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UNIVERSITY OF CALIFORNIA
ON BALANTIDIUM COLI (MALMSTEN) AND
BALANTIDIUM SUIS (SP. NOV.), WITH
AN ACCOUNT OF THEIR NEURO-
MOTOR APPARATUS

A THESIS ACCEPTED IN PARTIAL SATISFACTION OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
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BY
JAMES DALEY McDONALD

1922

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May 8, 1922

ON BALANTIDIUM COLI (MALMSTEN) AND
BALANTIDIUM SUIS (SP. NOV.), WITH
AN ACCOUNT OF THEIR NEURO-
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BY

J. DALEY McDONALD

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BALANTIDIUM SU

AN ACCOUNT OF THEIR NEURO-

MOTOR APPARATUS

J. DALEY McDONALD

by

J. Daley McDonald

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Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

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UNIVERSITY OF CALIFORNIA

ON BALANTHINE GOLF (Balanthinidae) AND BALANTHINIDAE
WITH AN ACCOUNT OF THE NEUROTONIC REACTION

BY
A. DELIST-MCDONALD

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

Approved: W. H. Murray, President, University of California
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1931
Charles V. Dreyer
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ON *BALANTIDIUM COLI* (MALMSTEN) AND
BALANTIDIUM SUIS (SP. NOV.), WITH
 AN ACCOUNT OF THEIR NEURO-
 MOTOR APPARATUS

BY

J. DALEY McDONALD

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INTRODUCTION

The earliest observation of Protozoa of the genus *Balantidium* has in several instances been accredited to Antony von Leeuwenhoek (1708). During an attack of dysentery he detected motile organisms in the discharges. At that time no discrimination had been made between ciliated and flagellated protozoa and his account of his observations is not sufficiently complete to make possible the classification of the organisms which he found. However, he stated that they were about the size of red blood corpuscles, which would indicate that they were intestinal flagellates and not *Balantidium*, which is very much larger.

Malmsten (1857) was the first to describe *Balantidium coli*. This species has become better known than the other species of the genus, due to its being the cause of a specific dysentery known as balantidiasis. Two persons suffering from this disease came to Malmsten for medical attention during 1856-57. He was assisted in the study of protozoans which he found in the excreta from these two patients by the zoologist Loven who believed that the parasites were new to science and so prepared a careful description of them accompanied by figures. For the organism they suggested the name *Paramoecium* (?) *coli*. Since that time infections with *Balantidium coli* have been reported in increasing numbers and some cytological studies have been made, though much more attention has been given to the problems of prophylaxis and treatment of the disease which this species causes than to the parasite itself.

The first record of *Balantidium coli* as a parasite of pigs was made by Leuckart (1861). Stein (1862) also studied these forms from pigs and he was the first to assign them to the genus *Balantidium*. The genus had been established by Claparède and Lachmann (1858) with *Balantidium entozoön* from the frog as the type species. More recently Strong (1904), Brumpt (1909), Walker (1913), and others have carried on investigations on this parasite of pigs in order to become acquainted with the problems involved in the infection of man.

ACKNOWLEDGMENTS

It has been my privilege to study the morphology of *Balantidium coli* and *Balantidium suis* (sp. nov.) under the direction of Professor Charles A. Kofoed, to whom I am indebted for helpful suggestions and for oversight of the entire work. Acknowledgment is due Professor William W. Cort for many valuable criticisms. I also take this opportunity to express my appreciation of the courtesy of Mr. R. B. Brown, superintendent of the Oakland Meat and Packing Company, who kindly granted me permission to work in the company's *abattoir* and also facilitated the work in every possible way.

MATERIAL AND TECHNIQUE

The material for these studies was obtained almost exclusively from pigs killed by the Oakland Meat and Packing Company, Stockyards, California. At their *abattoir* I was permitted to work in the room where the pigs were dressed, which made it possible to obtain the material from the intestine before it had cooled below the normal body temperature. To determine the presence of the balantidia a small slit was made in the caecum and a drop of the contents withdrawn with a pipette. This drop was quickly placed on a warm slide and examined with a microscope. If the animals were present they would be detected very readily for they are exceedingly active; in most cases they occurred in numbers sufficiently large that from one to ten could be seen in every field when a 16 mm. objective was used. This method was rapid enough to allow all pigs to be examined as fast as they were killed and dressed.

A sample from the caecum was not relied upon as critical in the determination of infection until examination of the entire length of the intestine had been made in several instances. In order to discover the normal distribution throughout the intestine it was removed entire and taken to the laboratory of the *abattoir*. Incisions were made every one or two feet, beginning with the duodenum and continuing to the rectum, and samples examined from each of these incisions. In no cases were balantidia found more than three feet above the ileocaecal valve, and only in two or three instances were any at all present in

the small intestine. In the caecum and first three or four feet of the colon the balantidia were always more active and more numerous than elsewhere. Posteriorly from this region they were found in progressive stages of encystment until in the rectum the majority were completely encysted.

OCCURRENCE AND GEOGRAPHIC DISTRIBUTION

Approximately 200 pigs were examined. They had been raised in the Sacramento Valley, except for one lot from Los Banos, California, and a lot from the state of Nevada. Of the 200 pigs examined 68 per cent were infected. The examinations were made at nine separate times between September, 1913, and May, 1918, ten to sixty individuals being examined each time. In five of the nine lots every pig was found to be infected. The lowest percentage of infection was 13 per cent, in the lot shipped from Nevada. This indicates a very general infection of pigs with *Balantidium* in this region of the United States.

Stiles (cit. Strong, 1904), Bel and Couret (1910), and others have previously found the organisms in pigs in the United States. Leuckart (1861), working in Germany, was the first to find *Balantidium* in pigs. Since then Stein (1862), Ekecrantz (1869), and Prowazek (1913) have reported them from the same country. In 1871 Wising noted their occurrence in pigs in Sweden. Grassi (1882) and Calandruccio (1888) have found the parasites in swine in Italy. Rapchevski (1882) reported the occurrence of balantidia in Russia. In France they have been found in pigs by Railliet (1886), Neumann (1888), and Brumpt (1909). Strong (1904), Walker (1913), and several others have noted the occurrence in pigs in the Philippine Islands. Similar reports from China have been made by Maxwell (1912), and Mason (1919); from Cuba by Taboadela (1911); and from South America by Bayana (1918). These citations indicate that *Balantidium coli* is probably as widely distributed geographically as is its host, the pig.

STUDIES OF LIVING ORGANISMS

The balantidia are very sensitive to changes of temperature. When the medium in which they are swimming is cooled a few degrees they slow up their movements very decidedly. After a time they become almost perfectly spherical, in which form their activity is restricted to a rotary motion with little or no progression. In this condition they will live for six or eight hours at ordinary room temperature.

In order to avoid the deleterious effects caused by cooling and by increased bacterial action, most of the studies on living organisms were made at the *abattoir*. When continuous observation over a long period was desired, however, the material was conveyed to the laboratory in thermos bottles and kept in the incubator at 37.5° C. In this manner material could be kept for three days. Ultimate degeneration of the organisms seemed to be due more to the increase in the bacterial content of the medium than to any other cause. During observation either an electric warm stage or the microscope warm oven designed by Long (1912) was employed. Of the several vital stains used, neutral red proved most satisfactory in the differentiation of the neuromotor apparatus.

Fixation and staining.—The following fixatives were used: Schaudinn's fluid, Zenker's fluid, formalin, osmic acid, and picromercuric fluid (according to the formula by A. D. Drew, used by Yocom, 1912). Quick action was one of the most important factors in the fixation, and was usually obtained by having the killing fluid hot (60–80° C.) and using an amount at least equal to the amount of the material to be fixed. Frequently the action was so nearly instantaneous that the cilia on the killed animals retained their exact relative position (see fig. N). After fixation the material was thoroughly washed, iodine alcohol being used if mercurial salts were present. Material was preserved in 70 per cent alcohol.

Before staining, the preserved material was usually concentrated by elimination of lighter *débris* by centrifuging and the heavier by sedimentation. Water was found to be a more satisfactory medium for these operations than either alcohol or salt solutions. In case sections were to be made, additional care was taken in the concentration process and then the material was handled according to the methods employed by Metcalf (1909) and by Sharp (1914).

Iron haematoxylin gave uniformly the best results in staining. For cysts, however, on account of their imperviousness, it was necessary to use Delafield's haematoxylin to which had been added a small amount of acetic acid. In addition to the first mentioned stain, Mallory's connective tissue stain was used on sections.

SYSTEMATIC POSITION OF GENUS AND SPECIES

Claparède and Lachmann (1858) removed *Bursaria entozoön* from the genus in which it had been placed by Ehrenberg (1838) and created for it the new genus *Balantidium*. This genus was of the family Bursaridae and the order Heterotricha. The twenty-two species of the genus that have been described to date are listed below.

KNOWN SPECIES OF THE GENUS BALANTIDIUM

Species	Original description by	Hosts
<i>Balantidium entozoön</i>	Ehrenberg, 1838	<i>Rana esculenta</i> <i>Rana temporaria</i>
<i>Balantidium coli</i>	Malmsten, 1857	<i>Sus scrofa</i> <i>Homo sapiens</i>
<i>Balantidium duodeni</i>	Stein, 1862	<i>Rana esculenta</i>
<i>Balantidium elongatum</i>	Stein, 1862	<i>Triton cristatus</i> <i>Triton alpestris</i> <i>Triton marmoratus</i> <i>Rana esculenta</i> <i>Rana temporaria</i>
<i>Balantidium medusarum</i>	Mereschkowsky, 1879	<i>Bougainvillea</i> , <i>Obelia</i> , <i>Eucopa</i> , <i>Broda</i> sp.?
<i>Balantidium amphitenides</i>	Entz, Sr., 1888	<i>Amphitenis</i> , <i>Turbellaria marina</i>
<i>Balantidium gyrans</i>	Kellicott, 1889	Aquatic worm
<i>Balantidium viride</i>	Willach, 1893	<i>Columba</i> sp.?
<i>Balantidium minutum</i>	Schaudinn, 1899	<i>Homo sapiens</i>
<i>Balantidium giganteum</i>	Bezenberger, 1903	<i>Rana esculenta</i>
<i>Balantidium helenae</i>	Bezenberger, 1903	<i>Rana cyanophlyctis</i> <i>Rana tigrina</i> <i>Rana limnocharis</i> <i>Rana hexadactyla</i>
<i>Balantidium gracile</i>	Bezenberger, 1903	<i>Rana cyanophlyctis</i> <i>Rana hexadactyla</i>
<i>Balantidium rotundum</i>	Bezenberger, 1903	<i>Rana esculenta</i>
<i>Balantidium falciformis</i>	Walker, 1909	<i>Rana palustris</i>
<i>Balantidium ovale</i>	Dobell, 1910	<i>Rana tigrina</i>
<i>Balantidium hyalinum</i>	Dobell, 1910	<i>Rana tigrina</i>
<i>Balantidium littorinae</i>	Chagas, 1911	<i>Littorina</i>
<i>Balantidium testudinis</i>	Chagas, 1911	<i>Testudo graeca</i>
<i>Balantidium hydrae</i>	Entz, Jr., 1913	<i>Hydra olygaetis</i>
<i>Balantidium piscicola</i>	Entz, Jr., 1913	<i>Piaretus brachypomus</i>
<i>Balantidium caviae</i>	Neiva <i>et al.</i> , 1914	<i>Cavia aperea</i>
<i>Balantidium orchestia</i>	Watson, 1916	<i>Orchestia agilis</i> <i>Talorchestia longicornis</i>

The wide diversity of hosts, ranging from hydroids and crustaceans to the warm-blooded vertebrates, including man, must demand a wide versatility on the part of the parasite. Considerable structural variation is apparent even on cursory examination, and some of these structural differences might be sufficiently marked to serve for generic differentiation. A new generic division would seem desirable, but the suggestions of Bütschli (1884) and Schweier (1900) in this direction have not been generally accepted.

BALANTIDIUM COLI MALMSTEN (1857)

SYNONOMY:

Paramoecium (?) *coli* Malmsten, 1857.

Plagiotoma coli, Claparède and Lachmann, 1858.

Leucophyra coli, Stein, 1860.

Holophyra coli, Leuckart, 1861.

Balantidium coli, Stein, 1862.

Up to the present time only one species, *Balantidium coli*, has been described as parasitic in pigs. It was first described by Malmsten (1857) who, noting its likeness to *Paramoecium colpoda* (Ehrenberg), suggested the name *Paramoecium* (?) *coli*. During the following year, Claparède and Lachmann (1858) reproduced one of Malmsten's original figures and after considering his description transferred the species to the genus *Plagiotoma*. In 1860, Stein, using the description by Malmsten (1857), pointed out that the organism was not a *Paramoecium* and believed that it properly belonged in the genus *Leucophyra*. Leuckart in 1861 discovered a ciliate in the intestine of pigs which he concluded was identical with the one already described, but he was not satisfied with the genus to which it had been assigned by Stein (1861) and believed that its closest relation was with *Holophyra* in which genus it should be placed. In 1863 he still retained this view but suggested the appropriateness of the establishment of a new genus. But Stein (1862) had already recognized those characters of the species which showed its close relation to *Balantidium entozoön* and had placed it in the genus *Balantidium*.

During the present investigation the following specific characteristics have been found very constant. The individuals of the species *Balantidium coli* are ovoid in form, the more pointed end being anterior; length varies from 30μ to 150μ ; breadth varies from 25μ to 120μ ; in the majority of individuals the length is 1.3 times the breadth; the greatest transverse diameter intersects the longitudinal axis poster-

ior to its midpoint; the adoral zone is approximately terminal, and the anterior tip of the body lies within it; the plane of demarcation between the apical cone of the ectoplasm and the endoplasm is approximately at right angles to the long axis of the body; the macronucleus is elongate but the length usually does not exceed three times the breadth; two contractile vacuoles are present, a smaller one located anteriorly and a larger one located posteriorly; a posterior cytopyge is usually distinctly visible.

BALANTIDIUM SUI SP. NOV.

Early in the work of examining pigs for *Balantidium coli* it became evident that this protozoan showed extreme variation in shape. In many instances the diversity occurred among individuals from the same host. Further observations led me to believe that there were two fairly distinct types, the one, longer and more slender as compared with the other which was distinctly ovoid. Measurements have been recorded by various writers of *Balantidium coli* from man. Malmsten (1857) in the original description gave the length as 60–100 μ ; breadth, 50–70 μ . Solojew (1901) recorded the length as 65 μ , the breadth as 40 μ . Wising (1871) states that the length varies from 50–100 μ , while the breadth varies from 40–50 μ . Prowazek (1913) gave the length as 52–71 μ , the breadth, 40–58 μ . Leuckart (1861) measured balantidia from swine and found the length to be 75–110 μ and the breadth 70 μ . Still others give dimensions, but all are inadequate for the determination of the occurrence of types with distinct proportions. First, with one or two exceptions all dimensions have been taken of balantidia found in man, and from these it might not be safe to draw conclusions regarding diversity among those found in pigs. In the second place, the measurements given are either averages or else represent extreme limits. In either case they are practically useless in determining individual variations, for even in the case of extreme types the range between limits is so great that two, or even more, distinct types, based on proportions of breadth to length, might be included. Nowhere has there been found a series of individual measurements which would make it possible to determine whether variations were continuous or discontinuous. To obtain such a series of measurements was the purpose of the phase of the work about to be described.

Material which was to be used in taking the measurements was killed and preserved with all possible care. Hot Schaudinn's fluid was used in all cases, the material being quickly and thoroughly mixed

into a large quantity of it so that action would be as nearly instantaneous as possible, thus avoiding distortion. Osmic acid vapor was tried but Schaudinn's fluid gave equally good results and was more convenient for manipulation. Material was never allowed to cool before fixing, for on cooling the individuals tend to become spherical. Several attempts were made to measure living organisms but their ceaseless activity at normal temperature (37.5° C) made this almost impossible and slowing them up by the use of Irish moss or by cooling, as mentioned above, caused them to become distorted. If there were changes due to fixation, the logical expectation would be that the error would be on the side of conservatism for such changes would tend to obliterate rather than accentuate the division into two groups; for the shape of the elongate forms would be more changed by the fixative, the tendency being for them to shorten and broaden and thus approach the ovoid type. However, in the method of fixation used, I am sure that distortion was so slight as to be negligible.

Following fixation the material was carefully washed and carried slowly through the lower grades of alcohol to 70 per cent in which the material was kept for measuring. A drop of the material from which measurements were to be taken was placed on a slide, covered with a coverglass, the excess of fluid removed, and the edges sealed with vaseline to prevent evaporation. Just enough fluid was removed from under the coverglass to reduce the depth of the medium so that the majority of the animals would lie flat, and yet not enough to allow the coverglass to exert any pressure. The exertion of pressure on the animals, however, would ordinarily be prevented by the presence of large particles of foreign material. The object of having animals lie flat on the slide was to avoid the error which would otherwise be caused by foreshortening. A slight elevation of one end would make considerable error in the determination of the length of the animal.

The slide was then placed on the microscope and systematically examined by the use of the mechanical stage. Beginning at the upper left-hand corner and progressing as one would in reading a book, every individual encountered in the survey was measured. The only exceptions made were in case the animal was not lying flat or showed marked signs of distortion. This procedure avoided selection which might unconsciously be made by the observer. For making the measurements a 4 mm. objective was used in combination with an ocular-micrometer inserted in 9x compensating ocular. With the magnification given by this combination the limit of error did not exceed one micron.

The longitudinal axis and the longest transverse axis of each individual were measured, and the ratio of length to breadth computed (see Table I).

TABLE I

COMPARATIVE MEASUREMENTS OF ONE HUNDRED INDIVIDUALS EACH OF
BALANTIDIUM COLI AND BALANTIDIUM SUIS

INDIVIDUALS FROM PIG No. 1 (<i>Bal. suis</i> , with five exceptions)			INDIVIDUALS FROM PIG No. 4 (<i>Bal. coli</i> , with one exception)		
Length in microns	Breadth in microns	Ratio of length to breadth	Length in microns	Breadth in microns	Ratio of length to breadth
114	42	2.71	99	75	1.32
108	51	2.11	96	81	1.19
63	36	1.75	126	75	1.68*
90	45	2.00	118	87	1.36
93	48	1.94	111	87	1.28
72	39	1.85	87	75	1.16
126	69	1.83	90	66	1.36
87	48	1.82	105	75	1.40
108	57	1.90	78	66	1.18
102	54	1.89	90	63	1.43
117	51	2.30	87	72	1.21
120	57	2.10	87	72	1.21
96	42	2.29	99	75	1.32
84	39	2.12	105	75	1.40
120	48	2.50	89	75	1.19
117	48	2.42	81	63	1.28
93	51	1.83	81	66	1.23
96	51	1.89	75	63	1.19
72	57	1.26*	84	69	1.22
75	54	1.39*	69	60	1.15
111	51	2.14	90	66	1.36
111	54	2.03	93	75	1.23
84	36	2.31	87	69	1.26
69	39	1.77	90	81	1.11
78	51	1.52*	105	90	1.16
81	45	1.80	87	69	1.26
111	45	2.42	84	72	1.17
81	45	1.80	66	58	1.14
84	45	1.86	75	60	1.25
90	45	2.00	81	63	1.29
84	42	2.00	81	60	1.35
90	42	2.12	99	78	1.27
117	57	2.03	87	69	1.26
60	33	1.82	78	63	1.24
108	57	1.90	81	60	1.35
108	57	1.90	105	75	1.40
96	48	2.00	90	60	1.50
75	42	1.79	93	78	1.18

* Other characters showed that these individuals were of the other species represented in the table.

TABLE I—(Continued)

INDIVIDUALS FROM PIG No. 1 (<i>Bal. suis</i> , with five exceptions)			INDIVIDUALS FROM PIG No. 4 (<i>Bal. coli</i> , with one exception)		
Length in microns	Breadth in microns	Ratio of length to breadth	Length in microns	Breadth in microns	Ratio of length to breadth
105	57	1.85	69	60	1.15
99	57	1.74	81	54	1.50
105	54	1.95	87	66	1.32
54	27	2.00	84	69	1.22
57	30	1.90	78	60	1.30
36	24	1.50*	66	51	1.29
81	31	2.23	66	54	1.22
75	42	1.78	96	66	1.45
66	33	2.00	81	54	1.50
78	36	2.08	84	66	1.28
93	39	2.39	114	87	1.31
63	36	1.74	87	69	1.26
102	48	2.06	93	72	1.29
78	39	2.00	84	72	1.17
81	42	1.93	102	65	1.58
87	48	1.82	87	63	1.38
93	45	2.03	99	66	1.50
84	42	2.00	81	72	1.13
81	39	2.04	78	63	1.24
75	39	1.93	102	75	1.36
78	45	1.74	66	48	1.38
75	37	2.01	75	57	1.32
100	45	2.11	84	66	1.27
81	39	2.04	93	66	1.40
72	39	1.85	72	58	1.24
84	40	2.06	96	72	1.33
90	42	2.07	90	68	1.32
78	36	2.08	60	39	1.54
102	48	2.06	87	69	1.26
81	37	2.10	69	54	1.28
66	37	1.78	90	69	1.31
87	36	2.41	69	54	1.28
78	42	1.86	72	60	1.20
84	42	2.00	75	57	1.47
90	38	2.37	75	62	1.21
66	35	1.89	72	63	1.14
78	42	1.86	90	75	1.20
97	44	2.20	74	66	1.12
79	37	2.07	72	54	1.33
98	42	2.32	60	50	1.20
90	45	2.00	84	58	1.45
87	49	1.78	84	64	1.31
90	36	2.50	88	60	1.47
81	35	2.31	99	75	1.32
69	48	1.44*	75	54	1.39

* Other characters showed that these individuals were of the other species represented in the table.

TABLE I—(Continued)

INDIVIDUALS FROM PIG No. 1 (<i>Bal. suis</i> , with five exceptions)			INDIVIDUALS FROM PIG No. 4 (<i>Bal. coli</i> , with one exception)		
Length in microns	Breadth in microns	Ratio of length to breadth	Length in microns	Breadth in microns	Ratio of length to breadth
90	54	1.68	81	69	1.18
66	33	2.00	66	52	1.27
76	38	2.00	93	63	1.48
78	42	1.86	81	75	1.08
90	57	1.58	96	75	1.28
66	40	1.65	114	90	1.27
81	39	2.04	75	60	1.25
96	39	2.43	87	72	1.21
75	38	1.98	84	60	1.40
84	39	2.18	87	63	1.38
51	30	1.70	84	63	1.33
75	39	1.92	72	60	1.20
84	40	2.05	90	66	1.36
116	47	2.48	84	72	1.16
84	36	2.32	93	72	1.29
81	39	2.04	87	58	1.50
75	45	1.68	93	72	1.29
Average	86	1.99	86	66	1.30

While taking the measurements of each individual, observations were made regarding the position of the mouth, the type and size of macronucleus, number and location of contractile vacuoles, and any other characters which might aid in differentiation.

In the handling of the data on dimensions I have followed in a general way the method used by Jennings (1908) in differentiating races of *Paramecium*. For the purpose of this work, however, the results seemed more lucid if, instead of plotting length and breadth along separate axes, the ratio of length to breadth was computed for each individual and if these ratios were then plotted on the abscissa while the numbers of individuals having each of these ratios were plotted on the ordinate. In computing the ratios the quotient was carried to the second decimal place. But in the construction of the curves only intervals of tenths (or first decimal place) were used; thus, for example, all ratios occurring between 1.25 and 1.34 inclusive were grouped as if they were 1.3. This had two advantages: first, it produced a smoother, steeper curve than would result if smaller intervals were taken, using the same number of individuals measured, and emphasized group rather than individual variations. Second, this grouping reduced any error which might result from the observer showing a preference for one graduation of the micrometer when

an individual measured more than one but less than another whole division of the scale; e.g., such a preference might result in the tabulation of several individuals having a length of 72μ , and of none with a length of 71μ , though in reality all lay within these two limits and as many were as near to one as to the other.

As previously mentioned, the graduations along the ordinate represent the number of individuals, each small interval representing one individual. In Jennings' (1908) work these intervals represent percentages of the total number of individuals. But it happens that

Fig. A. Graphic representation of the variation in the ratio of length to breadth among 200 *Balantidium* chosen at random from samples of material taken from several different pigs. The number of individuals is measured on the ordinate, the ratios on the abscissa. The dotted line is the curve resulting from the combination of the two curves shown in figure B, superimposed here to facilitate comparison.

in the graphs shown in figures B and C, the number of individuals showing a certain ratio is identical with the percentage of the total, for in these cases the total is 100 individuals.

Figure A represents graphically the result of the first attempt to determine the existence of different types. Measurements were made of 200 individuals. At least ten slides were used in getting these measurements and they were prepared from samples taken from nearly as many different pigs. It will be noted that the curve produced by plotting the ratios of these individuals is decidedly bimodal. One mode represents those individuals which are approximately 1.2 times as long as wide, while the other represents those which are 1.6 to 1.8 times as long as wide.

These findings seemed to fully justify my early suspicions that there were two very different types of balantidia parasitic in pigs.

But it was decided to conduct one more experiment, for corroboration, under slightly different conditions and with especial care in fixation of the material. It had been noted that though often both types occurred in the same pig (which was the case in most of the samples used in the first measurements), still one type might be greatly in excess, or there might be only one type present. In getting material

Fig. B. Graphic representation of the difference in ratio of length to breadth between two carefully selected lots, of 100 individuals each, of *Balantidium* from separate hosts. The continuous line represents those from one pig (nearly all are *Balantidium coli*); the broken line, those from the other pig (nearly pure infection with *Balantidium suis*).

Fig. C. This graph shows the variation in the ratio of length to breadth among 100 *Balantidium* secured from a case of balantidiasis in man.

for this second set of measurements, it seemed best to take it from pigs which had, as nearly as could be determined, pure infections of the respective types. Fortunately these requirements were fulfilled in the next lot of pigs examined. Both samples of material, the one containing the ovoid and the one containing the elongate type, were treated in exactly the same way. They were killed at the same time

with the same fluid (in separate containers) and in the same water bath. Thence to 70 per cent alcohol the treatment continued identical. One hundred individuals from each sample of material were measured.

From these measurements the graphs shown in figure B were constructed in the same manner as the previous one, except that the curves of the separate samples were plotted separately on the same axis. The continuous line represents the individuals from one pig and the broken line those from the other. The mode of the first curve occurs at 1.3. If the ratios 1.2 and 1.4 be included with 1.3, it is found that 80 per cent came within these limits. Of the entire number of individuals in the lot only one showed a ratio of 1.6 and one as high as 1.7, while there were none with a higher ratio. The second curve reaches its highest point at 2.0, while 71 per cent of the entire number measured is included between 1.8 and 2.2. A number of individuals have ratios above 2.2, while one had a ratio as great as 2.7. Five individuals have ratios below 1.6, at which point the curves begin to overlap; but in practically every one of these individuals there were observed characters (which are discussed below) that made it quite evident that they were really of the type represented by the other curve.

Upon comparing figures A and B their likeness is very striking, the second being corroborative of the results shown by the first. That both are bimodal is evident. However, the low points, the point of demarcation of the two groups, do not occur at the same place; in the first it is at 1.4, while in the second it occurs at 1.6. Also the median of the first mode in figure A occurs at 1.2 while in figure B it is at 1.3, and the median of the second mode in figure A is at 1.7 while in figure B it occurs at 2.0; that is, in figure A the entire curve is shifted to the left, meaning that all ratios are decreased or that all individuals approach nearer to the spherical shape. This shifting is greatest in the case of the second mode. This in conjunction with the greatest breadth of the second curve in figure B is what would be expected if the premise in regard to the effect of fixation discussed above (page 251) is correct. Extra care was used in the fixation of the latter lot of material whereas in the former only the ordinary precautions were taken. At any rate these differences between the two groups do not detract from the evidence which they offer that there are two distinct types of balantidia parasitic in pigs.

The value of these curves in showing race or species differentiation is directly proportional to the extent to which any other factors which

might produce a bimodal curve are non-operative. Factors involved in faulty technique were eliminated as far as possible. Over the effect of growth or age variation, however, the observer has no control. The possibility of these variations producing such curves as the one above is precluded by reference to the data from which the curves are constructed. Among the individual measurements recorded in Table I it will be noted that there are small individuals measuring $27 \times 50\mu$, and others measuring $42 \times 50\mu$; and that among the larger individuals, some measure $50 \times 120\mu$, and some, $90 \times 150\mu$. Further study of Table I shows that the two types are found among *all* sizes of individuals; consequently the variation of body proportions represented by the graphs is not correlated with variations of size, and probably not with the age or growth of the individuals.

That the variation could be accounted for by the occurrence of fission seems unlikely. Individuals might continue to elongate until binary fission occurred, and then by this process they might be shortened and the body proportion changed. Two considerations oppose this explanation. In figure A nearly equal numbers are of the respective types; in figure B each example contained almost exclusively one type of individual. In the former material very few dividing individuals were found, while in the latter not a single individual was seen in fission in either sample of material. But to accord with the above explanation one would expect to find many dividing forms among the elongate individuals represented by the broken line. In the second place, if this explanation were valid one would expect curves showing variation of body proportions to be continuous. Such is not the case, as is shown in figures A and B where the curve is bimodal due to a decided decrease of individuals having proportions intermediate between the two types.

The possibility of any effect from gametic variation, was eliminated by the study of conjugating forms, through which it was determined that isogamy was the rule. Likewise the possibility of influence of the quality of intestinal content of the host was eliminated by the frequent occurrence of both types in the same host. Other factors it would seem must be of minor importance and should give way for more positively corroborative evidence which may be found in correlated morphological difference.

Other specific characters.—In addition to the differences in relative lengths of the axes, one notes a distinct difference in the points of intersection, due to the variation in the shape of the types as pictured

in figures D, E, F, G, and H. The one form resembles very closely a hen's egg, the small end being anterior. In this case the longest diameter crosses posterior to the midpoint of the longitudinal axis. In the elongate type, usually the posterior end is as much drawn to

Figs. D-H. Camera lucida drawings of *Balantidium suis* sp. nov. (figs. D and E), and *Balantidium coli* (figs. F, G, and H), showing specific differences.

a point as is the anterior, and often more so. In these cases the longest diameter intersects the longitudinal axis at or anterior to its midpoint.

Coincident with the taking of measurements a careful search was made to detect other morphological differences which might occur between the two forms and be of aid in distinguishing one from the other. The earliest difference to be noted related to the macronucleus. The macronucleus in the ovoid type is relatively short, being approximately $\frac{1}{3}$ the length of the entire organism. It is customarily bean-shaped in appearance, but may be almost straight or so sharply bent

at its middle as to form a short V (fig. I), and its width averages about 0.4 to 0.5 of its length. In the slender types the macronucleus is relatively long and slender, being approximately $\frac{1}{2}$ of the entire length of the organism. It is ordinarily sausage-shaped, but it may also be in the form of a straight rod slightly enlarged toward the ends, or it may be so curved as to form an almost complete ring. In contrast to the form described above, the width in this case is about 0.2 to 0.3 of its length. Differences so great as these, viz., a length 2 to 3 times the breadth in one case and 4 to 5 times the breadth in the other are easily recognizable without actual measurement. These figures represent the average and do not mean that the limits of the two never overlap. This difference in nuclei serves as one of the easiest and surest ways of distinguishing the two types, for though the organism during locomotion may modify its proportions tremendously this does not noticeably affect the nucleus. It has been impossible to determine any difference between the micronuclei of the two types.

A very noticeable difference concerns the relative position of the cytostome. In the ovoid type the cytostome is almost, though never quite, terminal (see figs. F, G, and H). As mentioned previously, the anterior end is ordinarily drawn out to form a fairly decided point which lies within the area enclosed by the adoral cilia. In the slender type the cytostome is more laterally placed. The posterior limit of the right lip of the cytostome may extend ventrally to a point $\frac{1}{5}$ of the length of the animal, in which case the dorsal portion of the adoral circle of cilia may pass approximately through the terminal point of the body, but in normal form this point will never be within the adoral area. The parts which make up the adoral region of the animals show no fundamental differences except variation in the relative position of parts due to the lateral displacement of the cytostome. For example, the plane of demarcation between ectoplasm and endoplasm, which is approximately vertical to the long axis of the animal in the ovoid form, is at a decided angle, the ventral edge lying farther posterior in the elongate forms (see figs. D and E).

In the opinion of some authors the ventral displacement of the mouth is very significant (Delage and Hérouard, 1896; Minchin, 1912). These authors believe that the ventral displacement of the cytostome is progressive with evolution of the organism; i.e., that in the more primitive types the cytostome is terminal while in advanced groups it is successively displaced farther ventrally. Viewed in this light, the position of the mouth is here of considerable significance as

a specific character. Attempts to discriminate between the two forms on the basis of ctyopyge or vacuoles were without result.

The specific differences just discussed have seemed to indicate a sufficient degree of separation of the two types to warrant the division of the ciliates of the genus *Balantidium* which occur in the pig into two distinct species. The description by Malmsten (1857), in conjunction with the figures (Malmsten, pl. 1, figs. 1-6) which he published, make it practically certain that the ovoid type is the one originally described by him and to which he gave the name *Paramoecium* (?) *coli*.

So far as I have been able to determine, the elongate species above described has never before been distinguished from *Balantidium coli*. For this new species I suggest the name *Balantidium suis*. As a summary of the specific characters discussed above I give the following description:

Balantidium suis sp. nov.—Body elongate; length approximately twice the breadth and varies from 35 to 120 μ ; breadth from 20 to 60 μ ; usually tapers more posteriorly, is blunter anteriorly, longest diameter transects longitudinal axis anterior to its midpoint; adoral region ventrally placed, cytostome $\frac{1}{5}$ of way posteriorly along ventral surface; nucleus rod or sausage-shaped, at least one-half the length of the entire organism, its width about one-fourth of its length; the species is parasitic in the pig.

The specific name, *Balantidium suis*, seemed fitting since it indicated the common host, *Sus scrofa*. Whether or not this species occurs in man it has not been possible to determine conclusively. A review of published case records of balantidiasis seems to show that it does not, but only a few of these records are accompanied by figures or descriptions of the organisms which are adequate for making positive discrimination. Fortunately, I have been able in two cases to make some direct observations.

Through the kindness of Mr. W. H. Barnes, of the Department of Pathology of the University of California, I was permitted to study sections of the human intestine which he had obtained at an autopsy following a fatal attack of balantidiasis. Imbedded in the serous and subserous layers were numerous balantidia. Measurements to show proportions were of little value under the conditions, for the form of each organism was largely determined by pressure, exerted by surrounding tissues. But from other characters, the type of nucleus especially, it was conclusively determined that the species there present was *Balantidium coli*. No individuals of *Balantidium suis* were found.

Measurements of balantidia as they occur free in the human intestine were made possible through the kindness of Dr. E. L. Walker, of the Hooper Institute of Medical Research, who loaned me several slides which he had prepared while in the Philippine Islands. This material had been stained with haematoxylin. Using the same precautions as in previous work, a total of 100 individuals was measured. The data were handled as before and the resulting graph is shown in figure C. In comparison with previous graphs it will be noted that this graph closely approaches coincidence with the curves representing *Balantidium coli*, for its mode is at 1.3 while the extreme portion of length and breadth is 1.5.

In addition to slides of human material, there was also loaned material from one pig and from one monkey. It is interesting that each showed a pure infection with *Balantidium coli*. The monkey (Monkey No. 10, Table I; Walker, 1913) had been experimentally infected by feeding it cysts from a pig, but not the pig from which the above-mentioned material was taken. Therefore this material yields no evidence regarding the validity of the specific differentiation nor the possibility of *Balantidium suis* becoming established in monkeys or in man.

There is no likelihood of confusing the new species, *Balantidium suis* with *Balantidium minutum* (Schaudinn, 1899). The differences are very marked. The body of the latter is oval, pointed anteriorly, more like *Balantidium coli*. The peristome reaches to the equatorial plane. There is but a single vacuole while there are two in each of the species considered here. The macronucleus is spherical, whereas it is elongate in both *Balantidium coli* and *Balantidium suis*.

MORPHOLOGY

Balantidium coli (Malmsten) and *Balantidium suis* sp. nov. are ciliated protozoans, barely visible to the unaided eye, and are in a general way sac-shape (balantidium, *little bag*). Viewed through the microscope they appear grayish green in color. The homogeneity of the cell contents is broken by the presence of the nuclei, the contractile vacuoles, the food vacuoles, and sometimes by the presence of highly refractile bodies, the paramylum bodies. The entire surface of the body, except that of the oral plug, is covered with fine cilia. The cell contents are retained by a thin transparent pellicle which is protective

in function. The cytoplasm is distinctly differentiated into ectoplasm and endoplasm. The former constitutes a thin layer just underneath the pellicle and in it is situated the basal apparatus of the cilia. The layer of ectoplasm thickens greatly at the anterior end of the animal, to form, as it were, a matrix for the cytostome and its accessory apparatus. Within the ectoplasm, but not set off from it by a sharp line of demarcation, is the endoplasm. In the endoplasm are numerous food inclusions, often present in the form of starch or paramylum bodies. The macronucleus and the micronucleus are also within it, but seem to have no constant position in the cell. The macronucleus is either bean-shaped (as in *Balantidium coli*) or elongate and sausage-shaped (as in *Balantidium suis*). There are two contractile vacuoles, the larger being situated anteriorly and the smaller posteriorly. They lie closely beneath or may be entirely surrounded by ectoplasm, thus belonging really within that layer.

So far as can be determined the animals show no modification with respect to a substratum, yet the lateral and posterior displacement of the cytostome has lead to the designation of that side, toward which displacement occurs, as ventral, and the opposite surface as dorsal. This terminology is very nearly universal in the literature on *Balantidium*, and the correlated terms of right and left are used in the original description of the family Bursaridae (Stein, 1867) and the genus *Balantidium* (Claparède and Lachmann, 1858). The dorsal side may be somewhat more convex than the ventral; this occurs not infrequently in *Balantidium suis*, though, due to the plasticity of the organism, this is by no means constant. No part of the body is differentiated for skeletal purposes. The anal aperture or cytopyge is at the posterior tip and may be present as an actual aperture or only as an extreme thinning of the ectoplasm and pellicle at this point. In connection with it there is usually a rectal vacuole which serves as a storage reservoir for solid waste awaiting extrusion.

ECTOPLASMIC STRUCTURES

Pellicle.—The entire body is covered by an extremely thin but resistant pellicle (*pel.*, figs. I and L). The pellicle seems to be somewhat thickened, as shown by a higher degree of refractibility, along the margin of the lips of the cytostome where it turns in to form the lining of the oesophagus and the groove in which the oral cilia are set; otherwise, it is nowhere noticeably specialized. It shows alternating

Fig. I. *Balantidium coli*. Ventral view showing principal structures. The adoral cilia, with exception of one at either side, are indicated by basal granules only. The adoral membranelles are also represented solely by the basal apparatus. Ectoplasm is shaded, and ciliary rootlets are shown on one side only. $\times 1250$. *ad. cil.*, adoral cilia; *ad. cil. f.*, adoral ciliary fiber; *ad. memb.*, adoral membranelles; *ant. c. v.*, anterior contractile vacuole; *bg.*, basal granule; *bg. ad. cil.*, basal granules of adoral cilia; *cil.*, cilia; *cil. r.*, ciliary rootlet; *cir. oes. f.*, circumoesophageal fiber; *cytpg.*, cytoppyge; *ect.*, ectoplasm; *enl. ad. cil. r.*, enlargement of adoral ciliary rootlet; *end.*, endoplasm; *gr. b.*, granular band of ectoplasm; *long. f.*, longitudinal fibers; *mac.*, macronucleus; *mic.*, micronucleus; *mot.*, motorium; *nuc. memb.*, nuclear membrane; *oes.*, oesophagus; *or. pl.*, oral plug; *or. pl. f.*, oral plug fibers; *pel.*, pellicle; *per.*, margin of peristome; *post. c. v.*, posterior contractile vacuole; *rad. f.*, radial fiber; *ret. v.*, rectal vacuole.

ridges and grooves, the cilia passing through the bottom of the latter, but this condition is not due to longitudinal thickenings in the pellicle itself, but to the fact that it is closely applied to the ectoplasm which is thus furrowed. It can often be separated in "blisters" from the ectoplasm by tannic acid or weak alcohol, and when thus removed it shows regular longitudinal rows of perforations through which the cilia pass out from the ectoplasm. In this condition its transparency is very evident. The pellicle is not extremely impervious. For instance, when the active animals are introduced into normal salt

Fig. J. The neuromotor apparatus of the adoral region, anterior view. $\times 2000$. *ad. cil. f.*, adoral ciliary fiber; *ad. memb.*, adoral membranelles; *bg. ad. cil.*, basal granule of adoral cilia; *cir. oes. f.*, circumoesophageal fiber; *cyst.*, cytostome; *mot.*, motorium; *or. pl.*, oral plug; *or. pl. f.*, oral plug fibers; *per.*, margin of peristome.

solution plasmolysis takes place very quickly, and they present a grotesque appearance as they swim about with several huge depressions in their surfaces due to the shrinkage. *Intra vitam* stains such as neutral red and Janus green also penetrate very quickly. Resistance to pressure and mechanical change, however, is very marked, and is due to its tenacity and flexibility, both of which qualities are shown when the animal forces itself through an opening much smaller than the normal diameter of its body (fig. K) and also by the extreme flattening which it withstands under the increasing pressure of the cover-glass when evaporation of the preparation is allowed. As previously mentioned, these qualities indicate that the pellicle is protective and retentive in function rather than supportive or skeletal.

These organisms show remarkable mobility when observed under conditions as nearly normal as possible. I have attempted to depict something of this plasticity in figure K. As they travel amid the débris in the intestinal contents, which has been removed with them, a tendency to penetrate is much more noticeable than any avoiding reaction. Instead of reversing the ciliary action, backing away and taking a new direction as would paramaecia, the balantidia, when they come in contact with a solid object, rather apply themselves to the surface, round up, and seem to roll along it. After a moment of such slow contortion, they may swim away in a new direction,

Fig. K. Diagrammatic illustration of the plasticity of the organism, resulting in ability to pass through remarkably small openings.

determined by the direction of the anterior end. They avail themselves of the slightest opportunity to force their way through or between any obstacles. The anterior end, especially the thickened ectoplasmic portion, becomes at such times decidedly elongate and conical (fig. K, b). The cilia of this region beat spirally producing a boring action as this anterior tip is protruded into any slight opening. This action has in many instances been observed to cause two obstacles, either of which was larger than the organism itself, to separate sufficiently to allow it to pass between. The aperture need not be one-half of the diameter of the animal for the latter will constrict (fig. K, c) and the fluid contents flow through anteriorly as it progresses, resembling the process of putting a bag of beans through a small hole in a board. Throughout observations of the activity of these organisms, one is impressed with their fitness for penetrating the mucous lining of the intestine and the underlying tissues. Its thigmotropic response, its boring action, and its extreme plasticity, all seem to be adaptations for the function of penetration.

Ectoplasm.—Immediately underneath the pellicle, the cytoplasm is differentiated to form the ectoplasm (*ect.*, fig. I; pl. 27, figs. 1-7). The ectoplasmic layer, except at the anterior end, does not exceed two

microns in thickness. The anterior end of the animal, that is, all anterior to a transverse plane which would transect the body at a point $\frac{1}{6}$ to $\frac{1}{5}$ of the way to the posterior end, is composed entirely of ectoplasm. In this cone-shaped area is located the cytostome and a large part of the neuromotor apparatus. The protoplasm of this region seems to be homogeneously granular in fundamental structure. It stains very deeply with haematoxylin; so deeply in fact, that in differentiation it is necessary to destain other parts of the body almost completely before this part reaches a degree of transparency suitable for study. With Mallory's connective tissue stain this region also stains very densely, taking on both the brilliant red and the deep blue elements of the stain, in different structures, as will be explained below. This extensive thickening of the ectoplasm at the anterior end is clearly shown in the figures by Leuckart (1861) and has been noted by nearly all who have studied the animal more recently, but I have failed to find any discussion of its significance. This same phenomenon occurs in the Ophryoscolecidae, as pointed out by Sharp (1914) in his work on *Diplodinium* and by Braune (1913) in *Ophryoscolex*. In these cases the change seems to be correlated with the high degree of activity and specialization of the anterior end of the animal; in *Diplodinium* in connection with its selective feeding, and in *Balantidium* in connection with both feeding and activity of this entire region in penetrating the mucosa of the intestine. The centering of the neuromotor apparatus in this region gives additional evidence in regard to this question which will be discussed further in connection with the description of that apparatus.

Throughout the entire investigation of the minute structure of this animal, I have been unable to demonstrate the presence of any definite plane of demarcation between endoplasm and ectoplasm, such as the "ectoplasmic boundary layer" described by Sharp (1914) in *Diplodinium*, and shown in plate IV, figure 3 of his paper. Many of the fixed preparations used in the search for such a layer were sections of the animal, treated as nearly as possible according to the technique used by him and stained, as were his preparations, with Mallory's connective tissue stain. So far as it was possible for me to determine, any sharp boundary line between ecto- and endoplasm is lacking. On the contrary, they merge into one another and only in a general way can it be said where one terminates and the other begins.

Prowazek (1913, fig. 2) describes, in *Balantidium coli*, "ein Art von Zwischenmembran" which appears as wavy lines in optical sec-

tion. According to his interpretation this "Querlinie" separates the protoplasm of the cell body into two regions, the "apical zone," which I have described above as a thickening of the ectoplasm, and the rest of the cell protoplasm. The extent of this "Zwischenmembran" he does not note, but his text figures (1 and 2) do not show it as extending quite to the pellicle, but instead as stopping short of the pellicle at a distance about equivalent to the thickness of the ectoplasm at that point. This is significant in my interpretation of this region, namely, that what appears as a continuous line or plane when viewed from the side is in reality, as shown in cross-sections (pl. 27, figs. 6 and 7), a set of diverging fibers. These fibers take origin from dark-staining

Fig. L. Portion of the peripheral region of a cross-section of *Balantidium coli*, showing the structure of the ectoplasm and arrangement of cilia, somewhat diagrammatic. $\times 1500$. *b. g.*, basal granule; *cil.*, body-cilia; *cil. r.*, ciliary rootlet; *end.*, endoplasm; *gr. b.*, granular band of ectoplasm; *hy. b.*, hyaline band of ectoplasm; *pel.*, pellicle.

enlargements on the longitudinal fibers in the wall of the gullet and, diverging, pass peripherally until they turn posteriorly at the very inner edge of the thin layer of ectoplasm which covers the remainder of the body (pl. 28, figs. 9-12). Even in lateral view careful focusing will often show that the apparent "membrane" is really discontinuous, showing breaks and irregularities as one focuses on different levels and hence can not be considered as a true membrane. The arrangement of these fibers will be described more exactly under the discussion of the neuromotor apparatus.

The ectoplasm which constitutes a layer less than 3 microns in thickness around the remainder of the periphery of the cell shows a definite and somewhat complex structure. In tangential sections of the surface, which are so thin that they do not include much of the underlying endoplasm, one detects alternating light and dark longitudinal spiral bands (*gr. b.*, *hy. b.*, fig. N). These bands are parallel to the rows of cilia and very nearly equal in width. In cross-sections (fig. L) they are seen to extend nearly, if not quite the full depth of

the ectoplasm. As one follows these bands (or "stripes," as they are named by Johnson (1893, in his work on *Stentor*) anteriorly they seem to lose their distinctness when they become continuous with the apical cone. In some individuals, however, one can follow them some distance into this cone, but never is there the same degree of differentiation of the two areas in this region.

In the living animals, which are often quite opaque due to inclusions, it is nearly impossible to distinguish these longitudinal light and dark bands. With neutral red the dark or granular band stains faintly. With the haematoxylin stains used in thin sections of fixed material the dark band seemed to be finely granular in fundamental structure. The granularity in this case must be determined largely by the general appearance and stainability, for the individual granules are so small as to defy identification. There is no indication, however, of alveolar structure, so that the term *granular* is probably the more applicable and will be used to distinguish this from the light band. The latter takes only faintly the stains used and seems hyaline in structure. The granular bands lie directly beneath the ridges in the cuticle which occur between the rows of cilia. Or more correctly, the ridges on the surface of the animal are produced by the projection of these granular bands outwardly beyond the hyaline bands. These latter are directly beneath the grooves of the surface where the cilia pass through the cuticle and attach with the basal granules which lie in longitudinal rows; a single row in each hyaline band. The ciliary rootlets (*cil. r.*, figs. I and L) extending in from the basal granules proceed diagonally inward and pass into the interior margin of the granular band.

Stein as early as 1876 pointed out these alternating dark and light stripes in *Stentor*. To the granular and bright stripes, Bütschli (1889) gave the names "Riffenstreifen" and "Zwischenstreifen," respectively. Johnson (1893) gives a careful description of these bands as they occur in *Stentor coeruleus*. He notes that they vary greatly in width, and this is true in *Balantidium*; but both bands are much narrower than in *Stentor*, the combined width of the two not exceeding two microns. In *Stentor coeruleus* Johnson (1893) gives the width of the granular band as 22μ and that of the bright band as 7μ , these measurements being taken just under the adoral zone. It is interesting to note that in *Balantidium coli* the granular band is also slightly wider than the bright band. These bands become narrower from the region of the greatest circumference of the animals toward

each end, which is comparable with the arrangement in *Stentor*, though in the latter the narrowing must necessarily take place in the posterior direction only. The above author mentions the branching of stripes, but this does not occur in *Balantidium* so far as I have been able to determine. As the bands pass posteriorly, however, they become less distinctly differentiated and are hard to follow, and it might be that further study with more intensive stains would reveal a union in the region of convergence at the posterior end. More recent work has added to the number of Heterotricha that show this sort of differentiation of ectoplasm. Maier (1903) shows the striped nature of this layer in *Prorodon* and *Spirostomum*, while Neresheimer (1903) confirms the structure found by Johnson (1893) in *Stentor*. Schuberg (1887) also indicates a comparable plan of structure in *Bursaria*. The granular ridges of ectoplasm between the furrows in which the anal cirri are situated in *Euplotes patella*, discovered by Yocom (1918), may be comparable with the bands which occur in Heterotricha.

Cilia.—The entire surface of *Balantidium coli*, with the exception of the oral plug, is thickly beset with cilia (*cil.*, *ador. c.*, fig. I). These are of two kinds, viz., those which make up the adoral row of cilia and which measure from 8 to 12μ in length, and those covering the body, which vary from 4 to 6μ . Those covering the apical cone form an intergradation between the two. On this surface the cilia which occur immediately posterior to the adoral row are only slightly shorter and slightly more slender than the adoral cilia themselves. Passing posteriorly they gradually become shorter and less cirrus-like until they reach the base of the apical cone. Thence posteriorly they retain the uniform size.

The body cilia are comparatively short and very slender. So small are they in fact that to observe a single one is nearly impossible. In slides prepared by the usual methods no stain remains in the cilia if destaining is carried sufficiently far to differentiate other structures. Iodine (Weigert's solution) gives a fairly satisfactory stain for temporary mounts. The arrangement of cilia may be determined most readily by using a heavier stain and then observing the distribution of basal granules. Neutral red proves very satisfactory for this purpose. The cilia occur in longitudinal, slightly spiral rows, following the grooves between the ridges in the pellicle. These rows originate immediately posterior to the groove in which the adoral cilia are set and for a very short distance pass almost meridionally; very soon,

however, they turn toward the left, that is, in a counter-clockwise direction when the animal is viewed from a point exactly in front. They continue their spiral direction until almost to the posterior end when they again follow a meridional path to their termination. In passing the entire length of the body any single row of cilia twists to the left approximately 120° , or one-third the entire circumference. Whether some rows terminate or become continuous with contiguous rows before reaching the posterior tip of the animal, I have been unable to determine, for both basal granules and granular bands become very indistinct in this region even in the best preparations. The number of rows was counted with difficulty in several cross-sections from the equatorial regions of different animals, and it was found to vary from about 60 in small individuals to 120 in larger ones. No correlation between the variation in the number of rows of cilia and the species of the animal could be determined, but this is possibly due to the limitation of observation.

Basal apparatus.—The cilia perforate the pellicle and attach to the basal granules which lie immediately underneath (fig. L). The latter are small and apparently spherical or oval. They stain very deeply black or blue with haematoxylin. In the living animal they are readily emphasized by the use of neutral red, and less so by Janus green. In preparations stained with Mallory's connective tissue stain these granules show brilliantly red with the acid fuchsin, as do the other parts of the neuromotor apparatus and also the micronucleus. Longitudinally the granules are so closely placed that it is impossible to observe whether they are actually connected by a fibre. Cross-sections show that the cilia of one row have no transverse connection by any sort of stainable fiber with those of the next row. The rows of basal granules lie close beneath the depression in the pellicle in the hyaline or bright band of the ectoplasm. A ciliary rootlet (*cil. r.*, figs. I and L) extends from each basal granule centrally toward the endoplasm. It does not proceed in an exact radial line but rather diagonally toward the right until it enters the granular band near the inner surface of the latter. The ciliary rootlets in some cross-sections appear to have an exactly radial direction. In such cases, however, the granular band is somewhat diagonal in the opposite direction. This variation is probably produced either by torsion of the animal or by the direction of the effective beat of the cilia at the instant of fixation. The diagonal direction of the rootlets is readily detected in tangential sections. In focusing down through such a sec-

tion, the rootlet is invariably seen to run from the basal granule toward the observer's right into the contiguous granular band on that side. Thus, to avoid any confusion that might arise from the terms right and left, in a cross-section of the animal viewed from the anterior surface (such a view is shown in fig. L) the ciliary rootlets swerve in the counter-clockwise direction and enter the granular band lying immediately in that direction.

As the rootlet enters into the granular band it apparently enlarges thus forming a secondary basal granule. In some cases this may stain even more deeply than the basal granule itself, and appear as a definite body somewhat elongated in the direction of the circumference of the animal. It was thought at first that this might be the cross-section of a longitudinal fiber or myoneme. But the study of numerous tangential sections has failed to show the presence of any longitudinal fiber within the granular band. The stainability varies greatly and in preparations which have stained lightly the ciliary rootlets appear to fray out and merge into the granular band, while still retaining deeper color than the rest. Which interpretation is correct it is difficult to say, but the latter seems the more probable, especially in view of certain relations with the neuromotor apparatus which will be discussed later.

Pütter (1903) reproduces a figure from Studnicka (1899) showing in a schematic way five types of attachment of the cilia with their basal apparatus. Of these, two, at least, represent cases in which two basal granules or a diplosome are present. Saguchi (1917) in his studies on ciliated cells of Metazoa says in part regarding the basal granules of certain ciliated cells from amphibian larvae, "With favorable staining the basal corpuscles appear as diplosome or dumbbell shaped granules. One of these is situated at the upper the other at the lower border of the cuticle." In *Balantidium coli* the arrangement with respect to protoplasmic layers is quite different, though the cilia seem to follow somewhat the same plan of structure even to the presence of rootlets.

The most fruitful comparison may be made with the basal apparatus of cilia in *Isotricha prostoma* as described and pictured by Braune (1913). In this organism he describes diplosomic structure of the basal apparatus, in which the basal granule lies directly beneath the pellicle. The cilia, however, extend beyond the basal granule into the underlying layer—the "Zwischenschicht" of Eberlein, and terminate in the "Grenzschicht" with a second granule, which upon

maceration remains attached to the basal end of the cilium. So the basal apparatus in *Balantidium coli* even to the relative location of the basal granules is almost identical with that in *Isotricha prostoma*. In *Balantidium*, however, as mentioned above, the "Grenzschicht" seems to be lacking. The comparison becomes more significant in view of the fact that both ciliates are parasitic in the digestive tract of mammals, and both are in much the same state with reference to the degree of specialization and degeneration correlated with habits of the parasitic mode of life. So that in general morphology they seem to be more alike, though they are in separate orders, than do *Diplodinium ecaudatum* and *Balantidium coli*, which are in separate suborders only.

Ciliary Movements.

Locomotion is the chief function of the cilia except for those of the adoral zone. The balantidia swim in approximately a straight line and not in a spiral course as do paramoecia. They do, however, rotate on their axis as they progress. This rotation is generally from left to right, that is, in a counter-clockwise direction when viewed from a point in front of the animal. A few instances of reversal of the direction have been seen, but it is not at all common. The direction of rotation, i.e., from left to right, seemed at first inexplicable, since this was not compatible with the direction of the rows of cilia. The rows of cilia, as described above, are comparable to the threads of a left-hand screw. In order to penetrate, such a screw must be turned in a clockwise direction (when viewed from the point, not from the head). Such, also, is the direction of rotation of balantidia which one would expect to find if the arrangement of the cilia were the controlling factor, but the rotation is in the reverse direction. In the further study of the problem, I fortunately obtained some very thin tangential sections of animals on which the fixative had acted so quickly that the cilia were stopped instantly and left in the relative positions assumed in normal ciliary action.

Figure N is a camera lucida drawing of such a section. By analysis of the position of the cilia on this and other like sections, it was possible to determine the complete cycle of a single cilium. This cycle is diagrammatically represented in figures M and O. Figure N includes about two and one-half cycles of action as represented by the waves. The dark portions are produced by the prostrate position of the cilia at the termination of the effective stroke. The lighter

areas between are due to the fact that the cilia are recovering their vertical position and hence are viewed very nearly endwise. The position of consecutive cilia in any single row, from one point in a wave to a similar point in a following wave will fairly represent the successive positions taken by a single cilium in making one complete cycle. Figure M was made in this way. The arrow represents the long axis of the animal. From this diagram it is seen that the cilium at the end of the effective stroke lies rather close to the surface of the body and not along the row but decidedly to the left from it. In

Fig. M

Fig. N

Fig. O

Fig. M. Diagrammatic representation of the successive steps in one complete beat of a cilium of *Balantidium coli*. It also illustrates the positions of the respective cilia of a single row between the points *a* and *b* in fig. M, at which points the cilia are in a prostrate position at the end of their effective stroke. The arrow indicates the long axis of the animal.

Fig. N. Tangential section of *Balantidium coli*. The cilia still retain respective positions which they had in the normal swimming movements of the organism. $\times 1500$.

Fig. O. Diagram illustrating the effect of the ciliary action in the rotation of the organism. The arrow represents the long axis of the animal; *ab*., the direction of the rows of cilia; *cd*., the direction of effective stroke of a cilium attached at the point of intersection of the three lines; the ellipse is described by the tip of the cilium.

recovery it straightens up and passes anteriorly, thence to the right, crossing the row, of which it is a part, at right angles. At this point the cilium leans anteriorly only slightly from the vertical. The effective beat is produced by the quick stroke of the cilium posteriorly and to the left, and continues until the cilium has crossed the row again and lies close to the surface and extends to the left as represented by the position of the last cilium shown in figure M. According to the classification given by Pütter (1903), the movement of the cilia of *Balantidium* would be called infundibular. As will be noted from

figure O, the funnel described by the complete beat of the cilium is somewhat irregular, the rim outlined by the tip of the cilium is elliptical, and the base of the cilium, i.e., the neck of the funnel, is not central but is situated below the anterior focus of the ellipse. The ellipse described by the tip of the cilium lies in a plane which is not parallel to the surface of the animal, but which approaches it much more closely posteriorly. It was possible to corroborate the action of the cilia in observations on the living material. The cilia of the apical cone are somewhat larger than the other cilia of the body, and are closely coördinated in their action. In balantidia which were allowed to cool until action had slackened considerably, these cilia were observed to make their effective stroke at a decided angle to the rows of cilia. For instance, in the boring action in connection with the process of penetration described above, these cilia will beat in an almost exactly transverse direction, always from right to left. In further corroboration of this interpretation of the movements of the cilia is the fact that it explains the rotation of the animal during progression. It will be seen from figure O that the effective stroke of any single cilium will be in the direction *cd*. This line crosses both the arrow, representing the long axis of the animal, and the line *ab*, representing the rows of cilia, making an angle of approximately 20° with the latter. That is, the line *cd* forms an angle with the arrow on one side about equal to the angle on the opposite side made by the line *ab*. It clearly follows that if the effective stroke is in the direction *cd* then rotation will be from left to right and not vice versa as would be the case if the cilia beat in the direction of the rows in which they are arranged. The structure of the basal apparatus is significant in view of the direction of the effective stroke of the cilia, viz., posteriorly and to the left. The cross-section shown in figure L is viewed anteriorly and shows that the ciliary rootlet from each basal granule passes to the right and enters the granular band on that side of the hyaline band in which the basal granule lies. Without giving to the ciliary rootlets any motor or skeletal function, it still seems logical that they conform in a general way to the axis of the cilium, for the latter during the greater part of its movement inclines to the posterior and left of the row of which it is a constituent.

The peristome (*per.* fig. J) may be said to comprise all that part of the organism which lies within the row of adoral cilia. In the active animal it is variable in shape. At times it is almost round while at other times it may become a mere slit or groove. In what

seems to be its more normal proportions, however, it is approximately pear-shaped with the stem end of the pear directed ventrally. In *Balantidium coli* the adoral zone commonly includes the most anterior point or apex of the animal—at least its dorsal margin passes through this point. In *Balantidium suis*, the anterior tip of the animal lies wholly outside of this zone, the latter having migrated too far ventrally to include it.

The *cytostome* (*cytst.*, fig. J) does not occupy the whole interior of the peristome, but is situated at its ventral end. There is some indication that this aperture may be completely closed by the oral plug (*or. pl.*, fig. I) which comprises the rest of the peristome within the adoral row of cilia. This oral plug bears no cilia. It is exceedingly mobile, adapting itself readily to the almost constantly changing shape of the apical cone. It lies dorsal to the cytostome and is not exactly bilaterally symmetrical since it is pushed somewhat to the left to make room for the oesophagus. It extends inward, thinning as it does so, until it terminates about the beginning of the endoplasm. It is ectoplasmic, but very finely granular as compared with the rest of the ectoplasm. Mallory's connective tissue stain ordinarily gives it a decidedly bluish tinge with slight spots of red only where there are certain neuromotor fibers. Its action in feeding is very hard to follow, but its high degree of mobility impresses one when watching the activities of the organism, and may be demonstrated with fixed material by its extreme protrusion (pl. 28, fig. 14). In addition to this, the fact that it is intimately connected with the neuromotor apparatus would indicate that it functions in selective feeding. The oesophagus (*oes.*, fig. I; pl. 27, figs. 4–8) beginning at the cytostome, passes inwardly, not quite radially but swerving slightly to the right. It may be followed definitely through the ectoplasm and for a very short way into the endoplasm where it ends blindly. So far as I have been able to determine, it is a uniform tube-like opening without evident enlargements or constrictions. Prowazek (1913), however, gives in part the following description, “. . . es sehnt sich jedoch nicht direct trichterformig in die Tiefe, da man von der drei scharfe Konturen noch nachweisen kann (fig. 1).” While studying living forms stained with neutral red, I have often observed specimens in the exact position of the one shown by Prowazek (1913, p. 7, text fig. 1). The lines shown by him were easily recognizable, deeply stained with the neutral red, but I could interpret them only in the following way. The most anterior line which he shows seems to be

clearly the ventral lip of the peristome; the second line was identical with the deep staining motorium (fig. I) in the forms which I studied, and the most posterior line seemed identical with the ring of enlargements on the rootlets of the adoral cilia which lie close about the oesophagus. The margin of the peristome is slightly raised, forming a ridge or lip. This ridge is most pronounced from the midventral point, dorsal along the right margin of the cytostome, but as it proceeds around it becomes rather inconspicuous and wholly disappears on the left side. Thus the cytostome has the appearance of opening under a ledge, the ledge formed by the lip of the right side.

The peristome is delimited by an almost complete spiral circlet of cilia, the adoral cilia (*ad. cil.*, fig. I). The exact point of origin of the row is hard to determine but it is approximately at the ventral edge of the peristome, i.e., on the ventral lip of the cytostome. From here it proceeds in a sort of groove, along the right margin, around the dorsal margin, and down the left margin of the peristome. A short distance in advance of the ventral point, the row of adoral cilia turns into the cytostome and continues in a spiral course down the oesophagus; entering at the left dorsal side and ending in the ventral wall about halfway down. Thus these cilia in their entire course make one complete left-hand spiral, beginning on the ventral lip, passing around the peristome and down the oesophagus, terminating in its ventral wall.

Over the lip of the cytostome between the point where the adoral cilia turn into the oesophagus and the midventral point where this row of cilia has its origin, the longitudinal rows of body cilia turn in. Each of these rows, of which there are ten or twelve altogether, continues down the oesophagus until it meets the row of adoral cilia. Since this latter enters spirally, there is a ciliated patch on the ventral wall of the funnel-shaped oesophagus which is roughly the shape of a right triangle, the base of which is the lip of the cytostome. The hypotenuse is the row of adoral cilia which makes an acute angle with the longitudinal row of body cilia which enters in the mid line and which represents the third side of the triangle.

The adoral cilia, except where they lie within the oesophagus, are completely separate each from the other. This is easily verified by watching their action especially in a disintegrating animal, or one cooled to slow up ciliary movement, under which condition coördination is frequently interrupted, and a single cilium will be seen acting independently of its neighbor. Additional evidence is to be found in

the individuality of each basal granule. There is no evidence of fusion of granules, though adjoining granules are connected by a neuromotor fiber. However, in the triangular area mentioned above, there is considerable evidence that the cilia are united to form membranelles. It is very difficult to watch the action of these cilia, and the above conclusion was reached largely through a study of fixed material. In cross-sections (pl. 27, fig. 4) the cilia of the region seem to be very close together and quite regularly to be connected by, or to form, a sheet of almost transparent substance. The regularity of this occurrence would lead one to believe that it is the normal structure and not due to entanglement of the cilia in foreign matter. In addition, the basal granules of this region (pl. 27, figs. 4 and 5) do not stand out separately, but are so closely packed that they give the appearance of a single deeply stained mass. I have been unable to distinguish separate granules in the inner portion of the area and it seems likely that actual fusion of the granules may have occurred. The basal apparatus of this region is so densely packed and takes stain so readily that in certain views it may easily be mistaken for the motorium. As a result, it seems plausible to interpret this area as the basal apparatus of membranelles which lie in a plane transverse to the axis of the oesophagus. If this region be interpreted as a primitive oral groove or cytostome, a forerunner of such an elaborate arrangement as occurs in *Euplotes patella* (Yocum, 1918) or in *Stentor*, then comparison is very significant, for in the latter two the membranelles also run transversely in the cytostome. The only difference, then, between these organisms and *Balantidium* in this regard would be the difference in shape and extent of the area.

The adoral cilia (*ad. cil.*, fig. 1) are approximately double the length of the body cilia, i.e., from 6μ to 8μ in length or, in the very large individuals, they may reach a maximum length of 10μ . In fundamental structure they are like the body cilia, but the relative position of parts is somewhat different. Immediately beneath the pellicle each cilium bears a basal granule and from this a fiber continues inward which passes between the adoral plug and the surrounding ectoplasm. The sum of all the fibers from adoral cilia marks off very distinctly the conical cytostomal region from the surrounding ectoplasm. They seem to constitute the only partition between the protoplasm of the two areas, for I have not been able to detect any membrane in this region making a complete separation of the adoral region from the rest of the apical cone. Where the fibers pass from

the ectoplasm of this cone into the endoplasm, each bears an enlargement which stains very deeply. This enlargement is undoubtedly homologous with the inner enlargements of the basal apparatus of the body cilia for it bears the same relation to the basal granule and the cilium. It is farther removed from the basal granule but it maintains exactly the same relation to the ectoplasm and endoplasm, that is to say, in either case this enlargement lies in the plane between the two. From each enlargement a radial fiber extends laterally along the base of the apical cone to the periphery of the cell. The portion of the ciliary rootlet which continues inward from this enlargement is very distinct and may often be traced almost to the posterior end of the animal (fig. I). In no instance has it been possible to demonstrate any attachment or connection of the posterior ends of these rootlets. They often cross near the center of the organism and then fade out, becoming indistinguishable in the endoplasm. For a short distance inward from the oesophagus they are so closely placed dorsally and laterally that they form a kind of wall, but ventrally they are less numerous and likely to be much shorter, so that the pseudo-wall is not complete. It is sufficient, however, to direct the food for some distance into the endoplasm. There is no such high degree of differentiation here as Sharp (1914) found in the oesophagus of *Diplo-dinium ecaudatum*.

The homology of the adoral and body cilia is evidenced by the complete series of gradations from the one to the other which exist in the cilia of the apical cone (fig. I). The cilia which are proximal to the adoral cilia are almost identical with them. They are slightly smaller, but each has the basal granule beneath the pellicle, a fiber connecting this with an enlargement at the plane of differentiation of ectoplasm from endoplasm, and the rootlet extending posteriorly. The inner enlargement is much smaller and the ciliary rootlet shorter than those of the adoral cilia. These enlargements are connected with the enlargements of the adoral cilia by the radial fibers mentioned above. Passing posteriorly the cilia become progressively smaller, the basal granule and the enlargement closer together (due to the approach of the transverse plane of demarcation between ectoplasm and endoplasm to the pellicle), and the ciliary rootlets become progressively shorter and less distinct, until the cilia of the marginal area of the apical cone become identical with the body cilia. This apical cone then shows us a complete gradation of differentiation between the body cilia and the adoral cilia, and proves their homology.

Feeding is the chief function of the adoral cilia. They are closely coördinated in action, as is shown by the fact that in degenerating individuals they will beat in unison after other cilia beat only erratically or have ceased entirely. Coördination is most pronounced in the cilia of the left lip. The action of the adoral cilia produces a strong eddy in the surrounding medium with the vortex of the eddy within the peristome. In the above description it was noted that the right lip projected slightly forming a ledge or bank. The current produced by the cilia strikes against this ledge and solid objects are deflected into the underlying cytostome. The natural rotation of the animal on its longitudinal axis as described above aids in this process, for the right lip thus acts as the blade of an auger. The food particles pass down the oesophagus and collect at the inner end in a sort of droplet. After several bits have been collected, the droplet begins its circulation in the endoplasm as a food vacuole.

The closure of the cytostome in all probability is effected by the *oral plug*. The latter is very mobile and contains fibers of the neuro-motor apparatus. It has frequently been seen to project anteriorly in a knoblike protrusion (pl. 28, fig. 14). This same phenomenon was observed by Wising (1871). In view of its situation, its sensitivity, and its mobility, it seems plausible to interpret it as an oral plug with essentially the same function as the oral disk of *Diplodinium ecaudatum* (Sharp, 1914).

The discharge of indigestible portions of the food takes place at a constant point at the posterior end of the animal, the cytopyge (*cyt.*, fig. I). It is an opening, however, only at the time of discharge. At other times there is only a thinning of the cortical layer which can be identified with comparative ease in fixed material. In the living organism the process of defecation was frequently observed. The undigested particles become segregated at the extreme posterior end in a sort of vacuolè, the rectal vacuole (*rect. v.*, fig. I; pl. 28, fig. 13). After this vacuole has become of considerable size (often filling one-third of the cell in degenerating forms) the pellicle over the cytopyge ruptures and the contents are discharged. The pellicle quickly forms again closing the aperture, collection of indigestible particles in the rectal vacuole continues, and the process is repeated.

The *contractile vacuoles* (*post. c. v.*, *ant. c. v.*, fig. I) are two in number, usually situated on the dorsal side; one anteriorly, well up toward the apical cone, the other in the posterior one-third of the organism. There is a great deal of variation in their location in different animals, though in the individual their situation seems to be

very constant, at least throughout the limit of possible observation. These vacuoles seem clearly to originate within the ectoplasm. When fully distended they encroach far upon the region of the endoplasm and it becomes impossible to tell whether or not they are entirely surrounded by ectoplasm; but from their origin such might be suspected to be the case. Also such is the case in many related forms, for example, *Diplodinium ecaudatum* (Sharp, 1914) and *Euplotes patella* (Yocom, 1918).

The pulsation of the vacuoles was observed in several instances for a long period of time. The rate of pulsation varies considerably, occurring as rapidly as once in every thirty seconds under some conditions, while under others a complete cycle from discharge to discharge occupies a period of five minutes. In degeneration the pulsation is likely to be very much retarded or may cease entirely, the vacuoles becoming enormously distended and breaking together thus forming one large vacuole occupying fully one-half of the interior of the organism. Following this the animal ruptures and disintegrates. The observation of a considerable number of normal individuals has shown the usual cycle to be as follows. At two points in the ectoplasm small droplets of clear liquid appear. These increase in size and become the vacuoles usually seen. Contributing vacuoles or channels such as occur in *Paramaecium* have never been noted. When they have reached sufficient size (10μ to 15μ in diameter in the ordinary individual), they change from their spherical shape and begin to bulge, each on the side toward the other. These bulges elongate until they meet at a midpoint. At this midpoint a new vacuole arises and into it, through the channels thus formed, the two vacuoles discharge their contents. This large middle vacuole almost immediately discharges to the exterior through the pellicle, and at the same time the other two vacuoles re-form. However, the discharge of the middle vacuole may be delayed until the other two are well formed and then the individual has three vacuoles present. This and other variations are not uncommon and should be taken into account in the use of the number of vacuoles as a basis for classification. Leuckart (1861) described a third contractile vacuole though he did not give its relative position to the other two and he did not describe the process of contraction. He reported that he had observed the vacuoles "drop-like" through the cytoplasm and wandering from place to place. Solojew (1901) described the two vacuoles and observed a canal connecting the two, but he did not explain its function in discharge of the contents.

ENDOPLASMIC STRUCTURES

Endoplasm.—Within the ectoplasm the body is composed of the endoplasm (*end.*, pl. 27, figs. 7 and 8; pl. 28, figs. 9 to 12) and the inclusions therein. The endoplasm is less dense than the ectoplasm and more coarsely granular. It is quite fluid, having a fairly definite circulation in the active organism. From the inner end of the oesophagus the direction of flow is posteriorly along the ventral surface dorsalward just before reaching the posterior end, thence anteriorly along the dorsal surface. Just as it reaches the ectoplasm of the apical cone, it is deflected posteriorly down through the central portion of the body dorsal to the rootlets of the adoral cilia. This course can be followed readily by observing the circulation of the food vacuoles.

The *food vacuoles* are customarily globular and may contain starch granules, bacteria, or indigestible particles, of the food of the host. The ingestion of bacteria is probably abnormal since it seldom occurs except when the number of bacteria in the medium has increased greatly during incubation. The starch granules may occur in enormous numbers especially when the host has recently been fed on grain. Red blood cells have not been noted in any of the balantidia observed during the work.

That *Balantidium* may be cannibalistic is the only possible interpretation of several findings. In these instances small individuals were lying within the endoplasm of extremely large individuals ($100 \times 125\mu$) and were in a state of disintegration. The larger individuals were *Balantidium coli* in every case and the smaller may have been *Balantidium suis*, for both species were present in the material; but disintegration of the latter had progressed so far that specific identification was uncertain. The possibility of interpreting this phenomena as sporulation, which was described by Walker (1909), is precluded by the disintegration of the included organism and the fact that the normal vegetative phase of the nuclei of the large individual is unmodified. Further evidence is to be found in the fact that never more than a single individual has been found inside another, while sporulation would produce several.

Macronucleus.—As is generally the case in ciliates, balantidia are binucleate, having a large macronucleus and a small micronucleus. The macronuclei of *Balantidium coli* and *Balantidium suis* are slightly

different with respect to size and proportions as noted above (page 259), but structurally they are so closely alike that one description will suffice for both. The macronucleus (*mac.*, fig. I, pl. 28, figs. 13 and 14) always lies in the endoplasm but otherwise is not constant in location. Immediately surrounding it is an area in which the endoplasm is less granular and less dense in appearance, due perhaps as Yocom (1918) has suggested of *Euplotes patella* to more rapid oxidation in this region. It is elongate and may be straight and rodlike or it may be sharply bent into a horseshoe shape. In any case its diameter increases toward either end, giving it something of a dumb-bell shape. This constriction in the central region and enlargement at each end is more marked in *Balantidium coli* than in *Balantidium suis*. The nucleus is delimited by a definite nuclear membrane (*nuc. m.*, fig. I) which is especially apparent in material in which the macronucleus has shrunk due to faulty technique. Within this membrane are packed rather densely the masses of chromatin. The chromatin never occurs in equal sized regular granules but rather in unequal very irregular masses, sometimes of considerable size (1 to 2μ in greatest dimension). Often there is a sort of vacuolated area, usually near one end of the macronucleus, which is free from chromatin. The significance of this vacuolated area I could not determine. It does not seem to be due to degeneration and is not related to any phase of reproduction. It is in no way comparable to the "reconstruction band" described by Griffin (1910) and by Yocom (1918) in *Euplotes worcesteri* and *Euplotes patella*, respectively. The chromatin stains black with haematoxylin so that the macronucleus is the most conspicuous structure in a stained individual. When Mallory's connective tissue stain is used, the macronucleus takes on an orange hue.

Micronucleus.—The micronuclei (*mic.*, fig. I) of both species of *Balantidium* parasitic in pigs are exceedingly small, not exceeding 5μ in diameter. In the resting or vegetative phase the micronucleus is subspherical and its flattened side lies close against the nuclear membrane of the macronucleus. It may even lie in a depression in the macronucleus in which position it is scarcely distinguishable from the granules of the latter. It is surrounded by a nuclear membrane readily recognized when the micronucleus is undergoing mitosis. In a few instances the chromatin has appeared indistinctly granular but it is customarily so closely packed that it looks like a single solid mass.

NEUROMOTOR APPARATUS

The term neuromotor apparatus denotes an integrated system of fibers, with a coördinating center, which is present in some Protozoa, and which is credited with the power of conductivity of nervous impulses, and hence functions in the coördination of the motor organelles of the cell. The term was first used for ciliates by Sharp (1914) in his account of the structure of *Diplodinium ecaudatum*. Since then it has been employed by Kofoid and Christiansen (1915), Kofoid (1916) for flagellates, and by Yocom (1918) and by Taylor (1920) in their studies of the morphology and behavior of *Euplotes patella*.

The neuromotor apparatus of *Balantidium coli* can scarcely be considered apart from the motor organelles of the cell. To insure clarity in the description and discussion which follows, the motor organelles, previously described in some detail, will be briefly reviewed. With the exception of the oral plug which surrounds the cytostome the organism is thickly beset with cilia. Of these, the adoral cilia are largest. They are distributed about the margin of the peristome in a row which ends in the membranellar region in the ventral wall of the oesophagus. The rootlets of these cilia are exceedingly long, reaching well into the posterior third of the cell, where they end without any connection or attachment. They have two enlargements, the first being the basal granule which lies just beneath the pellicle, and the second being located at the junction of ectoplasm and endoplasm. The remainder of the cilia are arranged in longitudinal spiral rows. The most anterior cilia of these rows, i.e., those nearest the adoral cilia, are nearly as sturdy as the adoral cilia themselves. But, progressing posteriorly in the rows, the cilia become continuously smaller until they reach their minimum size at the base of the apical cone of ectoplasm. Likewise the ciliary rootlets become shorter, and the distance between the basal granules and the secondary enlargements of the rootlets becomes less and less as one approaches the base of the apical cone. This is shown in figure I. The remainder of the way posteriorly the cilia are of uniform size, and the secondary enlargement of the basal apparatus of each cilium is the termination of the ciliary rootlet and lies in the granular band of ectoplasm near its plane of junction with the endoplasm.

In *Balantidium coli* five distinct parts constitute the complete neuromotor apparatus, namely (1) a motorium or coördinating center, embedded in the ectoplasm close by the oesophagus, and from it fibers pass out to the oral plug and the motor organelles; (2) a circum-

oesophageal fiber, beginning and ending in the motorium and giving off branches as it passes through the oral plug; (3) a fiber connecting the adoral cilia and the cytostomal membranelles with the motorium; (4) the adoral ciliary rootlets passing posteriorly from the basal granules of the adoral cilia, each bearing an enlargement where it passes from ectoplasm into endoplasm, and finally ending without attachment in the endoplasm of the cell; (5) the radial fibers taking origin from the enlargements of the adoral ciliary rootlets and passing radially to the ectoplasmic layer where they turn posteriorly into the granular band.

The *motorium* (*mot.*, figs. I and J) when viewed in ventral aspect has somewhat the appearance of a reversed letter J. It lies within the ectoplasm of the apical cone, close to the ventral and right walls of the oesophagus. The part of the motorium corresponding to the vertical shaft of the J lies along the right wall of the oesophagus, and its anterior terminus is situated just inside and slightly dorsal from the point of origin of the right lip of the cytostome. The curved end of the J is posterior and passes ventrally and anteriorly around the oesophagus and ends close to the inner termination of the rows of adoral cilia. In the middle region a sharp slit-like constriction is often very conspicuous. It is with the part of the motorium anterior to this constriction, that the circumoesophageal fiber and the adoral ciliary fiber have their origin. The portion posterior to this constriction is quite variable both in size and in stainability. This variation is very suggestive of the behavior of the parabasal body of certain flagellates as described by Kofoed and McCulloch (1916), Swezy (1916), Kofoed and Swezy (1919). These authors have shown that the parabasal body is not kinetic in function but instead is a reserve or reservoir of material easily transformed into energy, which reservoir fluctuates according to the physiological condition of the animal. No direct evidence can here be advanced establishing such a function for this posterior portion of the motorium, but the fact of its fluctuation in volume and its variation in chemical nature (as shown by stains) would lead one to suspect that such an interpretation is correct.

The *circumoesophageal fiber* (*cir. oes. f.*, figs. I and J) takes origin from the anterior extremity of the motorium and passes into the oral plug. Its course is very close to the inner, i.e., oesophageal, surface of the plug. It encircles the oesophagus completely but in the membranelar area it becomes very hard to follow. Certain sections (pl. 27, fig. 3), however, seem to show that it unites again with the motorium. It is not a smooth fiber but bears irregular enlargements from which

fibers pass both posteriorly and anteriorly into the ectoplasmic mass of the oral plug. These branches make no observable connections, but seem to fade out in the ectoplasm. Morphological evidence would indicate that this portion of the neuromotor apparatus is concerned solely with the activities of the very mobile oral plug. The *adoral ciliary fiber* (*ad. cil. f.*, figs. I and J) also arises from the motorium. This fiber attaches to the motorium just anterior to the constriction. It passes directly to the basal granule of the first cilium of the adoral row from which it passes on and makes connection with each of the basal granules of the entire row. It turns inward following the row of adoral cilia along the dorsal or left-hand margin of the membranelle area. Here its course is exceedingly hard to determine, but like the circumoesophageal fiber, some sections seem to show its connection with the posterior end of the motorium.

The remainder of the neuromotor system is not directly connected with the motorium. The *adoral ciliary rootlets* (*ad. cil. r.*, fig. I) pass inward from the basal granules of the adoral cilia through the ectoplasm of the apical cone into the endoplasm and well into the posterior third of the organism where they end, not abruptly but by fading out. There is absolutely no indication of any attachment of their inner ends. Just posterior to the middle of the cell there is usually a very distinct crossing of the ciliary rootlets from the opposite sides of the peristome. Each of these adoral ciliary rootlets bears a decided enlargement at the point where it passes from the ectoplasm into the endoplasm. The aggregation of these enlargements gives the appearance in cross-sections through this region (pl. 27, fig. 6) of a zone of large, deeply staining granules about the oesophagus. Each of these enlargements gives rise to a fiber which passes radially outward in a transverse plane. These have been termed the *radial fibers* (*rad. f.*, fig. J; pl. 27, fig. 6). Each of these radial fibers as it passes peripherally connects with small enlargements of the rootlets of the cilia of the apical cone. The rootlets of the cilia of the apical cone are like those of the adoral cilia except that they shorten progressively as they near the periphery, at which point they scarcely extend into the endoplasm at all, becoming identical with those of body cilia. Since the radial fibers and the enlargements of the ciliary rootlets lie in the plane of contact between the ectoplasm and the endoplasm they very clearly mark the limit of the two. At the periphery the radial fibers turn posteriorly and become lost in the granular band. This last fact is very suggestive, since the terminal enlargements of the rootlets of the body cilia lie in these granular bands.

It has not been possible to demonstrate a posterior continuation of the radial fibers within the granular bands. If they do not continue posteriorly, making connections with the enlargements of the rootlets of the body cilia, then the logical alternative seems to be to attribute the function of conductivity to the granular band of ectoplasm itself. There must be some means of conduction of stimuli, for the body cilia are concerted in their action, though not to so high a degree as in the case of the cilia of the apical cone. Protoplasm is generally conceded to have the power of conducting stimuli. So here in *Balantidium coli* there seems to be a transition from conduction by the undifferentiated protoplasm which serves as a matrix for the ciliary apparatus, to a condition in which there is a differentiation of the protoplasm into fibers or strands to serve the purpose. Moreover, the increasing degree of differentiation is directly correlated with the increasingly high degree of coördination of the motor organelles.

It will be seen from the figures and the above description that *Balantidium coli* is equipped with a notably integrated system of fibers bearing such morphological relations as would make the assigning to them of the function of coördination perfectly logical. The motorium is connected directly only with the oral plug and the adoral cilia. But through ciliary rootlets and the radial fibers all of the cilia of the apical cone are in connection with the adoral cilia. And if the function of conductivity may be attributed to the granular bands of the ectoplasm, into which the radial fibers turn and become lost to view, and in which the enlargements of the ciliary rootlets of the body cilia lie, then all of the motor organelles of the organism are reached by the neuromotor apparatus, and by it the coördination of these organelles may be explained.

Attributing to the various fibers described above the quality of conductivity and to the whole neuromotor apparatus the function of coördination of all of the locomotor organs in swimming and feeding, microdissection experiments carried on by Taylor (1920), we may consider the functions of the various parts from that point of view. The main rôle of the motorium is to act as a coördination center. The circumoesophageal fiber would serve to correlate the movements of the oral plug with those of the adoral cilia in feeding and in avoiding-reactions. The ciliary rootlets and radial fibers make possible the coördination of all of the locomotor organs in swimming and feeding, and especially the cilia of the apical cone which are employed in the boring movement of the organism.

DISCUSSION

Up to the present time systems of intracytoplasmic fibers and accessory neuromotor masses comparable to that found in *Balantidium coli* have been fully described in several flagellates and a few ciliates. The very primitive type occurring in *Naegleria gruberi* (Schardinger) has been described by Wilson (1916). Kofoed (1916) and Swezy (1916) have made a critical comparative study of the motor systems of those flagellates, in which they have been most carefully studied. That there is a striking similarity in the neuromotor systems of flagellates and ciliates is clearly pointed out by Yocom (1918).

Among the ciliates, intracytoplasmic fibers have been known for some time and there have been several descriptions of them and several conflicting views as to their function (Engelmann, 1880; Bütschli, 1889; Schuberg, 1891; Maier, 1903; Prowazek, 1903; Griffin, 1910; Braune, 1913). Sharp (1914), however, was the first to describe fully a completely integrated fibrillar system with a central neuromotor mass, to which he applied the term neuromotor apparatus.

Of the neuromotor apparatus of ciliates that of *Balantidium coli* is the third to be quite fully worked out. In 1914 Sharp described the neuromotor apparatus of *Diplodinium ecaudatum* (Fiorentini). This apparatus consists of six parts. The central motor mass, or motorium, lies in the area of thickened ectoplasm at the anterior end of the animal between the dorsal and adoral membranelle zones. A fiber connects the motorium with the basal granules of the dorsal membranelles, a branch from which runs along the base of the inner dorsal lip. Another fiber connects the motorium with the basal granules of the adoral membranelles. A set of opercular fibers leave the motorium and pass along underneath the operculum. Lastly, the motorium has a definite connection by means of a fiber with what Sharp called the circumoesophageal ring. There is also a set of fibers in the wall of the oesophagus, which he termed the oesophageal fibers, and which he believed took their origin from the circumoesophageal ring. All of these structures as well as the micronucleus Sharp found had an affinity for the acid fuchsin when Mallory's connective tissue stain was used.

In *Euplotes patella* (O. F. Müller) the neuromotor apparatus, as described by Yocom (1918), is made up of five distinct parts. The

motorium is a somewhat bilobed mass and lies in the ectoplasm at the anterior end of the organism close to the right anterior corner of the triangular cytostome. From the left end of the motorium five main longitudinal fibers pass posteriorly, diverging slightly, and each joins with one of the five anal cirri. The exact relation of these fibers to the basal plates of anal cirri has been very carefully determined by Taylor (1920). Leaving the right end of the motorium a fiber passes to the membranelles of the adoral zone. Directly connected with this fiber is the "sensory structure" of the anterior lip. Lastly, there are dissociated fibers in connection with the frontal, ventral, and marginal cirri. In *Euplotes*, as in *Diplodinium*, all parts of the neuromotor apparatus as well as the micronucleus stain brilliant red with acid fuchsin.

For facilitating comparison, it might be well to summarize briefly the neuromotor apparatus of *Balantidium coli*, as described above. It consists in this organism of five distinct divisions. The motorium is a J-shaped mass situated in the thickened ectoplasm of the anterior end of the animal close to the cytostome and oesophagus. From it arises the circumoesophageal fiber, which gives off branches to the oral plug. A second fiber takes its origin from the motorium and connects with the basal granules of the adoral cilia. The adoral ciliary rootlets pass inward from the basal granules of the adoral cilia, and may extend well into the posterior third of the cell. The radial fibers take their origin from enlargements of the adoral ciliary rootlets, at the point where they enter the endoplasm. They pass outward radially, making connection with the ciliary rootlets of the cilia of the apical cone, and at the periphery turn posteriorly and cannot be traced farther in the granular bands of the ectoplasm. As in the previous forms just summarized, the neuromotor apparatus as well as the micronucleus of *Balantidium coli* is selective for acid fuchsin.

Of the three examples of neuromotor systems so far fully worked out and described, each represents a different order of the class Ciliata: *Diplodinium* being of the order Oligotricha; *Euplotes* being of the order Hypotricha; and *Balantidium* being of the order Heterotricha. Yet in spite of the diversity of forms there is a remarkable similarity in the neuromotor system. The presence of a motorium is common to all three. In each of the three organisms it is located at the anterior end of the animal and lies wholly within the ectoplasm near the cytostome. By means of fibers it is connected with a part or all of the motor organelles of the animal. Another feature of the

fibrillar portion of the apparatus common to all three forms is the strand connecting the motorium with all of the adoral cilia or membranelles, as the case may be. Finally, the system, in all cases, shows an affinity for acid fuchsin.

The circumoesophageal ring present in *Diplodinium* is represented in *Balantidium* by the circumoesophageal fiber running through the oral plug. The oral plug forms the wall of the greater part of the oesophagus, and the fiber lies very close to the oesophageal surface. From this fiber there are given off fibers which pass both anteriorly and posteriorly in the mass of the oral plug. These fibers are strikingly similar in location to the oesophageal fibers, described by Sharp (1914) in the wall of the oesophagus of *Diplodinium* and believed by him to arise from the circumoesophageal ring. Sharp points out that these fibers approach very close to the micronucleus though there was no demonstrable connection with it. In *Balantidium* this possibility is precluded by the shortness of the oesophagus which in no case extends inward to a point anywhere near the micronucleus. At times when the organism is viewed from the proper angle, the ciliary rootlets of the adoral cilia may have the appearance of ending in the proximity of the micronucleus and suggesting a connection with it. No such connection exists, however, as may be readily demonstrated in large numbers of whole mounts and still better in sections where these rootlets may be traced far posterior to the micronucleus, passing it some considerable distance away. In the vegetative phase, it is certain that no structural connection exists between any part of the neuromotor apparatus and the micronucleus.

Of the three neuromotor systems of ciliates here considered, that of *Balantidium* is clearly the least centralized, though none the less a unified structure. In *Diplodinium* all motor organelles connect directly with the motorium, and in *Euplotes* the same is true of all except the marginal cirri which have no connection whatever, whereas in *Balantidium* only the adoral cilia have direct connection with the motorium while the cilia of the apical cone (and those of the body, too, if they have any connection whatever) are connected with it only indirectly through the radial fibers and the rootlets of the adoral cilia. This lesser degree of centralization of the neuromotor apparatus may perhaps be explained by the lesser degree of specialization of locomotor organelles. Whereas in *Diplodinium* the body is devoid of cilia and the dorsal and adoral zones of membranelles are the sole motor organelles, and in *Euplotes* the locomotor organelles are restricted to the cytostomal membranelles and a few cirri on the ventral surface, in

the case of *Balantidium* the entire organism, with the exception of the oral plug, is covered with cilia. So the slightly lesser degree of specialization in the neuromotor apparatus would be the logical expectation if modification of intracytoplasmic structures is correlated with modification of external structures with which they have a direct connection or of which they form an integral part.

From the point of view of efficiency, also, the arrangement in *Balantidium* is readily explainable. The locomotor activities of the organism may be separated into three main sorts, swimming, feeding, and boring, this last very likely being used in penetration of the intestinal wall of the host. In swimming the coördination of the entire locomotor apparatus is necessary. In feeding, and in boring, particularly, the coördination of the adoral cilia with those of the apical cone is extremely essential. Such coördination would be most effectively brought about by a direct connection of the parts concerned, and this direct connection is accomplished by the uniting of all of the rootlets of the cilia of these two regions by means of the radial fibers, without the interpolation of the motorium.

Throughout the above discussion the assumption of a neural function for the neuromotor apparatus, i.e., the power of conductivity of stimuli resulting in coördination of parts, has been based on two general types of evidence, morphological and experimental. The chemical evidence, that is, the affinity for acid fuchsin, as presented by Sharp (1914) for *Diplodinium* and by Yocom (1918) for *Euplotes*, seems slightly less convincing in the case of *Balantidium*. In the last named organism not only does the micronucleus, which has no connection with the neuromotor apparatus, show an affinity for acid fuchsin but so also do certain cytoplasmic inclusions, which if not food particles are at least undoubtedly concerned in some way with metabolism and have no morphological relation to the neuromotor apparatus. Yocom (1918) states that there is more of the orange G in the micronucleus giving it a different shade from the parts of the neuromotor apparatus in the case of *Euplotes*; but in *Balantidium* I have been unable to detect any such differentiation.

Morphological evidence for attributing neural function to the neuromotor apparatus has been clearly presented by Yocom (1918) in his discussion of the apparatus in *Euplotes*. The evidence found in *Balantidium* is not strikingly different. There is in the latter organism the same intricate relationship between the neuromotor apparatus and the motor organelles. The most active cilia, i.e., the adoral cilia, are directly connected by the adoral ciliary fiber with

each other and with the motorium. The cilia of the apical cone are connected with the adoral cilia by the radial fibers and so indirectly with the motorium. In this case, however, coördination with the adoral cilia is most essential and this corresponds with their intimate connection. These morphological interrelations all point to a neural function for the neuromotor apparatus.

Very convincing experimental evidence of the neural function is to be found in the results of Taylor's (1920) microdissection experiments on *Euplotes patella*. In *Euplotes* there is a fiber connecting the adoral membranelles with the motorium, and also a fiber connecting each of the five anal cirri with it. In a series of experiments Taylor severed various ones of these fibers and observed very carefully the effect on the movements of the animal. He then compared these movements with the normal movements which he had previously carefully analyzed and classified. Severing of these fibers resulted in lack of coördination of the parts thus disconnected and resulted in abnormal movements. Incision made in other parts of the cell, but which did not sever neuromotor fibers did not so result. In the words of the author, "It is apparent, then, that the destruction of the motorium or the severing of some or all of its attached fibers is alone accountable for modification in the perfect and efficient coördination between the series of membranelles and the anal cirri. We may, therefore, regard these normal morphological relationships as conditioning the animal's usual behavior both in creeping and in swimming."

The general occurrence among Protozoa of protoplasmic modification to form organelles for locomotion, feeding, digestion, excretion, and protection has been known almost as long as have the Protozoa themselves. The functions of such organelles have not been difficult to determine. One might equally well expect to find modifications correlated with the conduction of stimuli, but the establishing of neural function is not so easy. This difficulty in conjunction with the traditional idea of the simplicity of the Protozoa has resulted in conservatism in crediting any intracellular structures with the function of conductivity. The recent detailed morphological studies on Protozoa and the experimental work of Taylor (1920), however, leave little doubt regarding the matter. This account of the neuromotor apparatus of *Balantidium coli* and *Balantidium suis* presents additional evidence of the likelihood of a quite general occurrence, in the Protozoa, of intracytoplasmic specialization resulting in a more or less integrated system for purposes of coördination.

SUMMARY

1. Pigs are very generally infected with balantidia as shown by the findings in previous investigations, many of which were carried on in foreign countries, and by the finding of 68 per cent infection among the two hundred pigs examined during the present investigation.

2. There are two species of the genus *Balantidium* that are parasitic in the intestinal tract of pigs, namely, *Balantidium coli* and *Balantidium suis* (sp. nov.).

3. *Balantidium coli* is the species first described by Malmsten (1857) from man, and later by Leuckart (1861) as a parasite in pigs.

4. *Balantidium suis* (sp. nov.) has not hitherto been distinguished from *Balantidium coli*. The former differs from the latter in being more elongate and being broadest anterior instead of posterior to the equatorial plane; in having a more slenderly proportioned macronucleus; and in having the mouth displaced ventrally, instead of being almost terminal, which causes the plane of demarcation between ectoplasm and endoplasm in this region to slant posteriorly toward the ventral surface instead of being perpendicular to the longitudinal axis, as is the case in *Balantidium coli*.

5. So far as recorded facts will justify conclusions, it seems unlikely that *Balantidium suis* occurs as a parasite of man, but instead that *Balantidium coli* is the cause of balantidiasis. Whether or not such is the case, it is very desirable that the occurrence of the two species in pigs be taken into account in future work on experimental infection, for to a failure to distinguish between the two species may be due the seemingly conflicting results of previous experiments.

6. The cilia of the two species are homologous. Variation occurs in size and relative position of parts only. The basal apparatus is essentially diplosomic, consisting of a basal granule connected by a ciliary rootlet to a secondary enlargement. The former is situated just beneath the pellicle; the latter lies in the plane of demarcation between ectoplasm and endoplasm. The adoral cilia are largest, the basal granule and secondary enlargement are farthest removed from one another, as the ectoplasmic thickening is greatest in this region, and the ciliary rootlet may extend far into the endoplasm. The cilia of the apical cone intergrade between the adoral cilia and the body cilia. As one

progresses posteriorly, they become smaller in size, the secondary enlargement approaches the basal granule as the ectoplasmic layer becomes thinner, and the extension of the rootlet into the endoplasm becomes shorter. The body cilia are smallest and their rootlets terminate in the secondary enlargement. The cilia of the anterior end are highly concerted in action. They beat in such a way as to give the animal a remarkable boring motion which probably serves in the penetration of the mucosa of the intestine.

7. Both species possess a neuromotor system. This is a highly developed and integrated system consisting of five correlated parts. The motorium, lying within the ectoplasm near the cytostome, gives rise to a circumoesophageal fiber. In addition, there is a heavier fiber which connects it with the basal granules of the adoral cilia. The rootlets of the adoral cilia extend far into the endoplasm, usually well into the posterior one-third of the cell. Where they pass from ectoplasm into endoplasm each adoral ciliary rootlet bears an enlargement and from this arises a radial fiber which passes to the periphery, turns posteriorly, and disappears in the granular band of ectoplasm. In passing to the periphery these radial fibers connect with enlargements of the rootlets of the cilia of the apical cone.

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EXPLANATION OF PLATES

PLATE 27

Camera lucida drawings of a series of transverse sections of *Balantidium coli* (Malmsten). The series begins at the anterior end and progresses posteriorly about one-third the length of the organism. $\times 1000$.

Fig. 1. This section shows dorsal portion only of peristome. The adoral cilia are removed showing their basal granules clearly.

Fig. 2. A portion of the oral plug is shown with the circumoesophageal fiber and the connected oral plug fibers. The deeply-staining region between protoplasm of oral plug and the surrounding ectoplasm, so marked in this individual, is rarely found.

Fig. 3. The continuation of cilia over the ventral lip and down into the cytostome are to be noted particularly; the beginning of the motorium on the (reader's) left of the cytostome; and the connection of the adoral ciliary fiber with the motorium.

Fig. 4. In this figure the cilia of the cytostome give the appearance of membranelles; the circumoesophageal fiber makes connection with the motorium.

Fig. 5. This shows the beginnings of the enlargements of the adoral ciliary rootlets.

Fig. 6. Here the oesophagus is completely surrounded by the enlargements and radial fibers may be seen arising from some of them.

Fig. 7. The extreme posterior tip of the motorium appears within the circle of enlargements; the dorsal half of this section is through the endoplasm.

Fig. 8. This section is posterior to the adoral apparatus with the exception of the rootlets of the adoral cilia which in cross-section can not be distinguished from the granules of the endoplasm.

PLATE 28

Figs. 9-12. Series of longitudinal sections through the anterior end of *Balantidium coli* (Malmsten). $\times 1000$. These sections are oblique, being more nearly frontal than sagittal, however. The section shown in figure 9 is tangential to the margin of the peristome some distance to the right of the most dorsal point of the latter (see fig. J in text). The remainder progress toward the left ventral lip. The oral plug fibers, the radial fibers, and the adoral ciliary rootlets are shown clearly in these sections.

Fig. 13. A sagittal section through *Balantidium suis* (sp. nov.). $\times 1000$. The specific characters, viz., elongate body and macronucleus, and *oblique* plane delimiting apical cone of ectoplasm, are shown in this section. The cytopyge is distinct and open to the exterior. A paramylum body of considerable size is present in the endoplasm.

Fig. 14. An oblique longitudinal section through *Balantidium coli* (Malmsten) showing the protrusion of the oral plug. $\times 750$.

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