FURTHER RESULTS ON THE ISOLATION OF ORGANISMS FROM FAECES BY A NEW METHOD[1].

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In a previous communication (Wordley, 1921) I fully described a method, introduced by Dudgeon, for isolating organisms from faeces and sputum. The material is dried to a powder and this dried powder spread over convenient culture media. From my observations this method, for faeces, was found to be superior to those generally in use for isolating the dysentery and enterica group of organisms. Further, this method is equally suitable whatever infection may be present and the procedure is the same in every case. Hitherto, different methods have been advocated for different infections, so that in cases in which the infection is doubtful or unknown, several different methods of isolating the causal organism might be necessary, while if only one method was used because of an erroneous preliminary diagnosis, a negative result would be obtained. Further, it was found that this dry method was most satisfactory for isolating faecal streptococci, especially if blood agar was used, for, using this medium, streptococci were isolated in every instance from any specimen of faeces, often in great abundance, and in addition a separation is obtained between haemolytic and non-haemolytic varieties of streptococci. Briefly the procedure is as follows. A portion of faeces about the size of a hazel nut is evenly spread over a sterile porous tile and allowed to partially dry; the material is then transferred to another tile and dried completely, so that it can be scraped off as a dry powder. This dry powder is then spread over a number of plates containing suitable culture media. Liquid stools and those containing much mucus can be dealt with just as easily in this manner. Further, during the process of drying, small pieces of mucus are shewn up and can be picked off for microscopy and the stool concentrated for protozoa, whereas they might have been missed on inspection of the whole stool. This method of drying on tiles will be found of great value in obtaining excellent separation of colonies from sputum, the drying does not appear to injure the most delicate organisms.

In my paper already referred to, the number of *B. typhosus* infections examined was limited, and since it has been impossible to secure any large number of stools from typhoid patients, recourse has been had, in the experiments detailed below, to artificial mixtures of faeces with *B. typhosus*. This organism was employed, as it is comparatively difficult to isolate, whereas *B. paratyphosus* B. is very readily isolated under all conditions. As an alter-

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native method, in order to provide a comparison, the brilliant green enrichment method of Browning (1919) was employed. A further comparison with the brilliant green method was advisable, as in my former paper the brilliant green tubes were only incubated for four hours before plating, instead of for 24 hours as recommended by Browning.

In all 100 stools have been examined by these two methods, the procedure adopted being as follows. Faeces were collected in small sterile pots from patients with no symptoms of intestinal disease. About a small teaspoonful of the sample was mixed with a sufficient quantity of sterile saline to make a thick emulsion. To this was added three drops of an emulsion of typhoid bacilli, prepared by adding 2 c.c. of saline to a 24 hours' growth of the organism on an agar slope. The mixture of faeces and typhoid bacilli was well mixed and one large loopful added to each of two brilliant green tubes. These tubes contained 5 c.c. of peptone water to which was added 0·1 c.c. and 0·2 c.c. of a 1/10,000 solution of the dye (Grübler's manufacture). The remainder of the typhoid-faeces mixture was spread on tiles and dried, and the resulting powder spread over plates. The peptone water brilliant green tubes were incubated at 37° C. for 24 hours and then plated, the plates incubated another 24 hours and any suspicious colonies picked off; similarly the plates inoculated with the dried powder were incubated at 37° C. for 24 hours, after which suspicious colonies were picked off and further tested. The media used for plating were MacConkey's lactose neutral-red bile salt agar, and litmus lactose agar. The results obtained are summarised in the following Table:

Table I.

No. of specimens examined	Positive results by dry method	Positive results by brilliant green
100	27	5

Among the 27 positive results obtained by the dry method are included two in which positive results were also obtained by the brilliant green method, while the five positive results from brilliant green include two examinations in which positive results were also obtained by the dry method. The results obtained in 100 examinations are also shown in Table II where a comparison is made between MacConkey's medium and litmus lactose agar.

Table II.

	No.
Positive results on litmus lactose agar only ... ... Dry method	19
Positive results on MacConkey's medium only ... ... Dry method	3
Positive results on both litmus lactose agar and MacConkey's medium Dry method	3
Positive results by brilliant green method	3
Positive results by brilliant green method and dry method	2
Total positive results	30

The brilliant green tubes were plated on both MacConkey's medium and litmus lactose agar, but the number of positive results is too small to separate.

It will be seen from the two Tables that the method of drying on tiles and plating direct gives much superior results compared with the brilliant green method; a similar conclusion was reached in my earlier paper (1921). Further, litmus lactose agar gives much better results than MacConkey's medium by this dry method. The one disadvantage of litmus lactose agar is that if the plate is heavily inoculated with faeces so much acidity is produced that all colonies appear red from a diffusion of the dye and colonies of *B. typhosus* have to be recognised by their characteristic naked eye appearance apart from any special colour of the colony, though where one is fairly constantly dealing with "enterica" infections, this presents less difficulty.

From constant use of the above method, it has been found superior to any of the methods commonly in use for isolating organisms from faeces. It effects a great saving in culture media since it is a method that is equally applicable to any infection or for the isolation of any organisms that may be present in faeces. Also it is equally serviceable for sputum, giving very much better separation of colonies than other methods and thus effecting much saving of time in obtaining pure cultures of individual organisms such as may be necessary in preparing vaccines for infections of the respiratory tract.

REFERENCES.

BROWNING, C. H. (1919). *Applied Bacteriology.* Oxford Medical Publ. p. 95.

WORDLEY, E. (1921). A new method for the isolation of organisms from faeces and sputum, with some observations on Haemolytic streptococci in faeces obtained by this method. *Journ. Hygiene*, XX. p. 60.