THE LABORATORY DIAGNOSIS OF DYSENTERY OCCURRING IN SOUTH AFRICAN TROOPS IN THE MIDDLE EAST.

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The primary purpose of this communication is to outline the methods used in a South African Hospital Laboratory in the Middle East for the diagnosis of dysentery. These methods are based on D.M.S., M.E., Technical Instructions, 1941, No. 59, and on methods used by Colonel J. S. K. Boyd, and have proven very satisfactory in our hands.

Dysentery in the Middle East, as in South Africa, may be caused by helminthic, protozoal or bacterial infection.

(1) Helminthic Dysentery.—Our experience of this condition is limited to one case which was discovered in the course of a routine examination of fifty-five kitchen personnel for dysentery carriers. The patient, a native from the Tzaneen District of the Transvaal, was infected with Schistosoma mansoni. The large lateral spine eggs of the parasite were very numerous and were easily detected in the mucus in the stool under the low power objective.

In rare cases S. haematobium has been known to cause dysenteric symptoms. Although seven cases of infection of the genito-urinary system with S. haematobium were detected, no evidence of dysentery caused by this organism was obtained.

(2) Protozoal Dysentery. (a) Amoebic Dysentery.—Eleven cases of infection with E. histolytica were encountered. In all but one of these cases, the naked-eye appearance of the stools was similar and characteristic of amoebic dysentery. These stools consisted of semi-solid brown faecal matter with some blood and brown mucus. The stools were very foul smelling. The one exception was a stool from a case of double infection with E. histolytica and B. dysenteriae, Shiga. This stool consisted of glairy mucus and blood and could best be likened to “sago with red-current jelly.” No difficulty was found identifying an E. histolytica infection microscopically. For microscopic examination, a portion of mucus from the stool was washed in warm saline and any area containing dark necrotic patches carefully examined under a cover glass sealed with vaseline. The absence of any definite cellular exudate such as is present in acute bacillary dysentery stool was an important feature. Whilst few polymorphs were seen, the number of mononuclears, macrophages and red cells varied in different specimens. In a few specimens Charcot-Leyden crystals were seen.

The one characteristic feature was the presence of actively motile amoebae containing red cells. Generally, when E. histolytica were found, they were
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Numerous. Sometimes a specimen of mucus from a stool disclosed one amoeba. In such cases a second or third specimen often showed an area containing numerous active amoebae.

Two important technical points were strictly observed. Firstly, all stools were brought to the laboratory in bed-pans within half an hour of the stool being passed. Secondly, a bottle of saline was kept constantly in the incubator, so that the mucus could be washed with warm saline immediately on arrival in the laboratory.

In three cases the presence of E. histolytica cysts was diagnosed. These cases had a previous history of E. histolytica infection. The examination for cysts was carried out by rubbing up a small portion of feces in a watch glass with a 1 per cent solution of iodine and potassium iodide. A drop of this suspension was mounted in the usual manner and examined for the presence of cysts.

In diagnosing the presence of cysts no cell over 14 microns in diameter was considered and examination with the micrometer eyepiece was found extremely useful.

(b) Flagellate Dysentery.—Two cases of infection with Giardia lamblia were observed. In neither case was any blood or mucus noticed in the stool nor was there any evidence of cellular exudate. Trichomonas hominis and Chilomastix mesnili were frequently observed in stools and on occasion along with E. histolytica. None of these flagellates could be accused of causing a true dysenteric condition.

(3) Bacillary Dysentery.—This condition accounted for the majority of the dysentery stools examined. In many cases the condition was of a mild nature and amounted to a "diarrhoea" or what is known in the Cape as "Appelkoos siekte." The naked-eye appearance of the stools was characteristic. In these mild cases the stools were of large volume, watery and pale brown in colour. Small flakes of mucus were present but no microscopic blood was evident.

In the more severe conditions the stool consisted of blood and mucus. Few of our cases showed much microscopic blood. The presence of copious blood is usually an indication of Shiga infection. Only one case of B. dysenteriae Shiga infection was encountered.

In a few cases of more severe dysentery, the stools were watery, of a yellow brown colour, and contained flakes of mucopus and greenish shreds of mucus.

The microscopic appearance of the stools was extremely characteristic. In a high proportion of cases an immediate diagnosis of the condition was made from the microscopic examination and a report forwarded at once to the ward. In this way correct treatment was instituted at the earliest possible stage of the infection. A flake of mucus was selected from the stool and examined in saline under a cover glass. The outstanding feature of the microscopic appearance was the cellularity of the field. The number of red cells present varied with the mucus selected, e.g. a clear piece of
mucus showed few red cells, a blood-stained shred showed many. Apart from the red cells, at least 90 per cent of the remaining cells were pus cells. A small number of epithelial cells, macrophages and eosinophils was also present, but the characteristic picture was that of an acute inflammatory exudate and was a typical "bacillary exudate."

In more advanced cases the number of pus cells decreased, more mononuclear cells were seen and erythrocytes became fewer. This type of exudate was known as an "indefinite exudate" and was of no diagnostic value as an indication of bacillary dysentery in view of its resemblance to the exudate found in amoebic dysentery. Stools showing an "indefinite exudate" were, however, cultured and from a certain portion dysentery bacilli were isolated.

It is essential that absolutely fresh stool specimens should be submitted for examination if a high percentage of isolations is to be obtained. Instructions were, therefore, issued that all "diarrheic" stools should be submitted immediately to the laboratory in a bed-pan and that on no account should more than thirty minutes elapse between the passing of the stool and its arrival at the laboratory.

The presence of cresolic disinfectant in the bed-pan was found at one stage to inhibit bacterial growth. This was remedied by thorough washing of the pans in hot water and substituting chloride of lime for cresolic disinfectant.

Cultures were made on MacConkey's medium, which was preferred to litmus-lactose-taurocholate agar. A portion of mucus was fished from the stool and well washed in sterile saline to get rid of excess *B. coli*. It was then rubbed on the surface of the plate. The platinum loop was then flamed and the rubbed area touched with the loop, with which a second area of the plate was stroked. Frequently the loop was again flamed and the second stroked area touched with the loop and, from this inoculation, a third area stroked. It was found that with this technique one half of a 3½-inch MacConkey plate could be used for each stool, although it was preferable to use one plate for each stool. The need for washing mucus before plate inoculation cannot be over-emphasized. Unless this procedure was carried out, the isolation rate dropped almost to zero. The plate was incubated overnight at 37° C. The colonies of dysentery bacilli were usually easily recognized as non-lactose fermenting, small, translucent, delicate colonies with a regular outline. Occasionally wholly irregular colonies of *B. dysenteriae* Sonne were observed. On some plates the colonies were so numerous that it was possible to carry out a direct agglutination test. In the majority of cases selected colonies were picked off and inoculated on to a second MacConkey plate, using a method suggested by Captain Manifold, I.M.S.

In this method a MacConkey plate is divided up into thirty-six sections by cross ruling the glass with a grease pencil. One strip containing six sections is used for each sub-culture and one of six colonies from each
original MacConkey plate is inoculated on each section. Thus six colonies from each of six stools can be inoculated on one MacConkey plate. The plate is incubated overnight and non-lactose fermenting colonies can be clearly distinguished. Direct agglutination tests using group sera can be carried out and the type of organism readily identified. Confirmatory biochemical reactions using lactose, glucose and mannite peptone water can then be done using inoculations from the plate colonies.

The direct agglutination tests were carried out by making a thick suspension of the organisms in saline. Five drops of this suspension were placed on a glass slide. One drop each of Shiga, Schmitz, Sonne, Flexner 1 and Flexner 2 polyvalent anti-sera respectively was added and mixed with the drops of suspension. The slide was then rocked for a few minutes. Positive results, which were easily seen naked-eye, developed in about five minutes. The mixtures which did not agglutinate served as controls.

The "Flexner" polyvalent sera used were prepared against the following strains:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Flexner 1</th>
<th>Flexner 2</th>
<th>Boyd</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. dysenteriae</td>
<td>...</td>
<td>...</td>
<td>1</td>
</tr>
<tr>
<td>B. dysenteriae</td>
<td>...</td>
<td>...</td>
<td>1</td>
</tr>
</tbody>
</table>

Occasionally strains were isolated which did not agglutinate with any of the type sera although they conformed in morphology and biochemical reaction with organisms of the dysentery group. Such strains were further investigated by inoculating tubes of lactose and saccharose peptone water, sealing with paraffin wax and incubating for three weeks. Most strains fermented lactose before the end of this period. One strain investigated in this manner proved to be B. dysenteriae Sonne.

The following table summarizes the results of an investigation of 125 cases of dysentery by the methods outlined above:

**TABLE.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of suspected cases investigated</td>
<td>125</td>
</tr>
<tr>
<td>Number of stools examined</td>
<td>134</td>
</tr>
<tr>
<td>Number of cases in which &quot;bacillary exudate&quot; was present</td>
<td>38</td>
</tr>
<tr>
<td>Number of cases in which &quot;indefinite exudate&quot; was present</td>
<td>57</td>
</tr>
<tr>
<td>Number of cases in which no exudate was present</td>
<td>30</td>
</tr>
<tr>
<td>Number of cases infected with B. dysenteriae Shiga</td>
<td>1</td>
</tr>
<tr>
<td>Number of cases infected with B. dysenteriae Schmitz</td>
<td>4</td>
</tr>
<tr>
<td>Number of cases infected with B. dysenteriae Sonne</td>
<td>2</td>
</tr>
<tr>
<td>Number of cases infected with B. dysenteriae Flexner</td>
<td>42</td>
</tr>
<tr>
<td>Total number of dysentery strains isolated</td>
<td>49</td>
</tr>
<tr>
<td>Percentage of cases showing exudate from which dysentery strains were isolated</td>
<td>53 per cent</td>
</tr>
<tr>
<td>Number of cases showing &quot;bacillary exudate&quot; from which dysentery strains were isolated</td>
<td>32 or 84 per cent</td>
</tr>
<tr>
<td>Number of cases showing &quot;indefinite exudate&quot; from which dysentery strains were isolated</td>
<td>17 or 30 per cent</td>
</tr>
</tbody>
</table>
It will be noted that, although no special selective medium was used, the isolation rate from bacillary exudates was very satisfactory.

Carriers.—A routine examination of kitchen personnel was carried out in an attempt to detect enteric and dysentery carriers. Three stools from each member of the hospital kitchen staff were examined. Altogether fifty-five members of the staff were investigated. The majority of the stools were formed or semi-formed and were, therefore, useless for examination for the presence of dysentery organisms. A few “loose” stools were further investigated by the methods described and from two of these B. *dysenteriae* Flexner was isolated. Neither of these carriers, one a native and the other an Italian P.O.W., reported ill at any time or appeared to consider their stools to be in any way abnormal.

Chronic Dysentery.—In twelve cases of chronic dysentery on whom sigmoidoscopic examinations were carried out, the bowel appearance suggested a chronic bacillary infection. Scrapings of the bowel wall were placed in a small bottle containing 30 per cent glycerine in normal saline adjusted with phosphate buffer to pH 8, and sent to the laboratory. MacConkey plates were inoculated immediately on arrival of the specimen at the laboratory. In some cases the MacConkey plates were inoculated directly at the bedside. Two strains of *B. dysenteriae* Flexner were isolated from scrapings left in glycerol-saline and one strain of the same organism was recovered from a plate made at the bedside.

**Discussion.**

The methods used in the examination of 134 stools from 125 patients suspected of dysentery have been outlined above. It is claimed that these methods have proven very satisfactory in view of the results obtained. The criteria used in the diagnosis of amebic dysentery, viz. (a) the presence of actively motile amebae and (b) the presence of erythrocytes in these amebae, led to the discovery of eleven cases of active amebic dysentery or 9 per cent of the total number of cases investigated. This high proportion of amebic dysentery is explained by the fact that the majority of the patients gave a previous history of amebic dysentery contracted in the Union or in the East African Campaign.

Only three patients were found excreting *E. histolytica* cysts in the faeces. All these patients presented evidence of previous *E. histolytica* infection. It is very doubtful if amebic cysts found in the stools of patients who have given no previous history of *E. histolytica* infection can be regarded as pathogenic. Many protozoologists are of the opinion that different races of *E. histolytica* exist, some of which are non-pathogenic to man. Thus Brumpt (quoted by Manson-Bahr, 1940), recognizes a “physiological species” morphologically indistinguishable from pathogenic *E. histolytica*.

The majority of cases of dysentery were bacillary, and organisms of the Flexner group formed the highest proportion of isolations. By carefully
washing flakes of mucus before inoculating plates of media, a high percentage of isolations was obtained in cases showing a "bacillary exudate." This isolation rate compared well with that reported by Anderson and Cruikshank (1941). These workers investigating an outbreak of Flexner dysentery and, using a specially selective medium for Flexner’s bacillus, reported an isolation rate of 97 per cent from the stools of thirty-five patients examined during the second to the fourth day of the disease and 83 per cent isolation from the stools of eighteen patients examined during the fifth to the seventh day of the disease. As the majority of our patients arrived in hospital from the third to the seventh day of the disease, our isolation rate of 86 per cent from thirty-eight patients showing bacillary exudate may be considered satisfactory.

The isolation of B. dysenteriae Flexner from the stools of two members of the kitchen personnel in the course of a routine examination was of considerable interest. Both patients exhibited stools showing an "indefinite exudate." Neither appeared ill nor appeared to consider his stools abnormal. These cases were probably suffering from chronic dysentery and they were obviously not suitable for employment in the hospital kitchens. Sporadic cases of dysentery had occurred at intervals amongst the hospital staff. Although it was not possible to trace any direct relationship between these carriers and such cases as did occur it was noted that fewer cases of dysentery occurred amongst the hospital staff after the two carriers had been isolated and cured.

The case of Schistosoma mansoni infection detected in a South African native in the course of routine examination for carriers raises an interesting problem in public health. This native came from the Tzaneen district of the Transvaal and apparently S. mansoni infection of natives is not uncommon in this district. Just as S. haematobium infection has gradually spread down the East Coast of the Union, so may we expect S. mansoni infection to spread. if a suitable snail host becomes infected with this helminth. It would appear worth while, whilst this infection is comparatively localized as it is at present, to take strenuous measures in an attempt to stamp out S. mansoni infection in the Union.

**Summary.**

1. 125 cases of dysentery were investigated in a South African General Hospital in the Middle East. 38 cases showed a "bacillary exudate" diagnostic of bacillary dysentery. 57 cases showed an "indefinite exudate," whilst 30 cases showed no exudate.

2. 11 of the cases examined, or 8.8 per cent, were found to be infected with E. histolytica.

3. 3 cases were found to be passing E. histolytica cysts. These cases all showed evidence of previous E. histolytica infection.

4. 32 of the 38 cases showing "bacillary exudate" yielded dysentery bacilli on culture.
B. dysenteriae Flexner was isolated in the majority of cases. B. dysenteriae Shiga, Schmitz and Sonne were also encountered.

A routine examination of 55 suspected carriers yielded two cultures of B. dysenteriae Flexner and in one case S. mansoni ova were identified.

(7) B. dysenteriae Flexner was isolated from three of twelve sigmoidoscope specimens submitted.

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BIBLIOGRAPHY.