An improved method of isolating salmonellae from contaminated desiccated coconut

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SYNOPSIS A report is given on results obtained in the examination of desiccated coconut from Ceylon and the Philippines using two or three media in parallel, the aim being to investigate the efficacy of the enrichment medium introduced by Rappaport, Konforti, and Navon (1956). Although the claims of Rappaport *et al.* related only to examination of faeces the Rappaport enrichment medium has been found to give higher recovery rates of salmonella from desiccated coconut than the selenite and tetrathionate media. The differences are so striking as to justify an expansion of this work.

In a report on the diarrhoeal diseases of England and Wales, Joan Taylor (1960) claims that the salmonella carrier rate in the general population in England is 0.29% and Cockburn and Vernon (1961) state that salmonellae were responsible for 61% of the 6,428 cases of food poisoning occurring in Britain in 1960. It is probable, however, that this is a significant underestimate of the true seriousness of the problem. Newell (1959) considers that at present no country in the world can be said to possess either a reasonable picture of the occurrence of this disease within its borders or an organization which has brought it under control. The problem is particularly grave in the developing tropical countries.

It is not difficult to find reasons for the failure accurately to assess the incidence of salmonellosis. With rare exceptions the salmonellae are enteric pathogens, present in the faeces in relatively small numbers amidst great numbers of non-pathogens of close cultural characteristics. In any attempt to identify the few salmonellae the difficulty is to prevent, on the one hand, overgrowth of them by non-pathogens and, on the other hand, to prevent inhibition of salmonella growth by using a too-selective medium aimed at suppressing non-pathogens.

Many media have been introduced for the purpose of allowing growth of salmonellae and at the same time inhibiting that of non-pathogenic enteric bacteria. Through the years the selenite enrichment medium of Leifson (1936) and the tetrathionate broth of Muller (1923), modified by Kauffmann (1930, 1935),

became widely accepted as a reliable combination for the routine isolation of salmonellae from faeces. Important modifications include the modified selenite broth of Hobbs and Allison (1945) and the modified tetrathionate of Knox, Gell, and Pollock (1942), of Rolfe (1946), of Preuss (1949), of Hajna and Damon (1956), and of Lang (1960).

Selenite and tetrathionate media have been widely used for the isolation of salmonellae from egg products, dried milk, poultry food, fish meal, fertilizers, mesenteric glands, sewage, sewer swabs, both with and without modification, depending on the material being examined. North and Bartram (1953) found that the addition of cystine to selenite broth gave improved recoveries with certain egg products, a finding confirmed by Taylor, Silliker, and Andrews (1958). Other suggested modifications of the media for use with foodstuffs include the selenite-brilliant green broth of Stokes and Osborne (1955) and the selenite-sulphapyridine-brilliant green broth introduced by the same authors. Similarly, in the examination of dried foodstuffs, North (1961) used pre-enrichment in lactose broth before enriching in selenite cystine broth and in tetrathionate. On occasion also the pH of the material under test may necessitate modifications of technique, as in the isolation of salmonellae from fertilizers containing superphosphate (Dixon and Wilson, 1960).

In this question of foodstuffs contaminated by salmonellae, desiccated coconut is of much importance. In 1953, in Australia, Kovacs (1953) isolated seven strains of *S. nyborg* and 30 strains of *S. senftenberg* from 146 samples of Papuan coconut.

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Also, in 1955 in Australia, Wilson and Mackenzie reported an outbreak of typhoid fever and of salmonellosis from desiccated coconut imported into Australia from Papua. This led to the virtual ending of the desiccated coconut industry in Papua, leaving Ceylon and the Philippines as the producers of most of the world's supply. However, Kovacs (1959) was able also to isolate salmonellae, including *Salm. paratyphi B*, from nine out of 35 samples of Ceylon coconut and there is increasing awareness that desiccated coconut both from Ceylon and from the Philippines is often infected with salmonellae. Galbraith, Hobbs, Smith, and Tomlinson (1960) reported that they were able to recover salmonellae from 9.0% of 851 samples of desiccated Ceylon coconut: these authors used two 20 to 25 g. samples from each batch, with one of the samples added to 100 ml. of nutrient broth and the other added to 100 ml. of single-strength selenite medium. After overnight incubation at 37°C. the samples were subcultured on to Wilson and Blair and desoxycholate-citrate-agar plates then incubated for another two days and subcultured again if necessary. Suspicious colonies were then picked off for identification. Similarly, Semple, Graham, and Dutton (1961) reviewed the findings of a bacteriological investigation of desiccated coconut discharged at Liverpool during a 10-month period, and reported that of 6,567 samples, 380 (5.8%) were found to harbour salmonellae: in all 36 serotypes were identified, with *Salm. paratyphi B* present in 33 of the samples. Later, Semple, Parry, and Graham (1961) reported three patients who had developed paratyphoid fever from infected coconut. Unfortunately they did not give details of their techniques. However, it is interesting to note that the 9% recovery rate of Galbraith *et al.* (1960) agrees very closely with our recoveries from the same material using selenite and tetrathionate enrichments as a routine, the tetrathionate enrichment having about a double yield in this material compared with the yield using selenite enrichment.

However, none of the above reports gives an exact gauge of the degree of contamination of the imported desiccated coconut: not only are the salmonellae irregularly distributed in any infected coconut but the numbers of salmonellae present are very low and may therefore be missed. Galbraith *et al.* (1960), for example, estimate the approximate numbers to be less than 0.03 per g. The irregularity of the distribution within the bags is well shown in an unpublished experiment in which we re-checked six random samples from each of five bags of desiccated coconut previously reported by us to show no salmonellae: we found that from one bag all six later samples showed salmonellae, two other

bags each showed two of the six samples to be contaminated with salmonellae, and the remaining six samples from each of the last two bags were all again found to be free from salmonellae. Conversely, bags earlier found positive for salmonellae were negative on re-checking.

In view of the unsatisfactory results obtained by selenite enrichment, we began an investigation into the use of other enrichment media in the course of which we tested the Rappaport medium.

Rappaport *et al.* (1956) claim that their medium permits unrestricted development of salmonella yet inhibits the growth of coliform bacteria, due to the contained 4% magnesium chloride and the 0.012% malachite green.

We cannot trace any record of Rappaport enrichment being applied to material other than faeces, and following a short series of experimental trials on desiccated coconut, this method of enrichment was tested alongside our usual enrichment procedures: the results obtained seemed so promising as to justify publication.

MATERIALS AND METHODS

A total of 1,169 samples of Ceylon coconut taken at random from about 12,000 bags, and 416 samples of Philippine coconut from approximately 4,000 bags, were examined during 1960/61. Comparative tests using Rappaport medium (Rappaport *et al.*, 1956) were made on a total of 1,049 samples. The composition of Rappaport medium is as follows:—

Solution A

Bacto tryptone (Difco)	0.5 g.
NaCl	0.8 g.
KH ₂ PO ₄	0.16 g.
Bi-distilled water	100 ml.

Solution B

40 g. of MgCl₂ dissolved in 100 ml. water

Solution C

0.4% solution of Malachite green in distilled water

For use, to each 100 ml. solution A was added 10 ml. of solution B and 3 ml. of solution C. Throughout the investigation the medium was distributed in 150 ml. volumes in 8 oz. wide-neck screw-cap jars. The medium was sterilized by steaming for 30 minutes, and may be held at room temperature for at least one month without deterioration.

The method used to investigate the efficiency of Rappaport medium in detecting salmonella contamination in coconut was to run parallel tests with Rappaport medium, selenite F (Mackie and McCartney formula), and/or tetrathionate broth (Difco). In each test 25 g. of the desiccated coconut was added to 150 ml. of enrichment medium, incubated for 24 to 48 hours and subcultured on to SS (Difco) and bismuth sulphite-agar (Difco) plates.

In all, a total of 1,049 samples was tested in the above

fashion: 757 of these samples were from a total of approximately 8,000 bags of Ceylon coconut and the remaining 292 samples were from a total of 3,000 bags of Philippine coconut.

The series is a relatively small one for a test of this nature but the findings are significant.

RESULTS

The findings are set out in Tables I to III.

TABLE I

RESULTS USING ONLY RAPPAPORT AND SELENITE ENRICHMENT

Enrichment Media	Origin of Coconut	
	Ceylon (534 samples)	Philippines (292 samples)
Rappaport only positive	89	43
Selenite only positive	6	12
Both positive	10	41
Total positive	105	96

TABLE II

RESULTS USING ONLY RAPPAPORT AND TETRATHIONATE ENRICHMENT IN 117 SAMPLES FROM CEYLON

Enrichment Media	Results
Rappaport only positive	14
Tetrathionate only positive	1
Both positive	6
Total positive	21

TABLE III

SUMMARY OF RELATIVE EFFICIENCY¹

	Origin of Coconut	
	Ceylon	Philippines
Rappaport positive, with or without positive findings in other media	93	88
Rappaport negative, one or both of the other media positive	7	12
Selenite positive, with or without positive findings in other media	18	55
Selenite negative, one or both of the other media positive	82	45
Tetrathionate positive, with or without positive findings in other media	27	Not done
Tetrathionate negative, one or both of the other media positive	73	Not done

¹Based on 138 positive samples from Ceylon coconut and 96 positive samples from Philippine coconut.

The serotypes recovered from 1,169 samples of Ceylon coconut are listed below. The numbers in brackets indicate the number of strains recovered from each serotype.

S. paratyphi B (33), *S. typhimurium* (12), *S. thompson* (4), *S. waycross* (13), *S. perth* (22), *S. hvittingfoss* (3), *S. kotte* (4), *S. rubislaw* (1), *S. chittagong* (17),

S. weltevreden (6), *S. bareilly* (18), *S. senftenberg* (9), *S. ferlac* (26), *S. angoda* (9), *S. newport* (7), *S. nchanga* (2), *S. chester* (1), *S. butantan* (1), *S. muenster* (1), *S. chingola* (4), *S. oranienburg* (1), *S. braenderup* (1), *S. san diego* (1), *S. solna* (3), *S. tennessee* (2), *S. Group B* unidentified (2).

In addition *Salmonella senftenberg* (96) and *Salmonella lexington* (1) were recovered from 416 samples of Philippine coconut.

DISCUSSION

From Tables I to III it will be seen that Rappaport enrichment medium gives greatly increased recovery rates compared with the selenite and tetrathionate media. The difference is even more striking than that reported by Collard and Unwin (1958) in the examination of 1,000 consecutive stool cultures: they found Rappaport to be twice as good as the others but commented that in their experience Rappaport enrichment did not seem to be as effective as selenite and tetrathionate media for the isolation of group E salmonella. It may be seen from our results that a wide range of serotypes has been isolated from Rappaport medium, and in contrast to the results of Collard and Unwin, it was highly satisfactory for group E, as a considerable number of strains of different serotypes of this group were isolated. The medium was also particularly useful for the detection of *Salmonella paratyphi B* in coconut.

As will be seen from Table III, with the Ceylon material the Rappaport medium failed in 7% of samples compared with the 82% failure of selenite and the 73% failure of tetrathionate in the total specimens recovered. It is suggested that the 7% of failures in the Rappaport could well be explained by the light inoculum and its irregular distribution throughout the bags but this cannot explain the disturbing failures with the other media. This problem of patchy distribution has also been raised by Anderson and Woodruff (1961).

The superiority of Rappaport medium for enrichment can also be shown by comparison with the findings of Galbraith *et al.* (1960) and of Semple, Graham, and Dutton (1961). Galbraith *et al.* showed a 9% recovery rate from 851 samples of Ceylon coconut and Semple *et al.* from a total of 6,567 samples found 380 positive (5.8%) compared with our findings of 138 positive samples (18%) in 757 specimens. Semple *et al.*, from a series of specimens six times larger than ours, identified a total of 36 serotypes compared with our total of 25 serotypes, and it is of particular interest to note that although the Semple series was larger than ours, we recovered the same number of *Salm. paratyphi B*.

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REFERENCES

- Anderson, K., and Woodruff, P. (1961). *Med. J. Aust.*, **1**, 856.
 Cockburn, W. C., and Vernon, E. (1961). *Mth. Bull. Minist. Hlth Lab. Serv.*, **20**, 160.
 Collard, P., and Unwin, Marion (1958). *J. clin. Path.*, **11**, 426.
 Dixon, J. M. S., and Wilson, F. N. (1960). *Mth. Bull. Minist. Hlth Lab. Serv.*, **19**, 79.
 Galbraith, N. S., Hobbs, B. C., Smith, M. E., and Tomlinson, A. J. H. (1960). *Ibid.*, **19**, 99.
 Hajna, A. A., and Damon, S. R. (1956). *Appl. Microbiol.*, **4**, 341.
 Hobbs, B. C., and Allison, V. D. (1945). *Mth. Bull. Minist. Hlth Lab. Serv.*, **4**, 63.
 Kauffmann, F. (1930). *Zbl. Bakt., I Abt. Orig.*, **119**, 148.
 — (1935). *Z. Hyg. Infekt.-Kr.*, **117**, 26.
 Knox, R., Gell, P. G. H., and Pollock, M. R. (1942). *J. Path. Bact.*, **54**, 469.
 Kovacs, N. (1953). Yearly Report of Public Health Laboratory, Perth, 1953.
 — (1959). *Med. J. Aust.*, **1**, 557.
 Lang, K. (1960). *Zbl. Bakt., I Abt. Orig.*, **180**, 221.
 Leifson, E. (1936). *Amer. J. Hyg.*, **24**, 423.
 Muller, L. (1923). *C. R. Soc. Biol. (Paris)*, **89**, 434.
 Newell, K. W. (1959). *Bull. Wild Hlth Org.*, **21**, 279.
 North, W. R. Jr. (1961). *Appl. Microbiol.*, **9**, 188.
 —, and Bartram, M. T. (1953). *Ibid.*, **1**, 130.
 Preuss, H. (1949). *Z. Hyg. Infekt.-Kr.*, **129**, 187.
 Rappaport, F., Konforti, N., and Navon, Betty (1956). *J. clin. Path.*, **9**, 261.
 Rolfe, V. (1946). *Mth. Bull. Minist. Hlth Lab. Serv.*, **5**, 158.
 —, Graham, A. J., and Dutton, E. M. (1961). *Med. Offr.*, **105**, 59.
 Semple, A. B., Parry, W. H., and Graham, A. J. (1961). *Lancet*, **2**, 364.
 Stokes, J. L. and Osborne, W. W. (1955). *Appl. Microbiol.*, **3**, 217.
 Taylor, Joan (1960). *Bull. Wild Hlth Org.*, **23**, 763.
 Taylor, W. I., Silliker, J. H., and Andrews, H. P. (1958). *Ibid.*, **6**, 189.
 Wilson, M. M., and Mackenzie, E. F. (1955). *J. appl. Bact.*, **18**, 510.

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