Clinical and other Notes.

A NOTE ON THE PROTOZOA OF THE INTESTINE.

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(Report to the Medical Research Committee, by Major H. R. Dean, R.A.M.C., T.F.)

The following notes comprise a brief account of the intestinal parasites which have been met with in the routine examination of dysentery convalescents during the last fourteen months, together with a short description of the methods which have been found most useful for the recognition of Entamoeba histolytica, the detection of which has been the principal object of our work. The methods employed for the detection of this parasite fall under two headings:—

(a) Methods which aim at the detection of amœboid forms as well as cysts.
(b) Methods which rely entirely on the recognition of encysted forms.

If it is desired to detect the amœboid forms it is of course necessary to work with fresh preparations. As will be seen later, in about thirteen per cent of our cases amœboid forms were found, but no cysts, and important as is the recognition of cysts, it is impossible to neglect the examination for amœboid forms without introducing into the results a very appreciable error.

The following table shows the incidence of infection by protozoan parasites in a series of 153 cases examined during October and November, 1916. Two examinations were made in each case.

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>E. histolytica</th>
<th>Lamblia intestinalis</th>
<th>E. coli</th>
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<tbody>
<tr>
<td>153</td>
<td>24·1 per cent</td>
<td>13 per cent</td>
<td>16·8 per cent</td>
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The percentage of cases infected with E. histolytica is higher than that recorded by other workers, a result which is probably due to the large number of cases in which amœboid forms only were found.

- Cases in which cysts and amœbæ were found... 10·4 per cent
- Cases in which amœbæ only were found... 13·7%

We attribute this result to the fact that the specimens were examined within a few hours of being collected, and to the use of neutral red saline as a medium for the emulsification of the faeces.

METHODS.

A purgative was administered the night before the examination was to be made. Specimens of faeces were put up in small tubes and sent with the least possible delay to the laboratory. The examination was carried out as soon as possible, as specimens of faeces more than six hours old are useless if it is desired to detect amœbic forms.

A solution of 2½ per cent iodine has been recommended by many workers for facilitating diagnosis. The nuclei of the cysts are most certainly made clearer by the solution, but the amœbæ are still difficult to differentiate from other cells lying in the faeces.

The method we have employed throughout our work is a slight modification...
of one advocated by Stitt and others. This consists in the use of a \( \frac{1}{4} \) per cent solution of neutral red, using saline as a solvent. The solution we have employed is more dilute, being but 1 in 10,000. The advantage of this method over others is that the endoplasm of _E. histolytica_ takes up the neutral red, while that of _E. coli_ usually refuses it, and is easily recognized by the pink colour. _E. coli_ has on rare occasions reacted to the stain, but to such a slight extent that no difficulty arose. Any colour which was present in _E. coli_ was confined to the edge of the vacuoles and was not uniformly distributed.

In confirmation of the validity of our method we have made permanent preparations, always with the result that the amoeba suspected of being those of _histolytica_ have proved to be so. As a fixative we have used Schaudinn's solution and stained with Heidenhain's iron haematoxylin. The preparations were left in the fixative from ten to fifteen minutes, then thoroughly washed with thirty per cent iodine so as to remove any crystals of corrosive sublimate.

The slides were then put into 2\( \frac{1}{2} \) per cent iron alum for half an hour, washed, and finally stained by a two per cent solution of hematoxylin for twelve hours. This long period of staining is an advantage, because the chromatin is strongly affected and does not easily wash out during the process of differentiation with the iron alum. When sufficient colour has been extracted the slides were dehydrated and mounted in Canada balsam in the usual way.

**Description of Parasites.**

_Entamoeba coli._—This organism is the commonest inhabitant of the intestine and occurs frequently in both amoeboid and encysted condition, though the former seems to be the phase of the life-history usually found. In the Medical Research Committee's report on amoebic dysentery, Dobell estimates that three-quarters of the cases investigated were infected by this parasite.

The amoeba are very variable in size, ranging from small forms about 7 microns in diameter to large ones of 25 microns or more. The average size is about 15 microns. They are oval or round in shape and the ectoplasm is not sharply marked off from the endoplasm, indeed it is difficult to differentiate the two regions unless the animal is seen in the active condition when the ectoplasm can be observed in the pseudopodia.

Vacuoles are present in practically all cases; there may be a single large one or many small ones distributed throughout the endoplasm. Wenyon and others have described as a constant feature the presence of bacteria in the endoplasm, but our experience has been that these bodies are rare, the endoplasm usually appearing fairly homogeneous.

The nucleus measures from 5 to 8 microns and is excentric; it can usually be seen in the living animal. In stained preparations it is found to be rich in chromatin, the nuclear membrane is thick and upon the periphery there are situated chromatin granules. In the centre there is a fairly large karyosome. Great variability is found as regards the arrangement of the chromatin in the nucleus, often the condition is that described above, but frequently the karyosome is large and the peripheral granules few in number, the whole complex recalling the arrangement met with in the nucleus of _Amoeba limax_. On occasion a large amoeba may be observed, which from its size might be mistaken for that of
Entamoeba histolytica, except that there is no trace of the neutral red reaction. Investigation by staining will demonstrate, however, that there are eight nuclei in the cytoplasm. This form is a stage in the life-history when the animal is preparing to multiply by schizogony. The nucleus has divided into eight, and round each of these nuclei the cytoplasm will aggregate to form eight small amoebae which will grow into adults. Intermediate conditions may also be seen with from two to eight nuclei in the body.

The encysted condition is also frequently encountered. The cysts are usually large, 10 to 20 microns, and contain eight nuclei, some of which can be seen in the unstained specimens; the cyst also appears to have a double contour. The shape is round or slightly oval, the former being the commoner.

Entamoeba histolytica.—Under this head we include the species known as Entamoeba tetragena and Entamoeba minuta. The first is undoubtedly a stage in the life-history of E. histolytica; the second species we incline to regard as a further stage in the same life-history; though the figures given by some workers (Popoff, 1911) would seem to indicate that we were dealing with a stage in the life-history of E. coli. The percentage of cases infected with E. histolytica is given by Dobell as probably from eighteen to twenty-five per cent.

The amoebae are easily distinguished by means of the neutral red stain; they are variable in size, ranging from small forms of 7 microns to large ones of 30 microns or even larger, on the average the size is greater than that of E. coli. The ectoplasm is also sharply marked off from the endoplasm as an unstained hyaline substance. Vacuoles are uncommon though they may occur; when present, however, they are small. Inside the endoplasm there are numerous particles and bacteria; blood corpuscles may also be found. Great stress is sometimes laid upon the presence of these last mentioned bodies and some workers go so far as to refuse to diagnose E. histolytica unless they are seen. Our experience has been that blood corpuscles are by no means so common as is supposed, and if we relied upon them as a means of differentiation our percentage of positive results would have been much smaller. Perhaps the fact that the cases we have examined have been convalescent has a good deal to do with the relative absence of ingested blood corpuscles.

The nucleus, unlike that of E. coli, is not visible in the living animal; when stained it is found to be eccentric in position, sometimes pressed against the periphery of the endoplasm. It is by no means so regular in shape as is that of E. coli. A further point of difference is the scanty amount of chromatin which it contains, represented by small peripheral granules on the thin nuclear membrane. A small ventricle can often be observed lying in the middle of the nucleus. The encysted stage of the life-history was formerly thought to be part of the life-history of another species of amoeba, E. tetragena. The size of the cysts varies from 10 to 18 microns; they are smaller than those of E. coli. Also the nuclei, which are four in number, are not seen in an unstained preparation, which is in contrast with the visible nuclei of E. coli cysts. Lying in the endoplasm of the histolytica cyst is a peculiar body, thought to be of chromidial origin, and known as the chromatin block. Two or more of these bodies are often present in one cyst, and as it is possible by careful focusing to observe them in the unstained condition they are of great use in differentiating between the cysts of this species and E. coli amoebae, which they superficially resemble in fresh
preparations. Our experience is that it is more easy to confuse these two conditions than any others and it has been our habit, whenever there was any doubt, to make stained preparations which at once settled the question.

Bodies resembling the chromidial blocks have been recorded from time to time as occurring in the cysts of *E. coli*, indeed we have ourselves found them on one occasion, but as eight nuclei were also visible there was no doubt as to the species of animal with which we were dealing.

*Lambia intestinalis* (*Giardia intestinalis*). *L. intestinalis* or *G. intestinalis* as this parasite should be called, is perhaps the most characteristic of all infections: This flagellate, rarely seen in the active condition, is pear-shaped, 12 to 18 microns long, with two large nuclei at the anterior end and four pairs of flagella, which spring from two supporting rods or axostyles running down the middle of the body. Near the nuclei are two suckers by means of which the animal attaches itself to the epithelial lining of the intestine. The form in which the parasite is usually seen in the faeces is the encysted one and according to Dobell's recent report more than one-third of the patients examined were infected.

The cysts are oval, measuring about 14 microns by 7 microns, though occasionally round forms may be seen. In all cases, however, the axostyles are visible as light lines running down the longitudinal axis of the cyst: Four nuclei are situated at the anterior end.

*Macrostoma mesnili* (*Tetramitus mesnili*).—This small flagellate is easily recognized when in the active condition by the rapidity of its movements. The body generally is pear-shaped, measuring about 12 microns along its longitudinal axis. Small round forms of about 5 microns are, however, often encountered.

At the anterior end there arise from a small basal granule three flagella which produce in preparations of the living parasite the appearance of a single whip. A longitudinal split, the cytosome, is seen at the anterior end as a light band, and inside this groove a small flagellum is found which appears to be attached to the body by an undulating membrane. No axostyle is developed in this species of flagellate.

The endoplasm has many vacuoles, which take up the neutral red stain. The cysts are small and very refractile, they are rounded in shape and measure 7 to 8 microns. The cytosome can in most cases be seen. Of the other parasites infecting the human intestines little need be said because of their comparative rarity, they are also well described by Wenyon.

*Trichomonas intestinalis* is very similar to macrostoma and of about the same size and shape. It differs, however, in the possession of an undulating membrane running the whole length of the body, and of an axostyle. The cytosome in this form is very small. *Cercomonas crassicauda* is a flagellate with a single flagellum at the anterior end, the posterior one often being drawn out into long tail-like processes. Besides these animals coccidian cysts and the eggs of a parasite worm, *Trichocephalus dispar*, may be seen on rare occasions.

It is interesting to note that the ciliates, *Balantidium coli* and *Nycotherius faba*, have not as yet been recorded from dysentery patients examined in the hospitals in this country. In every specimen of faeces examined the cysts of vegetable organisms, together with yeasts, etc., are met with. One of the commonest is *Blastocystis hominis*; superficially it seems to resemble a small
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E. coli amoeba, but it has a much flattened appearance and an enormous vacuole, the protoplasm appearing as a thin peripheral band. Epithelial cells and undigested vegetable matter abound, but no confusion should arise between these structures and the parasites already described above.

REFERENCES.


A SHORT NOTE ON CERVICAL LAMINECTOMIES, WITH AN ILLUSTRATIVE CASE.

BY CAPTAIN R. B. BLAIR.
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The number of cases of gunshot and shell wounds involving the spine, which are operated on at a Casualty Clearing Station, is extremely small. Many of them, notably those with paraplegia, are often considered to be hopeless, and indeed there are, as a rule, other cases waiting attention, in which operative interference would be more justifiable. In short, it is a disagreeable fact that in many cases of spinal injury operation is a useless waste of time and energy. There are cases, however, carefully selected, in which an operation should be considered.

The deciding factors may be:

1. Incomplete paralysis.
2. Accessibility of the missile.

Incomplete paralysis or paresis determined by a thorough examination of the case in question justifies a more favourable prognosis.

Accessibility of the missile discoverable by X-ray examination would indicate surgical interference. Pain, due to pressure on nerve roots, may be so severe as to justify an operation on humanitarian grounds alone, and a combination of all three or any two of the above reasons ought to remove all doubts from the surgeon's mind as to operation.

It may be a debatable point whether cases of spinal injury should be operated on at a Clearing Station or whether the operation should be delayed until the patient reaches a Base Hospital. It is certainly beyond question that if there is pressure on the cord by bone fragments or missile, the sooner that pressure is removed the more hopeful will be the outlook. If the pressure is not relieved, permanent changes will take place in the cord, and the hopes of recovery become reduced to a minimum. Should a missile be lodged, to delay the operation may be to invite the advent of sepsis, a very grave danger to the life of the patient from spinal meningitis or myelitis.