PROTOZOOLOGICAL EXPERIENCES DURING THE SUMMER AND AUTUMN OF 1916.

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I am indebted to the courtesy of the D.M.S. for permission to publish the following note:—

(*a*) Dysentery and Diarrhœa.

The cases dealt with in this note occurred during the months of June to November, inclusive. The opportunity has been afforded of studying the types of dysentery and diarrhœa prevalent amongst both British and Indian troops, and a comparison between the two has proved most instructive. The total number of different cases examined was 659, of which 378 were British and 281 Indians. A general classification of the stools, according to the presence or absence of pathological elements, is given in Table I.

TABLE I.—General Characters of the Stools.

Nationality	Totals	A. Blood, mucus and pus	B. Mucus and pus, but no blood	Total "dysenterics"	C. Diarrhœa alone
British ..	378	174 = 46 per cent	116 = 30·7 per cent	290 = 76·7 per cent	88 = 23·3 per cent
Indians..	281	49 = 17·4 ,,	56 = 20 ,,	105 = 37·4 ,,	176 = 62·6 ,,
Total ..	659				

Nearly all the stools under the heading C were loose or thin, but occasionally one would be pasty or semi-solid. The majority of the 176 Indian diarrhœal stools were of trichomoniasis cases, and of a characteristic creamy or lemon-yellow colour. Most of the British cases were examined only once, or at most twice, because they were at a Stationary Hospital, from which the patients were soon as a rule transferred to a General Hospital elsewhere; but most of the Indian cases have been examined at least six or eight times, frequently more, at approximately weekly intervals.

Apart from *Entamœba coli*, included for purposes of comparison with histolytica, account is taken in Table III only of those parasites known or considered to be of pathogenic importance.

TABLE II.—Occurrence of E. histolytica.

Nationality	Total findings of *E. histolytica*	Percentage of total cases	Histolytica-form	Percentage of total "dysenterics"	Percentage of blood and mucus stools	Tetragena-form and (or) cysts	Percentage of diarrhœal stools
British..	7	1·9 per cent	7	2·4 per cent	4 per cent	—	—
Indian..	44	15·7 ,,	13	12·4 ,,	26·5 ,,	31	17·6 per cent

TABLE III.—Other Protozoan Infections.

Nationality	Number of cases	*E. coli*	Trichomonas	Macrostoma	Lamblia	Balantidium
British	378	6=1·6 per cent	3=0·8 per cent	2=0·5 per cent	7=1·8 per cent	—
Indian	281	80=28·5 ,,	122=13·4 ,,	33=11·7 ,,	10=3·5 ,,	2=0·7 per cent

Notes on Results.

(1) *Dysentery.*—A marked disparity has been found between the British and Indian stools as regards the occurrence of protozoan infections. This difference stands in relation with the difference, as a whole, in the character of the stools with two sets of cases (cf. Table I).

Dysentery among the British cases examined was, with few exceptions, *not* amœbic, but due to some bacillary infection. This was true in regard to the stools coming under both the two headings A and B. For undoubtedly the great majority of the stools under B (with mucus and pus alone) were also of dysenteric character, chronic or approaching convalescence. Many of them consisted chiefly of thick, ropy mucopus, whitish or yellow, sometimes bile-stained and with little fæcal material. My work has not dealt with the bacteriological aspect of the question, so that I am unable to give details of the bacillary findings. But *Entamœba histolytica* occurred in only seven cases (all of them blood, mucus and pus stools); that is to say, only 2·4 per cent of the total "dysenterics" were amœbic. In no instance among the British cases were pre-encysting forms or cysts found.

Among the Indians, the proportion of dysenteric stools was, in the first place, much less (cf. Table I), and of these relatively fewer were bacillary. On the other hand, amœbic dysentery occurred far more frequently than among the British, twenty-six per cent

of the blood and mucus stools, or twelve per cent of the total dysenterics, yielding *E. histolytica*. In addition, of the remaining stools, mostly diarrhœal, thirty-one (or seventeen per cent) showed tetragena forms and (or) cysts. In one form or another, histolytica was found in nearly sixteen per cent of the total Indian cases during the period under review.

Only in three cases altogether was a mixed amœbic and a bacillary dysentery found; one of these was British, the other two being Indians. Active histolytica were found at the first examination, and dysenteric bacilli were also isolated.

In every case except one, where the histolytica form was found, the parasites were present in the first stool examined, the exception being a loose, diarrhœal one. The reason, probably, for the absence of the amœbæ at the first examination was that this case was also one of trichomoniasis (the amœbic dysentery not being acute). As is seen from Tables I and III, trichomonas diarrhœa was responsible for a large proportion of the Indian stools sent in, and owing to this factor pre-encysting tetragena forms or cysts (in convalescent or "carrier" cases) were sometimes not found at the first examination, but were observed on a subsequent occasion. (A few such "carrier" cases may have been missed at first, owing to some patients being invalided back to India before more than one or two examinations had been made.)

The presence or absence of amœbæ in a dysenteric stool is the only *safe guide* on which to base a diagnosis of the type of dysentery from the characters of the stool-sample alone. Many cases, to all appearance similar, both macroscopically and as regards the nature of the cell exudate, have been met with coming under the category either of amœbic or bacillary dysentery. In a stool of dysenteric character examined within three or four hours of its being passed, *there will be no difficulty in finding histolytica, if this parasite is the cause of the dysenteric attack.* If the amœbæ are not found after careful search, the attack is not one of amœbic dysentery. It may happen occasionally that in a case of dysentery no histolytica are found in the stools during the acute period, but later on tetragena-cysts are observed. This signifies a mixed infection, the dysenteric stools in such a case being mainly or entirely (if the patient is an amœbic carrier) due to a bacillary infection.

(2) *Normal Carriers of E. histolytica.*—Up to date, the stools of 134 normal Indians have been examined for amœbic cysts. This series has included healthy men in camp and also men in

hospital for surgical treatment, malaria, etc., but in all cases men with normal bowel conditions. No fewer than twenty-seven (or twenty per cent) contained tetragena-cysts. The men were drawn from different sources (regiments, stations, etc.); many of them had only recently come from India and none of them had been on Gallipoli. The average number of positives kept fairly uniform right through; there was no question of many coming from one batch or source and few from another. So far as it goes, this result points to about twenty per cent of this class of Indians being naturally (i.e., in ordinary circumstances) infected with histolytica. *It is probable that on this account Indian troops were largely responsible for the outbreak of amœbic dysentery among the troops in Egypt and on the Peninsula in* 1915. A corresponding series is being undertaken, as opportunity avails, upon men of the Egyptian Labour Corps in the same area, and so far as this has progressed the percentage of infections with tetragena cysts is very much less, barely four per cent. Moreover, a certain number of non-dysenteric British stools (more than fifty altogether) sent in for "enterica," and not included of course in the above tables, have been specially examined for amœbic cysts and in no single instance were any cysts found[1]; i.e., among the British, at any rate in this area, very few appear to be carriers, and this agrees with the scarcity of amœbic dysentery amongst them.

(3) *Emetine Treatment.*—Including twenty-three normal carriers, the definite results of emetine treatment in over sixty cases are now known, and have proved excellent. Generally, the routine course of twelve grains of emetine hydrochloride, was followed. The care and treatment of these cases was in the hands of Captain F. H. Salisbury, I.M.S., and I desire to thank him very much for the interest taken and especially for his valuable co-operation in regard to the trichomoniasis cases (see below). Out of all the cases, only in three were the parasites still present when the course was finished. One of these was originally a dysenteric case, in which pre-encysting forms and cysts were then found (after the treatment was finished); the other two were carriers, in which a few cysts were also still present. But all three cleared up completely after a further four or five grains of emetine followed by a week's course of ipecacuanha. With these exceptions the results were straightway favourable. Thirty-five cases, including twelve

[1] Many of these stools were pasty or semi-formed, and likely to show cysts if an infection had been present.

amœbic dysenteries, the remainder being diarrhœal with tetragena forms or cysts, were all examined at least four times, often five or six, at approximately weekly intervals after the course was finished; in none of these was histolytica in any form ever found again. The carriers were usually examined twice, but a few three times, similarly, also with negative results. From conversation with two or three workers (I.M.S. officers and others) who have had experience of amœbic dysentery in India or China, I gather that larger initial doses may be given with advantage, say $1\frac{1}{2}$ grains the first four days, or two grains for the first three days, followed up with one grain daily, till the usual total is attained. One worker in China had had uniformly good results by this method. There can be no doubt that it is all-important to give as much emetine as possible quickly, so as to kill all the amœbæ before they acquire tolerance to the drug and resistant forms are developed.

(4) *Remarks on the Amœbæ.*—I think that even at the present day it may be useful to summarize certain conclusions at which I have arrived, particularly as I have now had ample opportunity of comparing the unencysted forms of both histolytica and coli.

(*a*) If a stool consists of blood, mucus and pus and amœbæ are found in it, they will always be histolytica. Coli never occurs in the mucus and pus of a purely dysenteric stool; though of course, if the stool consists partly of fæcal matter in addition to slime, coli may be present in the former.

(*b*) In a dysenteric stool, there is more likelihood of an inexperienced person mistaking large macrophages for resting histolytica; this has probably occurred by no means infrequently in the recent past and I think it is still advisable to lay stress upon this point. Otherwise there is a danger of the number of amœbic dysentery cases being erroneously inflated. A large macrophage may be as large as a histolytica individual, not infrequently contains red-blood cells, and may show a clearer portion simulating ectoplasm. But it *very rarely* changes in shape, and if so, only very slightly, never actually progressing. On the other hand, when a resting histolytica wakes up, which some can usually be found to do, if watched for a time, the rate of change of shape or movement is rapid. The cytoplasm of a macrophage is more coarsely granular than that of a histolytica, and usually contains bright refringent granules of varying size. The nucleus of a macrophage, or portions of it (for the nucleus may be broken up) can usually be seen, contrary to what is the case in histolytica.

(*c*) The distinction between histolytica and coli: In loose diar-

rhœal stools it is often considered difficult to distinguish between these two types in the unencysted condition. But there are certain points which are of very great assistance in making the distinction. *One is the pronounced inactivity of coli.* Coli has been found in eighty different cases and few days have passed when I have not seen it in one of my repeat examinations. A large number of these cases have been loose stools in which unencysted forms of the parasites were generally present, either with or without cysts. In fifteen of the cases *E. coli*, in the unencysted form alone, was diagnosed at the first examination: in all these cases, coli cysts were observed at later examinations, and in none of them was histolytica, either active or encysted, ever found. (In some other cases, of course, both histolytica and coli were present together.) Now, even in freshly passed loose stools, unencysted coli individuals *are nearly always rounded and inactive.* If any do move, the movement consists mainly of change in shape, taking place gradually. Coli never show the quick-flowing movements which histolytica, even in the tetragena stage (the one usually met with in such stools) does.

The other point is the large conspicuous nucleus, with a well-marked ring of prominent refringent granules, possessed by coli. In the tetragena stage of histolytica the nucleus is sometimes readily visible (not by any means always) in life; but it is relatively small and has no such conspicuous grains of chromatin on the membrane. Now and again, in diarrhœal stools which show no obvious blood cells, tetragena individuals may be found containing one or two red corpuscles; the finding of amœbæ containing red cells in such a stool is conclusive evidence of the presence of histolytica. I have never found coli containing red blood cells in spite of the hundreds of occasions on which this form has been seen. But it is quite possible that coli may in certain circumstances (e.g., when the stool is a mixed fæcal and blood and slime one) ingest red cells; for I have several times found trichomonas under these conditions containing red blood corpuscles.

To sum up the matter, coli ought never to be mistaken for histolytica; histolytica in the intermediate tetragena form, occurring together with coli in loose stools, may now and then be liable to be overlooked unless attention is given to the above points, *when a correct decision can in nearly all cases be reached.*

(*d*) The meaning of the terms *tetragena* and *minuta*. It is important to note that there is no hard-and-fast line of demarcation between histolytica forms and tetragena (sometimes called minuta)

forms. I have had stools of amœbic dysentery cases, temporarily diarrhœal, in which a regular gradation and transition could be observed—all intermediate stages from moderately large histolytica forms down to small pre-encysting forms being present, and even one or two early cysts were once found. The origin of the pre-encysting forms is not to be regarded as a kind of special budding-off process of small daughter forms from a large histolytica individual; it is rather by the ordinary process of successive binary fission taking place rapidly in certain conditions, and leading to a diminution in size. The term histolytica is best applied to those forms in the submucosa living entirely on blood-cells and possessing the well-known characters: the term tetragena, on the other hand, may be conveniently retained for those forms living in the bowel which ultimately give rise to the pre-encysting individuals and the tetranucleate cysts in the above manner. These forms usually live, of course, in the same way as *E. coli*, feeding on bacteria, etc., though, as noted, they may ingest red cells, if there are any available. In many normal carriers pre-encysting forms and cysts are quite numerous, indicating a heavy infection with this intermediate tetragena stage of the amœba[1]; just in the same way as the presence of *E. coli* cysts, with or without pre-encysting forms, indicates the existence of the ordinary large coli individuals in the bowel which are giving rise to them (although in a semi-formed or stiff stool the latter may not be actually present).

The term minuta should not be used for the ordinary pre-encysting form. As a matter of fact, the latter is not "minute"; it is frequently scarcely any smaller than a pre-encysting coli may be, because it usually shows still some relatively well-marked ectoplasm, up to the time when it contracts, rounds itself off and encysts. The name "minuta" should be reserved for the distinctly small type or variety, the cysts of which are only about eight microns in diameter, which is occasionally met with, unaccompanied by the usual form (*vide* Woodcock and Penfold, *Brit. Med. Journ.*, 1916, vol. i, March 18). I have also found this variety here on two occasions.

(5) *Flagellate Infections.*—Flagellates, either in the active or the encysted form, were met with only on eleven occasions (i.e., in three per cent) among the British troops, whereas amongst the Indians they occurred in 142 different cases (i.e., fifty per cent).

[1] It is unlikely that these large numbers could originate from histolytica forms in the bowel-wall without the latter being first of all numerous enough to produce manifest dysenteric symptoms.

Lamblia was found very seldom, whether among Indian or British. In four instances the infection was intense, patches of the shed mucosa being present in the stool, with enormous numbers of parasites attached, giving the appearance of a regular mosaic. Two of these cases were also found to be bacillary dysentery, but from neither of the others were any pathogenic bacteria recovered. A point to note about the cysts is that they are not always ovoid, but may be at times practically spherical. In such a case, usually the majority of the cysts are of a relatively short and broad oval form, only a small number being spherical, and with transitions between the two. But on one or two occasions I have seen only rounded cysts present. In such a case a little care is required in order not to mistake them for the cysts of histolytica.

Trichomonas was by far the predominating flagellate. This form occurred in no fewer than 122 cases among the Indians; most frequently it was the only flagellate present, but in twenty-two stools macrostoma (syn., tetramitus) was found in addition. Most of the stools in which trichomonas occurred were loose, or thin, diarrhœal ones. Sometimes a little thin mucus and scattered pus cells were present, while in a few cases manifestly dysenteric stools also contained the parasites. But the great majority of the stools, it should be noted, never showed a "dysenteric" character on any examination, and from none of them were any pathogenic organisms ever isolated. It may be repeated that these cases were purely trichomonas diarrhœa, or trichomoniasis. They were often very troublesome and chronic, and in many cases resisted all attempts at treatment. I may be able, in collaboration with Captain F. H. Salisbury, I.M.S., to give fuller details about these cases later, and will only add here, that while nothing like a specific drug for these parasites has been obtained so far, oil of turpentine, given in ten-minim doses three times daily for a week or so, the course being occasionally repeated, proved the most efficacious. Several cases were quite cured thus, but not, unfortunately, the majority.

The parasites were usually numerous to abundant, at times swarming. Occasionally large clumps or masses of still actively motile trichomonas occurred, resembling an agglomeration of trypanosomes in a culture. Notwithstanding the hundreds of times this parasite has been observed, I have never been able to find any cysts. It is known that trichomonas can live in the unencysted condition for several days in the external environment (e.g., the moist fæces). Moreover, I have succeeded in cultivating

this form in "pure mixed" culture in dilute peptone solution, though only for a week so far unfortunately. Fresh sub-cultures were made every second day and the parasites throve and multiplied vigorously until the fourth or fifth day, but after then they began to degenerate and by the seventh day were nearly all dead. On every occasion the bacterial growth appeared to swamp them. If the bacteria could be held in check, I have no doubt trichomonas could be cultivated for any length of time. Owing to the great pressure of routine work through the summer and autumn I was not able to give as much time to these experiments as could have been desired.

In the case of macrostoma, on the other hand, although this parasite was found in far fewer cases (only in thirty-five altogether), the cysts were seen several times, occurring usually together with some active forms. Macrostoma degenerates and dies more quickly outside the body than does trichomonas (resembling lamblia in this respect), and although I tried to cultivate it, I failed to do so. On one occasion I used a mixed infection of trichomonas and macrostoma, but only the former succeeded.

On the above grounds, I consider that *trichomonas may have lost the power to produce cysts.* Although if this is the case, and cysts are lacking in trichomonas, it will be a very unusual feature amongst intestinal parasites, I do not think it is by any means an impossibility. Woodcock and Lapage (*Proc. Roy. Soc.*, B. 88, 1915, p. 353, and *Phil. Trans.*, B. 207, 1916, p. 375) have shown that under conditions of intensive culture certain fæces inhabiting flagellates entirely lose their (normal) capacity to form cysts; and a heavy infection of trichomonas may aptly be compared with an intensive culture. I have found also that trichomonas will live and remain active for five and a half hours, both in ·066 HCl solution (estimated absolutely) and also in pancreated solution in the same proportion which has been found (*Brit. Med. Journ.*, 1916, i, May 20) to produce the excystation of *E. histolytica.* Therefore, I consider it quite likely that *infection with trichomonas can take place by means of the active, unencysted forms*, the parasites being probably taken in with water and passing quickly through the stomach.

(6) *Some General Considerations on Infections with Intestinal Protozoa.*—There is a prevalent idea that if a protozoan is pathogenic, its presence must always be associated with symptoms; that if a particular parasite can be found in normal persons, that fact is sufficient to disprove its pathogenicity. This view is quite contrary

to the biological principles determining the relation between a parasite and its host. The series of Indian normals I have investigated (see above) is most instructive in this connexion. No less than twenty per cent are carrying normally the cysts of *E. histolytica*, the protozoan universally recognized as the cause of one severe type of dysentery. Now it is extremely improbable that anything like that proportion have suffered, or will suffer, from amœbic dysentery. And this result only bears out, on a larger scale, the observation already recorded by Woodcock and Penfold (*l. c.*) of the occurrence of carriers among the British invalided from Gallipoli, who had never suffered from dysentery or even diarrhœa. The conclusion may safely be drawn, therefore, that in a certain percentage of cases—no inconsiderable one, probably, in endemic areas, such as India—this pathogenic amœba lives normally in the gut, without necessarily ever giving rise to symptoms. As above explained, it lives in the tetragena-form, behaving exactly as *E. coli*. For the amœba to give rise to dysentery there must be some lesion or derangement of the bowel—at least, some non-normal condition—lowering its resistance and giving the amœba its opportunity.

Precisely the same reasoning applies to the intestinal flagellates (e.g., trichomonas, lamblia) and the ciliate, balantidium. Like histolytica, they may occur without causing any symptoms; but on the other hand they are *potentially* harmful. If there is any disturbance of the normal bowel condition, and its resistance is impaired, the balance is no longer maintained, as between host and parasite, the flagellates are able to thrive more vigorously, multiply more actively, and in their turn cause a more intense reaction on the part of the host, setting up, as it were, a vicious circle. It is not yet known if definite toxins, in the strict sense of the term, are produced by these intestinal protozoa, but it is certainly to be expected that the presence of a vast number of parasites in the intestine, with the concomitant production of waste metabolic substances, is sufficient to irritate the mucosa and give rise to deleterious and toxic effects. Fortunately the flagellates appear to be unable to penetrate the mucosa, in the way that *E. histolytica* and *Balantidium* can (given favourable conditions), and therefore, never (?) produce true dysenteric symptoms, the derangement being generally limited to severe or mild diarrhœa.

(*b*) Malaria.

A few words may be added in conclusion with regard to the malarial examinations for which I was responsible. During the

period from August 1 to November 30, 857 cases were examined for parasites, not counting more than 100 repeats. Until July there was scarcely any malaria, but during that month it began to increase. The worst months were September and October. Parasites were found in forty-three per cent of the cases, and especially among the Indians; quite five or six per cent in addition were obviously malarial bloods, although no parasites could be found. The findings are summarized in Table IV.

TABLE IV.—Cases of Malaria, August 1 to November 30 inclusive.

	Examinations made	Number positive	Malignant tertian	Benign tertian	Quartan
British	463	178	66=37 per cent	112=63 per cent	0
Indians and Egyptian Labour Corps	374	192	153=79·7 ,,	37=20·2 ,,	2=1 per cent
Totals ..	837	370	219=59·2 ,,	149=40 ,,	2=0·5 ,,

Among the British, benign tertian was twice as frequent as malignant tertian (pernicious), but among the Indians and Egyptian Labour Corps the malignant form was by far the most common. The rarity of quartan cases was noteworthy, only two being met with, both occurring in Indians. I was struck by the scantiness of the parasites in quite a number of the benign tertian cases among the British. Probably insufficient prophylactic dosage of quinine was responsible, checking but not completely inhibiting the development of the parasites.

In all the British malignant cases, the parasites (in the ring-form) were frequent or numerous, whereas the contrary was often the case among the Indians. On two or three occasions, malignant parasites very bacilliform in character were seen, but a few typical rings could always be found by searching. Crescents were never found in the British cases, as nearly all of these were new infections. Remarkably few mixed infections occurred, not more than three or four altogether. Only three fatal cases came under my notice, one of them being British and two Egyptians. These occurred at the onset of the season and were rapidly fatal cerebral cases. They were, in fact, only diagnosed post-mortem by finding abundant malignant parasites in the spleen.

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*J R Army Med Corps*1917 29: 290-300
doi: 10.1136/jramc-29-03-03