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A. Malins Smith

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MEASUREMENTS OF AND OBSERVATIONS UPON THE CYSTS OF *ENTAMOEBIA HISTOLYTICA* AND OF *ENTAMOEBIA COLI*

BY

A. MALINS SMITH, M.A. (Cantab.)

From the Liverpool School of Tropical Medicine

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From April 22nd, 1917, until March 4th, 1918, the writer measured the dimensions of 2,833 cysts of two of the intestinal entamoebae of man, *E. histolytica* and *E. coli*. The results of these measurements are given in the present paper. At the time each measurement was made the nuclei of the cyst were counted and their number recorded. A note was also made of the presence or absence of chromatoid bodies and vacuoles. The main results of these observations are recorded here also.

METHODS

The majority of the stools from which the cysts to be measured were taken were those of convalescent dysenterics, and were sent to the laboratory for the usual routine examination for dysentery. Cysts were also measured from a small number of carriers of *E. histolytica* who had not had dysentery. Stools in which cysts had been found by the routine examination were taken at random and set aside for measurement. Fifty-nine per cent. of the cysts were measured on the same day as they were sent in, twenty-two per cent. on the following day, eleven per cent. two days afterwards, and the remainder later. With very few exceptions the cysts were measured *in iodine* (Weigert's solution), in order that their nuclei could be readily counted. A very small number of the cysts was measured in normal saline. The number of individuals whose stools

were used for cyst measurement was eighty. In thirty-four of these cases the cysts of *E. histolytica* alone were measured; in thirty cases only *E. coli* cysts were measured. In sixteen cases a double infection with *E. histolytica* and *E. coli* was present, and cysts of both were measured. Thus, in all, the cysts of ninety-six infections were measured. In one or two cases, though infections of both *E. histolytica* and *E. coli* were present, only cysts of one species were measured.

All the cysts of *E. histolytica* and the large majority of the cysts of *E. coli* were measured under the 1/12in. objective and No. 2 eyepiece. A comparatively small number of *E. coli* cysts was measured with the 1/6in. objective and No. 2 eyepiece. Each division of the scale of the ocular micrometer measured 1.7μ when the 1/12in. objective was used, and 3.7μ with the 1/6in. objective. It was considered possible to estimate by the eye one-tenth of the micrometer division, i.e., 0.17μ , and values were actually recorded in this way, e.g., 7.1 divisions, 11.6 divisions. It is not claimed that the measurement thus obtained is accurate to the last figure. After considerable experience, however, it is estimated that measurements can be taken correct to the nearest 0.5μ , i.e., to about one-third of a scale division. This means that a cyst recorded as measuring 10.5 scale divisions may equally well be 10.6 or 10.4 divisions, but most probably is not 10.7 or 10.3 divisions. In the later part of the work 245 cysts of *E. histolytica* were measured with a scale whose divisions were exactly half of the width of the previous scale, i.e., the value of each division with the 1/12in. objective was 0.85μ , and with this scale the measurements were recorded to one-half a division, roughly 0.5μ , i.e., a similar accuracy to that claimed for the first scale.

In order to make sure that the figures of the curves given later should be significant, the figures recorded to one-tenth of a scale-division were added in groups of one-half a scale-division. Thus each group corresponds to a range of 0.85μ , and there is no question that accuracy of a higher order than this can be attained in the actual measurement.

As a general rule all the cysts encountered were measured in the order in which they were met. No omissions were made, so that a completely random selection of the cysts was ensured. The

insignificant departures from this rule were noted, so that their effect could be allowed for, if necessary, in making any deductions from the results obtained.

The species to which each cyst belonged was noted at the time of its measurement and a list of the criteria employed in diagnosis is here given, as the matter is a difficult and even controversial one. The difficulty was increased in this particular work by the following consideration. It was one of the objects of this work to establish a curve of the dimensions of the cysts for each of the species. It was therefore obviously inadmissible to use the size of the cyst as a basis of diagnosis. If, for example, all cysts below 14μ had been labelled *E. histolytica*, and all above 14μ *E. coli*, then the resulting curve of the dimensions of the species would have been valueless, as it would have been predetermined. It has been considered that a diagnosis could be reached on other points apart from size, and the points relied on are as follows:

1. *The number of nuclei in the cyst*

All cysts with more than four nuclei were diagnosed as *E. coli*. Cysts with one to four nuclei may belong to either *E. histolytica* or *E. coli* as far as the number of their nuclei is concerned. As, however, four is the characteristic number of nuclei of the mature cyst of *E. histolytica*, it is present much oftener in *E. histolytica* cysts than in *E. coli* cysts. If, therefore, an infection had tetra-nucleate cysts prevalent in it, this was taken as an indication that *E. histolytica* cysts were present. In the case of individual cysts, however, the diagnosis could not be made on this point.

2. *The character of the nuclei*

The nucleus of the cyst of *E. histolytica* has typically a smaller quantity of chromatin than has that of *E. coli*. The peripheral ring of chromatin is usually composed of small evenly distributed granules, while in *E. coli* the granules are larger and may even be irregular masses distributed irregularly round the nuclear ring. The general consequence is a greater visibility in the case of the nucleus of the *E. coli* cyst. The typical distinction is best seen in the mononucleate and binucleate cysts, and in these considerable reliance can be placed upon this character in the diagnosis. Even in the

tetranucleate cyst the greater visibility of the nuclei in *E. coli* is pretty obvious, though the differences in the structure of the peripheral chromatin ring between the one species and the other are perhaps not so marked in this stage.

A noteworthy effect of keeping stools containing *E. histolytica* cysts for a time is that changes occur in the nuclei of the cysts which tend to obliterate the distinction just pointed out between the cysts of the two species. The peripheral chromatin of the nuclei loses its typical fine granulations and seems to collect into larger, less regular masses. Even in fresh stools, cysts of *E. histolytica*, possibly somewhat degenerate, are encountered in which the peripheral chromatin of the nucleus seems more abundant than the normal, thus approaching to the kind of nucleus typical of the cyst of *E. coli*. This feature cannot therefore be relied upon alone in every case to distinguish the species. It is, however, most useful as a general diagnostic character.

3. *The character of the cytoplasm*

In *E. histolytica* cysts the cytoplasm as a whole is typically distinct from that of *E. coli*. Its colour seen fresh in normal saline is greenish-grey in *E. histolytica*, while in the cyst of *E. coli* it is paler. Though this is rather a fine distinction, yet it is one on which a good deal of reliance can be placed after considerable experience. In iodine the distinction of colour still persists, though it cannot be expressed in quite the same terms. The cytoplasm of the *E. coli* cyst is uniform and its granulations small and regular. In *E. histolytica* cysts there is a greater irregularity in the cytoplasm. Not only does the *E. histolytica* cyst more often contain chromatoid bodies and vacuoles, but the granulation of the cytoplasm itself is less uniform than in *E. coli* cysts. Taken altogether the cytoplasmic characters are of great importance in diagnosis.

4. *The chromatoid bodies*

Chromatoid bodies occur more frequently in *E. histolytica* cysts, and in these are characteristically rod-shaped. They are less frequent in *E. coli* cysts, and in these are much more irregular in shape, being divided, splintered, or laminated. Regular oblong

chromatoid bodies are very rare in *E. coli*, and even when present are usually needle-like, i.e., longer and narrower than the rod-shaped chromatoid bodies of *E. histolytica* cysts.

5. *The vacuoles*

Vacuoles are more frequent in *E. histolytica* cysts, and as a rule, as Dobell and Jepps (1917) have pointed out, take on a paler colour in iodine than do the vacuoles of *E. coli* cysts. They are also, as a rule, less sharply outlined than in *E. coli* cysts. There are, however, fairly frequent exceptions to these generalisations.

6. *The cyst wall*

The wall of the cyst of *E. coli* is thicker than that of the *E. histolytica* cyst. This point is, however, in my opinion not of much practical value, the thickness of the wall being so difficult to measure or estimate that very little diagnostic value can be attached to it. In common with Dobell and Jepps (1917), I can attach no meaning to the assertion made by some authors, e.g., Brug (1917), that the wall of the cyst of *E. coli* has often a double contour, while in *E. histolytica* the contour is single.

The greatest difficulty of diagnosis has arisen in my experience over tetranucleate and occasionally binucleate cysts without chromatoid bodies or vacuoles. In their absence the sole points of distinction are the colour and uniformity of the cytoplasm and the greater or smaller amounts of chromatin in the nucleus. Both these last distinctions may break down and the individual cyst be undeterminable.

In practice, as the sequel will show, the difficulty is not great, for the study of cases passing *E. coli* cysts only has shown that the tetranucleate cyst is very infrequent in undoubted *E. coli* infections, and in the vast majority of cases, even in mixed infections of the two species, the tetranucleate cyst has the characters of *E. histolytica*.

It may perhaps be well to sum up in parallel columns the points used in diagnosing the cysts of the two species. At the end of the paper a similar table will be given showing how the measurements of the present paper enable a more precise list of diagnostic points to be drawn up.

	<i>E. histolytica</i> cysts	<i>E. coli</i> cysts
No. of nuclei	1 to 4	1 to 16.
Character of nuclei ...	Peripheral chromatin of small granules, more or less evenly distributed. Nuclei in consequence less distinctly visible	Peripheral chromatin of larger unevenly distributed masses. Nuclei, therefore, more distinctly visible
Cytoplasm	Colour greenish, typically not uniform in appearance	Paler, greyish, typically uniform in appearance
Inclusions	Chromatoid bodies more frequent, typically rod-shaped, with square or rounded ends. Vacuoles more frequent, one or more in the cyst, usually faintly stained by iodine, with less sharply defined edges	Chromatoid bodies less frequent, irregular in shape, with pointed or splintered ends. Vacuoles less frequent, generally single in the cyst, usually deeply stained by iodine, and with more sharply defined edges
Cyst wall	Thinner	Thicker

Using these criteria I found it impossible to diagnose only eighteen cysts in obtaining the two thousand whose measurements are given in the two principal curves of the present paper. As the work was done by one person only, there was no checking of the diagnosis by another observer, but I believe that the proportion which need be diagnosed as doubtful by an experienced observer is very small.

The morphological differences summarised in the above table have been figured by various authors. The plate accompanying Matthews' (1918) paper in the present number of this journal illustrates them well. For differences in the character of the nuclei, for instance, cf. figs. 5, 6 and 8 (*E. histolytica*) with figs. 15, 16 and 14 (*E. coli*). The difference in the structure of the cytoplasm is seen on comparing figs. 5 and 6 with figs. 16 and 17. The two types of chromatoid bodies are shown in figs. 9 and 18, while the vacuoles are contrasted in figs. 5 and 6 as compared with fig. 15.

THE SIZE OF THE CYSTS

Frequency curves for the cysts of E. HISTOLYTICA and of E. COLI

The cases for examination were taken at random from the material examined by routine during the ten months of the work. It was thought that thus a sufficiently large number of the cysts (say 1,000) being taken from a sufficient number of cases, curves would be obtained representative of the two species.

The curve for E. HISTOLYTICA

We will consider first the frequency curve for the cysts of *E. histolytica*. The curve is given in fig. 1, p. 39, and represents the following measurements :

Scale-divisions ...	3·2	3·7	4·2	4·7	5·2	5·7	6·2	6·7
Number of cysts ...	2	17	71	65	36	8	60	156
Scale-divisions ...	7·2	7·7	8·2	8·7	9·2	9·7	10·2	10·7
Number of cysts ...	264	157	110	34	11	8	4	3

The thousand cysts whose measurements are shown in the curve were obtained from thirty cases. No case has contributed less than ten or more than fifty measurements to the total shown in the curve. Thus, although the contributions of all the cases are not the same, yet no one case predominates to any great extent over the others. The exact number of cysts from each case and their average diameter are given in Table I in ascending order of size.

The curves throughout this paper have the diameter of the cysts in μ as abscissae and the numbers of cysts as ordinates. The cysts were grouped first in groups of half a scale-division, i.e., $0\cdot85\mu$, and the curves were drawn with this figure as the unit for the abscissae. The readings in 1μ units were substituted for the $0\cdot85\mu$ unit after the curves were drawn, and it is for this reason that the points on the curve so rarely correspond to an exact number of μ . Cysts which were spherical had their diameter recorded in a single measurement. In the case of non-spherical cysts, their longest and

TABLE I.

No. of case	Number of cysts measured	Average diameter (μ)
1	43	7.1
2	31	7.3
3	27	7.9
4	37	7.9]
5	43	8.0
6	13	8.3
7	15	11.6
8	27	11.7
9	33	11.9
10	19	11.9
11	24	12.0
12	22	12.0
13	39	12.1
14	50	12.1
15	27	12.2
16	39	12.2
17	28	12.3
18	50	12.3
19	50	12.3
20	30	12.4
21	29	12.4
22	29	12.4
23	28	12.5
24	50	12.6
25	20	12.6
26	37	12.6
27	50	12.7
28	11	12.8
29	49	13.6
30	50	14.3
Total ...	1,000	

shortest diameters were both measured and the average of the two was taken as the diameter of the cyst. It should be noted that, wide as is the range of variation in the diameter of the cysts, the volume and weight of the cysts show an even wider range of difference, being proportional to the cube of the diameter. Thus, for example, the diameter of the largest cyst of *E. histolytica* measured (18μ) is approximately three and a quarter times the diameter of the smallest cyst measured (5.5μ). The actual size (volume) of the former cyst is, however, approximately thirty-five times that of the latter.

The extreme measurements are, as has just been stated, 5.5μ and 18μ for *E. histolytica* cysts. Dobell and Jepps (1917) have given 5μ and 20μ as the extremes, Wenyon and O'Connor (1917) record cysts of 6μ to 18μ , numbers which agree well with my own. As always, however, the extremes occur but rarely, and the essential point of interest is the distribution of the sizes between the two extremes. A most important feature of the curve is that it is bimodal. There is, moreover, a very marked separation between the two portions of which the curve consists. The first portion, with mode at 7.1μ , contains 193 cysts and their average diameter is 7.68μ . The second portion, with mode at 12.2μ , contains 807 cysts and their average diameter is 12.58μ . There seems to be no doubt from the curve that the cysts of this species divide themselves naturally into two strains, differing only in size, with dimensions as indicated in the curve. The smaller strain has been recognised recently (see James (1914), Woodcock and Penfold (1916), Wenyon and O'Connor (1917)), and a full account of it is given by Dobell and Jepps (1917). In my experience, cysts with diameters of 9.5μ and thereabouts, i.e., intermediate between the two strains, are very rare, as shown by the curve. I have encountered no case with cysts whose average diameter lies between 9μ and 10μ , nor any case showing even a considerable number of such cysts. The few cysts with diameters between 9μ and 10μ recorded in the curve are at the extreme limit of the range of size shown by the infections comprising them. They are either the very largest cysts of infections of the 'small' strain or the smallest cysts of infections of the 'ordinary' strain. Wenyon and O'Connor (1917), however, record one case (Kettlewell) most of whose cysts were 9μ to 10μ in diameter, and

the average diameter of fifty of whose cysts was 9.2μ . If such cases were at all frequent the curve given in fig. 1 would take on a very different appearance, for the separation between the two parts of the curve would be obliterated and the 'small' strain thus be no longer distinct from the 'ordinary' strain. Such infections must, however, be extraordinarily rare. The general agreement of all the older observations, that the lower limit of size for *E. histolytica* cysts was 10μ (see, for example, Elmassian (1909), Wenyon (1915), Kuenen and Swellengrebel (1913)), could scarcely have been reached if infections with an average size of about 9μ were at all common. In the thirty infections in my curve there was no such case, nor did such a case appear among the cases omitted from my curve because of the small number of measurements in each, nor in the infections which I have measured since the figures of this curve were completed. These infections number fifty in all, and had been acquired in various regions of the globe.

There is therefore *prima facie* evidence that a 'small' strain and an 'ordinary' strain exist differing from each other in size. They are not distinguished from each other in any other morphological feature, nor, as far as is known, in any respect save size. The subject of size strains will be discussed somewhat more fully later. The proportion of the 'small' strain in the curve obviously depends upon how many infections of this strain have been set aside for measurement. In this respect, records show that in a period when the greater part of this work was done there were among seventy-five infections of *E. histolytica* found in the routine examinations twenty-one of the 'small' strain, i.e., 28 per cent. Table I shows that of the thirty infections included in the curve, six (20 per cent.) were of the 'small' strain. Of the 1,000 cysts in the curve, 193, or 19 per cent., were of the 'small' strain. This strain has therefore a somewhat smaller representation in the curve than it is entitled to. There is no doubt, however, that the salient features of the curve would have been substantially the same if the representation of the two strains had been in strictly accurate proportions.

Treating separately the part of the curve which refers to the 'ordinary' strain, the following facts may be recorded. The curve is not a typical symmetrical frequency curve, in which the mode and the mean coincide, but extends further on the right of the mode than

on the left. The average diameter of the 807 cysts represented in it is 12.6μ , while the mode is at 12.2μ . The point representing the mode in the curve is of course really an average for the group 11.8μ — 12.65μ , and is more accurately represented by a horizontal line. The asymmetry of the curve is indicated in the fact that the average is at the right hand end of this modal group of measurements. With regard to the fact that the curve extends further to the right of the mode than to the left, it is worth noting that, of the twenty-eight cysts with diameters above 15μ , eighteen were contributed by one case alone (Case 30), whose average measurement is very high (14.3μ). Not only is the curve for the 'ordinary' strain asymmetrical, but it is not so regular as would be expected for a curve containing 800 measurements. Further reference will be made to this point. The curve for the 'ordinary' strain has a probable error of $\pm 0.86\mu$.

The curve for E. COLI

The curve for 1,000 *E. coli* cysts is given in fig. 2, p. 39, and represents the following measurements:

Scale-divisions ...	...	7.3	7.8	8.3	8.8	9.3	9.8	10.3	10.8	11.3	11.8
Number of cysts	...	1	23	51	125	143	173	145	111	93	56

Scale-divisions ...	...	12.3	12.8	13.3	13.8	14.3	14.8	15.3	15.8	16.3	...
Number of cysts	...	28	24	10	8	3	3	1	1	1	...

The cases from which the 1,000 *E. coli* cysts represented in Fig. 2 were obtained are shown in Table II, where they are arranged in ascending order of size.

The curve for *E. coli* differs from the curve for *E. histolytica* in being unimodal with mode at 16.7μ or, rather more accurately, the modal group is 16.25μ to 17.1μ with centre at 16.7μ . There is no clear indication in the curve of more than one strain being present in the cysts of this species. The extreme measurements are 12.5μ and 27.5μ . Apart from this series of measurements the writer has a

TABLE II.

No. of case	Number of cysts measured	Average diameter (μ)
1	22	15.5
2	12	15.6
3	51	15.6
4	50	15.8
5	30	16.0
6	17	16.1
7	33	16.2
8	25	16.3
9	50	16.3
10	50	16.4
11	19	16.7
12	50	16.8
13	14	16.8
14	10	16.9
15	10	17.1
16	50	17.4
17	26	17.4
18	10	17.5
19	51	17.5
20	50	17.6
21	10	17.7
22	13	17.8
23	19	17.9
24	13	18.1
25	20	18.5
26	50	18.6
27	50	18.6
28	50	18.8
29	34	19.0
30	11	19.2
31	11	20.0
32	36	20.1
(14 cases Miscellaneous)	53	16.8
	Total 1000	General average 17.3

FIG. 1. 1000 cysts of *E. bistolytica*.

FIG. 2. 1000 cysts of *E. coli*.

FIG. 3. *E. bistolytica* 'ordinary' strain 807 cysts. *E. coli* 1000 cysts.

record of a cyst of *E. coli* measuring 34μ in diameter. These extremes agree fairly closely with those recorded by Dobell and Jepps (1917), and also with those cited by them from other authors. I have not observed any cyst of *E. coli* as small as 11μ , the smallest size recorded by Dobell and Jepps, but have no doubt that in a very extensive series of measurements *E. coli* cysts of this size might occur. They must, however, be very rare. Although the largest cysts extend to 25μ or 30μ , and even very rarely beyond this, yet the curve shows clearly that cysts beyond 22μ are very infrequent. Ninety-seven per cent. of all the cysts in the curve are 22μ or below in diameter. The curve for *E. coli* is like that for *E. histolytica* in being somewhat asymmetrical. While the mode is at 16.7μ the average of the 1,000 measurements is 17.3μ , i.e., it does not lie in the modal group of measurements at all. The curve is even less regular than the *E. histolytica* curve, and certainly less so than might be expected from so large a number of measurements as 1,000. The probable error of this curve is $\pm 1.49\mu$.

Overlapping curves of E. HISTOLYTICA and E. COLI

A study of the two previous curves drawn so as to overlap each other, fig. 3, p. 39, brings out a point of great importance, namely, the value of size as a criterion of the species. It is seen at once that the curves overlap each other from 12.5μ (the smallest measurement of *E. coli*) to 18μ (the largest measurement of *E. histolytica*). According to Dobell and Jepps (1917) these extreme points may be even extended by a wider series of observations to 11μ and 20μ . It might be at once deduced that for all cysts between 11μ and 20μ in diameter size is useless as a diagnostic character, as cysts between these extremes may be either *E. coli* or *E. histolytica*. While this deduction may be strictly correct in an absolute sense, yet a study of the curves will show that these limits may be reduced very much and yet the diagnosis from size may have a very high probability of accuracy. It is really only between 13μ and 15.5μ that any considerable numbers of both species of cysts occur. Below 13μ there is a very high probability in favour of *E. histolytica*, above 15.5μ the probability is very high in favour of *E. coli*. Thus at a diameter of 16.7μ the chances are 25 : 1 (approximately) that any cyst will

be *E. coli*.^{*} Similarly the chances are 75:1 (approximately) in favour of a cyst of 12.5μ being *E. histolytica*.^{*} Contrasted with this we find that the chances are even that a cyst of 14.3μ is *E. histolytica* or *E. coli*. It is therefore quite justifiable, except between the fairly narrow limits 13μ to 15.5μ , to allow considerable weight to size as a diagnostic character, and this result is one of the most important deductions from the present series of measurements.

Although the sizes of individual cysts of the two species are often coincident, yet it may be worth while to point out that, if averages of fifty cysts are taken, the two species do not overlap, so far as my measurements go. The highest average measurement that I have obtained for a sample of 50 or more cysts of *E. histolytica* is 14.3μ . Wenyon and O'Connor (1917) give one case in which fifty cysts of *E. histolytica* averaged 15.1μ . Even this, however, is smaller than my lowest average for *E. coli*, which is 15.6μ for a sample of fifty-one cysts. Though further research may reveal *E. coli* infections whose average diameter is smaller than this, yet it can be said that, so far as the present evidence goes, the average diameter of fifty cysts is always distinctive of the species. Of course, in practice this statement is not of any great value, as the measurement of fifty cysts in every doubtful case would be laborious or even impossible. It would in any case give no help in the case of a mixed infection.

THE SHAPE OF THE CYSTS

It is well known that both in *E. histolytica* and in *E. coli* the cysts are usually more or less spherical. Cysts occur, however, in both species in which the shape is not spherical but ovoid or more or less elongated. When such a lack of symmetry was obvious in cysts occurring in the present series of measurements, the size of the cyst was expressed by the mean of its longest and shortest diameters. The difference between these two diameters in the cysts thus obviously not spherical varied very much. Sometimes it was

^{*} These chances apply to a series of cases in which *E. histolytica* infections and *E. coli* infections are equally common. In the usual series of cases of convalescent dysenterics where *E. coli* infections are about twice as common as infections of *E. histolytica* the chances would be 50:1 and 37:1 respectively.

small. Some of the records show a difference of 0.33μ between the two diameters, while differences of 0.5μ and 0.8μ are commonly recorded. On the other hand, the cyst may be very much elongated, the extreme record showing a difference of 9μ (approximately) between the two diameters in the case of a cyst of *E. coli*. No doubt if very carefully examined, and if differences smaller than those here recorded were noted, a very large percentage of the cysts would be found to be not perfectly spherical. The following figures refer to cysts in which the asymmetry was obvious. These in practice included all those cysts in which the two diameters differed by 0.5μ or more, and even some which differed by as little as 0.33μ .

Of 1,233 cysts of *E. histolytica* measured for this record, 260 were definitely recorded as asymmetrical, a percentage of 21. The extreme of asymmetry in *E. histolytica* was reached in this series of observations in the case of a cyst measuring 18.7μ by 11.6μ , a difference of 7μ between the longest and shortest diameters. Other extreme records are 17μ by 10.5μ (difference 6.5μ), 15.5μ by 9.5μ (difference 6μ), and 15μ by 9.5μ (difference 5.5μ). Individual infections vary very much in their relative asymmetry. In one infection twenty-seven cysts were measured without any asymmetrical cyst being recorded, in another only two asymmetrical cysts were noted in twenty-eight, while on the other hand an infection occurred in which twenty-one cysts were recorded as asymmetrical out of forty-five measured, or very nearly 50 per cent. There is some evidence that these differences persist and are characteristic of the different infections, but there is not as yet sufficient proof of this.

In the case of *E. coli*, 1,170 cysts were measured, and 193 of these (16.5 per cent.) were asymmetrical. This is lower than the corresponding figure for *E. histolytica* cysts, thus confirming the statement of Wenyon (1915) that the cysts of *E. histolytica* 'are not so accurately spherical as those of *E. coli*.' Differences of 0.33μ and 0.5μ between the diameters of *E. coli* cysts are recorded, while at the other extreme are records of cysts 26μ by 17μ (difference 9μ), 27μ by 19μ (difference 8μ), and 23μ by 15μ (difference 8μ). As instances of differences in the records of individual infections there may be given one case where no asymmetrical cyst was recorded out of fifty measured, while in another case twenty-seven were asymmetrical out of seventy-five measured.

THE NUMBER OF NUCLEI IN THE CYSTS

1. *E. histolytica*

As it seems clear that two size strains of cysts exist in the species *E. histolytica*, the two will be considered separately in the remarks which follow.

TABLE III.

<i>E. histolytica</i> 'ordinary' strain				<i>E. histolytica</i> 'small' strain		
Number of nuclei in the cyst	Number of cysts	Percentage of total	Average diameter in μ with probable error	Number of cysts	Percentage of total	Average diameter
1	326	32.2	$12.74 \pm .05$	82	45.0	7.5
2	136	13.4	$12.55 \pm .08$	53	29.1	7.5
3	13	1.3	12.30	1	...	...
4	536	53.0	$12.40 \pm .03$	46	25.3	7.8
Total	1011	...	...	182	...	...

Table III gives the results of observations of the number of nuclei present in each cyst. The 1,011 cysts of the 'ordinary' strain in the table include a number which are not included in the curve previously given of cyst diameter. They do not, however, include all those given in that curve. In spite of certain omissions and additions the material is substantially the same as that already dealt with. It is clear at once from Table III that, as is well known, the mature tetranucleate cyst of *E. histolytica* is accompanied in the faeces by considerable numbers of the mono- and bi-nucleate cysts. A little over half of all the cysts encountered were tetranucleate. The mononucleate cyst comes next in frequency, making up about one-third of all the cysts. The stage with three nuclei is very rarely encountered, only occurring in about one per cent. of the cysts. The 'small' strain shows some differences from the 'ordinary' strain in the incidence of the various nuclear stages, but too much importance must not be attached to the figures in this part of the table which represent much smaller numbers of cysts.

Table IV gives the particulars for 1,132 cysts of *E. coli*. The outstanding feature of the table is the enormous preponderance of eight-nucleate cysts in the total. Though every experienced observer has known that eight-nucleate cysts were encountered the most frequently, yet it is interesting to have a more exact idea of the extent of this preponderance. It must be at once stated that it occurs in cysts from stools which are for the most part formed or

TABLE IV.

<i>E. coli</i>			
Number of nuclei	Number of cysts	Percentage of total	Average diameter (μ)
1	6	...	17.7
2	77	6.8	17.3
3	8	...	17.5
4	31	2.7	17.3
5	4	...	18.1
6	7	...	17.3
7	5	...	18.3
8	983	86.8	17.4
12	2	...	23.7
16	9	1.0	23.4
Total ...	1132		

semi-formed. It is well known, and I have been able to confirm the fact by notes taken on several cases, that in loose stools the stages with a smaller number of nuclei are more frequent, as also are the amoebae. Thus Case 26 of Table II had a heavy and persistent infection of *E. coli*. The cysts of this infection were observed on six different dates. On three of these occasions the stool was loose after administration of a saline purgative. On the other three the stool was formed. The following results were obtained:—

Number of nuclei ...	1	2	3	4	5	6	7	8	16	Total
In the formed stools	...	3	2	3	...	...	1	115	...	124
In the loose stools ...	1	15	1	3	...	1	...	40	1	62

In the formed stools the cysts with eight nuclei or more make 93 per cent. of the total. In the loose stools those with eight or more nuclei make only 66 per cent. of the total. Though, even in the loose stools the eight-nucleate cysts are in the majority, yet they do not show such a great preponderance as in the formed stools. In formed and semi-formed stools, then, as Table IV shows, the eight-nucleate cysts form 87 per cent. of all the cysts seen. Two consequences of importance in diagnosis follow: (1) The rule that all cysts with more than four nuclei are diagnosed as *E. coli* becomes of very great practical value. In similar material to my own, 89 per cent. of the *E. coli* cysts can at once be certainly diagnosed. Forty-one samples of over ten cysts each were examined from a series of thirty-one cases. Of these, eleven samples of 10, 13, 50, 12, 32, 10, 10, 50, 16, 32 and 20 cysts, respectively, were entirely composed of eight-nucleate cysts. In addition to these, five other samples of 30, 20, 20, 24 and 25 cysts, respectively, were entirely composed of cysts with more than four nuclei. Not one of the samples was altogether without eight-nucleate cysts, and of course in almost all, these were in the majority. (2) The general impression of *E. coli* cysts obtained from such material as was used for this work is based overwhelmingly upon the mature cyst, the eight-nucleate cyst. In *E. histolytica* one's general impression is obtained from mononucleate and binucleate cysts as well as from the mature tetranucleate cyst. It is certain that if mononucleates and binucleates were numerous among *E. coli* cysts, a different impression of the cysts of the species would be obtained. The following instances of this may be given from the diagnostic table earlier in the paper:

E. coli cysts

Colour paler, greyish. If binucleate cysts were common or in preponderance this distinction of colour might have to be given up or more lightly stressed. Often the colour of the binucleate *E. coli* cyst, and particularly of the vacuolated binucleates, approaches very nearly to that of the *E. histolytica* cyst. It is the eight-nucleate cyst in particular which has the paler colour.

Cytoplasm more uniform. The typical uniform cytoplasm is best seen in the eight-nucleate cyst, though it occurs also in every stage.

Vacuoles less frequent. If mononucleate and binucleate *E. coli* cysts were common this distinction would certainly disappear, for in these stages the cyst is very frequently vacuolated. Probably the majority of such cysts are vacuolated.

The average diameter of the cysts at all stages of nuclear division is given in the last column of Table IV. There is no significant difference in any stage until one comes to the cysts with more than eight nuclei. Although these are few in number, yet the very large difference (about 6μ greater) in their average diameter from that of the cysts with eight nuclei or less is certainly significant. They seem to be in some way monstrous or abnormal forms.

Inspection of Tables III and IV shows that those stages of nuclear division which involve the absence of simultaneous division of all the nuclei in the cyst are rare. This is particularly marked in cysts of *E. histolytica* where only about one per cent. of all the cysts encountered were three-nucleate. In *E. coli* cysts the stages which show that all the nuclei of the previous stage have not divided simultaneously are those with three, five, six, seven and twelve nuclei respectively. It is to be noted that when these numbers of nuclei occur in the cyst, one or more of the nuclei are usually larger than the rest, the larger ones being presumably those which have lagged behind the others in division. These stages are all encountered very infrequently, in less than 1 per cent. of the cysts in each case. It is noticeable, however, that the stages with two and four nuclei are not very common either, being found in approximately 7 and 3 per cent. only of the cysts, respectively. The four-nucleate stage is only about four times as common as the three-nucleate stage, the one probably indicating simultaneous and the other certainly showing successive division of the two nuclei of the previous binucleate stage.

The seventy-seven cysts of *E. coli* in the binucleate stage are of two kinds, vacuolated and non-vacuolated. These two kinds are illustrated in the plate accompanying Matthews' (1918) paper, figs. 15 and 16. Of the vacuolated kind there were forty-one, of the non-vacuolated thirty, while in six there is no record as to this point. Twenty-eight of the thirty-one tetranucleate cysts were non-vacuolated. It is thus seen that the number of non-vacuolated binucleate cysts is approximately equal to the number of non-vacuolated tetranucleate cysts. This fact perhaps lends some support

to Wenyon and O'Connor's (1917) hypothesis that the peculiar vacuolated binucleate *E. coli* cyst is abnormal and not a part of the ordinary development. The vacuolated cysts are those which provide the extra number of binucleates over the tetranucleates, and they possibly do not develop further. The great scarcity of mononucleate *E. coli* cysts in my material is noteworthy. In the case in which the largest number of *E. coli* cysts was measured, the pre-cystic forms were binucleate. It is possible that this is very generally the case, and that the first division of the nucleus usually takes place before encystment. Further observation of infections in which numerous precystic forms and immature cysts are present is necessary to settle this point.

THE CHROMATOID BODIES

These are undoubtedly more prevalent in *E. histolytica* cysts than in *E. coli* cysts. Of 1,162 cysts of the 'ordinary' strain of *E. histolytica* (my data for the presence or absence of chromatoid bodies in the cysts of the 'small' strain are very incomplete, but I have seen nothing which leads me to think that this strain differs from the 'ordinary' strain in respect of chromatoid bodies) chromatoid bodies were present in 319 (27 per cent.), absent in 760 (65 per cent.), and doubtful in 83 (8 per cent.). The cysts with chromatoid bodies were, however, well distributed through the cases. Samples of ten cysts or more were observed from twenty-nine different cases. In only four cases did the samples throughout show no cysts containing chromatoid bodies. Samples of ten or more cysts were examined from forty-eight different stools from these cases. In ten of these samples there were no cysts with chromatoid bodies. In practice, therefore, one sees chromatoid bodies in the majority of infections where fair numbers of cysts can be examined. If only one to five cysts are seen, however, they may very often show no chromatoid bodies. Samples of fewer than ten cysts were examined from twenty-seven different stools from the present series of cases. Seventeen of these samples were entirely without chromatoid bodies. Since scanty infections in which one cannot observe more than ten cysts without great labour are fairly common, one quite frequently must diagnose in practice without the aid of chromatoid bodies. The infections so far referred to were examined primarily with the object of measuring the diameter of the cysts. They were selected

infections in which cysts were more abundant than the average. In order to have figures bearing upon the average infection as met with in routine examinations, a further series of observations was made on all the infections of *E. histolytica* appearing in the routine examinations over a certain period. These were examined in saline, in which medium the chromatoid bodies are more distinctly seen than in iodine. Notes were made of the abundance of cysts in the infection and whether chromatoid bodies were absent or present in the sample examined, also, if present, whether the cysts containing them were a majority or a minority of the cysts present. The following results were obtained. Samples were taken from one hundred and forty-four different stools from twenty-three different cases. In 51 per cent. of these samples the cysts were entirely without chromatoid bodies. In 28 per cent. the cysts containing them were fewer than half of the whole number observed, in 11 per cent. about half the cysts contained chromatoid bodies, and in 10 per cent. the cysts containing chromatoid bodies were in the majority. The samples examined were of the size usually seen in our routine examinations, i.e. they comprised in each case all the cysts present under two coverslips. It appears from this that in routine examinations there are just about equal chances of infections without any chromatoid bodies being encountered as of those in which chromatoid bodies are present. The following table shows the actual figures for some of the cases examined most often. Only one sample was taken from each stool.

TABLE V.

Case	Number of samples without chromatoid bodies	Number of samples in which fewer than half the cysts have chromatoid bodies	Number of samples in which about half the cysts have chromatoid bodies	Number of samples in which cysts with chromatoid bodies are in the majority
1	15	5	...	1
2	5	3	2	2
3	5	1	1	5
4	6	1	1	...
5	10	11	1	1
6	9	10	2	...

Only once in both series of observations, i.e. the series in iodine as well as the series in saline, was an infection encountered in which every cyst observed contained chromatoid bodies.

It is possible that in cysts containing very small chromatoid bodies these might be overlooked in iodine, and that only counts from stained preparations would give absolutely accurate results. It is, however, from saline or iodine preparations that the usual routine diagnosis has to be made, and to such cases the present observations are applicable.

Of the three hundred and nineteen *E. histolytica* cysts containing chromatoid bodies 30 per cent. were mononucleate cysts, 19 per cent. were binucleate cysts, and 50 per cent. were tetranucleate cysts. The cysts themselves occur, as Table III shows, in the proportions mononucleate cysts 32 per cent., binucleate cysts 13 per cent., tetranucleate cysts 53 per cent. The fairly close agreement between these figures seems to show that the supposition of some writers (Hartmann* 1912, James† 1914) that chromatoid bodies tend to disappear as the cyst becomes mature is not borne out by the facts. The chromatoid bodies seem indeed to be present indiscriminately in all the stages.

Chromatoid bodies are undoubtedly less frequent in cysts of *E. coli*. Out of 1,240 cysts observed during the course of this work 1,159 (93 per cent.) were without any chromatoid bodies. In sixty-nine (5.5 per cent.) chromatoid bodies were present. The sixty-nine cysts with chromatoid bodies came from nine different cases out of the forty-six cases observed, so that at one time or another about 20 per cent. of all the cases with an infection of *E. coli* showed cysts with chromatoid bodies. All the cysts with chromatoid bodies were eight-nucleate cysts with one exception (a tetranucleate cyst).

The general result of these observations is to diminish the value of chromatoid bodies as a diagnostic character for *E. histolytica* cysts because they are so frequently absent from the cysts (two-thirds

* Hartmann states, 'Die Bedeutung dieser Chromidien und Chromidialkörper ist nicht vollkommen klar. Wahrscheinlich handelt es sich um einen Reservestoff; denn im weiteren Verlauf der Cystenbildung und der sich anschliessenden Cystenruhe werden diese Körper in der Regel ganz oder fast ganz aufgebraucht.'

† James says, that in many of the adult cysts there are but few chromidia, and the four nuclei show very plainly.

of all the cysts being without them), at any rate when observed in saline or iodine. It has been shown also that in such samples as are commonly taken for routine examination the whole sample is without chromatoid bodies on about 50 per cent. of the occasions of examination.

With the conclusions here reached as to chromatoid bodies may be contrasted the statements of Mathis and Mercier (1917a). These authors state that the typical mature (tetranucleate) cysts of *E. histolytica* always contain chromatoid bodies and that such typical cysts are 75 to 80 per cent. of all the cysts encountered. My observations show that of tetranucleate cysts, as of mononucleate, only about 25 per cent. contain chromatoid bodies.

SIZE STRAINS IN THE TWO SPECIES

We have seen already that the frequency curve for *E. histolytica* cysts indicates the existence of two strains differing in size, which I have referred to in this paper as the 'small' strain and the 'ordinary' strain respectively. These two strains have already been noted in previous literature. Wenyon and O'Connor (1917) and Dobell and Jepps (1917)* have carried this idea further, and have indicated the existence of other size strains. The evidence given is that when the average diameter of a number of cysts is obtained from any one infection, this average diameter remains constant, or nearly so, from day to day, for that infection, so that a sufficient

* While the present paper was passing through the press, a paper by Dobell and Jepps (1918) appeared in which the theory of the presence of various size strains in the species *E. histolytica* was much more fully elaborated. By the measurement of large samples (500 cysts) of seven different infections the authors claim to have established the existence of five size strains in this species. The cysts occurring with greatest frequency in each of these five races are approximately of the following sizes: 6.6μ , 8.3μ , 11.6μ , 13.3μ , and 15μ . The measurements are the fullest and most accurate that have yet appeared, and I am in full agreement with their conclusion that infections of these sizes were found in the material on which they worked. It is probable, also, that the infections of the five different sizes mentioned correspond to five real strains or races in *E. histolytica*. I do not, however, think that, so far, the authors have given complete proof of this supposition. They say, 'For the complete demonstration of this fact [the existence of strains in *E. histolytica*] it is necessary to prove that the mean diameter of the cysts from any patient is not subject to any considerable variation from day to day, but remains constant.' In proof of this they appeal to their general experience, but only furnish actual measurements from one case, their E42. In this case two samples of 500 cysts each were taken at dates about a month apart, and the average diameter of the two samples differed by only 0.25μ . This difference and differences similarly obtained in my own work are very small compared with the difference between the two strains which I have called the 'small' (7.7μ) and the 'ordinary' (12.6μ), and I consider the existence of these two strains to be sufficiently established. The difference found by Dobell and Jepps, and differences similarly obtained

sample obtained at any time gives an average size characteristic of that infection and different from the average of other infections. Wenyon and O'Connor say: 'Starting from the strain with small cysts, a series of strains occur with gradually increasing average size of cyst. There are strains in which the cysts measure 9μ to 12μ , others 10μ to 14μ , others 12μ to 16μ , and finally large strains with cysts measuring 14μ to 18μ . As is to be expected, each strain is associated with "minuta" forms of amoebae of corresponding size. It seems very improbable that these strains represent different species of amoebae, for we cannot be sure that a strain of amoebae which will produce cysts of small size at one time will never at another time produce larger ones. We have noted, however, that in case Healy, in which cysts of large average size were found for a long time, towards the end of the period of observation a certain number of smaller ones began to appear. The point, however, can only be definitely decided by following individual untreated cases for long periods.'

In the course of the present work samples from different stools of certain individual cases were measured on several different occasions, and it might have been thought that in this way proof would have been forthcoming as to whether the average size of the cysts of an infection varied or not from time to time. It has to be remembered, however, that unless fairly large numbers of cysts are measured on each occasion the variations due to errors of sampling will be large, and no strict proof will be obtained. For the main purpose of this work large samples were not required, and as has been indicated, fifty cysts was usually the largest number measured at one time.

by me on smaller samples are, however, not insignificantly small when compared with the difference between such strains as the 11.6μ and 13.3μ strains. It seems to me, therefore, that much more evidence than can be supplied by two samples from but one case, however large the samples may be, is necessary to prove the constancy of size of races as close together as this in average size. Successive random samples of 500 cysts from the same stool do not differ by as much as 0.25μ , so that such a difference, small as it is, may be significant of change rather than constancy. When, too, the authors suggest that further detailed work would no doubt reveal other races possessing cysts of other mean diameters, they are elaborating a system of strains within the species whose mean diameters are so near together that it becomes almost impossible to prove their existence. If it is taken as probable, rather than fully proven, that the five strains of Dobell and Jepps exist, there are indications that in the present work I have found, besides the 'small' strain, a strain corresponding to the 11.6μ strain of Dobell and Jepps in my Infection I of Table VII, shown in Fig. 4, and one corresponding to their 13.3μ strain in Infections 12, 13, and 14 of Table VII, shown in Fig. 7. It seems clear from my Infections 7 to 11 of Table VII that either a race intermediate between these two exists or that many infections consist of the 11.6μ and 13.3μ strains in roughly equal proportions. I have found no infection with an average as high as 15μ .

TABLE VI.

Case	Date of examination	Number of cysts measured	Average diameter (μ)
1	17.1.18	21	14.4
	19.1.18	33	13.9
	29.1.18	23	13.4
	25.2.18	60	13.7
2	13.12.17	45	11.6
	21.1.18	25	11.4
	22.1.18	30	11.8
3	1.12.17	37	12.6
	16.2.18	50	12.0
4	2.7.17	50	12.3
	16.7.17	28	12.6
5	25.7.17	20	12.2
	10.8.17	19	12.1

Table VI gives for five cases of *E. histolytica* the average diameter of the cysts at different dates in the same case, together with the number of cysts measured. Mere inspection, however, does not enable us to decide how far the variations which occur in the average diameter are significant. In Cases 2, 4 and 5 the agreement seems close, while it is somewhat less so in Cases 1 and 3, but one cannot tell whether these differences are, or are not, due to random errors of sampling in such small numbers. I have applied Karl Pearson's (1911) method of 'goodness of fit'* to Cases 1 and 3. By this method it is found that the variations in Case 1 are such as are to be expected in samples of this size. In Case 3 the difference is such that only once in fifty trials would such a difference occur without real change in the size of the cyst population. The apparent change in average size in Case 3 appears therefore to be significant, but much further work on these lines is necessary before this point can be settled. It is interesting, apart from definite proof on this point, to see if any indications occur in my measurements pointing to the existence of a greater number of size strains than the two already mentioned.

In Table VII are found in ascending order of size the average diameters of all samples of cysts larger than fifty of the 'ordinary' strain.

* I am much indebted to Dr. James Johnstone for drawing my attention to this method and for other help with the statistics of this paper.

TABLE VII.

Infection	Number of cysts measured	Average diameter of the cysts (μ)
1	100	11.6
2	50	11.7
3	60	11.8
4	50	11.9
5	51	12.1
6	87	12.2
7	92	12.4
8	73	12.4
9	63	12.6
10	51	12.7
11	80	12.7
12	49	13.6
13	140	13.8
14	55	14.3

It would be expected, if strains exist among these, that the averages would tend to group themselves round two or three nodal points according to the number of strains existing. Instead of this, however, we find a continuous series of eleven infections from 11.6μ to 12.7μ . Then comes a distinct break in the series, the remaining three infections having averages of about 1μ higher than the highest of the previous eleven infections. There is some indication therefore of two strains in this series, a larger and a smaller.

Fig. 4, p. 57, shows the curve of frequency of one of the smaller infections (average 11.6μ) and also the curve for one of the larger infections (average 13.8μ). The measurements represented in the curves are:—

Scale-divisions	...	...	...	...	5.7	6.2	6.7	7.2	7.7	8.2	8.7	9.2	9.7	10.2	10.7
Number of cysts	11.6 infection	...	...	...	3	17	37	26	12	5	...	...	...	...	...
	13.8 infection	...	...	...	...	...	1	11	34	38	33	16	5	1	1

The comparison seems to show clearly that the strains are different, there being a difference of about 2μ between the modes, 1.5μ between the minimal readings and about 4μ between the maximal readings. In each case, too, a fairly smooth and symmetrical curve is produced. These infections are those shown in Cases 1 and 2 of Table VI, and it is seen that the variations from day to day are considerably smaller than the difference between the average of one curve and another. If the readings from the two infections, however, are added together, the curve of fig. 5, p. 57, is obtained with mode at 13.1μ . This curve, too, if smoothed a little between the third and fourth reading becomes a typical symmetrical frequency curve. When, therefore, it is remembered that between these two infections there are many others of intermediate sizes, it will be seen that the proof of the existence of two separate strains corresponding to the two infections in fig. 4 depends upon the most rigid proof that variations in the size of the cysts do not occur from one day to another which are at all significant in comparison with the difference in size between the two strains. Such proof is not yet forthcoming in this series of measurements, and the question must be left open for the present.

It may be recorded, however, that infections of the 'ordinary' strain* of *E. histolytica* from those cases who have never been out of England and who have never had dysentery are prevailingly of the smaller-sized cysts. In fig. 6, p. 57, the curve represents the measurements of all the cysts measured from carriers of *E. histolytica* who had never left England. The figures represented in the curve are:—

Scale-divisions ...	...	...	5.7	6.2	6.7	7.2	7.7	8.2	8.7
Number of cysts ...	...	...	5	43	85	95	47	22	3

It will be seen that a symmetrical curve results, with average of 12μ (300 cysts measured) and with no cyst over 15μ . The mode of the curve is at 12.2μ and the mean is 12μ , so that these coincide closely enough. What significance attaches to the fact that all the infections of the 'ordinary' strain which have come to my notice in persons who have never left England and who have not had

* Infections of the 'small' strain are infrequent in carriers who have never left England.

dysentery are smaller than the average in size I cannot say. Further investigations are being made into this question.

In fig. 7, p. 57, the curve represents the measurements of the three infections with large cysts shown in Table VII. The figures shown in the curve are :—

Scale-divisions	5·7	6·2	6·7	7·2	7·7	8·2	8·7	9·2	9·7	10·2	10·7
Number of cysts	1	2	2	23	54	66	52	25	11	4	4

The curve is smooth and symmetrical, with mode $13\cdot9\mu$ and mean $14\cdot1\mu$, and such as would be obtained if it represented a single strain. It was noted in describing the main curve (fig. 1) for the 'ordinary' strain of *E. histolytica* that it was not quite as smooth or symmetrical as it might have been expected to be, containing as it does the measurements of 807 cysts. Both the curves obtained by separating out certain cases from the large group, viz., the curves of fig. 6 and fig. 7 are smoother and more symmetrical (as is seen by the closeness of the mode to the mean) than the large curve of fig. 1. This would be the case if the curves of figs. 6 and 7 represented separate strains, while the large curve represented a mixture of strains. The facts about these curves may therefore be an indication that separate strains exist within the 'ordinary' strain, but of course they constitute no proof of this hypothesis.

The existence of the 'small' and the 'ordinary' strains is better grounded. The present large series of measurements has revealed very few cysts indeed intermediate in size between these two strains, and no single infection in which the majority of cysts are of this intermediate size. The variations which occur in the average size of the cysts of an infection (see Table VI) are small as compared with the difference between the average size of the 'small' strain $7\cdot7\mu$ and that of the 'ordinary' strain $12\cdot6\mu$. Observations of many more cases than are included in the measurements of this paper show that cases with the 'small' strain show that strain persistently for a long time if they are not cured, and that the same applies to the 'ordinary' strain. The two strains do not replace each other.

To summarise this section, it has been shown that there are

indications that the ordinary strain of *E. histolytica* cysts may be further sub-divided into at least one smaller and one larger strain. No proof of the existence of these strains can be established until it can be shown that the size of the cysts in one infection does not vary outside the limits of sampling errors from one day to another. It would also be of great interest to know whether a change of host would have any effect on the size of the cysts of an infection. The investigation of this question is accompanied, however, with obvious difficulties. The existence of two strains, the 'small' (7.7μ) strain and the 'ordinary' (12.6μ) strain, is considered to be established.

Size strains in E. coli cysts

In Table VIII are given in ascending order of size the average diameters of all the *E. coli* infections of which fifty or more cysts were measured.

TABLE VIII.

No. of infection	Number of cysts measured	Average diameter (μ)
1	51	15.6
2	50	15.8
3	50	16.3
4	50	16.4
5	100	17.0
6	52	17.4
7	51	17.5
8	53	17.6
9	190	18.6
10	50	18.6
11	50	18.8

Just as in the case of *E. histolytica* the averages are fairly evenly distributed between the two extremes. There is a considerable interval (1μ) between Infection 8 and Infection 9, which may indicate that the species is split up into at least two size strains, but nothing definite can be said until more infections and larger samples of these

FIG. 4. Two infections of *E. histolytica* 'ordinary' strain
100 cysts and 140 cysts.

FIG. 5. The two infections of Fig. 4 in one curve. 240 cysts.

FIG. 6. 300 cysts from all the cases who have never left England.

FIG. 7. Three infections (244 cysts) of large cysts of *E. histolytica*.

FIG. 8. Two infections of *E. coli*. 100 cysts and 190 cysts.

have been measured. Table IX gives the variation from one day to another in the size of the cysts of a few infections.

TABLE IX.

No. of infection	Date of examination	Number of cysts measured	Average diameter (μ)
9	10.7.17	20	18.6
	11.7.17	65	18.6
	25.7.17	24	19.3
	26.7.17	21	18.2
	30.7.17	20	18.8
	1.8.17	40	18.2
5	14.11.17	50	16.8
	29.11.17	25	16.6
	24.1.18	25	17.7
6	17.9.17	28	16.8
	18.9.17	24	18.1
8	26.11.17	21	17.1
	27.11.17	32	17.9

The evidence upon the important question as to whether change in cyst size occurs from one day to another is insufficient. The samples measured are usually too small. In Infection 8 the agreement is fairly close between one day and another. In Infections 5, 6 and 9 there are differences of over 1μ between the extremes of the average measurements. In Infection 9, however, the extremes are connected by averages intermediate between them, so that the whole infection remains fairly uniform. Further measurements of large samples are required before it can be stated whether variations do or do not occur outside the limits of error of sampling.

In fig. 8, p. 57, the measurements for the two largest samples, one hundred from Infection 5 and one hundred and ninety from Infection 9, are given in the form of curves. The curves represent the following figures:—

Scale-divisions ...		8.0	8.5	9.0	9.5	10.0	10.5	11.0
Number of cysts	Infection 9 ...	...	1	10	15	32	28	42
	Infection 5 ...	2	10	11	19	21	17	10

Scale-divisions ...		11.5	12.0	12.5	13.0	13.5	14.0	14.5	15.0	15.5	16.0
Number of cysts	Infection 9	26	16	6	5	3	2	1	0	1	1
	Infection 5	6	3	1	...	...	...	...	...	...	...

Taken by themselves these would seem to belong to two distinct strains, but as there are infections apparently intermediate between them in size, the matter cannot be settled without further data. If two, or even three, different size strains of *E. coli* occur, then cases would probably exist having an infection of the pure strains only, while other cases would doubtless show mixtures of two or more of the pure strains. In such a state of things the measurement of samples from a large number of cases would reveal a series of infections ranging pretty evenly between the two extremes of size, and differing from each other by comparatively small amounts, such a series in fact as Table VIII shows. It becomes thus exceedingly difficult to establish the existence of strains which lie so near together in average size as, say, 1.5μ . Only by measuring large samples of a large number of infections could the matter be settled. It would be very desirable also to settle the question as to the effect of a different host upon the average size of the cysts of an infection. Until these two lines of enquiry have been followed up the question must be left open.

It may be recorded that in one case there appeared to be an effect of emetine bismuth iodine treatment upon the size and shape of the cysts of an *E. coli* infection. Before the treatment fourteen cysts were measured on November 8th, 1917. The average size was 17μ . The treatment caused a disappearance of *E. coli* cysts from the stools for a period. Their first reappearance in the stools was on November 30th. On this date twenty cysts were measured, and their average size was 20.4μ . This increase of size is quite outside the usual limits of variation for such samples. Not only were the cysts large, but they were much more irregular in shape. Before the treatment only one cyst out of fourteen was recorded as asymmetrical. Afterwards fourteen out of twenty were recorded as asymmetrical.

SUMMARY

It will be of interest to summarise the results of the present work in their bearing upon the question of the diagnosis of the cysts of *E. histolytica* and *E. coli*.

The size of the cysts

It has been shown to be possible, relying upon other characters than that of size for the diagnosis of the species, to obtain two curves

showing the frequency of the various sizes of cysts in the two species. These two curves give information as to the amount of overlapping in size of the cysts of the two species. It is shown that between 12μ and 18μ (other authors give 11μ to 20μ) cysts of both species may occur. The present curves show, however, that the extreme sizes are so rare that the probability of their occurrence is very small. It is only between 13μ and 15.5μ that any considerable numbers of both species of cysts occur. Consequently, except between these limits, the factor of size may be given considerable weight in the diagnosis.

The shape of the cysts

E. histolytica cysts are somewhat more often distinctly asymmetrical than are *E. coli* cysts. Judged by the same standard of asymmetry, 21 per cent. of *E. histolytica* cysts are asymmetrical, while only 16.5 per cent. of *E. coli* are so.

The number of nuclei

It has been shown that the two species differ very much in the comparative frequency of the mature (tetranucleate for *E. histolytica*, 8-nucleate for *E. coli*) cyst in the stools. For *E. coli*, at any rate, it has been proved that the comparative rarity or abundance of cysts in the early stages of nuclear division depends upon the character of the stool, the immature cysts being more frequent in loose stools. Though no similar proof is forthcoming in the case of *E. histolytica*, yet it becomes important to state that the figures obtained refer to infections for the most part from formed and semi-formed stools. In such material mature cysts of *E. histolytica* formed 53 per cent. of all those encountered, while in *E. coli* 87 per cent. of all the cysts were mature. Since the stools were similar for the two species, it is clear that *E. histolytica* cysts more frequently escape to the exterior in the faeces while in the early stages of nuclear division. Problems of diagnosis are much simplified by this difference between the two species, for the rule that all cysts with more than four nuclei are diagnosed as *E. coli* at once settles the diagnosis of approximately 90 per cent. of all the cysts of *E. coli* encountered. At the same time, I cannot agree with the statement of Wenyon and O'Connor (1917) that 'the presence of four nuclei in the fully-developed *E. histolytica* cysts is so universal that one can state with

certainly that this is a reliable feature for diagnostic purposes.' It is true that the fully-developed cyst of *E. histolytica* contains four nuclei, but this only helps the diagnosis in so far that cysts with more than four nuclei can be certainly referred to *E. coli*. The presence of four nuclei in a cyst is not, however, in itself a reliable feature for diagnosis, since an *E. coli* cyst obviously may contain four nuclei. Moreover, it has been shown in the present series of observations that only about half of the cysts of *E. histolytica* are in the tetranucleate stage, the remainder being mono- or binucleate. Since *E. coli* cysts may also be mono- or binucleate, a large number of cysts must remain doubtful if regard be paid solely to the number of their nuclei. It can be said, now that the present work has shown that mono- or tetranucleate cysts of *E. coli* are comparatively rare in the stools of dysentery convalescents, that if these numbers of nuclei occur in a cyst, there is a considerable probability that the cyst in question is that of *E. histolytica*. Also if an infection occurs in which anything like half the cysts are tetranucleate, *E. histolytica* must be present. Such considerations, however, cannot be decisive; for instance, an infection such as the one just mentioned, though it must contain *E. histolytica*, may also contain *E. coli*. The fact that I have not encountered a single infection of *E. coli* which did not contain some 8-nucleate cysts is more decisive. It appears from my experience that the 8-nucleate cyst is present in every infection of *E. coli* cysts from similar material to that used in the present work. In spite of the exception taken to the above statement of Wenyon and O'Connor, I completely agree with them in their contention that the cysts of the two species are distinct. Taking all characters into account, I have no doubt that a correct diagnosis can be given in the overwhelming majority of cases. As was stated earlier, the chief difficulty in diagnosis occurs with tetranucleate, and to a less extent with binucleate cysts. Nuclear characters, described previously, usually suffice to distinguish *E. histolytica* from *E. coli* in the mononucleate stage (the great rarity of mononucleate cysts of *E. coli* being also taken into account). The vacuolated binucleate *E. coli* cyst, with its large, deeply-staining vacuole and usually lenticular nuclei, is very characteristic and cannot be mistaken. The non-vacuolated binucleate *E. coli* cyst can usually be diagnosed by the large amount of peripheral chromatin in its nuclei, and also

by its colour and by the uniformity of its cytoplasm. Sometimes, however, a single binucleate cyst is undeterminable. The tetranucleate cyst without chromatoid bodies is the most difficult to diagnose, for the nuclear characters are occasionally not decisive at this stage. The nuclei of a tetranucleate *E. coli* cyst, however, are of larger size both actually and relatively to the cyst than in *E. histolytica*, and the tetranucleate *E. coli* cyst has usually the colour and texture of the cytoplasm characteristic of this species, so that a diagnosis can usually be made. Considering the comparative rarity of tetranucleate *E. coli* cysts, the difficulty in practice is not of great importance. The differences between the tetranucleate cyst of *E. histolytica* and that of *E. coli* are seen in figs. 8 and 14 of the Plate accompanying Matthews' (1918) paper.

The chromatoid bodies

The chief result of this work as to the chromatoid bodies is to reduce their importance as a character for diagnosis on account of their comparative infrequency. It has been shown that in the average *E. histolytica* infection, as seen in the routine examination of convalescent dysenterics, slightly over half of the samples do not show any cysts containing chromatoid bodies. If a case can be subjected to continued examination, my figures show that cysts containing chromatoid bodies will be found in the vast majority of cases. Since, however, it is usually required, and is certainly desirable, that an absolute diagnosis shall be given on a single examination, the former figures, which show that in half the examinations the diagnosis must be made without the help of chromatoid bodies, are the more relevant. When we consider cysts, rather than infections, we see that only about one-quarter of all the cysts of *E. histolytica* contain chromatoid bodies. Even if all the doubtful cases are included as positive for chromatoid bodies, there still remain two-thirds of the cysts of *E. histolytica* which do not contain chromatoid bodies.

In *E. coli* only 5 to 6 per cent. of all the cysts have chromatoid bodies, though at one time or another about 20 per cent. of the cases with an infection of *E. coli* show cysts containing chromatoid bodies. Though these bodies occur rather rarely in *E. coli* cysts, yet when they do occur they are of great help in diagnosis, being almost always characteristic for the species.

A diagnostic table will now be given adding the results of measurement and enumeration to those given earlier in the paper.

	<i>E. histolytica</i> cysts	<i>E. coli</i> cysts
Size	5 to 20 μ . Two size strains exist of average diameter, 7.7 μ and 12.6 μ respectively. Of the 'smaller' strain 93 % of the cysts lie between 6 and 9 μ in diameter. Of the 'ordinary' strain 93 % lie between 10 μ and 14 μ and 96 % between 10 μ and 15 μ .	12 to 34 μ : 97 % lie between 13 and 22 μ in diameter, 84 % lie between 14 and 20 μ .
Number of nuclei ...	1 to 4: 53 % tetranucleate. 33 % mononucleate.	1 to 16: 87% 8 nucleate, 89 % with more than 4 nuclei.
Character of nuclei ...	Peripheral chromatin of small granules more or less evenly distributed. Nuclei in consequence less distinctly visible.	Peripheral chromatin of larger unevenly distributed masses. Nuclei, therefore, more distinctly visible.
Cytoplasm	Colour greenish, typically not uniform in appearance.	Colour paler, greyish, except in the vacuolated binucleates which form about 4 % only of the cyst population. Typically uniform in appearance.
Inclusions	Chromatoid bodies more frequent, present in $\frac{1}{2}$ of the cysts, but in almost all the infections. In about $\frac{1}{2}$ the examinations all the cysts are without chromatoid bodies. Rod-shaped, with square or rounded ends. Vacuoles more frequent, one or more in the cyst, usually faintly stained by iodine, with less sharply defined edges.	Chromatoid bodies less frequent, present in about 5 % of the cysts, and 20 % of the infections. In the great majority of the examinations all the cysts are without chromatoid bodies. Irregular in shape, with pointed or splintered ends. Vacuoles less frequent, generally single in the cyst, usually deeply stained by iodine, and with more sharply defined edges.
Cyst wall	Thinner	Thicker.
Shape	More often asymmetrical	Less often asymmetrical.

CONSIDERATION OF THE RESULTS OF OTHER WORKERS

Certain results of previous workers have been quoted in the course of this paper, but it may be useful also to comment on other work on the same lines as the present. Since the rule that all cysts with more than four nuclei belong to *E. coli* has proved to be of

such fundamental importance, it is necessary to say something on the question whether *E. histolytica* cysts ever contain eight nuclei. Kuenen and Swellengrebel (1913) and Swellengrebel and Schiess (1917) have affirmed the existence of 8-nucleate cysts in *E. histolytica*. I fully agree with Mathis and Mercier (1917) and with Dobell and Jepps (1917) in thinking the evidence brought forward by these authors insufficient to establish the existence of such cysts. More recently Brug (1917) has brought forward a case in which the evidence for the existence of a cyst of *E. histolytica* with eight nuclei is somewhat stronger. The cyst in question occurred in an infection in which all the remaining cysts were 1- to 4-nucleate and in which the majority were 4-nucleate. The stools of the patient producing this specimen had been often examined microscopically, and no *E. coli* had ever been seen. The size of the 8-nucleate cyst was 12μ , and it had a thin wall and small nuclei. The evidence, even in this case, is not complete. Brug unfortunately does not state how many times the stools of this patient were microscopically examined. Dobell (1916) has instanced a case in which an *E. coli* infection was not found until the thirty-seventh examination, and this case was only positive three times in one hundred and seven examinations. Carter, Mackinnon, Matthews and Malins Smith (1917) give a case in which an infection of *E. coli* was not detected until the forty-fifth examination. Since undoubted *E. coli* infections may show themselves as infrequently as in these cases, it becomes no longer surprising that an infection of *E. coli* should show itself very rarely while an accompanying infection of *E. histolytica* might be present much more regularly. It is, therefore, not possible to assert on this account alone that Brug's single 8-nucleate cyst was a cyst of *E. histolytica*. Neither can it be absolutely diagnosed by its size, 12μ . Though *E. coli* cysts of this size are rare, yet they undoubtedly do exist, and therefore size alone cannot give a certain diagnosis. The thickness of the wall is not in itself a character to which much importance can be attached. It seems, therefore, that though Brug's instance is better founded than previous ones, it is still possible to interpret it as a case in which a very scanty and infrequent infection of *E. coli* accompanied the infection of *E. histolytica*. A case comparable to that given by Brug, but with the *E. coli* infection appearing still

more infrequently, was that of a patient who was examined during the present work and who had an infection of *E. histolytica* which proved very refractory to all kinds of treatment. In consequence his stools were examined a very large number of times. The stools, in fact, were examined on one hundred and sixty-six occasions, on one hundred and twelve of which cysts of *E. histolytica* were present. On the seventy-second and on the one hundred and thirty-eighth examination *E. coli* was recorded. On the second of these occasions the record was based on a single 8-nucleate cyst. On the first occasion not more than two *E. coli* cysts were seen, but no record was made of the number of nuclei. On the ground of the infrequency of the appearance of 8-nucleate cysts in a frequent *E. histolytica* infection, this is a very strong case for the occurrence of an 8-nucleate cyst of *E. histolytica*. But such a consideration is not a reliable basis for diagnosis, and the 8-nucleate cyst was regarded as being undoubtedly *E. coli*. If 8-nucleate cysts of *E. histolytica* occur, they must be so rare as scarcely to affect the question of diagnosis, and certainly their occurrence has not yet been proved.

*Previous records of the extremes of size in cysts of
E. histolytica and E. coli*

My own records are 5μ to 18μ for *E. histolytica* cysts, 12μ to 34μ for *E. coli*. They may be compared with the following:—

	<i>E. histolytica</i>	<i>E. coli</i>
Dobell and Jepps (1917)	5 to 20μ	11 to 33.5μ
Kuenen and Swellengrebel (1913)	11 to 19μ	13 to 28μ
Mathis and Mercier (1917b)	11 to 15μ	14 to 28μ
Brug (1917)	7 to 20μ	10 to 25μ
Wenyon and O'Connor (1917)	6 to 18μ	13 to 36μ
Craig (1913)	7 to 20μ	...
Woodcock and Penfold (1916)	7 to 8 and 10.5 to $?$ μ	...

Besides these records, James (1914) has recorded a case in which the 8-nucleate cysts averaged only 10μ , and Wenyon (1913) has

recorded 8-nucleate cysts of 9μ in diameter. These are, however, isolated records, and in neither case is it distinctly stated that the cysts were measured in the fresh state. Apart from these, all the evidence points to 11μ being the extreme lower limit of size of *E. coli* cysts. Undoubtedly those observers whose lower limit for *E. histolytica* cysts is 10μ or 11μ have failed to observe what has been called in this paper the 'small' strain, infections of which have of late constituted about one-quarter to one-third of all the records of *E. histolytica* at the Liverpool School of Tropical Medicine.

Frequency curves for E. histolytica and E. coli cysts

Frequency curves have been given by Kuenen and Swellengrebel (1913) and Mathis and Mercier (1917). The curves given by the former, though based on far too few measurements, one hundred cysts only in each species, are in close agreement with the curves given in the present paper. Except for the fact that the 'small' strain of *E. histolytica* has been entirely omitted, their curves take the same general course as my own. With the curves given by Mathis and Mercier (1917) on the other hand, my curves, based on one thousand cysts, show no agreement whatever. The presence of two marked peaks in their curve for *E. histolytica* and three peaks in their curve for *E. coli*, with very few records between the peaks, is quite inexplicable to me. I am convinced from the measurement of much larger numbers than theirs that the dimensions of both species of cysts increase by infinitely small stages from one extreme to the other, and that, though the curves may not be perfectly smooth, there are no marked breaks in the series. I am, therefore, also unable to support their theory (Mathis and Mercier 1917c) of the occurrence in *E. histolytica* of 'kystes gamogoniques' of two types, microcysts and macrocysts, and in *E. coli* of 'kystes gamogoniques' or 'sexués' and 'kystes schizogoniques' or 'asexués,' the former kind being divided as in *E. histolytica* into microcysts and macrocysts. This theory is so closely associated with their peculiar frequency curves for the sizes of the two species of cysts and depends so much upon those curves for evidence, that the theory cannot very well stand unless the curves are accurate. Whatever may be the truth upon this latter point, it is quite clear that the numbers measured by these authors were far too few.

The possibility of distinguishing the two species of cysts

It is clear that all the work of the present paper implies a belief that *E. histolytica* and *E. coli* are separate species which can be distinguished from each other in the encysted stage.

In this belief the writer is supported by Wenyon and O'Connor (1917), Dobell and Jepps (1917), Mathis and Mercier (1917), as well as by the majority of less recent writers. Gauducheau (1915) has, however, stated the opposite view on what appear to me quite insufficient grounds. He acknowledges the reality of the presence in certain stools in Tonkin of two types of cysts, the larger with eight nuclei and the smaller with four nuclei. It appears to me that the separation into these two types (species) is possible, in spite of the fact that any particular diagnostic criterion may occasionally break down. More recently Knowles and Cole (1917) have enunciated the view that *E. coli* and *E. histolytica* are one species, and have even suggested for this species the name *E. coli communis*. Their chief evidence for this conclusion is that when they separated the species according to 'text-book teaching as to differentiation between amoebic species and after a full and careful examination of all accessible information with regard to each patient,' they could find no clear differential characters of any kind which would serve to separate the one species from the other. This conclusion is supported by several curves and tables showing the results of large numbers of careful measurements and observations. These all appear to support the deduction that neither in size, number of nuclei, character of nuclei nor pathogenicity do the two species show any clear differences. Since these curves and tables are entirely opposed to my own conclusions, it is desirable to put forward an explanation which would reconcile the two sets of measurements. I suggest as a tentative explanation of the work of Knowles and Cole a modification of a criticism which suggested itself to the authors themselves, namely, that the great majority of the cysts they measured were cysts of *E. coli*, and that only a small proportion were really *E. histolytica*. Probably only the infections which they separated out as *E. minuta* were really *E. histolytica*. The hypothesis that the stools in general, and particularly the series used for the measurements, contained fewer *E. histolytica* infections and more *E. coli* infections than the authors recorded would explain the difference between their results and those

of other workers. It would bring the general results of their examinations (see their Table III) more into line with what is usually found as to the relative frequency of *E. coli* and *E. histolytica* infections. It would explain the paucity of tetranucleate cysts and their large average size (15.5μ , see their Table IV), which might well be the average size of a mixed lot of cysts of the two species in which *E. coli* predominated. If, too, the real *E. histolytica* cysts were excluded from the figures on which Charts C1, C2, and C3 were based, by being separated out under the name *E. minuta*, then one would expect the remaining cysts, however divided artificially into two species, to give almost coincident graphs such as are presented in these charts. The great rarity of chromatoid bodies in the cysts also points in the direction of the cysts being prevailingly cysts of *E. coli*. The fact that many of the cases were those of acute or convalescent dysenterics does not dispose of this criticism, for Wenyon and O'Connor (1917) record that in nine hundred and sixty-one patients admitted to hospital in Egypt for dysentery, diarrhoea and related intestinal disorders, a single examination revealed the cysts of *E. histolytica* in only 2.2 per cent. of the cases. Unfortunately the authors nowhere state definitely what morphological criteria they relied upon in making diagnoses. The statements in the text-books are so conflicting and unreliable that one cannot gather their criteria from the recorded fact that they followed those given in the text-books. Until the authors state clearly on what morphological grounds—entirely apart from the dysenteric history of the patient, which ought to carry no weight whatever, since bacillary dysentery is so prevalent—their diagnoses were made, the simplest hypothesis which will bring their results into line with those of other workers is to suppose that the great majority, though not all, of their cysts were those of *E. coli*.*

Brug (1917) has considered the question of the differential diagnosis of the two species, and has concluded that there is no one single character which taken alone is decisive in diagnosis, but that by the totality of characters shown by a number of cysts one can arrive at a definite diagnosis. This is substantially the position taken up in the present work, though, as already stated, I do not

* A paper by Brug (1918) has appeared since the above was written, making substantially the same criticism of the work of Knowles and Cole.

think Brug's instance to the contrary is sufficiently well founded to invalidate the rule that cysts with more than four nuclei are those of *E. coli*. Undoubtedly, as Brug states in opposition to Mathis and Mercier, and as is also stated by Dobell and Jepps (1917) and Chatton (1917), the cysts of both species contain chromatoid bodies. I disagree with Brug, however, in thinking that the form of these is similar in the two species of cysts. Rod-shaped chromatoid bodies typical of *E. histolytica* are at any rate exceedingly rare in *E. coli*, and when they occur are usually narrower and more needle-like than in *E. histolytica*.

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