now and then he throws back to a typical Bronze Age man like Darwin, or to a typical Neolithic man like Jonathan Wild.

THE QUESTION OF MODIFICATION.

In conclusion, I should like to ask a question of those who hold that the modifications of skull shape are not necessarily racial, but are produced in any race as adaptations to similar functional needs. In other words, that if a Norseman, a Slav, and a member of the Neolithic or Mediterranean race

FIG. 7.

FIG. 8.

were to eat the same kind of food in the same way from infancy their skulls would take on the same type. I should like to ask them whether there is the least reason to believe that the habits or food of either Wild or Nichol differed from those of their fellow-countrymen; or whether there is sufficient difference in the food and habits of modern Englishmen to account for the English skull shape being nearly pure Nordic while the German is nearly pure Slav?

Is it not a more reasonable explanation that in our island, as in Norway, the brachycephalic, Alpine, or Slav blood has not had the chance of superseding and stamping out the Nordic?—which it has done, if German prisoners are any test, throughout the greater part of Germany.

THE LATE DR. ROBERT STEVENSON.—Robert Stevenson, M.D. Glasg., whose death occurred last week, was honorary anaesthetist to the David Lewis Northern Hospital, Liverpool, and had a wide private practice as an administrator of anaesthetics. He was also surgeon to the Post Office, the Diamond Match Works, and other industrial businesses, as well as medical referee to several important companies. As a sound medical witness in county-court cases his services were very much in demand. As a medical colleague he was ever ready to help any professional brother, though, like several other practitioners in Bootle, he was an uncompromising opponent of the panel system. Since the outbreak of the war his voluntary work at military hospitals proved a severe tax on a none too robust constitution, and the last duty he performed was giving anaesthetics at the hospital, practically dying in harness after a very short illness. The funeral took place at Bootle Cemetery on April 11th in the presence of a large and representative gathering of his colleagues, patients, and friends. He leaves a widow to mourn her loss.

DYSENTERY BACILLI:

THE DIFFERENTIATION OF THE TRUE DYSENTERY BACILLI FROM ALLIED SPECIES.

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IN the bacteriological examination of cases of suspected dysentery and of convalescents it is a common experience to meet with organisms evidently related to the dysentery bacilli but not fully identical with them. Sometimes the difference is one of sugar reactions, at others it is serological, the organism either being inagglutinable, or agglutinating only to low titre, with stock antidyenteric sera. Such strains are often returned as "atypical" or "inagglutinable" Shigas or Flexners, as the case may be, but much doubt has existed as to their significance. Caution is necessary before assuming a causal relation with the disease, even when such atypical organisms are present in the stools in large numbers.

Very little work has been carried out on the bacterial species allied to the dysentery bacilli, and since many laboratory workers have been troubled in deciding how to return these aberrant forms in their reports, it appeared desirable that their characters should be more fully studied. By arrangement with Colonel Sir W. B. Leishman cultures were forwarded to me at St. Bartholomew's Hospital from several laboratories in France, and many have been obtained from home dysentery hospitals. Upwards of a hundred strains have been examined, including as many aberrant ones as possible, but with a sufficiency of genuine dysentery bacilli for comparison. It was hoped to investigate the pathogenicity of all the strains examined, but owing to the extreme difficulty of procuring rabbits only a few of the main types could be studied in this way. For the same reason only the stock sera in general use could be employed in the serological investigations; this side of the subject will be pursued as soon as a supply of animals is available. Meanwhile the cultural results, now almost completed, suggest certain conclusions which are put forward in this preliminary communication in the hope that they may be of service to workers in military laboratories.

I have to express my thanks to those who kindly sent me cultures; to Dr. Joekes, who helped me to carry out many of the sugar reactions; to Dr. R. G. Canti for invaluable assistance in making and titrating new media; and to Captain A. C. Inman, R.A.M.C., who undertook most of the agglutinations, and to whose experience and help I am much indebted.

Nomenclature.

Just as we name the "coli group" from its most prominent member, the general term "dysentery group" may be employed for the Gram-negative, non-motile bacilli which do not liquefy gelatin and which form acid but no gas in glucose media.

The true dysentery bacilli form only a section of this group. So far as present evidence goes, there are two distinct species of dysentery bacilli: (a) the Shiga or Shiga-Kruse bacillus which seems a fairly homogeneous entity; and (b) the group comprising the Flexner and Y types, which are apparently races of one species. To these it is possible that a third should be added—namely, the "Strong" type; but the material on which this paper is based does not allow of any conclusion on the matter. The sugar reactions supposed to distinguish the Flexner-Y races are now known to be inconstant in subculture, and the serological differences which undoubtedly exist are probably racial rather than specific. In the case of the Shiga and Flexner-Y bacilli the necessary criteria for assuming a causal relation with bacillary dysentery are satisfied. Not only are they present in the diseased intestine, but specific antibodies are developed in the patient's blood, while intestinal lesions can be produced in the rabbit by intravenous or subcutaneous injection of pure cultures.

These criteria are not at present satisfied in the case of other members of the "dysentery group," even when such organisms have been found present in considerable numbers in the stools. Some of them, however, are undoubtedly pathogenic for the rabbit, though without the definite production of true "dysenteric" lesions, and a relation with human disease may be demonstrable in the future; others appear quite non-pathogenic.

The great majority of the organisms which pass as "atypical" dysentery bacilli fall under three species or groups of species, which, so far as present evidence goes,

derive their importance from their liability to be confounded with genuine dysentery bacilli.

Since the characters of two at least of these three groups are fairly constant and well defined it is clearly desirable to give them specific names in place of referring to them as atypical or inagglutinable dysentery bacilli. The following names are therefore provisionally suggested, though they may have to give way should it be shown that prior descriptions exist.

1. *Bacillus ambiguus*. An organism resembling Shiga's bacillus, but forming indol and otherwise separable from Shiga by tests which will be given later. It has no demonstrable relation with human disease.

2. *Bacillus alkalescens*. An organism simulating Flexner's bacillus, but fermenting dulcitol and producing alkali with much vigour. It appears quite non-pathogenic.

3. *Bacillus dispar*. This term is suggested as a convenient one for the lactose-fermenting organisms of the dysentery group. It is probable that more than one species comes under this heading, as some ferment lactose early and some very late. The latter are specially liable to be confounded with Flexner's bacillus. Some members of this group are decidedly pathogenic for the rabbit.

Descriptions of these three forms will be given later in fuller detail.

Tests Employed.

It will be convenient first to mention the tests employed in the study of this series of organisms, with details as to the precise technique.

Preservation of cultures.—Dysentery bacilli soon die out on ordinary agar. They may be kept alive for months in sealed tubes of Dorset's egg medium. The simplest method of checking evaporation is saturation of the cotton-wool plug with paraffin wax.

1. Sugar reactions.

As a basis for these reactions broth and peptone-water prove quite satisfactory. An extensive trial has been made of an "ovomucin" basis prepared according to Winter's suggestion.¹ Bleached acid fuchsin has been used as the indicator. This medium is cheap and easy to make. The formula is as follows:—

Take 45 g. of dried commercial white of egg, make up to a litre with tap water and dissolve in the cold. Steam at 100° C. to precipitate the albumin and globulin and filter while hot. The filtrate should be almost clear. Add 0.5 per cent. sodium chloride, 0.5 per cent. of the desired sugar, and a sufficiency of the acid fuchsin indicator. Bring the whole to boiling point and run in N/10 HCl till the fluid is still just pink while hot but becomes colourless on cooling. Subsequent steaming, after tubing, diminishes the acidity somewhat.

Media thus prepared should be transparent and colourless, readily becoming crimson on development of acid. The ovomucin basis has proved quite as good as the ordinary peptone basis; indeed, it appears more delicate as certain very slight reactions may be appreciable in it which are usually missed with peptone—e.g., the power of some strains of Shiga's bacillus to give slight acidity with maltose and even lactose, as first stated by Winter.

The fermentable substances used in the present research have been six in number—viz., glucose, lactose, maltose, saccharose, mannite, and dulcitol. The essential ones in dysentery work are four only—viz., glucose, lactose, mannite, and dulcitol. Glucose refers the organism provisionally to the dysentery group, by giving acidity without gas; lactose is needful for excluding late lactose fermenters, for which purpose the tubes must be kept under observation for three weeks or more, though an earlier clue may be afforded by persistent and progressive acidification of litmus milk. Mannite is necessary to distinguish Shiga from the Flexner group, while dulcitol is useful, since a positive result suggests the *alkalescens* group; genuine dysentery bacilli do not appear to ferment dulcitol. The maltose and saccharose tests are of little real service; the subgrouping of the Flexner races by sugar reactions seems beset with fallacies.

2. Indol formation.

Of the various methods tried for this test that advocated by Glynn—viz., extraction with ether—seems much the best. A 48-hours culture in broth or peptone water is shaken with 1 c.cm. of ether. Some of the ether is then pipetted off on to a few drops of water in an ordinary agglutination tube, after which a few drops of Ehrlich's paradimethyl-reagent are run down the side of the tube and settle between the ether and the water. Here the rosy violet colour appears at once, or at most in a couple of minutes. No persulphate is needed. The virtue of this method of testing is that no subsidiary colour reactions occur, and there is never the smallest doubt as to the result of the test. Alternatively the paradimethyl-reagent may be run into the original broth tube after shaking with ether; this is quicker, but not quite so clean and decisive.

The indol test serves only one really useful purpose in dysentery work—namely, in distinguishing Shiga from *B. ambiguus*. Shiga never forms indol; *B. ambiguus* and *alkalescens* form it freely; different strains of Flexner-Y are variable in this respect, as are the lactose fermenters.

3. Alkali formation in relation to initial acidity.

All members of the dysentery group form alkali by the cleavage of protein, though this is masked in presence of a fermentable sugar. The true dysentery bacilli form it somewhat less vigorously than most other members of the group. *B. ambiguus* resembles the dysentery bacilli in this respect, but *B. alkalescens* and *B. dispar* form alkali with more rapidity.

The ordinary test revealing alkali formation is culture in litmus milk, which in the case of *B. alkalescens* becomes dark slaty-blue in the course of a week or two. In the case of *B. dispar* the reaction is masked by lactose fermentation. *B. ambiguus* and the true dysentery bacilli give slight initial acidity, followed by slight alkalinity later, but the changes in colour are sluggish and often difficult to follow; in the case of most of the dysentery strains examined the litmus milk at the end of two or three weeks was much the same colour as at the beginning.

An attempt was made to devise a medium which would offer a more sensitive registration of alkalinity.

This proved easy, for all that is needed is a sugar-free medium and a suitable indicator. The latter was found in phenol-sulphone-phthalein (phenol red), which gives a striking series of colour changes about the point of absolute neutrality. At 6.0 on the P_H scale it gives a slightly greenish-yellow, which passes to full yellow, orange, and apricot, the last-named indicating the point of absolute neutrality. The colour then becomes yellowish-pink, cherry-red, bluish-pink, and, finally, a full crimson at about 9.0 P_H. The precise degree of alkali formation within these limits can thus be read off easily. Both peptone and ovomucin have been tried as a basis, but the latter proved the better because it does not become so turbid as peptone water and the colour readings are clearer.

The medium is made thus: Prepare ovomucin as above described with the addition of 0.5 per cent. NaCl. To each 100 c.cm. add 4.6 c.cm. N/10 HCl, steam, and filter. Add 1 c.cm. of a 0.25 per cent. suspension of phenol red in water to each 100 c.cm. of medium. Tube and steam for 30 minutes at 100° C. on each of two successive days. The reaction should now be +2.5 on Eyre's scale and the colour yellow with a very faint greenish tinge. (The steamings reduce the acidity from +5 to +2.5.) It is easy to tell whether the reaction is too alkaline, by inoculating a tube with Shiga's bacillus as a test. This should show no growth in less than a week.

A hundred or more strains have been submitted to this test, which has proved useful in bringing out additional differences amongst the members of the dysentery groups, though not quite in the way that was expected.

The differences in the rate of alkali formation, when it has once begun, were not so great as was anticipated, but great variations were found in the "incubation period" which elapsed before there was any visible sign of growth or alkali formation, these two going hand in hand. The optimum reaction for growth is about +1.5 Eyre, and if the initial acidity is no greater than this all species begin to form alkali in one or two days and have reached a full carmine in a week or so. But if the initial acidity is put back to +2.5 Eyre the more delicate pathogenic organisms are placed at a disadvantage. The test thus becomes not merely one for alkali formation but largely one for ability to grow in ovomucin at this point of acidity.

It is probable that further experience may improve the method. Meanwhile it has afforded the following results, the figures given being the averages of many strains tested, because the variations of individual strains are sometimes considerable.

B. Shiga has never shown growth or alkalinescence before the seventh day, and the average time of commencing change is over ten days. Some strains never grow.

B. Flexner exceptionally begins to grow in two or three days, but the average is six to seven days. One or two strains have shown no growth in four weeks, and one only began on the forty-third day.

B. ambiguus begins to grow on an average in five days.

B. alkalescens gives vigorous alkalinity in two, or at most three, days.

B. dispar gives vigorous alkalinity in two days.

When once growth and alkalinity have commenced the usual time occupied in running through the gamut of tints offered by the indicator is from eight to ten days, but some strains of *B. alkalescens* and *dispar* can accomplish the task in five days.

The chief point of importance brought out by these observations is the greater vigour of growth, and consequently of alkali formation, shown by the two last-named species in comparison with the genuine dysentery bacilli. It must be admitted that the test is one more fitted for research work than for routine laboratory use, for the differences shown by the different species are best brought out when a large number of strains are simultaneously tested on the same batch of medium.

The Acid Agglutination Test.

The flocculation and precipitation of bacterial emulsions in presence of certain H-ion concentrations is a phenomenon which has long been known.

Different bacterial species are agglutinated at different H-ion concentrations, and Michaelis has shown how this fact may be used for differentiating *Para. A* and *B* from *B. typhosus*. In a recent paper² he claims that in presence of a trace of protein the dysentery bacilli may be readily distinguished from their allies. The claim has been tested upon a large number of strains of Shiga and Flexner-Y on the one hand, and *B. ambiguus*, *alkalescens*, *dispar*, and other miscellaneous organisms on the other, with the result that Michaelis's statements seem fully confirmed. The test is so simple and rapid, and furnishes such useful information, that it deserves to be widely known and used. The method is as follows:—

Six stock solutions are made up with normal soda and normal acetic acid in the following proportions—

	Normal NaOH.	Normal acetic acid.	Distilled water.
No. 1	5 c.cm.	7.5 c.cm.	87.5 c.cm.
No. 2	5 "	10.0 "	85.0 "
No. 3	5 "	15.0 "	80.0 "
No. 4	5 "	25.0 "	75.0 "
No. 5	5 "	45.0 "	50.0 "
No. 6	5 "	85.0 "	10.0 "

affording a constant strength of sodium acetate with an excess of acetic acid ranging from 1/40 N in No. 1 to 4/5 N in No. 6. For the test a 24-hour agar culture of the bacillus is emulsified in 20 c.cm. of distilled water; broth cultures cannot be used on account of the sodium chloride present. Three parts of emulsion are measured into each of six test-tubes or agglutination tubes, with the addition to each tube of

¹ Zeitschr. f. Hyg., lxx., 283.

² Deutsch. med. Wochenschr., 1917, xliii., 1506.

a little protein (for a small test-tube holding 1 or 2 c.cm. a drop of a tenfold dilution of normal human serum suffices). One part of each stock acid solution is now added to the six tubes respectively and well mixed. The tubes are incubated for two hours at 37° C. and the results read with the naked eye, preferably after standing at laboratory temperature for half an hour.

True dysentery bacilli show no macroscopic agglutination.

Nine strains of Shiga and 29 strains of Flexner-Y have been subjected to the test. No Shiga was agglutinated; in the case of one or two strains one could see a slightly granular appearance with a lens, though not with the unassisted eye. One Flexner-Y, when first tested, showed marked agglutination; it was plated out and found to be impure, so the test was repeated with cultures from two isolated colonies. These still showed feeble agglutination, just perceptible to the naked eye. Another strain showed weak agglutination when first tested, but 6 colonies tested after replating showed no macroscopic agglutination. With these exceptions all Flexner-Y's remained unagglutinated.

All other members of the dysentery group (*B. ambiguus*, *B. alkalescens*, *B. dispar*, and a few others unplaced) were without exception vigorously agglutinated at some point in the six tubes, usually in the last 5, sometimes in all 6.

Altogether 42 strains were tested—viz., 13 *B. ambiguus*, 11 *B. alkalescens*, 11 *B. dispar*, and 7 miscellaneous. One strain of *alkalescens* was at first negative, but on repeating the test after a few passages through broth it agglutinated well, while a second strain of the same organism, isolated from the same case a little later, agglutinated vigorously from the first.

On one occasion 10 proved strains of dysentery were mixed with 11 known not to be true dysentery, though they had in practice been confounded with them. They were numbered consecutively and handed to me by Captain Inman. I put them through the acid agglutination test in ignorance of their individual nature. The true dysenteries were picked out with no exception.

Judging from this experience it would seem that the test, though an empirical one, is likely to be of much service.

It enables the bacteriologist who is searching for dysentery bacilli forthwith to discard any culture which agglutinates within the H-ion concentrations of Michaelis's six solutions. Failure to agglutinate, however, does not alone prove an organism to be true dysentery; the ordinary cultural and serological tests should be applied in confirmation. A strain of *B. coli* from a "dysentery" case was found not only to resemble true dysentery in the absence of acid agglutination, but clumped to full titre with a Y-serum, and this after it had been in cultivation for a year. This case is of interest as suggesting an antagonism between acid agglutination and agglutination with anti-dysenteric sera. Major McNee isolated a number of strains of a form of *B. coli* from suspected dysentery cases, all of which agglutinated well with Flexner-Y serum. After being kept for a year they still clumped with this serum though to lower titre. He kindly sent me 12 cultures of the organism. All were tested against Flexner-Y sera by Captain Inman, and only one was now found to agglutinate to titre. This was the only one which proved negative to acid agglutination; all the rest clumped vigorously. It should be added that five strains of supposed dysentery, not included in the above figures, although they gave the cultural reactions of Flexner, proved totally inagglutinable with stock specific sera, but showed vigorous acid agglutination. These strains are still under investigation.

Agglutination with Specific Sera.

For reasons already given it has not been possible to make the number of monovalent sera required for an adequate serological study of the strains under investigation. All strains, however, have been tested against the various stock sera available.

The method employed has been the macroscopic one at a temperature of 55° C., readings being taken at the end of four to five hours. Sometimes young living broth cultures were used, but more often formalinised broth cultures which had been kept in the ice chamber.

Captain Inman, whose experience in dysentery agglutination is second to none, has been so good as to undertake this part of the work in the case of a large number of strains. He has used the strict Dreyer technique, but I myself have departed from it to the extent of employing a graduated pipette instead of the drop method in making the dilutions and a hot-air oven in place of a water-bath. In cases of difficult agglutinability the organism was passed through a series of daily broth cultures, starting from a single colony, and then formalinised and put on ice for a few days; one or two strains which at first agglutinated badly became readily agglutinable when thus treated.

Although the detailed serological study of the dysentery group has been unavoidably deferred, certain general conclusions appear to emerge from this preliminary work and may shortly be stated as follows:—

(1) Shiga's bacillus is readily agglutinated to end-titre by any of the stock Shiga sera. No instance of an inagglutinable Shiga has been met with in the course of this work.

(2) *B. ambiguus* is not agglutinated by Shiga sera, even in low dilution.

(3) Flexner-Y bacilli are usually agglutinated to end-titre, or its neighbourhood, by one or another of the stock sera on the market, but different strains may respond to different sera.

Certain sera—e.g., the Oxford-Y—are sharply specific as regards different races, and the same seems true of the R.A.M.C. sera prepared at Milbank. Others, and especially the polyvalent ones, are wider in

their embrace: such are the Y serum supplied by Burroughs and Wellcome, and above all the Lister Institute Y serum, which brings down Flexners better than Y's.

Failure to agglutinate with any one serum thus does not prove that a bacillus is not a Flexner-Y, but when a bacillus does not agglutinate, or agglutinates only to 1/4 titre, with a series of authentic sera, there is a *prima-facie* presumption against its dysenteric nature, though all available tests must be employed in arriving at a decision. Certain strains giving the cultural reactions of Flexner undoubtedly fail to agglutinate with any stock serum; two or three such have been met with in the course of this work, and are apparently separable from true dysenteries by the acid agglutination test also. They are at present under further investigation.

There seems no necessary relation between agglutination by specific Y or Flexner sera and the fermentation of maltose; the classification by sugar reactions is not parallel with that by racially specific sera. Finally, agglutination even to end-titre with a Flexner-Y serum does not necessarily prove an organism to have any relation with dysentery. Certain strains of *B. coli* may behave thus.

(4) *B. alkalescens* fails to agglutinate with Flexner-Y sera within the usual time limit of 4 to 5 hours.

If the tubes are left at 55° C. for 24 hours, some agglutination often occurs up to 1 in 500 or even 1 in 1000, but always short of end-titre. This agglutination seems of a different order from that given by the true dysentery bacilli. In a few absorption tests it was found that *B. alkalescens* failed to absorb agglutinins for Flexner-Y, though completely removing its own agglutinin fraction from Flexner-Y serum. This bacillus seems a poor agglutinator and has very feeble antigenic powers, contrasting in this with Flexner-Y.

(5) *B. dispar* is in much the same case.

Flexner-Y sera will not agglutinate it in 4 or 5 hours, and after 24 hours at 55° C. only in low dilutions. Like *B. alkalescens* it is a poor agglutinator. A serum was prepared which clumped the homologous strain quite well up to 1 in 12,000 or more, and in characteristic fashion, with an inhibition zone in the lower dilutions, but this agglutination was manifested only after 24 hours at 55° C., scarcely at all in 4 hours.

The serological relations of *B. alkalescens* and *B. dispar* with the Flexner-Y bacillus seem thus remote, though there is a suggestion of some small element in common.

Pathogenicity.

Inasmuch as the pathogenic powers of the true dysentery bacilli are well established, the limited number of rabbits available for experiment were all employed in testing other members of the dysentery group of bacilli. The following ten observations were made.

1. Pathogenicity of *B. ambiguus*.

A living suspension in saline of about one quarter of a 24-hours agar slope was injected intravenously into a rabbit of 1650 g. The animal became ill in an hour or two and was found dead next morning. No visible lesions could be found in the body; the intestines showed no sign of congestion. The heart's blood yielded *B. ambiguus* on culture.

Two further animals were injected intravenously a few days later with a similar living agar suspension from the same slope culture as the preceding. The doses were much smaller, but still large; one animal received approximately 800 million and the other 1200 million bacilli. Both animals remained quite unaffected.

While, therefore, this bacillus, injected intravenously in overwhelming dose can kill a rabbit, no suggestion of intestinal lesion was produced. The large living doses borne without ill-result in the remaining two cases make it clear that the pathogenicity of the organism is at best of a low order and very different from that of Shiga's bacillus.

2. Pathogenicity of *B. alkalescens*.

A formalinised broth culture, later proved not to have been entirely killed, was injected intravenously into a rabbit of 2030 g. in a dose of 1 c.cm. Neither this dose nor succeeding larger doses ever produced the smallest sign of illness; the animal gained weight.

A similar treatment of a rabbit with dead formalinised broth cultures of another strain, presumably a variety of *B. alkalescens*, but differing from the type in its sugar reactions, also produced no sign of illness.

A third strain of typical *B. alkalescens*, isolated a day or two previously from the bloody stool of a case of mucous colitis (probably not a genuine case of dysentery), was injected intravenously into another rabbit. The dose was 1 c.cm. of a living broth culture three days old. The animal was unaffected and gained weight.

These experiments, few as they are, suggest that *B. alkalescens* is devoid of pathogenic power and separate it sharply from the Flexner-Y group.

3. Pathogenicity of *B. dispar*.

Two strains were tested. The first was a very late lactose fermenter, but otherwise gave the cultural reactions of a Flexner, though agglutinating strongly with Michaelis's acid test. One c.cm. of a formalinised broth culture, injected intravenously into a rabbit, killed the animal in less than 24 hours. The small intestine was congested and the contents of the large intestine fluid. The heart's blood was sterile on culture.

A second rabbit was injected with the same formalinised broth culture in the attempt to procure an antiserum. Doses of 1/20 c.cm., gradually increasing to 1 c.cm., were given intravenously and produced no ill-effect. One c.cm. was then given subcutaneously, when the animal began to lose weight and finally showed paresis of the hind limbs, whereupon it was killed. The titre of the serum on the homologous bacillus was practically nil.

A further strain of *B. dispar* was tested on two other rabbits. The first, an animal of 2.60 kg., received an intravenous dose of 1 c.cm. of a formalinised broth culture which had not been completely killed. No further dose was given. The animal wasted from the first, and presently became paralysed in the hind limbs and then in the fore limbs. It died on the 13th day, the bacillus being recovered from the heart's blood. The colon was thin-walled and injected and presented doubtful appearances of ulceration. There were no hæmorrhages.

The second rabbit was treated with small weekly doses of a dead formalinised broth culture, ranging from 1/10-1/5 c.cm. intravenously, and finally with doses of 1 and 2 c.cm. subcutaneously. It did not waste much, and when killed, after six weeks, yielded a high titre serum.

These experiments show that some, at least, of the late lactose-fermenting members of the dysentery group possess considerable pathogenic power for the rabbit. The relation to human dysentery remains unproven for the present.

Description of the Three Species or Groups Allied to the Dysentery Bacilli.

B. ambiguus, *B. alkalescens*, and *B. dispar* have certain characters in common with other members of the dysentery group.

Their size and shape are the same. Measured in the wet living condition, in 24-hour broth cultures, the diameter is about 0.9 μ and the length 1 to 3 μ . In stained dried films the size is less, the diameter being about 0.6 μ . *B. alkalescens* is rather stouter than the other two, being 1 μ or a little more in diameter when living. They are all non-motile and presumably non-flagellate. There is nothing to suggest that they form spores. All are negative to Gram's stain and none of them liquefy gelatin. The special characters which differentiate them from other members of the dysentery group are these:—

The special characters of *B. ambiguus* are:—

Produces acid without gas in glucose media, usually in 24 hours, occasionally not till the second day (1 out of 13 strains). Some observers speak of traces of gas being formed; this has not been observed here. The disaccharides are never fermented, with the exception of an occasional late acid formation in saccharose (4-8 days) seen in 3 out of 13 strains. Mannite and dulcitol are not fermented. Indol is formed readily in peptone water or broth: two strains, otherwise typical, failed to form it out of 13 examined. In litmus milk there is slight initial acidity, the colour returning later to the starting point or becoming slightly alkaline. Alkali formation in peptone is not very early in onset. Lieutenant-Colonel C. J. Martin states that broth cultures have a stale fishy smell. The organism agglutinates strongly with Michaelis's acid agglutination test; no exception has been found to this. It is inagglutinable with Shiga sera or Y serum; no specific antiserum has been made in this laboratory. No record is to hand of any case in which the organism has been agglutinated by the blood of the patient from whom it had been isolated. It is sometimes noted as abundant in the stools of "blood and mucus" cases, more often in small numbers. Lieutenant-Colonel Martin notes its presence in stools which also contained Flexner-Y bacilli in abundance. The bacillus appears non-pathogenic to the rabbit unless injected in overwhelming dose.

The special characters of *B. alkalescens* are:—

Produces acid without gas in glucose and maltose and in mannite and dulcitol media. Lactose and saccharose are not fermented. The reactions are manifest at latest on the second day. A few strains have been met with, possibly referable to this species, in which the dulcitol reaction was absent and some in which saccharose was fermented at the end of seven days. Indol has been formed in all the strains examined. Litmus milk gives a characteristic reaction; after a week or ten days it becomes dark blue, and gradually passes into a slaty tint resembling that seen with *B. alkaligenes fecalis*. Alkali formation occurs early and runs its course quickly in an ovumucin or peptone medium in the absence of sugars. *B. alkalescens* clumps strongly with Michaelis's acid agglutination test. The organism is not agglutinated by specific Shiga or Flexner-Y sera within the ordinary time limit of 4-5 hours at 55° C. Only when the tubes are left at this temperature for 20-24 hours does agglutination occur with some strains, and then well below the titre of the serum. It seems at best a bad agglutinator and an organism of feeble antigenic power. No case has been met with in which it has been agglutinated by the serum of the patient from whom it has been isolated; in one instance in which it was obtained in almost pure culture from the stools this test was twice negative, even in a serum dilution of 1 in 10, although the patient had been ill for several months. It appears quite non-pathogenic for the rabbit.

The special characters of *B. dispar* are:—

This name is tentatively suggested for the lactose-fermenting members of the dysentery group without prejudice as to the number of types or species which may be thus included. Three strains fermented lactose in 24 hours, others only after 8-10 days, and one not until the 24th day. Of 11 strains examined all fermented glucose, maltose, and mannite readily, six fermented saccharose, and two fermented dulcitol, in no case with gas production. Indol was formed by four strains only. Litmus milk is acidified and ultimately clotured, but the clotting may not occur for a fortnight or more. In media containing no sugars alkali formation is as early and vigorous as in the case of *B. alkalescens*. Agglutination has always been vigorous with Michaelis's test. In its relations with specific Flexner-Y sera it also resembles *B. alkalescens*. There is no agglutination within the ordinary time limit, but in 20-24 hours at 55° C. some clumping may occur in lower dilutions. Even with a serum prepared against itself, the poor agglutinability of one strain was manifest in the same way, 24 hours at 55° C. being required. There is no record of the agglutination of these lactose-fermenting bacilli by the serum of the case from which they have been obtained. Some types at least are pathogenic

for the rabbit, already related. The study of the lactose-fermenting members of the dysentery group appears to be worth pursuing in fuller detail in relation to human disease.

It will be convenient to put the chief facts just stated in a tabular form, contrasted with the corresponding data for the true dysentery bacilli:—

	Sugar reactions				Inoculation	Alkali formation	Michaelis's acid agglutination	Agglutination with specific dysenteric sera	Pathogenicity for rabbit
	Lact.	Gluc.	Mann.	Dulc.					
<i>B. Shiga</i> ...	-	+	-	-	-	Slow.	-	To titre.*	Very high.
<i>B. ambiguus</i> ...	-	+	-	-	+	"	+	Negative.*	Prac. neg.
<i>B. Flexner-Y</i> ..	-	+	+	-	±	Mod.	-	To titre.†	Marked.
<i>B. alkalescens</i> ..	-	+	+	+	+	Vig.	+	Negative.†	Negative.
<i>B. dispar</i> ...	+	+	+	-	±	"	+	"†	Marked.

* With Shiga serum. † With one or another Flexner-Y serum. Mod., Moderate. Vig., Vigorous. Prac. neg., Practically negative.

This table shows the common reactions of the types concerned; the occasional exceptions which have been noted have been already mentioned.

Conclusions.

Although it is plain that this short study of the dysentery group is very incomplete, it has been thought advisable to bring forward the conclusions which seem to emerge at this stage, not only for the assistance but much more for the criticism of other workers at the subject. Should the conclusions be confirmed by others a distinct step in advance will have been made, since it appears that a large proportion of the forms which have masqueraded as atypical dysentery bacilli can readily be distinguished by appropriate tests.

The species described as *B. ambiguus* and *B. alkalescens* seem fairly well defined and can be rejected as having no connexion with dysentery. With regard to the lactose-fermenting forms which I have grouped under the name *B. dispar*—more for the sake of a convenient general term than because I believe them to be a single entity—much more work is necessary. There is a general belief that the lactose fermenters can be excluded in dysentery work, and this is very likely correct; at least, they cannot be returned as dysentery bacilli.

Even when all these simulators of *B. dysenteriae* are rejected there will doubtless remain a certain proportion of aberrant strains which cannot be thus accounted for, and some of these may be really atypical members of the Flexner-Y group. Judging, however, from this series of 100 strains, the majority of which were sent as presenting abnormal features, such a residue would be very small.

The acid agglutination test will, I think, be found of great practical value in routine laboratory work with dysentery bacilli. It seems hardly too much to say that it will do what no one specific serum can do, and that the margin of error is perhaps less than when even a number of specific sera are used.

One or two points remain for comment. Before returning a dysentery bacillus as inagglutinable it is always desirable to pick out a colony, pass it through a few daily subcultures in broth, and then formalinise the last broth culture and keep it for a few days in the cold. Some strains, which at first agglutinate poorly, come down freely after such treatment. Again, in returning a bacillus as agglutinable it is desirable to state whether it agglutinates to the full titre of the serum or only to half or a quarter titre. In order to do this it is necessary for the worker to have tested for himself the titres of the sera which he uses by the actual method which he is accustomed to employ. A given serum will differ widely in the results it yields with varying techniques and the titre stated on the bottle may give an erroneous impression. The method employed should be mentioned in the returns.

Finally, it is greatly to be desired, in the case of all doubtfully pathogenic forms isolated from cases of suspected dysentery, that careful observations should be made on the agglutinating power of the patient's serum upon the organism isolated, as this is an important link in the chain of evidence.