should be used for this purpose. There is barley and malt used for the production of alcohol, an inefficient form of food at the best and a deadly poison in excess. According to the Food Controller there are one million quarters of brewer's malt in this country at the present time; it is not to be used for food, but for the production of beer. The convictions for drunkenness in the Metropolitan Police District and the City for the first quarter of this year were 6,176. The Food Controller says that the food value of beer is not disputed, and the Government does not consider that it is necessary to save the millions of quarters of barley, malt, and other foodstuffs which are to be used in the manufacture of alcohol. There may be reasons of policy for allowing alcohol to men in the services or employed in some essential occupations, but there is none for its widespread consumption.

Oats can be used as a substitute for wheat, but there is difficulty in obtaining a ready and cheap supply; the oats are being kept for the horses. A horse may consume 40 to 80 lb. of oats per week, and hunters and racehorses receive the best. Hunting and racing have been and are allowed; only now is the Government taking steps to consider whether savings in this respect should be effected in the interests of the food supply of the people. There are thousands of hunters, racehorses, riding horses, and carriage-horses, used only for pleasure in this country; the consumption of oats, maize, beans, hay, straw, and carrots by these animals must be enormous. With the exception of the hay and straw, which would be useful for cows, all these tons of food could be used by man himself; the objection that the horses are needed as a reserve for breeding and for the army has no force, for all these animals could be kept at grass, and under these conditions would liberate many men for essential work.

TABLE II.—Percentage Composition of Certain Cereals.

	Protein.	Fat.	Carbo- hydrate.	Fuel Value per lb.
Wheat, flour ...	8.3	1.0	73.5	Calories. 1615
Barley, pearled ...	6.6	1.0	76.1	1630
Maize, flour ...	5.8	1.2	76.3	1625
Oat, meal ...	13.4	6.6	65.2	1795

All of these substances have been used even in times of peace as substitutes for wheat. In some respects oats are a better food than wheat and the fuel value of 1 lb. is greater than the total for one day of the voluntary ration suggested by the Food Controller. A horse eating daily 10 lb. of oats consumes an amount of cereal food which would be sufficient for ten to twenty men.

Sugar and potatoes are foods which were introduced into this country in times relatively recent, and it is obvious that they are not so essential as some people imagine. The children should have the first claim on sugar, and they could also obtain milk sugar in milk and malt sugar in malt, besides those essential substances known as "vitamines."

The supply of milk is another difficulty, and it has received the attention of the Government. A maximum price has been fixed for the retailer and the producer; the farmers have been selling their cows and giving up the production of milk, notwithstanding the fact that it was a very lucrative business according to many writers to the daily press. For young children, invalids, and our sick and wounded soldiers milk is a food for which no efficient substitute can be found. In the case of infants the practice of wet nurses should be revived if the mother cannot or will not suckle her child.

During the spring and summer months there may be no serious shortage, for those are the best months; when the cows are at grass the supply is large and feeding is cheap. There is a shortage at the present time, and the autumn and winter will show the effects of the reduction of the milking herds. Action, however, must be taken now. Heifers cannot be made into milch cows under ten months. The Government have revised their "milk orders"; the farmers ask for the recall of these orders. In any case a reserve of cows could be provided by the end of next winter if the Government purchased at once numerous heifers and turned them out to graze with

young bulls on land owned or taken over by the Government. Even if money were an object, the transaction would be probably a very profitable one. A supply of milk should be safeguarded, and its cost should be low for the sake of the children, even if it be necessary for the State to give a subsidy.

If the scarcity of food is general, the true remedy lies in an extension of the supply. Action has been taken at last, pasture has been ploughed up, but the highly fertile ground of the hop gardens will apparently in many cases still grow hops for beer, instead of corn or potatoes for bread. The hop growers should have compensation, if necessary, but food they should grow, for it is food which is needed.

Another source of food which is undeveloped is the salt-water and the fresh-water fish, but this, owing to the supply of cattle, is not so urgent as the question of cereals.

If the cost of food continues to rise, difficulties of a serious kind must occur, for a strong man is liable to become dangerous when he is hungry. A corresponding rise in wages does not meet every difficulty. The wage is not adjusted to the size of the breadwinner's family. A great relief and a true safeguard for the present and the future would be the free supply of dinner and tea to school-children; the necessary machinery is simple, if the expense is a general one borne by the State. The health of the future generation could be secured, a great burden would be taken from many hard-working and deserving parents. Foodstuffs which the labouring classes cannot obtain readily or cook properly could be used in these free meals and bread would be saved. There would be no danger that the children would be overfed, for even with active and growing children the law of averages as regards food would hold good. Many children, as well as men and women, are doing more work than they ever did before; this means an increase in the consumption of food, especially cheap food, such as the cereals.

Petty orders, such as the prohibition of Sunday-school treats and cakes, show no appreciation of the laws of nutrition, and cause an amount of irritation out of all proportion to the result obtained. The owner of poultry is not allowed corn for his fowl; the hen does not return in eggs and flesh the food value of the grain consumed; no animal ever did or ever will, for it must have some for its life. When allowance, however, is made for the consumption of refuse the hen is economical. The owner of racehorses, hunters, and other horses used for pleasure, is allowed in unlimited quantities the very best oats, maize, and beans that he can buy. He takes the food from the mouths of the poor by outbidding them in the purchase of some of the best articles of food which are scarce at the present time.

ON THE THREE COMMON INTESTINAL ENTAMOEBAE OF MAN, AND THEIR DIFFERENTIAL DIAGNOSIS.

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At the present day the belief is widely held that there are only two amoebic parasites of the human intestine—the harmless *Entamoeba coli*, and the dysentery amoeba, *E. histolytica*. Although it is certain that nearly all the amoebae hitherto described from human faeces actually belong to one or other of these species, whatever names may have been bestowed upon them, nevertheless it is now possible to say with equal certainty, we believe, that there are not two, but in reality three different *Entamoebae* commonly inhabiting the human bowel. Our own observations have convinced us personally of this fact, and in conjunction with those of others recently published have, we believe, placed the matter beyond all doubt. As many people are now engaged in the diagnosis of amoebic infections, and as they are still in many cases—as we have good reason to believe—confining their attention to certain forms of the two well-known species only, we think no excuse is needed.

for offering the following summary of our present knowledge of this matter. It is clearly of considerable importance that the facts should be widely known, as there are still many convalescent dysenteric patients in the military hospitals and dépôts in this country who are being examined and treated for infection with *Entamoeba histolytica*. A correct diagnosis of such infections is not possible unless the three species of *Entamoeba* commonly occurring in such patients are clearly differentiated from one another, and from other organisms.

We may begin by briefly recording our own experiences, indicating later how they agree with, or differ from, those of others.

Not so very long ago it was generally supposed that the cysts of *Entamoeba histolytica* could be readily distinguished from those of other *Entamoebae* by their size. It was—and still is—stated that the cysts of this organism have an average diameter of about 12μ , individual cysts varying from this mean by only one or two microns as a rule. We have long known that this is not correct, and that there is a far greater range of size observable in cysts passed in the faeces of different persons. A case observed by one of us at the beginning of 1916 regularly passed cysts of *E. histolytica* having an average diameter of only 7 to 8μ . Since then we have observed many similar cases. In examining the stools of patients for these small cysts we frequently encountered other small cysts containing one, two, or four nuclei, but differing in structure from those of *E. histolytica*, which at times gave us considerable trouble in diagnosis. These cysts had been figured by Wenyon (1915), and attributed by him to *Chilomastix* (= "*Tetramitus*") *mesnili*. That they did not belong to this organism we soon convinced ourselves, as we have long been familiar with its very characteristic, and always uninucleate, cysts. But to what organism they really belonged we were long unable to decide. Meanwhile we discovered on many occasions small dead or degenerate organisms, associated at times with the cysts in question, and other "cysts" or "bodies" characterized by the presence within them of a large mass of glycogen. We further discovered a small amoeba which in some ways resembled the small free-living amoebae of the *Amoeba limax* type, but which, however, appeared to be a true intestinal parasite, and not a coprozoic form, as it invariably died very quickly after leaving the body of its host. We have now been able to prove that all these cysts and organisms are stages in the life-history of the same form—a small parasitic amoeba which is by no means uncommon in the class of cases examined by us.

In the meantime, descriptions of this organism have been published by Swellengrebel and Winoto (February, 1917) and by Wenyon and O'Connor (March, 1917). To the descriptions and figures of the latter authors we can add but little. They have called the new organism *Entamoeba nana*, and have noted that it is a comparatively common parasite in Egypt. Its frequent occurrence in dysenteric and other patients in this country has not, we believe, been hitherto noted.

We propose now to describe very briefly our observations on *E. nana* and its cysts; to describe then the chief points of difference between these and those of *E. histolytica*—with especial reference to small cysts of the latter; to discuss very briefly certain relevant details concerning *E. coli*; and to add a few notes on the methods which we have found most useful in identifying these species, and distinguishing them from other organisms, in the routine examination of human faeces for the detection of protozoal infections.

Entamoeba nana (Wenyon and O'Connor).

E. nana is a very small amoeba measuring, when rounded, about 6 to 12μ in diameter, the majority of individuals being about 8μ . In general structure it somewhat resembles many amoebae of the so-called *limax* group. The protoplasm is generally much vacuolated, the vacuoles containing bacteria upon which the animal feeds. There is no contractile vacuole. The pseudopodia are few, blunt, and hyaline. The very characteristic nucleus is rather small, and not always easily observable in the living organisms. Its karyosome is smaller than that in the nucleus of a typical *A. limax*, and usually occupies an apparently excentric or peripheral position. It is usually irregular in shape and often segmented into several pieces of unequal size united by slender threads. (See Fig. 2.)

The amoeba moves very sluggishly on a cold stage, and very soon becomes rounded up and dies. Attempts at cultivation on amoeba agar have always failed.

The cysts are oval or spherical structures, usually measuring 8 to 10μ in length or 7 to 8μ in diameter. The nuclei are 1, 2, or 4 in number in the vast majority of cysts, closely resembling in structure those of the uninucleate amoebae, except for their smaller size in late stages of development. (See Figs. 7, 8, 9.) In the quadrinucleate cyst the nuclei usually lie near to one another, and frequently in close contact. Very rarely cysts have been observed containing as many as eight nuclei.

In addition to the nuclei the cysts contain a variable number of highly refringent granules which give some of the reactions of volutin; and sometimes—especially in the binucleate stage—a large dull inclusion is also visible in the living cyst. This is composed of a substance insoluble in alcohol, soluble in water, coloured dark brown by iodine, and bright red with Best's carmine, from which we conclude that it is a mass of glycogen.

The cysts of *E. nana* appear to be very resistant. Although they cannot withstand drying, they remain apparently unchanged in moist faeces for more than three weeks. We have stained specimens made after an interval of twenty-four days which are as good as those made from the fresh stool.

From the fact that persons infected with *E. nana* sometimes pass diarrhoeic stools which contain only cysts, it seems to us not improbable that the habitat of this amoeba is the small intestine. (A similar phenomenon is seen in the case of *Lambia*, which inhabits the small intestine, but is very unusual with the protozoal parasites of the colon.)

We have no evidence to show that *E. nana* is pathogenic. The majority of infections studied by us were in convalescent dysenteric cases; but we have no reason to correlate the infection with the dysentery, and have found the amoebae in persons with no dysenteric history. As the organism resembles *E. coli* in its food habits, it seems likely that it is equally harmless. Many of our cases have been treated for *E. histolytica* infection with emetine bismuthous iodide, but in no case have we observed that the treatment removed the *E. nana*. In this respect also, therefore, this organism resembles the harmless *E. coli*, and differs from *E. histolytica*.

E. nana is in our experience a very common parasite. It is only recently that we have kept a careful record of infected cases, and we have not always determined small infections and those in which we found only dead and degenerate organisms. Moreover, most of our recent cases have been examined only twice each, which we know is insufficient to detect all infections. Nevertheless we have recorded over 15 per cent. of the last four or five hundred cases examined as infected with *E. nana* (73 infections in 478 cases).

It seems clear that the organism just described is identical with that described by Wenyon and O'Connor, although they do not mention the occurrence of glycogen in the cysts—a feature which we find very constant and characteristic. Such cysts have, however, been described by Swellengrebel and Winoto for their so-called "*Amoeba limax*," which, as already noted, we also believe to be identical with the amoeba studied by us. Wenyon and O'Connor state (1917) that the organisms previously described by Wenyon (1916) as *Amoeba limax* were in reality *E. nana*; but a still earlier reference to this organism appears to us to be contained in a paper by James (1915). The description and figures of his "free-living amoebae from the human intestinal tract" seem to us to refer almost certainly to *E. nana*, although he noted the occurrence of uninucleate cysts only. James himself identified his organisms with those previously described by Wenyon (1913) and Chatton and Lalung-Bonnaire (1912). Wenyon's amoebae may possibly have been *E. nana*,* but those described by the French authors are certainly not, as they are undoubtedly typical amoebae of the *limax* group.

E. nana appears to have a wide geographical distribution. The infections we have studied were in soldiers invalided to England from Egypt, Gallipoli, Salonica,

* Colonel Wenyon's return to England since these lines were written has enabled us to determine this point. He has very kindly allowed us to examine preparations of the amoebae, which he first described in 1912 (*Journ. London School of Trop. Med.*, vol. ii, p. 31). He agrees with us that the amoebae are *E. nana*; and this appears, therefore, to be the first case of infection with this organism on record.

DESCRIPTION OF FIGURES.

All figures, except 13 and 14, depict fixed specimens stained with iron haematoxylin.

FIG. 1.—*E. histolytica*, amoeba of "minuta" form belonging to race forming small cysts.

FIG. 2.—*E. nana*, two free amoebae; showing nuclei and numerous vacuoles containing ingested bacteria.

FIG. 3.—Amoeba of the *A. limax* group from human faeces some days old. Note the contractile vacuole, nucleus, and food vacuoles.

FIGS. 4, 5, 6.—Uninucleate, binucleate, and quadrinucleate cysts respectively of *E. histolytica*, race producing small cysts. The black masses in the cysts are the deeply-stained chromatoid

bodies; the clear spaces in Figs. 4 and 5 the vacuoles, from which the glycogen has been extracted in making the preparations.

FIGS. 7, 8, 9.—Uninucleate, binucleate, and quadrinucleate cysts respectively of *E. nana*. Compare Figs. 4, 5, 6.

FIG. 10.—Cyst of typical *Amoeba limax*, showing nucleus, chromatoid masses, and thick corrugated cyst wall.

FIGS. 11 and 12.—Cysts of *Chilomastix mesnili*, oval and typical lemon-shaped forms respectively; showing nucleus, remains of buccal structures, and volutin grains.

FIG. 13.—Binucleate cyst of *E. nana* in iodine solution. The black mass represents the deeply-stained glycogen vacuole.

FIG. 14.—Cyst of *Ch. mesnili* containing large mass of glycogen, as it appears in iodine solution.

Mesopotamia, France, and South Africa. The cases studied by Wenyon and O'Connor were in Egypt, James's cases were in Panama, and the three cases of Swellengrebel and Winoto were a European in Sumatra, a Javanese in Holland, and a European from the Dutch Indies. It is not, however, possible to state that all the infections were contracted in the places mentioned.

We have followed Wenyon and O'Connor in placing the amoeba just described in the genus *Entamoeba*. It differs, however, so considerably from other members of the genus—especially in its nuclear structure—that we have done so with some hesitation. We do not yet think it possible to define the limits of the genus *Entamoeba* with precision, owing to our ignorance of the complete life-history of most parasitic amoebae.

Entamoeba histolytica (Schaulinn emend.).

This organism is now so well known that it is unnecessary to redescribe it. We propose here merely to enter into some detail concerning the dimensions of its cysts. For a considerable time we have kept records of the size of the cysts in all infections which we have studied, and have thus collected particulars of 200 cases. We will now summarize these as briefly as possible.

In 75 of the infections cysts below 10μ in diameter occurred; and in 62 of these no cysts larger than 10μ were ever found. In the remainder (13 infections) the size of the cysts ranged both above and below this diameter. A few cases showed a variation in size from

7 to 15μ . In all these infections cysts measuring 7 to 9μ were by far the commonest.

In 127 infections, on the other hand, only cysts above 10μ in diameter were seen. The diameter of the cysts in 123 of these varied from 10μ to not more than 15μ , the majority of cysts ranging round 12μ . In the four remaining infections cysts as large as 16μ , 16.5μ , and 17.6μ were recorded. (In other cases we have also observed cysts—undoubtedly of *E. histolytica*—which attained a diameter as great as 20μ . These diameters in all cases refer to spherical—not oval—cysts measured in saline or iodine solution.)

It will be noted that we have referred to 200 cases and 202 infections. This is because two of the cases appeared each to have two separate infections; each passing cysts measuring either 7 to 8μ or 12 to 14μ , with none of intermediate size.

We see no reason to doubt that all these cysts belong to *E. histolytica*, and represent simply those formed by different strains or races of a single species. The occurrence of numerous strains, differing from one another in size alone, has been observed within almost all species of protozoa hitherto subjected to systematic study. Moreover, we have no reason to believe that these strains of *E. histolytica* differ from one another in pathogenicity or any other character save size. We may also note that all strains behave in a precisely similar manner towards treatment with emetine, given either hypodermically or as the double (bismuthous) iodide *per os*.

Although we suppose that those cases which pass cysts of two different sizes, with no intermediates, represent instances of simultaneous infection with two different strains, we are of course unable to determine whether cases passing cysts of all sizes from 7μ to 15μ represent multiple infections, or infections with strains of amoebae which form cysts of varying size; since it is clear that either interpretation is equally possible.

We have detected carriers of small cysts of *E. histolytica* among men coming from France, Egypt, Gallipoli, Salonica, Mesopotamia, and India, so that the strains producing small cysts do not seem to be confined to any particular locality.

The pre-cystic amoebae ("*minuta*" forms) sometimes found associated with the small cysts differ only in their smaller size from those commonly met with in the larger strains. When rounded they rarely exceed 10μ in diameter. But whether the tissue-invading ("*histolytica*") forms are likewise smaller we are unable to say, since we have not been able to study the acute condition in any of our carriers of small cysts.

We may perhaps observe that the name "*Entamoeba minuta*" is not properly applied to the strains of *E. histolytica* forming small cysts. There can no longer be any doubt that the organism described as *E. minuta* by Elmassian (1909) was the ordinary strain of *E. histolytica* forming cysts of the larger size. The small amoebae which he found associated with these cysts were merely the small pre-cystic forms characteristic of subacute infections. Woodcock and Penfold (1916), and others, are therefore in error when they apply the name *E. minuta* to the strain producing small cysts.

Small cysts of *E. histolytica* appear to have been first noted by James (1915) in Panama. Since then they have been found by Woodcock and Penfold (1916). They have recently been carefully studied by Wenyon and O'Connor (1917) in Egypt, with whose conclusions regarding them we are in complete agreement. Mathis and Mercier (1917), in discussing the size of the cysts of *E. histolytica*, have recently stated that the usual dimensions are 12.5μ or 14μ . They cite no instances of cysts measuring less than 8.5μ or more than 15μ . We ourselves have seen cysts of all sizes from 5μ to 20μ , in fresh preparations. And we believe that the French observers, if they would study the cysts from a large number of cases rather than a large number of cysts from a few cases, would reach conclusions identical with those of Wenyon and O'Connor and ourselves. We think it probable that Kuenen and Swellengrebel (1913) and numerous other authors who have found the majority of cysts of *E. histolytica* to measure 11 to 14μ in diameter have overlooked all infections with the races producing only small cysts (10μ or less). Others have recorded these infections as rarities—for example, Woodcock and Penfold (1916), and Smith and Matthews (1917). Nevertheless, since in no less than 62—that is, over 30 per cent.—of the last 202 *E. histolytica* infections which we have studied no cysts larger than 10μ were ever found, it appears that such infections are far from uncommon. The importance of this from a practical point of view need not be further emphasized.

It should be added that the 200 cases of *E. histolytica* infection referred to above were made up as follows: 155 carriers detected at a dysentery dépôt; 21 carriers described in a former paper by one of us (C. D., 1916), from a general military hospital; and 24 carriers studied at another military hospital by the other (M. W. J.), and about to be described in a forthcoming article. Fourteen of the cases in the last two categories have no history of dysentery.

We give figures of the small pre-cystic amoeba (Fig. 1) and of the small cysts (Figs. 4, 5, and 6) of *E. histolytica* for comparison with the corresponding forms of *E. nana* (Figs. 2 and 7-9).

Entamoeba coli (Lösch) Schaudinn.

Concerning this familiar organism we here propose to make only a few observations, chiefly on the size of the cysts and the number of their nuclei.

It is usually supposed that cysts of *E. coli* can be distinguished from those of *E. histolytica* by their larger size. Most authors have regarded 15μ as the minimum diameter of *E. coli* cysts. Kuenen and Swellengrebel (1913) have recorded cysts measuring as little as 13μ ,

however, and other authors have given even smaller measurements. Quite recently Wenyon and O'Connor (1917) state that they have seen no cysts less than "thirteen or fourteen microns" which they could attribute with certainty to this organism. Mathis and Mercier (1917) place their lower limit of size between 12μ and 13μ . We ourselves have, however, on several occasions measured undoubted *E. coli* cysts as small as 11μ . As we have already seen, the cysts of *E. histolytica* are frequently of this size and sometimes very much larger. It is therefore clearly impossible to use the size of the cyst as a crucial character for the differentiation of these two species. We consider the only certain means of distinguishing the cysts of the two amoebae from one another is the study of the structure and number of the nuclei. Although we have studied a very large number of cysts of both species we have never found those of *E. histolytica* containing more than four nuclei; and consequently we regard the occurrence of cysts containing more than four nuclei of the *coli-histolytica* type as the most certain criterion of an *E. coli* infection. In this we agree entirely with Mathis and Mercier, and differ from Swellengrebel and Schiess (1917).

From time to time we have, like other observers, encountered *E. coli* cysts of very large size. These contain as a rule more than the eight nuclei of the typical fully developed cyst. We have counted various numbers of nuclei up to sixteen, and in one cyst at least eighteen, and probably more, were present. The largest spherical cyst that we have found measured 33.5μ in diameter, though the long diameter of oval cysts has sometimes slightly exceeded this value.

These figures approximate closely to those given by Wenyon and O'Connor (1917), whose maximum measurements are 32μ and $38\mu \times 34\mu$.

Mathis and Mercier (1917) regard the large cysts of *E. coli* containing sixteen nuclei as normal stages in the life-history. For our part, however, we think, it most probable that they merely represent double cysts formed from an originally binucleate amoeba. Such amoebae, which presumably have been arrested in division, are sometimes found in stools. We would remark that if similar double cysts are ever formed by *E. histolytica*, to distinguish them from the 8-nucleate cysts of *E. coli* would probably be extremely difficult, if not impossible.

DIAGNOSIS.

So much has already been written about the differential diagnosis of *E. coli* and *E. histolytica* that we shall confine ourselves, after a few general remarks, to a consideration of certain less discussed difficulties which have arisen since the discovery of *E. nana* and of the small cysts of *E. histolytica*.

Concerning the unencysted forms of *E. coli* and *E. histolytica* we shall say but little. These have been discussed so exhaustively by Wenyon and O'Connor (1917), and our experience is in all points of importance so entirely in agreement with theirs, that we have nothing to add to their conclusions. We will merely emphasize once more the fact that, in the absence of encysted forms, the specific diagnosis of an entamoebic infection is frequently very difficult, and not seldom impossible. From the encysted forms, however, a correct diagnosis can usually be made with certainty by a practised observer.

The main differences between typical *E. coli* cysts and the larger-sized cysts of *E. histolytica* are now sufficiently well known for a detailed description to be unnecessary here. As already noted, the size of the cysts is a character on which too much reliance should not be placed. Another character sometimes regarded as distinctive of *E. histolytica* is the presence of the so-called "chromidial" or "crystalloid" bodies in the cysts. These bodies, which we shall call chromatoid bodies (since in our opinion they have nothing to do with the chromidium of other rhizopods and are not crystalloid), are, it is true, very generally—though by no means invariably—present in *E. histolytica* cysts; but on the other hand we find they are by no means uncommon in the cysts of *E. coli*, as several authors have already noted. We do not understand why Mathis and Mercier (1917) deny their occurrence in *E. coli* cysts. A much more distinctive character is the vacuole, which, however, is not usually present in fully mature cysts of either species. It is formed in the early stages of encysta-

tion and persists as a rule in *E. histolytica* until the 4-nucleate stage is reached. In *E. coli* it is seen up to the 4-nucleate stage, but is very uncommon in cysts containing 8 nuclei. It attains its maximum size in *E. coli* during the binucleate stage, when it frequently occupies the greater part of the cyst. Such cysts we—contrary to Wenyon and O'Connor—regard as normal stages in development. Unlike those of *E. coli*, *E. histolytica* cysts often possess more than one vacuole. The vacuoles are best studied in cysts mounted in iodine solution. As those of *E. coli* contain abundant glycogen, they stain dark brown; whereas *E. histolytica* vacuoles, which contain but little of this substance, are far less deeply coloured. In iodine solution the edge of the vacuole is ill-defined in *E. histolytica*, but usually clear cut in *E. coli*. These characters are not absolutely constant; we have, for instance, seen cysts of *E. histolytica* with vacuoles quite as darkly stained and sharply outlined as those of *E. coli*.

That the substance in these vacuoles is really glycogen there can hardly be any doubt. In addition to the iodine reaction above noted, we find that it gives characteristic reactions with Best's specific carmine stain for glycogen, and displays the usual solubilities of this substance.

As already noted, we regard the number and structure of the nuclei as the most reliable characters for distinguishing cysts of *E. coli* from those of *E. histolytica*. Some observers attach considerable importance to the thickness of the cyst wall—for example, Kuenen and Swellengrebel (1913), Woodcock and Penfold (1916)—a character which we, with Mathis and Mercier (1917), regard as quite unreliable. The assertion that the cyst wall of *E. histolytica* has but "a single contour," while that of *E. coli* is double, appears to us to rest upon malobservation or an error in microscopy.

When we come to consider *E. histolytica* cysts of 10 μ or less in diameter we can rule out *E. coli* as a possible source of confusion, but we encounter several new difficulties. It should be borne in mind that the small cysts (10 μ or less) of *E. histolytica* differ from the larger in no respect save size, so that anybody familiar with the latter should have little trouble in identifying the former. The chief additional difficulty arises from the circumstance that the smaller the cysts are the more troublesome they are to find, especially when present in the faeces in small numbers and accompanied by other cysts and organisms of comparable size and similar appearance. The chief cysts likely to lead to confusion are those of *E. nana* and *Chilomastix*. The former are readily distinguished from those of *E. histolytica* by the structure of their nuclei, the absence of chromatoid bodies, and the character of the glycogen vacuoles. Moreover, although great variation is observable in the shape of the cysts of both species, those of *E. nana* are generally oval, while those of *E. histolytica* are typically spherical. The nuclei of small *E. histolytica* cysts are seldom distinctly visible when the cysts are examined in saline solution; while those of *E. nana* cysts are, for all practical purposes, completely invisible in this medium. In iodine solution the nuclei of *E. histolytica* cysts can generally be clearly made out, while this is seldom the case with cysts of *E. nana* on account of the numerous minute granules (? volutin) which are generally present. As a rule the chromatoid bodies of *E. histolytica* cysts are readily seen in saline or iodine (especially in the former), and when present serve at once to distinguish them. The glycogen vacuoles, as already noted, should be studied in iodine solution, in which the small, faintly stained, and ill-defined vacuole of *E. histolytica* is easily distinguished from the relatively large, deeply stained, and sharply defined glycogen mass of *E. nana* (see Fig. 13). When the determination of the species still remains in doubt the only safe procedure is to fix and stain wet cover-glass preparations, and examine them systematically with the oil immersion. The cysts are easily fixed and stained by any good cytological method; but by far the most useful and rapid method for routine purposes is, in our experience, fixation with Schaudinn's fluid, followed by staining in haemalum. Other rapid methods of staining which have given us good results are Weigert's iron-haematoxylin, carmalum, and paracarmine. Though the best preparations are obtained with Heidenhain's iron-haematoxylin stain, it is quite unnecessary to use this laborious and lengthy method for mere diagnosis.

As noted above, the cysts of *E. histolytica* may also be confused with those of *Chilomastix* (= *Tetramitus*), which are frequently present in human faeces. As published accounts of these are remarkably divergent, and in some cases incorrect, a redescription here may not be superfluous. Wenyon's original account (1910) of this organism ("*Macrostoma mesnili*") describes and figures its cysts as oval structures. Although oval and even spherical cysts undoubtedly occur, by far the commonest form is that which possesses a protuberance at one end, so that in shape it closely resembles a lemon (see Figs. 12 and 14). Such cysts were first figured by Alexeieff (1912) for *Chilomastix cauleryi*—a parasite of amphibia. Prowazek and Werner (1914) figure a similar cyst as a "possible early stage in encystation" of the human parasite. Wenyon (1915) further figures an oval 4-nucleate cyst as belonging to this species; but this, as we have seen, is really the cyst of *E. nana*—an error which Wenyon and O'Connor (1917) have since corrected. Nevertheless, Swellengrebel and Winoto (1917) claim to be able to distinguish these cysts from one another. Finally, Lynch (1916) has boldly described the cysts of *Chilomastix mesnili* as those of *Trichomonas intestinalis*. The only complete description which we have seen is that just published by Wenyon and O'Connor in the work quoted above.

The cysts of *Chilomastix mesnili*, whether of the typical lemon shape (Fig. 12), round, or oval (Fig. 11), measure about 7 to 10 μ in greatest diameter. They never contain more than one nucleus, which is of large size and very characteristic structure (Figs. 11 and 12). As its chromatin is usually concentrated at one pole, it is typically in the form of a signet ring. Smaller masses of chromatin are sometimes visible at other points on the nuclear membrane, but rarely in the centre. For this reason the nuclei of encysted *Chilomastix* often resemble those of the uninucleate cysts of *E. nana* (cf. Figs. 11 and 7). This resemblance is most striking in stained preparations; for in cysts examined in saline the nuclei of both are usually invisible, whilst in iodine, although the *Chilomastix* nucleus can usually be made out as a definite signet ring (Fig. 14), only the karyosome of the nucleus of *E. nana* can be distinguished with ease. The most distinctive feature, however, of the *Chilomastix* cyst is a complex structure which represents the persistent part of the mouth of the free flagellate. This structure resembles a sling, its outline representing the fibre forming the lip of the mouth. The mouth flagellum, which in the flagellate borders an undulating membrane, can sometimes be seen lying within the sling (see Figs. 11 and 12). The relation of these parts to the nucleus and blepharoplasts is similar to that seen in the free flagellate; but in the encysted forms the connexion between these structures is often severed. The outline of the mouth, though usually invisible in cysts examined in saline, can as a rule be clearly seen in iodine preparations. Another feature characteristic of the *Chilomastix* cyst is the presence of a few small and brightly refractile granules. These, so far as we have been able to investigate their reactions, appear to be composed of volutin. The similar granules in the cysts of *E. nana* are usually smaller, less refractile, and more numerous. Another character, which does not appear to have been previously noted, is the occasional presence in the *Chilomastix* cyst of a mass of glycogen. This mass, which stains very deeply in iodine solution, is sometimes of very large size (see Fig. 14), and is present, we believe, in newly-formed cysts only. The oval cysts of *Chilomastix* containing glycogen may be mistaken for similar cysts of *E. nana* (Fig. 13); and both must be distinguished from the usually much larger cyst-like bodies described as "I-cysts" by Wenyon (1916) and Wenyon and O'Connor (1917), which are not uncommon in human faeces.

Wenyon and O'Connor (1917) have just described a new intestinal flagellate which they call *Tricercomonas intestinalis*. We have never found this flagellate ourselves, and from the published account we are unable to state how its cysts can be distinguished with certainty from those of *E. nana*. We have seen cysts of this organism which very closely resemble their figures of the cysts of *Tricercomonas*.

From the foregoing descriptions it will be evident that, when the cysts are examined in saline and iodine solutions, the most outstanding characters differentiating

those of *E. histolytica*, *E. nana*, and *Chilomastix* from one another are: the chromatoid bodies of the first, the apparently uniformly granular contents of the second, and the mouth of the third. It must always be remembered, of course, that the cysts here described form only a part of the numerous small "bodies" commonly present in human faeces. Some of these are probably vegetable organisms resembling yeasts, others possibly cysts of protozoa as yet unidentified. Although we are familiar with several different kinds of these, we are not yet able to determine and describe them satisfactorily.

Although we are usually able to arrive at a certain diagnosis from a study of the cysts, it is sometimes impossible to reach definite conclusions when unencysted forms only are found, since these are all too often dead and degenerate. As already noted, *E. nana* dies very quickly after leaving the host. This is also the case with the small "*minuta*" forms of *E. histolytica*, and often also with flagellate *Chilomastix* and *Trichomonas*. The flagellates, when they have degenerated to such an extent that they are merely small rounded masses of protoplasm, devoid of flagella, are not distinguishable with certainty from similarly dead and degenerate amoebae.

An unencysted *E. nana* can easily be distinguished, when alive, from the small "*minuta*" forms of *E. histolytica* by its pseudopodia, which are never so hyaline and blade-like, and by the numerous food vacuoles (containing bacteria) which are not seen in *E. histolytica*. In both forms the nuclei are very difficult to observe in the living organism, though they serve to differentiate them with certainty in good stained preparations (Figs. 1 and 2). Degenerate *Chilomastix* flagellates can usually be distinguished from degenerate small *E. histolytica* amoebae of the "*minuta*" type by their ingested bacteria and the persistent outline of the mouth. It is often impossible to determine whether a rounded-up and degenerate organism, in which nothing but vacuoles containing bacteria can be clearly distinguished, was originally a *Chilomastix* or an *E. nana*. Such forms are almost certainly not "*minuta*" amoebae, but, in the absence of cysts, a further diagnosis cannot be made with certainty. The degenerate forms of *Trichomonas* can usually be distinguished by the peculiar undulating "amoeboid" movement which they often continue to display for a long time.

Although *E. nana* could hardly be mistaken for the much larger *E. coli*, with its similar inclusions but characteristic nucleus, it might be confused with one of the small so-called "*limax*" amoebae sometimes found in stale stools. If the stool is more than a day old and the amoebae are actively motile on a cold stage, it is certain that they belong, not to any species of *Entamoeba*, but to some free-living form. Although it is usual in medical works to include all these small free-living amoebae under the term "*Amoeba limax*," there are probably many different species belonging to quite different groups which may occur in human faeces. A common form is shown in Fig. 3 for comparison with *E. histolytica* (Fig. 1) and *E. nana* (Fig. 2). The most distinctive characters of this and related forms are the nucleus, with its large central karyosome, and the presence of a contractile vacuole. Some free-living amoebae possess more than one contractile vacuole, but such structures are never found in any species of *Entamoeba*. All the common "*limax*" amoebae form uninucleate cysts, which, in typical forms, cannot be mistaken for those of any *Entamoeba*. The cyst wall is always thick and frequently—but not always—very irregular in outline and brown in colour (see Fig. 10). The single large nucleus usually lies near the centre, surrounded by numerous deeply-staining masses, probably homologous with the chromatoid bodies of some entamoebic cysts. Most of these small free-living amoebae can be readily cultivated on agar.

The figures accompanying this paper have been selected in order to illustrate the more important points considered above. We have thought it superfluous to give new figures of the more familiar forms. Accordingly, of the three common intestinal amoebae with which this paper chiefly deals we have figured only certain forms of *E. histolytica*, and *E. nana*, whilst *E. coli* is unrepresented and two other organisms are added.

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POST-OPERATIVE TYMPANITES:

ITS NATURE AND SOME POINTS IN ITS TREATMENT.

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IN the Cavendish lecture of 1915¹ Professor Arthur Keith described a neuro-muscular tissue specialized from Auerbach's plexus of nerves and situated where delays in the passage of the bowel contents normally take place. He regarded the myenteric plexus of the intestine as similar in nature and functions to the nodal and conducting system of the heart, each specialized nervous area in the alimentary canal being the regulator of peristaltic activity in the part of the intestine immediately beyond it. According to this view, a want of co-ordination between the muscular activity of the various parts may give rise to effects comparable to those of heart-block, and it was concluded that the condition of intestinal stasis is brought about by such a want of co-ordination of function.

Keith's teaching supports certain views which I have long held. At the meeting of this section in October, 1914,² I attributed the tympanites following some abdominal operations to an enfeebled peristaltic action, and especially to delay in the large bowel. I suggested then that the condition might be described as one of acute intestinal stasis. Five cases were published in which an otherwise irremovable tympanites following an operation upon the abdomen was treated by making an intestinal fistula, and four of the patients recovered. The fifth died, and it seemed to me that in this case the second operation was too long delayed, and that it could not have been undertaken too early. I argued that if a persistent tympanites can be cured by making a fistula its onset should be preventable by the same means, and that treatment by prevention in suitable circumstances would be more certain of success than any method of cure. This view was put into practice, and two cases were recorded³ in which a caecal fistula was made when intestinal difficulties seemed likely to arise. Recovery was uncomplicated by any bowel symptoms, and I have since treated eleven cases in this way with two failures, and assisted at one successful operation for the relief of a fully developed tympanites. In the ten successful cases the fistula was closed after a short time.

To save space only four cases are here briefly recorded.

CASE I.

On the second day of acute symptoms a diffuse peritonitis was found affecting the pouch of Douglas, the Fallopian tubes and the intestine and mesentery in the lower portion of the abdomen. Much lymph floated in free fluid or adhered in thick layers to the parts named, except the Fallopian tubes. These were of a deep blue colour, and greatly enlarged by oedema. They contained no pus and were not removed. All the intestines in the middle and upper abdomen were red and moderately distended. The vermiform appendix showed signs of old inflammation and was removed, but it lay outside the

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