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# The isolation of salmonellas from British pork sausages and sausage meat

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## SUMMARY

Between 1969 and 1974, 1467 packets (3309 samples) of pork sausages and sausage meat produced by two large and two medium sized manufacturers and several local butchers were examined for the presence of salmonellas. Of these, 435 packets (786 samples) were found to contain salmonellas, but there was a wide variation in the isolation rates according to the producer. The salmonella incidence in samples from several small and two medium sized producers was low (0–11 %) while the results from the two large producers investigated showed a striking difference, the rate of salmonella contamination in the product of one was low (about 2 %) and in that of the other consistently high (40–60 %).

A comparison of liquid enrichment media, incubation temperatures and selective agar media was also carried out to determine the most efficient combination for the isolation of salmonellas from minced meat products. The results showed that (*a*) incubation of enrichment cultures at 43° C. yielded a consistently greater number of salmonella isolations than at 37° C., regardless of plating medium, (*b*) tetrathionate broth A (Rolfe) was superior to selenite broth as an enrichment medium at both 37 and 43° C. and (*c*) brilliant green agar gave better results than deoxycholate citrate sucrose agar and bismuth sulphite agar as a selective medium.

## INTRODUCTION

The role of animals as a reservoir of salmonellas has long been recognized (Savage, 1932; Buxton, 1957; Hobbs, 1961; van Oye, 1964) and it is well established that pigs are a major reservoir of these organisms. Food poisoning incidents have occurred in which pork and pork products were the suspected food vehicles of infection (Bevan Jones, Farkas, Ghosh & Hobbs, 1964; Burns, Mair & Hooper, 1965).

There are reports of many surveys on the prevalence of salmonellas in pigs. Scott (1940) isolated salmonellas from 3·8 % of mesenteric lymph nodes from 1000 healthy pigs; Reports (1947, 1955) give isolation rates of 2·5 % of 5285 pigs

and 0·4 % of 5166 samples of lymph nodes and other tissues; Smith (1959) found salmonellas in 12 % of mesenteric lymph nodes of 500 pigs; Edel & Kampelmacher (1970) isolated salmonellas from 30·1 % of 700 pigs examined in 7 Dutch slaughterhouses; the PHLS Working Group, Skovgaard & Nielsen (1972) found salmonellas in 7 % of 5637 caecal samples and 6 % of 2483 mesenteric gland samples of English pigs and 3 % of 296 caecal samples and 4·2 % of 359 lymph node samples of Danish pigs. Galton, Smith, McElrath & Hardy (1954) showed that the excretion rate of live hogs increased from 7 % on the farm to 25 % in the holding pens at the abattoirs, and by the time they reached the killing floor, 51 % were positive. Williams & Newell (1970) also demonstrated the effect of stress on the pattern of salmonella excretion in pigs.

This study, which began in 1968 and terminated in 1974, was designed to determine the prevalence of salmonellas in pork sausages and sausage meat prepared by some of the main manufacturers in England. The survey also provided an opportunity to carry out a comparison of liquid enrichment media, incubation temperatures and selective agar media to determine the most efficient combination for the isolation of salmonellas from minced meat products.

## MATERIALS

Packets of sausage and sausage meat from four British manufacturers and several local butchers were examined for the presence of salmonellas. Two of the manufacturers operated on a large scale (A and B) and two on a small scale (C and D). The local butchers had a limited production for a single shop or a small chain of shops. Samples were received either directly from the manufacturers (A and B) or via local public health inspectors or other persons who purchased the sausages or sausage meat at regular intervals from normal retail outlets.

Samples of pork sausage meat and pork sausages were supplied by large manufacturer A from three different sources. Material from source 1 contained sulphite as preservative while samples from sources 2 and 3 were sulphite free. The samples were examined at regular intervals, usually weekly, over a period of 16 months from March 1969 to July 1970.

Sausages from the large manufacturer B were supplied packed and ready for retail sale from 2 factory sources; approximately two-thirds of the samples came from one of the factories. They were examined at weekly intervals almost continuously from November 1969 onwards. A small number of samples examined during 1968 were purchased from normal retail outlets.

Sausages and sausage meat from manufacturers C and D and from local butchers were collected at weekly intervals for various periods of time between 1968 and 1974.

Over the five year period a total of 1467 packets of sausages or sausage meat (3309 samples) were examined at a rate of approximately 6 packets each week.

## METHODS

For the media trial each packet of sausages or sausage meat was divided into two or more samples; each sample comprised a single small sausage or an equivalent portion of a larger sausage or sausage meat. From March 1969 to July 1971 three jars of enrichment media were used for the samples from manufacturer A, two jars of Selenite F broth (a modification of the medium of Liefson, 1936) for incubation at 37 and 43° C. and one jar of tetrathionate broth A (Rolfe, 1946) for incubation at 43° C. The trial fell into two parts with the samples from manufacturer B, from March 1969 to June 1970 three jars of enrichment media were used as described for manufacturer A. For the latter part of the trial, June 1970 to July 1971 two additional jars were added, Rolfe tetrathionate A incubated at 37° C. and Muller Kauffmann tetrathionate (Kauffmann, 1935) incubated at 43° C. After completion of the trial all samples until the end of the survey were examined by a much shorter method (Rolfe tetrathionate A incubated at 43° C. and subcultured to BGA or BGA and DCA). The modified selenite broth contained mannitol instead of lactose and was sterilized by Seitz filtration. The Rolfe tetrathionate broth was prepared to formula A described by Rolfe (1946), but Oxoid nutrient broth No. 2 (CM67) was substituted for the 1% Lemco and 1% Evans peptone in the broth base. The broth and chalk base was prepared and sterilized in advance of needs and could be stored at ambient temperature for a long time. The iodine and thiosulphate mixture and brilliant green solution were normally added as required for use. The complete medium could be stored in the refrigerator for up to 7 days. The Muller Kauffmann tetrathionate broth consisted of the Oxoid tetrathionate broth base (CM29) with the addition of 4·75 g. ox bile (Oxoid L50), 19 ml. iodine solution (iodine 200 g., potassium iodide 250 g., distilled water 1000 ml.) and 9·5 ml. of 0·1% brilliant green solution (BDH or Chroma) per litre as described by Edel & Kampelmacher (1969). The basal medium could be stored for a long time but on addition of the other ingredients the complete medium was used immediately.

The enrichment media were distributed in 100 ml. volumes in sterile screw-capped 1 lb. honey jars (300 ml. capacity), immediately before use. Approximately 10–15 g. of sausage or sausage meat were distributed into each jar and mixed well by breaking up the meat with a sterile wooden spatula, the jars were then incubated at 37 or 43° C. Subcultures were made from 37° C. enrichments after 24 and 72 hr. incubation and from 43° C. enrichments after 24 and 48 hr. incubation on bismuth sulphite agar (Oxoid CM 201) (BSA), deoxycholate citrate agar (Liefson, 1935) as modified by Hynes (1942) and further modified by the addition of 1% sucrose (DCA), and brilliant green agar (Oxoid CM 263) (BGA). Subcultures on BSA and DCA were made in the usual manner. Two plates of BGA were used for each enrichment culture, they were streaked according to the method described by Edel & Kampelmacher (1969). All plates were incubated at 37° C. and examined after 24 and 48 hr. incubation except for the BGA plates which were examined after 24 hr. only

One colony with the morphological characteristics of a salmonella was picked for identification from each plate. These organisms were identified by biochemical

Table 1. *Salmonella isolations from packets of pork sausage meat and sausages from large and small manufacturers*, 1968–74

| Source of samples | Packets examined | Salmonella isolated | % | Serotypes |
|---|---|---|---|---|
| Large manufacturer A | | | | |
| Sausage meat 1969–70 | 177 | 4 | 2·3 | *S. bahati* 2<br>*S. dublin*<br>*S. typhimurium* |
| Sausages 1970–1 | 135 | 3 | 2·2 | *S. dublin*<br>*S. panama*<br>*S. typhimurium* |
| Large manufacturer B | | | | |
| Sausages 1968–74 | 854 | 413 | 48·4 | See Table 4 |
| Medium manufacturer C | | | | |
| Sausages 1968, 1972, 1974 | 101 | 4 | 4·0 | *S. eppendorf*<br>*S. typhimurium* 2<br>*S.* 4,12:d:- 2 |
| Medium manufacturer D | | | | |
| Sausages 1968, 1972, 1974 | 100 | 11 | 11·0 | *S. agona* 4<br>*S. anatum* 3<br>*S. derby*<br>*S. panama* 2<br>*S.* 4,12:d:- |
| Small manufacturers 1968, 1971, 1972, 1974 | 100 | 0 | 0 | — |
| Total | 1467 | 435 | 29·7 | — |

tests (Cowan & Steel, 1970) and those conforming to the reactions of the salmonella group were examined serologically. Preliminary identification was made by slide agglutination tests using polyvalent and monovalent O and H antisera prepared by the Standards Laboratory for Serological Reagents, Colindale. Some strains were referred to the Salmonella and Shigella Reference Laboratory, Colindale for complete serological analysis.

Strains of *Salmonella typhimurium* and *S. enteritidis* were sent to the Enteric Reference Laboratory, Colindale for phage typing.

## RESULTS

The numbers of salmonella isolations made from sausages and sausage meat from all the manufacturers are summarized in Table 1. A total of 1467 packets were examined of which 435 yielded salmonellas. Most of these isolations (95%) came from the products of large manufacturer B, although less than 60% of the total samples examined came from this source. No salmonella isolations were made from the samples of butchers' sausages. Of the samples from the medium sized manufacturers, 4% of packets (2·7% samples) from manufacturer C and 11% (7% samples) from manufacturer D were found to contain salmonellas.

It is interesting to note that *S. dublin* was isolated twice from the samples of manufacturer A. This salmonella serotype is known to have cultural peculiarities.

Table 2. *Salmonella isolations from packets of pork sausage meat and pork sausages with and without sulphite. Large manufacturer A* (1969–71)

| Source and nature of samples | Packets examined | Salmonella isolated | % |
|---|---|---|---|
| 1 with sulphite | 135 | 5 | 3·7 |
| 2 without sulphite | 92 | 1 | 1·1 |
| 3 without sulphite | 85 | 1 | 1·2 |
| Total | 312 | 7 | 2·2 |

Table 3. *Salmonella isolations from packets* of pork sausages from large manufacturer B*, 1969–74

| Source of samples | Packets examined | Salmonella isolated | % |
|---|---|---|---|
| Factory 1 | 524 | 295 | 56·3 |
| Factory 2 | 281 | 92 | 32·7 |
| Total | 805 | 387 | 48·0 |

* 49 packets (26 positive) examined in 1968–August 1969 not included as the factory of production could not be identified.

Harvey & Price (1967) state that it is best isolated on DCA incubated at 37° C. or on SS agar at 40° C. On both occasions the serotype was isolated from Rolfe tetrathionate broth incubated at 43° C., the first isolation was made on DCA and BSA and the second on all three agar media, i.e. DCA, BSA and BGA.

The results of examination of samples of sausage meat and sausages produced by manufacturer A are shown in Table 2. The incidence of salmonellas in these samples was low from all three sources. Isolation rates ranged from 1·1 % to 3·7 % of packets (0·3–2·3 % samples) with a total rate of 2·2 % of packets (1·2 % samples) containing salmonellas. Although the isolation rates from all three sources were low, samples from source 1 containing sulphite as preservative yielded more salmonellas than the samples from sources 2 and 3 which did not contain sulphite.

Table 3 gives the number of packets of sausages with salmonellas from manufacturer B over the period November 1969 to October 1974. The isolation rate from products manufactured by factory 1 (56 %) was almost twice as high as from factory 2 (33 %). The number of isolations of different serotypes of *Salmonella* made over the period 1968 to October 1974 from both factories owned by manufacturer B are shown in Table 4. The figures are divided roughly into six separate years. A total of 38 different serotypes were isolated. Some samples yielded more than one serotype even though only one colony from each plate was picked for identification; the largest number of serotypes found in a single packet or sample was four.

*Salmonella infantis* was most frequently isolated although almost wholly from factory 1. *S. agona* was isolated regularly from both factories. *S. derby*, *S. anatum*, *S.* unnamed (4,12; d:-) and *S. panama* were also isolated frequently and mainly

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Table 4. *Salmonella serotypes isolated from large manufacturer B over a 6-year period*

| Serotype | Number of isolations made over period | | | | | | |
|---|---|---|---|---|---|---|---|
| | Jun. 68–Aug. 69 | Nov. 69–Oct. 70 | May 71–Dec. 71 | Jan. 72–Dec. 72 | Jan. 73–Dec. 73 | Jan. 74–Oct. 74 | Total |
| *S. infantis* | 41 | 42 | 28 | 3 | 18 | 24 | 156 |
| *S. agona* | — | 12 | 27 | 25 | 10 | 25 | 99 |
| *S. derby* | — | 7 | 5 | 4 | 30 | 20 | 66 |
| *S. anatum* | — | 12 | 13 | 17 | 4 | 7 | 53 |
| *S.* 4,12:d:- | 1 | 11 | 11 | 12 | 1 | — | 36 |
| *S. panama* | 4 | 14 | 7 | 4 | 1 | — | 30 |
| *S. typhimurium* phage type | | | | | | | |
| 1 var. 5 | — | — | — | — | — | 2 | 2 |
| 3a | — | — | 1 | 1 | — | — | 2 |
| 4 | — | 1 | — | — | — | — | 1 |
| 12a | — | 3 | — | 1 | — | — | 4 |
| 17 | — | — | — | — | 1 | 1 | 2 |
| U20 | — | 2 | — | — | — | — | 2 |
| U24 | — | — | — | 1 | — | — | 1 |
| 31 | — | — | — | — | 1 | — | 1 |
| U71 | — | — | 6 | 1 | — | — | 7 |
| U180 | — | — | — | 1 | — | — | 1 |
| *S. indiana* | 1 | 7 | — | 12 | — | 1 | 21 |
| *S. brandenburg* | — | 5 | — | 3 | 6 | — | 14 |
| *S. bredeney* | — | — | 4 | 7 | 2 | 1 | 14 |
| *S. haifa* | — | — | — | — | 4 | 7 | 11 |
| *S. newport* | — | 8 | — | — | 3 | — | 11 |
| *S. stanley* | — | 10 | — | 1 | — | — | 11 |
| *S. livingstone* | 5 | 1 | 1 | 1 | — | — | 8 |
| *S. worthington* | — | 6 | — | 1 | — | 1 | 8 |
| *S. muenchen* | 1 | 4 | — | — | — | 2 | 7 |
| *S. heidelberg* | — | 4 | 2 | — | — | — | 6 |
| *S. saint-paul* | — | — | — | — | 4 | 2 | 6 |
| *S. enteritidis* phage type | | | | | | | |
| not typed | 1 | — | — | — | — | — | 1 |
| 8 | — | 1 | — | 3 | — | — | 4 |
| *S. takoradi* | — | 1 | — | 3 | — | — | 4 |
| *S. chester* | — | 1 | — | — | 1 | 1 | 3 |
| *S. duisburg* | — | 2 | 1 | — | — | — | 3 |
| *S. give* | — | — | 1 | 1 | 1 | — | 3 |
| *S. kentucky* | — | 3 | — | — | — | — | 3 |
| *S. london* | 1 | — | — | — | 2 | — | 3 |
| *S. reading* | — | — | 3 | — | — | — | 3 |
| *S. eimsbuettel* | — | — | — | — | — | 2 | 2 |
| *S. isangi* | — | — | — | 2 | — | — | 2 |
| *S. kiambu* | — | 2 | — | — | — | — | 2 |
| *S. kottbus* | — | 2 | — | — | — | — | 2 |
| *S. bradford* | — | — | — | 1 | — | — | 1 |
| *S. cerro* | — | — | 1 | — | — | — | 1 |
| *S. epicrates* | 1 | — | — | — | — | — | 1 |
| *S. hadar* | — | — | — | — | 1 | — | 1 |
| *S. kapemba* | — | 1 | — | — | — | — | 1 |
| *S. stanleyville* | — | — | 1 | — | — | — | 1 |
| *S. thompson* | — | — | — | 1 | — | — | 1 |
| *S. virchow* | — | — | — | 1 | — | — | 1 |
| Total | 56 | 162 | 112 | 107 | 90 | 96 | 567 |
| Proportion of packets positive | 26/49 (53·0%) | 97/173 (56·0%) | 77/145 (53·0%) | 86/223 (38·6%) | 60/158 (38·0%) | 67/106 (63·2%) | 413/854 (48·4%) |

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Table 5. *Salmonella isolation rates on different media. Pork sausage meat from large manufacturer A*, 1969–70

| Enrichment medium and incubation temperature | Proportion of samples positive on | | | |
|---|---|---|---|---|
| | DCA | BSA | BGA | All media |
| Selenite 37° C. | 4/534 (0·8) | 2/534 (0·4) | ND | 4/534 (0·8) |
| Selenite 43° C. | 2/526 (0·4) | 1/526 (0·2) | 4/494 (0·8) | 4/526 (0·8) |
| R. tet. 43° C. | 7/534 (1·3) | 7/534 (1·3) | 7/506 (1·4) | 7/534 (1·3) |
| All enrichments and all plating media | | | | 8/534 (1·5) |

Figures in parentheses are percentages.

DCA, Deoxycholate citrate sucrose agar; BSA, bismuth sulphite agar; BGA, brilliant green agar; R. tet., Rolfe formula A tetrathionate; MK. tet., Muller Kauffmann formula tetrathionate; ND, Not done.

Table 6. *Salmonella isolation rates on different media. Pork sausages from large manufacturer A*, 1970–1

| Enrichment medium and incubation temperature | Proportion of samples positive on | | | |
|---|---|---|---|---|
| | DCA | BSA | BGA | All media |
| Selenite 37° C. | 1/411 (0·2) | 1/411 (0·2) | 1/411 (0·2) | 1/411 (0·2) |
| Selenite 43° C. | 0/354 | 0/354 | 0/354 | 0/354 |
| R. tet. 43° C. | 1/417 (0·2) | 1/417 (0·2) | 1/417 (0·2) | 2/417 (0·5) |
| All enrichments and all plating media | | | | 3/417 (0·7) |

For notes see Table 5.

from factory 1. The isolations of *S. typhimurium* included 10 different phage types.

Comparative results for various media and incubation temperatures are given in Tables 5–8. Although only a small number of salmonella isolations were made from the pork sausage meat samples from manufacturer A (Table 5), the largest number was isolated using Rolfe tetrathionate broth incubated at 43° C. and subcultured on BGA. There were too few salmonella isolations from pork sausages (Table 6) to allow conclusions to be drawn on the relative efficiency of the different media.

Salmonellas were frequently found in samples from manufacturer B and the efficiency of the different combinations of liquid and solid media and incubation temperatures could be assessed. Tables 7 and 8 compare isolation rates from individual samples of sausages.

Table 7 compares the results from three sets of enrichment conditions, and Table 8 gives the results from five sets of enrichment conditions. A greater number of salmonella isolations was made from selenite broth incubated at 43° C. than at 37° C., but higher isolation rates were obtained from Rolfe tetrathionate at 43° C. than from selenite at both temperatures. When Rolfe tetrathionate was incubated at both 37 and 43° C. the highest total isolation rate was obtained after the 37° C. incubation (Table 8). However, there was considerable variation between the efficiency of the different plating media, BGA producing

Table 7. *Salmonella isolation rates on different media. Pork sausages from large manufacturer B, November* 1969–*June* 1970

| Enrichment media and incubation temperature | Proportion of samples positive on | | | |
|---|---|---|---|---|
| | DCA | BSA | BGA | All media |
| Selenite 37° C. | 3/341 (0·9) | 7/341 (2·1) | 16/168 (9·5) | 23/341 (6·7) |
| Selenite 43° C. | 13/323 (4·0) | 14/323 (4·3) | 39/309 (12·6) | 42/323 (13·0) |
| R. tet. 43° C. | 91/341 (26·7) | 96/341 (28·2) | 97/327 (29·7) | 109/341 (32·0) |
| All enrichments and all plating media | | | | 124/341 (36·4) |

For notes see Table 5.

Table 8. *Salmonella isolation rates on different media. Pork sausages from large manufacturer B, June* 1970–*July* 1971

| Enrichment medium and incubation temperature | Proportion of samples positive on | | | |
|---|---|---|---|---|
| | DCA | BSA | BGA | All media |
| Selenite 37° C. | 11/225 (4·9) | 7/225 (3·1) | 24/225 (10·7) | 29/225 (12·9) |
| Selenite 43° C. | 35/174 (20·1) | 15/174 (8·6) | 42/174 (24·1) | 48/174 (27·6) |
| R. tet. 37° C. | 44/231 (19·0) | 47/231 (20·3) | 95/231 (41·1) | 114/231 (49·4) |
| R. tet. 43° C. | 96/242 (39·7) | 87/242 (36·0) | 99/242 (40·9) | 105/242 (43·4) |
| MK. tet. 43° C. | 6/111 (5·4) | 4/111 (3·6) | 5/111 (4·5) | 7/111 (6·3) |
| All enrichments and all plating media | | | | 148/243 (60·9) |

For notes see Table 5.

approximately 21% more positive samples than DCA and BSA. Rolfe tetrathionate at 43° C. although giving a lower total isolation rate gave consistently high results on all three agar media. With all the combinations of selective enrichment media and incubation temperatures BGA appeared to be the best selective agar medium, but it must be examined after 24 hr. incubation. The Muller Kauffmann tetrathionate broth gave poor results in comparison with the other liquid enrichment media and it was used only for a proportion of the samples examined during the second part of the media trial.

## DISCUSSION

The number of salmonella isolations from samples varied according to the manufacturer. The total figure of 29·7% of packets from which salmonellas were isolated is comparable with the results reported by other workers. Galton, Lowery & Hardy (1954) found salmonellas in 23% of fresh sausages, Weissman & Carpenter (1969) reported an isolation rate of 38·3% and Surkiewicz, Johnston, Elliott & Simmons (1972) found 28% of samples of pork trimmings and 28% of finished sausages contaminated with salmonellas.

Our results suggest that the salmonella incidence in sausages produced by small manufacturers and butchers is low. The large manufacturers investigated showed a striking difference, the incidence of salmonellas in the product of one was low (about 2%) and of the other consistently high (40–60%). Galton *et al.*

(1954) found salmonellas in only 7·5% of nationally distributed sausages while 25% and 57·5% of samples from local markets and local abattoirs respectively were positive.

Many measures have been shown to play important roles in reducing infection in animals or the contamination by salmonellas of carcass meat and comminuted meat products (Williams & Spencer, 1973). They include attention to methods of transport, reduction of stress in the animals, improved systems of lairaging and improved methods of slaughter, particularly in evisceration. The method of removing intestines is important and in particular the method of detaching the rectal end of the alimentary tract, so that the interior of the carcass is not contaminated. Also the design, construction and hygiene of lairages, and the relation between the lairage, abattoir and meat products plant are all important in reducing the incidence of salmonella contamination.

The comparison of media and incubation temperature for the isolation of salmonellas shows three main features: (*a*) the elevated incubation temperature of 43° C. yielded a greater number of isolations from selenite broth, (*b*) tetrathionate broth is superior to selenite broth as an enrichment medium, although the Muller Kauffmann formula used in this survey was inferior to both selenite and Rolfe tetrathionate and (*c*) BGA was superior to DCA and BSA as a selective medium, although with optimum enrichment conditions the differences between the isolation rates on the three agar media were small.

Although Rolfe tetrathionate broth incubated at 37° C. yielded more salmonella isolations than the same medium incubated at 43° C. there was greater variation between the efficiency of the plating media, many more isolations were made on BGA than on DCA and BSA. After enrichment in Rolfe tetrathionate at 43° C. the differences between the results on the three plating media were much smaller suggesting that these were the optimum enrichment conditions. Other workers (Harvey & Thomson, 1953; Harvey & Price, 1968; Edel & Kampelmacher, 1968, 1969) have also obtained better salmonella isolation rates from various materials using 43° C. rather than 37° C. for the incubation temperature of liquid enrichment culture.

The poor results obtained with the Muller Kauffmann tetrathionate broth do not agree with the findings of Edel & Kampelmacher (1968, 1969). However this emphasizes the importance of variations in the different ingredients of a medium, in particular the brand and batch of brilliant green appears to be critical. The amount of heating and storage will also affect the efficiency of a medium, and a good source of peptone is also essential.

The Muller Kauffmann tetrathionate broth differs from the Rolfe version in that it contains bile salts, a lower concentration of tetrathionate and an excess of thiosulphate. The bile salts allow enteric organisms to grow while inhibiting other common non-intestinal organisms (MacConkey, 1908). The concentration of tetrathionate in the Muller Kauffmann medium (0·018 M) is not great enough to be highly inhibitory; the high selectivity of the medium must be attributed to a combination of tetrathionate, iodide, thiosulphate and chalk, but the large excess of thiosulphate and the chalk operate in favour of the *Proteus* group

(Knox, Gell & Pollock, 1943). The medium of Rolfe is based on the findings of Knox *et al.* (1943) who suggested that a medium containing a high enough concentration (about 0·03 M) of balanced tetrathionate was nearer to the ideal conditions which will combine good growth of *S. typhi* and reliable inhibition of *Proteus*. Rolfe's medium contains a minimum of free thiosulphate and it is important that the volumes of thiosulphate and iodine solutions should be measured accurately. Medium A (0·03 M tetrathionate) allows the growth of salmonellas including most strains of *S. typhi* and also *Proteus* strains, while medium B (0·039 M tetrathionate) allows the growth of few organisms other than salmonellas. However, according to Harvey (personal communication) medium B must be used at 37° C.

At the higher concentrations as in the Rolfe media, Knox *et al.* (1943) considered that the tetrathionate acts as a selective inhibitor of growth while in lower concentrations as in the Muller Kauffmann medium it behaves as a selective promoter of growth.

The elevated temperature suppresses many competing gram-negative bacteria and permits the salmonellas to grow in relatively pure culture; it may also aid the dispersal of fat in the sausages more than incubation at 37° C., thus allowing better distribution of the meat throughout the enrichment broth. Morris & Dunn (1970) added tergitol No. 7 (sodium heptadecyl sulphate) to tetrathionate brilliant green enrichment broth for the isolation of salmonellas from pork sausage incubated at both 37 and 43° C. Without tergitol more salmonellas were isolated at 43° C. than at 37° C. but with tergitol there was no difference. The tergitol dispersed and emulsified the fat which improved the isolation of salmonellas when the cultures were incubated at 37° C. At 43° C. the fat problem was not as troublesome and no advantage was derived from the addition of tergitol at this temperature. These workers also found that brilliant green sulphadiazine agar was superior to BSA. The BGA used in this survey, although it did not contain sulphadiazine, gave better results than either DCA or BSA.

Our thanks are due to the two large companies and to the public health inspectors who provided us with samples from the smaller manufacturers for their co-operation throughout the survey.

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