THE BACTERIOLOGICAL EXAMINATIONS OF THE FAECES IN FOUR CASES OF TYPHOID FEVER, MADE AT FREQUENT INTERVALS FOR A PERIOD OF ONE MONTH.

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Reasons for Investigation.

The investigations described in this paper were designed in order to observe whether changes in the diet of patients suffering from typhoid fever were accompanied by any changes in the faecal flora, and also to observe whether pyrexia, diarrhoea or constipation occurring during the course of the disease altered the numbers of typhoid bacilli in the faeces.

The investigations were confined to four cases of the disease, which were under the care of Dr Foord Caiger at the South Western Fever Hospital. This fact precluded any chance of there being any articles of diet administered other than those prescribed.

The observations were controlled by examinations of the urine and the specimens of faeces were very carefully taken so as to avoid urinary contamination.

Classification of the intestinal flora.

The intestinal flora has been classified in many ways by various investigators. Cushing and Livingood (1900) divided it into two groups, (1) permanent or obligatory, and (2) transient or facultative. The first group being always present, the second unable to flourish in the presence of the first, unless it encounters or produces a pathological lesion of the mucosa. The conditions set up by infection with the *B. typhosus* are, these workers maintain, of such a nature as to encourage additional members of this second group to flourish.

Torrey (1919) divided intestinal flora into two groups, (1) fermentative, and (2) putrefactive; with *B. coli* mid-way between and having the power of passing from one to the other main group. He classed *B. Welchii*, *B. acidophilus*, *Streptococcus* (including *Enterococcus*) and some others in the first group, while he placed *B. sporogenes*, *B. proteus*, *B. pyocyaneus* and *Staphylococci* in the second.

Morris, Porter and Meyer (1919), in an elaborate and comprehensive investigation, divided the intestinal flora into three groups, (1) fermentative or

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saccharolytic, (2) putrefactive or proteolytic, and (3) facultative or normal. In the main these workers supported the views of Torrey (1919), but they sharply differentiated the faecal flora of young children from that of adults, the former, according to them, being chiefly of group 1, the latter tending, as age advances, to become more and more of the type of group 2. They maintained that this fact bears greatly on the course and prognosis in cases of typhoid fever.

THE BACILLUS TYPHOSUS.

Typhoid bacilli make their appearance in the intestinal canal as a result of the ingestion of infected food or water. Penetrating the mucosa they invade the blood stream and an extensive infection of the intestinal lymphoid tissue takes place.

OTHER ORGANISMS.

The commonest inhabitant of the intestine, both normally and in cases of typhoid fever, is the *B. coli*.

Lembke is quoted by Dudgeon (1924) as having found that the *B. coli* alone remained constant and independent of diet in 89 cases examined. This, however, was before various strains of *B. coli* were recognised and before it was realised that this organism is capable of assuming either a fermentative or proteolytic character, lying, according to Torrey (1919), mid-way between these two great groups of intestinal bacteria.

Dudgeon, Wordley and Bawtree (1921 and 1922) have further classified the *B. coli* into two other groups, namely haemolytic and non-haemolytic. The non-haemolytic colon bacilli occur in the intestinal tracts of 85 to 90 per cent. of normal persons, whereas in such conditions as colitis and diarrhoea the haemolytic variety may be found in as many as 35 per cent. of the specimens of faeces examined.

Streptococci are very common normal inhabitants of the intestinal tract, and in this series of four cases of typhoid fever an abundance of these organisms was found, which however diminished rapidly as the amount of milk in the diet was decreased. The cause of this is, in all probability, as is pointed out by both Torrey (1919) and by Dudgeon (1924), directly due to the rich supply of streptococci in the milk.

Kufferath (1921) in an investigation extending over four years found streptococci in 21 per cent. of the specimens of dairy milk submitted to him. Frost and Bachmann (1922) found haemolytic streptococci in 28 per cent. of specimens of high grade milk obtained from 412 cows.

Many other organisms may be isolated from the stools of typhoid patients, the commonest being *Staphylococcus albus* and *aureus*. According to Torrey (1919), these belong to the proteolytic type and would be expected more in putrefactive than in fermentative stools.

The significance of secondary organisms.

It is necessary to determine as far as possible what conditions cause changes in the flora of the intestine, and what effect such changes have on the patient, especially in cases of typhoid fever.

A quotation from Herter (1907) in this connection may not be out of place. "Our attention has, perhaps, been too exclusively fixed on the specific excitants and the rôle played by associated bacteria must receive more study, for it is clear that they sometimes play a significant part in determining the outcome of an infection. The difference that decides whether a man shall live or die must frequently be a slight one looked at from the standpoint of the processes of battle within the body."

It is well known that there are many septic conditions which occur as complications of typhoid fever from which it is possible to cultivate the *B. typhosus*. A great majority of the abcesses and boils, however, are caused by staphylococci. It is interesting to note that in two of the cases which I investigated boils developed during the attack of typhoid fever. The stools from case number 2 had previously contained a large number of *Staphylococcus aureus* with an entire absence of *S. albus*; the pus from the boils in this case showed a pure culture of *S. aureus*. The faeces from case number 4 showed an abundant growth of *S. albus*, and the boils in this case contained this organism also in pure culture.

Prof. L. S. Dudgeon has shown me records of some of his cases of typhoid fever in which suppurative conditions occurred in various parts of the body due to *S. aureus*, which organism was in each case isolated in abundance from the faeces. One case in point was diagnosed from blood-culture as typhoid fever, but no typhoid bacilli, haemolytic *B. coli* or *S. aureus* were found in the stools; fifteen days later a large abscess developed in connection with an acute osteo-myelitis of the right femur; from this abscess *S. aureus* was grown in pure culture while the stools contained this organism in abundance.

The relation of diet to flora.

The types of intestinal flora are chiefly controlled by the chemical character of the food. Carbohydrates favour on the whole fermentative organisms, protein, on the other hand, favours putrefactive ones.

The most powerful of the carbohydrates in suppressing the proteolytic bacteria are lactose and dextrin. A diet of bread and milk, which contains these two carbohydrates, sets up a flora of the fermentative type in a very short space of time, provided always, as is shown by Torrey, that the calorie content is high enough. Torrey (1915) with great care and detail examined 100 stools from 22 typhoid patients. He shows that with a diet rich in carbohydrates (carbohydrate 250–300 gms., protein 50–100 gms., fat 75–100 gms. per diem) a fermentative state can be induced in the canal, which, by the acid reaction that it induces, is inhibitory to the growth of the proteolytic

bacteria and thus aids in preventing absorption of toxic bodies to which these organisms give rise. He shows that the ordinary milk diet, with its low calorie content, is not able to support a fermentative state, let alone induce one, in a subject having a tendency to a proteolytic flora. He points out that although the state of the flora does not influence the susceptibility to attack, yet, in those patients who, on admission to hospital, had a flora of the fermentative type, the disease tended to run a milder course. He considers this to be the reason why young children often have milder attacks of typhoid fever than adults. It would seem from this investigation by Torrey that diet has a very marked influence on the intestinal flora, and bearing in mind the remarks of Herter (1907) quoted above, it is reasonable to think that in the past the relation between diet and intestinal flora has not received due attention.

It was the observations of Torrey (1915) and of Dudgeon (1924) that led to the investigation of this series of four cases of typhoid fever in an endeavour to ascertain whether there are any modifications indicated in the diet usually given to typhoid patients in this country.

CASES.

The four cases which I investigated occurred in members of one family. The first individual to be affected was a child, and there followed a fortnight later an infection in the mother and in two other children. The father and the two remaining children were not attacked. The cause of the outbreak was believed to be certain infected clothing in the marine-store kept by the family. I am indebted to the kindness of Dr Foord Caiger for permission to carry out my investigations on these patients under his care.

SUMMARY OF THE CLINICAL HISTORIES OF THE PATIENTS.

Case 1. S. U., aet. 13, female, admitted to hospital 20. xii. 23 with 14 days' history of illness diagnosed clinically as typhoid fever.

Patient looked ill; abdomen distended but not tender; spleen not palpable. Temp. 102·5, pulse 130.

Patient remained acutely ill for 12 days. Serum taken on the 22nd day agglutinated *B. typhosus* 1/100, but not *B. paratyphosus* A, B and C. Temp. 28th day, normal. Uninterrupted recovery.

Case 2. E. U., aet. 41, mother of the other patients, admitted 5. i. 24 with 10 days' history of illness; abdomen distended but general condition good. Temp. 101·2, pulse 109.

Serum on 18th day agglutinated *B. typhosus* 1/400, but not *B. paratyphosus* A, B and C. General course of the disease mild; temp. 20th day, normal.

Case 3. C. U., aet. 11, daughter of above, admitted 5. i. 24 with 7 days' history of illness. Patient looked ill but had no distension. Temp. 103, pulse 100.

Spleen palpable 12th day; rash appeared 15th day; serum taken on 16th day agglutinated *B. typhosus* 1/500. General course mild; temp. 18th day, normal.

Case 4. K. U., aet. 9, sister of above, admitted 5. i. 24 with 8 days' history of illness. Patient looked ill, but had no distension. Temp. 103·5, pulse 109.

Serum taken on 15th day agglutinated *B. typhosus* 1/100, but not *B. paratyphosus* A, B and C. The general course was more severe than the preceding cases; recovery however was uninterrupted. Temp. 28th day, normal.

Treatment.

All four cases were treated on the same lines. The diet consisted of: milk, 2 oz.; lime water, 2 oz.; glycerine of pepsin, ½ dr. and cinnamon oil, 5 m. every two hours, during the febrile period. Distension was treated with Ol. Terebinth, 5 m. every four hours. After the temperature had subsided liquid paraffin was given in each case.

Investigation of cases.

The results of the investigations which I carried out are tabulated below.

Case 1. 18 January, 1924.

Day of dis.	*B. typhosus*	Strep. and staph.	Diet etc.	Purgatives	Stools	Temp.	Urine
43	0	+ + +	Semi-solid with milk	Paraffin liquid	Normal	Normal	*B. coli anaerogenes* + +
44	0	+ + +	,,	,,	,,	,,	
46	0	+ +	,,	,,	,,	,,	
50	0	+ +	Chicken, milk less	,,	,,	,,	
54	0	+ +	,,	,,	Slight diarrhoea	,,	
56	0	+	Milk less	,,	Normal	,,	*B. coli*
62	0	+ *S. aur.* +	,,	,,	,,	,,	*anaerogenes* + +
64	0	+ − *S. aur.* + +	,,	,,	,,	,,	
67	+ +	+	,,	,,	Slight diarrhoea	,,	
69	0	+ −	,,	,,	Normal	,,	
75	0	+ −	,,	,,	Slight diarrhoea	,,	*B. coli anaerogenes* +

Case 2. 18 January, 1924.

Day of dis.	*B. typhosus*	Strep. and staph.	Diet etc.	Purgatives	Stools	Temp.	Urine
23	+ −	+ + + *S. aur.* + −	Milk	Nil	Constipated	100 99·8	*B. typhosus* +
28	+	+ + + *S. aur.* + +	,,	,,	,,	Normal	
30	+ +	+ + + *S. aur.* + +	,,	,,	,,	,,	
35	+ +	+ + +	Semi-solid with milk	Paraffin liquid	,,	,,	*B. coli* + +
37	+ +	+ + *S. aur.* + −	,,	,,	,,	,,	

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Case 2. 18 January, 1924 (*continued*).

Day of dis.	*B. typhosus*	Strep. and staph.	Diet etc.	Purgatives	Stools	Temp.	Urine
42	+ −	+	Semi-solid with milk	Paraffin liquid	Constipated	Normal	
44	+	+ −	,,	,,	,,	,,	
47	0	+ −	Solids, milk less	,,	,,	,,	
49	0	+ −	,,	,,	,,	,,	
51	0	Nil	,,	,,	,,	,,	
54	0	,,	,,	,,	,,	,,	*B. coli* + −

Remarks:—1. Enema simplex given daily.
2. 35th day *S. aureus* isolated from boils (right leg and back).
3. 47th day some haemolytic *B. coli* in the stools.

Case 3. 18 January, 1924.

Day of dis.	*B. typhosus*	Strep. and staph.	Diet etc	Purgatives	Stools	Temp.	Urine
20	0	+ + +	Milk	Nil	Diarrhoea	Normal	*B. coli* + +
21	0	+ + +	,,	,,	,,	,,	
23	0	+ + + *S. alb.* + +	,,	,,	,,	,,	
25	0	+ + + *S. alb.* + +	,,	,,	,,	,,	
27	+ +	+ + + *S. alb.* +	,,	,,	,,	,,	
31	0	+ + + *S. alb.* + −	,,	,,	,,	,,	
32	+	+ +	Semi-solid	,,	,,	,,	*B. coli* + +
37	+ +	+	,,	,,	Slight diarrhoea	,,	
44	+ +	+	Solids, milk less	Paraffin liquid	Normal	,,	
46	0	+ +	,,	,,	,,	,,	
48	0	+	,,	,,	,,	,,	
51	0	+ −	,,	,,	,,	,,	*B. coli* +

Case 4. 18 January, 1924.

Day of dis.	*B. typhosus*	Strep. and staph.	Diet etc.	Purgatives	Stools	Temp.	Urine
21	0	+ + +	Milk	Nil	Diarrhoea	101·5 101	*B. coli* + +
22	0	+ + + *S. alb.* +	,,	,,	,,	101·5 101	
24	0	+ + +	,,	,,	,,	101 100	
26	0	+ + + *S. alb.* + +	,,	,,	Normal	101 99·8	
31	+ +	+ + +	,,	,,	Constipated	Normal	
33	+ +	+ *S. alb.* + + +	,,	,,	,,	,,	*B. coli* + −
36	+ +	+ + *S. alb.* + +	,,	,,	,,	,,	
38	+ +	+ *S. alb.* + + +	Semi-solid with milk	,,	,,	,,	
40	0	+ −	,,	,,	,,	,,	
45	0	+ + +	,,	Paraffin liquid	Normal	,,	
47	0	+ −	,,	,,	,,	,,	
49	0	+ −	Solids, milk less	,,	,,	,,	*B. coli* +

Remarks:—1. 36th day *S. albus* isolated from boils (shoulder and back).

In the above tables: + + + indicates "in abundance."
+ + ,, "many."
+ ,, "few."
+ − ,, "1 to 3 colonies only."

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Methods employed.

The examination of the specimens from these four patients was carried out on the following lines.

The specimens passed in the morning were examined about two to three hours later. The general character of the stool, and its reaction to litmus was noted, a small portion of the stool was then dried by the method devised by Dudgeon and described by Wordley (1921).

This method consists of taking a specimen of faeces and transferring a portion thereof to the surface of a dry, sterile porous tile on which it is evenly spread with a knife. The tile is then placed under a bell-jar and left to dry at room temperature for two or three hours after which the material is scraped off and transferred to another similar tile and left until it is quite dry. When dry the material is scraped as a powder into a small heap in the centre of the tile, thoroughly mixed, and a little is placed on the various media upon which it is to be cultivated.

In order to increase the percentage of successful attempts to isolate the *B. typhosus*, various methods have, from time to time, been devised, such as the "Brilliant Green" method of Browning. Lactose bile has also been tried as a selective medium, but according to Tonney, Caldwell and Griffin (1916) it is directly antagonistic to the growth of the *B. typhosus*.

The tile method has a great advantage over these other methods in that it enables the maximum number of typhoid bacilli to be obtained without retarding the growth of the remainder of the flora. It is thus possible to study the complete flora with one method, and if desired it is possible to use blood-agar as a plating medium for the growth of the streptococcus and other haemolytic organisms. Another advantage is that, since the material placed on the media is a dry powder, comparisons from day to day have a common and stable basis, no matter whether the patient is constipated or diarrhoeic.

Isolation and cultivation of *B. typhosus* and other flora from typhoid fever patients.

The cultivation of the *B. typhosus* from the faeces, except in the later stages of the disease, may not be of importance in the actual diagnosis of typhoid fever, owing to the earlier and more certain methods of blood-culture and agglutination tests. It is of importance, however, in the stage of convalescence and in the case of a suspect carrier.

The media employed in this investigation were:

1. Two plates of litmus lactose agar. ("Lemco," 1 per cent., NaCl, 0·25 per cent.; peptone, 1 per cent.; agar, 2·5 per cent. and litmus 10 per cent.)
2. One plate of blood-agar (10 per cent. whole human blood).
3. One plate of agar.
4. One tube of litmus milk.
5. One tube of starch medium (1 per cent. starch in "Lemco" broth).

To each of the above a small quantity of the powdered faeces was added and all were incubated at 37° for 24 hours.

McConkey's medium was not used with the tile method because it was not desired to retard the growth of any organism, but rather to encourage every organism present in the faeces to grow on the medium so that a complete picture of the flora might be obtained. Litmus lactose agar was used therefore as a plating medium.

After incubation any blue translucent colonies on the litmus lactose plates were transferred into tubes of lactose broth and on to agar slopes. Any other colonies required for further examination, such as haemolytic colonies from the blood-agar plates, were transferred to agar slopes.

The milk tubes were examined for clot and the presence of foaming, and the starch tubes were tested microscopically for yeasts on the 1st and 3rd days.

Any gram-negative, motile organism, which after 24 hours' incubation had not produced gas in lactose, was tested by the coarse agglutination method against anti-typhoid serum and further inoculated into tubes of "Lemco" broth containing 1 per cent. of glucose, mannite, dulcite, cane-sugar, and lactose; and also into tubes of litmus milk and peptone water.

Organisms which produced gas in lactose were tested for haemolysis against a reagent composed of human red cells suspended in peptone water containing 0·5 per cent. and 0·85 per cent. NaCl. They were also tested by the coarse agglutination method against the anti-coli sera prepared by Dudgeon, Wordley and Bawtree (1922). Three of these were of a non-haemolytic, and two of a haemolytic strain.

All the sugar reactions were read on the 1st and 7th days and the litmus lactose plates were examined at the end of 24 and 48 hours.

An approximate differential count was made of the colonies on each plate.

CONDITIONS AFFECTING THE ISOLATION OF TYPHOID BACILLI.

The conditions bearing on the isolation of the bacilli from the stools are (1) the stage of the disease, (2) the state of the stools at the time of the investigation, and (3) the methods employed.

Attempts to isolate the bacilli from the stools are successful, according to an average of the figures given by various writers, in about 50 per cent. of cases by the end of the third week of the disease. The state of the stools is important as regards (1) whether they are fluid or formed, (2) whether they are acid or alkaline, (3) the time that has elapsed since they were passed, and (4) the question of urinary or other contamination.

There is no doubt that the last two are the most important; the time interval between collection and examination should be as short as possible and never exceed 12 hours, and great care must be taken to avoid contaminating the faeces with urine in order that urinary organisms may not be ascribed

to the faeces. As regards whether the stool should be fluid or not to get the best results, Tonney, Caldwell and Griffin (1916) maintain that elaterin when administered to a patient acts as a powerful cathartic without being in any way an antiseptic, and they found a much higher percentage of positive results when using this drug than they did without it.

Results of the investigation.

The appearance of the *B. typhosus* in the faeces did not show any relationship to changes in the diet or treatment in any of the four cases.

The later in the disease that the bacilli appeared, the shorter was the duration of their appearance.

Accompanying the appearance of the bacilli in Case 1 on the 67th day there was an abundant growth of slow lactose fermenting *B. coli*, forming very large colonies.

The disappearance of the *B. typhosus* from the stools was followed in each case by a marked simplification of the intestinal flora.

Typhoid bacilli were isolated in all 15 times from these four cases. Case 2, who was constipated during the whole investigation, gave seven consecutive positive results, and I think that this fact indicates that constipation *per se* is not detrimental to success when the tile method is used.

The mildest case clinically (Case 2) had most typhoid bacilli, both faecal and urinary.

Streptococci diminished in number in all these cases *pari passu* as the milk in the diet was reduced.

Haemolytic streptococci and staphylococci were found on nine occasions. Yeasts were never isolated, but sarcinae were frequently found.

The finding of the same organism in the bowel and in the skin lesions has already been referred to.

A few special points may be worthy of notice such as the frequent presence of *B. coli anaerogenes* in the stools and urine of Case 1. Another interesting point was the occurrence in all four cases, at or about the same time, of an abundant growth of the slow lactose fermenting *B. coli* referred to above; of these the organisms isolated from Case 2 were strongly haemolytic and were agglutinated by one of the haemolytic anti-coli sera in the series referred to above.

The bacilluria of typhoid bacilli in Case 2 when treated with hexamine gave place to one of *B. coli*.

The daily changes in flora in all the cases were slight and the general type was simple. *B. coli* was the only organism which was invariably present, though at times more abundant than at others. *S. aureus* was present on only six occasions. The stools throughout were slightly alkaline to litmus. Had these patients been given the diet advocated by Torrey (1919) what changes might we have expected, either clinical or bacteriological? I fail to see that

any material change would have resulted, for presumably the milk and bread (*i.e.* lactose and dextrin) received by these patients was sufficient for their needs in keeping excessive numbers of proteolytic organisms in check. The diet given to these patients would therefore seem to have been satisfactory.

Toxaemia in severe cases would appear to indicate the need for examination of the stools for putrefactive organisms, and, if these are present, treatment with increased carbohydrate diet might be advisable, but in the milder cases the diet given on similar lines to that prescribed for these four cases would appear to be satisfactory.

SUMMARY OF CONCLUSIONS.

1. The appearance and disappearance of typhoid bacilli in the faeces in these four cases bore no relation to changes in the diet nor to the physical state of the stools, but the later they appeared the shorter was the duration of their appearance.

On the disappearance of typhoid bacilli from the stools the intestinal flora tended to become more simple.

2. *B. coli* was the only organism invariably present at every examination.

3. Streptococci were very much more abundant in the earlier stages of the disease, when milk formed the greater part of the diet, than in the later stages.

4. In two cases where boils occurred on the body the causative organism had previously been isolated in large numbers from the faeces.

5. With stools slightly alkaline to litmus the flora in these cases was relatively simple and fermentative in type. There is no apparent advantage, therefore, in giving a high carbohydrate diet except in cases of marked alkalinity and putrefaction.

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