$)

- C. *E. insolitum* forma *insolitum*. Contracted specimen seen from the dorsal aspect. ($\times c. 1000.$)

- D. *E. insolitum* forma *insolitum*. Contracted specimen seen from the right side. ($\times c. 1000.$)

the oral area, which ranges in extent from 0.28 to 0.46 dorsoventral diameters. The posterior end is rounded, its curvature being interrupted, however, by the smooth outline of the fleshy caudal lobe, 0.05 to 0.17 dorsoventral diameters in length, into which the ventral surface is produced posteriorly (see text-fig. 1 A). In about 6 per cent. of the individuals examined the caudal process was found to have a more angular outline with a sharply pointed termination, like a hook directed dorsalwards (see text-fig. 1 B). It was

not found possible to correlate this with any other distinctive feature, and hence it was concluded that this constituted a "forma." Anteriorly the body shows a shallow concavity on either side of the dorsal ridge, the lower border of which extends in a smooth curve from near the anterior end to about the middle of the dorsal surface. This feature is especially clearly seen in transverse sections of the anterior part of the body (see text-fig. 3 E, G, J).

Size.

The range of length observed for this species is from 43μ to 68μ . The dorsoventral diameter varied from 27μ to 45μ , and the transdiameter from 22μ to 35μ . The diameter of the oral zone varies from 10μ to 16μ , while the length of the caudal lobe varied from 2μ to 7μ . The length of the macronucleus shows greater variation than any other dimensions, ranging from 8μ up to 29μ .

Since the size of the individual ciliates varies, of course, considerably, according to the interval which has elapsed since the last division, the dimensions given above were all taken from "adult" individuals, recognizable by the more elongate form of the body and the relative positions of the macronucleus and micronucleus (see text-figs. 1 A & D and 2 E).

The size and proportions of the body are indicated in tabular form in Table I.

TABLE I.

	Microns.	Ratio.	Standard dev.	Coefficient of variation.
Body length	52 (43-68)	1.49 (1.21-1.81)	5.74	11.05
Transdiameter	31 (22-35)	0.77 (0.69-0.85)	3.39	11.02
Dorsoventral diameter ..	35 (27-45)	1.00	3.59	10.45
Mouth	13 (10-16)	0.38 (0.28-0.46)	2.16	16.72
Macronucleus	14 (8-29)	0.42 (0.21-0.85)	3.96	36.50
Caudal lobe	4 (2-7)	0.11 (0.05-0.17)	1.15	30.75

Oral Apparatus.

In an expanded specimen the oral opening is circular in outline and occupies the greater part of the anterior surface of the body, but owing to the thickness of the outer lips this is not readily apparent from the measurements (see text-figs. 1 A and 3 I). The outer adoral furrow is deep, and both outer and inner adoral lips are thick and conspicuous (see text-figs. 1 A and 3 I). The material having been preserved in formalin for several years, the membranellæ in all fully expanded specimens had broken up into their component cilia, so that it was not possible to determine the original grouping. In sections, however, it can be clearly seen that the membranellæ form a left-hand spiral commencing on the left side of the body and running inwards to disappear in the mouth-opening (see text-fig. 3 I). In contracted specimens the oral opening is reduced to such an extent that the mouth is represented only by a narrow transverse slit in the anterior projection, which leads into a wide conical chamber containing the oral apparatus (see text-figs. 1 D and 3 B & C).

In hæmatoxylin-stained specimens, and to a less extent in dry silver preparations, a small neuromotorium can be distinguished just below and in front of the anterior end of the macronucleus. An exceedingly thin motor strand, with about 20 membranellar rootlets, extends ventrally from the neuromotorium (see text-fig. 1 A).

Gullet and Œsophagus.

The gullet is a wide cylindrical tube extending through the oral apparatus. The Œsophagus, which is most readily seen in contracted specimens, takes

the form of an inverted cone truncated anteriorly, which curves slightly to the right and leads upwards into the endoplasm posteriorly at an angle of about 25° to the horizontal longitudinal axis. In the wall of the oesophagus numerous fine fibrillæ may be seen running longitudinally. These fibrils, which were most clearly visible in specimens stained with Noland's fluid or crystal violet, may possibly have a contractile function and assist in the retraction of the oral apparatus, in addition to giving added strength to the walls in connection with the engulfing of large food particles (see text-figs. 1 A, B, D and 3 B, C, D).

Endoplasmic Sac.

The boundary layer is sharply distinct, both in stained and unstained preparations, and the limits of the endoplasmic sac are therefore clearly visible. It commences abruptly just behind the oral apparatus and rapidly widens to its maximum diameter, which is maintained back to the level of the commencement of the rectum. The endoplasmic sac is thus abruptly truncated posteriorly, the general plane of its hinder boundary lying almost at right angles to the main longitudinal axis of the body. Ventrally, however, the posterior wall curves back a little way beyond the level of the commencement of the rectum, so forming a small shallow pocket, the "diverticulum posterius" of Dogiel (1927) (see text-figs. 1 A and 3 H). The dorsal boundary of the endoplasmic sac is ventrally depressed, usually in the anterior half of the sac, by the presence of the macronucleus. In some individuals there is a slight projection of the ventral surface of the sac about midway along its length (see text-fig. 1 D). The surrounding ectoplasm is relatively thin laterally, but relatively thick along the dorsal and ventral boundaries.

Rectum and Anus.

The thin-walled rectum arises from about the centre of the posterior boundary of the endoplasmic sac, whence it runs, making an angle of 35° to 40° to the horizontal longitudinal axis, back to the anus, which lies just dorsal to the caudal lobe. The rectum is much compressed dorsoventrally and considerably expanded laterally, so that the anus is a wide, slit-like aperture. In hæmatoxylin stained preparations five elliptical myonemes may be detected surrounding the rectum (see text-fig. 1 A & D). Of these, one, surrounding the exit of the rectum from the endoplasmic sac, is much thicker than the other four, which lie nearer the anus, and perhaps functions as a sphincter. The membrane forming the wall of the rectum is relatively thick, a feature which may perhaps be correlated, as Kofoed and MacLennan (1930) have suggested for other species of this genus, with the fact that the food often consists of large particles. Moreover, the slit-like form permits easy defæcation of large flat particles like grass, which are unchanged in form during digestion, however they may be altered chemically (Dogiel, 1925). Fibres, similar to those occurring in the wall of the oesophagus, are present in the rectal membrane also, but are not so conspicuous (see text-fig. 1 C).

Macronucleus.

The macronucleus is usually located just anterior to the middle of the body in the mid-dorsal ectoplasm. It is of the massive type characteristic of the genus. There is a well-defined nuclear membrane enclosing a densely packed mass of polygonal chromatin granules, extending up to the inner surface of the membrane. Both in form and dimensions it shows greater variation than any other part of the body, the coefficient of variation being 36.50 per cent. (see Table I.).

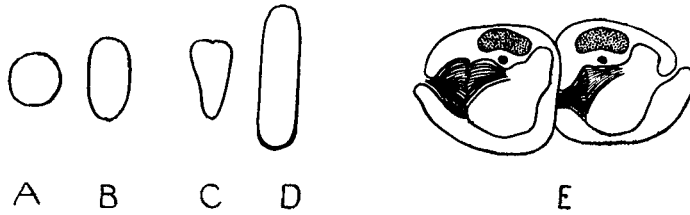
The most common form, which occurs in about 43 per cent. of individuals in a random sample, is elliptical (see text-fig. 2 B). Generally about twice as long as broad, such nuclei vary in length from 8μ to 23μ , with a mean of

13.86 μ . Expressed as a fraction of the dorsoventral diameter, the size of this type ranges from 0.26 to 0.57, with a mean of 0.40. Elliptical nuclei lie at an angle to the main longitudinal axis of the body, being twisted clockwise about 25° in a horizontal plane.

Next in order of frequency comes the spherical type (see text-fig. 2 A), which occurs in about 23 per cent. of individuals in a random sample. The diameter varies from 8 μ to 17 μ , with a mean of 11.35 μ , or, in terms of dorsoventral diameter, 0.23 to 0.38, with a mean of 0.30.

Slightly less common is the elongate type (see text-fig. 2 D), found in about 20 per cent. of individuals in a random sample. Of sausage-like form, and generally from three to four times as long as broad, it varies in length from 12 μ to 29 μ , with a mean of 19.24 μ . Thus, on the average, it is decidedly the longest of the four varieties, not merely in absolute measurement, but

Text-figure 2.



Appearance of the nuclei.

- A. Spherical type of macronucleus. E. Dividing specimen showing position of
 B. Elliptical type of macronucleus. micronucleus relative to macronucleus
 C. Wedge type of macronucleus. during binary fission. (\times c. 500.)
 D. Elongate type of macronucleus.

also proportionately, ranging from 0.40 to 0.85 dorsoventral diameters, with a mean of 0.56. Like the elliptical form, the elongate type of macronucleus is twisted clockwise horizontally, relative to the main longitudinal axis of the body, and at about the same angle.

The wedge or conical type of macronucleus occurs in only 14 per cent. of individuals in a random sample (see text-fig. 2 C). It is broad and wedge-shaped in side view, being thicker at the anterior than at the posterior end and slightly flattened dorsoventrally. Seen from above, however, it is elliptical in outline and set at a similar angle to the main longitudinal axis of the body, as are the elliptical and elongate types. In size and proportions it closely resembles the elliptical type, of which it is probably a modification, ranging from 11 μ to 21 μ in length, with a mean of 15.69 μ , or 0.29 to 0.59 dorsoventral diameters, with a mean of 0.45. Its length is usually almost exactly double its greatest dorsoventral diameter (thickness).

The measurements and proportions of these types of macronucleus are summed up in Table II.

TABLE II.

Type.	Frequency per 100 in sample.	Microns.	Ratio.	Mean length of individuals in dorsoventral diameters.
Elliptical	43	8-23 (13.86)	0.40 (0.26-0.57)	1.48
Spherical	23	8-17 (11.35)	0.30 (0.23-0.38)	1.44
Elongate	20	12-29 (19.24)	0.56 (0.40-0.85)	1.56
Wedge	14	11-21 (15.69)	0.45 (0.29-0.59)	1.51

From this table it may be seen that the larger nuclei tended to occur in the larger individuals, and statistical tests showed that there was a significant correlation between length of macronucleus and length of body, despite the discrepancy in shape. Moreover, it was possible to trace a complete series of intergradations between the types, by examining a sufficiently large number of individuals. It may therefore be concluded that these various forms and sizes of macronucleus do not indicate the existence of races or formæ, but are merely a further instance of the high degree of variability which is characteristic of the Ophryoscolecidae.

Micronucleus.

Generally elliptical or spherical in shape, the micronucleus lies close against the left ventral border of the macronucleus, near its anterior end. It is homogeneous in appearance, no chromatin granules or nuclear membrane being distinguishable (see text-fig. 1). Preparatory to nuclear division, the micronucleus moves ventralwards, away from the macronucleus, and takes up an independent position opposite the middle of the ventral surface of the latter. The daughter micronuclei retain this position until division is completed (see text-fig. 2 E).

Contractile Vacuole.

The contractile vacuole is situated directly anterior to the macronucleus in the dorsal mid-line. Its posterior surface usually rests against the anterior surface of the macronucleus when it is fully expanded, and presumably in consequence of the pressure which it exerts, the anterior surface of the macronucleus becomes flattened or even depressed at the point of contact. In a small proportion of specimens, however, especially those with a small macronucleus, the contractile vacuole may be separated from the anterior border of the macronucleus by a space almost equal to its own diameter. The contractile vacuole is normally spherical, but when fully expanded takes the form of a convex lens with a short radius of curvature, being circular when seen from the side, owing to compression between the concavities of the antero-lateral surfaces. When fully expanded, it has a diameter varying from 0.30 to 0.42 dorsoventral diameters.

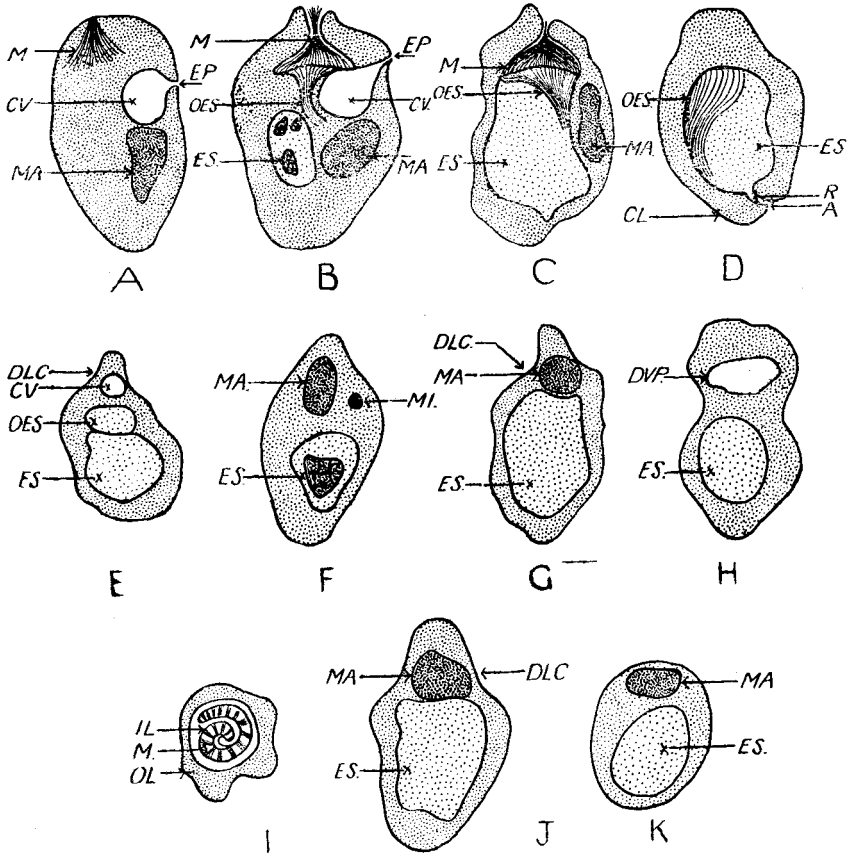
A short funnel-shaped excretory canal runs from the antero-dorsal border of the contractile vacuole to open on the dorsal surface of the body (see text-figs. 1 A and 3 A, B, E).

Food.

This ciliate is able to ingest food particles of very discrepant size, ranging from bacteria and starch grains to large fragments of husk of wheat. Dogiel (1925) and Weineck (1934) have suggested that probably the smaller particles are swept into the gullet by the current created by the movements of the membranellæ, while the larger particles are pushed into the gullet by the lips and dragged into the endoplasmic sac by contractions of the œsophagus. Bretschneider (1934), however, denies that the œsophageal fibres are contractile.

The food seems to consist principally of starch grains and plant débris of various kinds, together with small quantities of bacteria. A large number of specimens were examined, and particles consisting of various types of vegetable fragments were observed. Some of these fragments were of remarkable size and could only be wedged diagonally into the endoplasmic sac, which shows remarkable plasticity and readily adapts itself to large and irregular food particles (see text-fig. 4 J). Among the materials identified in the endoplasmic sac (by the aid of Denison, 1942) were the epidermal cells and palisade cells of bean seeds, parenchyma cells of carrot, epidermal cells of apple, bast fibres of apple, fragments of husk of wheat, fragments of skin of banana,

Text-figure 3.



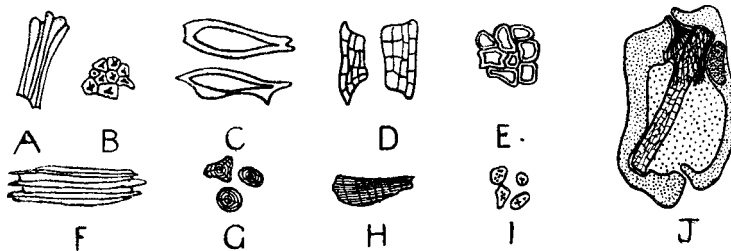
Sections of *E. insolitum* in various planes. (All $\times c. 500$.)

A-D. Vertical longitudinal sections.

E-K. Transverse sections.

(For key to lettering see text-figure 1.)

Text-figure 4.



Food of *E. insolitum*. (All $\times c. 500$.)

- A. Palisade cells of bean.
- B. Epidermal cells of bean.
- C. Parenchyma cells of carrot.
- D. Fragments of husk of wheat.
- E. Epidermal cells of apple.

- F. Bast fibres of apple.
- G. Starch grains.
- H. Skin of banana.
- I. Aleurone grains.
- J. Specimen distorted by large food-particles.

aleurone grains, starch grains, chloroplasts, parenchyma of grass and bacteria (see text-fig. 4 A-I). Enquiry and observation confirmed that these food materials could reasonably be expected to be present in the faeces of the rhinoceros. It is curious that few identifiable grass fragments occur, since hay forms part of the food of captive rhino; perhaps the diarrhoea was due to excess of apple and other special items of diet, and at the same time this proved an exceptionally suitable food for the *Entodinium*, which was thus present in unusually large numbers. Or, of course, the *Entodinium* may always be present in large numbers and merely have been flushed out as a result of the diarrhoea. It is clear that *E. insolitum* is a remarkably catholic feeder, and apparently exercises little discrimination either in the nature or the size of its food particles; probably the bacteria present in the gut in great abundance are ingested accidentally, together with vegetable particles. There is no sign of that specialization in food habits which has been noted for certain species of *Entodinium* (e. g. *E. longinucleatum*) and other Ophryoscolecidae (Dogiel, 1925 and 1927).

TAXONOMY.

Before coming to a definite decision with regard to the systematic position and status of the new ciliate, it will not be out of place to discuss its affinities with the existing species of the genus.

It would be not unnatural to consider first the species occurring in wild Indian ungulates. Unfortunately, with the exception of three species from the mouse-deer (*Tragulus meminna*), all the Indian species have been recorded only from domestic animals, principally goats and cattle; indeed, Bhatia (1936) records only two other species in the whole family from wild hosts, namely *Polydinium mysoreum* and *Elephantophilus zeta* from the Indian elephant. I shall therefore consider the morphological characteristics seriatim, in relation to any described species of the genus to which resemblance may be observed, no matter from what part of the world they come.

Thus in form and proportions the closest resemblance is to *E. biconcavum* and *E. anteronuclatum* forma *monolobum*, but the anterodorsal concavity of the *Entodinium* of the rhinoceros is not found in either of these species, nor does the posterior concavity of *E. biconcavum* occur in the new ciliate. In size the closest resemblance is to *E. anteronuclatum*, but the similarity must be treated with caution in view of the marked discrepancies between the measurements given in several instances for the same species by Dogiel (1927), Eberlein (1895) and Kofoid and MacLennan (1930). With regard to the digestive system, neither the oral apparatus nor the endoplasmic sac shows any marked difference between the various species of the genus. The distinctive conical oesophagus, with its prominent longitudinal fibrils, resembles that found in many species of the genus, among them *E. anteronuclatum*, *E. bursa*, *E. curtum*, *E. ovinum*, *E. nanellum* and several of the spiny forms. The characteristic rectum, combining encircling myonemes with a broad slit-like section, is unique. Though similar myonemes are found encircling the cylindrical rectum in the members of the "biconcavum" group (i. e. *E. biconcavum*, *E. bifidum*, *E. acutum*, *E. aculeatum*), and a slit-like rectum occurs in *E. ellipsoideum*, *E. longinucleatum*, *E. nanellum*, *E. acutonucleatum*, *E. ovoideum* and *E. rhomboideum*, the two features never occur together. In passing, it may be remarked that neither in the rectum nor in the oesophagus does there appear to be any obvious connection between the structure and the nature of the food. In respect of the nuclear complex, the closest resemblance is to members of the "biconcavum" group, in which the macronucleus is set at an angle to the long axis of the body, considerable variation of size and shape occurs in the macronucleus, and the micronucleus (in *E. biconcavum* in particular) occupies the same relative position. It must, however, be noted that the elliptical type of macronucleus occurs in *E. ekendræ* and *E. ovoidonucleatum*, the spherical type in *E. setnai* and *E. chaterjeei*, and the wedge type (in addition

to the members of the "biconcavum" group) in *E. laterale*, *E. rectangulatum*, *E. rhomboideum*, *E. simplex* and *E. anteronucleatum*. The only species in which the micronucleus occurs in the same relative position (in addition to the members of the "biconcavum" group) are *E. rhomboideum*, *E. nanellum*, *E. contractum*, *E. brevispinum*, *E. longinucleatum* and *E. acutonucleatum*; in all other species the micronucleus is either ventral to the macronucleus or is placed nearer the posterior end of the macronucleus. The position of the contractile vacuole anterior to the macronucleus in the centre, instead of on the left-hand side, is unusual in the genus, and is only found in *E. pisciculum*, *E. rostratum* and *E. setnai*; the first two species also possess a conspicuous excretory canal.

With regard to the species occurring in non-ruminant hosts, it may be noted that there are no obvious points of resemblance between the new ciliate and either *E. lemni* or *E. paludicolæ*; but that in *E. mammilatum* the general shape and the caudal lobe are similar, and the contractile vacuole and the micronucleus occupy the same positions relative to the macronucleus, which is of the elongate type.

There are also resemblances to some of MacLennan's (1935) new species, especially *E. medium*, *E. costatum*, *E. rotundum*, and *E. pristinum*, in the slit-like form of the rectum and in the possession of a distinct excretory canal.

Thus, while there are many points of resemblance between the new ciliate and other non-caudate species of the genus, especially *E. biconcavum* and *E. anteronucleatum*, these resemblances are only partial, and in view of the unique combination of structural features possessed by the *Entodinium* of the rhinoceros, it must be concluded that on morphological grounds alone it is a distinct species, quite apart from the unusual character of the host.

I therefore propose to constitute it a new species: *Entodinium insolitum*, sp. n.

The degree of variability shown by the new species in certain characteristics (*e. g.* body-length, size and shape of macronucleus) is by no means unusual in the members of the family Ophryoscolecidae, a fact which has been stressed by Dogiel (1927) in his monograph on the family, and has since been investigated experimentally and statistically by Poljansky and Strelkow (1938). Thus, in particular, Dogiel points out that the macronucleus tends to develop from spherical, through conical, to elongate in the members of this family. Moreover, Poljansky and Strelkow point out that variability in the size of the macronucleus may be partly attributed to the age of the individual and partly to the fact that there frequently occurs unequal division of the macronucleus between daughters. This was also observed in the present material. The limits of variation, found by Poljansky and Strelkow in their experimental clones, were very wide in respect of both body-length and the size and form of the macronucleus. It may at once be stated, therefore, that the size of the body and the size (and, to some extent, the form) of the macronucleus are factors which vary so greatly, even in members of the same clone, that systematic conclusions and specific definitions based upon them and unsupported by ample alternative evidence, should be treated with great reserve. It is for this reason that I have avoided making the macronucleus a basis for the establishment of several species or subspecific categories in the present case. Statistical tests showed that, in spite of the high degree of variability in the dimensions of the body and of the macronucleus, the distribution curve obtained by plotting the measurements of a hundred specimens picked out at random was normal, which would hardly have been so had several species been involved.

On the basis of the shape of the caudal lobe, a feature which shows no gradation of form between the normal rounded type and the hooked type, it is proposed to divide the species into two formæ, namely, *E. insolitum* forma *insolitum*, forma n., with rounded caudal lobe, and *E. insolitum* forma *uncinatum*, forma n., with hook-like caudal lobe. Kofoid and MacLennan,

in their excellent monograph on the species of *Entodinium* from *Bos indicus* (1930), have abolished Sharp's (1914) category "forma" in the genus *Entodinium* on the grounds that "it does not add a taxonomic unit of any value, but, on the contrary, serves to confuse nomenclature," and further state that it "adds to nomenclature a name for the individual differences incidental to division." The adoption of subspecific units is a very common practice throughout the class Ciliophora, as may be seen by glancing through the recent exhaustive revision of the free-living forms by Kahl (1930-1935), and provides a convenient method of labelling the constant differences of structure, in such variable genera as *Entodinium* and *Metopus*, as are not of sufficient magnitude to justify the establishment of separate species. In fact, so far from confusing the nomenclature, this subspecific category, by reducing the number of species based on trivial characteristics, actually simplifies it, and precludes the necessity of establishing such loose and unsatisfactory subgeneric categories as the "species groups" of Kofoid and MacLennan. If, subsequently to the description of a forma or variety, experimental work such as that of Poljansky and Strelkow (1938) shows that it is, indeed, a group of individuals of such stable and distinct characteristics as to be at all times sharply and readily separable from other individuals of the same population, then it can duly be raised to a higher rank. To establish species, as Kofoid and MacLennan have done, on any morphological difference, however trivial, unsupported by experimental work, is to run the serious risk that many of the so-called species may subsequently prove to be races or even durable modifications, with resultant confusion in the taxonomy of the genus.

Dogiel (1927) himself admitted that the formæ or varieties of ophryoscolecid species were of very unequal taxonomic value, some being modifications, while others were races or biotypes. In other words, the term "forma" indicates a non-heritable aberration comprising different categories of polymorphism in different species, and to put all these on the same level as substantive species, while producing an apparent simplification, actually complicates the systematics of a group.

HOST-PARASITE RELATIONS.

The principal interest of the discovery of this new species of *Entodinium* is to be found in what may be termed its ecological aspects.

Very little work has been done on the intestinal parasites of the rhinoceros. A few species of ciliates have been described from the African rhinoceros by Buisson (1923), but, as far as I have been able to discover, no investigation of the gut fauna of the Indian rhinoceros has ever been attempted, even in individuals confined to zoological gardens, to say nothing of wild specimens. The only recorded intestinal parasite from this species is *Triplumaria hamertoni* Hoare, which was found in the same faecal sample as *E. insolitum*. Hence there is, at first sight, nothing remarkable in the discovery of a new species of *Entodinium* in this host. But the uniqueness of the occurrence lies in the fact that, with the exception of very small numbers of three species occurring in the cæcum of rodents [*E. mammilatum* Da Cunha (1914) in *Cavia apera*, *E. lemni* Kopperi (1935) in *Lemmus lemnus* (L.), and *E. paludicolæ* Kopperi (1935) in *Arvicola scherman* (Shaw)], all the species of *Entodinium* so far described have been found in the rumen and reticulum of ruminant Artiodactyla. No species of *Entodinium* has been found to occur in the gut of any Perissodactyla, even in the horse, which has been thoroughly investigated from this point of view by a number of workers (cf. Hsiung, 1930).

The question immediately arises as to whether *E. insolitum* is a primary parasite of the rhinoceros or whether it is a secondary parasite acquired from its original host by contagion. Unfortunately this question is one which it is impossible to decide with certainty, but a consideration of some of the points involved is interesting. Thus, in the first place, the *Entodinium* was present

in the faeces in very large numbers, the sample which was handed to me (already much diluted with fixative) containing just under 100,000 individuals per c.c. This seems a very high concentration, especially as the species reported from rodents and lemmings were present in small numbers; but Becker (1932) and Poljansky and Strelkow (1938) have pointed out that the numbers of ciliates in experimental animals depend primarily on the nature of the diet. Mowry and Becker (1930), by adding grain to the diet, were able to obtain a concentration of no fewer than 3,000,000 ciliates per c.c. Moreover, trypanosomes and other parasites are often more abundant in laboratory animals than in their original hosts.

There is nothing inherently unlikely in the acquisition of an infection such as the one under consideration, either by direct or indirect contact, between the rhinoceros and other animals at some period during its life-history. Becker and Hsiung (1929) have shown that infection with *Entodinium* occurs by mouth contact or by eating food contaminated with infected saliva before the latter has had time to dry, the ciliates being brought up into the mouth with the regurgitated food from the rumen. Infection may also possibly take place by drinking contaminated water. In a zoological garden possibly the "salt-licks" are also a source of infection. Although desiccation is invariably fatal to *Entodinium* (Becker and Hsiung, 1929), according to Wertheim (1934) the ciliates of this genus show remarkable powers of resistance to changes of temperature, and are well able to survive cooling to as low a temperature as 14° C. Fantham (1922) even claims to have found living specimens of *Entodinium* on the grass in pastures. Hence, it is obviously possible, theoretically, for the rhinoceros to have become infected from some ruminant. It would be manifestly impossible to trace all the possible contacts, even since the animal was acquired by the Zoological Society, not to mention those which occurred previously. Some species of *Entodinium* from cattle, sheep and goats have been found not to be host-specific, but can be established in other ruminant hosts. Dogiel (1927), indeed, has gone so far as to state that the original ciliate fauna of the alimentary canal is lost in animals confined in zoological gardens, and is replaced by forms from cattle and other domestic animals. This is, however, altogether too sweeping a generalization, and there is much evidence that it is not always true. To take a single instance, the individual rhinoceros in which *E. insolitum* occurred was introduced into the Regent's Park Gardens of the Zoological Society of London in 1924, yet in 1933 it was found to have living in its intestine a cyclopothiid ciliate new to science (*Triplumaria hamertoni* Hoare); seven years had evidently not sufficed to eradicate this form and replace it with ciliate species from domestic ruminants.

In this connection it has to be borne in mind that the ciliate fauna of the horse, and probably of other Perissodactyla, is quite different from that of domestic ruminants (*cf.* Hsiung, 1930), and all attempts to introduce ciliates of other domestic ruminants into horses have failed (Becker and Hsiung, 1929; Dogiel and Winogradowa-Federowa, 1930). Under the circumstances it seems improbable that a member of a genus which has not succeeded in establishing itself in the horse, despite the ample opportunities for cross-infection from domestic ruminants which must have occurred during several thousand years of collateral domesticity, could establish itself in the rhinoceros. Furthermore, most gut parasites of wild animals show a high degree of host specificity. Hence, it may be concluded with a fair degree of probability, on ecological grounds alone, that *Entodinium insolitum* is specific to the Indian rhinoceros, since were it possible for a form from a domestic host to establish itself in so different an animal, overcoming the difficulty of passing through the acid gastric juices (as the active forms of certain other parasitic protozoa have been shown to do by Hegner in rats and guinea-pigs), it would be extremely unlikely to flourish and be present in such enormous numbers.

With regard to the site of infection, there is, unfortunately, no direct evidence. There are three distinct possibilities, namely, the colon, the cæcum and the cardiac diverticulum of the stomach. In the only species of *Entodinium* known to occur in non-ruminant hosts (*E. mammillatum* in *Cavia aperea*, *E. lemni* in *Lemmus lemmus*, and *E. paludicola* in *Arvicola scherman*), the site of infection is stated to be the cæcum. Kofoid found the ophryoscolecoid species *Polydinium mysoreum* and *Elephantophilus zeta* in both the cæcum and the colon of the Indian elephant. According to Buisson (1923) cycloposthiid ciliates are generally found in the cæcum of horse and tapir, although Hsiung (1930) states that all the Oligotricha of the horse occur in the colon with the exception of *Cycloposthium* itself. No help is to be obtained from Buisson's work (1923) on the ciliates of the African rhinoceros, since these are merely stated to have occurred in the "intestine." The Indian rhinoceros possesses a pouch of the stomach, the cardiac diverticulum, which is lined by a much plicated mucous membrane, as contrasted with the smooth lining of the rest of the stomach. It lies within the bounds of possibility that this may be the site of infection; but Dogiel (1927) has stated that *Entodinium* is only found in the rumen and reticulum of ruminants, because the reaction there is weakly alkaline, the only digestive juice present being saliva, and it seems probable that the reaction in the cardiac diverticulum of the stomach of the rhinoceros is strongly acid. Acidity is fatal to *Entodinium*; a fall in the pH of the rumen contents in domestic hosts from the normal figure of pH 7.6 to 7.8 (Mowry and Becker, 1930) to 6.9 results in a sharp drop in the numbers of ciliates (Ferber, 1929), while a further fall to 6.0 produces complete defaunation (Mangold and Usuelli, 1930), a fact which is the basis of the milk-diet method of producing azoic (*i. e.* defaunated) experimental animals. In view of the fact that the cæcum of the rhinoceros is very short and broad, it is improbable that ciliates present in it would be absent from the colon and *vice versa*—hence it may be concluded that most probably both these regions form the site of infection.

At the time that the infested faecal sample was obtained the rhinoceros was suffering from severe diarrhoea and wasting. It is, however, extremely unlikely that the ciliates were in any way responsible for this condition, in view of the fact that healthy cattle, sheep and goats are generally more or less heavily infected with various species of *Entodinium*. Opinion is divided as to whether the members of this genus are deleterious, merely harmless commensals, or actually beneficial to the host. Dogiel (1925) has stated that they play an important part in breaking up the vegetable particles in the food into smaller fragments, and so making them more readily susceptible to bacterial and enzyme action. In a later paper, Dogiel and Winogradowa-Fedorowa (1930) emphasized the rapid division of ciliates which occurred in the rumen and reticulum of ruminants, and formed the conclusion that many must pass into the psalterium and beyond, and be digested as animal protein. This has recently been confirmed by Baker (1943). On the other hand, Margolin (1930) considered that the ciliates subsisted on the products of cellulose fermentation of bacteria, and that they therefore robbed the host of food. Undoubtedly, ophryoscolecoid ciliates readily digest starch and sugars (Trier, 1926), a fact which explains the enormous increase in their numbers which occurs when corn or oats are added to the diet of the host (Mowry and Becker, 1930; Poljansky and Strelkow, 1938). They also digest protein and chlorophyll (Ferber, 1929). More recent work by Weineck (1934) has shown that some Ophryoscolecidae also possess enzymes which enable them to digest cellulose in the endoplasmic sac. Hungate (1943), as a result of careful experimental work, has recently concluded that while the species of *Diplodinium* possess enzymes which enable them to digest cellulose, the smaller ciliates, including *Entodinium*, are unable to do so. Van der Wath and Myburgh (1941) have pointed out that rumen ciliates inevitably ingest both host-secreted and bacterially-secreted enzymes, as well as starch and

cellulose-digesting bacteria, and that these probably continue their activities inside the endoplasmic sac.

In a very careful series of experiments, in which the growth and progress of a series of young domestic ruminants heavily infected with Ophryoscolecidae was compared with the growth and progress of a parallel series of azoic animals, Becker and his collaborators (1929) were able to show conclusively that, despite these facts, the infusoria in the rumen conferred no benefit whatsoever on the host, but, on the other hand, neither did they appear to harm it in any way. Hungate (1943) reached the same conclusion by a different experimental approach. In Perissodactyla the situation is not quite parallel, since it is difficult to imagine that ciliates living in the colon and caecum can be digested as animal protein, although they may play some part in accelerating bacterial fermentation of cellulose by breaking up the vegetable particles into smaller fragments. On the other hand, the ciliates occurring in the caecum and colon of the horse apparently have no deleterious effect on the host. List (*vide* Becker, 1932) has even suggested that the ciliates benefit their host by ingesting pathogenic bacteria. Hence it may be fairly assumed that the rhinoceros also supports *Entodinium insolitum* and *Triplumaria hamertoni* without detriment to itself.

DIAGNOSIS.

Form and Proportions.—Body of approximately oblong outline in side view, 1.21 to 1.81 dorsoventral diameters in length, compressed laterally to 0.69 to 0.85 dorsoventral diameters with dorsolateral concavities anteriorly. Anterior end sharply truncated, posterior end smoothly rounded, with fleshy caudal lobe 0.05 to 0.17 dorsoventral diameters in length. In the forma *uncinatum* the caudal lobe is pointed and hook-like.

Size.—

Length	43 μ to 68 μ (mean 52 μ).
Transdiameter	22 μ to 35 μ (mean 31 μ).
Dorsoventral diameter . . .	27 μ to 45 μ (mean 35 μ).
Caudal lobe	2 μ to 7 μ (mean 4 μ).

Digestive Apparatus.—Oral apparatus with about twenty membranellæ; neuromotorium and circular fibril with membranellar rootlets. Elongate funnel-shaped oesophagus leading obliquely posteriorly and dorsad, with conspicuous longitudinal fibrils. Endoplasmic sac almost rectangular in outline in side view, distensible, with “diverticulum posterius.” Thin-walled rectum in the form of a transverse slit, surrounded by five elliptical myonemes, of which the most anterior is thicker than the rest.

Macronucleus.—Massive, densely packed with polygonal chromatin granules of variable size and form. Generally elliptical, but may be spherical, sausage-shaped or wedge-shaped, varying from 0.21 to 0.85 dorsoventral diameters in length (mean 0.42). Size 8 μ –29 μ (mean 14 μ).

Micronucleus.—Homogeneous, elliptical or spherical, lying close against left ventral border of macronucleus near its anterior end.

Contractile Vacuole.—Spherical when partly expanded, lenticular when fully expanded, lying directly anterior to macronucleus in dorsal mid-line. With anterodorsal funnel-shaped excretory canal leading to dorsal excretory pore.

Food.—Plant débris and bacteria.

Habitat.—Alimentary canal of the Indian rhinoceros.

Locality.—Unknown: possibly India.

SUMMARY.

(1) An account is given of the structural features of a new ciliate, *Entodinium insolitum*, sp. n., from the alimentary canal of the Indian rhinoceros (*Rhinoceros unicornis*). It is represented by two forms, namely,

E. insolitum forma *insolitum* and *E. insolitum* forma *uncinatum*. The material was obtained from a diarrhoeic stool, but it is considered to be improbable that the presence of the ciliate was in any way responsible for the condition of the host.

(2) The new ciliate is unique in being the first species of *Entodinium* to be recorded from the Perissodactyla. The possibility of cross-infection of the rhinoceros from some other ungulate is discussed and dismissed.

(3) The site of infection is not known, but is probably the colon or cæcum.

(4) The resemblance and possible relationship of *E. insolitum* are discussed.

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