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The examination of samples infected with multiple salmonella serotypes

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INTRODUCTION

In diagnostic medical bacteriology the object is to isolate and identify a pathogenic organism. The result is used to aid diagnosis and control infection. In enteric fever or salmonella food poisoning one does not normally expect to find more than one pathogen in a sample, but it sometimes happens that several serotypes can be demonstrated in a patient (Hormaeche, Surraco, Peluffo & Aleppo, 1943; Harvey, Price, Davis & Morley-Davies, 1961; Taylor, 1960).

In the study of the epidemiology of salmonella infections, however, it is advisable to attempt to demonstrate the presence of all serotypes in a foodstuff as salmonella infections are spread by food. If this is not done, the epidemiological picture may be confused (Winkle & Rohde, 1958). Salmonellas can be isolated from a great variety of both human and animal foods and the latter are of interest because of the possibility that salmonella infections can be initiated and maintained in animals through contamination of their food-stuffs. The extent of this problem is debatable. Painstaking care is needed to succeed in isolating all, or nearly all, of the species present. An appreciation of the technical difficulty is important because one particular species might grow quickly in culture and appear to be the dominant pathogen, or even the only pathogen present. This paper sets out to discuss several methods of approaching the problem of multiple salmonella contamination which we have found helpful.

TECHNIQUES STUDIED

The methods examined were:-

(1) Use of colonial character to isolate certain serotypes.

(2) Picking of large numbers of suspicious colonies from a selective agar.

(3) Use of multiple subculture from selenite F broth (Harvey, 1957).

(4) Splitting the sample into several equal parts and selectively culturing each part in selenite F broth.

(5) Use of agglutinating sera to remove serotypes from a mixture in an orderly and premeditated manner (Harvey & Price, 1962).

(6) Use of a physical or chemical method to encourage the growth of one serotype rather than another. Here we should also consider the avoidance of a method that will effectively discourage the isolation of certain serotypes which grow less abundantly than the majority.

METHODS

1. *Use of colonial character to isolate certain serotypes*

Most salmonellas usually produce colonies on solid media which are indistinguishable from each other. A few serotypes, however, have colonies which *may* be characteristic on certain selective agars.

Such organisms are: *S. paratyphi B* (Schleimwall-Versuch, Müller 1910), *S. pullorum* (very small characteristic colonies on brilliant green MacConkey agar), *S. dublin* (small colonies on brilliant green MacConkey agar, normal-sized colonies on S.S. agar and deoxycholate citrate agar and often marked inhibition of growth on Wilson and Blair's agar). *S. typhi* sometimes, but not invariably, produces characteristic colonies on Wilson and Blair's medium and brilliant green MacConkey agar. The recognition of such minor degrees of differentiation can aid considerably in the search for these serotypes in difficult materials. When other serotypes are also present in such samples, all such assistance is extremely valuable. Colonial differences between certain salmonellas have been discussed in some detail by Stokes & Bayne (1957). The number of serotypes mentioned in this paper could be extended from our own experience.

Familiarity with the colonies of *S. pullorum* came with the weekly examination of many samples of Australian frozen egg in 1956 and 1957. We should hesitate to accept the implication that many medical bacteriologists are unfamiliar with the culture of this serotype (Lancet, 1965). Report (1958) records the fact that more than fifteen medical laboratories co-operated in the examination of egg products of various origins. From nearly 20,000 samples, 2414 strains of *S. pullorum* were isolated. We do not think that many infections in man are due to this serotype in South Wales and our close liaison with the local veterinary laboratory encourages us in this belief.

S. dublin has been searched for in the local abattoirs over the last decade and its frequent successful isolation is largely due to the recognition of its cultural peculiarities. On one occasion a single Moore's swab from an abattoir (Moore, 1948; Harvey & Phillips, 1961) produced colonies on brilliant green MacConkey agar (Harvey, 1956) of two salmonella serotypes: *S. dublin* and *S. typhimurium*. The colonial characters of the two varieties appeared distinctive, *S. dublin* colonies being much smaller than those of *S. typhimurium*. To test our ability to recognize *S. dublin* visually we picked 24 characteristic colonies for identification. Of these 20 were found to be *S. dublin*.

Polluted water supplies are examined weekly in Cardiff for salmonellas and we always incubate the brilliant green MacConkey plates to encourage slime-layer development on *S. paratyphi B* colonies. This usually allows differentiation between *S. paratyphi B* and other salmonellas growing on the same plate (Harvey, 1956). It also sometimes allows the recognition of slime-layer-negative strains of *S. paratyphi B*. The isolation of such organisms is of interest (Wilson & Miles, 1964*a*). The majority of isolations of *S. paratyphi B* from abattoirs and meat markets in South Wales have been slime-layer-negative. All the isolations from Indian crushed bone have also failed to produce slime layers. One recent culture of *S. paratyphi B*

from meat and bone meal said to be of British origin was slime-layer-positive. Outbreaks of gastro-enteritis in man caused by slime-layer-negative *S. paratyphi B* have been recorded recently (Report, 1964: Kingsley Smith & Thomas, 1966) and the subject of animal to human spread has been discussed in the past (Medical Officer, 1951). *S. paratyphi B* is not to be regarded as entirely host-specific for man (Buxton, 1957).

Sewage is so frequently contaminated with many salmonellas that a search for a single variety can be difficult and time-consuming. If foci of *S. paratyphi B* are being surveyed in the sewage of a large city (Harvey & Phillips, 1955) then the slime layer test is a necessity.

Although the colonial characters of other salmonellas are often indistinguishable from each other, this is not always the case. Sometimes on Wilson and Blair's medium different serotypes can be picked by differences in colony size and appearance. This has also been noted by Arnold (1956). This phenomenon can be useful in the examination of sewage, meat, natural water and animal feeding-stuffs.

2. *Picking and identifying large numbers of suspicious colonies from a selective agar*

This is the most usual method of examining specimens containing multiple serotypes. It has worked well in the hands of Hormaeche *et al.* (1943) and Juenker (1957). It is also applicable to the isolation of multiple phage-types of *S. paratyphi B* from a single patient (Sloan, Wilson & Wright, 1960).

Table 1. *Serotype distribution in* 50 *colonies picked from Wilson and Blair medium*

Serotype	no. of colonies identified as this serotype
S. anatum	11
S. bronx	1
S. jodhpur	5
S. karachi	10
S. kirkee	9
S. oranienburg	13
S. richmond	1

In our earlier work on the isolation of salmonellas from imported crushed bone we used this method, picking colonies to the water of condensation of small agar slopes in bijou bottles. These slopes were incubated for 6 hr. when the turbidity in the water of condensation was usually sufficient for slide agglutination to be attempted with H agglutinating sera. The picks were made direct from Wilson and Blair plates with a straight platinum wire. Contamination with other organisms was seldom experienced. A single example suffices as illustration. A subculture was made to Wilson and Blair agar from crushed bone cultured in selenite F broth; 50 colonies from the selective agar were isolated and identified. The serotypes found are shown in Table 1. Had only ten colonies been picked, only four serotypes would have been identified.

3. *The use of multiple subculture from selenite F broth*

The picking and identification of 50 colonies is not necessarily an impossible task even in routine practice. Yet the typing of this large number of colonies at a single subculture time may not be able to reveal the presence of some of the serotypes in the sample. The identification of fewer colonies from plates inoculated at different subculture times from the same enrichment broth may often give a more satisfactory result. This technique was first used as a routine in 1955 in the examination of swabs placed in open floor drains in bakeries (Harvey & Phillips, 1961). Topley & Fielden (1922) pointed out that, in an ordinary broth culture from a specimen of faeces, various bacteria succeed one another as the dominant viable organisms. In selenite F broth inoculated with material containing several salmonella serotypes, the ratio of one serotype to another is a function of the time of subculture (Harvey, 1965). Dixon (1959) found in a study of competitive growth of mixed coliform cultures in nutrient broth, that the organism inoculated in smallest numbers increased proportionately after 18 hr. incubation but there was no tendency for it to become dominant.

The type of result to be expected from the multiple subculture technique is best illustrated by a single experiment. Naturally infected crushed bones after 1 hr. incubation in broth at 37° C. were separated from the supernatant fluid. This fluid was then diluted to approximately 50 ml. with tap water, and 50 ml. double strength selenite F broth was added. The 100 ml. of fluid was incubated at 43° C. (Harvey & Thomson, 1953). Subcultures were made to Wilson and Blair's medium at 5, 18, 24, 48 and 72 hr. The plates were incubated at 37° C. for approximately 48 hr. and examined. The plate inoculated from selenite broth at 5 hr. incubation

Table 2. *Interdependence of serotype proportion and subculture time from enrichment medium*

	Percentage of 48 colonies picked at subculture times				
Serotype	5 hr.	18 hr.	24 hr.	48 hr.	72 hr.
S. derby	0	0	2	29	4
S. enteritidis (jena)	0	27	0	0	0
S. newport	0	0	0	10	0
S. oranienburg	0	15	17	4	6
S. reading	0	2	4	8	81
S. typhimurium	0	56	77	47	8

showed no salmonella-like colonies. All the other plates appeared positive and 48 suspicious colonies were picked from each to small agar slopes. The agar cultures, after incubation, were investigated and six serotypes were identified. The percentage (of 48 colonies) representing each serotype at the five subculture times is given in Table 2.

Obviously more serotypes could be isolated by multiple subculture than by relying on a single subculture time.

The technique finally chosen for routine practice was to pick 12 colonies from a plate representing each subculture time. Four subculture times were used. The

method was of great service in the earlier stages of our crushed bone investigation but has latterly been replaced by other techniques (4 and 5) which have been found more efficient.

4. *Splitting the infected sample into several equal parts and culturing each part in selenite F broth*

This technique is simply an adaptation of the first method of obtaining pure cultures from mixed cultures (Lister, 1878) and carries with it the same criticisms (Wilson & Miles, 1964*b*).

If a litre of water containing several salmonella serotypes is divided into ten equal parts and each part is cultured separately in selenite F broth, it is likely that the serotypes isolated may not be identical in each 100 ml. portion.

This method has been found to be most convenient for weekly examination of the River Taff, which drains a large area of Glamorgan county. With the pasteurization of egg products, overt human infections caused by salmonellas have declined in this region. There is still, however, a great deal of interesting latent infection not coming to the attention of the laboratory. As a measure of this hidden salmonellosis, we examine weekly 1 l. of water from the Taff, for salmonellas. The sampling site is carefully chosen and we believe that the isolations reflect the latent salmonella infection in part of our area. The results of some of these examinations are given in Table 3. Isolations from the meat market and abattoirs in Cardiff and from local infections in man of serotypes corresponding to those found in the river are given for comparison.

It will be noted that this technique is equally suitable for the isolation of individual serotypes and separate phage-types of *S. paratyphi B* and *S. typhimurium*. The method is particularly convenient for the routine examination of animal feeding-stuffs (Harvey & Price, 1967).

5. *The use of agglutinating serum to remove serotypes from a mixture in an orderly and predetermined manner*

This technique differs from those in the preceding sections in that the element of chance is partially eliminated. The bacteriologist is in control of the decision which serotypes to remove from his mixture and to some extent which serotypes he wishes to recover. We will therefore consider this technique in some detail.

The use of agglutinating serum in the selective isolation of salmonellas is not new. It was tried unsuccessfully by Loefler in 1906 and with greater success by Wassén (1930), Bailey & Laidley (1955) and Juenker (1957). The method was employed in a relatively simple form (Harvey, 1957) and was later developed for the examination of crushed bone fragments imported from India and Pakistan (Harvey & Price, 1962; Harvey, 1965).

The method of serological separation as used by Juenker (1957) involved the identification of one serotype from a selective agar. Numerous salmonella-like colonies were then picked to semi-solid agar containing antisera for phase I and phase II flagellar antigens of the serotype identified. If, on incubation, no spread through the soft agar occurred, it was assumed that all the colonies picked were of

the same serotype as that originally identified. Where, however, spread did occur the growth was examined for other antigens. In this study precautions were taken to avoid the criticism of production of serologically induced variants. We have

Table 3. *Isolations of salmonellas from consecutive samples of River Taff*

Figures in parenthesis after *S. paratyphi B* and *S. typhimurium* are phage-types. All dates are in 1966.

No.	Date (w.e.)	Serotypes and phage-types isolated	Corresponding isolations from man*	Corresponding isolations from meat and abattoirs*
1	23. iv.	*S. liverpool*, *S. reading* *S. paratyphi B* (1)	*S. liverpool*, 7. iv.	*S. oranienburg*, 28. iii.
2	30. iv.	*S. brandenburg*, *S. liverpool*, *S. reading* *S. minnesota*	*S. paratyphi B* (Taunton), 13. v.	*S. panama*, 3. v. *S. liverpool*, 16.v.
3	7. v.	*S. liverpool*, *S. paratyphi B* (1)	*S. schwarzengrund* 28. vi.	*S. paratyphi B* (1) 24. v.
4	14. v.	*S. brandenburg*, *S. oranienburg*, *S. paratyphi B* (1)	*S. brandenburg*, 25. vii	*S. panama*, 13. vi. *S. schwarzengrund*, 25. vii.
5	21. v.	*S. panama*, *S. paratyphi B* (1), *S. paratyphi B* (untypable)		
6	11. v.	*S. liverpool*, *S. reading*, *S. panama*, *S. oranienburg* *S. paratyphi B* (1)		
7	18. vi.	*S. tennessee*,. *S. typhimurium* (32), *S. paratyphi B* (1)		
8	25. vi.	*S. indiana*, *S. panama*, *S. schwarzengrund*, *S. typhimurium* (1 var. 5), *S. typhimurium* (32)		
9	30. vi.	*S. schwarzengrund*		
10	9. vii.	*S. liverpool*, *S. reading*, *S. schwarzengrund*		
11	16. vii.	*S. oranienburg*, *S. panama*, *S. schwarzengrund*, *S. paratyphi B* (1), *S. paratyphi B* (Taunton)		

* Date of isolation shown after serotypes.

employed this technique in a prolonged examination of 50 g. of crushed bone. During the course of the examination 617 suspicious colonies were picked to agar slopes and twelve different subgenus I salmonellas and four different subgenus III salmonellas were identified. One subgenus I serotype gave trouble in that we were uncertain whether it was *S. reading* or *S. saintpaul*. There were 114 such cultures whose antigenic formula was doubtful. All 114 cultures were examined by the phase change method of Harvey & Price (1961), but the H agglutinating sera added to the semi-solid agar in the pasteur pipette included both phase I factor

eh and Phase II factor 5. Of the 114 cultures introduced into the pipettes, 113 were immobilized and representatives of these were shown to be *S. reading*. The single culture that migrated through the soft agar was identified by the salmonella reference laboratory as *S. saintpaul* (1, 4, 5, 12: eh–1, 2).

To test further the specificity of separation we introduced a mixture of *S. typhimurium* and *S. agama* into the distal end of a pipette containing 0·15 % nutrient agar mixed with H Phase I, factor i, and H Phase II, factor 2, agglutinating sera. Incubation overnight at 37° C. resulted in growth to the upper surface of the serum-agar column. This surface growth was plated and after incubation of the plate only colonies of *S. agama* were identified. The experiment was repeated with *S. poona* and *S. bristol*. No difficulty was encountered in the serological separation of these two antigenically similar organisms. *S. enteritidis* and *S. dublin* were also separated from each other by this method. The manipulation of the agar filled pipettes has already been described (Harvey, Mahabir & Price, 1966).

Experiments were then conducted to demonstrate that a very small minority of one serotype could be separated from a different serotype dominant in a mixture. For convenience of observation a sucrose fermenting salmonella (Dixon & Curtis, 1960) was used as one of the pair of competing serotypes and a suitable indicator plating medium was employed. The result was invariable: the minority species could easily be separated from the dominant species.

Further observations showed that mixtures of many serotypes could be progressively separated into their constituent parts and each salmonella species isolated and identified. Later we found it possible to emulsify the entire bacterial growth present on a selective agar and to use this dense suspension as test material for examining for multiple serotypes. At first, like Wassén (1930), we added selenite to the semisolid agar + serum mixture, but subsequently this was found unnecessary and undesirable. The mere passage of a mixed growth of salmonellas and other organisms through a column of 0·15 % nutrient agar increased the ratio of salmonellas to other organisms present. (Harvey *et al.* 1966).

The following technique was finally adopted; for clarity an actual examination will be described. A 15 g. sample of crushed bone was placed in an 8 oz. wide-mouthed screw-capped jar. Sufficient nutrient broth was added to cover the bone fragments and the jar and contents were incubated at 37° C. for 24 hr. Double-strength selenite F broth was then added equal in volume to the bone fragments + broth. The jar was incubated in a 43° C. water bath for 24 hr. The enrichment broth was subcultured to Wilson and Blair's medium and this was incubated at 37° C. for 48 hr. Freshly poured Wilson and Blair was used prepared according to the formula of de Loureiro (1942). After incubation the plate showed many salmonella-like colonies with typical surrounding sheen. A colony was removed to a nutrient agar slope which was incubated for 24 hr. The Wilson and Blair plate was put on one side. The colony subcultured to agar was identified as *S. poona* (13, 22: z–1, 6). The entire remaining growth on the Wilson and Blair plate was wiped off with a sterile throat swab and emulsified in 0·2 ml. peptone water. Two loopfuls (2 mm. diameter) of H phase I, factor z, serum and two loopfuls of H phase II, factor 6, serum were added to 20 drops (20 × 0·02 ml.) of 0·15 % nutrient agar.

A pipette identical with that described by Harvey *et al.* (1966) was filled with this serum–agar mixture and the suspension from the Wilson and Blair plate was sucked into the distal bulb of the pipette underneath and in contact with the lower surface of the soft agar. This type of pipette was very easily prepared. An ordinary pasteur pipette was taken and the barrel heated in a bunsen flame at a point $\frac{1}{2}$–$\frac{3}{4}$ in. proximal to the junction of the neck and barrel. When molten, the glass was pulled out into a robust capillary 5 in. long. The capillary distal to the bulb so formed was cut off $\frac{3}{4}$–1 in. below it. The lower end of the filled pipette was sealed and the whole was incubated at 37° C. for 24 hr. Growth was now visible at the upper surface of the soft agar in the barrel. This growth was subcultured to a second Wilson and Blair plate which was incubated at 37° C. for 48 hr. The second plate was treated like the first and the process was serially repeated until no further serotypes were isolated. From the 15 g. of bones, *S. poona*, *S. gaminara*, *S. london*, *S. reading* and *S. hvittingfoss* were isolated one after the other. In practice 4 × 15 g. of bones were examined in this way simultaneously. This involves a combination of methods 4 and 5. In one sample of 4 × 15 g. of bones twenty different serotypes were found. Part of this work has already been published (Harvey & Price, 1962). It should be noted that none of the previously undiscovered serotypes reported in that publication was isolated by the serological method. The final list of serotypes isolated is given in Table 4.

It is worth noting that *S. typhimurium* was the sixth commonest serotype in this list and that 18 % of samples were infected with this organism. Similarly a relatively high percentage of specimens of fish meal imported into Holland were found to contain *S. typhimurium* by Jacobs, Guinée, Kampelmacher & van Keulen (1963). We think this point is worth emphasis. Indian and Pakistani material does not appear to be a constituent of animal feeding-stuffs owing to the danger of anthrax infection (Davies & Harvey, 1953, 1955). It does, however, raise the possibility that *S. typhimurium* may be more commonly present in animal feeding-stuffs than was previously thought. The search for a specific serotype in a sample is probably a question of adapting the serological technique so as to favour the isolation, or of adapting the present method of fluorescence microscopy to the same end. Crushed bone from India and Pakistan is a complex material for study. Almost 100 % of samples are infected with salmonellas. Salmonella counts are high (Smith, 1960) and many serotypes are present in each sample. The serological technique can, however, be used routinely for the examination of other samples such as polluted water, and swabs from abattoirs and from table surfaces and machinery in wholesale-meat premises. In such specimens the number of serotypes present is usually not more than two and the process is therefore much simpler.

6. *Use of two cultural techniques each designed to favour the isolation of separate salmonella serotypes*

The salmonella group is culturally inhomogeneous. *S. choleraesuis* is best isolated by means of a fluid or solid brilliant green medium. *S. typhi* will not grow at 43° C. but in our hands has shown itself to be resistant to 0·8 % selenite (Harvey, 1957; Harvey & Price, 1964). *S. pullorum* grows poorly at temperatures above

40° C. (Stokes & Bayne, 1957), but is well isolated from selenite F broth incubated at 37° C. and subcultured to brilliant green MacConkey. Such cultural idiosyncrasies have to be taken into account in the investigation of specimens containing these organisms especially if they are mixed with other salmonellas more easily cultured. Two separate cultural techniques may have to be used in such instances.

Table 4. *Salmonellas isolated from crushed bone imported from India and Pakistan*

Subgenus I		Subgenus I		Subgenus I	
Serotype	Times isolated	Serotype	Times isolated	Serotype	Times isolated
S. senftenberg	22	*S. kandla*	4	*S. havana*	1
S. anatum	21	*S. saintpaul*	4	*S. huvudsta*	1
S. newport	20	*S. seigburg*	4	*S. karachi*	1
S. cubana	19	*S. worthington*	4	*S. kirkee*	1
S. reading	16	*S. sp* (unidentified)	4	*S. lansing*	1
S. poona	14	*S. adelaide*	3	*S. marylebone*	1
S. typhimurium:		*S. derby*	3	*S. montevideo*	1
type 1 b (4a) 1		*S. enteritidis* (jena)	3	*S. newington*	1
type 22 1		*S. magwa*	3	*S. simsbury*	1
type 1 a (U57) 1		*S. muenchen*	3	*S. singapore*	1
type 2 (11) 1		*S. onderstepoort*	3	*S. taksony*	1
type U 131 1	14	*S. paratyphi B*:		*S. waycross*	1
type 1 1		type 1, var. 12 1		*S. urbana*	1
type 2 c (14) 2		type 1, var. 3 1	3	Percentage of samples positive for subgenus I	99
untypable 3		untypable 1			
untyped 3		*S. stanley*	3		
S. gaminara	13	*S. telhashomer*	3		
S. butantan	12	*S. adamstown*	2		
S. cerro	12	*S. alachua*	2		
S. richmond	12	*S. champaign*	2		
S. tennessee	12	*S. godesberg*	2		
S. kentucky	10	*S. grumpensis*	2		
S. oranienburg	10	*S. jodhpur*	2		
S. westhampton	10	*S. matopeni*	2		
S. chester	9	*S. pomona*	2		
S. give	9	*S. sandiego*	2		
S. hvittingfoss	8	*S. schwarzengrund*	2		
S. bredeney	7	*S. treforest*	2		
S. london	7	*S. virchow*	2		
S. minnesota	6	*S. charity*	1		
S. meleagridis	5	*S. chingola*	1		
S. bere	4	*S. chittagong*	1		
S. bronx	4	*S. dublin*	1		

Subgenus III	
Serotype	Times Isolated
26:23–30	4
26:23–21	2
26:26–25	2
9a, c:29–31	1
16:22–31	1
20:24–28	1
29:24–31	1
29:33–21	1
30:23–31	1
30:27–28	1
Percentage of samples positive for subgenus III	15

In this laboratory we examine weekly specimens of stream water for *S. typhi*. Occasional samples are contaminated with *S. typhimurium*. We therefore culture the material in double strength and four times strength selenite F broth (Harvey & Price, 1964). The former medium encourages the growth of *S. typhimurium*, the latter that of *S. typhi*. Similarly when we were examining samples of Australian frozen egg, it was found that *S. pullorum* was best isolated at 37° C. and *S. typhi-*

murium at 43° C. Both procedures were therefore used. *S. dublin* is also a serotype whose cultural peculiarities have to be recognized. It is best searched for on deoxycholate citrate agar incubated at 37° C. or on S.S. agar incubated at 40° C. (Livingstone, 1965). These two plates are always used in the culture of abattoir specimens thought to contain this organism. The same samples are also cultured on Wilson and Blair's agar which effectively reveals other salmonella serotypes.

DISCUSSION

This paper may seem to overemphasize technical detail. To a practical bacteriologist, it is difficult to accept this as a valid criticism. Sir Almroth Wright prefaced his work on *The Technique of the Teat and the Capillary Glass Tube* (1912) with a quotation from Carl Ludwig—'Die methode ist alles'. The quotation is still apt today when standardization of technique is held to be of paramount importance. Method standardization was recently discussed by Hilbert (1966) and the danger of rigidity of technique was noted. As both the present authors were trained by early workers in Sir Almroth Wright's laboratory, the influence of his book on our technical approach is not unnatural.

In this paper we have recorded various methods which have been found valuable over a period of almost twenty years. During that time we have become familiar with a great many members of the salmonella group, and most Public Health Laboratory Service laboratories in England and Wales have had similar experience.

It is difficult to put forward an optimum technique for the examination of samples containing multiple serotypes. To some extent the time available for the examination is the deciding factor. Where this is short, method 4 is the most convenient. Where the full range of serotypes in a material is being investigated and time is not important, a combination of methods 4 and 5 is best. Where less complex material is examined, method 5 is the one of choice. Methods 2 and 3, though effective, are rather tedious. We seldom employ these techniques today. As already indicated, method 6 is suitable for a restricted class of sample where two serotypes are present with distinctive cultural characters.

It might be thought that the serological technique described here could be criticized on the grounds that serologically induced variants might be produced. This type of criticism is always difficult to counter, but if it were valid, we should expect our serotype isolations from Indian crushed bones to differ somewhat from serotypes actually isolated in the Indian subcontinent. Reference to the literature will show that this is not so (Ganguli, 1958; Sharma & Singh, 1961; Agarwal 1962).

SUMMARY

Attention is drawn to the confused epidemiological picture experienced in investigating outbreaks of salmonellosis due to multiple serotypes. Public Health Laboratories are often faced with the examination of specimens containing several serotypes and in this paper six techniques are described which have been found useful in dealing with this problem. The choice of technique depends on the time available for the examination and the epidemiological importance of obtaining an

accurate result. A preference is expressed for an immuno-isolation technique, in which the bacteriologist is in technical control of the isolation of specific serotypes. The method is possibly open to objection on the grounds of serological induction of new serotypes, but we should regard this danger as slight in the majority of samples examined.

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