

# Further study of strontium selenite and selenite F broths for the isolation of *Salmonella typhi*

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**SYNOPSIS** The efficiency of strontium selenite and selenite F broths in the isolation of *S. typhi* from 625 clinical stool specimens were compared. A total of 126 strains of *S. typhi* were detected. Of these, 96 (76%) were isolated with selenite F broth and 118 (94%) were isolated with strontium selenite broth. At the same time, the number of false positive isolates accruing to strontium selenite broth was less than that accruing to selenite F broth. The superiority of strontium selenite broth over selenite F broth was more obvious when relatively few typhoid bacilli were present in faeces, eg, after antibiotic therapy, or when stools had been left for prolonged periods at room temperature. In the isolation of *S. typhi* with strontium selenite broth, salmonella-shigella agar was found to be a better plating medium than xylose lysine desoxycholate agar.

Enrichment media are generally employed as a useful adjunct for the isolation of salmonellae from stools. Three enrichment media, viz., selenite F broth, tetrathionate broth, and Rappaport medium, are widely used in most laboratories. It is generally accepted that selenite F broth is the most suitable for the isolation of *S. typhi*, which is markedly inhibited by Rappaport medium and often to some degree by tetrathionate broth, particularly if brilliant green is added.

Iveson and Mackay-Scollay (1969) introduced a new enrichment medium, strontium selenite broth, for the isolation of salmonellae. They found that it was superior to selenite F broth, particularly in the isolation of *S. typhi*. The same results were also obtained in our laboratory (Chau and Huang, 1970). However, studies relating to the isolation of *S. typhi* were obtained on limited numbers of stool specimens; further study on a greater number of clinical stool specimens was therefore carried out and the results obtained are reported here.

## Materials and Methods

### STOOL SPECIMENS

From March 1971 to May 1972, 625 stool specimens collected daily or at intervals of one or two days from 43 typhoid fever cases and three typhoid carriers during their stay in an infectious disease hospital

were examined. Of the 43 typhoid fever cases, 37 were bacteriologically confirmed whereas six were clinically diagnosed as typhoid fever. The majority of the typhoid patients were admitted to the hospital during the second or third week of their illness. The three typhoid carriers were admitted to the hospital for antibiotic treatment.

### ENRICHMENT AND PLATING MEDIA

Strontium selenite broth was prepared according to Iveson's revised formula (personal communication) as follows:

Bacto tryptone (Difco)	.. ..	0.5 g
Sodium chloride	.. ..	0.8 g
Di-sodium hydrogen phosphate	.. ..	0.05 g
Strontium hydrogen selenite (Ajax, Australia)	.. ..	0.2 g
Distilled water	.. ..	100.0 ml

The medium was dispensed in 10 ml volumes and sterilized by steaming for 30 minutes. The final pH was 6.8.

Selenite F broth (Leifson, 1936) and Rappaport medium (Rappaport, Konforti, and Navon, 1956) were prepared in our laboratory according to methods of the authors. Tryptone and peptone used in these media were obtained from Oxoid Co (London) and sodium hydrogen selenite and malachite green were from BDH Co (Poole, England). When not in use the media were kept at 4°C for not more than one month.

Salmonella-shigella agar and xylose lysine de-

soxycholate agar were prepared from dehydrated media of Oxoid Co.

#### METHOD OF STUDY

1 Stool specimen of approximately the size of a pea was emulsified in 10 ml of sterile saline by agitation with a pasteur pipette. One drop of the emulsion delivered from the pasteur pipette was (a) streaked directly onto salmonella-shigella agar and (b) inoculated into the enrichment broths, respectively. After 18 to 24 hours' incubation at 37°C, the enrichment broth cultures were subcultured onto salmonella-shigella agar. Suspicious colonies of salmonella were submitted to preliminary biochemical tests and all the presumptive salmonella strains were identified by conventional biochemical and serological methods.

2 After the preliminary examination, stool specimens were kept in the refrigerator. If positive for *S. typhi*, they were inoculated again into strontium selenite and selenite F broths and subcultured onto both salmonella-shigella and xylose lysine desoxycholate agars for further isolation. The specimens were then left at room temperature and re-examined as mentioned above for three successive days. A total of 84 stool specimens known to be positive for *S. typhi* were examined in this way.

#### Results

##### PRIMARY ISOLATION OF *S. typhi* FROM CLINICAL STOOL SPECIMENS

In the primary isolation, 126 out of 625 stool specimens yielded *S. typhi*. Strontium selenite broth

alone gave 118 positive isolates (94%) whereas selenite F broth alone yielded 96 positive isolates (76%), a difference in efficiency of 18% (Table I). *S. typhi* was isolated in scanty growth from two of the specimens by Rappaport medium alone, whilst selenite F and strontium selenite broths of the same yielded proteus and pseudomonas only.

After antibiotic therapy (sulphamethoxazole/trimethoprim or chloramphenicol) fewer *S. typhi* organisms were isolated. Under these circumstances, the superiority of strontium selenite broth over selenite F broth was more evident. Before antibiotic therapy, strontium selenite broth alone yielded *S. typhi* from 72 out of 78 cultures, or 92% of the total isolates, whilst selenite F broth alone yielded 62 cultures only, or 80%, giving a difference of 12%. After antibiotic therapy the strontium selenite medium yielded 46 *S. typhi* cultures out of 48 total isolates (96%), whilst the latter one yielded 34 (71%), bringing about a difference of 25%. The data are presented in Table II.

Furthermore, fewer non-lactose fermenting, salmonella-like false positive colonies were obtained after subculture from strontium selenite broth than from selenite F broth. A total of 452 lactose-negative, non-salmonella strains were obtained from selenite F broth; these included 160 lactose slow or non-fermenting coliform bacilli, 214 proteus species (mainly *Proteus mirabilis*), and 78 pseudomonas species. On the other hand, only 297 lactose-negative, non-salmonella strains were obtained from strontium selenite broth in which 129 were lactose slow or non-fermenting coliform bacilli, 117 proteus species, and 51 pseudomonas species.

	Total	SX-SF	SX-R	SF-R	κX	SF	R	Direct Plating on SS Agar
No. of positive isolates from patients	99	97	94	78	92	75	15	37
No. of positive isolates from carriers	27	27	26	21	26	21	7	13
Total no. of positive isolates	126	124	120	99	118	96	22	50
Percentage efficiency	100	98	95	78	94	76	17	40

Table I Relative efficiency of strontium selenite, selenite F, and Rappaport medium alone or in combination in isolating *S. typhi* from faeces.

SX = Strontium selenite; SF = Selenite F; R = Rappaport medium.

		Total	κX	SF	R	Direct plating on SS agar
Stools examined before antibiotic therapy	No. of positive isolates	78	72	62	15	34
	% efficiency	100	92	80	19	44
Stools examined after antibiotic therapy	No. of positive isolates	48	46	34	7	16
	% efficiency	100	96	71	15	33

Table II Relative efficiency of strontium selenite and selenite F broths in isolating *S. typhi* from faeces examined before and after antibiotic therapy

SX = Strontium selenite; SF = Selenite F; R = Rappaport medium.

Out of the 43 typhoid fever cases, 35 were stool culture positive with the use of strontium selenite broth alone as enrichment, while only 30 cases were stool culture positive when selenite F broth was used. In the five cases which showed positive stool cultures with strontium selenite broth but not with selenite F broth, positive stool cultures were obtained only in the first one or two specimens but not in further ones during the remaining period of their illness.

SUBSEQUENT ISOLATION OF *S. typhi* FROM STORED POSITIVE STOOLS

When 84 positive stool specimens were kept at room temperature for repeated examination (see Method of study 2), the number of positive isolates decreased rapidly, no matter whether strontium selenite broth or selenite F broth was used in the isolation. The decreasing rates were, however, different between the two enrichment media used (Fig. 1). In the case of selenite F broth the number of positive isolates decreased from 64 to 18 after being kept at room temperature for three days, with a reduction of 72%;

while in the case of strontium selenite broth, the number decreased from 76 to 33 with a reduction of 57%.

From the data presented in Fig. 1, it was also clear that xylose lysine desoxycholate agar was less efficient as the plating medium than salmonella-shigella agar for the isolation of *S. typhi*. A total of 340 positives were obtained when salmonella-shigella agar was used while a total of 299 positives were obtained with the use of xylose lysine desoxycholate agar.

Discussion

Since Leifson (1936) first introduced selenite F broth as an enrichment medium for the isolation of salmonellae, it is generally agreed that this medium is the best enrichment in the isolation of *S. typhi*. Various modifications of this medium have been developed. Hobbs and Allison (1945) reported that the replacement of lactose with mannitol improved the efficiency, although Smith (1952) found the difference to be very little. Banič (1963) reported that

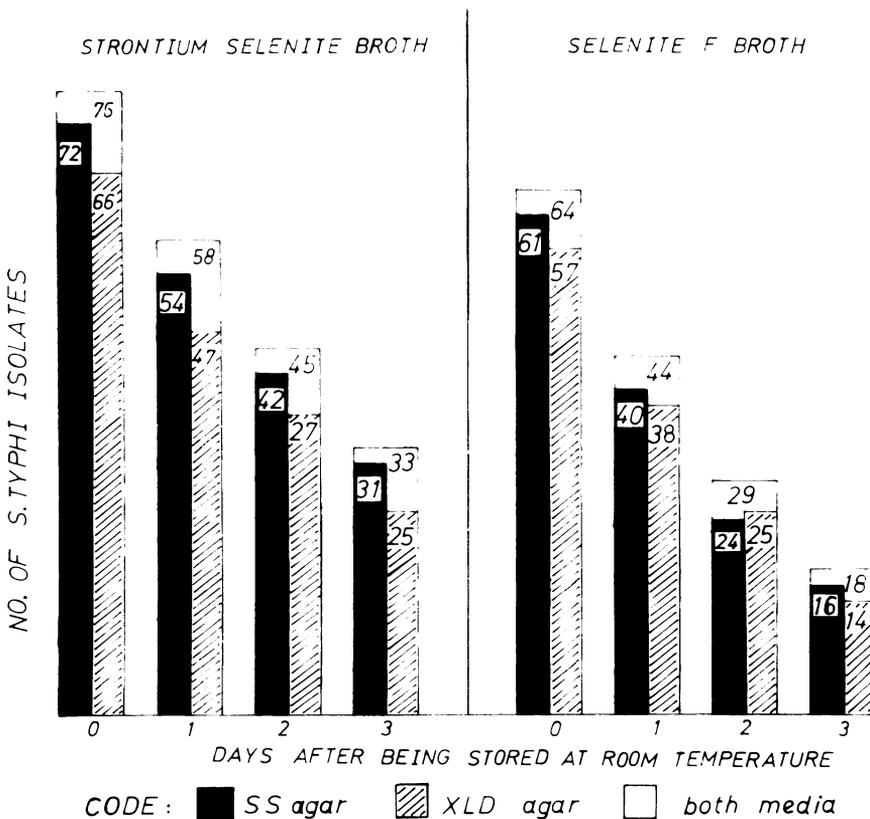


Fig. 1. Comparison of the efficiency of two enrichment broths and two plating media in the isolation of *Salmonella typhi* from 84 positive stool specimens stored at room temperature for three successive days

better results could be obtained when the amount of selenite salt in the selenite F broth was reduced with the incorporation of 3.4%  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ . However, in our hands, Banič's claim could not be substantiated. In 1969 another new modification, the strontium selenite broth was introduced by Iveson and Mackay-Scollay who found that it was more satisfactory than the original selenite F broth. Their results have been confirmed in our present study as well as in a previous investigation (Chau and Huang, 1970).

The composition of strontium selenite broth is different from that of selenite F broth in several ways: the deletion of lactose, a decreased amount of phosphate and selenite which is in the form of  $\text{Sr}(\text{HSeO}_3)_2$ . In order to find out what factors were responsible for the improvement in efficiency of this medium, a modified enrichment broth, similar to strontium selenite broth in all respects except the replacement of strontium hydrogen selenite by equivalent sodium hydrogen selenite, was prepared and compared with other enrichment broths in a limited number of trials in our laboratory. The results revealed that the modified enrichment broth was less effective than selenite F broth. Thus the higher efficiency of strontium selenite broth was, perhaps, in some way related to the presence of strontium. It was also noticed that after culturing of the stool specimens, less selenite was reduced to selenium, i.e. less reddish precipitate formed, in strontium selenite broth than in selenite F broth. This indicated that the amount of selenite in strontium selenite broth was relatively more stable than that in selenite F broth after the growth of enteric bacteria.

From the data presented here, the combined use of strontium selenite and selenite F broths yielded the highest number of positive isolates. In practice, however, it is better to combine the use of strontium selenite broth with Rappaport medium, especially when isolation of *S. choleraesuis* is anticipated. As already mentioned, Rappaport medium is not a good enrichment medium for the isolation of *S. typhi*, yet *S. typhi* was isolated from this medium alone on two occasions. This was quite possibly due to the abundance of pseudomonas and proteus present in selenite F and strontium selenite broths on these two

occasions whereas these commensals were strongly inhibited in the Rappaport medium.

Xylose lysine desoxycholate agar was reported to be better than salmonella-shigella agar for the isolation of shigellae and salmonellae in giving more positive isolates and less false positive cultures (Taylor and Schelhart, 1971). We found, however, that xylose lysine desoxycholate agar was less satisfactory than salmonella-shigella agar in the isolation of *S. typhi*, and that colonies of *S. typhi* on xylose lysine desoxycholate agar were smaller than those on salmonella-shigella agar while colonies of the commensal enteric bacteria such as *E. coli* were of about the same size or even a little larger than those on salmonella-shigella agar. It might suggest that xylose lysine desoxycholate agar was less selective, though it gave better differentiation of colonies than did salmonella-shigella agar.

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