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# Enrichment procedures for the isolation of *Salmonella*, *Arizona*, *Edwardsiella* and *Shigella* from faeces

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## SUMMARY

Strontium selenite A broth incubated at 37° C., and strontium chloride B broth incubated at 43° C., have been found an effective combination for the isolation of *Salmonella*, *Arizona* and *Edwardsiella* from human faeces.

Direct plating on deoxycholate citrate agar was superior to enrichment methods for the isolation of *Shigella* species.

Strontium selenite A broth was suitable for the isolation of *Salmonella* and *Arizona* bacteria at both 37° and 43° C.

Strontium chloride B broth incubated at 43° C. was best for the isolation of *Salmonella* and *Edwardsiella*.

Modified bismuth sulphite agar, although superior to D.C. agar for the growth of *Salmonella* and *Arizona* bacteria, was unsuitable for *Edwardsiella* and *Shigella* species.

A considerable difference was observed in the distribution of *Salmonella*, *Arizona* and *Edwardsiella* serotypes isolated from humans in remote areas, when compared with isolations from more densely populated urban and agricultural centres.

Epidemiological and zoogeographical aspects of host–parasite associations between humans, animals and pathogenic Enterobacteriaceae in Western Australia are discussed.

## INTRODUCTION

Strontium hydrogen selenite and strontium chloride have been found effective as selective ingredients in *Salmonella* enrichment media (Iveson & Mackay-Scollay, 1969, 1972). In comparative tests, strontium chloride malachite green broth (strontium chloride M) was found suitable for the isolation of salmonellas from human and animal faeces, foodstuffs, meat processing and abattoir effluents. The medium compared favourably with Rappaport's broth and, except for the isolation of *S. typhi*, was superior to selenite F. Strontium selenite broth was superior to selenite F, tetrathionate, Rappaport and strontium chloride M media for the isolation of *S. typhi*.

Similar results were reported by Chau & Huang (1971), who found strontium selenite broth superior to selenite F for the isolation of *S. typhi*. The method was also suitable for the isolation of the host-specific serotypes, *S. paratyphi* A, *S. paratyphi* B, *S. paratyphi* C, *S. abortusequi* and *S. sendai* from the faeces of

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hospital patients who were admitted without fever or enteric symptoms. Strontium chloride M and Rappaport were satisfactory for the isolation of *S. choleraesuis* but unsuitable for the recovery of *S. typhi*. Strontium chloride M was also found suitable for the isolation of *Salmonella* from bovine specimens (Miller, 1971).

In further investigations it was shown that the selective qualities of strontium chloride M broth were only slightly reduced when the malachite green dye was omitted, furthermore the modified medium (strontium chloride B broth) was found suitable for the isolation of *Edwardsiella tarda*, as well as *Salmonella* and *Arizona* bacteria (Iveson, 1971). The medium was well suited to the elevated temperature technique, and multiple salmonella serotypes were recovered from abattoir effluents using strontium chloride B broth incubated at 43° C. (Iveson & Mackay-Scollay, 1972).

In the present investigations, comparisons of media have been undertaken to evaluate the performance of strontium selenite A and strontium chloride B enrichment media, for the isolation of *Salmonella*, *Arizona*, *Edwardsiella* and *Shigella* from human faeces. Specimens were collected from both urban and remote areas and the techniques involved and results obtained are presented in this report.

## MATERIALS AND METHODS

### *Specimens*

A total of 12,807 faeces specimens were examined from patients with gastro-enteritis or from selected contacts throughout Western Australia. Faeces samples of 2–10 g. were collected into 10 ml. volumes of Sachs (1939) faeces transport medium, and occasionally into dry sterile bottles. Approximately 5000 of the samples were collected in the sparsely populated semi-tropical Kimberley region in the far north of Western Australia, and were transported by air to the central laboratories. The transit period varied from 1 to 3 days.

### *Culture procedure*

Faeces samples were mixed with the transport medium and inoculated direct on Oxoid deoxycholate citrate (D.C.) agar, and approximately 1·0 ml. was added to 10 ml. volumes of the particular enrichment media used in a series of six complementary studies.

The first, study I, involved the testing of 4958 faeces specimens using direct culture on D.C. agar, strontium selenite broth prepared with potassium dihydrogen phosphate, and strontium chloride B enrichment broth incubated at 37° C. Study II was directed particularly to investigate the performance of strontium chloride B broth incubated at 43° C., and a total of 1950 faeces samples were examined by the elevated-temperature technique, as well as by the methods used in the first study.

Study III was undertaken to evaluate the performance of strontium selenite broth prepared with di-sodium hydrogen phosphate (strontium selenite A broth). A total of 5465 faeces specimens were examined using strontium selenite A broth incubated at 37° C. and strontium chloride B broth incubated at 43° C. In study IV

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particular attention was directed to assess the performance of strontium selenite A and strontium chloride B, when used in parallel with G.N. broth (B.B.L.).

Studies V and VI involved the repeat testing of selected faeces samples from which Salmonella, Arizona or Edwardsiella species had been previously isolated. The repeat examinations were performed on specimens stored at room temperature for periods of 7–14 days in the laboratory. In study V, 131 positive samples were repeat-tested by direct culture and strontium selenite, strontium chloride B, strontium chloride M and selenite F (Oxoid) incubated at 37° C, and in the final study, 140 positive specimens were repeat-tested using strontium selenite A, strontium chloride B and Rappaport enrichment media incubated at 37° and 43° C.

## *Media*

*Strontium selenite A enrichment broth*

| | |
|---|---|
| Bacto tryptone (Difco) | 0·5 g |
| Sodium chloride | 0·8 g |
| Di-sodium hydrogen phosphate ($Na_2HPO_4$) | 0·05 g |
| Strontium hydrogen selenite (Ajax) | 0·2 g |
| Distilled water | 100 ml |

The strontium hydrogen selenite, $Sr\ (HSeO_3)_2$, was dissolved in water without heating, the remaining ingredients were added, and the medium distributed in 10 ml. volumes and sterilized by steaming for 30 min. The pH was 6·8 and did not require adjustment. The medium remained stable during storage at room temperature for periods up to 6 months.

*Other enrichment broth media*

The strontium chloride B, Rappaport and selenite F media were prepared as reported by Iveson (1971). The strontium selenite and strontium chloride M broths were prepared as reported by Iveson & Mackay-Scollay (1972). Gram-negative broth (B.B.L.) was prepared as recommended by the manufacturer.

Subcultures from the enrichment broths were performed after 18–24 hr incubation, on D.C. agar (Oxoid) and modified bismuth sulphite agar (Iveson, 1971). An average of three suspect colonies were selected from each of the plating media, and examined biochemically and serologically.

## RESULTS

*Salmonella* organisms were recovered from 1042 faeces samples, *Arizona* from 11, *Edwardsiella* from 40 and *Shigella* from 738 specimens.

Direct culture procedures recovered 315 (30%) *Salmonella*, 1 (9%) *Arizona*, 9 (23%) *Edwardsiella* and 702 (95%) *Shigella*, whereas enrichment methods recovered 1042 (100%) *Salmonella*, 11 (100%) *Arizona*, 37 (93%) *Edwardsiella* and 139 (18%) *Shigella*. The relative efficiency of the direct and enrichment culture methods used are detailed in Table 1.

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Table 1. *Relative efficiency of strontium selenite and strontium chloride* B *Enrichment in four experiments in the isolation of* Salmonella, Arizona, Edwardsiella *and* Shigella *from* 12,807 *faeces samples*

| Expt. and no. of samples | Culture method | Temp. (C.) | Species and isolations: *Salmonella* | | *Arizona* | | *Edwardsiella* | | *Shigella* | |
|---|---|---|---|---|---|---|---|---|---|---|
| I (4958) | Direct | 37° | 121 | (334) | 1 | (1) | 6 | (21) | 393 | (406) |
| | Strontium chloride B | | 267 | | 1 | | 20 | | 52 | |
| | Strontium selenite | | 289 | | 1 | | 0 | | 21 | |
| II (1950) | Direct | 37° | 68 | (287) | 0 | (0) | 0 | (7) | 75 | (76) |
| | Strontium chloride B | | 197 | | 0 | | 7 | | 7 | |
| | Strontium selenite | | 198 | | 0 | | 0 | | 0 | |
| | Strontium chloride B | 43° | 257 | | 0 | | 6 | | 3 | |
| III (5465) | Direct | | 112 | (379) | 0 | (10) | 3 | (9) | 212 | (229) |
| | Strontium selenite A | 37° | 307 | | 10 | | 0 | | 53 | |
| | Strontium chloride B | 43° | 324 | | 1 | | 7 | | 15 | |
| IV (434) | Direct | 37° | 14 | (42) | 0 | (0) | 0 | (3) | 22 | (27) |
| | Strontium selenite A | | 37 | | 0 | | 0 | | 10 | |
| | G.N. Broth | | 18 | | 0 | | 0 | | 6 | |
| | Strontium chloride B | 43° | 37 | | 0 | | 3 | | 3 | |
| Totals (12,807) | | | | (1042) | | (11) | | (40) | | (738) |

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Table 2. *Relative efficiency of strontium selenite, strontium chloride, Rappaport and selenite* F *for the isolation of* Salmonella, Arizona *and* Edwardsiella *from* 271 *faeces specimens*

| Expt. | Samples | Culture method | Temp. (C.) | Species and isolations | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Salmonella* | | *Arizona* | | *Edwardsiella* | |
| V | 131 | Direct | 37° | 30 | | 0 | | 3 | |
| | | Strontium selenite | 37° | 80 | | 1 | | 0 | |
| | | Strontium chloride B | 37° | 70 | (100) | 0 | (1) | 13 | (13) |
| | | Strontium chloride M | 37° | 70 | | 0 | | 0 | |
| | | Selenite F | 37° | 55 | | 0 | | 0 | |
| VI | 140 | Direct | 37° | 26 | | 0 | | 2 | |
| | | Strontium selenite A | 37° | 90 | | 4 | | 0 | |
| | | Strontium chloride B | 37° | 76 | | 0 | | 5 | |
| | | Rappaport | 37° | 91 | (113) | 2 | (7) | 0 | (6) |
| | | Strontium selenite A | 43° | 98 | | 6 | | 0 | |
| | | Strontium chloride B | 43° | 95 | | 1 | | 5 | |
| | | Rappaport | 43° | 77 | | 0 | | 0 | |
| Totals | 271 | | | | (213) | | (8) | | (19) |

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*Salmonellas*

In Expts. I and II there was no significant difference in isolations by strontium chloride B and strontium selenite broths incubated at 37° C. In Expt. II the isolations obtained by strontium chloride B incubated at 43° C. were significantly greater than those by either strontium chloride B or strontium selenite incubated at 37° C.

In Expts. III and IV there were no significant differences in isolations by strontium selenite A incubated at 37° C. and strontium chloride B broth incubated at 43° C.

*Arizonas*

Only 11 strains were isolated, and of these 10 were recovered in Expt. III during a single outbreak of infection associated with Arizona 26:26–25. Strontium chloride B isolated only 1 strain, whilst strontium selenite A incubated at 37° C. isolated 10 strains.

*Edwardsiellas*

A total of 40 isolations were obtained – 9 by direct plating and 37 by enrichment in strontium chloride B broth incubated at 37° or 43° C. There were no Edwardsiella isolations by any other enrichment broth.

*Shigellas*

In the four experiments direct culture on D.C. agar was greatly superior to enrichment methods. In a total of 738 Shigella isolations, 599 were recovered by direct culture alone, and 103 by both direct and enrichment culture. A total of 36 (4·9 %) isolations were recovered exclusively by enrichment methods. In Expts. I and II the strontium selenite and strontium chloride B enrichment methods increased the direct culture isolation's total from 468 to 482 (3 %), compared with an increase of 234–254 (8·5 %) by the strontium selenite A broth used in Expts. III and IV. In Expt. IV two Shigella isolations were recorded exclusively from G.N. broth and three from strontium selenite A broth.

Multiple *Salmonella* infections were detected in 71 (7 %) positive specimens – 13 samples yielded 3 serotypes, and 58 samples 2 serotypes, on a single examination. *Salmonella* and *Shigella* were recovered together from 22 samples, *Salmonella* and *Edwardsiella* from 9, *Salmonella* and *Arizona* from 5, and *Shigella* and *Edwardsiella* species from 2 samples. A total of 5 specimens also yielded 2 *Shigella* serotypes.

Most of the mixed *Salmonella* infections, and all infections by *Arizona* and *Edwardsiella*, were diagnosed in humans living in remote areas. The geographical distribution and relative frequency of *Salmonella*, *Arizona*, *Edwardsiella* and *Shigella* isolations recovered from the more densely populated urban and agricultural centres located south of the 28° latitude, and in the remote sparsely populated northern regions of Western Australia, are shown in Table 3.

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Table 3. *Distribution and relative frequency of* Salmonella, Arizona, Edwardsiella *and* Shigella *isolations in southern and remote northern areas of Western Australia*

| Serotype | Isolations from Southern areas | Isolations from Northern areas | Serotype | Isolations from Southern areas | Isolations from Northern areas |
|---|---|---|---|---|---|
| *S. abony* | 0 | 12 | *S. paratyphi* A | 1 | 0 |
| *S. adelaide* | 14 | 28 | *S. paratyphi* B | 6 | 1 |
| *S. anatum* | 11 | 8 | *S. poona* | 1 | 4 |
| *S. bahrenfeld* | 0 | 36 | *S. potsdam* | 3 | 0 |
| *S. ball* | 0 | 1 | *S. ramat-gan* | 0 | 2 |
| *S. bleadon* | 0 | 2 | *S. rubislaw* | 0 | 15 |
| *S. bovismorbificans* | 46 | 6 | *S. saintpaul* | 6 | 10 |
| *S. bredeney* | 1 | 3 | *S. senftenberg* | 0 | 16 |
| *S. brisbane* | 0 | 1 | *S. singapore* | 2 | 0 |
| *S. bukavu* | 0 | 3 | *S. taksony* | 0 | 1 |
| *S. charity* | 0 | 3 | *S. tennessee* | 2 | 32 |
| *S. chester* | 33 | 44 | *S. treforest* | 0 | 3 |
| *S. derby* | 38 | 7 | *S. typhi* | 2 | 0 |
| *S. eastbourne* | 0 | 11 | *S. typhimurium* | 266 | 44 |
| *S. emmastad* | 0 | 6 | *S. urbana* | 0 | 3 |
| *S. enteritidis* | 3 | 1 | *S. wandsbek* | 0 | 7 |
| *S. fremantle* | 0 | 10 | *S. wandsworth* | 0 | 37 |
| *S. gaminara* | 0 | 1 | *S. welikade* | 0 | 5 |
| *S. give* | 10 | 6 | *S. zehlendorf* | 0 | 1 |
| *S. havana* | 27 | 13 | *S. species* | 0 | 8 |
| *S. hvittingfoss* | 0 | 23 | A. 9a9b:29:31 | 0 | 1 |
| *S. jangwani* | 0 | 14 | A. 26:24:25 | 0 | 1 |
| *S. java* | 0 | 3 | A. 26:26:25 | 0 | 9 |
| *S. kisarawe* | 0 | 1 | *E. tarda* | 0 | 40 |
| *S. kottbus* | 0 | 1 | *Sh. flexneri* I | 27 | 4 |
| *S. lansing* | 0 | 3 | *Sh. flexneri* II | 22 | 10 |
| *S. lexington* | 2 | 0 | *Sh. flexneri* III | 21 | 0 |
| *S. litchfield* | 0 | 5 | *Sh. flexneri* IV | 126 | 167 |
| *S. livingstone* | 2 | 1 | *Sh. flexneri* VI | 101 | 136 |
| *S. manila* | 0 | 1 | *Sh. flexneri* X | 3 | 1 |
| *S. muenchen* | 57 | 77 | *Sh. flexneri* Y | 2 | 22 |
| *S. newbrunswick* | 2 | 5 | *Sh. boydii* IV | 5 | 9 |
| *S. newington* | 1 | 1 | *Sh. schmitzii* | 6 | 5 |
| *S. ohlstedt* | 0 | 3 | *Sh. sonnei* | 54 | 22 |
| *S. oranienburg* | 13 | 32 | Totals | 922 | 998 |
| *S. orientalis* | 2 | 0 | | | |
| *S. orion* | 4 | 10 | | | |

The results of the repeat tests performed on samples found positive during routine testing are detailed in Table 2. In Expt. V, strontium selenite broth prepared with potassium dihydrogen phosphate was superior to strontium chloride B, strontium chloride M and selenite F, for the isolation of *Salmonella* when samples were incubated at 37° C. The three strontium media were each considerably better than selenite F.

In Expt. VI strontium selenite A and Rappaport broth were both superior to strontium chloride B medium for the recovery of *Salmonella* when enrichment media were incubated at 37° C., but in tests at 43° C. strontium selenite A and

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strontium chloride B were considerably better than Rappaport's medium. Strontium selenite A was best for the recovery of *Arizona* species.

Strontium chloride B was the only enrichment procedure to recover *Edwardsiella* in the repeat tests, and was superior to direct culture when samples were incubated at 37° and 43° C.

## DISCUSSION

Salmonella enrichment media have been designed with the aim of modifying a selected portion of a microbial-ecosystem and achieving, at least for a limited period of time, a cultural schema suitable for the rapid multiplication of the desired bacterial species. At the same time, during the incubation period, undesired competitors must be eliminated or their growth suppressed.

The problems of achieving an efficient single enrichment or plating medium for the isolation of *Salmonella*, *Arizona*, *Edwardsiella* and *Shigella* organisms, has been complicated by the cultural diversity of the desired species, the close association of the culturally similar non-pathogenic Enterobacteriaceae and, in mixed infections, by competition between the desired species which has reduced the chances of detecting the full range of pathogens.

An early insight into the problems of isolating different varieties of bacteria was revealed by Beijerinck (1901), who observed that under the selective conditions of a single enrichment process, it was impossible in most experiments to achieve a relative increase in one species without leading to a disappearance of other organisms. It was also observed that different bacterial species were dominant at different stages of the enrichment culture, however, it was noted that the method made it possible to isolate a large variety of bacteria that were adapted to different environmental conditions.

Salmonella bacteria have been isolated from a wide range of vertebrate hosts, foodstuffs, and contaminated environments, and in an ever increasing species approximately 1600 serotypes have been identified. It has been remarkable in a species of such diversity that only a few serotypes have failed to grow in selenite F or tetrathionate enrichment media, which were introduced primarily to facilitate the isolation of *S. typhi*. Significantly, it has been those serotypes infecting a restricted host range, e.g. *S. typhi*, *S. paratyphi A*, *S. choleraesuis* and *S. abortus-ovis*, that have proved difficult to isolate. On the other hand, if the selective cultural requirements of an important host-specific strain have been met, it has usually followed that the serotypes capable of infecting a wider host range have also grown satisfactorily.

Selenite F broth has been widely accepted as a reliable medium for the isolation of *S. typhi* and the majority of food poisoning serotypes from humans and animals. However, both selenite F and tetrathionate enrichment media have been frequently modified in attempts to improve their performance, and in a recent comprehensive study of the bacterial aetiology of human diarrhoea (Sakasaki, Tamura, Prescott & Bencic, 1971) Rappaport's medium, G.N. broth, and selenite cystine enrichment, together with direct culture on S.S. agar, were preferred for the isolation of *Salmonella*, *Arizona* and *Edwardsiella*.

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With the exception of *S. typhi*, Rappaport broth has been superior to both selenite F and tetrathionate for the isolation of *Salmonella* (Collard & Unwin, 1958; Iveson, Kovacs & Laurie, 1964; Hooper & Jenkins, 1965; Iveson & Kovacs, 1967; Iveson & Mackay-Scollay, 1969, 1972). However, the method was found unsuitable for the isolation of *S. dublin* and *S. pullorum* from animals and was not suited to the elevated temperature of incubation (Harvey & Price, 1968).

Enrichment culture methods combined with subculture to solid plating media have been superior to direct-plate cultures for the isolation of the majority of *Salmonella* serotypes. The increase achieved by the selenite F and tetrathionate combination has varied from 33 % (Cook, Frisby & Jebb, 1951) to 164 % (Galton & Quan, 1944). The selenite F and Rappaport combination has increased the direct culture isolations 144 % (Hooper & Jenkins, 1965). Salmonella isolations were increased from 238 to 583 (145 %) by Iveson & Kovacs (1967), who used Rappaport, selenite F and tetrathionate enrichment, and from 34 to 108 (218 %) by Chau & Huang (1971), who used strontium selenite, strontium chloride M, Rappaport and selenite F enrichment, as well as direct plating on S.S. agar.

In the present investigation enrichment methods increased the *Salmonella* isolations obtained by direct plating from 315 to 1042 (231 %), *Arizona* from 1 to 11 (1000 %), *Edwardsiella* from 9 to 40 (344 %), and *Shigella* from 702 to 738 (5 %).

The combination of strontium selenite A broth incubated at 37° C. and strontium chloride B enrichment broth incubated at 43° C. increased *Salmonella* isolations from 112 obtained by direct plating to 379 (238 %), *Arizona* isolations were increased from 0 to 10, *Edwardsiella* 3 to 9 (200 %) and *Shigella* from 212 to 229 (8 %). The new combination was also superior to G.N. broth for the recovery of *Salmonella*, *Edwardsiella* and *Shigella*.

Salmonella infections with multiple serotypes were detected in single samples submitted from patients in both urban and remote areas. A total of 42 (59 %) samples from remote areas yielded more than one serotype, compared with 29 (41 %) from urban centres.

*S. typhimurium* was the most common serotype recovered from humans in urban areas, but there was a strong bias against the distribution of the organism in mixed infections. For example, *S. typhimurium* with 310 (28 %) isolations was recovered along with other serotypes in only 17 (24 %) mixed infections, whereas *S. derby* with only 45 (3·5 %) total isolations was associated with multiple infections on 18 (25·3 %) occasions. A high frequency of *S. derby* in mixed infections has also been observed by Cherubin & Winter (1970), who suggested that *S. typhimurium* was more frequently involved in person to person transmission, while other serotypes, including *S. derby*, were directly or indirectly foodborne. In the present study, *S. typhimurium* occurred sporadically throughout the 2-year period, and was not directly associated with infected foodstuffs. On the other hand, infected meat products and food-handlers were directly implicated in an outbreak of *S. havana* infections, and *S. derby* was recovered in four of the six mixed infections diagnosed during the outbreak. The two serotypes were also frequently detected in effluent samples from the abattoir supplying the meat centre.

It was also observed that there was a marked difference in the geographical

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distribution of serotypes. In the more densely populated and agricultural areas south of the 28° latitude a total of 551 *Salmonella* isolations and 26 serotypes were identified, and of these *S. typhimurium* with 266 (48 %) isolations was the predominant serotype. By contrast, in a total of 571 isolations and 50 serotypes from remote northern areas, only 44 (8 %) *S. typhimurium* isolations were recorded.

A total of 50 (89 %) *Salmonella* serotypes were identified from remote areas, furthermore 30 (54 %) *Salmonella* serotypes and all the Arizonas and Edwardsiellas were isolated exclusively in these areas, a remarkable distribution for a sparsely inhabited region almost 600,000 square miles in area and containing only 50,000 (5 %) of the state's population. There was also a close relationship between the *Salmonella, Arizona* and *Edwardsiella* serotypes isolated from Aboriginal patients in remote areas, and strains that have been recovered from reptiles in Western Australia (Iveson, Mackay-Scollay & Bamford, 1969; Iveson, 1971; Iveson & Mackay-Scollay 1972). Surprisingly, 25 of the *Salmonella* serotypes isolated from humans in remote areas have not been detected in humans or domesticated animals in the more closely settled urban and agricultural centres located in southern areas of the state. Differences in the distribution patterns of *Salmonella* and *Arizona* serotypes recovered from reptiles, when compared with isolations from foodstuffs, humans and sewage from nearby urban areas, have been reported by Zwart, Poelma & Strik (1970), who commented that reptiles carry their own pattern of *Salmonella* and *Arizona*, characterized by an impressive spread over a great variety of serotypes.

The distribution pattern of *Salmonella* serotypes identified in the present investigation has suggested that differences between the serotypes occurring in humans and reptiles are less marked in geographically isolated regions inhabited by indigenous human and animal populations, particularly in areas where the effects of agriculture are minimal. Intensive agriculture, including irrigation processes and large-scale mining centres, have only recently been established in the northern Kimberley and Pilbara regions of Western Australia.

The relative absence of *S. typhimurium*, together with increased isolations of salmonellas classified serologically in numerically higher somatic groups, and strains of subgenus II, III and IV in remote areas has been observed previously in Australia (Iveson *et al.* 1969). It was also reported that the geographical distribution of serotypes suggested that many species were established in Australia before the invasion of the region by European man, his introduced fauna and parasites.

Arizona infections were diagnosed mainly in young Aboriginal children, but on one occasion *Arizona* 26:26:25 was isolated from a nurse who had attended an infected child. The predominant symptoms have been acute gastro-enteritis with or without fever, and in several cases admission to hospital has been necessary to effect treatment. Arizona 26:26:25 and Arizona 26:24:25 have also been isolated from humans, reptiles or animal products from the Indian subcontinent (Bhat, Shanthakumari & Myers, 1969; Harvey & Price, 1962; Kaura *et al.* 1971; Sharma, Kaura & Singh, 1970).

Edwardsiellas were isolated from both children and adults with symptoms of gastro-enteritis and occasionally from healthy individuals. From 29 patients

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edwardsiellas alone were isolated, whereas from 9 patients salmonellas were also isolated and from 2 shigellas. A total of 11 patients were infected, in addition to *Edwardsiella*, with *Giardia lamblia*, *Ancylostoma duodenale*, *Strongyloides stercoralis* or *Hymenolepis nana*.

Of the 40 infections 34 (85 %) were diagnosed in Aboriginal patients and 8 infections occurred in hospital visitors or their children.

The *Edwardsiella* genus has shown considerable serological diversity and, in the 2 years since strontium chloride B broth was introduced 50 serotypes, including 10 serotypes isolated from humans in the present study, have been identified so far, in a total exceeding 500 isolations in Western Australia.

The mechanisms underlying the selective action of strontium hydrogen selenite and strontium chloride, have not been fully investigated. However, strontium selenite A broth has shown a pattern of selenite reduction similar to selenite F. The reduction of selenite to selenium has been slightly less intense, but the characteristic brick-red colour associated with bacterial growth was clearly evident after 16–24 hr. incubation. A concentration of 0·2 % strontium hydrogen selenite and 0·05 % di-sodium hydrogen phosphate has been found optimum for the recovery of *Salmonella* and *Shigella*, compared with 0·4 % sodium hydrogen selenite as used in selenite F. It was found unnecessary to incorporate a fermentable carbohydrate. Both media contained Bacto tryptone and isotonic sodium chloride.

Strontium selenite broth was first prepared using potassium dihydrogen phosphate (Iveson & Mackay-Scollay, 1969), and the pH was adjusted to 6·8 with sodium hydroxide. However, it was observed that improved results were obtained with the strontium selenite A modification, furthermore, at the reduced concentration of 0·05 % di-sodium hydrogen phosphate, the optimum pH was achieved without further adjustment.

The ability of certain chloride salts to favour selectively the growth of certain salmonellas was first reported by Gray (1931), who used lithium chloride added to peptone water, and Rappaport, Konforti & Navon (1956), who used a combination of magnesium chloride and malachite green. The dye was added to increase the selectivity of the medium but, although suppressing the growth of undesired competitors, the dye rendered the medium unsuitable for the isolation of *S. typhi*. It was found essential to use Bacto tryptone in order to recover the more fastidious *S. paratyphi* A. Bacto tryptone has been used continuously in the strontium chloride B medium, and has been found particularly effective in promoting the growth of *Salmonella* and *Edwardsiella*. The pH 5·3 combined with the 3·6 % strontium chloride concentration has also efficiently suppressed coliforms and *Proteus* species, and the selective action was further improved when samples were incubated at 43° C.

Strontium selenite A and strontium chloride B enrichment media have replaced the enrichment procedures previously used in our laboratories, and have been found an improved combination for the isolation of *Salmonella*, *Arizona*, *Edwardsiella* and *Shigella* from humans, animals and environmental samples. The media have efficiently suppressed the non-pathogenic Enterobacteriaceae and have responded favourably to the elevated temperature of incubation. It has been

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found unnecessary to add carbohydrates, accessory growth factors, dyes or antibiotics, and both media have been stored ready for use at room temperature for several months without adverse effects.

Throughout the investigation, strontium chloride B broth incubated at 37° or 43° C. and subcultured to D.C. agar was found best for the isolation of *Edwardsiella* species. Occasionally, however, strains isolated by direct plating were not isolated after enrichment.

Strontium selenite A broth incubated at 37° or 43° C. and subcultured to modified B.S. agar was best for the isolation of *Arizona* species.

Modified B.S. agar was greatly superior to D.C. agar for the isolation of *Salmonella* and *Arizona* species. *Edwardsiella* and *Shigella* species were, however, isolated exclusively on D.C. agar.

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