STUDIES ON *GIARDIA MICROTI*

BY

WILLIAM CHARLES BOECK

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CONTENTS

THE CYCLE OF ENCYSTMENT IN *GIARDIA MICROTI*

INTRODUCTION

Most of the Protozoa which have been studied intensively for a long period of time have been shown to possess rhythms or cycles of what might be termed general vitality. These rhythms may be seen in the sensitiveness to environmental conditions of the individual organism or races of organisms, as in the case of *Paramoecium aurelia* (Woodruff), but more often the rhythms or cycles are evident in the reproductive activity of the protozoan. Cycles in the fission rate of *Paramoecium* were found by Calkins (1904) and by Woodruff (1905). Gregory (1909) showed that there were cycles of high and low vitality in *Stylonychia mytilus* and *Tillina magna*. The cycles were found to be fairly regular and the last work of Woodruff on *Paramoecium aurelia* (1917) has shown that even changes in the culture media and in temperature fail to modify the cycles of endomitis which are characteristic for the species. There may be a slight initial influence on the cycle of the *Paramoecium* when the ciliates are suddenly placed in a changed environment, but after a short period, during which a readjustment of the organisms takes place, the endomitic interval regains its normal length.

Cycles in the life history of *Haematozoa* are also known, the most classical examples of which are those of the various species of *Plasmodium*. In these protozoans not only is the reproductive process rhythmic in nature, but also that of sporulation.

In the flagellates the presence of cycles of vitality expressed in reproductive activities is also known. The haemoflagellates in their life history present striking examples of cyclic development, and one has only to watch the various free living flagellates in an ordinary aquarium to notice that a rhythm or cycle must exist for them in view of the fact that their numbers are seen to fluctuate from day to day and week to week.

For some time a cycle of encystment was suspected in *Giardia* found in the intestine of rodents, but it was during an investigation of mitosis in *Giardia microti* (Boeck, 1917) that evidence of such a cycle was found. A table in the paper referred to above showed that cysts were found upon examination in only five out of nineteen cases of infection. This fact raised the suspicion of the presence of a cycle of encystment, for if encystment occurs continuously in the life history

of each flagellate, then cysts should be present in every one of the hosts in which the examination of the large intestine had been made. (The large intestine is the region of the digestive tract in which cysts occur in greatest numbers.) Again, if encystment occurs at all times then cysts should always be found in faeces of rats infected with *Giardia,* and the number of cysts in the faecal sample of a rat should be approximately the same for each day. The process of encystment would then be an even, regular process from day to day and would not show evidence of a sudden rise and fall in the number of cysts found in the faeces.

The enumerations of the cysts of *G. intestinalis* in human dysenteric faeces made by Porter (1916) gave for the first time critical evidence of a cycle of encystment in this flagellate, which is a species allied to *G. microti.*

The importance of knowing whether or not there is a cycle of encystment in these flagellates cannot be overestimated. It would be of great significance in the therapy of dysentery caused by these organisms as well as a considerable factor to reckon with in the diagnosis of dysenteric patients by the daily examination of their stools, if a more or less regular cycle should be found to occur. It would be necessary to make a longer series of examinations in order to determine whether or not a patient were infected if a cycle of encystment is present in the life history of the flagellates causing the dysentery than it would if no such cycle were present. Accordingly daily examinations of the faeces of fifteen rats were begun and carried on throughout a period of twenty-eight days for the purpose of securing if possible sufficient additional evidence to definitely determine the facts.

MATERIALS AND METHODS

The faecal pellets of the rats vary in size from the average, about twelve millimeters in length and six millimeters in diameter, to about four millimeters in length and two millimeters in diameter. Constipation was present when the pellets of the smallest size were defaecated. The small size of the pellets could not be correlated with the absence or presence of cysts within them. At no time were the faeces liquid in consistency. There is no evidence of diarrhoea in the rats infected with *Giardia* like that caused by *G. intestinalis* in man and mice. The color of the faeces was usually a dark brown. Variations in the color from darker shades of brown to very light yellow were of no signi-

ficance as a diagnostic factor in the detection of cysts. The amount of stools defaecated each day varied and on no day did the rats fail to pass any stools.

In the examination of the stools for the first four days a modification (Boeck, 1917) of the method of faecal examination described by Cropper and Row (1917) was used. The method in its modified form is as follows: To at least one gram of faeces add thirty cubic centimeters of normal salt solution and stir with a Hamilton-Beach "cyclone" mixer for ten minutes. Then add five cubic centimeters of ether and stir for two minutes longer. The suspension is then placed in a separatory funnel and allowed to stand for five to seven minutes, during which time the two liquids will separate, the ether carrying most of the débris to the top while the cysts remain in the normal salt solution below. The normal salt solution is then drawn off into a centrifuge tube of a capacity of fifteen cubic centimeters and centrifuged for three minutes. The cysts are concentrated at the bottom of the tube and the supernatant fluid is drawn off with a pipette. A drop of neutral red solution, one part to ten thousand parts of distilled water, is added to a drop of the residue from the bottom of the tube and transferred to a slide for microscopic examination. The cysts are readily detected with a one-inch eye-piece and a four millimeter objective. The cysts measure about fourteen microns in length and six to seven microns in diameter.

The other examinations were made by making a suspension of the stools in distilled water, and stirring them until the mixture appeared uniform in density. Again a drop of neutral red solution of the same dilution as in the previous method, was added to a sample of the faecal suspension, which was then examined under the microscope for cysts. The neutral red is of great service in that it differentiates the cyst from the yeasts and débris, which in most cases are partially or totally stained while the cysts are very seldom affected by the stain and so stand out in the preparation as clear, transparent, ovoid bodies. The cysts also reveal in many instances their two or more nuclei, also the remains of the axostyle, intracytoplasmic flagella, and the parabasal bodies.

There is no doubt that the concentration of the cysts by the ether-centrifuge method is superior in accuracy to the simple microscopical examination described in the preceding paragraph, but because of the number of rats under observation this shorter method was used in this work. A count of the cysts was also undertaken at the time examinations were made. At first a haemocytometer was used, but it was

found that the cysts were never in sufficient numbers to make the use of this instrument practicable. The number of cysts were then counted in any twenty fields of the microscope, using a one-inch ocular and four-millimeter objective. The following table (1) shows the occurrence of the cysts in the faeces and the count that was made. The examinations commenced October 25, 1917, and were concluded November 21, 1917.

This table is a record of the daily examination of the faeces of the different rats, with the number of cysts counted in any twenty fields of the microscope. A negative sign signified that no cysts were found in the faeces for that day.

From a study of table 1 it will be seen that rat number 7 was negative for all the examinations. It received five treatments of magnesium sulphate in a twenty-five per cent solution and no cysts or living *Giardia* were seen in the semi-fluid stools. It was concluded that this rat was not infected with *Giardia*. Cysts were found in the faeces of rat 3 for three successive days, after which there was no recurrence. In rat 15 cysts appeared November 8 and not again until November 20 and 21, when the examinations for all the rats were concluded.

In the study of the rats in table 1, to determine whether or not there was evidence for a cycle of encystment, the data for rats 3 and 15 were not considered since neither showed two complete periods when cysts were ejected and consequently no interval could be determined between successive appearances of cysts. The data for rats 1, 2, 4, 5, 6, 8, 9, 10, 11, 12, 13, and 14 are the only data, then, that were used in this study of periodicity in the appearance of the cysts.

The length of the period during which the cysts were defaecated with the faeces varied from one to fourteen days, and the interval during which no cysts were found in the faeces varied from one to eleven days. There is a common feature seen in the records of most of the rats in that the ejection of cysts occurred at three or four periods during the twenty-eight days of daily examinations. The highest count of cysts was recorded on November 21 in the examination of the faeces of rat 14. There were eighteen cysts in twenty fields of the high power objective.

The data for each one of the rats was plotted so that each graph resulting would represent more clearly the evidence for the presence of a cycle of encystment in *Giardia microti*. The points on the abscissa represent the days when the examinations were made, while the points on the ordinate represent the number of cysts counted in twenty

TABLE 1

TABLE SHOWING THE NUMBER OF CYSTS OF *Giardia* IN THE FAECES COLLECTED DAILY FROM FIFTEEN RATS FROM OCTOBER 25 TO NOVEMBER 21, 1917.

The number of cysts were counted in any twenty fields of the microscope with a four millimeter objective and a one-inch ocular.

	October							November																				
	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Rat 1					4	7	2			6	6	7	4				1	1		5	4	7	3	3	5	2	3	10
Rat 2					2	3	5		2	3	3	9	4	1		2	2	2		2		4	5	5	1	5	1	
Rat 3										1	1			2														
Rat 4																	4	4	2	1	1							
Rat 5						1	5			1	1	2			6	1	2	1	1	1			2	2	3	5	10	5
Rat 6		5					4	10	5																			
Rat 7																												
Rat 8	3	5	7	6	2	2		2	2	5					5	5	3	3	3	1	5	3			4	1	3	5
Rat 9					2	2	3	2	2	1										4	1				3	2		4
Rat 10					3	3	4			3	3						1	1	1	1		6	3	3	2	2	3	7
Rat 11								2	2						1	2	1	1		1	2	3	1	1	13	9	6	15
Rat 12			2	2								3				3	3	3			2	9	9	9	2	4		
Rat 13		5								5	5			2		5												
Rat 14			10	12	5	4		4	4					5	2				1	2	3	2	10	10	5	10	7	18
Rat 15															3												5	7

fields of the high power objective.   Each one of these graphs will now be discussed.

Rat 1. This graph is plotted from the data of rat 1.   Its modes are very sharply defined since the periods of depression are characterized by an absence of cysts.   The first period when cysts were found in the faeces lasted from October 29 to November 1.   The mode of this part of the curve was reached on October 30.   A depression period of two days when no cysts were found in the faeces ensued.   This period endured for three days.   The mode recording the maximal number of cysts occurred on November 5.   A period when no cysts were found
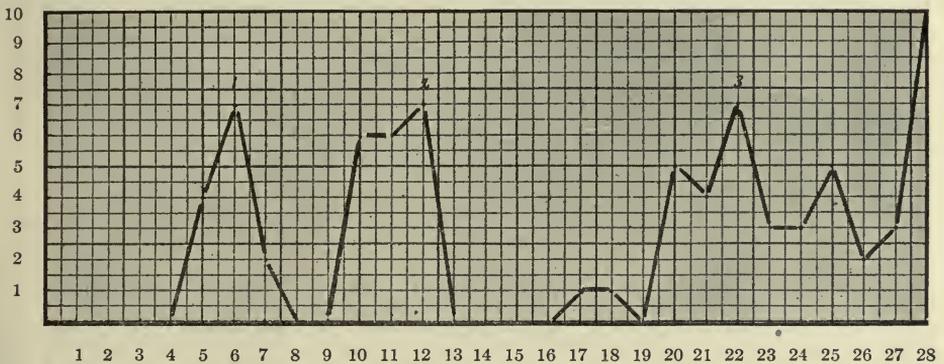
RAT 1

Fig. 1

in the faeces followed for four days, then there were two days when one cyst was detected, followed again by an examination in which no cysts were detected.   Another period of cysts in the faeces took place from November 12 up to November 21, when the last examination was made.   The mode of this period came on November 15, while another mode was in the process of formation because the number of cysts was increasing when the last examination was made.   The interval between mode one and two . is six days, and the second and third modes are separated by an interval of ten days.

Rat 2. This graph, made from the data of rat 2, is very similar to the graph in figure 1, there being three modes in the curve.   The first mode occurred on October 31 when four cysts were counted in twenty fields and the period of depression followed, during which the number of cysts decreased to two on November 2.   The cysts still continued to be found in the faeces and their number began to increase until another mode was reached on November 5.   A sharp falling off

in the number of cysts then took place, but the cysts were not absent from the stools until November 12. Two cysts were found on the following day, but none occurred on November 14. The third period of cysts in the faeces began November 15, and although there was a decrease in the number of cysts on November 18, to rise again on the following day, this period may best be regarded as continuing from

Rat 2
Fig. 2
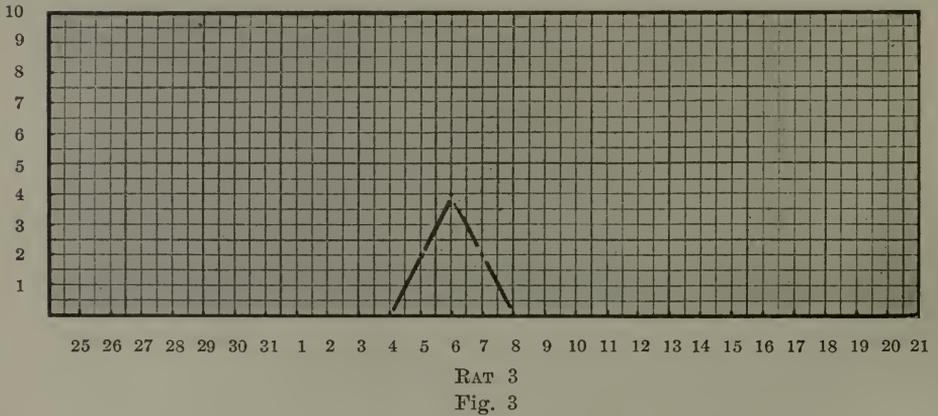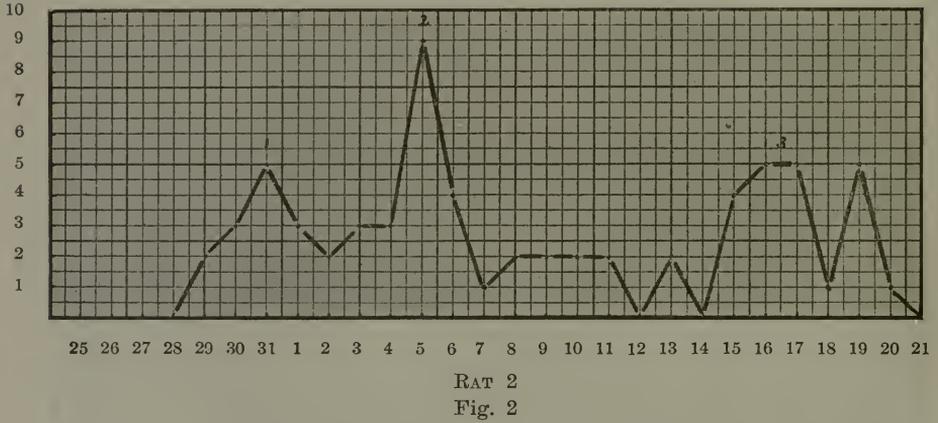
Rat 3
Fig. 3

November 15 to 21, when no cysts were found in the last faecal examination. The intervals between modes one and two, two and three, are five and eleven days, respectively.

Rat 3. There was only one period when cysts were ejected, November 5 to 7, and because they never recurred again the data from this rat could not be used to contribute any evidence on our problem. The rat was found free from infection at autopsy on January 9, 1918; the organs were normal, and none of the lesions of the intestine were present which characterize an infection by *Giardia*. The fact that

this rat was infected and then became free, showed that it was capable of throwing off the infection.   There was no reinfection as can be seen from the records, which run negative for thirteen days.

Rat 4. The degree of infection was very light in this animal for the greatest number of cysts found in the faeces was four on November 10 and 11.   There were two periods when cysts were found in the
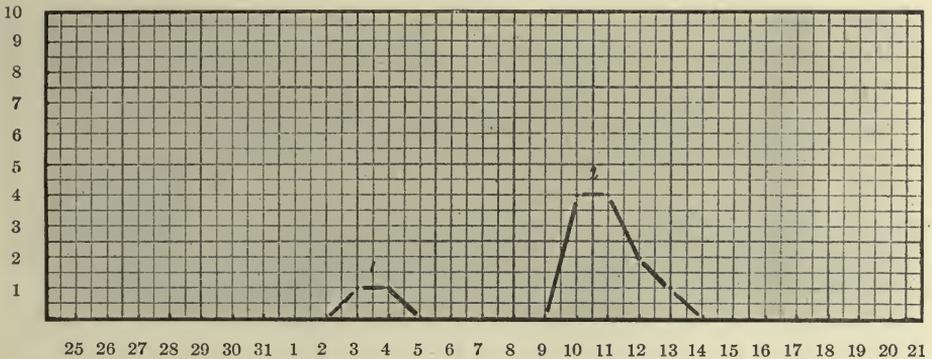
RAT 4
Fig. 4
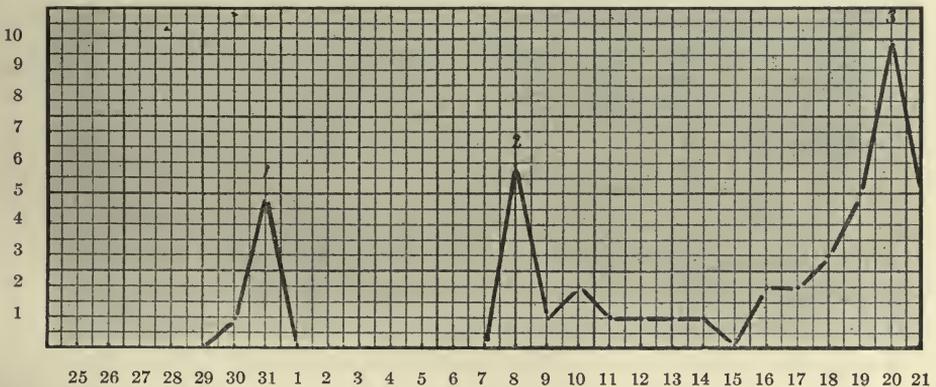
RAT 5
Fig. 5

faeces.   The first period was two days in length and only one cyst was found on each day.   Then came an interim of depression of six days in which the examinations revealed no cysts.   The mode of the second period of cysts in the faeces came on November 10, and the period ended November 19.   The interval between the two modes is seven days.

Rat 5. The first period of cysts in the faeces, October 30 to November 1, results in a portion of the graph sharply set aside from the

remainder, for after this period there follows an interval of depression when eight negative examinations occurred. The next period of cysts in the faeces came on November 5, when the number of cysts increased very suddenly to six, then fell to one on the next day, and remained at two or one for the five following days. The mode of this period came on the first day, when six cysts in twenty fields were counted. No cysts were counted on November 15, but on the next day the number of cysts in the faeces began steadily to increase until the mode of this period was reached on November 20, when ten cysts were counted. The number began to decrease when the last examination was made. The intervals between modes one and two, two and three, are eight and twelve days, respectively.
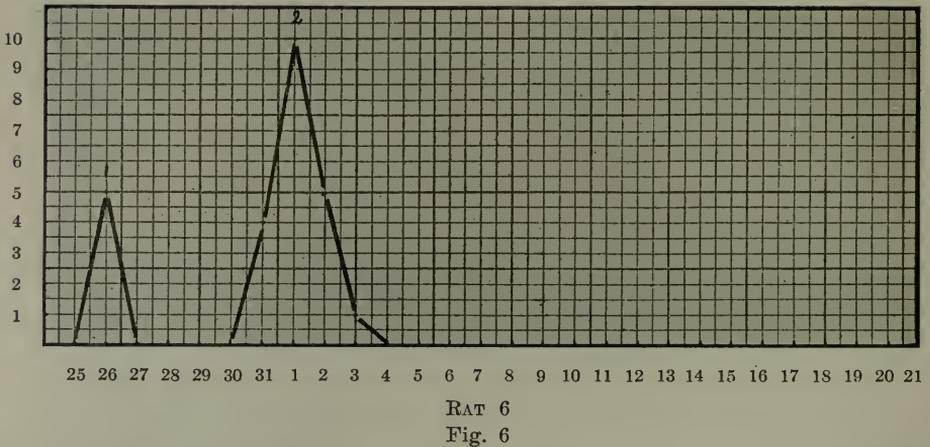
RAT 6
Fig. 6

Rat 6. This graph shows that two periods of cysts in the faeces occurred. These two periods were recorded in the first eleven examinations; the remaining examinations did not reveal any cysts. The first period lasted only one day, while the second period lasted four days. The interval between the modes of these two periods was six days.

This rat at autopsy on January 9 was still infected, even though it had eighteen consecutive negative examinations. The organs were normal except that there was a small amount of gas in the jejunum. In other respects this autopsy was identical with that of rat 3. The fact that this rat was still infected after having had eighteen consecutive negative examinations showed that reinfection may have taken place between the day when the last examination was made and the day the autopsy was made. Reinfection was possible for the cages

were not cleaned of their faeces each day after the last faecal exam-
ination had been made. The cysts which might have caused this
reinfection may have entered the cage by dropping through the wire
netting with faecal pellets. On two instances an infected rat became
free from its cage and ran over tops of the others and it is very likely
that a few of its pellets could have dropped into the cages of the
other rats.

Rat 8. There are three periods and an incomplete fourth when
the cysts were found in the faeces. The mode of the first period occur-
red on October 27. The period of depression between this mode and
the mode of the next period when cysts were in the faeces is very
well defined in that the curve decreases abruptly from mode one until
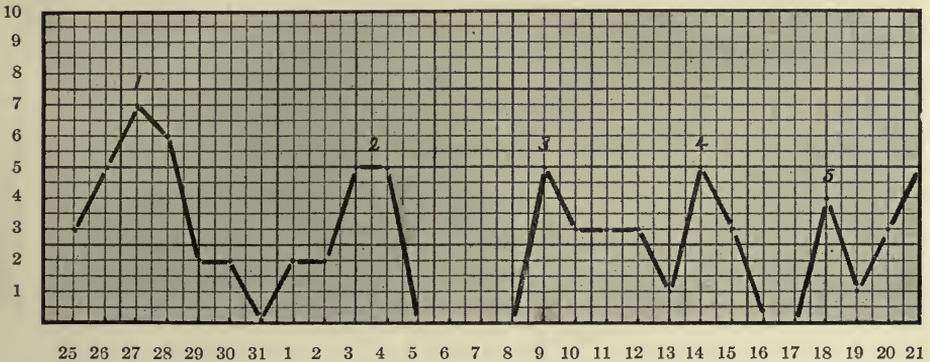
RAT 8

Fig. 7

on October 31 no cysts were found in the faeces, then the curve rises
again until the second mode is reached on November 3. The period
of depression between the second and third mode is defined by four
negative examinations. The third period of cysts in the faeces com-
mences on November 4 and continues until November 16. Two maxima
appear in the portion of the graph, and both of them have been termed
modes since there is a distinct period of depression on November 12,
but it is possible that we have in reality a single mode in this period.
The next period starts on November 18 and was continuing when the
last examination was made; but because of this last mode five may not
be the true mode of this period; instead the last one may be only a
stepping-stone to another mode which would have been reached if the
examinations had continued a greater length of time. The intervals
between the modes are 7½, 5½, 5, and 4 days, respectively.

Rat 9. There are three distinct periods when cysts were found in the faeces in this rat. This condition, we have seen, has been common to many of the curves already discussed. The first period of cysts in the faeces extended from October 29 to November 4. The number of cysts reached three in twenty fields at the mode on October 31. A period of depression then took place, from November 4 to 12, during which no cysts occurred in the faeces. From November 12 to 15 is

RAT 9

Fig. 8

RAT 10

Fig. 9
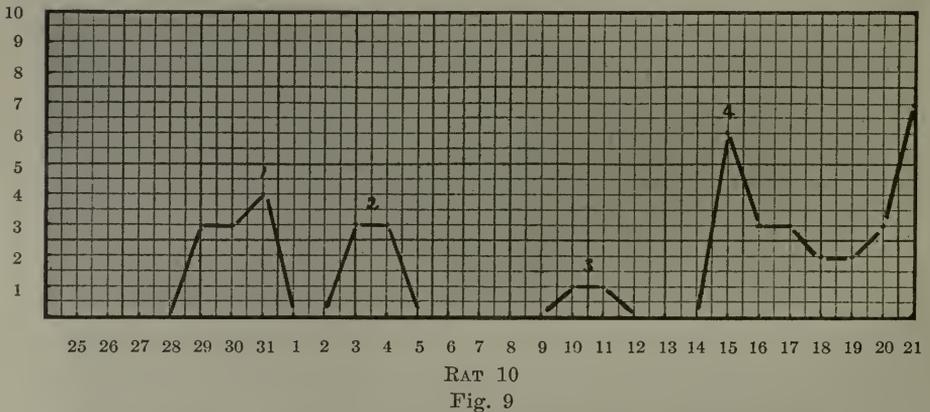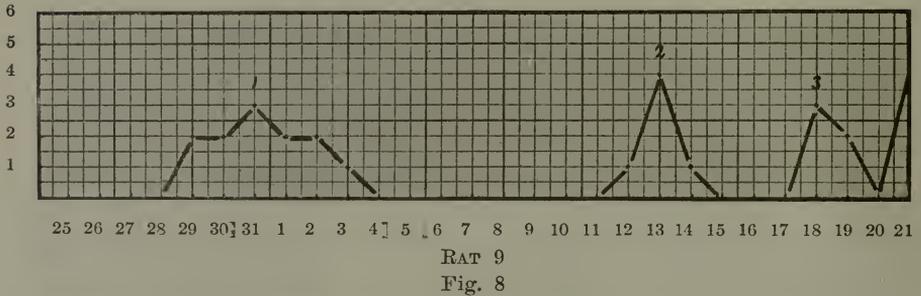
the next period of cysts in the faeces, with the mode on November 13. A period of depression of three days followed; no cysts were counted when the examinations were made. The cysts then appeared again on November 18, on which day a third mode is reached. But as in mode five, shown in figure 7, mode three in this figure may have proved to be a part of the rising curve following it, had the examinations been continued for a few days longer. The intervals between modes one and two and two and three are thirteen and five days, respectively.

Rat 10. There are four modes representing the days when the highest number of cysts were counted in four distinct periods. The first period, October 29 to November 1, was three days in length and

the mode came on October 31. The depression period between the
first two cycles of encystment was two days in length during which no
cysts were found in the faces. The next period of cysts in the faeces
was only two days in length, November 3 to 5, and four cysts were
counted on each of these two days. No cysts were then found for
five successive days when on November 10 and 11 one cyst only was
found. A depression period of two days with no cysts precedes the
last cycle of encystment, which was continuing when the last exam-
ination was made, to form another mode. The intervals between
modes one, two, three, and four are 3, 2, 7, and 4 days, respectively.
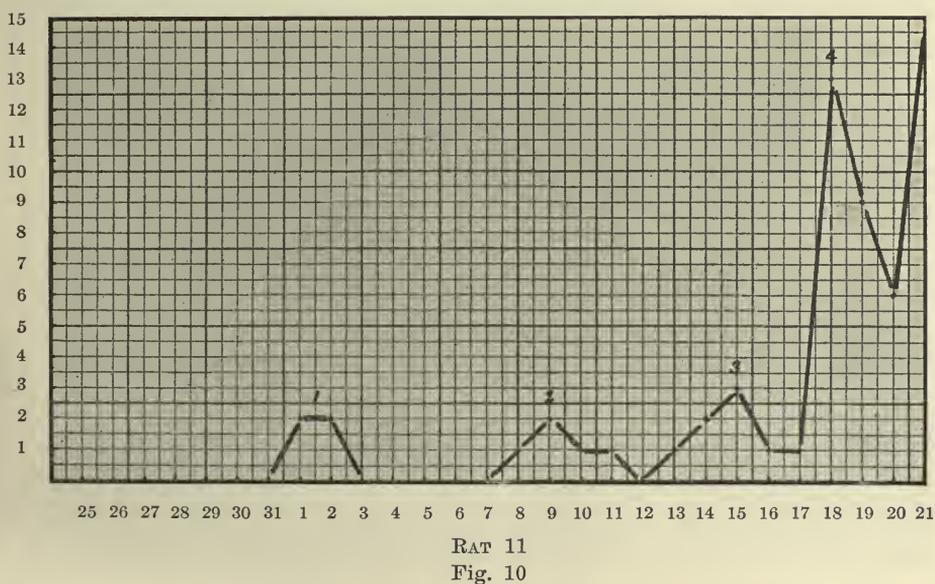
Rat 11
Fig. 10

The interval between the last mode and incomplete mode on November
21 is six days.

Rat 11. The first two periods or cycles of encystment were of
short duration and the number of cysts was never high. The first
cycle took place from November 1 to 3 and two cysts were counted
on each day. A depression period of five days followed this cycle
and no cysts were found in the faeces. The next cycle, from November
8 to 12, although longer than the first cycle, contained only a small
number of cysts. The mode of this cycle appeared on November 9.
Another mode is designated on November 15, which may be a true
mode since a period of depression follows it for two days, or this
portion of the cycle may be a part of the period of cysts in the faeces

which follows the period of depression on November 18, when a pronounced mode is seen in the curve. After November 18 a short period of depression is characterized by a.falling off in the number of cysts found, but their number begins to increase again, and had reached fifteen cysts when the last examination was made. The intervals between the modes are 7½, 6, and 3 days, respectively.
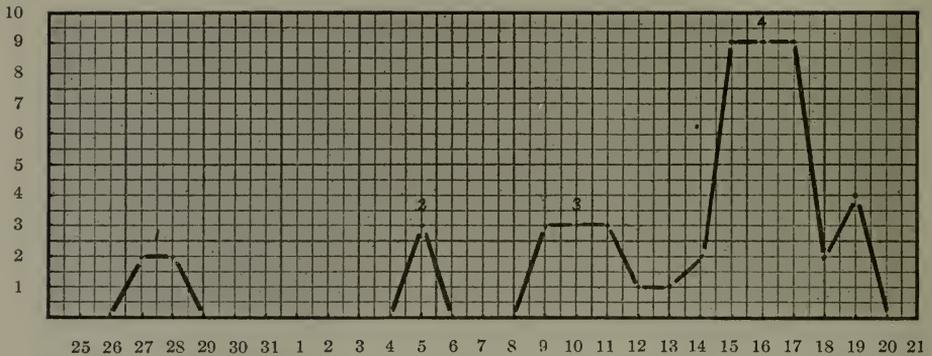
RAT 12
Fig. 11
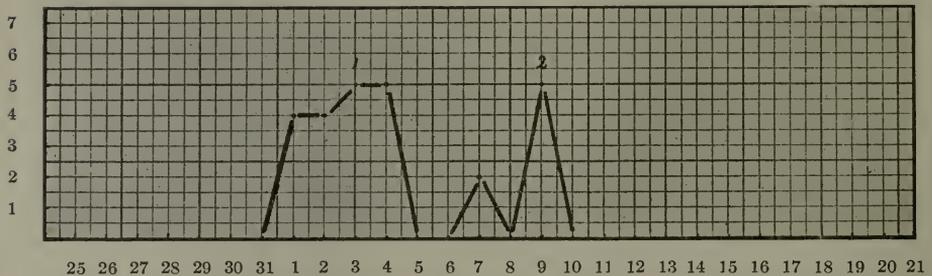
RAT 13
Fig. 12

Rat 12. There are four cycles of encystment represented in the graph which was made from the data of rat 12. The cysts were found during two successive days in the first cycle October 27 to 29. A period of depression then continued for seven days when no cysts were found in the faeces. The cycle which followed was only one day in length and only three cysts were counted in the examination of the faeces. The third cycle is preceded by two days when no cysts were found. The mode of the third cycle was reached on November 10 when three cysts were found in twenty fields. A couple of days when the number of cysts decreased to one in twenty fields then ensued, to

be followed by a cycle when the number of cysts reached nine for three successive days. The cysts had disappeared from the faeces when the last examination was made. The intervals between the modes one, two, three, and four are 8½, 5, and 6 days, respectively.

Rat 13. The infection of the rat was light, for the number of cysts was small and there were only two cycles of encystment during the twenty-eight days. The first period when the cysts were in the faeces extended from November 1 to 5, with the highest number of
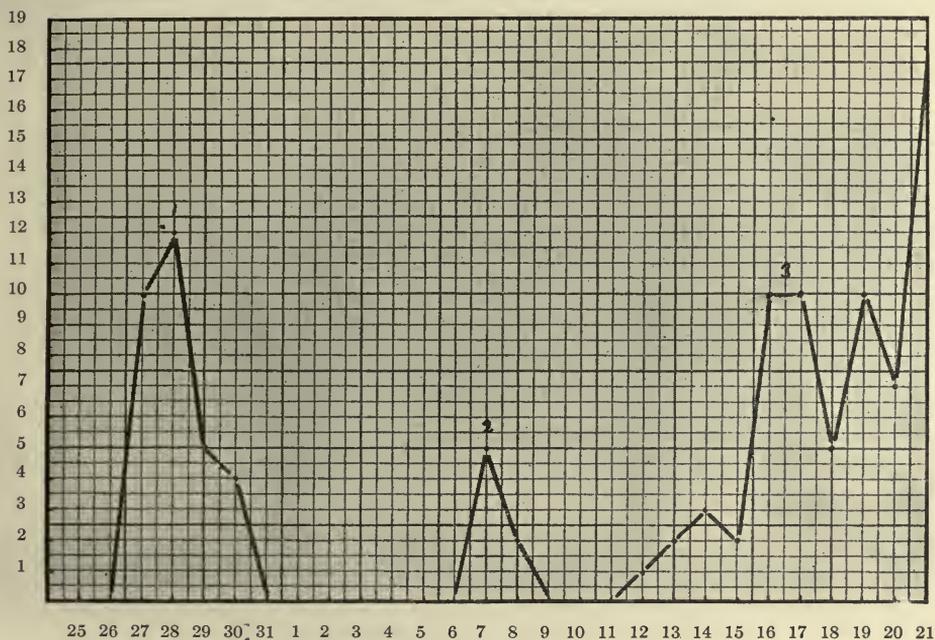
RAT 14
Fig. 13

cysts coming on November 3 and 4; then for a day no cysts were found. The next period of cysts in the faeces was from November 7 to 10. In this period there was one day, November 8, when no cysts were found; but since the number of cysts found in the other days was very small it is probable cysts were present on November 8 and escaped detection because of their small numbers. The mode of this second period was reached on November 9 when five cysts were counted in twenty fields. The interval between the two modes is six days in each case.

Rat 14. The infection in this rat was the heaviest for all the rats examined. The maximum numbers of cysts detected ran higher than

the numbers of the other rats. There were three distinct periods when the cysts were found in the faeces. The first period lasted four days, October 27 to 31. The mode of this period came on October 28, when 12 cysts were counted. A period of depression of eight days when no cysts were found in the faeces preceded the next period when the cysts recurred in the faeces. This period was short, lasting only two days, with the mode on November 7. Two days followed during which the cysts disappeared from the faeces. Then the third period when cysts recurred again in the faeces began November 12. This period extended to November 18, when a period of depression marked by a fall in the number of cysts took place. The mode was reached on
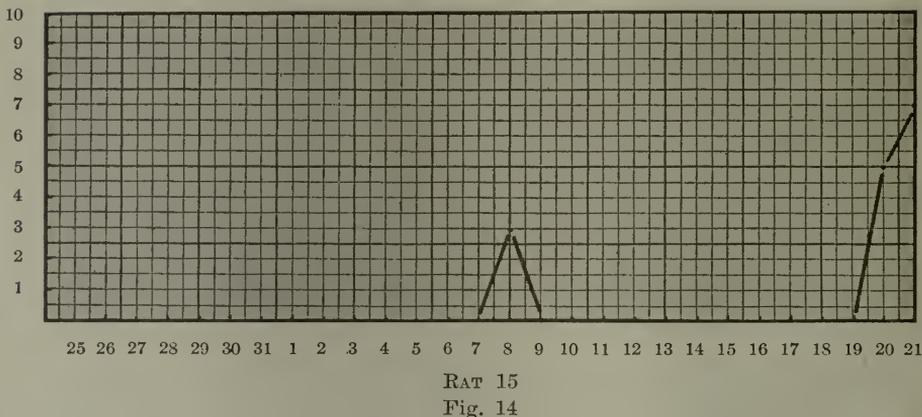
Rat 15
Fig. 14

November 16, when 10 cysts were recorded for twenty fields. After the single day when the number of cysts decreased to five the number immediately increased and when the last examination was made the number had reached eighteen in twenty fields. This was the largest number of cysts found in twenty fields for all the rats examined. The intervals between the modes one and two and two and three, are 10 and 9½ days, respectively.

Rat 15. The infection in this rat was very light, for during the twenty-eight days cysts occurred only three times and in small numbers, though the number of cysts was increasing when the last two examinations were made. It will be seen that there are not two complete periods when cysts were found in the faeces, and because of this fact these data of rat 15 could not be used to determine the cycle of encystment in *Giardia* of the rat.

The series of graphs just presented may be divided into two groups. In the first group may be placed those curves which show positive

examinations comprising less than one-half the total number of exam-
inations. The graphs of figures 3, 4, 6, 12, and 14 fall into this class.
In the second group may be placed all the other groups; in these more
than one-half the total number of examinations were positive.

What caused this great difference in the degree of the infection
in the rats is not known at the present time for there is no evidence
at hand with which to attack this problem; but it is very significant
that, even when the positive examinations comprised less than half
the number of all the examinations which were made, there are still
distinct periods into which the positive examinations fall.
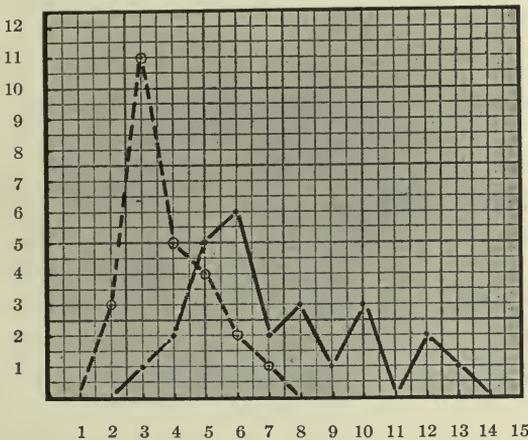
Fig. 15

Frequency curve of modal intervals.

Curve (solid) plotted by single-day units (on abscissa) of the day intervals
between the modes of all the figures 1, 2, 4, etc.
Curve (broken) plotted by two-day units, of the day intervals between modes
of all the curves. It is a more typical frequency curve.
The interval (average) between modes is shown to be seven days. Seven
days is the interval then, between the maxima number of cysts in the faeces.

This graph represents the combined plot of the intervals between
the modes of all the curves described in the preceding pages. The
units on the abscissa stand for the length, in days, of each interval;
the units on the ordinate stand for the frequency with which any
given interval occurred.

The solid line is a frequency curve whose descent is characteris-
tically lytic in nature; the latter feature is eradicated if only the out-
side points are represented in the curve. The broken line curve was
made from the same data as the solid line curve except that the units
on the abscissa are two days in length instead of a single day. This

doubling of the units on the abscissa results in the curve being one-half as long on the abscissa, while the height of the mode is far more increased; furthermore, it serves to straighten out the descent of the curve, giving a more typical frequency graph.

This graph presents striking proof of the existence of a cycle of encystment in *Giardia* in the rat, for we see that the average interval between the modes of the cycles of encystment is about seven days. Most of the intervals were about six days in length. In other words, the maximum number of cysts were found in the faeces about every seven days. This curve indicates that encystment falls into regular periods, the climax of each recurring period being reached about every seventh day.

If a series of curves be so placed one above the other that the first mode of each curve lies on the same ordinate line and the rest of the curve be allowed to fall as it will, if there is a similarity or a close identity of the interval between the other modes of each curve, the modes of all the curves should, in the majority of the cases, fall along as many common ordinate lines as there are modes. Since most of the curves have three or four modes we should be able by such a handling of the graphs as that given above to detect four common ordinate lines upon which the majority of all the modes of the curves will be located. The distance between these lines would be the true interval between the crest of each mode, or the interval between the days when the maximum numbers of cysts were found in the faeces.

Such a treatment of the curves is the most conclusive proof of the presence of similar intervals common for all the curves. Obviously a high degree of exactness is impossible with the curves made from a study of the cycle of encystment in rats, since the incidence of infection varies in the case of each rat and there is also a margin of error in the detection of the infection. However, there is, as we have seen, evidence for an interval between the ejection of the maximum numbers of cysts, and this interval can be seen by superimposing the curves one above the other with their first modes coinciding, in order to determine whether or not the other modes in the curves will in the majority of cases also coincide on other common ordinates.

In figure 16 the curves have been placed one above the other so that their first modes lie on the ordinate line at point 6 on the abscissa. In this figure of superimposed curves it will be seen that the second mode for the majority of the curves is on an ordinate line, at a place between points 11 and 12 or 13 on the abscissa; the third
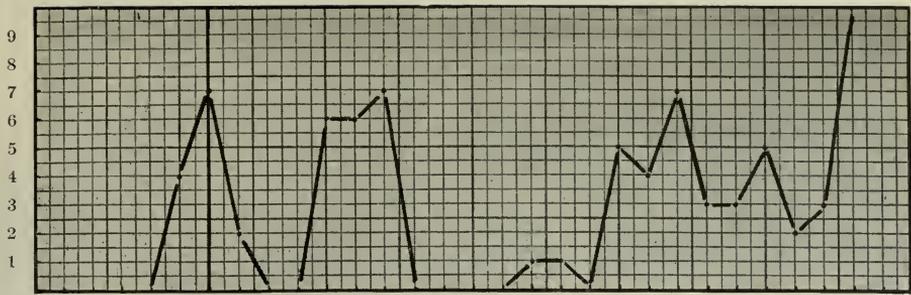
RAT 1
Fig. 1
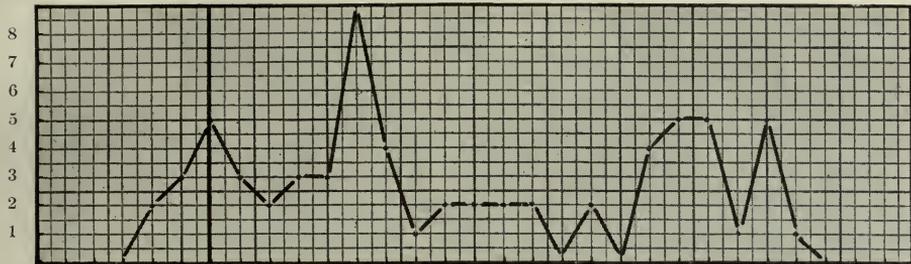
RAT 2
Fig. 2
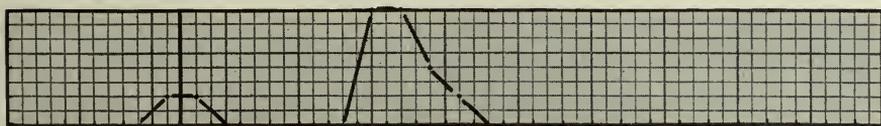
RAT 4
Fig. 4
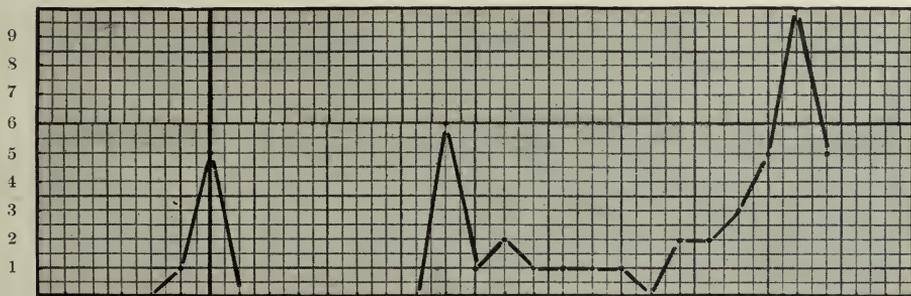
RAT 5
Fig. 5
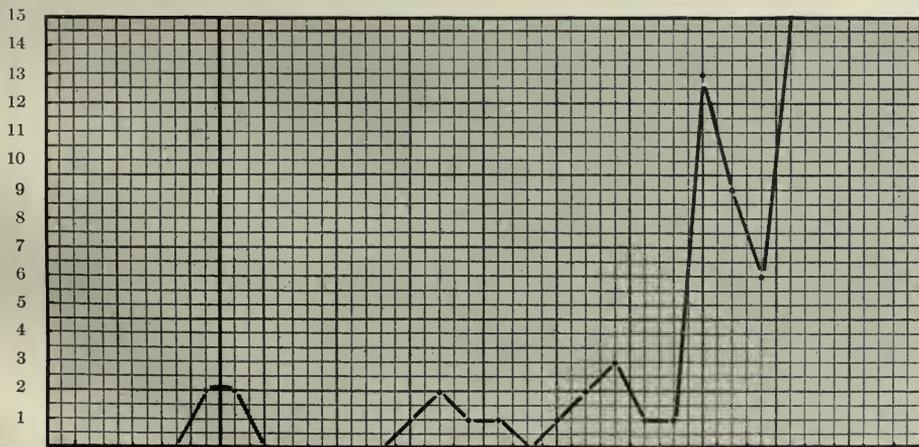
RAT 11
Fig. 10
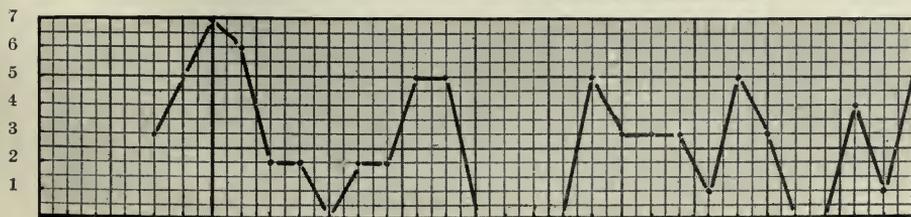
RAT 8
Fig. 7
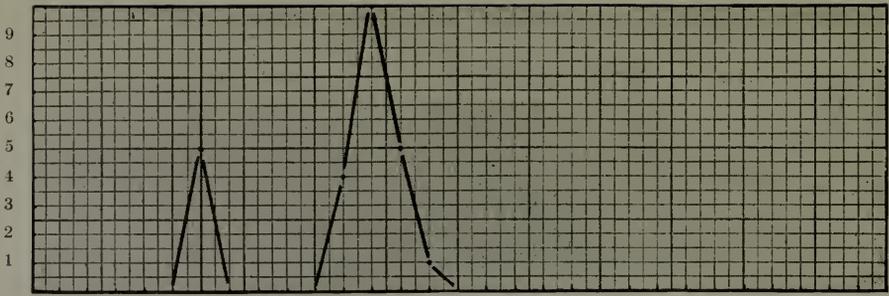
Fig. 16

Fig. 17
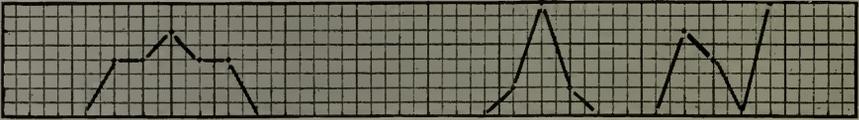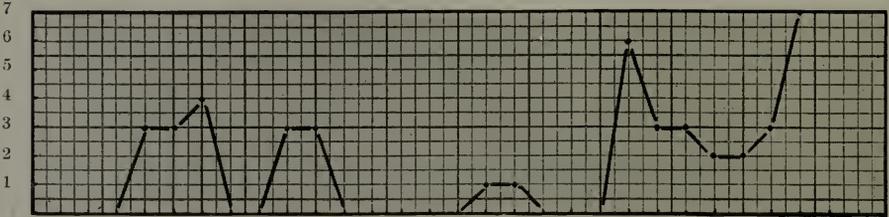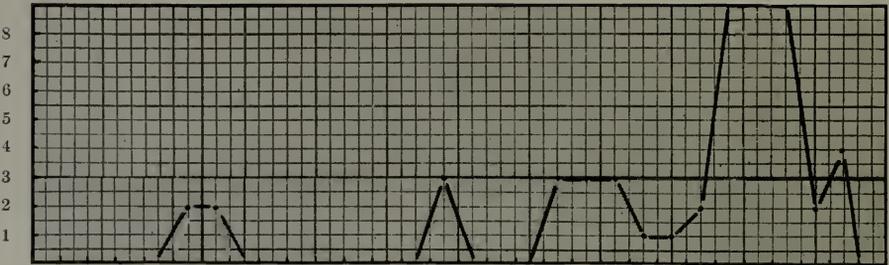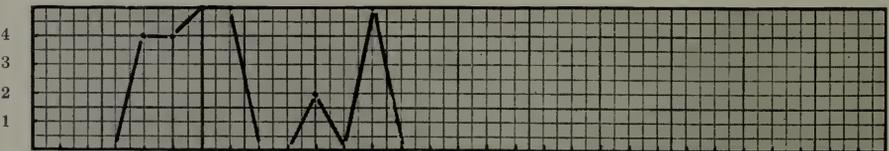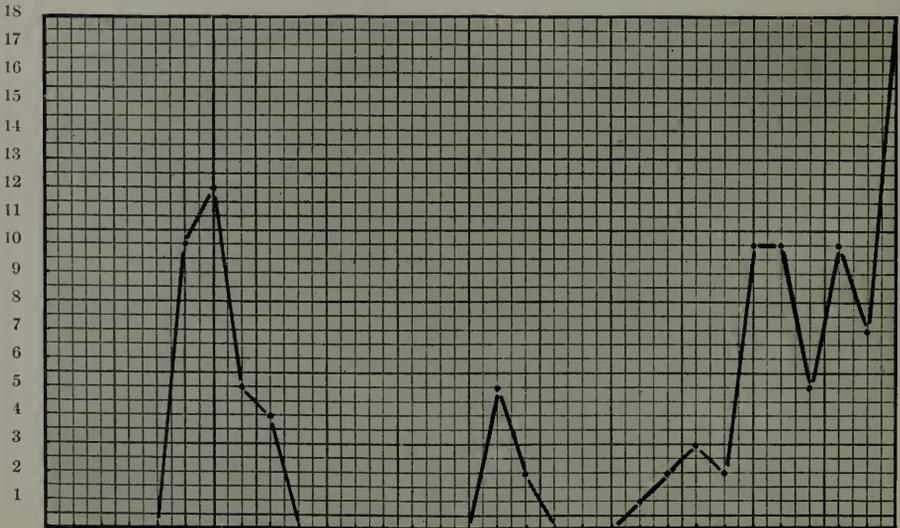
mode of those curves having three or more modes lies on the ordinate line in the region of points 19 and 20 on the abscissa, while the fourth mode of the curves which have four modes is seen to lie on the ordinate line at points 25 or 26 on the abscissa.

In all cases these ordinate lines determined above were selected because the majority of the modes of all the curves fell on these four lines. Thus ten curves had at least two modes, and of these ten six fell on the ordinate line in the region of points 11, 12, or 13 on the abscissa. Five curves showed a distinct mode in the region of points 19 and 20 on the abscissa, and eight curves showed definite modes on the ordinate line in the region of points 25 and 26 on the abscissa. From this evidence it is justifiable to conclude that there is a common interval between the modes of all the curves when they are placed one above the other so that their first modes lie upon a common ordinate line. This interval is approximately equal between all the ordinate lines. The intervals between the ordinate lines are 6, 7, and 7 days; their average interval is 6⅔ days.

The average interval of 6⅔ days as obtained from the super-imposition of the curves, is almost identical with the average interval of 7 days obtained by plotting the frequency of all the modes (see fig. 15). Thus by two different methods approximately the same interval has been obtained, setting the interval between the maximum numbers of cysts ejected in the faeces at about every seven days. In other words, the cycle of encystment of *Giardia* occurred about every seven days in the rats under observation during a period of nearly one month.

In the superimposition of the curves and in the consequent study of the data to determine the common ordinate lines no consideration was taken of the terminal point of all the curves, because the curve was in the process of forming another mode in most cases when our observations were suspended. Therefore, because of its incompleteness this portion of the curve was useless in the determination of the cycle of encystment.

In order to substantiate the evidence for these common ordinate lines which mark the days when the maximum number of cysts occurred in the faeces of most of the rats examined I plotted the occurrence of the average number of cysts for each day when the curves were thus superimposed. This is the average number of cysts that occurred on each ordinate line in figure 17. For example, on ordinate line 4 in figure 16 two cysts were found twice, three cysts

twice, and four cysts once. The average number of cysts on this ordinate was three cysts, and this was the number plotted in figure 18 on ordinate line 5 as representing the average number of cysts for that day. In like manner the average number of cysts was determined for the other days and plotted in figure 17.

The modes 1, 2, and 4 are at once very evident and it will be found that they lie on the same ordinate lines that were determined in figure 16. The third mode is not so conspicuous, which I interpreted to mean that the incidence of encystment was small and disturbed, possibly by some environmental factor for all the rats whose modes go to make up this *common mode*. But it will be seen, as was pointed out in connection with figure 16, that 6 rats showed a distinct mode at this area in their curve. Of the other six rats one was showing a small number of cysts at this time (fig. 5), three were negative, and no cysts were found in the following examinations (figs. 4, 6, 12), so these curves could not be considered; the other two rats were negative during these days. Therefore, *out of eight possible cases six* of them showed a distinct mode at point 19 (fig. 17), which made it justifiable to pick out this line as representing a *common mode* for most of the curves. It was the day when a maximum number of cysts was found.

The mode at 26 (fig. 17) was chosen rather than the mode at 28 because more individual curves showed a distinct mode at this day, and so represented more truly the day when the maximal number of cysts was found for the majority of all the rats.

The average interval between the modes 1, 2, 3, and 4 (fig. 17) is found to be 6⅔ days, which corresponds to the interval between the ordinate lines determined in figure 16.

DISCUSSION

From the data just presented the conclusion is derived that there is a cycle of encystment in *Giardia* in the rat, that this cycle is regularly periodic, and that the interval between successive maximum numbers of cysts is about seven days.

It is fully realized that the possibility of error is fairly large in the study that has been made in determining this cycle. In the first place because of the time required for examination only one examination was made to determine whether or not a rat was negative for any given day. To eliminate this error at least three ordinary examinations should be made to determine whether a rat is negative. Had it

been possible to continue the examinations by means of the ether-concentration method, adopted later, then three ordinary examinations would not have been necessary, one examination by this method would have sufficed.

Again, in all the studies of this nature the more cases one works with the safer are his conclusions; therefore, if a greater number of rats could have been handled the conclusions would have a greater degree of certainty. But even with this relatively small number of cases, when the results are to a great extent uniform, the conclusions are at least highly significant and to a considerable degree may be relied on as presenting the truth of the situation. The data from
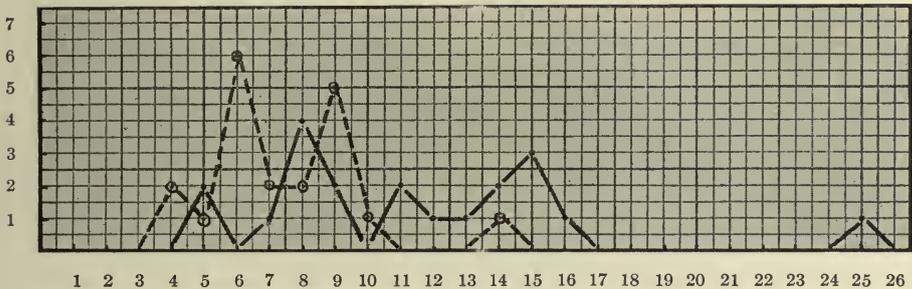
Fig. 18

Frequency curve of mode intervals; data taken from charts submitted by Porter (1916).
Solid line, interval plotted by single-day units, modes at 8, 15, 24.
Broken line, interval plotted by two-day units, modes at 6, 9, 14.
The broken line curve, a typical bi modal polygon.
The interval between the maximal number of cysts is about 7–8 days. Fourteen days represents two intervals, and the small mode at 24 represents three intervals.
These results from studies of seven cases of *Giardia intestinalis* are very similar to those given by me for *G. microti*.

the fourteen rats presented by several methods yield in every case the same fairly uniform and equivalent cycle of encystment.

Another source of evidence is available for comparison to demonstrate the value of these results in this small number of cases. In determining the cycle of encystment for *Giardia intestinalis* from man and mice Porter (1916) noticed that there was a period of about a fortnight between the maximum number of cysts recurring in the faeces. She did not, however, call attention to the fact that there was also a period of about seven or eight days when another maximum number of cysts was to be found in the faeces. This she would have found apparently if the day-interval between the modes in all her figures had been plotted.

It is significant that a plot made of analogous data in my own study reveals only one mode (fig. 15) and sets the period at seven days as the interval between maximum numbers of cysts in the faeces. The curve is a typical frequency curve with a single mode.

In plotting the interval between the modes in the curves submitted by Porter (1916) there are two distinct modes present (fig. 18). It is a bimodal frequency curve. The solid line in figure 18 represents the interval plotted by single-day units on the abscissa, just as was done in figure 15 of my own study. Two distinct modes are seen at points 8 and 15 and a small mode at point 24. There was only one instance of an interval of 24 days (see chart 1, Porter, 1916). When these same data were plotted by two-day units on the abscissa, the
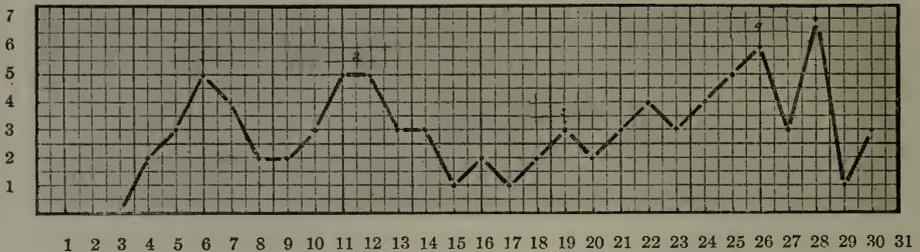
Fig. 19

A plot of the *average* number of cysts for each day for rats 1, 2, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, leaving out of consideratoin the number of cysts which occurred on the last examination in each rat.

Modes 1, 2, and 4 are very definite; mode 3, although not so pronounced as the other modes, was selected since in six rats this point represented the days of a maximal number of cysts in the faeces. Mode 4 at point 27 on the abscissa was selected instead of the mode at point 29 because it was more representative of all the curves; five curves had a mode at or near point 27, while only two curves had a mode at point 29.

The average day-intervals between modes 1, 2, 3, 4, is 6⅔ days are almost identical with the intervals found in figure 15.

broken curve results, and again we find that there are two modes present. This curve thus plotted represents two, if not three (point 24), distinct periods at which time maximum numbers of cysts were found in the faeces for *Giardia intestinalis*.

From this study of Porter's charts and the plottings of the modes of her graphs it is evident that the cycle of encystment in *G. intestinalis* has an interval of about seven to eight days between the maximum number of cysts in the faeces. The mode at point 24 (fig. 18) is the locus of a third occurrence in one instance only of a maximum number of cysts, which suggests that there was a tendency at least for the cycle to continue at the second interval of fourteen days on the interval

of the first two maxima. The results are very striking and uniform in this series of only seven cases, and taken together with the data derived from my study of the cycle of encystment in *Giardia* of the rat give ground for concluding that there is a regular periodicity in the appearance of the cysts of *Giardia* in the faeces of about six to seven days.

It is quite possible that the species of *Giardia* found in some of the rodents, especially in *G. microti,* is one and the same species as that found in man, namely, *G. intestinalis,* because of the similarity in structure. Another reason for believing that these species may be identical is revealed by the study of periodicity made by myself on *G. microti* and by Porter (1916) on *G. intestinalis,* and also by the manipulation of her data in the plotting of the curve representing the frequency of the interval between modes, or the days when the maximum number of cysts was detected. This study shows that both of these species have almost identical cycles of encystment. This feature may be common for all the species of *Giardia,* therefore a characteristic of the genus and not a peculiarity of a single species only; but in either case it is significant as evidence toward the solution of the true etiology of giardiasis (lambliasis) of man.

Summary

1. There is a cycle of encystment in *Giardia* in the rat; the recurring interval between the maximum numbers of cysts in the faeces is about seven days.

2. The cycle of encystment in *G. intestinalis* is about seven or eight days, as seen in the charts submitted by Porter (1916), and not a fortnight, as she has concluded. This in reality, if not fully plotted, represents two cycles of encystment.

3. Because the cycles of encystment of *Giardia* in the rat and of *G. intestinalis* are almost identical there is another reason to infer that these two species may be one and the same. This fact may lend some aid in the solution of the true etiology and prevention of giardiasis (lambliasis) of man.

DEVELOPMENT OF *GIARDIA MICROTI* WITHIN THE CYSTS

Introduction

The author (1917) has pointed out the presence of three distinct types of cysts for *Giardia microti*. These types were placed in three categories because their morphology warranted such a classification. The first of these types is the "single individual" cyst (pl. 1, fig. 1). This cyst harbors only one individual which has the usual two nuclei. The second type is the "binary" cyst (pl. 1, figs. 5–8), containing four nuclei. The cytoplasmic body may not have undergone plasmotomy, (pl. 1, figs. 4, 5) or plasmotomy may be completed with two daughter flagellates formed (pl. 1, figs. 7, 8). The third type is the "multinucleate" cysts, so called because it always contains more than four nuclei and never more than sixteen nuclei (pl. 1, figs. 12–16).

A study of these cysts and their location in the intestine of the mouse or rat at once suggest definite progressive development of the flagellate within the cyst. To give an account of this development with a view to supplying more evidence toward completing all the stages in the life history of this parasite, the following data are submitted.

Materials and Data

The first part of these data were gathered from a study of the material from five different meadow mice, *Microtus californicus californicus* (Peale). Other preparations were made but only five series revealed the presence of cysts. The following table shows the types of cysts found in the different regions of the digestive tract of the meadow mice.

Cysts were not always found in all the regions of the digestive tract (series 28, A4) and this was explained by virtue of a cycle of encystment. In series 28 the process of encystment had been going on for some time and the cysts had already progressed as far as the colon and rectum, while in series A4, the process of encystment had just begun and so the cysts were found only in the small intestine. In series 29 and 33 preparations made from all the regions of the intestine showed the presence of cysts. Encystment was in the midst of its cycle. Series 29 and 33 present two parallel lines of data, identical in all respects except that in series 29 only single and binary cysts were

TABLE 2

TYPES OF CYSTS OF *Giardia microti* IN INTESTINE OF *Microtus*

Series	Sex	Date	Small intestine	Caecum	Colon	Rectum
28	♀	March 2, 1916			single binary	single binary
29	♀	March 4, 1916	single	single	mostly binary	mostly binary
33	♀	March 8, 1916	single multinucleate	single	single mostly binary	single mostly binary multinucleate
36	♀	February 8, 1917	single multinucleate	no preparation made		
A4	♂	September 27, 1917	single			

found, while in series 33 not only were the single and binary cysts present but also multinucleate cysts. This latter type of cyst was found chiefly in the small intestine.

The probable reason why the cysts were found in so few of the infected mice at autopsy was the fact that a negative period in the cycle of encystment was then in progress.

In *Giardia microti* there are two types of reproduction, binary and multiple fission. In taking up the evidence for the development of this flagellate within the cysts a review will be made first of the data that are related to the reproductive method by binary fission and then the data concerned with the method of multiple fission.

Binary fission may occur in the free state of the flagellate, as has been shown by the work of Kofoid and Christiansen (1915) and by the author (1917), and also within the cyst; the latter fact has been known for a long time. Previous to the work mentioned above, describing binary fission taking place in the free state, it had been held that this method of fission took place only within the cyst. Schaudinn (1903) had noticed two individuals within a single cyst wall and had called such a cyst a "copulation cyst" because he thought the two flagellates were in syngamous union. It had been previously shown, however (Boeck, 1917), that these cysts were only binary cysts and that there is no evidence of sex in *Giardia* as Schaudinn had inferred.

Binary Fission Within the Cyst

When encystment takes place it involves the formation of a wall around a single flagellate. This process begins in the ileum and caecum of the meadow mouse. The greatest number of single cysts are consequently found in the ileum or caecum of the digestive tract. From table 2 it will be seen that these single cysts may also be found in the colon and rectum, but their number in these regions is very small compared with the number found in the small intestine. The ratio in series 29 in one preparation was fifty-five binary cysts to five single individual cysts in the colon and rectum.

The fact that there were great numbers of single cysts in the ileum and only a few in the large intestine, great numbers of binary cysts being present in their stead, coupled with the presence of nuclear changes within the cysts, is conclusive evidence of progressive multiplication within the cysts.

The single cyst (pl. 1, fig. 1) is strongly indicative of recent encystment because one can easily detect within it all the organelles of the

flagellate characteristic of its free state, the only exception being the absence of the extracytoplasmic parts of the anterolateral, postero-lateral, and caudal flagella. The intracytoplasmic portions of these last named structures are still present within the cyst.

In the metamorphosis of a single individual cyst into a binary cyst the nuclei undergo division. Distinct anaphase spindles were found in many cases (pl. 1, fig. 3), but no late prophase, metaphase, or telophase stage was found. The mitotic activity during these stages is probably very rapid while the anaphase is of longer duration. The telophase was detected in cysts of *Giardia* found in the rats (pl. 1, fig. 12). In no instance was the chromatin of either nucleus seen to have resolved itself into chromosomes. Centrosomes were dis-tinguishable in some of the cysts (pl. 1, figs. 2, 4), but in no case were they found to be divided with one daughter centrosome located at each pole of each nucleus.

In the cyst shown in plate 1, figure 1, the nuclei of the organism appear to be in the resting stage, for the karyosome is a single ovoid mass of chromatin. This stage in the development within the cyst is very typical of the nuclear constitution of all the single-individual cysts found in the ileum and the caecum.

In the caecum single-individual cysts were also found, which, however, present the first stage of definite progressive development beyond that described in the preceding paragraph. These cysts (pl. 1, figs. 2–4) show the chromatin of the nuclei dividing or already divided into two separate masses upon a spindle, with evidence of their migration to the poles of each nucleus. This stage corresponds to the anaphase in mitosis. Passing along the digestive tract from the caecum to the colon we find a few of the single-individual cysts are still to be found, even as far down as the rectum, but their number is very small.

In the colon the next stages of development within the cysts were found. The nuclei have divided to form four daughter nuclei (pl. 1, fig. 5), which are very close to each other and in the resting stage. The cytoplasmic body within the cyst is still in the condition of a single encysted individual, no plasmotomy having as yet taken place. The axostyle has partially divided, but only two distinct parabasal bodies are to be seen.

The next stage in the progress of binary fission within the cyst is also to be found in the cysts in the colon. In this stage (pl. 1, fig. 6) two of the nuclei have migrated to a position which was earlier the

posterior pole of the body of the parent, but no plasmotomy has as yet taken place. During this stage another set of intracytoplasmic organelles, namely axostyle, anterior peristomal fibrils, and postero-lateral flagella have been formed. The method of forming the axostyle is one of splitting of the parent structure (pl. 1, fig. 3), but there is no evidence at hand to determine the exact method of formation of the other organelles.

In the colon and in the rectum occur the binary cysts (pl. 1, figs. 7, 8) which represent the completion of binary fission. The method of plasmotomy appears to be a longitudinal division of the parent on a plane parallel to the major axis of the body and at first horizontal, at least in the matter of nuclear separation, for even before plasmotomy has begun (pl. 1, fig. 6) two of the nuclei and the axostyle are seen to be upon a different optical plane from that of their sister structures. The axostyle, however, appears to split in the sagittal plane. When plasmotomy has been completed a side view of the two flagellates within the cyst shows one individual above the other. The line of separation of the two flagellates may have no reference to any plane of plasmotomy. Kofoid and Swezy (1916) have shown that movement in trichomonads is very active during plasmotomy in the free state. It is quite possible that this also occurs within the cyst in *Giardia*. An oblique view (pl. 1, fig. 8) shows the two flagellates occupying such a position that their anterior ends are at opposite ends of the cyst; this is the position which they might assume if plasmotomy occurred by a longitudinal cleavage of the body in a plane horizontal to the major axis of the body. However, in typical binary cysts the two zoöids lie in an end to end, back to back position.

It is noteworthy that in all the cysts of the binary type the individuals resulting from binary fission of the parent flagellate lack their respective parabasal bodies. This absence has been shown by the author (1917) to be correlated with the depletion of the reserve material in the form of these bodies during encystment, and with excessive mitotic and motor activity of the flagellate while in the free state. These bodies at first hypertrophy during encystment, often they are scattered like a cloud of chromotoidal substance in the cytoplasm (Kofoid and Christiansen, 1915), but are always absent in the free somatella stage or when plasmotomy during binary fission within the cyst has been completed.

Infection is known to occur by the ingestion of the cysts; no intermediate host is necessary. The cyst wall in all probability is digested

off when the cysts reach the small intestine. Those cysts which contain two individuals, products of binary fission, would discharge two flagellates into the intestine when the cyst wall was digested away. It is also possible that cysts might be ingested which had not completed all the stages of binary fission, because such cysts were found in the rectum, and if this happened and the cyst wall was digested away then a somatella would be liberated which could continue its development in a free stage.

A word may be said here regarding these somatella stages described many times by Kofoid and Christiansen (1915) for *G. muris* and by the same investigators and myself for *G. microti*. Most of these somatella stages represent plasmodial bodies resultant from previous mitotic activity of individuals which had not encysted. Obviously the large number of individuals present in the intestine can be explainable on the ground of binary and multiple fission in the free stage of the flagellate, but another interpretation may be proposed for the somatella stages.

These stages may be interpreted to be somatellas liberated from cysts while the flagellate body within was in the course of binary fission. Especially does this seem plausible when we compare the free somatella described by Kofoid and Christiansen (pl. 8, fig. 55; 1915) and the encysted somatella described in this paper (pl. 1, fig. 6). Furthermore, these authors figure cysts with part of the cyst wall apparently digested away and the somatella in a process of binary fission (pl. 7, fig. 38; Kofoid and Christiansen, 1915). The interpretation that these somatellas may have originated from encysted individuals which have escaped from the cyst seems justifiable in view of the facts just presented. But most of these somatella stages no doubt originate from an individual undergoing binary fission in the free state.

Multiple Fission Within the Cysts

During the process of multiple fission within the cysts the two nuclei of the encysted flagellate go through three successive divisions to form a total of sixteen daughter nuclei. The method of division is that of mitosis, for anaphase spindles were found in many cases (pl. 1, fig. 10), but the other phases of mitosis were much obscured and were therefore detected with gerat difficulty. The chromatin of each nucleus was never seen to be divided into chromosomes. A somatella containing sixteen nuclei is found in every multinucleate cyst which has completed all its nuclear divisions (pl. 1, figs. 10, 11).

From table 2 we see that the greater number of these cysts were found in the small intestine, and in one series they were found in the rectum. This was interpreted to mean that encystment commenced in the small intestine (duodenum) and the cysts were in the rectum because they had been carried there by the peristaltic movement of the bowels.

Encystment, it appears, begins with the formation of a wall around a single individual, and the immediately subsequent stages are similar to those in binary fission preceding plasmotomy of the two-zoöid somatella. Many of the stages following the first division of the nuclei were not found. Some of the cysts showed twelve nuclei (pl. 1, fig. 9) due to the fact that some of the nuclei have not divided the third time. In some cysts (pl. 1, fig. 10) two of the nuclei were seen to be dividing for their last time (third division). No plasmotomy was seen to occur within the cyst, but from the evidence given by Kofoid and Christiansen (1915) it is more probable that plasmotomy takes place outside the cysts after the digestion of the wall of the cysts in the small intestine.

This method of reproduction within the cysts does not seem to be the common method employed by this flagellate, for the cysts were detected in only two cases in my preparations of *G. microti* from the meadow mouse and were never detected in some two hundred examinations of cysts of a species of *Giardia* found in the rat. It is a method which results in a greater proliferation of the individuals when compared with binary fission. In the rat the binary cysts were found in the faeces at regular intervals, showing that encystment was cyclic and not continuous or sporadic in nature. But since the multiple cysts were not found with such regularity it appears that this method of reproduction does not follow at regular intervals unless its interval of recurrence is much longer than that found for binary fission. It may be, then, that disturbance of the normal environment causes an increased fecundity on the part of the flagellates which results in the method of multiple fission for its expression. The possibility of multiple fission following the formation of a zygote by syngamy is not excluded.

These cysts when ingested would have their cyst walls digested away as in the case of the binary cysts. Since each one of the multinucleate cysts contained a somatella of sixteen nuclei an eight-zoöid somatella would be liberated to continue its plasmotomy in a free state. Cysts showing part of the cyst wall absent were figured by Kofoid and Christiansen (1915, pl. 7, figs. 42, 53) and these cysts

contained somatellas in various stages of multiple fission. The absence of part of the cyst wall is interpreted to be due to digestion and a continuance of the process would result in the liberation of the somatella. Somatella comprising as many as four zoöids were found by the investigators just mentioned, but in no case were eight-zoöid somatellas found. The case of the four-zoöid somatella appears to have resulted from the multiple fission of a flagellate which had never encysted, instead of representing a possible half of the eight-zoöid individual which might have come from a cyst.

In all the multinucleate cysts the axostyle had split to form two daughter axostyles. This is similar to the activity of the parent axostyle during binary fission, but the other organelles were not duplicated which is unlike the condition in binary fission. It is probable that the formation of the other organelles is deferred until the process of plasmotomy begins, that being the time that they appear in binary fission.

It is evident from all the data at hand that the processes of binary and multiple fission are very similar if not identical in their early stages. Both methods begin with the encystment of a single individual, and the subsequent stages up to the formation of a two-zoöid somatella are alike. The process of binary fission ceases when plasmotomy of the two-zoöid somatella results in the production of two daughter flagellates; but with the formation of the two-zoöid somatella the process of multiple fission still goes on. The nuclei have gone through one division to form four nuclei, the four nuclei go through another division and eight nuclei are formed, and these eight nuclei, when they divide, form sixteen nuclei. There are three successive divisions of the nuclei in multiple fission but only one division in binary fission. The two processes differ in that multiple fission does not appear to occur so often as binary fission since the cycle of encystment during which binary fission takes place is shorter than the cycle of multiple fission, if a cycle of multiple fission exists. It is probable also, as has been stated, that multiple fission is initiated by some environmental disturbances and, therefore, may not be cyclic but sporadic in nature.

The data for binary and multiple fission within the cysts of *Giardia microti* were taken from preparations made from the meadow mouse. A further collection of data will now be presented to corroborate the evidence for binary and multiple fission within the cyst. These further data were taken from a study of the cysts of a species

of *Giardia* found in culture rats. This species of *Giardia* resembles *G. microti* as far as the general form of the body and the organelles are concerned. The species is longer than *G. microti,* measuring 13 to 17 microns in length and 6 to 9 microns in width. The cysts, however, are approximately the same size as those of *G. microti;* they measure 11 to 13 microns in length and 5 to 7 microns in width (compare pl. 1, figs. 5, 13).

The locus of infection in the rat differs from that of the meadow mouse. In the meadow mouse the flagellates were found in all the regions of the small intestine, but in the rat the jejunum is the area that is most heavily infected. No flagellates occurred in the duodenum and only a few in the ileum. Again, it was found that the intestine of the meadow mouse was not discolored by an infection with *Giardia* (Boeck, 1917), but in the rat the jejunum is orange colored and filled with gas when *Giardia* are present. This latter condition is very similar to the condition found in culture mice and *Peromyscus gambeli* when infected with *G. muris.*

A study was made of the cysts found in the different regions of the small and large intestines in order to see if the method of development for this species of *Giardia* during encystment was similar to that of *G. microti* during its encystment.

The following table gives the types of cysts found in the different regions of the digestive tract of the mouse.

TABLE 3

TYPES OF CYSTS OF *Giardia* IN INTESTINE OF RAT

Series	Sex	Date	Small intestine	Colon	Rectum
A5	♂	Oct. 12, 1917	single binary	binary	binary
A6	♂	Oct. 21, 1917		binary	binary
200		Feb. 8, 1918	binary	binary	binary
304		Feb. 8, 1918		binary	binary
307		Feb. 8, 1918	single binary	binary	binary
313		Feb. 8, 1918	binary	binary	binary

It will be seen at once that the only types of cysts encountered in the preparations were the single-individual and the binary cysts; no multinucleate cysts were ever found. In the meadow mouse the binary cysts did not occur until the cysts had reached the colon on their transit through the intestine to the outside of the mouse, but in the rat the binary cysts occurred in the jejunum along with the flagellates in the free state. The single-individual cysts were few in number compared with the binary cysts, and so it would seem that the

division of the nuclei to form four daughter nuclei follows soon after the encystment of the flagellate. The cysts are defaecated as binary cysts, for over two hundred preparations from the faeces were examined and showed the cysts at a stage of development in binary fission the same as that found in the small intestine.

In the rat the binary cysts occurred throughout the large intestine and were found in the faeces in a stage of development which was not in advance of the stage that was found in the jejunum and ileum (pl. , figs. 14, 16). A few of the single-individual cysts were also found in the large intestine, but the number was almost insignificant compared with the number of binary cysts. The single-individual cysts of *Giardia* found in the rat resembled very closely those of *G. microti* which were found in the meadow mouse (cf. pl. 1, figs. 1, 12).

Mitosis, with its phases somewhat modified, is the method of nuclear division. At no time in the cysts examined could chromosomes be detected during the process of mitosis. Anaphase spindles (pl. 1, fig. 12) were very common. When the new nuclei are formed two remain in the anterior region of the cyst; but the other pair appear to migrate to a more posterior position, and they lie upon a different optical plane from that occupied by the other pair (pl. 1, figs. 13–16). This difference in optical planes of the two pairs of nuclei is due to the direction of the major axis of the spindle during the division of the parent nuclei. The major axis of the spindle is directed dorsoventrally, but in many cases the spindle may be tilted obliquely in an anteroposterior direction (pl. 1, fig. 12).

The fact that cysts found in the faeces are in the same stage of development as those found in the small intestine makes it appear that further development of these cysts in the intestine is arrested. The two-zoöid somatella present in the binary cysts was never found to have undergone plasmotomy while in the cyst, as was the case in *G. microti* found in the meadow mouse. It is very probable that further development is dependent on the cysts being ingested by another rat. The action of the enzymes of the stomach it is believed serves to prepare the cyst wall for digestion when the cyst reaches the small intestine; otherwise, we should expect those cysts found in the small intestine before the defaecation to lose their walls, without making ejection of the cysts from the rat and subsequent ingestion necessary. The reason why the development of this species of *Giardia* found in the rat should be arrested during encystment is a matter of

conjecture at this time. It is very probable that this species is different from *G. microti* although their structure is very similar. If it is a new species then it is very probable that there is simply a difference in the rate of development between *G. microti* and this new species. The first stages of development are similar in all respects and there is reason to believe that the following stages are also similar. The greatest difference is in the location of the different stages in the various regions of the intestine. The fact that binary cysts were found in the small intestine leads one to believe that the early stages of development, including the formation of the two-zoöid somatella in this new species, were more rapid than the corresponding rate in the formation of these stages in *G. microti*, but that after the cysts had gone through these stages of binary fission the rest of the development was slower or arrested when compared with *G. microti*, which continued to pass through all the other stages of binary fission even to the completion of plasmotomy within the cyst.

On the other hand, the presence of cysts in the faeces in an early stage of binary fission might be accounted for by a more rapid rate of peristalsis in the rat compared with the rate of peristalsis in the meadow mouse. In order to test this hypothesis the cysts which were found in the faeces were incubated at 31° C for several days continuously and examined at intervals of one, four, and five days. The temperature of 31° C was chosen because it was lower than the optimum of most of the bacteria found in the faeces. This would tend to prevent excessive proliferation on the part of the bacteria which would hasten the death of the encysted flagellates. This temperature is considerably lower than the body temperature of the rat, but it has been used for the cultivation of Protozoa with some degree of success.

Upon the examination of the cysts at the intervals stated above no noticeable changes in the development within the cysts were found. The condition of the body within the cyst was identical with that when the cysts were defaecated. The cysts decreased markedly in their number, which may be due to their death through the agency of the bacteria. The incubation failed to cause any further development but it cannot be said that this experiment disproves the supposition of the effect of peristalsis. Incubation with higher temperatures and with cultures of cysts free from bacteria should be tried before condemning the hypothesis.

Summary

From the data which has been reviewed in the foregoing pages the following facts regarding the life cycle of *G. microti* are established.

There are two distinct phases in the life history of the flagellate: the one a free vegetative phase; the other encystment.

During the vegetative phase the animal may pass through the following stages:

As an adult organism it may undergo mitosis in which case there is:

Subsequent formation of a two-zoöid somatella and later plasmotomy to form two daughter flagellates; or,

The formation of an eight-zoöid somatella, followed by plasmotomy to form eight daughter flagellates; or,

A single flagellate may encyst.

During encystment the animal may pass through the following stages:

By binary fission two daughter flagellates may form within the cyst which will be liberated when the cysts are ingested by another host.

By multiple fission an eight-zoöid somatella may be formed which will be liberated to complete plasmotomy upon the ingestion of the cyst by another host and the digestion of the cyst wall.

In case cysts should be ingested before they have completed either binary or multiple fission, a somatella would be liberated upon the digestion of the cyst wall which would continue its development as a somatella in a free state.

THE PARABASAL BODIES OF *GIARDIA MICROTI*

Introduction

In many of the parasitic Protozoa the process of nutrition does not always result in a growth of the cytoplasmic body, but instead there may result the formation of bodies in the cytoplasm, which act as reserve food materials. These bodies may be intimately connected with the motor activity of the organism, especially with the metabolic activity, in which case they will be used later during rapid growth, during reproductive periods, or during encystment, when the original source of nutrition is cut off.

These bodies have been called *metaplastic* bodies because they result from the metabolic activity of the organisms and are deposited in the cytoplasm. Among these bodies may be cited the paramylum grains of flagellates, the paraglycogen grains of gregarines and ciliates, the plastinoid granules of coccidia, and the *parabasal* bodies of flagellates.

Function of the Parabasal Bodies

In a previous paper by the author (1917) a short account was given of the behavior of the parabasal bodies of *Giardia microti*. It was pointed out then that these organs were metabolic reserve centers of food materials, which acted as ''conveniences on the part of the flagellate for coping with the intestinal medium in which it lives. They appeared to be more intimately connected with the metabolic activity of the parasite rather than its motor activity.''

This hypothesis was based only upon morphological aspects of these bodies during encystment and during the life of the parasite in the free state. For the sake of completeness the activity of these parabasal bodies during encystment and in the free state of the flagellate may be reviewed.

In the free-living adult (full-sized) flagellate there are two parabasal bodies located in the posterior third of the body and lying dorsal and usually across the axostyle. The bodies are usually elongate fusiform in shape. In the cyst the bodies may be two or more in number and are greatly hypertrophied at the beginning of encystment. In the cyst the bodies are located in various regions but always in the posterior part of the body. They often spread out like the tail of a

comet (pl. 1, figs. 1, 4, 5, 13, 15, 16), or they may appear as conglomerations of a material, cloud-like in character through the cytoplasm. Such was the condition of the parabasal bodies during multiple fission in *G. muris* (Kofoid and Christiansen, 1915).

During mitosis in the free state of *G. microti* the parabasal bodies are always present in the stages of the prophase and in the metaphase, but they were often lacking in the anaphase and telophase and almost without exception they are missing in the stages of plasmotomy. The only exception was an instance described in which a parabasal body was seen in one of the daughter flagellates in the process of plasmotomy of a two-zoöid somatella in the free state (Boeck, 1917). In the investigations upon *G. muris* during mitosis the parabasal bodies were absent in the late phases of mitosis and always absent in the two-zoöid somatellas resulting from binary fission (Kofoid and Christiansen, 1915). Likewise in the four-zoöid somatella resulting from multiple fission of a flagellate in the free state, the parabasal bodies were lacking.

With the encystment of *G. microti* the parabasal bodies hypertrophy to a great extent (pl. 1, fig. 1) and as the process of binary fission within the cyst continues the bodies disappear (pl. 1, figs. 6–8). In multiple fission within the cyst of *G. microti,* there also occurs an increase in size of the parabasal bodies (pl. 1, fig. 9), and when the sixteen nuclei have been formed in many cases the parabasal bodies are absent or they appear faint (pl. 1, fig. 11). The somatella stages found in a free state may have resulted from their liberation from a cyst by the digesting away of the cyst wall, and in these stages, it has been pointed out, the parabasal bodies have disappeared.

It was on these morphological aspects of the parabasal bodies during mitosis and during encystment that the conclusion was reached that these bodies were reserve food centers to be utilized during periods of reproduction and during encystment, when the original source of food supply had been cut off. At such periods as these there is an extra drain on the food supply of the flagellate, since the rate of metabolism during reproductive activity is great, and since during encystment not only are reproductive processes carried on, necessitating a drain on the food supply, but also encystment itself may extend over a long period of time, which necessitates an extra food supply. This food reserve is depleted at the end of encystment, when, for the most part, the reproductive processes within have also been completed.

BIOCHEMICAL AND STAINING QUALITIES OF THE PARABASAL BODIES

More evidence, not morphological but biochemical in nature, is required to determine the function of the parabasal bodies.

When either the free forms or cysts of *G. microti* are stained with Heidenhain's iron haemotoxylin the parabasal bodies appear very dark, and for this reason it was previously thought that these bodies were chromatoidal in nature and of probable chromatinic origin. In *Giardia microti,* however, there has been no evidence of chromidia within the nuclei or escaping from the nuclei. Again, increase in size of the parabasal bodies is not accompanied by a decrease in size of the karyosome of the nuclei, so this evidence would militate against the origin of the parabasal bodies from the nuclei.

Since iron haemotoxylin is a selective stain for both chromatin and cytoplasmic structures, which in most cases are of cytoplasmic origin, it was thought more prudent to use other stains substantively, in order to fix the chromatophilly of the parabasal bodies. When the preparations of flagellates and cysts were stained with acid fuchsin and methyl green the parabasal bodies appeared red and the chromatin of the nuclei appeared green. From this reaction the parabasal bodies were thought to be acidophillic, and so differed from the chromatin of the nuclei which was basophyllic. In about half of the flagellates the parabasal bodies appeared to be situated upon or in a definite area of the cytoplasm, limited by a membrane-like structure. In other cases the two parabasal bodies appeared as if stained by iron haemotoxylin, i.e., just two fusiform bodies. But when the area enclosed by a membrane-like structure appeared, that portion of the area not occupied directly by the parabasal bodies was lighter in staining reaction than the parabasal bodies or the cytoplasm surrounding this parabasal area. There seemed to be evidence of another substance along with the parabasal bodies, the two together constituting the parabasal complex, so to speak. This other substance might well be the ground substance, or parabasal-plasm within which the parabasal bodies are situated.

To substantiate the acidophyllic nature of the parabasal bodies preparations were treated with basic fuchsin. In these preparations no flagellate was seen in which the parabasal bodies or parabasal-plasm was stained, but in all cases the parabasal-plasmic region was identified as a more refractive area than the surrounding cytoplasm. It was certain, then, that the constitution of the parabasal bodies

differed from that of the chromatin of the nuclei, that their constitution was acidophyllic. Some of the very preparations which when
stained with basic fuchsin revealed no parabasals were subsequently
treated with iron haematoxylin and the parabasals reappeared; they
had been present but the basic fuchsin did not stain them.

Alexeieff's (1917) work with flagellates, especially *Trichomonas
augusta,* has shown that the parabasal rod is of mitochondrial constitution. Instead of the rod, in times of division there may be a row
of mitochondrial granules which he believes form the parabasal rod
by forming chondriomites. He has also shown that the mitochondria
are structures which secrete glycogen, which is utilized by the motor
and metabolic activity of the organism.

When preparations of free flagellates killed in Schaudinn's fluid
and fixed in ninety-five per cent alcohol were treated with Lugol's
solution to test for glycogen in the parabasal bodies two distinct
conditions of the parabasal-plasm were noted. The parabasal bodies
themselves did not appear but only the glycogen contained in the
parabasal-plasm. In the first place the glycogen was seen as a rectangular, mahogany colored mass filling all the parabasal-plasm for no
membrane-like structure could be detected; or the glycogen assumed a
thin rod-shaped mass, or was in two smaller masses. In the latter two
cases, however, these glycogen masses were lying the parabasal-plasm,
which was easily identified by the more darkly stained, ovoid, membrane-like structure which surrounded them. In the second place, other
individuals did not show any glycogen present in the parabasal-plasm,
but the area itself was clearly seen as a refractive, rectangular body
lying in a position identical with that occupied by the parabasal bodies.
The cases which revealed no glycogen in the parabasal-plasm were
about the same in number as those that showed glycogen present. Out
of fifty flagellates, twenty-three showed glycogen present, twenty-four
showed the absence of the glycogen, and three flagellates showed no
glycogen or parabasal-plasm present. These three casese were interpreted to mean the complete absence of the parabasal bodies and
parabasal-plasm.

It was thought at the time that perhaps those flagellates which
showed only the parabasal-plasm present actually lacked parabasal
bodies. Accordingly some of the preparations treated with Lugol's
solution were stained with iron haemotoxylin, and after examining fifty
flagellates forty-seven were found to have parabasal bodies and three
did not. This was identical with the ratio counted in the preparation

treated with only Lugol's solution. But out of the forty-seven flagellates showing parabasal bodies present twenty of them showed the presence of the parabasal-plasm lying between the parabasal bodies. This was attributed to previous treatment with iodine since preparations not treated with iodine previous to staining with iron haematoxylin, fail to show as distinctly the presence of the parabasal-plasm.

Some glycogen is lost through processes of killing and fixation of the flagellates, so that the glycogen which remains is only a portion of the original amount present in the parabasal-plasm. In view of this fact, in the case of those flagellates which showed only parabasal-plasm without glycogen, the lack of glycogen may have been due to the loss of the comparatively small amount present in the parabasal-plasm previous to the killing and fixation of the preparations.

Origin of Parabasal Bodies

The origin of the mitochondrial granules found in *Trichomonas augusta* around the nucleus, along the parabasal rod, and in the axostyle may be of nuclear origin since previous to mitotic activity a distinct chromidial cloud is seen about the nucleus. These chromidia may have been extruded from the nucleus since there is also an intranuclear chromidial cloud present at the same time. Alexeieff (1917) also finds that the mitochondrial granules are azurophyllic when stained with Giemsa. This reaction being characteristic of the chromatin of the nucleus Alexeieff believes that the reaction is further evidence of the probable nuclear origin of the granules. It seems safe to infer from this paper that if the mitochondrial granules form the parabasal rod indirectly this rod is also of nuclear origin.

It has been mentioned previously, however, that in *G. microti* there is no evidence of chromidial extrusion or chromidial clouds, and because the parabasal bodies are markedly acidophyllic there seems little evidence for attributing nuclear origin to these organs. Again, glacial acetic acid in the killing fluid failed to dissolve the parabasal bodies, which would have been expected if they were mitochondrial in constitution. From the evidence at hand it appears best to designate these parabasal bodies along with the parabasal-plasm as structures derived directly from the cytoplasm. They are metaplastic in nature, since they are formed from the anabolic processes of metabolism and tend to disappear when the katabolic activity exceeds anabolism. This is especially marked during the final stages of mitosis, plasmotomy

and encystment with its incident processes of reproduction. When the parabasal bodies have disappeared they are reformed from substances out of the cytoplasm, when metabolism is again normal.

Alexeieff (1917) has termed the parabasal bodies of flagellates the ''kinetoplaste;'' but it does not seem that this name is appropriate for the parabasal organs of *Giardia* since as we have seen that there are actually two substances which make up the parabasal complex. In cognizance of the presence of the parabasal bodies themselves lying in or upon another substance it seems to the author best to refer to the bodies as parabasal bodies which secrete glycogen. The glycogen is stored up in the other substance, the ground work or *parabasal-plasm*. The word kinetoplaste connotes the motor but not the metabolic significance of the parabasal bodies and because, as we have seen, the parabasal bodies are more intimately correlated with the metabolic activity of *Giardia* it is best to discard the word and to still refer to the complex as parabasal bodies and parabasal-plasm.

Summary

From the foregoing data the parabasal bodies were found to be:
Acidophyllic in constitution.
They are of cytoplasm origin.
Their function is to secrete glycogen which is retained for subsequent use in the parabasal-plasm. The glycogen constitutes a reserve food supply which is utilized during the reproductive period and during encystment.

THE THERAPEUTIC VALUE OF BISMUTH SUBNITRATE AND BISMUTH SALICYLATE IN THE TREATMENT OF GIARDIASIS (LAMBLIASIS) OF RATS

Introduction

The use of these two salts in the chemotherapy of giardiasis of man was attended with some success in England and consequently encouraged further treatments with these same chemical compounds in order to ascertain their true therapeutic value.

The account of the bismuth treatments of giardiasis of man was given by Porter (1916), who made an enumerative study of the cysts of *Giardia intestinalis* occurring in the stools of dysenteric patients.

Procedure

Finding a large number of rats infected with *G. microti*, a species similar to *G. intestinalis* (the history of which was followed for a period of one month by daily faecal examinations) six were selected which showed the heaviest infection. The degree of infection was determined by the number of positive examinations of the faeces made daily during the month. Three of the rats were treated with bismuth sub-nitrate and the other three with bismuth salicylate.

It was found that the best way to administer the dose is to spread the salt (powder) on water-soaked bread each day. The rats then ate the bread at the same time, receiving approximately a full dose of the salt. The rats did not object to the treatment, although at different intervals they appeared very nervous, slow in movement, often sluggish, to some degree ferocious, and their coats manifested a certain degree of roughness. Periods of constipation also occurred, and in the case of one rat no faeces were defaecated on one day. Otherwise constipation was indicated by the defaecation of very small pellets.

As has been said previously, a history of the six rats was known throughout a period of about a month. Table 4 shows the degree of infection of each rat for each day during a period of twenty-eight days when daily examinations had been made of the faeces. The number in each square represents the number of cysts counted for that day in any twenty fields of the microscope by the use of a one-inch ocular and four-millimeter objective. A negative sign in the square means that no cysts were detected in the stools for that day.

TABLE 4

DAILY NUMBER OF CYSTS IN THE FAECES OF THE SIX RATS TO RECEIVE BISMUTH TREATMENT

	October							November																				
	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Rat 1	…	…	…	…	4	7	2	…	…	6	6	7	…	…	…	…	1	1	…	5	4	7	3	3	5	2	3	10
Rat 4	…	…	…	…	…	…	…	…	…	1	1	…	…	…	…	…	4	4	2	1	…	…	…	…	…	…	…	…
Rat 8	3	5	7	6	2	2	…	2	2	5	5	…	…	…	…	5	3	3	3	1	5	…	…	…	4	1	3	5
Rat 9	…	…	…	…	2	2	3	2	2	1	…	…	…	…	…	…	1	…	1	4	1	…	…	…	3	2	…	4
Rat 10	…	…	…	…	3	3	4	…	…	3	3	…	…	…	…	…	3	1	1	1	…	6	3	3	2	2	3	7
Rat 12	…	…	2	2	…	…	…	…	…	…	…	3	…	…	…	3	3	3	…	…	2	9	9	9	2	4	…	…

The treatments began on December 18, 1917, and continued for three weeks, until January 8, 1918. The dose for man was a teaspoonful of either of the bismuth salts three times a day, and usually after five to ten days of treatments the infection appeared to be cured (Porter, 1916). This dose for man amounted to about 3.6 to 4 grams of the chemicals each day.

To arrive at dosage for the rats a dose was proportioned according to the ratio of the body weight of man (about 146 pounds) and the average weight of the rats (about 300 grams). This dose was found to be about 20 milligrams each day. This was the first dose tried with the rats, but was considerably increased as the treatments progressed (table 5).

In making the daily faecal examinations of these six rats faecal emulsions of the stools were prepared in distilled water each day during the course of treatment. A drop of this emulsion was transferred to a slide, adding to it a drop of neutral red solution $\frac{N}{10,000}$ (Boeck, 1917), and then the preparation was examined for the cysts of *Giardia*.

When the cysts were found their number was counted in any twenty fields of the microscope, using a one-inch ocular and a four-millimeter objective. A plus sign was placed in the square of the table (table 5) for that day and the number of cysts counted was placed above the sign. If no cysts were detected in the first coverslip preparation from the emulsion, after stirring the emulsion a second preparation was made and by the same procedure a third preparation if the second one turned out to be negative. At the end of three preparations no cysts were detected, then the stools were designated negative for that day, and a minus sign was written in the square of the table for that day. If, however, cysts were found in either the second or third preparation their number was counted and a plus sign written in the square for that day, placing the number of cysts counted above the sign and the number of the preparation below the sign. It took three negative examinations to make a rat negative for that day on which the stools were collected.

The dose of twenty milligrams was first administered to the rats. The dose was the same for the bismuth subnitrate and bismuth salicylate. After seven treatments with a dose of twenty milligrams the daily examinations still showed rats 1, 4, 9, and 12 to be infected, but rats 8 and 10 showed no evidence of infection, there being no cysts in

TABLE 5

TABLE SHOWING THE DOSAGE OF THE BISMUTH SALTS GIVEN TO THE RATS. IT ALSO SHOWS THE RESULT OF THE FAECAL EXAMINATION FOR EACH

								December							January						
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7
Dose in milligrams	20	20	20	20	20	20	20	30	30	50	50	50	0	0	30	60	30	50	0	0	60
1. Bismuth salicylate	0	…	…	2	11	8	…	4	4	4	4	6	2	0	…	…	…	…	…	…	…
8. Bismuth salicylate	0	…	…	…	…	…	…	…	2	1	2	…	…	…	…	…	…	2	2	2	2
12. Bismuth salicylate	0	2	2	3	10	18	5	5	5	11	6	2	1	…	…	…	…	2	5	4	4
4. Bismuth subnitrate	2	2	1	1	1	2	…	…	2	2	2	1	…	…	…	…	…	3	…	…	…
9. Bismuth subnitrate	0	…	2	4	5	7	1	*	2	…	2	2	2	…	…	…	2	2	2	2	2
10. Bismuth subnitrate	0	…	…	1	1	1	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…

* No faeces.

the faeces. On December 25 the dose was increased to thirty milligrams for two days and then to fifty milligrams for three days, for the fact that four rats remained infected led one to believe that the dose previously used in the treatments was insufficient to effect a cure.

After increasing the dose to fifty milligrams the crystals of the bismuth subnitrate were seen in the faeces of rats 4, 9, and 10. The dose certainly must have been adequate since the faeces contained a great amount of the crystals, and the chemicals must have come in contact with the flagellates.

The rats appeared quite ill as a result of the large dose and the treatment was dispensed with for two days. On January 1 a dose of thirty milligrams was again administered and this dose was increased to sixty milligrams on the following day. A dose of thirty milligrams followed by fifty milligrams was given the rats on the next two successive days. Treatment was suspended on January 5 and 6, and sixty milligrams were given on the last day.

At the end of this treatment with these two bismuth salts rats 1, 4, 9, and 12 were still infected. Cysts were found in the faeces of all these rats, except rat 4 on the last day. But rat 4 showed cysts in the faeces two days before the treatments were concluded. Only rat 8, treated with bismuth salicylate, and rat 10, treated with bismuth subnitrate, appeared to be cured.

Discussion

From table 4 we see that these two rats, 8 and 10, were heavily infected during the twenty-eight days that their faeces were examined. But during the treatment cysts were found in the faeces of rat 8 on only two different and successive days, while cysts were found in the faeces of rat 10 only on one day. Apparently the drug had cured these rats.

The rats were then posted and at autopsy no sign of pathological disturbance of the organs was seen. The jejunum, the seat of infection by *Giardia* in rats, was not discolored nor did it contain any gas. In cases of infection the jejunum is usually orange colored and contains gas. The upper and lower duodenum, jejunum, and ileum were examined for free forms and cysts of *Giardia* and none were found, nor were there any cysts in the colon or rectum. *Octomitus muris* was very abundant in the jejunum and ileum. The absence of *Giardia*

in both these rats at autopsy, showed them to be free from infection by this flagellate.

It would seem that these two rats, 8 and 10, were cured by the action of the chemicals, but such a conclusion is not altogether warranted when we consider that the other four rats remained infected. Another interpretation of these two cases is justifiable, viz.: In the study of the cycle of encystment in *G. microti* rat 3 showed only one period when the cysts were found in the faeces. At autopsy this rat proved to be free from an infection by *Giardia* and the conclusion reached was that the rat had been capable of ridding itself of the infection, one way or another.

It will be noticed that in the cases of rats 8 and 10, which appeared cured by the chemicals, treatment with these salts of bismuth did not take place until December 18. This date was twenty-six days, approximately four weeks, after the day when their period of infection of twenty-eight days, November 21, had been concluded. There was an interim, then, of nearly four weeks during which these two rats could have thrown off the infection.

If rats 8 and 10 threw off the infection previous to the treatment with the salts of bismuth then all the days subsequent should have showed no cysts in the faeces. This, however, was not the case for rat 8 showed cysts in the faeces on two successive days, December 27 and 28, and rat 10 showed cysts in the faeces on December 22. A possible explanation of the presence of these cysts may be that they are perhaps the result of a short period of reinfection.

Reinfection might have occurred by the transfer of pellets from rat 9 into the cages of rats 8 and 10. Rat 9 escaped from its cage one night and was found the next day running along the cages of the other rats. If reinfection took place then, according to the data both rats 8 and 10 must have thrown off the infection for the second time for no cysts were in the faeces after the single period of infection in either of the rats and they were not present at autopsy.

Even though reinfection might have taken place it is altogether probable that the salts of bismuth did cure these two rats; but the persistence of the infection in the other four rats certainly militates against the practicability of these chemicals as a specific cure for giardiasis.

Porter (1916) reports the cases of three men infected with *G. intestinalis* and treated with bismuth salts in which an apparent cure was affected. It is significant to note that daily faecal examinations of

these men were not made after the time the cysts disappeared from the stools, following treatment with the chemicals. These men were then discharged from the hospital as cured from giardiasis. Porter recognized the presence of a cycle of encystmtent in *G. intestinalis,* but this factor was not taken into account when the men were determined free from infection. It is very probable if these men who appeared cured could have been watched for several days longer and their stools examined daily, that the cysts might have recurred. The fact that the cysts were absent from the stools after several treatments with the salts of bismuth was not conclusive proof that the men were free from the infection of *Giardia;* this could only have been ascertained by a series of daily examinations for the purpose of determining whether or not the cysts recurred in the faeces at the proper time in the cycle.

Summary

From the experimental work conducted with the salts of bismuth on rats infected with *Giardia* and the results obtained with the treatment of giardiasis in man with the same chemicals it is certain that the therapeutic value of bismuth subnitraté and bismuth salicylate is negative in the treatment of giardiasis. The conclusion is supported by W. L. Yakimoff, W. J. Wassilevski, and N. A. Zwietkoff (1918), who state the inefficacy of these chemicals in the chemotherapy of this disease.

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EXPLANATION OF PLATE

All figures are of cysts of *Giardia microti* from the meadow mouse and of the cysts of *Giardia* found in the culture rats, drawn with camera lucida from smear preparations. Magnification, × 2750.

PLATE 1

Fig. 1. Ventral view, single-individual cysts found in small intestine and caecum; nuclei in resting stage. Two parabasals dividing.

Fig. 2. Dorsal view, single-individual cyst found in caecum; nuclei dividing, parabasals in division.

Fig. 3. Dorsal view, single-individual cyst found in caecum of meadow mouse; nuclei in anaphase, small parabasals, axostyle completely split.

Fig. 4. Dorsal or ventral view, single-individual cyst, chromatin in one nucleus divided into two masses. Parabasals dividing. Cyst from caecum of meadow mouse.

Fig. 5. Dorsoventral view, binary cyst from colon of meadow mouse; nuclei divided, two parabasals.

Fig. 6. Dorsoventral view, binary cyst from colon of meadow mouse; four nuclei, new organelles, no parabasals.

Fig. 7. Side view, binary cyst from colon of meadow mouse; shows plan of cleavage of the parent body to form two daughter flagellates. No parabasals.

Fig. 8. Side view, binary cyst from colon and rectum of meadow mouse. Two complete individuals with their organelles, no parabasals.

Fig. 9. Dorsoventral view, multinucleate cyst, two axostyles, large parabasals, twelve nuclei. Found in small intestine of meadow mouse.

Fig. 10. Dorsoventral view, multinucleate cyst, two axostyles, twelve nuclei, parabasals. Found in small intestine of meadow mouse.

Fig. 11. Dorsoventral view, multinucleate cyst from small intestine of meadow mouse; two axostyles, no parabasals, sixteen nuclei.

Fig. 12. Dorsal view, single-individual cyst from jejunum of rat; nuclei in anaphase, large parabasals, axostyle partially split.

Fig. 13. Dorsoventral view, binary cyst from jejunum of rat; four nuclei, large parabasals.

Fig. 14. Dorsoventral view, binary cyst from colon of rat; four nuclei and three large parabasals.

Fig. 15. Ventral view, binary cyst from jejunum of rat; four nuclei in resting stage, parabasals.

Fig. 16. Side view, binary cyst from jejunum of rat; four nuclei two exostyles, large parabasals.

Many of the cysts in the preparations show a shrinkage away from the cyst wall; this is attributed to plasmolysis at the time of fixation.

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