

THE NUMBER OF BACILLI HARBOURED BY ENTERIC CARRIERS

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In the course of experiments inquiring into the properties of selective culture media for salmonellae, including *Salmonella typhi*, it was desired to examine specimens of faeces containing small numbers of pathogens. For several reasons it was undesirable to use for the tests normal faeces to which small numbers of salmonellae had been added from an artificial culture. Accordingly, samples of faeces were collected from twenty-four typhoid and paratyphoid carriers and the pathogens were enumerated. The pathogens were found to be present in very large numbers often outnumbering *Bacterium coli*. Frequently there were more pathogens in the faeces of a carrier than in the faeces of a patient suffering from an attack of enteric fever. The numbers probably erred on the low side as all the specimens were at least 1 day old and some were 2 days old before they were examined. Most were collected from carriers who lived some distance from the laboratory, and the specimens had been sent by post.

METHODS

A small amount of faeces was weighed and enough water added to make a 10% suspension. Tenfold dilutions were made up to 10^{-7} using a fresh sterile dropping pipette for each step. The bacilli were enumerated by the technique of Miles & Misra (1938), one drop (1/50 ml.) of each dilution being allowed to fall on a culture plate. After incubation the colonies were counted and identified. Several culture media were used, namely, brilliant green MacConkey's medium (best for *S. paratyphi B*), deoxycholate citrate medium (best for *S. typhi*) and ordinary MacConkey's medium (to enumerate *Bact. coli* and *Streptococcus faecalis*). Wilson and Blair's medium was also used.

At the same time one drop of each dilution was added to a tube of ordinary nutrient broth and to selenite broth, in order to verify that the end-point of the titration by the Miles & Misra technique agreed with that obtained in fluid culture media. It was suspected that counts on solid culture plates would err on the low side because many salmonellae in faeces might be feebly viable.

RESULTS

The results were expressed as the number of bacilli per gram of faeces and are shown in Table 1.

Four carriers of *S. typhi*, nos. 9-12, had less than 500 bacilli per gram of faeces, and the full routine examination of the specimens revealed no *S. typhi*. Specimens from these carriers had been examined at monthly intervals for several years, and in the preceding year (twelve specimens) no. 9 had been found positive on four

occasions, nos. 10 and 11 on one occasion only, and no. 12 had not given a single positive result.

The other twenty carriers had enough bacilli in their faeces to be countable by the Miles & Misra technique, and most of them harboured very large numbers. Each carrier was examined on several occasions and the high counts were confirmed. It was known, however, that carrier no. 21 occasionally gave a negative

Table 1. *Number of S. typhi and S. paratyphi B in a gram of faeces of carriers*

(a) Typhoid carriers

Carrier	Known duration of carrier state (years)	<i>S. typhi</i>	<i>Bact. coli</i> , etc.
1	3	4,500,000	7,000,000,000
2	3	4,000,000	7,000,000,000
3	3	550,000	1,400,000,000
4	12	45,000,000	5,000,000
5	12	2,500,000	20,000,000
6	12	1,000,000	100,000,000
7	12	600,000	700,000,000
8	12	500,000	300,000,000
9	12	< 500	300,000,000
10	12	< 500	450,000,000
11	12	< 500	120,000,000
12	12	< 500	650,000,000

(b) Paratyphoid carriers

Carrier	Known duration of carrier state (years)	<i>Salm. paratyphi B</i>	<i>Bact. coli</i> , etc.
13	1	12,000,000,000	1,550,000,000
14	1	2,000,000,000	150,000,000
15	1	500,000,000	10,000,000
16	1	300,000,000	8,500,000
17	1	200,000,000	2,450,000
18	1	90,000,000	40,000,000
19	1	20,000,000	300,000,000
20	1	100,000	10,000,000
21	12	25,000,000	2,500,000,000
22	18	50,000,000	250,000,000
23	18	10,000,000	20,000,000
24	20	500,000	110,000,000

result in a routine examination. Carrier no. 20, when examined at the eighth month after infection, had shown 100,000,000 bacilli per gram of faeces, a result which was confirmed more than once. At the fifteenth month (when this article was written) the number had fallen to below 500 per gram.

There was no reason to believe that the carriers were not representative of their kind.

Possible advantages of a small inoculum

The colonies growing on the surface of the culture plates from the drops of the several tenfold dilutions of faeces were easily recognized. As a specimen of faeces was diluted the pathogens grew in purer culture. In the examination of the twenty found positive one drop (1/50 ml.) of 1:1000 dilution of faeces was not only clearly positive but was virtually a pure and rich culture of *S. typhi* or *S. paratyphi B* on one or other culture plate, and no elaborate methods of culture would have increased the number found positive.

Most bacteriologists agree that better results are obtained if a suspension of faeces is made before culture, and it sometimes happens that a small inoculum yields more colonies of pathogens than a larger one. As an example of this, the result of the full examination of specimen 1 is quoted. One drop of each dilution (including undiluted faeces), as used for the Miles & Misra count, was dropped on a separate culture plate using a whole plate (deoxycholate citrate) for each drop. With a glass spreader, the inoculum was distributed in the usual way to obtain a progressive thinning out of the growth in different parts of the plate. The culture made from undiluted faeces showed only four colonies of *S. typhi*. There was a rich growth of *Bact. coli* in one corner of the plate, but over the greater part of the plate there was no growth, the surface appearing as if uninoculated. The cultures made from dilutions 1:10, 1:100 and 1:1000 showed progressively fewer *Bact. coli* and more *S. typhi* relatively and absolutely, and colonies of the latter appeared in all parts of the culture plate. The culture made from 1:10,000 dilution was a pure culture of *S. typhi*, but the absolute numbers were fewer than had been obtained with the 1:1000 dilution.

Brilliant green MacConkey's medium for *S. paratyphi B*, however, is usually able to take a large inoculum of faeces and still yield a pure and rich culture of the pathogen. For this reason it is considered to be the most satisfactory for routine use for all salmonellae except *S. typhi*. A concentration of brilliant green can be chosen to make the medium useful for the isolation of *S. typhi* and *S. paratyphi B*: if increased appreciably it will inhibit *S. typhi* but produce a medium exceptionally selective for other salmonellae.

Comparison of end-points on solid and in fluid media

Tenfold dilutions of each specimen of faeces were made, and for the enumeration by the Miles & Misra method the inocula were single drops of each of the dilutions. The same series of inocula were placed in fluid culture media, both nutrient broth and selenite broth being used. It was thought possible that the counts by the Miles & Misra method would err on the low side, as many bacilli might be feebly viable, and thus the fluid cultures might reveal salmonellae in smaller inocula, i.e. higher dilutions, than those found positive on solid culture plates. This did not happen and it was concluded that the enumerations by the Miles & Misra technique were sufficiently accurate.

Two typical results are quoted, one for *S. typhi* (Table 2) and one for *S. paratyphi B* (Table 3).

Table 2. *Comparison of end-points*

Inoculum number	Number of bacilli (Miles & Misra enumeration)	
	<i>S. typhi</i>	<i>Bact. coli</i> , etc.
1	16	28,000
2	2	2,800
3	0	280
4	0	28
5	0	3
6	0	0

Selenite broth and ordinary broth cultures inoculated with inocula 1 and 2 yielded *S. typhi*. The others did not, and a tube of ordinary broth inoculated with inoculum 6 was sterile.

Table 3. *Comparison of end-points*

Inoculum number	Number of bacilli (Miles & Misra enumeration)	
	<i>S. para-typhi B</i>	<i>Bact. coli</i> , etc.
1	36	500,000
2	5	50,000
3	1	5,000
4	0	500
5	0	50

Selenite broths yielded *S. paratyphi B* from inocula 1 and 2: ordinary nutrient broth yielded *S. paratyphi B* from inocula 1-3.

SUMMARY

Salmonella typhi or *S. paratyphi B* were found in very large numbers in the faeces of enteric carriers. Of twenty-four carriers, four were found negative by the fullest examination. Of the twenty found positive almost all harboured many millions of bacilli per gram of faeces.

A minute inoculum of one drop (1/50 ml.) of a 1:1000 dilution of faeces on a culture plate only rarely failed to reveal all the positives without the use of an 'enrichment' medium and the result of such a procedure was a culture plate with virtually a pure culture of the pathogen.

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REFERENCE

MILES, A. A. & MISRA, S. S. (1938). *J. Hyg., Camb.*, **38**, 732.

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