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Isolation of salmonellas from sewage-polluted river water using selenite F and Muller–Kauffmann tetrathionate

By R. W. S. HARVEY and T. H. PRICE
Regional Public Health Laboratory, University Hospital of Wales, Heath Park, Cardiff

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SUMMARY

Selenite F broth and a modified Muller–Kauffmann tetrathionate broth were investigated using sewage-polluted natural water as inocula. The modification of the tetrathionate medium was necessary as commercial alternatives would not allow multiplication of small numbers of salmonellas.

The wide range of molar concentrations in different tetrathionate broths was emphasized.

Selenite F broth was more efficient than Muller–Kauffmann tetrathionate broth, in our hands, with the material tested. Direct inoculation of the enrichment media was used.

Each of the two media examined had a bias towards selection of certain serotypes. If possible, both enrichment broths should be used to obtain maximum information.

INTRODUCTION

When examining samples containing multiple salmonella serotypes, use of complementary cultural techniques has been briefly discussed (Harvey, 1957; Harvey & Price, 1967). The present communication records results obtained with two different enrichment broths in routine practice. Many of the samples examined contained more than one serotype, but the findings were of interest both in the total number of salmonella isolations and in the significant bias that each contrasted medium had on selection of particular serotypes. An extreme example of such bias has recently been recorded for the isolation of *S. dublin* (Harvey & Price, 1975).

MEDIA

Selenite F broth and tetrathionate broth are the enrichment media used most frequently in Europe for salmonella isolation. Problems of medical, veterinary and food bacteriologists differ and medical microbiologists with clinical responsibility must use a selenite broth to facilitate isolation of *S. typhi* and *Shigella sonnei*. Several different tetrathionate broths are available. They can be conveniently prepared by interaction of 2 N sodium thiosulphate with N iodine solutions to form sodium tetrathionate. In one class of media, neutralization of the two components is incomplete. In the second class (balanced tetrathionate), thiosulphate is

completely or almost completely neutralized by iodine (Knox, Gell & Pollock, 1943; Rolfe, 1946). Within both classes of media, a series of different molar concentrations of sodium tetrathionate is favoured by various authors. In the thiosulphate excess media, molar values may range from 0·018 M (Muller, 1923) to 0·02 M (Knox, Gell & Pollock, 1942). In the balanced tetrathionate class, concentrations vary between 0·015 M (Harvey, 1957, unpublished; Morgan, 1974), 0·03 M (Knox *et al.* 1943), 0·032 M and 0·039 M (Rolfe, 1946). There are, therefore, a minimum of six possible choices of tetrathionate broth available. Recently, a European group of microbiologists (Edel & Kampelmacher, 1969) produced evidence that commercial Muller–Kauffmann tetrathionate broth (Kauffmann, 1930, 1935) functioned well at the incubation temperature of 43° C. (Harvey & Thomson, 1953; Harvey & Price, 1968). The method has become the basis of a standard recommended technique. A Muller–Kauffmann tetrathionate broth was, therefore, included in our routine salmonella isolation. Some modification of the formula was essential as the commercial Muller–Kauffmann tetrathionates advocated (Oxoid CM 343 and modified Oxoid CM 29) were, in our hands, extremely inhibitory to multiplication of small numbers of salmonella (R. W. S. Harvey, unpublished; Harvey, Price & Crone, 1975). At that time we were collaborating with the bacteriological department of Bristol University and an alternative formula prepared from dehydrated Bacto nutrient broth (B3) and fresh ox bile was suggested (A. H. Linton, Personal communication). This medium permitted growth of small numbers of salmonellas and its selectivity incubated at 43° C. was encouraging (Morgan, 1974). We wished to use a medium that would conform to the standards of Stokes (1968) and the modified tetrathionate fulfilled these conditions.

Two enrichment broths, in common use, were thus selected for investigation. The first was selenite F broth, the second a modified Muller–Kauffmann tetrathionate broth. The preparation of these two media has been described elsewhere (Harvey & Price, 1974).

MATERIALS AND METHODS

The material used was sewage-polluted natural water – the River Taff. The sampling point was near Pontypridd. Another study of this river has estimated that at this point the coliform count is in the region of 4×10^5/100 ml. (Smith, 1970).

Two hundred ml. of polluted water were examined in each test. The sample was mixed by shaking. One hundred ml. were added to 100 ml. of double strength selenite F broth and 100 ml. to 100 ml. of double-strength Muller–Kauffmann tetrathionate broth (Harvey & Price, 1974). The inoculated media were incubated at 43° C. in a dry incubator and subcultured to brilliant green MacConkey agar (Harvey, 1956) at 24 and 48 h. Temperature checks were made by a thermometer kept inside the incubator; fluctuation between 42 and 43° C. was permitted. Selective agars were incubated at 37° C. for 24 h. and examined for suspicious colonies. One such colony was picked from each plate for further study and preliminary serotyping. Representative cultures were sent to the Salmonella and Shigella Reference Laboratory for complete serological identification and when necessary, to the Enteric Reference Laboratory for phage-typing.

Table 1. *Salmonella serotypes isolated from the River Taff by means of Selenite F broth and Muller–Kauffmann tetrathionate broth*, 1970–1974

Selenite F	+	+	−		+	+	−		+	+	−
Muller–Kauffmann	+	−	+		+	−	+		+	−	+
Serotype				Serotype				Serotype			
S. agama	3	6	4	*S. enteritidis*; 13	0	1	1	*S. newington*	0	1	0
S. agona	189	163	76	*S. enteritidis*: untypable	4	5	4	*S. newport*	2	2	13
S. alachua	0	1	0	*S. eppendorf*	0	1	0	*S. ohio*	0	1	2
S. amsterdam	0	1	0	*S. give*	0	1	2	*S. oranienburg*	3	54	15
S. anatum	9	19	21	*S. hadar*	3	12	10	*S. panama*	33	32	61
S. binza	0	1	0	*S. haifa*	0	1	2	*S. paratyphi B*; 1	12	68	62
S. bovismorbificans	5	2	5	*S. hartford*	0	1	0	*S. paratyphi B*; 1 var. 1	3	6	0
S. braenderup	0	1	0	*S. havana*	0	0	1	*S. paratyphi B*; 1 var. 7	0	0	1
S. brandenburg	1	14	5	*S. heidelberg*	4	5	10	*S. reading*	3	8	5
S. bredeney	16	6	27	*S. ibadan*	0	3	1	*S. rhydyfelin*	0	1	0
S. california	3	1	1	*S. indiana*	17	52	20	*S. riggil*	0	0	1
S. chester	0	0	2	*S. infantis*	4	15	17	*S. saintpaul*	2	8	14
S. coeln	0	0	1	*S. isangi*	0	0	2	*S. senftenberg*	3	3	1
S. corvallis	0	1	0	*S. java*	0	2	5	*S. stanley*	2	4	6
S. cubana	0	3	2	*S. javiana*	0	0	2	*S. takoradi*	0	3	0
S. derby	20	13	3	*S. kaapstad*	0	1	0	*S. tennessee*	0	1	0
S. dublin	0	2	0	*S. kiambu*	0	1	2	*S. thompson*	1	1	2
S. duisberg	0	0	2	*S. livingstone*	0	1	0	*S. typhimurium*	10	75	26
S. enteritidis; 2	0	2	0	*S. london*	0	0	1	*S. virchow*	2	4	5
S. enteritidis; 4	2	5	0	*S. montevideo*	0	6	33	*S. wien*	0	2	1
S. enteritidis; 8	4	1	2	*S. muenchen*	0	0	1	*S.* − 4, 12: d: −	8	14	11

Phage types of *S. enteritidis* and *S. paratyphi B* are shown after the serotype.

Table 2. *Isolation of different phage types of* S. typhimurium *from Selenite F broth and Muller–Kauffmann tetrathionate broth* 1970–1974

Selenite F broth	+	+	−		+	+	−
Muller–Kauffmann tetrathionate broth	+	−	+		+	−	+
Phage-type				Phage-type			
1	0	5	2	U71	0	0	1
1 var. 5	0	2	2	U121	0	1	0
1a	4	11	4	U129	1	0	2
3a	0	2	0	U149	0	1	0
4	0	4	1	U163	4	0	3
10	0	2	1	U165	0	1	0
14	0	9	0	U193	0	2	0
12a	0	0	1	U206	0	0	1
32	0	3	0	U234	0	1	1
U17	0	3	1	U267	1	7	1
U20	0	3	0	Untypable	0	14	5
U30	0	1	0	Untyped	0	2	0
U65	0	1	0				

Total isolations from selenite F only. 75
Total isolations from Muller–Kauffmann tetrathionate only. 26
Total identifiable phage types from selenite only. 10
Total identifiable phage types from tetrathionate only. 3
For total isolations of *S. typhimurium*, in this study, selenite F broth is more efficient than Muller–Kauffmann tetrathionate broth.
$P < 0{\cdot}001$.

RESULTS

The results are recorded in Tables 1–4. Table 1 shows the distribution of each serotype between the enrichment broths. Samples were paired, which allows simple tests of significance to be used. The test employed was

$$\chi_c^2 = (a-b-1)^2/(a+b),$$

where a and b are the numbers of successes and failures ($a > b$) (Fisher & Yates, 1963). Fifty-three separate serotypes or phage-types were isolated from selenite F and 46 from Muller–Kauffmann tetrathionate, 15 from selenite only and 10 from tetrathionate only. In this analysis, non-concordant results only were used.

In order to keep Table 1 within reasonable limits, phage-types of *S. typhimurium* are not listed. This information, however, is given in Table 2. Twenty-one phage-types were identified from selenite and 14 from tetrathionate. The total isolations irrespective of phage-type obtained from selenite broth only were 75 and from tetrathionate broth only 26. The selenite medium was, therefore, more efficient than the tetrathionate medium in the isolation of this serotype ($P < 0{\cdot}001$).

In Table 1, the incidence of certain other serotypes is distributed unequally between the two enrichment broths. These serotypes are: *S. agona*, *S. bredeney*, *S. derby*, *S. indiana*, *S. montevideo*, *S. newport*, *S. oranienburg* and *S. panama*. The

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Table 3. *Enrichment media bias towards certain* Salmonella *serotypes* S. typhimurium *excluded*

Serotype	Probability value indicating media are equally good
Excess isolations on selenite F	
S. agona	$P < 0{\cdot}001$
S. derby	$P < 0{\cdot}05$
S. indiana	$P < 0{\cdot}001$
S. oranienburg	$P < 0{\cdot}001$
Excess isolations on Muller–Kauffmann tetrathionate	
S. bredeney	$P < 0{\cdot}001$
S. montevideo	$P < 0{\cdot}001$
S. panama	$P < 0{\cdot}01$
S. newport	$P < 0{\cdot}01$

Table 4. *Salmonella isolations from Selenite F broth and Muller–Kauffmann tetrathionate broth irrespective of serotype*

		Muller–Kauffmann tetrathionate		
		+	−	Total
Selenite F	+	692	118	810
	−	41	32	73
	Total	733	150	883

A negative result against one of the two media compared in this table signifies that no salmonellas of any species were isolated from that medium

$$\chi^2 = 36{\cdot}3. \quad P < 0{\cdot}001.$$

probability values that both media are equally efficient in the isolation of these eight salmonellas are given in Table 3.

Table 4 records *absolute* success or failure to isolate salmonellas irrespective of serotype. The period over which these figures were collected was not identical with that from which the data in Table 1 were drawn. Selenite F broth, in this investigation, was the more efficient medium ($P < 0{\cdot}001$).

DISCUSSION

The emphasis in this study is primarily on the bias which two enrichment broths show in selection of salmonella serotypes. This bias may be absolute, or nearly so. Both selenite and tetrathionate broths are reported to be toxic for *S. cholerae-suis* and *S. abortis ovis* (Smith, 1952). Our experience with *S. cholerae-suis* would confirm this. Tetrathionate broth inhibits the growth of *S. paratyphi A* (Banwart & Ayres, 1953), while selenite F broth encourages isolation of *S. typhi* (Hobbs & Allison, 1945) and *S. dublin* (Harvey & Price, 1975). We have drawn attention to selection of serotypes before when recording results of culturing a suspension of tortoise faeces in selenite F and Muller–Kauffmann tetrathionate broth over a

3-year period. The animal belonged to one of the authors so that a follow-up was possible. In that study, 50 salmonella colonies were picked from selective agars subcultured from the two enrichment media (Harvey & Price, 1974). The phenomenon of bias is most likely to be encountered when material contaminated with multiple serotypes is examined as in the current study. Smith (1952), using tissue fluids containing salmonellas as inocula, found selenite to be better than tetrathionate as an enrichment medium. We have had similar experience in the present investigation. Table 4, which only records samples found positive or negative irrespective of serotypes, clearly demonstrates the significant advantage selenite F has over Muller–Kaufmann tetrathionate broth if water samples are inoculated directly into enrichment media without a pre-enrichment stage in an unselective fluid medium.

It must be clearly emphasized that both media studied here supported growth of minimal numbers of salmonellas contrary to the experience of Vassiliadis, Pateraki, Papadakis & Trichopoulos (1974), who used Oxoid (CM 343) Muller–Kauffmann tetrathionate. Roberts, Boag, Hall & Shipp (1975) have also found commercial Muller–Kauffmann a disappointing medium compared with Rolfe A tetrathionate (approximately 0·032 M).

We feel that the bias each medium shows towards selection of certain serotypes has been clearly shown in Tables 1–3. It is an advantage to use the two media in parallel if maximum information is to be obtained from a sample. This is important when attempting to correlate salmonella isolations from the environment with infection in man and animals.

If cheapness is of great importance it would seem that selenite F broth is the best choice for the medical microbiologist, and we have demonstrated here that this medium is also valuable to public-health bacteriologists in recovery of salmonellas from sewage-polluted natural water and elsewhere to the veterinary bacteriologist concerned with the diagnosis of infection caused by *S. dublin* (Harvey & Price, 1975).

We must emphasize that selenite F broth and the Cardiff version of Muller–Kauffmann tetrathionate broth have been compared in the current paper using *direct* inoculation of material into the enrichment broths. Use of a pre-enrichment stage alters the relative efficiency of these two media. This point will be made in a further study.

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REFERENCES

BANWART, G. J. & AYRES, J. C. (1953). Effect of various enrichment broths and selective agars upon the growth of several species of salmonella. *Applied Microbiology* **1**, 296.

EDEL, W. & KAMPELMACHER, E. H. (1969). Salmonella isolation in nine European laboratories using a standardized technique. *Bulletin of the World Health Organization* **41**, 297.

FISHER, R. A. & YATES, F. (1963). *Statistical Tables for Biological, Agricultural and Medical Research*, 6th ed. Longman: Edinburgh.

HARVEY, R. W. S. (1956). Choice of selective medium for the routine isolation of members of the salmonella group. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **15**, 118.

HARVEY, R. W. S. (1957). The epidemiological significance of sewage bacteriology. *British Journal of Clinical Practice* **11**, 751.

HARVEY, R. W. S. & PRICE, T. H. (1967). The examination of samples infected with multiple salmonella serotypes. *Journal of Hygiene* **65**, 423.

HARVEY, R. W. S. & PRICE, T. H. (1968). Elevated temperature incubation of enrichment media for the isolation of salmonellas from heavily contaminated materials. *Journal of Hygiene* **66**, 377.

HARVEY, R. W. S. & PRICE, T. H. (1974). Isolation of salmonellas. *Public Health Laboratory Service Monograph Series*, no. 8, London: H.M.S.O.

HARVEY, R. W. S. & PRICE, T. H. (1975). Studies on the isolation of *Salmonella dublin*. *Journal of Hygiene* **74**, 369.

HARVEY, R. W. S., PRICE, T. H. & CRONE, P. B. (1975). Quality control tests of two salmonella enrichment media using different inocula. *Journal of Hygiene* **74**, 375.

HARVEY, R. W. S. & THOMSON, S. (1953). Optimum temperature of incubation for isolation of salmonellae. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **12**, 149

HOBBS, B. C. & ALLISON, V. D. (1945). Studies on the isolation of *Bact. typhosum* and *Bact. paratyphosum* B. *Monthly Bulletin of the Ministry of Health and the Emergency Public Health Laboratory Service* **4**, 12.

KAUFFMANN, F. (1930). Ein kombiniertes Anreicherungs-verfahren für Typhus- und Paratyphusbazillen. *Zentralblatt für Bakteriologie, Parasitenkunde, Infectionskrankheiten und Hygiene* (I. Abt.: Orig.) **119**, 148.

KAUFFMANN, F. (1935). Weitere Erfahrungen mit dem kombinierten Anreicherungsverfahren für *Salmonella* bazillen. *Zeitschrift für Hygiene und Infectionskrankheiten* **117**, 26.

KNOX, R., GELL, P. G. H., & POLLOCK, M. R. (1942). Selective media for organisms of the salmonella group. *Journal of Pathology and Bacteriology* **54**, 469.

KNOX, R., GELL, P. G. H. & POLLOCK, M. R. (1943). The selective action of tetrathionate in bacteriological media. *Journal of Hygiene* **43**, 147.

MORGAN, J. (1974). Technical factors influencing salmonella surveillance I.M.L.T. Thesis, University Hospital of Wales.

MULLER, L. (1923). Un nouveau milieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. *Comptes Rendus des Séances de la Société de Biologie et de ses Filiales* **89**, 434.

ROBERTS, D., BOAG, K., HALL, M. L. M. & SHIPP, C. R. (1975). The isolation of Salmonellas from British pork sausages and sausage meat. *Journal of Hygiene* **75**, 173.

ROLFE, V. (1946). A note on the preparation of tetrathionate broth. *Monthly Bulletin of the Ministry of Health and the Emergency Public Health Laboratory Service* **5**, 158.

SMITH, H. W. (1952). The evaluation of culture media for the isolation of salmonellae from faeces. *Journal of Hygiene* **50**, 21.

SMITH, H. W. (1970). Incidence in river water of *Escherichia coli* containing R factors. *Nature, London* **228**, 1286.

STOKES, E. J. (1968). In *Clinical Bacteriology*, 3rd ed., p. 306. London: Arnold.

VASSILIADIS, P., PATERAKI, E., PAPADAKIS, J. & TRICHOPOULOS, D. (1974). Evaluation of the growth of salmonellae in Rappaport's broth and in Muller–Kauffmann's tetrathionate broth. *Journal of Applied Bacteriology* **37**, 411.