A NEW METHOD FOR THE ISOLATION OF ORGANISMS FROM FAECES AND SPUTUM, WITH SOME OBSERVATIONS ON HAEMOLYTIC STREPTOCOCCI IN FAECES OBTAINED BY THIS METHOD.

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Professor L. S. Dudgeon, when consulting bacteriologist to the Forces in the Balkans during the late war, had tried the effect of drying the stools of suspected dysentery patients on some porous material so as to leave only the mucus. In bacillary cases he found that a more abundant growth of dysentery bacilli was obtained from the dried mucus than from the untreated specimen, so that the drying had no harmful effect on the organisms. He used for this purpose unglazed red tiles such as were to be found locally, and suggested that the method should be given a more thorough trial with a more satisfactory type of tile made in this country. With this view the following experiments were made.

Dudgeon's Method.

A portion of the faeces, about a small teaspoonful, is evenly spread over a porous tile resting on an asbestos mat and allowed to dry for from 30 minutes to two hours, at room temperature, according to the amount of water or mucus in the specimen. When the faeces are dried to a stiff paste, this is scraped off and evenly spread on a second tile and allowed to dry for a further one to two hours or until it has become a fine dry powder when scraped off. A quantity of this dry powder is transferred to a plate, and evenly spread over the surface with a glass spreader. If the faeces are liquid, as much as possible should be poured on the first tile short of letting it run over the edge. By using two tiles one after the other, it will be found that however liquid the faeces are, or however much mucus is present, complete drying can be obtained. It is important, in order to get the best results, that the material should be completely dried on the second tile before plating, and when scraped off, be in the form of a sand-like powder, with no trace of stickiness.

By this method excellent separation of individual colonies is obtained over the whole surface of the plate with no tendency to run together so that suspicious colonies can be picked off with ease. It is quick, and since it is equally

[1] The expenses of this investigation were defrayed by a special grant from Mr Louis Oppenheimer, to whom I am greatly indebted.

applicable to any infection, is most economical with media since different methods do not have to be adopted according to the nature of the organism suspected of being present. Using other methods than the tile method on an erroneous supposition of the infecting organism, the latter may be easily missed, as will be shown later.

By this method of drying on tiles, it is extremely easy to isolate small portions of mucus, which would be missed if the solid stool were examined, and thus is a most suitable procedure in enabling one to examine likely portions for *Entamoeba histolytica* cysts. Naturally in this instance complete drying is not resorted to, but just sufficient to produce a stiff paste in which pieces of mucus are easily seen. The same procedure can be followed also in searching for the ova of parasitic worms.

The method is also very suitable in plating from sputum. Excellent separation of individual colonies is obtained and a delicate organism such as the pneumococcus can be recovered in abundance when present. The same procedure is adopted as with faeces. A thick layer of sputum is spread on a tile and allowed to dry to complete dryness. During the process of drying both with faeces and sputum, the tiles should be covered over with a bell jar to protect them from contamination from dust, etc. The dried sputum is scraped off and spread over some suitable plate. The dried sputum when scraped off will be found to be a light, flaky, very fine powder, in contrast to dried faeces from which the powder is like sand and much heavier. For this reason, because of the extreme lightness of dried sputum, it is advisable when scraping it off the tile, to work behind a screen. A suitable screen can be made from an old half plate size photographic plate fixed on a piece of wood as a base, a deep groove being cut in the wood block at an angle in which the plate is fixed and thus inclined slightly inwards.

The tiles used are white unglazed porcelain tiles about 6 inches square. For use each large tile is divided into four pieces which gives a more convenient size to spread the material on. When required for use they are sterilized by heat, an electric hot plate is very convenient, but this can be done equally well by placing them on an asbestos cooking mat supported on an iron tripod and heating them with a Bunsen burner. Such asbestos cooking mats can be obtained from any ironmonger or stores. When cooled the tiles are ready for immediate use. For spreading the faeces and for scraping off the dried residue an old knife, especially kept for the purpose, is very convenient, since it can be easily sterilized by heating to redness.

The spreading of the material used, and the scraping off should be done with the tiles on an asbestos mat, as it is difficult to be certain that no powder has fallen off the tile, while if an asbestos mat is used, the whole mat can be sterilized in the flame immediately after use. When the tiles have been used, it is convenient to drop them into a bucket of some suitable disinfectant. At intervals they are collected, and before being put into use again, burnt dry by heating them over a small crucible furnace. It will be found that with tiles

that have been used several times, drying of the material used is somewhat slower.

The method of drying on tiles was used in the following series of examinations of stools of patients known to be suffering from an infection of the Typhoid group. The large number of negative results is due to the fact that before discharge three consecutive negative examinations were made. In addition to the dry method a loopful of faeces was added to a series of 5 c.c. peptone water tubes to which 0·04, 0·08, 0·12, 0·22 of a c.c. of a 1/10,000 solution of brilliant green had been added. One loopful of the top layer of fluid from each of these tubes was plated after they had been incubated for four hours at 37° C. One plate was made from the dried faeces. This procedure was followed for every stool examined. MacConkey's neutral red bile salt lactose agar was used for plating on. It will be seen that incubation of the cultures in brilliant green peptone water was for 44 hours, and not 24 hours as recommended by Browning (1919), or 7 to 12 hours as practised by Mackie (1919).

The following results were obtained:

Total number of examinations	64
Negative results by both methods	42
Total positive results by dry method ...	19
Positive from peptone water brilliant green; negative by the dry method	2

Amongst the 19 positive results by the dry method are included ten examinations in which positive results were also obtained from brilliant green peptone water, leaving nine examinations in which positive results were obtained by the dry method only. These 19 positive results by the dry method are additionally favourable to this method, as even when positive results were obtained by the alternative method, the former means a considerable saving of media. This is not so important in the few cases examined in this instance, but would be so when dealing with large numbers of cases.

These 64 examinations were made from 18 cases of "Enterica," 15 cases of infection by *Bacillus typhosus* and two by *B. paratyphosus* B. By the term positive result is meant that one of these organisms was isolated from the faeces, the cultural reactions of the organism being confirmed by agglutination of a 24 hours culture on agar.

All cases were examined once a week so that in some cases the infecting organism was isolated on more than one occasion from the faeces, while there were four cases in which no bacilli of the "Enterica" group were isolated on any occasion by either method. Three of these latter cases were infections by *B. typhosus*. All contracted Typhoid while under observation, from the fourth case to be mentioned later. *B. typhosus* was recovered by blood culture in all three. They all had a very mild attack of typhoid fever, the duration of the fever was so short and they were so mildly ill, that but for the fact that the diagnosis was made bacteriologically, it is improbable that Typhoid would

have been suspected, clinically they were so atypical, though this difficulty is denied by Garrow (1920). One of the three only had fever for four days with sudden onset of backache and pains in the limbs, and resembled influenza more than anything. The last case in which no typhoid bacilli were found in the faeces, was a case of cystitis from which *B. typhosus* was obtained in pure culture from the urine.

Amongst the already mentioned cases was one of some interest which illustrates very well the value of the dry method. The patient was suffering from typhoid fever from whom on the first occasion on which the stools were examined, typhoid bacilli were isolated by the dry method and also from peptone water brilliant green. At the second examination *B. dysenterae* Shiga, confirmed culturally and serologically, was isolated by the dry method only and no typhoid bacilli by either method. It appeared that the patient had been a soldier and had contracted dysentery in the East during the war. He must have been still harbouring dysentery bacilli which the attack of typhoid fever had aggravated. There was no reason to suspect dysentery in this case, as he was a known case of typhoid, so that except by the dry method, it is doubtful if they would have been isolated.

In addition to the above cases, one case of Flexner dysentery was examined. In this case, in addition to the dry method, a loopful of faeces was inoculated into a broth tube, incubated for one hour at 37° C. and a plate spread with one loopful from this tube. The following results were obtained from this patient.

Total examinations made	5
Negative results by both methods ...	3
Positive results by dry method only	1
Positive result from broth only ...	1

Since cases of dysentery are comparatively rare in England, the following experiments were made, employing normal faeces to which a small amount of a pure culture of *B. Flexner* (Gallipoli) Dudgeon (1919) was added. The normal faeces were made into a thick emulsion with saline. Two c.c. of this emulsion were employed to which was added one drop of an emulsion of the Flexner bacillus prepared by adding 2 c.c. of saline to a 24 hours' growth on an agar slope.

The resulting mixture of normal faeces and dysentery bacilli was plated by the dry method and also from a broth tube inoculated with a loopful of the mixture and incubated for one hour at 37° C.

The following results were obtained:

Total examinations	23
Negative by both methods	8
Total positives by dry method	13
Positive from broth negative by dry method	2

Of these 13 positives by the dry method, positive results were also obtained from broth in four instances, leaving nine examinations in which the dysentery bacillus was isolated in these artificial mixtures by the dry method only.

The total figures for all examinations both from patients with a bowel infection from the enteric or dysentery bacilli, and the artificial mixtures, is as follows:

Total number of examinations	91
Negative results by both dry method and alternative method used	53
Total positive results by dry method ...	33
Total positive results by alternative method, *i.e.* either peptone water brilliant green for the enteric group, or from broth for dysentery infections with negative results from dry method	5

Of these 33 positive results by the dry method, positive results were also obtained in 14 instances by one of the alternative methods, according to the nature of the infection, 19 examinations in which the dry method was successful and the alternative method unsuccessful.

Observations on Streptococci in faeces especially with regard to Haemolytic varieties.

Attention was drawn to the ease of isolating streptococci from the faeces by the dry method from the fact that, in the series of examinations of typhoid patients already mentioned, streptococci were isolated on MacConkey plates in great abundance on 15 occasions. In nine cases the patient was a known case of typhoid, in two dysentery, one was a case of coeliac disease, one of sprue, and one a case of diarrhoea of one year's duration in a child of four.

For this reason further examinations were made on a medium more suitable for the isolation of streptococci. Since blood agar would also show the presence of haemolytic colonies, this medium was used throughout. In this manner streptococci can be grown in abundance from any sample of faeces whether from normal healthy subjects or from patients with intestinal disease. In the course of examining the stools of 60 patients, 39 of which were normal healthy people, using the dry method and plating on blood agar, numerous colonies of streptococci were found in 58. In this series haemolytic colonies of streptococci were frequently observed, in view of this fact, the streptococci isolated by this method were investigated further.

Haemolytic streptococci in faeces do not appear to have been very frequently observed. Davis (1920) investigated the fate of haemolytic streptococci in the intestinal canal. Plating from broth on blood agar, he examined the stools of normal rabbits and found no haemolytic streptococci. He then fed the rabbits with haemolytic streptococci and found that he could recover the same type of organism from the faeces in the day immediately after the first day of feeding, but that they quickly disappeared and were not recovered again, however long the feeding experiments were continued. He next examined human faeces from 53 cases of all varieties, but found no haemolytic streptococci, although Pilot and Davis (1919) had shown that these organisms were present in 100 per cent. of cases in the crypts of the tonsils. He also examined the faeces of four scarlet fever cases but found no haemolytic streptococci.

Oppenheim (1920) examined 55 stools from 15 patients from which 323 strains of streptococci were isolated. He inoculated blood agar plates directly from the faeces. All streptococci isolated were tested as regards their fermentation properties, with the following results:

Percentage fermenting		glucose	...	100
,,	,,	lactose	...	97
,,	,,	salicin	...	97
,,	,,	inulin	...	1
,,	,,	mannite	...	76

Amongst these 323 strains he found five that had haemolytic properties.

Moody and Irons (1920) examined the stools of patients suffering from scarlet fever. They also examined the throats of all their cases and found haemolytic streptococci in every case. They examined 309 stools from 85 patients. They employed saline emulsions of the faeces plating this on blood agar, *i.e.* agar + 10 per cent. of goat's blood. They found streptococci present in the faeces of 26 patients, and from these 26 cases isolated 22 strains of haemolytic streptococci. All these haemolytic organisms fermented lactose and salicin, but not mannite or inulin.

Dible (1921) studied 152 strains of streptococci all isolated from faeces. For isolation he employed either direct plating on agar plates, direct plating on MacConkey's bile salt lactose agar, or by methods advocated by Thiercelin (1902) for the isolation of streptococci from faeces, chiefly anaerobic cultivation in broth followed by plating on agar. He studied the fermentation properties of all strains using Hiss' serum water medium plus the addition of various "sugars." All strains were tested for haemolysis. For this purpose he used an equal volume of the culture of the organism to be tested and of 10 per cent. washed rabbit's corpuscles. The culture medium used was serum one part, broth four parts. The mixture of serum broth and rabbit's corpuscles was incubated for $2\frac{1}{2}$ hours at 37° C., placed in the cool overnight and read next morning. He found that the streptococci dealt with were of two types culturally, one forming minute pin-point colonies on agar, the other much coarser colonies with a tendency to coalesce. Further the coarse colonies were usually diplococci on examination, whereas the fine colonies showed chain formation. In differentiating streptococci from enterococci he tested the statement of Houston and M'Cloy (1916) that their enterococcus would withstand exposure to heat and tested all strains for their ability to withstand exposure to 60° C. for as long as 30 minutes. He finds an intimate relation between, on the one hand, heat resistance, diplococcal form, ability to ferment mannite and coarse colony formation, and on the other, sensitiveness to heat, failure to ferment mannite, a tendency to form chains with fine colony formation. He considers the typical enterococcus to be diplococcal in form with the above characteristics and the following reactions on sugars.

	Litmus milk	Lactose	Inulin	Salicin	Mannite	Dulcite
Type ...	+	+	−	+	+	−
Variation I	+	−	−	+	+	−
,, II	+	+	−	+	−	−
,, III	+	−	−	+	−	−

In the series of organisms examined, he only found two haemolytic organisms, both forming long chains and both non-mannite fermenters, and non-resistant to heat. In the total series 85 strains were heat resistant, 52 heat sensitive.

Percentage fermenting	lactose	... 78
,, ,,	salicin	... 93
,, ,,	inulin	... 17
,, ,,	mannite	... 53
,, clotting	litmus milk	... 72

Method. In the following series of examinations faeces were dried by Dudgeon's tile method in every instance. They were plated on blood agar made by adding 1 c.c. of human oxalated blood to 15 c.c. of melted agar at a temperature of about 50° C.

Haemolysis was noted on the plate and all strains were further examined for haemolysis by inoculating a 5 c.c. peptone water tube to which 0·1 c.c. of centrifugalized human red cells were added and well shaken to ensure a uniform suspension. These tubes were incubated at 37° C. overnight and read next morning. The cultural characteristics of all streptococci were examined with results noted in the table. The media employed were Lemco broth containing 1 per cent. of the various "sugars" used. All tubes were incubated for five days at 37° C.

Only one colony was examined from each plate, unless haemolytic colonies were present, when one of each was examined, namely, one haemolytic and one non-haemolytic. If haemolytic colonies were present they were never present in abundance, two or three per plate being the maximum, more often only one. The haemolysis on a blood agar plate was of two varieties, (i) a colony with a small diffuse somewhat opaque zone of haemolysis with a greenish colouration, and (ii) a sharply defined clear transparent completely haemolysed colourless zone 2–4 millimetres in diameter as defined by Brown (1919). The first variety never produced haemolysis in peptone water red cells, the second always did.

In the subjoined table the signs used have the following significance:

Against the sugar tubes	+	means	production of acid.
,, ,, ,,	−	,,	no change.
,, Litmus milk	+1	,,	acid only.
,, ,, ,,	+2	,,	acid plus clotting.
,, Resistance to heat	+	,,	resistance to exposure of 30 minutes at 60° C.
,, ,, ,,	−	,,	killed by exposure to 60° C. for 30 minutes.

Dible's observations on heat resistance were not seen until the majority of these results were completed, so that only a few organisms have been tested for this property. The method employed was to place a 24 hours' broth culture of the organism to be tested in the water bath for 30 minutes at 60° C. The tube was then stood at room temperature for one hour when three drops were inoculated on an agar slope and incubated for 24 hours at 37° C.

From a consideration of the table it will be seen that the diplococcal type is in the majority, few long chained streptococci being met with. In all 52

No.	Nature of case	Haemolysis on blood agar plate	Haemolysis in red cells peptone water	Morphology	Lactose	Dextrose	Mannite	Dulcite	Inulin	Salicin	Starch	Litmus milk	Character of colony on agar	Resistance to heat
1	Rheumatoid arthritis	+	+	Diplococci and short chains	−	+	−	−	−	+	−	+2	Fine	...
2	Myelaemia	+	+	,,	+	+	−	−	−	+	−	+1	,,	...
3	Diarrhoea in infant	+	+	,,	+	+	−	−	−	+	+	+2	,,	...
4	,,	−	−	,,	+	+	−	−	−	+	+	+2	,,	...
5	Typhoid	−	−	,,	+	+	−	−	+	+	+	+2	,,	...
6	,,	+	−	,,	+	+	−	−	+	+	+	+2	Coarse	...
7	,,	−	−	Diplococci chains of 8	+	+	−	−	−	+	+	+2	Fine	...
8	Melaena	+	+	,,	+	+	+	+	+	+	+	+1	,,	...
9	,,	−	−	Diplococcus	+	+	+	−	−	+	+	+2	Coarse	...
10	Colitis	+	+	,,	+	+	+	−	−	+	−	+2	,,	...
11	,,	−	−	,,	+	+	+	−	−	+	−	+2	,,	...
12	Amoebic dysentery	−	−	,,	+	+	−	−	−	+	−	+1	Fine	...
13	,,	+	−	Diplococci long chains	+	+	−	−	−	+	−	+2	,,	...
14	Melaena	−	−	Diplococci chains of 8	+	+	−	−	−	−	−	+1	,,	...
15	Sprue	−	−	Diplococcus	+	+	+	+	+	+	+	+2	,,	...
16	,,	−	−	,,	−	+	−	−	−	+	−	+2	,,	...
17	,,	+	+	Diplococci chains of 8	+	+	−	−	−	+	−	+2	,,	...
18	Normal	−	−	Diplococcus	+	+	−	−	−	+	−	+2	,,	...
19	,,	−	−	,,	−	+	−	−	−	+	−	+2	,,	...
20	,,	−	−	Diplococci	−	+	−	−	−	−	−	+2	,,	...
21	,,	−	−	,,	+	+	−	−	−	−	−	+2	,,	...
22	,,	−	−	,,	−	+	−	−	−	+	−	+2	Coarse	...
23	,,	−	−	Diplococci chains of 4	−	+	−	−	−	+	−	+2	,,	...
24	,,	−	−	Diplococci	+	+	+	−	−	+	−	+2	,,	...
25	,,	−	−	,,	+	+	+	−	−	+	−	+2	,,	...
26	,,	−	−	,,	+	+	+	−	−	+	−	+2	,,	...
27	,,	−	−	,,	+	+	+	−	−	+	−	+2	,,	...
28	,,	−	−	,,	+	+	+	+	+	+	+	+2	Fine	−
29	,,	−	−	,,	+	+	+	−	−	+	−	+1	,,	...
30	,,	−	−	,,	+	+	+	−	−	+	−	+2	,,	+
31	,,	−	−	,,	+	+	+	−	−	+	−	+2	,,	+
32	,,	+	+	,,	+	+	+	−	−	+	−	+2	Coarse	+
33	,,	−	−	Diplococci chains of 6–8	+	+	−	−	−	+	−	+2	Fine	+
34	,,	−	−	Diplococci	+	+	−	−	−	+	−	+2	,,	...
35	,,	−	−	,,	+	+	+	−	−	+	−	+2	,,	+
36	,,	+	+	Diplococci chains of 10	+	+	−	−	−	+	−	+2	,,	+
37	,,	+	+	Diplococci	+	+	+	−	−	+	−	+2	,,	+
38	,,	−	−	,,	+	+	+	−	−	+	−	+2	,,	+
39	,,	−	−	,,	+	+	+	−	−	+	−	+2	,,	...
40	,,	+	+	Diplococci chains of 20	−	+	−	−	−	−	−	−	,,	...
41	,,	−	−	Diplococci	+	+	+	−	−	+	−	+2	,,	...
42	,,	−	−	,,	+	+	−	−	−	+	−	+2	,,	...
43	,,	−	−	Diplococci chains of 6	+	+	+	+	−	+	−	+2	Coarse	...
44	,,	−	−	Diplococci	+	+	+	−	−	+	−	+2	Fine	+
45	,,	−	−	,,	+	+	−	−	−	+	−	+2	,,	−
46	,,	−	−	,,	+	+	+	−	−	+	−	+2		+
47	,,	−	−	,,	+	+	+	−	−	+	−	+2	,,	+
48	,,	−	−	,,	+	+	+	−	−	+	−	+2	,,	+
49	Pernicious anaemia	−	−	,,	+	+	−	−	−	+	−	+2	,,	+
50	Normal	+	+	,,	+	+	−	−	−	+	−	+2	,,	+
51	Pernicious anaemia	−	−	,,	+	+	−	−	−	+	−	+2	,,	−
52	Normal	−	−	Diplococci chains of 4–6	+	+	−	−	−	+	−	+1	,,	−

https://doi.org/10.1017/S002217240005991X Published online by Cambridge University Press

strains were examined, 13 being haemolytic colonies on blood agar, 11 producing haemolysis of red cells in peptone water. The same two types of colonies were observed as mentioned by Dible, many of the diplococci morphologically could not be distinguished from pneumococci.

In the eleven strains that produced haemolysis in peptone water plus red cells, four were of the long chained variety, seven were diplococci. Four fermented mannite, seven did not.

Briefly the cultural characteristics of all strains examined were as follows:

(1) A group fermenting dextrose, lactose, mannite, salicin, and not dulcite, inulin or starch. 19 in number.

(2) A group fermenting dextrose, lactose, salicin, and not mannite, dulcite, inulin, starch. 13 in number.

Lactose was fermented by	45	strains or	86·5	per cent of all			
Dextrose	,,	,,	52	,,	100	,,	,,
Mannite	,,	,,	24	,,	46·1	,,	,,
Dulcite	,,	,,	4	,,	7·7	,,	,,
Inulin	,,	,,	5	,,	9·6	,,	,,
Salicin	,,	,,	48	,,	92·3	,,	,,
Starch	,,	,,	9	,,	17·3	,,	,,

All with one exception, No. 40, acidified litmus milk, acidification and clot being produced by 45.

The term normal case in the table includes a variety of patients, the majority being cases of hernia in young adults, or patients admitted for the surgical treatment of deflected nasal septum, and a few cases for removal of tonsils, in which at the time of examination there was no obvious sign of tonsillitis.

REFERENCES.

Brown, J. H. (1919). The use of Blood Agar for the Study of Streptococci. *Monographs of Rockefeller Inst.* No. 9, p. 6.

Browning, C. H. (1919). *Applied Bacteriology.* Oxford Med. Pub.

Davis, D. J. (1920). Fate of *Streptococcus haemolyticus* in the Gastro-Intestinal Canal. *Journ. Infect. Dis.* xxvi. 171.

Dible, J. H. (1921). The *Enterococcus* and the Faecal *Streptococci*, their properties and relations. *Journ. Path. and Bacteriol.* xxiv. No. 1.

Dudgeon, L. S. (1919). Studies of Bacillary Dysentery occurring in the British Forces in Macedonia. *Med. Research Council, Special Report Series,* No. 40, p. 19.

Garrow, R. P. (1920). The Myth of Atypical Enteric Fever. *Lancet,* ii. 886.

Houston, T. and M'Cloy (1916). The Relation of the *Enterococcus* to Trench Fever and allied conditions. *Lancet,* ii. 632.

Mackie, T. J. (1919). The brilliant green enrichment method for the isolation of the paratyphoid bacilli. *Journ. Roy. Army Med. Corps,* xxix. 458.

Moody and Irons (1920). On the occurrence of Haemolytic Streptococci in the stools of Scarlet Fever. *Journ. Infect. Dis.* xxvii. 363.

Oppenheim, C. J. (1920). The Human Faecal Streptococci. *Ibid.* xxvi. 117.

Pilot and Davis (1919). Haemolytic Streptococci in the Faucial Tonsil, and their significance as Secondary Invaders. *Ibid.* xxiv. 386.

Thiercelin (1902). Procédés faciles pour isoler l'entérocoque des selles normalles; filtration des selles, culture préalable en anaérobie. *Compt. Rend. Soc. Biol.* S. 11, p. 1082.