DETECTION OF AMEBAE IN CASES OF CHRONIC SYSTEMIC AMEBIC INFECTION*

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The Endameba dysenteriae affects chiefly the intestinal tract. Heretofore disease caused by it has usually been regarded as limited to that tract except for occasional involvement of the liver by an abscess process and still more rarely by abscess formation in other portions of the body.

The interest shown during the past few years in the search for pathogenic protozoa in feces has demonstrated that amebae are frequently found and that infection with the Endameba dysenteriae is by no means limited to persons who have been in the tropics or even in the southern portion of the United States. Many of the soldiers who returned from Europe were infected with this pathogenic type of ameba. Kofoid and his associates report the finding of Endameba dysenteriae in 2,300 hospital cases of soldiers who returned overseas and in only 4.3 per cent of 576 soldiers who had seen only home service. On the other hand, Boeck and Stiles believe that from the evidence available it is not clear that the return of our soldiers from Europe has caused any material increase in the number of cases of amebic dysentery in this country.

Many persons who harbor this parasite are not ill. They are apparently carriers of the organism. Other persons in whom the parasites are found have not had any symptoms referable to the intestinal tract but, on the other hand, have had symptoms such as general indisposition, weakness,

lack of ambition, mental apathy and depression, neuralgic or neuritic pains, etc.—symptoms apparently due to a systemic toxemia. In many cases treatment with emetin will cause a disappearance of the symptoms. It has therefore been assumed that the symptoms were most likely caused by amebae.

A few years ago Kofoid and Swezy discovered Endameba dysenteriae in necrotic foci in the bone marrow of a case of arthritis deformans under the clinical care of Ely and associates. Since, that time the Endameba dysenteriae has been found in the feces of many cases of chronic multiple arthritis. Many of these cases of arthritis have been cured by specific antiamebic treatment, leading to the natural assumption that the amebae were the cause of the arthritis.

These observations will probably considerably extend the field of activity of the public health as well as clinical laboratory worker.

A search for Endameba dysenteriae should be made in many cases of illness, regardless of the absence of an epidemic of dysentery or of dysenteric symptoms in a given case. This should be especially true of all cases of arthritis and neuritis, accompanied by general indisposition, in which some other definite cause is not obvious.

Cases of systemic amebic infection are probably always accompanied by some involvement of the intestinal tract. Nevertheless the amebae may not be found in the feces, especially when only one or a few specimens are examined. Boyers in a personal communication states that

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a persistent search for the specific amebae will seldom fail to reveal their presence in cases of systemic amebiasis which are clinically positive.

Microscopic examination of feces represents the only practical means of identifying pathogenic amebae in cases of systemic amebic infection. A series of at least 6 specimens taken on 6 successive days should be examined before regarding the case as negative. Boyers claims that the administration of bile salts seems to favor the appearance of amebae in the feces. The enteric coated tablets called "Glycotauro" (Hynson, Westcott and Dunning) represent a convenient form of taking the bile salts.

The following directions for the collection of specimens are sent out by our laboratory:

**DIRECTIONS FOR THE COLLECTION OF SPECIMENS OF FECES TO BE EXAMINED FOR AMEBAE**

1. Give patient 18 5-grain tablets of Glycotauro (bile salts) with instructions to take 1 tablet 2 hours after each meal for 6 days.

2. Give patient 6 small wide-mouth well-stoppered bottles with instructions to collect a small portion of feces (about the size of a small marble) each day for 6 successive days, beginning the day after starting on the bile salts.

3. Send specimens to the laboratory as soon as possible. If the laboratory cannot be reached within a day, pour a small amount (about one-fourth volume of feces) of 10 per cent formalin (one part of the commercial formalin to 10 parts of water) over the feces.

The laboratory examination consists of a search chiefly for the cyst forms of the parasite, since these are, as a rule, very much more numerous than the motile vegetative forms. This is especially true of chronic amebiasis. In cases which present a rather definite clinical picture of amebiasis and cysts have not been found on repeated examination, it is advisable to make an examination for the vegetative forms also. Specimens to be examined for such should be kept at body temperature by means of a vacuum bottle and a warm stage.

**TECHNIC OF EXAMINATION**

We have followed chiefly the technic recommended by Kofoid and his associates. Since many laboratory workers are not familiar with their methods I present an outline of them herewith:

Two methods are used: (1) a direct smear of fresh fecal matter stained with a modified Donaldson's iodine-eosin, and (2) a fixed preparation stained with Haidenhain's iron-hematoxylin.

1. A drop of normal salt solution and one of iodine-eosin stain are placed close together on a slide but not touching. A round applicator stick or a tooth-pick is smeared with the feces, rolled in the drop of normal salt, then in the drop of iodine-eosin. A single cover slip is placed on both drops, half the material under it being stained and the other half unstained. Examine the unstained portion first for living flagellates and active amebae. In the stained portion the protozoan cysts stand out as bright spherules against the pink background and soon become tinged with the iodine to varying tones of yellow, with the nuclei becoming clearly defined as the iodine penetrates. If glycogen is present in the cysts, it becomes light or dark brown in color.

**IODINE-EOSIN STAIN**

a. Saturated aqueous solution eosin in normal salt .................. 2 parts

b. 5 per cent potassium iodide in normal salt solution saturated with iodine .................................. 1 part

c. Normal salt solution .................. 2 parts

The proportion of iodine solution used may be modified to advantage by adding a slight excess of that given in the formula if the nuclei do not appear after a few moments' application of the stain. The stain should be made up each day from the stock ingredients.

2. For fixed preparations a smear is made on a slide which has been previously
thoroughly cleaned in alcohol-ether and flamed. If the fecal material is too dry, moisten slightly with normal salt, make a thin smear with the applicator stick or the flat side of a toothpick or by using the edge of another slide or a cover slip, and immerse directly in fixing fluid without allowing the slide to become dry.

**FIXING AND STAINING METHODS USED**

1. Schaudinn's fluid (even if previously fixed by formalin) .................. 5 min.
2. 70 per cent alcohol tinged with Gram's iodine .......................... 5 min.
3. 70 per cent alcohol ........................................ 5 min.
4. 50 per cent alcohol ........................................ 5 min.
5. Tap water ........................................ 2 min.
6. 2 per cent iron alum—aqueous solution 5 to 12 hrs.
    Or 2 per cent iron alum—aqueous solution heated to 30° C. (never higher) .................................. 10 min.
7. Tap water—running ........................................ 5 min.
8. 0.5 per cent Hematoxylin—aqueous solution (Haidenhain's) .......... 12 to 18 hrs.
    or 0.5 per cent Hematoxylin—aqueous solution heated to 30° C. (never higher) .................................. 10 min. to 1 hr.
9. Tap water rinse
10. Differentiate in 1 per cent iron alum with careful watching under the microscope ................................ 3 to 30 min.
11. Wash in running water .................................. 10 min.
12. 50 per cent alcohol ........................................ 5 min.
13. 70 per cent alcohol ........................................ 5 min.
14. 90 per cent alcohol ........................................ 5 min.
15. 100 per cent alcohol ....................................... 5 min.
16. Xyloc ........................................ 5 min.
17. Mount in balsam—cover.

Schaudinn's fluid—2 parts saturated aqueous Hg Cl₂ in normal salt; 1 part absolute or 95 per cent alcohol. Add 4 c.c. glacial acetic acid to 96 c.c. of the mixture on using.

If a quick method is desired, the same outline can be followed with a shortening of the time of application of the iron alum and the hematoxylin. The slide is taken from the water, flooded with or in alum by a pipette and held over a flame or placed upon a heated plate for about five minutes, or until it begins to steam. Wash in water and treat in the same way with the hematoxylin, continuing the application of heat until a metallic scum appears on the top of the fluid on the cover slide. Differentiate in iron alum. Care must be taken throughout the entire process to avoid drying of the smear. Use American hematoxylin, standardized white crystals only. Use only violet crystals of iron alum; reject yellowish powder.*

Boeck and Stiles² prefer to use eosin and iodine separately for the fresh unfixed specimens.

It is obviously a matter of the greatest importance to be certain of a diagnosis of *Ameba dysenteriae*. There are so many other amebae of similar structure; other types of protozoa and other cells of somewhat similar morphology that the diagnosis of the specific ameba of the dysentery type should not be left to anyone not entirely familiar with their definite recognition.

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*Ordinarily only the iodine-eosin method is employed and the 1/6 dry objective used. For the confirmation of suspicious forms, stain with iron hematoxylin and examine with oil immersion. Use a binocular microscope.

**REFERENCES**