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Strontium chloride and strontium selenite enrichment broth media in the isolation of *Salmonella*

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INTRODUCTION

Two enrichment methods, the tetrathionate broth of Müller (1923) and the selenite F medium developed by Leifson (1936), are widely used in the isolation of *Salmonella* from faeces and other contaminated materials. Modifications to these media have included the addition of selective inhibitory agents or growth factors to increase isolations both of numbers and of serotypes. However, certain serotypes, particularly *Salmonella choleraesuis*, frequently fail to grow in these media, and overgrowth by *Proteus* species and other enterobacteria interferes with the recovery of salmonellas. The enrichment culture method introduced by Rappaport, Konforti & Navon (1956), although an improvement on other methods in the recovery of most salmonellas, was found unsatisfactory for the isolation of *S. typhi*, and its efficiency in the recovery of *S. choleraesuis* from clinical materials had not been evaluated (Iveson & Kovacs, 1967; Anderson & Kennedy, 1965; Hooper & Jenkins, 1965).

Attention was directed to the extended use of specific ions incorporated in an enrichment medium which would be capable of recovering the full range of salmonella serotypes likely to be encountered in men and animals. Eisenburg (1918) had reported on the apparent resistance of *S. typhi* to strontium salts, and Hotchkiss (1923) had outlined the necessary concentrations of various salts, which included strontium chloride, required to inhibit the growth of *Escherichia coli* in peptone water. Rappaport & Konforti (1958) noted that the inhibitory action of malachite green could be selectively modified by certain bivalent cations to inhibit the growth of non-pathogenic Gram-negative microorganisms, while allowing the growth of certain salmonellas. More recently, Banič (1964) and Zajc-Satler & Banič (1965) used a selective combination of magnesium chloride and sodium hydrogen selenite. This enrichment medium, although efficient, was inferior to tetrathionate infusion broth.

A study was undertaken to test the selective action of strontium ion, in the form of the soluble chloride, and this was later expanded to include strontium selenite salt. Preliminary experiments were performed on pure cultures of *S. typhi*, *S. paratyphi A*, *S. choleraesuis*, *S. typhimurium*, *Arizona* spp., *E. coli*, *Pseudomonas* spp. and *Proteus* spp. On the basis of the results obtained, two simple and stable enrichment media were developed. These were used in parallel with the routine methods for salmonella isolation, as reported by Iveson & Kovacs (1967). The technique involved and the results obtained are presented in this report.

MATERIALS AND METHODS

The investigation comprised three complementary studies.

The first, Study I, involved the testing of 3,336 human faeces specimens and 128 sewage samples, using strontium chloride, selenite F and Rappaport enrichment broth media. Study II was directed particularly to the isolation of *S. choleraesuis* and involved the examination of 98 faeces specimens and 69 macerated gland samples from healthy pigs using the same enrichment media. Study III was undertaken to evaluate the recovery of *S. typhi* from 83 faeces specimens from suspect or actual cases of typhoid fever or carriers, and these were tested by the use of strontium selenite broth in addition to the media used in Studies I and II.

Study I

Faeces specimens were obtained from patients throughout Western Australia suffering from gastro-enteritis. Samples of 1–5 g. were transported to the laboratory in 1 oz. screwcap bottles containing 10 ml. Sachs (1939) enteric transport medium. Each faeces sample was mixed well and examined by inoculation of approximately 0·5 ml. into 10 ml. each of strontium chloride, selenite F and Rappaport enrichment broth media.

Sewage samples were collected by using swabs (Moore, Perry & Chard, 1952) which had been immersed for 24–48 hr. After lifting, each swab was placed in 100 ml. 1/4 Ringer solution and transported to the laboratory where, after mixing, 20 ml. from each sample was added to 150 ml. of each of the three enrichment media.

Study II

Inoculations of approximately 0·5–1 g. into 10 ml. each of the three enrichment media were made from faeces specimens and from macerated gland samples from healthy pigs.

Study III

Faeces from typhoid cases, carriers or suspect cases, emulsified in Sachs' transport medium, were inoculated in approximately 0·5 ml. amounts into 10 ml. each of the three media, strontium chloride, selenite F and Rappaport, and into the same volume of strontium selenite broth. On occasion, as additional controls, the tetrathionate broth of Preuss (1949) modified by Iveson & Kovacs (1967) and commercial selenite F, were also used.

All faeces samples were inoculated with pasteur pipettes of 2–3 mm. internal diameter. All specimens, in addition to being inoculated into the enrichment broths, were sown, in approximately 0·1 ml. amounts, on MacConkey, deoxycholate-citrate-agar (DC), Shigella and Salmonella agar (SS, Difco) and modified bismuth sulphite agar (BS) solid media. Sub-cultures from enrichment media were made at 18–20 hr. on SS and BS agar media, and again at 48 hr.

Media

Strontium chloride enrichment broth

Solution A (base)

Bacto tryptone (Difco)	0·5 g.
Sodium chloride	0·8 g.
Potassium dihydrogen phosphate	0·1 g.
Distilled water	100 ml.

Solution B (Stock)

Strontium chloride (B.D.H.)	60 g.
Distilled water	100 ml.

Solution C (Stock)

Malachite green (Merck)	0·4 g.
Distilled water	100 ml.

For use, to each 100 ml. solution A, 10 ml. solution B and 1·0 ml. solution C were added. 10 ml. volumes were distributed and sterilized by steaming for 30 min. The final pH was 5·0–5·5. The medium remained suitable for use after storage at room temperature for one month.

Strontium selenite enrichment broth

Preparation of strontium selenite. Twenty-five g. selenous acid (B.D.H.) were dissolved in 300 ml. distilled water. Strontium carbonate was added in approximately 1 g. amounts at a time with constant stirring at 50° C until effervescence ceased and a small amount of the carbonate remained undissolved. The pH at this stage was about 3·4. The mixture was vacuum filtered through a sintered glass disk (porosity 3) and added to approximately four times its volume of absolute ethanol with stirring. The amorphous salt precipitated and was collected by vacuum filtration on a sintered glass disk and washed twice with absolute ethanol. As much ethanol as possible was removed by vacuum from the precipitate on the sintered glass disk, which was then air dried in an oven at 50° C. The salt was soluble to approximately 2% by weight in water.

Preparation of strontium selenite enrichment broth:

Bacto tryptone	0·5 g.
Sodium chloride	0·8 g.
2% Strontium selenite	10 ml.
Potassium dihydrogen phosphate	0·1 g.
Distilled water	100 ml.

The broth was adjusted to pH 7·8, distributed in 10 ml. amounts and steamed for 30 min. The medium was found suitable for use after storage at room temperature for 2 months. Slight strontium phosphate precipitate was noted during storage but this did not interfere with its performance.

Other enrichment broth media

Selenite F medium used was that of Leifson (1936) and the Rappaport medium was modified by Iveson & Kovacs (1967).

RESULTS

In Study I a total of 475 salmonella strains were isolated from faeces, of which 474 were recovered by enrichment and 209 by direct culture methods. One strain was isolated only by direct plating, compared with 33 only by one or other of the enrichment media methods: 16 of these by Rappaport only, 12 by strontium chloride broth only and five by selenite F broth only.

The following *Salmonella* serotypes were recovered (the figures in parentheses indicate the frequency of isolations):

Salmonella typhimurium (221), *muenchen* (68), *chester* (21), *adelaide* (13), *oranienburg* (13), *wandsworth* (11), *senftenberg* (10), *newington* (9), *abony* (8), *anatum* (8), *bovis morbificans* (8), *orion* (8), *tennessee* (7), *onderstepoort* (6), *rubislaw* (6), *enteritidis* (4), *give* (4), *havana* (4), *hvittingfoss* (3), *ball* (2), *bredeney* (2), *brisbane* (2), *jangwani* (2), *lansing* (2), *saintpaul* (2), *eastbourne* (1), *emmasted* (1), *fremantle* (1), *kimberley* (1), *litchfield* (1), *ohlstedt* (1), *poona* (1), *potsdam* (1), *singapore* (1), *typhi* (1), *welikade* (1) and salmonella group (17), *Arizona* 26:26–25 (3), *Arizona* 5:29–30 (1).

Table 1. *Relative efficiency of strontium chloride, Rappaport and selenite F enrichment in the isolation of salmonella from* 3336 *human faeces and* 128 *sewage samples*

Combinations of enrichment media			Faeces		Sewage	
SC	R	SF	Salmonella positive	% efficiency	Salmonella positive	% efficiency
+	+	+	266	100	46	100
+	+	.	256	96·2	42	91·3
+	.	+	238	89·5	37	80·4
.	+	+	247	92·9	35	76·1
+	.	.	223	83·8	29	63·0
.	+	.	230	86·5	29	63·0
.	.	+	151	56·8	18	39·1

SC = strontium chloride; R = Rappaport; SF = selenite F.

With the exception of the *S. typhi* isolation, which was made from selenite F medium, no preferential isolation of a particular serotype was made by any one enrichment method.

Of the four *Arizona* isolations, one was positive on direct culture, two were made through selenite F, four through Rappaport and four through strontium chloride enrichment broths respectively.

Considering the 266 specimens which failed to yield salmonellas on direct plating, it was shown (Table 1) that 230 were positive using Rappaport's method compared with 223 and 151 using strontium chloride and selenite F enrichment broths respectively.

Of 128 samples of sewage examined, 46 yielded salmonellas. Of these, 11 were

isolated by strontium chloride only, nine by Rappaport only and four by selenite F only.

The following *Salmonella* serotypes were recovered from the sewage samples:

Salmonella adelaide (1), *anatum* (3), *bareilly* (1), *bovismorbificans* (1), *bredeney* (1), *chester* (13), *emmasted* (1), *give* (2), *heidelberg* (1), *meleagridis* (1), *muenchen* (13), *newington* (2), *oranienburg* (1), *orientalis* (1), *orion* (3), *potsdam* (1), *paratyphi B* (1), *rubislaw* (2), *senftenberg* (6), *singapore* (2), *saintpaul* (2), *typhi* (2), *typhimurium* (17) *wandsbeck* (1), *Arizona* 26:26–25 (1).

The two *S. typhi* isolations were made through selenite F medium and the single *Arizona* isolation through strontium chloride broth.

Of the 46 positive specimens, 29 isolations were made through strontium chloride, 29 through Rappaport and 18 through selenite F enrichment broth. Table 1 shows the combinations of enrichment culture methods yielding salmonellas from human faeces and sewage in Study I.

In Study II 27 isolations of salmonella were obtained from 98 samples of pig faeces; 24 were made through strontium chloride enrichment, 19 through Rappaport and 15 through selenite F. Of 69 pig gland samples examined, 15 yielded salmonellas, 11 of the isolations being made through strontium chloride, 10 through Rappaport and two through selenite F.

Table 2. *Relative efficiency of strontium chloride, Rappaport and selenite F enrichment in the isolation of salmonella from* 98 *pig faeces and* 69 *pig gland samples*

Combinations of enrichment media			Pig faeces		Pig glands	
SC	R	SF	Salmonella positive	% efficiency	Salmonella positive	% efficiency
+	+	+	27	100	15	100
+	+	.	26	96·3	15	100
+	.	+	26	96·3	11	73·3
.	+	+	21	77·8	10	63·3
+	.	.	24	88·9	11	73·3
.	+	.	19	70·4	10	63·3
.	.	+	15	55·6	2	13·3

SC = strontium chloride; R = Rappaport; SF = selenite F.

The following *Salmonella* serotypes were recovered from pig faeces and glands:

Salmonellae adelaide (3), *bahrenfeld* (2), *birkenhead* (1), *bovismorbificans* (4), *bredeney* (2), *chester* (3), *choleraesuis* (11), *derby* (9), *give* (1), *litchfield* (2), *muenchen* (4), *orion* (4), *saintpaul* (1), *tennessee* (1), *typhimurium* (6).

Of the 11 isolations of *S. choleraesuis*, four were made through both strontium chloride and Rappaport media, four through Rappaport only and three through strontium chloride only. Both direct and selenite F enrichment culture methods failed to yield *S. choleraesuis*.

The combinations of enrichment culture yielding salmonellas from pig glands and faeces are shown in Table 2.

In Study III four methods of enrichment culture as well as direct plating were

used in parallel on 83 faeces specimens suspected of containing *S. typhi* (Table 3). Forty-six of these specimens yielded *S. typhi* by one or more methods: 45 (97·8 %) were positive by strontium selenite, 35 (76·1 %) by selenite F, 25 (54·8 %) by strontium chloride, two (4·4 %) by Rappaport and 21 (45·7 %) by direct plating methods of culture respectively.

In a further comparative experiment 19 faeces specimens suspected of containing *S. typhi* were tested by the tetrathionate broth enrichment method in parallel with the four enrichment methods described, as well as by direct plating. All 19 proved positive through strontium selenite, 14 through selenite F, 11 through tetrathionate, eight through strontium chloride, one through Rappaport and five by direct plating.

Table 3. *Relative efficiency of strontium chloride, Rappaport, selenite F and strontium selenite alone or in a combination in the isolation of* S. typhi *from faeces of suspected typhoid fever cases or carriers*

	SC	R	SF	SX	SC.SF	SC.SX	SF.SX	Direct plating
No. positive	25	2	35	45	35	45	46	21
% efficiency	54·8	4·4	76·1	97·8	76·1	97·8	100·0	45·7

SC = strontium chloride; R = Rappaport; SF = selenite F; SX = strontium selenite.

In tests on 12 sewage samples known to contain *S. typhi*, all 12 were positive through strontium selenite but only seven were positive through selenite F enrichment methods. No specimen was positive on direct plating. These specimens were not tested by the other methods reported on in the study.

DISCUSSION

In a comparison of enrichment media for the isolation of *Salmonella* from faeces, Hobbs & Allison (1945) reported that selenite F medium was the most satisfactory method for the isolation of *S. typhi*. Recoveries of other serotypes, including *S. paratyphi B*, were comparable in both selenite and tetrathionate media. It was also reported that selenite F appeared suitable for the isolation of *Salmonella* from faeces specimens and lymph nodes of pigs, although occasionally both media might fail to suppress proteus species.

Similar results were reported by Cook, Frisby & Jebb (1951) on salmonella recoveries, which included isolations of *S. typhimurium*, *S. paratyphi B* and *S. typhi*, from tests on human faeces. Smith (1952, 1959) reported on a like experience in examinations of both human and animal specimens. Smith also noted that both selenite and tetrathionate media were unsuitable for the isolation of *S. choleraesuis*. Moore *et al.* (1952) reported improved isolations with selenite broth from sewage samples, and McCoy (1962) found that selenite and tetrathionate media were fully comparable and he recommended their use for sewage examination. He noted, however, that selenite favoured the isolation of *S. typhi*.

In trials of the enrichment broth introduced by Rappaport *et al.* (1956), Collard

& Unwin (1958), Hooper & Jenkins (1965) and Iveson & Kovacs (1967) achieved higher recoveries of salmonellas from faeces with Rappaport's medium than with either selenite or tetrathionate broths. However, the relative failure of Rappaport broth in the recovery of *S. typhi* placed severe limitations on its general application.

The results obtained in the present investigation further confirmed the relative superiority of Rappaport's enrichment broth over selenite medium in the recovery of *Salmonella* from both human faeces and sewage. At the same time, they showed that strontium chloride was comparable to Rappaport's medium in that, of the 266 possible recoveries of *Salmonella* from 3336 specimens of human faeces, where the organisms present were insufficient in number to provide direct culture positives, 230 and 223 isolations were respectively made through Rappaport alone and strontium chloride alone.

In the examination of 98 pig faeces and 69 pig glands, 26 and 11 isolations of *Salmonella* were made using strontium chloride and Rappaport medium in conjunction; the isolations through each medium alone were 24 and 11 for the strontium chloride and 19 and 10 for Rappaport, with the faeces and gland specimens respectively. Again, the relative poor showing for selenite F medium was noteworthy.

The 11 isolations of *S. choleraesuis* were made through Rappaport and strontium chloride media alone or in combination, while the complete failure of selenite F in the isolation of this serotype would preclude its use in the sphere of public health and veterinary bacteriology.

In the third trial in the present investigation (Study III), where the recovery of *S. typhi* from the faeces of typhoid fever cases and carriers by the use of selenite F, Rappaport and strontium chloride and strontium selenite enrichment media were under test, it was shown that, with the combined use of strontium selenite and selenite F, no specimen found to contain *S. typhi* by any combination of methods failed to yield the organism. It was also shown that strontium selenite alone failed in the isolation of *S. typhi* from only one of the 46 positive faeces as opposed to 11 failures with selenite F, 21 failures with strontium chloride, 44 failures with Rappaport and 25 failures on direct plating.

In the limited trial with 19 faeces suspected of containing *S. typhi*, tetrathionate broth was instrumental in the recovery from only 14, compared with all 19 by strontium selenite medium.

The isolation of *S. typhi* through strontium selenite from all 12 sewage specimens examined in this study, as compared to seven isolations through selenite F under parallel conditions, provided further evidence of the superiority of strontium selenite over other enrichment broth media for the recovery of *S. typhi* from human faecal matter.

SUMMARY

Two new media, strontium chloride and strontium selenite broths, are described for the enrichment culture of *Salmonella* from human and animal material.

In comparative trials, strontium chloride was found to be comparable to Rappaport medium for the recovery of a wide range of *Salmonella* serotypes from

human faeces and sewage, and from pig faeces and glands. Both were superior to selenite F medium.

Strontium selenite was found to be superior to selenite F in the recovery of *S. typhi* from human faeces and sewage.

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