United Services Medical Society.

THE MICRO-ORGANISMS OF DYSENTERY.

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Much has been written on the causative agents of dysentery, but as most of the accounts of these organisms are far from complete, it may be interesting to summarise our present knowledge of this interesting subject. It is hardly necessary to point out that, notwithstanding the fact that dysentery is still shown without any subdivision in the new issue of the nomenclature of diseases, the clinical entities known for centuries under this name are not one disease, but a group of maladies of very varying degrees of severity, ranging from the acute dysentery so familiar to those of us who have served in tropical or subtropical countries, to the simple infective diarrhoea which occurs in infants and adults in temperate latitudes. This latter, which has hitherto been regarded as "simple diarrhoea," may be, and often is, caused by the bacilli of dysentery, as has been shown by the work of Duval and Bassett and other investigators. The dysentery group of diseases is remarkable as the only one in which two distinct maladies caused, in the one case by a group of bacilli, and in the other by a member of the animal kingdom, have been, until comparatively recently, hopelessly confused by climatologists, and are still actually included under one heading in all official statistical returns.

Proceeding on biological lines, I shall first consider the organisms associated with the name dysentery and derived from the vegetable kingdom.

Bacillary Dysentery.

The organisms which produce this type of disease belong to the coliform group of bacilli. The honour of first isolating a pathogenic bacillus from the stools of dysentery patients belongs to Shiga, but since his researches, published in 1898, a number of organisms which have been demonstrated to be causal agents of the disease have been found by observers in various parts of the world.

I have had the opportunity of working with a considerable number of these organisms, and in December, 1906, published the result of some experiments with six strains of the bacillus. Sir
Patrick Manson has done me the honour of publishing the table summarising my work in the last edition of his invaluable treatise on tropical diseases (pp. 436-439), but as the result of further investigation I consider that the time has arrived to abandon the cumbersome nomenclature associated with the names of individual observers and to group the bacilli of dysentery under two headings, viz.:

(1) Type “A,” including the organisms discovered by Shiga and Kruse; and (2) type “B,” including the organisms isolated at Manila by Flexner.

It will be found that all organisms, including the bacillus isolated by our French comrade M. Vaillard, will fall into one or other of these groups.

To economise space and for purposes of ready reference I have prepared the attached modification of my original table, which, I think, shows clearly the morphological and cultural characteristics of the dysentery organisms and their resemblance to and difference from the other members of the coli group of bacilli.

The following points merit brief special reference: (1) Flagella; (2) sugar media; (3) agglutination; (4) vitality; (5) clinical bacteriological examination; and (6) evidence of pathogenicity.

Flagella.—The dysentery organisms were at first considered to be non-motile, and most of the earlier monographs stated that although Brownian movements are marked flagella are not present. It is, however, now recognised that the bacillus is always motile when recently recovered from the stools, and only loses its motility in sub-cultures. Vedder and Duval were the first to demonstrate, by a modification of van Ermergen’s process, that the bacillus “possesses a number of lateral flagella of great fineness but considerable length.” These results have been confirmed by Birt and Eckersley,1 who have demonstrated flagella in eight strains of the organism. The best method of demonstrating flagella is Stephen’s modification of van Ermergen’s. The following is a description of this method: Three solutions are required, viz., A, B, and C. “A solution” consists of osmic acid, 2 per cent., 1 part; tannic acid, 10 to 25 per cent., 1 part. “B solution” consists of nitrate of silver, 1 per cent. “C solution” is composed of 5 per cent. gallic acid solution and 5 per cent. solution of ammonia, equal parts; this solution is best prepared immediately before

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<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Character</th>
<th>Motility</th>
<th>Flagella</th>
<th>Growth on nutrient agar</th>
<th>Gelatine stab</th>
<th>Peptone and salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) <em>Bacillus dysenteriae</em> (Type &quot;A&quot;)</td>
<td>Short rod with rounded ends; no spores. Length, 1-3 μ. Young cultures are very short, often show irregularity of staining, often arranged in clumps or short chains</td>
<td>Motile in recent cultures from stools, gradually loses motility in subcultures</td>
<td>2-6 μ. Mostly terminal; rather short and thick</td>
<td>Semi-opaque; resembles the growth of <em>B. typhosus</em>, but is more transparent. Has a characteristic odour, called by the Germans &quot;Spermgeruch&quot;</td>
<td>Similar to (4); but film which spreads out from puncture usually absent. Growth not seen till 48 hours, and then only slight white growth</td>
<td>Faint haziness, which rapidly clears; no indol</td>
</tr>
<tr>
<td>(2) <em>B. dysenteriae</em> (Type &quot;B&quot;)</td>
<td>Ditto</td>
<td>Ditto</td>
<td>Ditto</td>
<td>Ditto</td>
<td>Ditto</td>
<td>Ditto</td>
</tr>
<tr>
<td>(3) Pseudo-dysenteric bacillus</td>
<td>Generally somewhat larger than true dysentery bacillus</td>
<td>Ditto</td>
<td>Variable</td>
<td>Ditto</td>
<td>Ditto</td>
<td>No indol</td>
</tr>
<tr>
<td>(4) <em>B. typhosus abdominalis</em></td>
<td>Longer than either <em>B. dysenteriae</em> or <em>B. coli</em>; oval ends</td>
<td>Sub-cultures always very motile</td>
<td>8-12 μ</td>
<td>More opaque than those of <em>B. dysenteriae</em></td>
<td>Similar, but surface film usually present</td>
<td>Ditto</td>
</tr>
<tr>
<td>(5) Paratyphoid bacilli (&quot;A&quot; and &quot;B&quot;)</td>
<td>Ditto</td>
<td>Not so mobile as <em>B. typhosus</em>, but more so than <em>B. coli</em></td>
<td>Variable</td>
<td>Ditto</td>
<td>Ditto</td>
<td>Ditto</td>
</tr>
<tr>
<td>(6) <em>B. enteritidis</em> of Gaertner (Enteritidis Group)</td>
<td>Ditto</td>
<td>Similar to <em>B. typhosus abdominalis</em></td>
<td>8-12 μ</td>
<td>Ditto</td>
<td>Ditto</td>
<td>Ditto</td>
</tr>
<tr>
<td>(7) <em>B. coli communis</em></td>
<td>Shorter and thicker than <em>B. typhosus</em></td>
<td>Motility is not so marked as <em>B. typhosus</em></td>
<td>2-6 μ</td>
<td>More opaque than <em>B. typhosus</em></td>
<td>Whiter, thicker, more opaque, and showing gas bubbles</td>
<td>Indol production marked</td>
</tr>
</tbody>
</table>

All organisms of the *Coli* Group are
<table>
<thead>
<tr>
<th>CHARACTERISTICS OF THE COLI GROUP OF BACILLI.</th>
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</thead>
<tbody>
<tr>
<td><strong>Neutral red shake</strong></td>
</tr>
<tr>
<td>No appreciable change of colour</td>
</tr>
<tr>
<td>Ditto</td>
</tr>
<tr>
<td>Ditto</td>
</tr>
<tr>
<td>Ditto</td>
</tr>
<tr>
<td>&quot;A&quot; usually no change; &quot;B&quot; sometimes fluorescent</td>
</tr>
<tr>
<td>Similar result to <em>B. coli</em></td>
</tr>
<tr>
<td>Canary yellow colour produced and gas bubbles</td>
</tr>
</tbody>
</table>

Decolorised by Gram's method of staining.
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use. The technique is as follows: (1) Take a twenty-four-hour agar culture of an actively motile bacillus. (2) Prepare a thin emulsion of the bacilli. (3) Spread on a clean slide. The slide is best cleaned by heating over gauze. (4) Fix by aid of gentle heat and stain in solution A for about half an hour. (5) Wash off excess of stain but do not wash. (6) Add a few drops of B solution. (7) Knock off excess of stain and stain again. (8) Add a drop or two of C solution. Allow the solution to remain for a few minutes on the slide and then wash thoroughly. Repeat the procedure till a dark brown colour is produced. (9) Dry and mount. This procedure rarely fails, even in the hands of persons not very familiar with this line of work.

Sugar Media.—An alkaline preparation of casein called nutrose has been used by various German investigators and by an Austrian army surgeon, named Doerr, for preparing the special sugar media for differentiating the Bacillus dysenteriae from the other members of the coli group. The formula for these media is:

- Mannite or special sugar 1 part
- Sodium chloride 0.5 parts
- Nutrose 1 part
- Water 100 parts

Dissolve nutrose and sugar or mannite by aid of heat and add 5 cc. of neutral litmus to each litre.

The reaction of the various members of the coli group on these special media is shown on the table (p. 584.)

Agglutination.—Shiga attached much importance to the agglutination of his bacillus with the blood of a patient suffering from the disease, and it was shown that a precipitate was given in dilutions as high as 1 in 200. The reaction is usually given during the first week of the disease, and from the seventh day onwards increases rapidly in favourable cases. As in enteric fever, the dilutions which give agglutination, if charted, show a curve which indicates the progress of the disease. If a steady rise is shown, the progress is favourable, but if the agglutination power of the blood remains stationary, or diminishes, the case usually ends fatally. The value of the test was greatly discredited of recent years until it became generally recognised that the serum of patients will only clump with bacilli of the type which produces the disease. The test should therefore always be applied with both types of the bacillus.

Vitality.—All varieties of dysentery bacillus are killed by a temperature of 60° C. in about half an hour. They are also very readily destroyed by disinfectants, 5 per cent. carbolic acid or 1 in 2,000 perchloride of mercury destroying them in from three to five minutes. Bacilli have lived on rags for twenty-one
days, and Pfuhl states that it will survive in soil for one hundred and one days. The organism has lived for fifty-five days in tap water at ordinary temperatures, and for eighty-eight days at a temperature of 37° C. It has been recovered from milk after three weeks, from butter after nine days, and from fruit and vegetables after eleven days, so that the necessity for careful cooking of all food supplies during an epidemic is very apparent.

Clinical Bacteriological Examination.—Take a small quantity of the mucus and prepare a film in the ordinary way. In cases of dysentery it will generally be found that very few other organisms are present, and in this respect the dejecta differs very widely from normal stools or from those of enteric fever or cholera, which usually teem with various bacterial flora. To prepare cultures, transfer a small quantity of the mucoid material to about 5 cc. of sterile water. Shake up well and then seed out by sweeping a bent rod moistened with the fluid over MacConkey's agar placed in a Petri dish. Incubate at 37° C. for twenty-four hours. The colonies of B. coli will be readily excluded by the formation of acid, as the dysentery bacillus is not a lactose fermenter. All colourless colonies should therefore be transferred to nutrose-mannite and nutrose-sugar media for differentiation from other coliform organisms.

Pathological Evidence of Specificity.—The evidence of pathogenic effects of the various strains of this organism on man is, necessarily, somewhat small, but if not convincing it is certainly strongly presumptive. Strong reports a case in which a Philippine prisoner under a capital sentence was induced to swallow a culture of the organism. He died from acute dysentery, which set in forty-eight hours after swallowing the culture, but the value of this evidence is discounted by the fact that the disease was epidemic in Manila at the time. Shiga made an emulsion of one-twelfth part of an agar slope in sterile bouillon and had it injected into his own back. Slight pyrexia resulted and a small abscess formed at the site of the inoculation, but no diarrhœa resulted. Shiga's blood serum, however, showed active agglutination with the bacillus some days afterwards. Flexner records a laboratory accident in which one of his assistants aspirated a small quantity of fluid culture into his mouth. Notwithstanding prompt expectoration and the free
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use of a disinfectant mouth-wash, severe diarrhoea with bloody and mucous stools, tenoma, and tenesmus, developed in forty-eight hours. On laboratory animals marked enteritis, chiefly affecting the large bowel and showing various pathological changes identical with those of dysentery, has been produced. The lesions are most marked in rabbits, young pigs and dogs. Horses react strongly to inoculation with the bacillus, and have been killed by a small dose. The channel of experimental infection which produces the best results is subcutaneous injections. "Ingestion with food by an oesophageal tube, after preliminary neutralisation of the gastric contents or exhibition of an irritant, has not been successful, while even the direct introduction of a pure culture into the small intestine after laparotomy in a dog produced no appreciable result."

Conclusions.—We may take it as bacteriologically proven by various observers—

(1) That notwithstanding slight cultural differences the various strains of *B. dysenteriae* isolated conform to what must be regarded as two types of the same organism. In addition to these pathogenic strains of the organism there may be isolated from the stools a bacillus which is non-pathogenic to laboratory animals, but is otherwise with difficulty distinguished from the true bacteria of dysentery. Whether, however, the bacillus represents a degraded or transitional form of the true bacillus it is, as yet, impossible to say.

(2) That symptoms and intestinal lesions identical with those found in man supervene after the subcutaneous inoculation of rabbits with the cultures of the various strains of *B. dysenteriae*.

(3) That the dysentery organisms have considerable vitality. They will live on clothing for three weeks, and have maintained their virulence in soil even at a temperature of 1°C. for long periods. When spread on bread-crumbs, or similar articles of food, they survive for about a week.

(4) They are very readily destroyed by heat or by weak solutions of perchloride of mercury or of the higher phenols.

(5) The specific agglutination reaction with the serum of persons suffering from acute dysentery can generally be obtained within the first week following the onset of symptoms. It is of great value in the case of all patients suffering from a prolonged attack of diarrhoea, as it has been obtained in nearly all cases when the test has been applied with the type of bacillus isolated from the stools.
AMOEIC DYSENTERY.

Ascending the biological ladder, we find that the organism producing amœbic dysentery belongs to the animal kingdom, and has been placed by Leuckhart in the Rhizopoda class of the Protozoa, or one-celled animals. An amœba was first described by Lambli in 1859, and subsequently by Losch in 1875, but to Schaudinn is due our present exact knowledge of the genuine organism. He found that many kinds of amœboid organisms occur in the human intestine, and that some of these are not true amœbæ at all, but merely amœboid stages in the development of the higher forms of the protozoa, such as Trichomonas, Lamblia, and other infusorians. Genuine amœbæ he divided into two classes: (1) Those provided with a shell, the Thecamœbæ; and (2) those that have no covering, or Gymnamœbæ. "At least one of the former and two of the latter variety occur in the intestine." The two naked forms are genuine parasites, but one of them is harmless, and the other one of the most dangerous of pathogenic protozoa. Schaudinn has renamed the former, hitherto known as Amœba coli, the Entamœba, and the latter, known as the Amœba dysenterica, as the Entamœba hystolytica. He found the parasite in the excreta of about half the persons which he examined, and Schuberg afterwards demonstrated the fact that as the organism dwells in the upper region of the colon, by hurrying the onward passage of the fæces, the protozoon could nearly always be found in the dejecta of healthy persons.

The Entamœba has two well-marked methods of reproduction, one merely vegetable and the other clearly sexual in type. The asexual form consists in simple segmentation of the nucleus into eight parts, which surround themselves with protoplasm and separate into a "characteristic brood" of young amœbæ. In the sexual cycle of reproduction the organism comes to rest, contracts, and surrounds itself with a gelatinous coat which becomes the cyst wall. The nuclei, after undergoing reconstruction, divide, copulate, and then divide again, so that the parent nuclei eventually form eight young amœbæ which are enclosed within the cyst wall, and like the cysticercus of the tapeworm, cannot start in life on their own account until the cyst has been taken into the stomach of a new host and the gelatinous cyst wall dissolved by the intestinal juices.

The E. histolytica, as the A. dysenteriae is called by Schaudinn, differs widely in appearance and mode of production from the non-
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pathogenic type of the organism. It has a clear tough ectoplasm and ill-defined nucleus, and Schaudinn considers this tough outer layer enables the parasite to force its way between the epithelial layers and submucosa, where it undergoes development, destroys the tissue, and thus forms the ulcers characteristic of dysentery. In support of this view he states that he has actually observed the protozoa forcing their way between the cells in the freshly excised bowel of a cat which had died of amoebic dysentery.

*E. histolytica* in its asexual form multiplies by simple fission or forms new amoebae by budding; "the characteristic brood formation" of the non-pathogenic organism does not occur. The cystic stage is also produced in a different way, and it is of special interest to note that it is not formed till the patient is beginning to recover from his attack and the stools are becoming solid, a fact which is of the utmost importance in framing preventive measures against the disease.

The protozoon throws out from its surface a series of little knobs, about 3 \( \mu \) in diameter, each containing a particle of chromatin. These knobs break off and, having developed a firm capsule, ultimately become hard, opaque little bodies called, for want of a better name, "spores." These bodies are expelled in the faeces, and on being taken into the intestinal tract infect a new host. An experiment of Schaudinn’s seems to prove this conclusively. He allowed some of the faeces from a case of undoubted tropical dysentery to dry in air, and assured himself by careful microscopy that it contained nothing resembling the cysts of *E. coli*, but only the "spores" of *E. histolytica*. He took the material from the slides actually examined, and having made an emulsion with water, gave it in food to a healthy young cat, whose faeces had proved to be free from dysentery organisms. In three days the cat passed stools containing typical *E. histolytica*, and it died of dysentery on the fourth day. The autopsy showed typical dysenteric ulceration of the colon with *entamoebae* "in all stages of penetration of the intestinal wall."

Schaudinn gave large quantities of faecal matter from this case to another cat, but the animal remained healthy, showing that the ameboid form of the organism is innocuous. He then administered a little of the dried-up faeces containing some of the spore-like bodies from the tropical dysentery case, and the animal, which was larger and stronger than the first cat, developed dysentery in due course and died in a fortnight. Kruse and Paquale, as is well known, produced dysentery by the introduction of amebae *per
rectum, but McWeeney points out that this rectal transference can hardly be realised under natural conditions. Whether the entamoebae contained in the faeces from the acute stage will, if gradually dried, become converted into cysts outside the body is not clear, but it is obvious that the dejecta of dysentery must be prevented from getting access to drinking water or being conveyed by flies to food. The E. histolytica and Shiga's bacillus appear to be antagonistic, and both Schaudinn and Castellani have failed to find them in the same bowel. This is a point which is of particular interest and importance to those of us whose duty it is to endeavour to place the diagnosis of intestinal fluxes on a more scientific basis. In this relation, as a point of practical interest in the laboratory, I may remind you that amœbæ can only be found in the stools within twelve hours after being passed, as after that interval they begin to break up. They are found most readily in the smaller pieces of the mucus. The organisms disintegrate with heat, so that films cannot be placed in the Bunsen flame. They should be dried in air and then placed for five minutes in Gulland's solution, which is made up as follows: Absolute alcohol, 25 cc.; pure ether, 25 cc.; solution of corrosive sublimate in alcohol (strength, 2 grammes in 10 cc.), 5 cc. The films should then be immersed in carbol-fuchsir for three minutes, washed vigorously in water for thirty seconds, and dried in air.

The method of diagnosing dysentery without the microscopic appearance of the stools is, I maintain, as unreliable as diagnosing the variety of malaria from the character of the fever alone. It is hoped, therefore, that this paper, which may be described as a plea for the more systematic examination of the dejecta of diarrhœa patients, may succeed in interesting medical officers more deeply in the microscopic diagnosis of the remarkable group of diseases which has been the scourge of armies in the field since the days of Agincourt.

DISCUSSION.

Lieutenant-Colonel Leishman said that, while quite realising the difficulty of condensing so complicated a subject, he thought Major Blackham's paper hardly left a fair impression of its great complexity, and of the uncertainties which still existed as to the number of these

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micro-organisms, and the part which they played in dysentery. These and other points were at present the subject of much debate, and it could hardly be said that the causation of the disease was quite so simple and so clear as might be gathered from the paper. In the case of bacterial dysentery a large number of varieties, types and sub-types had been isolated and described; many of them, however, differed only in respect of their fermentative reactions with various sugars, and he thought that there was a tendency at the present moment to place undue stress on these fermentative reactions; at all events, in the case of some of the strains which had been described, some of these reactions had been proved to be inconstant. Still greater uncertainty prevailed as to the number of species of amoebae which might be encountered in normal or dysenteric stools and as to the association of some of these with tropical dysentery. The artificial cultures of these protozoa had shown that others existed beside the two described by Major Blackham. Schaudinn's work, too, was felt by many to stand in need of revision. A point of great practical importance seemed to him to lie in the fact that the preventive measures which had proved so successful in controlling the epidemic form of the disease were of equal value, whether the type was bacillary or amoebic.

Dr. Macnamara, R.N., said that he had observed that in amoebic dysentery the ulcers caused were of considerable size and showed much undermining, while in the bacillary type the ulcers were much smaller and closely crowded together, and asked if the reader's experience tallied in these respects with his.

Lieutenant-Colonel C. Birt stated that he had isolated Shiga's bacillus in twenty-six cases out of fifty-five examined in Pretoria. Shiga's bacillus, so far as its inability to ferment mannite is concerned, is constant. A culture isolated in February, 1905, still leaves unchanged mannite-nutrose-litmus tubes incubated for a week. The ulcers in the South African cases were usually very large, with thickened, sloughing, excavated bases; unlike the condition Dr. Macnamara had observed in his cases of bacillary dysentery.

Major Blackham, in his reply, expressed his agreement with Lieutenant-Colonel Birt's remarks, and said that clinically his experience coincided with that of Dr. Macnamara.

The Chairman moved a vote of thanks to Major Blackham for his interesting paper, with which the proceedings closed.