THE
British Medical Journal.

THE JOURNAL OF THE BRITISH MEDICAL ASSOCIATION.

LONDON: SATURDAY, JULY 3RD, 1915.

THE IDENTIFICATION OF THE PATHOGENIC
MEMBERS OF THE TYPHOID-COLON
GROUP OF BACILLI.

By J. HENDERSON SMITH, M.B., CH.B. EDIN.,
LISTER INSTITUTE OF PREVENTIVE MEDICINE, LONDON.

EVERY year an immense amount of work is done in this country on the isolation and examination of organisms belonging to the typhoid-colon group of bacilli. Much of it is concerned with the routine examination of water, but a considerable proportion is directly concerned with the examination of excreta, and in many laboratories an extensive experience has been gained of satisfactory methods of isolating and identifying the important pathogenic members of the group. This year, however, war conditions have brought about an enormous expansion in the amount of such work, and at the same time exceptional urgency for its rapid execution. Partly as a result of this, there are already engaged in the examination of excreta many workers whose previous bacteriological experience has been mainly concerned with other departments, and who have not hitherto had to deal with the routine examination of large quantities of such material; and their number is likely to increase still further, as we must anticipate a large and progressive increase in the volume of work with the arrival and continuance of the hot weather. Such men have not the time to look up the recorded experience of others, and perhaps not always the opportunity to consult those whose previous work has made them more familiar with the best methods to adopt. It is hoped that the present paper may be of service to them, based as it is not only on the literature but also on the very large experience of this Institute for many years past, both in the identification of the pathogenic organisms and in the rapid examination of large quantities of material.

The typhoid-colon bacilli are all Gram-negative rods which grow freely on ordinary media at 20°-37° C. and do not form spores, and they are subdivided into two main groups, according to their action on lactose. Those which ferment lactose, producing from it acid with or without formation of gas, are labelled with the general name of *B. coli*, and do not concern us further here. They are practically ubiquitous, and many of them are pathogenic in certain regions or under certain conditions, but they do not produce epidemic disease with predominantly intestinal symptoms. The lactose non-fermenters are again divisible into two main groups by their action on gelatin. The gelatin liquefiers pass into the great group of *B. proteus* (some of them, like *B. coli*, occasionally pathogenic), and with these also we are not further concerned. All the organisms of the group recognized as regularly producing epidemic intestinal disease are included amongst the lactose non-fermenters and gelatin non-liquefiers.

The application of these two tests excludes the great majority of the organisms which belong to the colon-typhoid group, and the first elimination is largely effected by the addition of lactose and a suitable indicator to the medium on which the material is plated in the first instance. Thus in MacConkey's medium any organism which has produced acid from the lactose affects the

neutral red in the medium, and the colonies are red lactose non-fermenters remaining colourless. But there is a source of fallacy in the primary subdivision which must be continually kept in mind. There is a fairly extensive group of organisms which are really akin to the lactose fermenters but do not ferment lactose in the first few days of growth. After an interval of perhaps as long as six to seven days' growth on the sugar they produce descendants which do ferment it, but in the first period they are lactose non-fermenters. In practice one must include in the first selection these late lactose fermenters along with the non-fermenters. Before the final diagnosis is made they will have declared themselves, and if it is realized that the first selection has not been absolutely exclusive of all *B. coli*, they rarely cause serious trouble in the subsequent procedure.

This difficulty of variable characteristics is one which runs through the whole of bacteriological classification. The classification is based on certain characters, which in this group consist of behaviour towards certain substances (carbohydrates, gelatin, milk, etc.) and of serological reactions. Probably none of the characters of any particular organism is so absolutely fixed that it cannot possibly be altered. The difficulty, however, is, so far at least as is known at present, only occasionally a practical one. It may be and is possible under certain conditions to induce a typhoid bacillus to ferment lactose, or to breed a Gaertner bacillus which does not produce gas from glucose; but in clinical typhoid or Gaertner infections the one is not in fact a lactose fermenter, and the other produces gas, and in the practical conditions of the present time any organism which does not conform to the characters described may be rejected as not one of the known pathogenic members of the group. (The only exception which is perhaps worth mention here is in the case of *B. paratyphosus* A. This organism normally produces gas from the carbohydrates it ferments, but occasionally the production of gas is very slight or absent on some or all of them. Suspicion will be aroused by the fact that acid is produced only in the sugars attacked by the typical bacillus, especially if gas is present in one or more of them, and an agglutination test will settle the point.)

The genuine lactose non-fermenters and gelatin non-liquefiers, then, are the important group, and of these a table is given. This explains itself. No attempt has been made to make it exhaustive or strictly systematic. It is intended only as a guide in practical work, and is in fact constructed in accordance with the steps recommended for actual procedure later on; but it contains all the diagnostic characters required for the recognition of the pathogenic bacteria, whatever the system of classification adopted. In two instances, namely, the mannite fermenting group of dysentery bacilli and *B. paratyphosus* B, further subdivisions are frequently recommended. These have not been included in the table, because no result of immediate practical importance is gained thereby, but they require some notice.

Mannite Fermenting Dysentery Bacilli.—This is a group where classification presents exceptional difficulties. In the case of infection with typhoid, Gaertner, or the other pathogenic bacilli of the typhoid-colon type, we have to deal with organisms which present a fairly definite and uniform combination of characters. But in the dysentery due to mannite-fermenters such uniformity does not exist.

They are all non-motile and all produce acid on mannite, and of course they do not ferment lactose or liquefy gelatin, but, beyond this, uniformity either of cultural or serological characters ceases. In different epidemics the causal organisms may conform completely to a recognized type, such as Flexner's original bacillus, or they may differ in one or more particulars from any of the types previously established. Under such circumstances detailed subclassification is of no value for our purpose here. If a table were constructed subdividing into groups by the successive application of different tests, it would have a certain value in systematic differentiation, and the separation of a subgroup, that of *B. dysenteriae* Y, by the use of maltose has become in some sort classical. But even the academic value of such differentiation is doubtful because of the uncertainty whether these organisms are stable in the characters they are found at one time to possess or may not change in one character or another from time to time. The system adopted in the table is to regard all organisms which are agglutinated by Y serum as possible causes of dysentery. *B. dysenteriae* Y is an organism very closely related to Flexner's bacillus, from which it differs in the fact that it does not ferment maltose at the time of isolation, and in its variable production of indol. Serum prepared with it is found to agglutinate all the tested strains of clinically pathogenic bacilli except the bacillus of Strong. It also agglutinates a number of organisms agreeing with the pathogenic bacilli in the mannite and non-motility characters, but differing from them all in one or more respects when other tests are applied, and not yet found in association with clinical dysentery. These minor differences are not sufficient, in view of the want of uniformity in the known pathogenic bacilli, to justify their exclusion from the dysentery organisms, and any organism agglutinating with Y serum should be regarded as a potential cause of the disease.

Strong's bacillus is of rare occurrence. It has apparently been isolated in only one epidemic hitherto (in the Philippine Islands), and can only be finally identified by use of a serum prepared from another strain of the same organism; but it should be looked for, and its characters are given in the table.

B. paratyphosus B.—It is claimed by several English and some foreign workers that a distinction is possible between this bacillus and an organism isolated from attacks of food poisoning. This bacillus, which is known under various names, as *B. suispestifer*, *B. aertryck*, etc., agrees in cultural and ordinary agglutination characters with paratyphoid B, and is considered by many authorities to be identical with it; but a distinction is said to be possible by means of the absorption technique. This procedure, however, can scarcely be carried out in the routine examination of excreta on a large scale, and certainly not by those who are inexperienced in such work.

Agglutination tests may be performed microscopically or macroscopically. The latter method is to be preferred. It takes little or no more time to make the preparations, makes subsequent examination easier and more definite, and allows a final examination to be made even after twenty-four hours without the risk of drying. The test serums should be used in high dilution in salt solution, at least 1 in 500, and preferably 1 in 1,000. Proper controls are essential. Spontaneous agglutination is not uncommon in this group—for example, especially with *B. paratyphosus* B. It should be remembered that with dysentery bacilli the processes of agglutination usually proceed much more slowly than is the case with typhoid, and even with strong immune serum it is advisable to wait several hours before deciding that a test is negative.

METHOD OF PROCEDURE.

This must vary according to the circumstances of the worker, the pressure on his time, the extent of equipment, the quantity of material to be examined, the hour of its arrival and so on, and must be modified to suit the individual. The method first given below is that recommended as desirable wherever possible. It gives a reasonably complete examination of the material, and will lead to the discovery of pathogenic organisms if present in as great a percentage of cases as is practically attainable. It involves a considerable but, except in periods of great stress, quite practicable expenditure of time and material on the first day of isolation of the colonies. By concen-

trating the greater proportion of the work on each sample or batch of samples on the day succeeding their receipt, it greatly lightens the subsequent labour and clears the way for the investigation of subsequent samples. In circumstances of great urgency or pressure full examination may not be possible. An abbreviated technique must then be adopted, but this involves missing a number of cases discoverable by the fuller method. Such abbreviated methods are given below.

1. Plate the suspected material on MacConkey's medium (bile-salt neutral-red lactose agar), and incubate at 37° for at least eighteen hours. If no colourless colonies are present by that time, replace in incubator for twenty-four further hours and examine again.

2. Colonies must be selected from uncrowded parts of the plates. It is best, until experience has been gained, to select colonies differing in appearance. Pick off five colourless colonies and emulsify each in a few drops of sterile broth. If time allows subsequent inoculation of the sugars, etc., the same day, incubate the broth emulsions for two to three hours before proceeding; if not, proceed at once to inoculate from each emulsion a set of eight tubes, containing mannite, glucose, saccharose, dulcitate, broth, litmus milk, gelatin, lactose. Grow the gelatin at room temperature, the others at 37°.

3. After twenty-four hours proceed to provisional diagnosis. If all colonies have given identical results, it is unnecessary to examine further more than two; if the results differ, specimens of each kind must be considered.

PROVISIONAL DIAGNOSIS.

Reject all organisms fermenting lactose or liquefying gelatin. The remainder fall into three groups according to their action on mannite.

A. No Change on Mannite.

The only important organism here is *B. dysenteriae* Shiga, and its identification may be completed almost at once. Morgan's No. 1, an organism frequently associated with epidemic summer diarrhoea, should also be looked for.

No Change on Glucose excludes Shiga; such an organism is probably *B. alcaligenes* (and may be definitely diagnosed if the milk is already alkaline, and no change has occurred in the saccharose and dulcitate tubes).

Acid on Glucose without Gas: makes Shiga probable, practically the only other possibilities being some members of the *alcaligenes* group and some water bacilli. Reject any organisms which have fermented saccharose or dulcitate, and test the others with Shiga serum. Negative result excludes, positive result identifies, Shiga; confirm by absence of motility on the broth tube.

Acid and Gas on Glucose: without change on saccharose or dulcitate makes Morgan's No. 1 probable; confirm by presence of motility.

There remains for subsequent examination in this section only the confirmation of organisms identified provisionally as Shiga.

B. Acid only on Mannite.

The important organisms here are *B. typhosus*, the group of dysentery bacilli of Flexner type, and *B. dysenteriae* Strong. Inoculate liberally from the broth tube a second broth tube, and incubate for four to five hours, and test for motility. Meanwhile test with typhoid serum all strains except those fermenting saccharose. Positive result identifies; confirm the same day by presence of motility. Test with Y serum those which ferment cane sugar as well as those negative to typhoid serum. Positive result identifies as a member of the Flexner group; confirm the same day by absence of motility.

Amongst the remaining organisms—that is, those negative to both Y and typhoid serum—there may be: (1) Inagglutinable typhoid and dysentery strains; (2) *B. dysenteriae* Strong. These cannot be recognized without further time, and each organism is to be subcultured daily on broth meanwhile.

For subsequent examination in this section there are thus: (1) Confirmation of identified strains; (2) the unagglutinated strains.

C. Acid and Gas on Mannite.

The important organisms are those of the paratyphoid-Gaertner group. Reject at once all which have fermented saccharose or clotted milk. Omit for

the present (but do not yet discard) all which have failed to produce acid and gas on dulcitate. With the remainder proceed as follows: Inoculate a salicin tube and add it to the set of eight tubes. Then test each organism with three serums in this order: Paratyphoid B, paratyphoid A, and Gaertner. Positive result identifies. Subculture the negative strains daily.

For subsequent examination in this section there remains: (1) Confirmation of identified strains; (2) consideration of unagglutinated strains and those which had not fermented dulcitate.

By this preliminary examination we have recognized within thirty hours of isolation the great majority of the pathogenic bacilli of the group and got rid of a very large number of organisms certainly not pathogenic. The remaining unidentified bacilli may include *B. dysenteriae* Strong, or inagglutinable strains of the other pathogenic organisms. Temporary inagglutinability is a phenomenon which while it occurs in only a small proportion of the cases is yet so frequent in the examination of large numbers that we must be prepared to meet it. It usually disappears on repeated subculture on artificial media.

The subsequent examination must be delayed till the result of the indol and milk tests can be obtained. The interval should be six further days—that is, a week from first inoculation of the sugars, etc. It must begin always with verification of the lactose and gelatin tests.

SUBSEQUENT EXAMINATION.

A. No Change on Mannite.

We have here only to confirm the diagnosis of the organisms identified as Shiga and Morgan. *B. dysenteriae* Shiga does not produce indol, produces no change in mannite, saccharose, or dulcitate, and initial acidity in milk is replaced by alkali; it is non-motile. Morgan's No. 1 produces indol, acid followed by alkali in milk, acid and gas on glucose, no change in other carbohydrates (except levulose and galactose), and is motile.

B. Acid only on Mannite.

Confirmation of Previous Diagnosis.

1. *Typhoid Bacillus*.—Acid only and permanently in milk, no indol, no action on saccharose, acid only on mannite and glucose, no change on dulcitate or late acidity, motile. (Non-motile strains occur, but are extremely rare.)

2. *Dysentery of Flexner Type*.—Test for agglutination with Flexner serum. Positive result places the organism among the close allies of Flexner. Typical Flexner produces indol, acid followed by alkali on milk, acid only on glucose and mannite, no change in saccharose or dulcitate, and is non-motile. For further subdivision, *vide supra*.

Examination of Unclassified Strains.

1. *Motile Strains*.—These cannot be dysentery; the only possibility is inagglutinable typhoid. Reject any organism whose characters do not agree completely with those just given for typhoid. Test the remainder with the last subculture on broth and typhoid serum. Positive result identifies definitely; but a negative result with an organism of correct cultural characters must be returned as inagglutinable typhoid. (Preserve such strains for later examination.)

2. *Non-motile Strains*.—Examine the last subculture with Y serum. Negative result leaves the possibility of Strong's bacillus (and non-motile typhoid; note characters). Reject those which have not produced acid only on saccharose. *B. dysenteriae* Strong produces indol, acid on glucose, saccharose and mannite, and should give late clot on milk (*vide supra*).

C. Acid and Gas on Mannite.

Confirmation of Identified Strains.—All these produce acid and gas on glucose, mannite and dulcitate, no change on salicin and saccharose, fail to produce indol, and are motile. Paratyphoid A produces acid only and permanently in milk; Paratyphoid B and Gaertner produce acid followed by alkali in milk.

Unidentified Strains.—Reject all which have failed to produce acid and gas on dulcitate, and all which do not conform exactly to the above cultural characters of Paratyphoid A or Paratyphoid B. Test with Paratyphoid A serum the last subcultures of those agreeing with Paratyphoid A; and with Paratyphoid B and Gaertner serum those agreeing with Paratyphoid B.

If still negative they are not to be identified as pathogenic, but the strains should be preserved. (*Vide supra* for subdivision of the Paratyphoid B organisms.)

ABBREVIATED METHODS.

These all fail to detect pathogenic organisms in some cases in which the fuller method would have been successful, and the proportion of such failures is greater the more rapid the method. All will detect the greater number, and in periods of stress it is better to examine by a short method in order to cover a larger number of samples. In all of them the first step is to plate on MacConkey's medium and incubate as before for at least eighteen hours.

I.

Pick off five (or more) colourless colonies direct into a mannite tube and incubate till next day. If the result is no change, or acid only, in all tubes, select one only and proceed as follows, according to the action on mannite; if the results differ, take one of each kind:

- No change*. Inoculate a broth tube, and in four to five hours test for motility. If non-motile, test with Shiga serum; positive result identifies.
- Acid only*. Inoculate a broth tube, and in four to five hours test for motility. If motile, test with typhoid serum; if non-motile, with Y serum. Positive result identifies as typhoid or mannite fermenting dysentery.
- Acid and gas*. So many non-pathogenic organisms have this action that it is advisable to inoculate all five organisms into saccharose, dulcitate, litmus milk, broth, and gelatin. Next day reject the organisms which ferment saccharose and do not ferment dulcitate; and test the others with paratyphoid B, paratyphoid A, and Gaertner serum.

Confirmation of identified strains may be carried out. This method will detect a high proportion of the pathogenic organisms. It misses all inagglutinable strains, and by assuming under (a) and (b) identity of all the five organisms from their action on one carbohydrate, misses a further proportion, and it delays the identification of the acid and gas producers for a further twenty-four hours. It saves the considerable expense of the carbohydrate examination of the (a) and (b) groups, and the labour thereby involved, though more agglutination tests are necessary.

II.

Inoculate five or more colourless colonies into broth, and examine for motility in four to five hours. If motile, test with typhoid, Paratyphoid B, Paratyphoid A, and Gaertner serums. Clinical information may help to determine the order of testing. If non-motile, test with Shiga and Y serums. This is probably the best method when the full examination is impossible. It omits more inagglutinable strains than the last, but is rapid, economical, and will detect most of the pathogenic organisms present.

III.

Test by agglutination with the six serums colourless colonies taken straight from the plate, guiding the order by clinical information. Examination of the preparation directly it is made will frequently show the presence of motility, and save the labour of examining with the dysentery serums, but the absence of motility under these conditions does not necessarily mean that the organism is non-motile. This method of examination is the most rapid of all, but the proportion of inagglutinable strains missed is still higher under these conditions.

Various modifications of these methods are, of course, possible, but the full method is recommended wherever practicable, and the second abbreviated method as an alternative. There are several ways in which the experienced can save time and increase the probability of finding any pathogenic organisms present. Most important of these is the initial selection of likely colonies, and the appearance of the plate is also of great assistance. The use of large plates or substitutes, on each of which two or more samples can be plated at the same time without mixing, saves a great amount of sterilizing, washing, pouring, etc. The use of plates already warmed on inoculation increases markedly the size of the colonies at the time of examination, and enrichment media for preliminary incubation may increase the chance of detecting the organisms

sought. But modifications of this kind cannot be discussed in this paper, and must be left to the individual himself to work out.

[Copies of this paper with, if desired, the table mounted on card, may be obtained from Dr. Henderson Smith, Lister Institute, Chelsea Gardens, London, S.W.]

The Croonian Lectures

ON

TRYPANOSOMES CAUSING DISEASE IN MAN AND DOMESTIC ANIMALS IN CENTRAL AFRICA.

DELIVERED BEFORE THE ROYAL COLLEGE OF PHYSICIANS OF LONDON,

BY SIR DAVID BRUCE, C.B., F.R.C.P., F.R.S.

SURGEON-GENERAL, A.M.S.; LATE DIRECTOR OF THE ROYAL SOCIETY'S COMMISSION ON SLEEPING SICKNESS.

LECTURE II.—GLOSSINA MORBITANS: TRYPANOSOMA BRUCEI: WILD GAME.

In my first lecture an attempt was made to introduce the subject—the trypanosome diseases of man and domestic animals in Central Africa—by a few introductory remarks on trypanosomes generally, and their classification; and also by a short description of their carriers, the tsetse flies. In this lecture I propose to deal with one of the species of trypanosomes more in detail, describing its morphology, its pathogenic action on various animals, its carrier—the tsetse fly, *Glossina morsitans*—and the reservoir of the virus—the wild game.

TRYPANOSOMA BRUCEI.

The first species of Group A is *Trypanosoma brucei*, and it will be convenient to begin with it, as it is also first chronologically. That is to say, it was the first pathogenic trypanosome discovered in Central or South Africa, and if you will allow a short autobiographical digression, I shall relate the circumstances leading up to the discovery.

Investigations in 1894-96.

Shortly after I arrived in South Africa in 1894, the late Sir Walter Hely-Hutchinson, the then Governor of Natal and High Commissioner of Zululand, asked me to go to the north of Zululand in order

to report on an outbreak of disease, called nagana, which had broken out among the native cattle.

Travelling at that time was no easy matter, as the railway into Zululand had not been constructed. My wife and I left Pietermaritzburg on October 27th, 1894, going by mule wagon; we arrived seven days later in Eshowe, the capital of Zululand. There an ox wagon was provided, and in it we trekked to Ubombo, a magistracy in the north of Zululand, and in the centre of the affected district. This was reached, after a month's journey, on November 24th, 1894.

Fig. 1 represents the small wattle and daub hut which was provided for living in. The verandah was used at first as the laboratory, but afterwards, with the aid of the natives, a special hut was built. Shortly after we arrived

some of the affected cattle were brought in by the natives. As I had just come from the Army Medical College, Netley, where I had been teaching bacteriology for five years, it was natural that a bacteriological examination of the blood and organs of the infected cattle should first be made. This proved negative.

About this time (1894) the study of the blood had become popular, thanks probably in great measure to Ehrlich, and it was the fashion to make elaborate examinations of the red and white blood corpuscles. To this the discovery of the nagana parasite was probably due. It must be remembered that these parasites are, as a rule, very few and far between in the blood of oxen, and also that our staining methods in those days were rather primitive. After some days of this blood examination it began to be remarked that a curiously-shaped object, different from anything previously found in blood, was sometimes seen lying among the blood corpuscles. At first it was thought to be accidental, due to the carbol-fuchsin stain which was being used, but soon it became evident that it might be a blood parasite. It was then thought that if it was it might be motile in the living state. Fresh preparations of blood were made, and after a long search a rapidly moving object was seen lashing about among the red blood corpuscles. At that time I knew nothing about trypanosomes, and at first thought that the wriggling object might possibly be a small filaria. There were few or no allusions to these haematozoa in medical literature at that time, but when I returned to Natal and had an opportunity of consulting books it soon became evident that the rapidly vibrating body was probably a trypanosome.

But there was as yet no proof that the organisms seen had any causal connexion with the disease. They might well be harmless blood parasites, and they were so scanty in numbers in the blood of the oxen that this might well be so. This led to trying the effect of injecting the blood of nagana cattle into horses and dogs. In these animals the disease is much more acute than in cattle, and the blood swarms with the parasites. In this way it began to be evident that these haematozoa had a causal connexion with the disease.

But at that time there was no suspicion that nagana and tsetse-fly disease were one and the same. It was believed by everybody that the tsetse fly killed horses and cattle by injecting a poison into them. Moreover, these cases of nagana were occurring among native cattle many miles away from the "fly belt." The work was being done on the top of the Lebombo, a range of hills some 2,000 ft. in height, running north and south, about 50 or 60 miles from the coast. Between the

Fig. 1.—Laboratory Hut, Ubombo, Zululand.

hills and the sea there was a low-lying coast plain, some parts of which were infested with tsetse flies. Now, I had read in Livingstone and other African travellers and hunters about the tsetse-fly disease, and was curious to know what it was like. Two young oxen and several dogs were therefore sent down into this "fly belt" and herded among the fly for a fortnight. At the end of this time they were brought back to the hill, and it was a great surprise to find the same parasites in their blood as that found in the nagana oxen. In this way the fact gradually unfolded itself that nagana and the "fly disease" of the travellers and hunters were identical. This, then, was the manner in which the discovery of the part played by trypanosomes in tsetse-fly disease was made.

I arrived at Ubombo on November 24th, 1894, and was