CULTURE METHODS IN THE DIAGNOSIS OF AMŒBIASIS.

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Entamoeba histolytica was first successfully cultivated in an artificial medium by Boeck and Drbohlav (1925). St. John (1926) drew attention to the value of culture of the organism in the diagnosis of amœbiasis, and Craig and St. John (1927) found that culture of faeces for E. histolytica gave a higher percentage of positive results than was obtained either by direct examination or by examination after concentration where one specimen only was examined. In a series of 71 cases E. histolytica was obtained in culture in 11 cases, while direct microscopic examination and examination after concentration gave positive results in 6 cases and 9 cases respectively.

A negative result from the examination of one specimen of faeces is, of course, insufficient evidence on which to exclude a diagnosis of amœbiasis. A series of six to seven specimens is required before reasonable assurance can be given that amœbe are absent from the stools. The purpose of the investigation to be described was to determine of what value culture of faeces for E. histolytica might prove when employed as a routine measure in a military hospital laboratory. That is to say, would culture for amœbe prove an effective alternative to the examination of the long series of specimens required from each case of suspected amœbiasis?

In this context it is appropriate to note the conditions in which laboratories situated in endemic amœbiasis areas function. Though the actual ratio of amœbic to bacillary dysentery may be low, comparatively, and though perhaps these two diseases may not account for a very large proportion of the total number of medical cases in the wards, yet the number of stools sent to the laboratory for examination for amœbe forms a very considerable proportion of all specimens received. This is due to the fact that a series of stools is required from each patient in whom amœbiasis is suspected, as has already been mentioned, and amœbiasis is always in the mind of the physician who works in an endemic area, as indeed it must be, when he examines patients complaining of a variety of symptoms, apart altogether from those who give typical histories. Furthermore, each patient who has been treated for amœbiasis undergoes tests of cure. These involve the examination of another series of specimens from each case.

The staff available to deal with this large number of specimens is usually inadequate. Though this cannot be rectified in these days of acute shortage of man-power, it does mean that the time given to the examination of a specimen of faeces depends on the total number of specimens to be disposed of and the pressure of other work. Thus less time is devoted to the examination of each specimen than is desirable. It will be readily appreciated, therefore, that any method of diagnosis of amœbic infection which would obviate to some extent the usual lengthy examinations would relieve a busy laboratory of much routine work.

The point need not be further laboured. It will be appreciated at once by any who have worked in laboratories in endemic areas. Though the results now to be presented form only a small series—the writer returning to this country before completion of the investigation—it is felt that their publication might be of interest, and possibly of some help, to those whose duties still bring them into contact with numerous cases of amœbiasis.

Technical Methods.

The Medium.—The original medium of Boeck and Drbohlav (1925) consisted essentially of inspissated egg and Locke's solution, the slopes being covered with a mixture of Locke's solution inactivated human serum. Dobell and Laidlaw (1926) modified the method, using slopes of inspissated horse serum covered with egg albumen solution. A small amount of rice starch added to the solution gave a more luxuriant growth of amœbe. Simpler media have also been devised. Craig (1934) reports favourably on a medium consisting of one part of inactivated human serum and seven parts of 0.85 per cent saline, the mixture being sterilized.
Culture Methods in the Diagnosis of Amœbiasis

by filtration. In the present investigation an altered version of Dobell and Laidlaw's method was used, the medium being prepared as follows:

1. Loeffler's serum slopes: These are made from ox serum in the usual manner.
2. Ringer's solution:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Sodium chloride</td>
<td>6·0 grammes</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0·5</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0·1</td>
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<tr>
<td>Sodium bicarbonate</td>
<td>0·1</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1,000 c.c.</td>
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Autoclaved at 15 pounds for twenty minutes.

3. Egg albumen: Two new-laid eggs are thoroughly cleansed with soap and water and with spirit. The shells are punctured and the egg white run into a sterile vessel. This is well beaten up and is then added to the Ringer's solution. Sufficient of this Ringer-egg solution is now added to each Loeffler's slope to reach the top of the solid medium. The tubes are tested for sterility in the incubator, where they may be stored.

4. Rice starch: A few grammes of rice are triturated in a mortar till a fine powder results. This is sterilized in the hot-air oven at 160° C. for twenty minutes. Before the medium is used a knife-point of the rice is added to each tube employed.

Inoculation.—Failure results if the methods normally employed in bacteriological technique are used. Amœbæ are never present in the faeces in numbers comparable to the number of micro-organisms in, say, a specimen of pus, or to the number of dysentery bacilli in the stool from a case of bacillary dysentery. For this reason the amount of material conveyed by a platinum loop is insufficient as an inoculum when amœbæ have to be cultured.

If the stool is solid or semi-solid, a portion about the size of a pea is introduced into the culture tube and gently rubbed up in the fluid, the medium having previously been warmed to 37° C. If the specimen is fluid, at least 0·1 c.c. should be added with a sterile pipette. The tube is then incubated at 37° C.

Examination of Cultures.—Here again, the usual bacteriological methods cannot be used. At least 0·1 c.c. of material should be removed for examination. This is effected by drawing up the sediment at the bottom of the slope with a sterile capillary pipette, at the same time gently scraping the surface of the slope with the end of the pipette. A coverslip preparation is then made in the usual manner and examined microscopically. The amœbæ exhibit similar appearances to those found in direct preparations. Ingested red cells are, of course, absent, their place being taken by the easily recognizable rice granules. The characteristic motility of the amœba is also observed. Since amœbæ other than E. histolytica may be present in cultures the usual criteria of differentiation must be borne in mind. The whole preparation must be examined. In some cases several amœbæ may be found in most fields, while in others considerable search must be made before a single individual is discovered. The microscopic examination is carried out after eighteen to twenty-four hours' incubation. Should this be negative another examination is made after a further twenty-four hours in the incubator.

Subculture.—If the second microscopical examination is negative a subculture should be made. Craig (1934) points out that amœbæ may be absent on first or second examination but yet be discovered in subcultures.

To effect subculture 0·1 c.c. of sediment is removed from the medium as described above and is transferred to a fresh tube which is then incubated for twenty-four hours.

Avoidance of Contamination.—While E. histolytica grows well in a medium containing bacteria derived from the same source as was the amœba itself, the addition of organisms from extraneous sources may cause the amœba to die out from the culture. For this reason a technique which is, relatively, aseptic should be maintained.

Results.

Specimens of faeces from 405 unselected cases were examined. In most cases the request accompanying the specimens was for microscopic examination for protozoa or cysts. In each case a direct film was made and examined microscopically, and the culture for amœbæ
was prepared. In practically every case successive specimens were sent from each patient until a diagnosis had been made in the ward or in the laboratory; or until it was considered by the medical officer in charge of the case that further examinations were unnecessary. These specimens were examined microscopically as received, though only the first specimen from each patient was cultured for amœbe. Cultures and subcultures were examined at the intervals already mentioned.

The results were as follows:

- Culture positive and direct preparation positive...18 cases
- Culture positive and direct preparation negative...24 cases
- Culture negative and direct preparation negative...363 cases

Total 405 cases

Cultures.—All the cases in which *E. histolytica* was found showed positive cultures. In all cases, too, the first culture was positive, and in all but 3 cases the amœbe were found on first examination after twenty-four hours' incubation. In these 3 cases first microscopic examination was negative, a positive result being obtained after forty-eight hours' incubation. In the 363 cases in which *E. histolytica* was not found, the primary culture and the subculture remained negative.

Direct Preparations.—Of the 42 positive cases, that is, the total number in which amœbe were detected by any method, *E. histolytica* was found in direct preparations in only 18 cases. The remaining 24 cases were diagnosed by culture alone. This is not to be taken as a direct demonstration of the superiority of cultural methods to ordinary microscopic examination. The circumstances must be considered. When examining a series of six to seven specimens from a suspected case of amœbic dysentery, *E. histolytica* may be found in the first specimen or in the second or in the third or, quite as commonly, the parasite may not be detected till later specimens are examined. In any event, the competent observer expects, sooner or later, to find the parasite or its cysts if the case be one of intestinal amœbiasis. But if the culture method is employed it is probable that only one or two specimens of faeces will have been examined by the time the culture is ready for the first observation. If the culture is then positive further examination of direct preparations is not required. Should the culture be negative, thus requiring further incubation, and even should a subculture be necessary, there still may be a saving in the number of direct examinations required.

DISCUSSION.

The results detailed in the foregoing paragraphs indicate that culture of faeces for *E. histolytica* as a routine measure in the diagnosis of amœbiasis can be of considerable value. Extended observations should show whether or not complete reliance may be placed on the method. It will be necessary to show that cultures are always positive when amœbe can be demonstrated by direct microscopy in an adequate series of specimens. It will also be necessary to demonstrate that cysts, if present in the specimen, exist or can be otherwise recognized in culture.

Should further observation confirm the results already obtained, the laboratory diagnosis of amœbiasis may become considerably less laborious. The long series of specimens previously essential will no longer be required, though it must be recognized that direct examination can certainly not be dispensed with *in toto*. It will still be necessary to examine fresh specimens for the presence and nature of cells, crystals, ova and other constituents with the same care and diligence as has always been required.

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REFERENCES.


