2. Keep the soldier fit by getting the full calorific equivalent out of his ration, thus keeping the blood pressure up and "warding off fatigue."

If congestion (Stage I) has occurred, I have always detained the men at the regimental aid post for twenty-four to thirty-six hours. If a regimental aid post has not existed in the part of the line to be held I have immediately made one, and have always been supplied with a fatigue by the commanding officer to erect it in minimum time. This dug-out will usually accommodate six to eight men, and timely massage with rest have rendered the men fit to go back to trenches in thirty-six hours. They report each day for three days for further massage, and at the end of this time, with instructions as to the keeping up of their legs whenever possible, I have had no further trouble.

By this means many men were kept in the firing line who would otherwise have been temporarily lost to the unit, and this is a point the importance of which need not be emphasized.

I have to thank my commanding officer for the kind help and co-operation he has shown in this endeavour against "trench foot," and I have every confidence that we shall be able to face another winter campaign with a minimum of men going down with this condition.

The remarks and suggestions made in this paper may not be new to many, but what I have written has been the result of careful and persistent investigation of my own cases, which at first were considerable in number, but which have, I am glad to say, been reduced to nil.

THE EXCYSTATION OF ENTAMOEBA HISTOLYTICA (TETRAGENA) AS AN INDICATION OF THE VITALITY OF THE CYSTS.

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In the course of an experimental investigation undertaken to ascertain the value of emetine as a prophylactic for amoebic dysentery we have found kittens extremely refractory to the cysts of *E. histolytica* (*tetragena*)—that is, only a very small proportion (one out of twelve, after a three weeks' incubation period) of those fed with faeces containing large numbers of cysts become infected. We were thus led to endeavour to cultivate the *Entamoeba* from the cysts in order to obtain, if possible, stock cultures of active forms for rectal inoculation. Owing to the fact that we are going abroad very shortly, this work has now to be broken off for the time being. As we have found a method of causing the cysts present in the faeces to excyst and liberate the contained *Entamoeba*, we think it worth while to indicate briefly the results obtained, since this method furnishes the most reliable, and, indeed, the only conclusive, evidence of the vitality of the cysts. This is a point of the utmost practical importance as bearing upon the duration of life and the infective power of the cysts under varying environmental conditions.

A sample of faeces containing a good infection with cysts is well emulsified with saline (or water) and filtered through three or four layers of fine gauze, to remove the larger faecal particles. The filtrate is then centrifugalized and the deposit washed three or four times, the supernatant liquid, which contains large numbers of bacteria but no cysts, being pipetted off each time. By this means toxic products are removed and the cysts are concentrated in a mass of fine faecal debris. As excysting agents we have tried pepsin, in an acid medium, bile, and pancreatic extract, either alone, consecutively, or together, as appeared indicated, but the only success we have had has been with pancreatic extract used alone. The preparation used is Benger's *liquor pancreaticus*. A mixture is made

up in the following proportions: Nutrient broth 5 parts, liquor pancreaticus 2 (or 3), cyst-containing sediment 1 part. This mixture is then incubated for five to seven hours at 37°C. At the same time a drop of the mixture is placed on a slide, covered, and the cover-slip ringed to prevent evaporation; this is also incubated. This observation-preparation serves as a control to the tube and can be taken out and rapidly examined whenever desired, to ascertain how excystation is progressing; after five or six hours excystation is usually taking place.

Even of cysts in freshly passed faeces, the majority of which appear quite normal, only a small proportion excyst. The first indication of approaching excystation is that the protoplasm becomes very slightly retracted from the cyst-membrane and appears somewhat crinkled (Fig. 1). By careful watching it can be seen that the contour of the body is very slowly changing its shape, that is to say, the *Entamoeba* is becoming slightly amoeboid (Figs. 2, 3). The nuclei are not visible; it may be pointed out that when the nuclear rings are clearly visible in a fresh *tetragena* cyst, it is a sign that the cyst contents are dead; in this respect the *tetragena* cyst differs from the cyst of *E. coli*. Next, the periphery of the body becomes applied to the thin cyst membrane in the region where the wall is about to be dissolved (Fig. 4). Dissolution of the cyst-membrane takes place over a small area only. It is brought about, we consider, by the action of external influences; for example, the pancreatic juice, probably helped to some extent also by bacterial ferments present in the intestinal contents.

In the next stage (Fig. 5) the *Entamoeba* is beginning to emerge from the cyst. A small protuberance, often finger-like, is thrust out through the aperture; it may appear clearer than the bulk of the protoplasm, and be

mainly ectoplasmic in character. The whole of the protoplasm now gradually flows out of the cyst (Fig. 6); the entire process of emergence may take only about ten minutes, though it may take longer. In every instance which we have observed, the entire protoplasm comes out as a single *Entamoeba*; there is no indication whatever of division while still inside the cyst. We are strongly of the opinion that this is the normal method of excystation. It may be mentioned that tyrosin was always present to saturation in the liquor pancreaticus which we used, the white deposit which is usually seen at the bottom of a bottle of this preparation (after keeping for a while) consisting of pure tyrosin needles and sheaves which have crystallized out. We are informed by Dr. Maclean that the amount of this amino-acid normally present in the duodenum is not likely to represent a greater concentration than we have

Excystation of *Entamoeba histolytica* (*tetragena*); from sketches made at the time of observation. Figs. 1, 2, and 3. Slight amoeboid changes within the cyst. Fig. 3 shows a slight development of ectoplasm (this is unusual, and only seen if excystation is apparently delayed). Figs. 5 and 6. Emergence of the *Entamoeba* through an aperture in the cyst membrane. Figs. 7 and 8. Two liberated *Entamoebae*, with their empty cysts (seen in optical section); in the latter figure the aperture in the membrane is in focus, and this *Entamoeba* possesses a "chromidial block" (the endoplasm is dotted to indicate its finely granular character; the ectoplasm is left clear).

used in our tubes. From our observations we do not consider that a division into four amoebulae takes place, as a rule, within the cyst. After the *Entamoeba* has completely emerged, the cyst-membrane left behind appears like a very delicate, single-contoured, usually spherical ring (Fig. 7); only by careful focussing at a particular level can the break in the wall be made out, which represents the dissolved area (Fig. 8).

We have seen excystation of amoebae which had no chromidial block in the protoplasm (Fig. 6), as well as of others which possessed this structure (Fig. 8); so that one of these conditions cannot be said, apparently, to represent a riper phase, as regards readiness for excystation, than the other. For a little while the liberated *Entamoeba* remains more or less rounded and sluggish; but soon it begins to throw out the typical clear, blunt pseudopodia of the *histolytica* type, these often welling out laterally in the characteristic manner. We have not yet been able to carry out direct observations further at this period, and cannot say, therefore, whether the *Entamoeba* proceeds to divide into four, and, if so, how soon this occurs. Indeed, from our living observations, we could not tell whether the amoebae which excysted had always four nuclei, or whether individuals with only one or two nuclei also had been able to excyst.

When we have ascertained that excystation was proceeding actively in the tubes, we have always subcultured from them into the expression-liquid of blood-agar tubes, to which either two parts of citrated blood or equal parts of citrated blood and fresh serum were added. These new tubes were then placed in a vacuum and incubated at 37° C. At from eighteen to twenty hours after the excystation period we have seen both small and moderate-sized active *histolytica* forms in these cultures, a few of the larger individuals containing red blood cells. It is a moot point whether any multiplication had occurred, beyond probably division of some of the originally encysted forms into four; and even in regard to this we do not feel certain. In spite of all our efforts so far, the growth of bacteria has always been excessive and gave no promise of successful cultivation of the *Entamoebae*. On one or two occasions, twenty-four hours later—that is to say, from forty-two to forty-four hours after excystation—we have found one or two amoebae still alive but moribund.

To our surprise, the above method, which has proved so successful for the excystation of *E. histolytica*, has entirely failed to produce excystation of *E. coli*, and by none of the other methods tried have we been able to induce this form to excyst. We have evidence, however, that the small "*minuta*" form, which we have recently described,¹ excysts in the same manner as *histolytica* (*tetragena*), using the same method; and this bears out our view as to the close relationship of these two forms.

In the case of *Lamblia*, also, we have been unable to induce excystation. From the appearances of the cyst contents which we have observed, we are strongly of opinion that only a single individual will be liberated and not two separate individuals. In other words, we consider, as we have previously stated,² that conjugation and not division occurs within the *Lamblia* cyst. Wenyon³ has expressed the view that these cysts are multiplicative, and represent, indeed, the endogenous form of multiplication shown by these parasites within the host. In our opinion this view is mistaken, and does not take into account the biological factors (environmental conditions) which influence both encystment and excystation (Cropper and Drew,⁴ Woodcock and Lapage⁵). Not to enter into details here, we will only point out that when permanent (resistant) cysts, such as are passed out with the faeces, are formed in the bowel, conditions other than those prevailing in the region where encystment is occurring are necessary for excystation and the commencement of a fresh cycle of activity. Equally as is the case with the cysts of the parasitic *Entamoebae*, for instance, we have little doubt that these *Lamblia* cysts only undergo excystation on passing down the alimentary tract of a fresh host and coming into contact with its digestive juices. From our experience of multiplication in flagellates we should say the process takes place very rapidly; and it is quite likely that it occurs mainly amongst those parasites which are attached to the epithelium.

Had time permitted we had intended to use the above described method of inducing excystation of the cysts of

E. histolytica to test the vitality, and consequently the infective power, of the cysts, when kept under different conditions for varying lengths of time; much more evidence is needed in regard to this point on account of its great hygienic and sanitary importance. The chief experiment which we have had opportunity to carry out, so far, in this direction has been to keep cysts in a very slowly running current of water for fifteen days and to ascertain that certainly some were still alive at the end of this period. By this time the debris was practically freed from faecal bacteria, apart from those normally occurring in the water, and free-living protozoa (ciliates and flagellates) were present. We should say, therefore, that water which has been contaminated with cyst-containing faeces may remain a source of infection for a considerable period.

REFERENCES.

- ¹ Woodcock and Penfold, BRITISH MEDICAL JOURNAL, 1916, i, p. 407.
² Ledingham, Penfold, and Woodcock, BRITISH MEDICAL JOURNAL, 1915, ii, p. 710. ³ Lancet, 1915, ii, p. 1296. ⁴ Researches on Induced Cell Reproduction in Amoebae. ⁵ Proc. Roy. Soc., B, 88, 1915, p. 353.

AMOEBIC DYSENTERY IN A MAN WHO HAD NEVER LEFT ENGLAND.

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AMOEBIC DYSENTERY occurring in the British Isles is remarkably rare. Most of the cases hitherto recorded have been conditions of intestinal ulceration associated with liver abscess which have only been diagnosed *post mortem*. Thus Dickinson,¹ in 1862, described the occurrence of a hepatic abscess of considerable size with ulceration of the large intestine in a woman who had never been out of England. In 1881 Dickinson² again recorded a second case, that of a man aged 36, who also had never left England. *Post mortem*, ulceration of the large intestine was found coexisting with a liver abscess. In the same year Moore³ described a further case, that of a child aged 3½, who died with two hepatic abscesses and extensive ulceration of the large bowel. She had never been out of London. Amoebae are not mentioned in connexion with any of these cases, though the organism is said to have been discovered by Lamb⁴ as early as 1859, subsequently being more fully described under the name *Amoeba dysenteriae* by Lösch in 1875.

Saundby and Miller,⁴ however, in 1909 recorded the case of a man aged 42, in whom, *post mortem*, were found extensive ulceration of the large intestine and a liver abscess. Amoebae were demonstrated both in the intestinal ulcers and in the abscess cavity.

In none of these cases was the diagnosis made until after death, and it remained for Marshall,⁵ in 1912, to report the first case occurring in the British Isles which was diagnosed during life. This was a man who had never left Scotland, and in whose stools amoebae were repeatedly demonstrated; he made a good recovery. An ex-soldier, living near this patient, had spent some time in India, but gave no history of dysentery. Amoebae were not found in this man's faeces, and feeding experiments on a cat gave negative results.

Our patient was a private, who enlisted at the beginning of 1915. Prior to enlistment he had always lived in or about Manchester. The great majority of the men in his unit had been abroad at some time, but none had been out of England during the present war. Of three men who were sleeping in the same room as the patient only one had been abroad. This man had served in the South African war, where he had had diarrhoea of three days' duration only, which never recurred. His faeces, as well as those of five others in the unit, including the cook, who were said to have had dysentery abroad some years ago, were examined, with negative results. One, however, showed encysted forms of *Entamoeba coli*.

The patient, rather a stout man, aged 39, but looking considerably older, was admitted to hospital on February 16th, 1916. He had had no previous illness up till the onset of his present