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Twenty-five meat-and-bone meal samples were enriched with either selenite-cystine or tetrathionate and incubated for 1 and 2 days. Seven were previously found to be positive; of the other 18, 16 were positive for salmonella. The number of somatic serogroups per sample ranged from 1 to 11 with a mean of 3.8. Significantly more ($P < 0.01$) group C₁ salmonellae were isolated using tetrathionate than selenite, whereas significantly more of groups G, 35, and Difco poly-valent D were isolated from selenite than tetrathionate. Seventy-six per cent of the presumptive colonies from Brilliant Green agar showed a positive lysine decarboxylase reaction, and there were no differences between media or times of incubation. Ninety-four per cent of the lysine decarboxylase-positive cultures showed a positive somatic antiserum response; again there were no differences between times or enrichments although there were significantly more total positive serogroups at 2 days than at 1 day from tetrathionate but not from selenite. There were indications that certain serogroups preferred either one or the other enrichment. There were no differences in total positive samples with the two enrichments although neither alone was sufficient to identify all positives. Several lactose-positive salmonellae were recovered.

The effects of the two enrichment media, tetrathionate and selenite, on salmonella isolation have been studied by a number of workers. Sharma and Packer (11) artificially contaminated 300 cow and pig fecal samples with four cultures of salmonellae, viz., *Salmonella anatum*, *S. typhimurium*, *S. newport*, and *S. choleraesuis*. They found more positive samples with tetrathionate than with selenite-F. Both media appeared to be unsatisfactory for isolating *S. choleraesuis*. They also found the optimal time of incubation for tetrathionate to be between 24 and 30 hr with an apparent decrease in recovery at 48 hr. Selenite did not show the decrease at 48 hr. Hurley and Ayres (4) found tetrathionate to be more inhibitory to *S. pullorum* than selenite, especially following the addition of egg albumin.

Banwart and Ayres (1) studied the effect of tetrathionate and selenite-F on the growth of several pure cultures of salmonellae. They found definite inhibition by tetrathionate of *S. paratyphi* and a decrease in the growth rate of *S. give* in the log phase. Selenite-F decreased

the numbers of *S. anatis* (*S. anatum*) during the initial incubation period. The addition of whole egg minimized these differences. Greenfield and Bigland (3) studied the effect of selenite on several salmonellae. *S. choleraesuis*, *S. gallinarum*, *S. pullorum*, and *S. typhi* did not grow well, especially when incubated at 43 C rather than at 35 C. *S. typhimurium*, *S. paratyphi B*, and several strains of *Arizona* were not inhibited by 1% sodium selenite at 35 C. Taylor (13) studied naturally contaminated albumin samples using both cystine-selenite-F and Brilliant Green tetrathionate broth. He indicated that the tetrathionate was superior to selenite. Other studies on the effect of incubation time were by Osborne and Stokes (9) who indicated that 2 days of incubation resulted in fewer viable salmonellae from enrichment media. Studies using meat-and-bone meal by Carlson et al. (2) did not show any differences between 1 or 2 days of incubation.

Taylor and Silliker (15) considered the use of either selenite or tetrathionate alone a calculated risk, feeling that neither by itself was

capable of identifying all positive samples. They used naturally contaminated dried egg albumin as test materials. Sen (10) also suggested that several isolation media would be preferable to one alone for isolating salmonellae from feces.

For the recommended procedure for isolating salmonellae from meat-and-bone meal (14), the tetrathionate is used as the selective enrichment. The Food Protection Committee of the National Academy of Sciences (8) recommends the use of both tetrathionate and selenite-cystine for isolating salmonellae from foods. They also suggest an 18- to 24-hr incubation period. In *The Bacteriological Analytical Manual of the Food and Drug Administration* (16) it is recommended that both media be used for isolating salmonellae from animal feeds.

The purpose of this study was to compare the efficacy of selenite-cystine and tetrathionate incubated at 1 and 2 days for isolating serogroups of salmonellae from naturally contaminated meat-and-bone meal samples.

MATERIALS AND METHODS

Twenty-five meat-and-bone meal samples were collected from a number of rendering plants throughout Pennsylvania, Ohio, Indiana, Wisconsin, and Iowa. Some plants were sampled twice, a year apart; these were no. 1 and 18, 2 and 14, and 3 and 16. The latter was known to purchase other renderers' products; in fact, the meat-and-bone meal from this plant's own production line was negative for salmonella. One plant was sampled five times over a 2-yr period and was known to harbor a considerable salmonella population (represented by samples 13, 15, 20, 21, and 22) and imported nearly all its material. Sample 12 was a composite of known positive samples from the Pennsylvania Department of Agriculture (courtesy of E. T. Mallinson). The rest of the samples were chosen at random. The composition of the samples was mostly beef (including meat scraps and dead animals) from all plants except no. 8 and 9 (mostly pork) and no. 4 (poultry).

Preparation of samples. The generally coarse meat-and-bone meal samples were sifted through a no. 12 sieve until approximately 700 g of material was obtained. This was thoroughly mixed and divided into two nearly equal portions. One portion was used for the tetrathionate series, and the other was used for the selenite-cystine. From each portion, nine 30-g portions were weighed into 8-oz (approx. 240-ml) wide-mouth screw-cap jars. The remainder of each portion was then remixed and divided into two portions; one was for a tenth 30-g sample, and the remainder was for ten 3-g samples. The 3-g samples were placed into 0.75 by 6 inch test tubes. The work area and equipment were thoroughly cleaned and sanitized before another sample was tested.

Enrichment media. The two enrichment media,

tetrathionate and selenite-cystine, were from Difco and were tempered to 37 to 39 C before addition to the samples of the meat-and-bone meal. One hundred ml was added to each of the 30-g samples, and 10 ml was added to the 3-g sample. The samples were mixed with stirring rods and incubated for either 24 or 48 hr \pm 1 hr at 37 C \pm 1 C. Ten-milliliter beakers were used for covering the test tubes. The caps of the bottles were left loose during incubation.

Selective agar. Brilliant Green agar (Difco) was used as the selective agar. At the end of the incubation period, the samples were thoroughly mixed by hand in the case of the 30-g samples and by the use of a vortex-type oscillating mixer for the tubes. About 5 to 10 min after mixing, a loopful of supernatant fluid was streaked carefully onto the agar. The Brilliant Green agar plates were incubated at 37 C for 24 hr \pm 1 hr.

Preliminary confirmation. Two colonies were picked from each plate. The colonies selected were, in general, those showing a pinkish or magenta hue; however, in cases where none of these colonies was evident, two green colonies were picked. The examination of the colonies was facilitated by the use of a fluorescent light (Glow-Box, I²R Co., Cheltenham, Pa.). Any colony showing any magenta color was considered to be salmonella-presumptive. The picked colonies were inoculated onto lysine-iron agar slants and incubated for 24 hr \pm 1 hr. Lysine decarboxylase-negative cultures were considered salmonella-negative. The remainder, either with or without an H₂S reaction, were tested further with somatic antisera.

Agglutination tests. The antisera used were purchased from Difco Laboratories and included the somatic groups A, B, C₁, C₂, D, E₁, E₂, E₃, E₄, F, G, H, I, poly A, poly A-1, poly B, poly C, poly D, poly E, poly F, and *Arizona* polydiphasic. The single factors 18, 20, 21, and 35 were also tested. The cultures on the lysine-iron slants were tested first with the polyvalent antisera and then with the appropriate group antisera. A positive agglutination reaction with any of the antisera confirmed the culture as *Salmonella* or *Arizona*.

Statistical analyses. A chi-square test for association (2 \times 2 contingency table) was used to determine the significance of any differences obtained. Since there was no previous indication of a superiority of either enrichment medium or day of incubation, a two-tailed test was used. The statistically stronger one-tail test would have been more powerful but would have necessitated invalid post hoc analyses. The probability of rejecting a true hypothesis (α) was set at 0.05 although some comparisons were also made with $\alpha = 0.01$.

RESULTS

Preliminary confirmation. The numbers of presumptive lactose-positive colonies, cultures showing preliminary confirmation with a positive lysine decarboxylase test and those showing final confirmation by somatic aggluti-

nation, are shown in Table 1. Sample 12 (known-positive composite) showed the lowest ratio of preliminary confirmed to presumptives (10.2%), whereas sample 2 showed 124% and sample 7, 136%. The greater-than-expected percentage of sample 2 was due to the isolation of eight cultures of salmonellae which formed green colonies (lactose-positive) on the Brilliant Green agar. Lactose-positive strains were also isolated from samples 1, 6, 14, and 15. The high confirmation rate of samples 7 and 17 was due to the isolation of pinpoint colonies (not classed as salmonella-presumptive) which produced an alkaline reaction without H₂S on lysine-iron. The overall preliminary confirmation rate was 76.1%. Three of the 25 meat-and-bone meal samples had no lysine decarboxylase-positive cultures and were not included in the tables.

Final confirmation. The final confirmation rate could not exceed 100% since the lysine decarboxylase-negative slants were discarded. Six samples showed 100% confirmation (no. 1, 5, 8, 12, 15, and 16), whereas the lowest, as expected, occurred with no. 7 and 17 which had the pinpoint colonies. The overall confirmation rate was 93.5%, with 14 samples over 90%.

Preliminary confirmation ratios. The ratios of the lysine decarboxylase-positive cultures to the presumptive salmonellae from the Brilliant Green agar plates are shown in Table 2. The only significant differences were between the 30- and 3-g sample sizes, the 30-g sample showing 81.4% confirmation compared to 65.7% for the 3-g sample. The difference between sample sizes was significant for both selenite and tetrathionate. Chi-square analyses were also made of all other pair-wise comparisons (i.e., selenite, 1 versus 2 days; tetrathionate, 1 versus 2 days; etc). None of these comparisons showed any statistical differences.

Final confirmation ratios. The percentages of somatic group positives from the lysine decarboxylase-positive cultures (Table 3) increased at 2 days incubation with both sample sizes and enrichments, although a significant chi-square was not obtained until the sample weights and enrichments were combined for the chi-square test. The comparison of 1 and 2 days of incubation gave a chi-square of 8.78, which was significant with $P < 0.01$. In these tests, a two-tailed chi-square test was used since there was no evidence prior to the start of the experiment for a superiority of either enrichment, day of incubation, or sample size

TABLE 1. Preliminary confirmed and fully confirmed cultures from individual meat-and-bone meal samples

Meat-and-bone meal sample	Lactose-negative colonies	Lysine decarboxylase-positive cultures	Somatic group positive cultures ^a	Lysine-positive/lactose-negative colonies (%)	Somatic-positive/lysine-positive (%)
1	156	106	106	67.9	100
2	90	112	95	124	84.8
3	10	8	4	80.0	50.0
4	12	7	6	58.3	85.7
5	50	45	45	90.0	100
6	98	74	69	75.5	93.2
7	14	19	2	136	10.5
8	120	115	115	95.8	100
9	110	107	106	97.3	99.1
10	68	46	44	67.6	95.6
11	16	8	4	50.0	50.0
12	78	8	8	10.2	100
13	128	115	110	89.8	73.3
14	58	53	52	91.4	98.1
15	32	10	10	31.2	100
16	121	108	108	89.2	100
17	18	19	4	106	21.0
18	26	18	17	69.2	94.4
19	77	22	16	28.6	72.7
20	76	67	66	88.1	98.5
21	80	61	60	76.2	98.4
22	160	79	78	49.4	98.7
Totals	1,638	1,246	1,165	76.1	93.5

^a Includes lactose-positive colonies from sample 1 (2 colonies), sample 2 (8 colonies), sample 6 (1 colony), sample 13 (1 colony), sample 14 (5 colonies), and sample 15 (6 colonies).

TABLE 2. Preliminary confirmation rates of presumptive colonies^a

Sample wt (g)	Enrichment	Incubation time (days)	Preliminary confirmation	
			Lysine-positive/presumptive colonies (no.)	Lysine-positive ^b
30	Selenite	1;2	439/525	83.6
3	Selenite	1;2	198/298	66.4
30	Tetrathionate	1;2	441/556	79.3
3	Tetrathionate	1;2	168/259	64.9
30	Selenite and tetrathionate	1;2	880/1081	81.4
3	Selenite and tetrathionate	1;2	366/557	65.7
30;3	Selenite and tetrathionate	1;2	1246/1638	76.1

^a Critical $\chi^2_{.995}$ (1df) = 7.88; critical $\chi^2_{.975}$ (1df) = 5.02.

^b Numbers in brackets are expressed as percentages; numbers to the right of brackets are χ^2 .

^c Significant difference at 1% level.

TABLE 3. Final confirmation rates of lysine-positive cultures

Sample wt (g)	Enrichment	Incubation time (days)	Final confirmation	
			Somatic group positive/lysine-positive (no.)	Somatic group positive ^a
30	Selenite	1	203/214	94.8
30	Selenite	2	222/225	98.7
3	Selenite	1	86/100	86.0
3	Selenite	2	88/98	89.8
30	Tetrathionate	1	198/212	93.4
30	Tetrathionate	2	224/229	97.8
3	Tetrathionate	1	69/83	83.1
3	Tetrathionate	2	75/85	88.2
30;3	Selenite	1	289/314	92.0
30;3	Selenite	2	310/323	96.0
30;3	Tetrathionate	1	267/295	90.5
30;3	Tetrathionate	2	299/314	95.2
30;3	Selenite	1;2	599/637	94.0
30;3	Tetrathionate	1;2	566/609	92.9
30;3	Selenite and tetrathionate	1	556/609	91.3
30;3	Selenite and tetrathionate	2	609/637	95.6
30	Selenite	1;2	425/439	96.8
3	Selenite	1;2	174/198	87.9
30	Tetrathionate	1;2	422/441	95.7
3	Tetrathionate	1;2	144/168	85.7
30	Selenite and tetrathionate	1;2	847/880	96.2
3	Selenite and tetrathionate	1;2	318/366	86.9
30;3	Selenite and tetrathionate	1;2	1165/1246	93.5

^a Numbers in brackets are expressed in percentages; numbers to the right of brackets are χ^2 . Critical $\chi^2_{.995}$ (1df) = 7.88; critical $\chi^2_{.975}$ (1df) = 5.02.

^b Significant difference at 1% level.

as far as the percentages of confirmed cultures were concerned. Some of the other 1- versus 2-day incubation comparisons in this table (i.e., selenite, 30-g; and tetrathionate 30- and 3-g) would have been significant with a one-tailed test (chi-square for one-tail test = 3.84). There were no significant differences between enrichments, but there was a highly significant difference between sample sizes with the 30-g

samples showing a 96.2% final confirmation rate compared to 86.9 for the 3-g size. The sample size difference was apparent for both the selenite and tetrathionate.

Numbers of somatic groups. Table 4 shows the total numbers of recovered somatic groups from the 22 samples. The most frequently isolated was C₁ from 68% (15) of the samples. E₄ and the E₄ variant (positive H₂S) were present

TABLE 4. Individual meat-and-bone meal samples showing different somatic groups^a

Sample no.	Somatic group															Total ^c				
	B	C ₁	C ₂	E ₁	E ₂	E ₃	E ₄	E ₄ ^b	G	H	35	18	30	21	Ari-zona		poly A	poly A-1	poly D	poly B
1	-	+	-	-	-	+	+	-	+	-	-	-	-	+	-	-	-	-	5	
2	-	+	-	-	+	+	-	+	+	-	-	-	-	+	-	-	-	-	6	
3	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
4	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
5	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	2	
6	+	+	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	6	
7	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	1	
8	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	7	
9	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	+	-	10	
10	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	2	
11	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
12	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	2	
13	+	+	+	+	+	-	+	-	+	+	+	-	-	+	-	-	-	+	11	
14	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	4	
15	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	3	
16	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	3	
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	1	
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	1	
19	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	2	
20	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	2	
21	+	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	5	
22	-	+	-	-	-	+	+	+	+	+	-	-	-	-	+	-	+	-	8	
Totals ^d	7	15	4	4	4	8	9	4	7	3	2	2	1	2	3	3	1	4	1	84
% ^e	32	68	18	18	18	36	41	18	32	14	9	9	5	9	14	14	5	18	5	

^a Plus indicates recovery; minus indicates no recovery.

^b This E₄ serogroup was H₂S positive.

^c Average number of somatic groups per sample is 3.8.

^d Total numbers of recovered somatic groups.

^e % = per cent recovery of somatic groups

in 59% (13) of the samples. Three other groups (E₃, G, and B) were each found in about 33% of the samples. Groups A, D, F, I, poly C, poly E, and poly F were not found in any samples. There also were no isolations of *Proteus*. This table also shows the total number of somatic groups isolated. These ranged from 1 group in 6 of the samples to a high of 11 distinct groups in no. 13 and 10 in no. 9. The average number of somatic groups found was 3.8.

Chi-square analysis of differences in somatic groups. The different somatic groups were further analyzed by determining differences caused by days of incubation, type of enrichment, and weight of sample. The results are shown in Table 5. This table shows the numbers of each somatic group in the samples without regard for the frequency with which the serotypes occurred within the samples. There were no significant differences except when comparing 30- versus 3-g sizes; the 30-g samples gave a larger number of somatic

groups. A nearly significant χ^2 value (3.50) was obtained with group C₁ when the comparisons were 1 day versus 2 days, selenite 3- and 30-g. There was also a near-significant difference in group G when 3- and 30-g were compared by summing days and enrichments ($\chi^2 = 3.86$). In this table as in Table 4, most of the samples' serogroups were represented by C₁ and E₄ including the E₄ variant.

Analysis of individual colonies. Table 6 is a compilation of all the presumptive-positive colonies which were selected with an analysis of somatic group differences by chi-square. The chi-squares for individual somatic groups were calculated by using the total of all groups as the marginal totals for the 2 x 2 contingency tables. The chi-squares for differences between enrichments and days were made using the total number of presumptive-positive colonies as the marginal totals. The table shows a highly significant ($P < 0.01$) increase in the frequency of isolation of group C₁ from

TABLE 5. Total number of meat-and-bone meal samples showing different somatic groups^a

Somatic group	Selenite 30 and 3 g		Tetrathionate 30 and 3 g		Selenite and tetrathionate 30 and 3 g		1 and 2 days 30 and 3 g		1 and 2 days with selenite and tetrathionate	
	1 Day	2 Days	1 Day	2 Days	1 Day	2 Days	Selenite	Tetrathionate	30 g	3 g
Group B	3	4	5	9	8	13	7	14	14	7
C ₁	11	19 ^b	18	20	29	39 ^c	30	38	50	26
C ₂	3	2	0	1	3	3	5	1	6	0
E ₁	4	3	3	5	7	8	7	8	11	4
E ₂	3	4	6	6	9	10	7	12	11	8
E ₃	6	7	5	5	11	12	13	10	17	6
E ₄	11	19	9	10	20	19	20	19	22	17
E ₄ ^d	2	1	3	2	5	3	3	5	6	2
G	5	5	5	4	10	9	10	9	14	5 ^e
H	2	0	0	1	2	1	2	1	3	0
35	4	3	1	2	5	5	7	3	5	5
18	2	2	3	2	5	4	4	5	5	4
30	0	1	0	0	0	1	1	0	1	0
21	1	0	1	1	2	1	1	2	3	0
Arizona	4	3	2	4	6	7	7	6	7	6
Poly A	3	3	0	1	3	4	6	1	3	4
Poly A-1	1	1	1	1	2	2	2	2	4	0
Poly D	3	4	2	2	5	6	7	4	9	2
Poly B	0	0	0	1	0	1	0	1	0	0
Total Positive ^f	28	28	27	29	56	57	56	56	73	39 ^g

^a Critical χ^2 .975 (1df) = 5.02.

^b χ^2 = 3.50.

^c χ^2 = 2.59.

^d E₄, positive H₂S.

^e χ^2 = 3.86.

^f These were used as marginal totals for chi-square.

^g Difference was very significant ($P < 0.01$).

tetrathionate as compared to selenite. Highly significant increases in the frequency of isolation of groups G, 35, and poly D were found with selenite. It should be noted that group 35 was very infrequently isolated (only two samples were positive); thus, the chi-square difference might be viewed as biased when all 22 samples were considered. The chi-square for 2 days versus 1 day tetrathionate using 30- and 3-g was significant at the 5% level (favor of 2 days).

DISCUSSION

The use of lysine-iron agar slants alone for identification of salmonellae worked very well in these studies. Triple-sugar iron agar, although providing a good preliminary separation of lactose and nonlactose fermenters, would have required the extra step into lysine-iron agar for preliminary salmonella grouping. The lysine decarboxylase reaction seems to be a better first-selection criterion than the nonability to ferment sucrose or lactose; indeed, apparently lactose-positive salmonellae, as isolated in this study, would have been missed if

triple-sugar iron had been the first differential medium.

The inability of either selenite-cystine or tetrathionate alone to pick up all positive samples was another confirmation of the results or opinions of other workers using a number of different raw materials (5, 6, 9, 15, 8). This study showed a significant difference in the individual somatic groups depending on whether selenite-cystine or tetrathionate was used.

This study also showed the relatively high contamination rate of meat-and-bone meal samples. Disallowing six samples which were suspected of being positive and one previously found to be negative and retested here, there were 16 positive samples out of the 18 picked at random. This study was, however, more thorough than the usual analysis where only one selective enrichment medium is incubated for 1 day. Leistner et al. (5) found 37% of meat-and-bone meal samples to be positive for salmonellae, whereas Leistner et al. (6) found 61% positive. Both of these studies were conducted using tetrathionate and selenite-cystine media.

TABLE 6. Numbers of somatic group positive colonies

Somatic group	Selenite 30 and 3 g		Tetrathionate 30 and 3 g		Selenite and tetra- thionate 30 and 3 g		1 and 2 days 30 and 3 g	
	1 Day	2 Days	1 Day	2 Days	1 Day	2 Days	Selenite	Tetrathionate
Group B	5	11	10	17	15	28	16	27
C ₁	118	138	135	136	253	294	256	291
C ₂	3	3	0	1	3	4	6	1
E ₁	13	11	14	16	27	27	24	30
E ₂	14	16	14	11	28	27	30	25
E ₃	24	27	23	24	47	51	51	47
E ₄	28	19	28	25	56	44	47	53
E ₄ ^b	3	2	6	4	9	6	5	101
G	27	26	10	11	37	37	53	21 ^a
H	3	0	0	1	3	1	3	1
35	13	20	1	5	14	25	33	6 ^a
18	8	4	6	4	14	18	12	10
30	0	2	0	0	0	2	2	0
21	1	0	2	3	3	3	1	5
Arizona	8	7	9	10	17	17	15	19
Poly A	5	4	0	1	5	5	9	1
Poly A-1	5	4	4	4	9	8	9	8
Poly D	11	16	5	5	16	21	27	10 ^c
Poly B	0	0	0	1	0	1	0	1
Total somatic-posi- tive ^d	289	310	267	299 ^c	556	609	599	566
Total presumptive- positive ^e	399	424	408	407	807	831	823	815

^a Significantly different at 1% level.

^b E₄, H₂S positive.

^c Significantly different at 5% level.

^d Marginal totals for χ^2 for serogroups.

^e Marginal totals for χ^2 for somatic-positive totals.

The number of serotypes isolated (an underestimate in this study since no H agglutination was used) ranged from 1 to 11 with an average of almost 4. Leistner et al. (6) found that 70% of their positive samples had more than one serotype; one of 47 of their samples had six serotypes. The study reported here showed that 16 of 22 positive samples (73%) contained more than one serogroup; 1 had 10 and another had 11. The average number of serogroups was nearly 4. A common practice is to pick two suspect colonies for further identification. Therefore, if the figure of four serotypes is typical of the entire meat-and-bone meal population, it would be impossible to determine the entire gamut of serotypes with only two colonies. On the other hand, to be fairly certain that a sample contains salmonellae of any serotype, the two colonies would appear to be adequate.

The significantly higher isolation rates of the C₁ from tetrathionate and of G, 35, and poly D from selenite underscores the importance of using both these selective media in salmonella isolations. Leistner et al. (5) also showed that

certain serotypes preferred one or the other of these two enrichment media.

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These tests did not show any advantage of a 2-day incubation period as far as detecting the positive meat-and-bone meal samples was concerned. There was, however, a very significant increase in the percentage of fully confirmed cultures at 2 days. This might be an indication that the selective media inhibited

the ability of the salmonellae to compete with the false positive types. Such a competition was also indicated from the higher confirmation ratios obtained from the 30-g as compared to the 3-g samples. The apparently higher numbers of false positives in the 3-g samples might also be a reflection of the nonrandom distribution of salmonellae in meat-and-bone meal especially when the particle size becomes a factor. A single particle, for instance, may be of tooth or bone (no salmonellae except on the surface) or may be of dried blood or meat (salmonellae throughout).

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TABLE 4. Individual meat-and-bone meal samples showing different somatic groups^a

Sample no.	Somatic group																Total ^c			
	B	C ₁	C ₂	E ₁	E ₂	E ₃	E ₄	E ₄ ^b	G	H	35	18	30	21	Ari-zona	poly A		poly A-1	poly D	poly B
1	-	+	-	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	-	5
2	-	+	-	-	+	+	-	+	+	-	-	-	-	-	+	-	-	-	-	6
3	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
4	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
5	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	2
6	+	+	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	6
7	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	1
8	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	7
9	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	+	-	10
10	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	2
11	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
12	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	2
13	+	+	+	+	+	-	+	-	+	+	+	-	-	+	-	-	-	-	+	11
14	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	4
15	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	3
16	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	3
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	1
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	1
19	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	2
20	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	2
21	+	+	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	5
22	-	+	-	-	-	+	+	+	+	+	-	-	-	-	+	-	+	-	-	8
Totals ^d	7	15	4	4	4	8	9	4	7	3	2	2	1	2	3	3	1	4	1	84
% ^e	32	68	18	18	18	36	41	18	32	14	9	9	5	9	14	14	5	18	5	

^a Plus indicates recovery; minus indicates no recovery.

^b This E₄ serogroup was H₂S positive.

^c Average number of somatic groups per sample is 3.8.

^d Total numbers of recovered somatic groups.

^e % = per cent recovery of somatic groups

in 59% (13) of the samples. Three other groups (E₃, G, and B) were each found in about 33% of the samples. Groups A, D, F, I, poly C, poly E, and poly F were not found in any samples. There also were no isolations of *Proteus*. This table also shows the total number of somatic groups isolated. These ranged from 1 group in 6 of the samples to a high of 11 distinct groups in no. 13 and 10 in no. 9. The average number of somatic groups found was 3.8.

Chi-square analysis of differences in somatic groups. The different somatic groups were further analyzed by determining differences caused by days of incubation, type of enrichment, and weight of sample. The results are shown in Table 5. This table shows the numbers of each somatic group in the samples without regard for the frequency with which the serotypes occurred within the samples. There were no significant differences except when comparing 30- versus 3-g sizes; the 30-g samples gave a larger number of somatic

groups. A nearly significant χ^2 value (3.50) was obtained with group C₁ when the comparisons were 1 day versus 2 days, selenite 3- and 30-g. There was also a near-significant difference in group G when 3- and 30-g were compared by summing days and enrichments ($\chi^2 = 3.86$). In this table as in Table 4, most of the samples' serogroups were represented by C₁ and E₄ including the E₄ variant.

Analysis of individual colonies. Table 6 is a compilation of all the presumptive-positive colonies which were selected with an analysis of somatic group differences by chi-square. The chi-squares for individual somatic groups were calculated by using the total of all groups as the marginal totals for the 2 x 2 contingency tables. The chi-squares for differences between enrichments and days were made using the total number of presumptive-positive colonies as the marginal totals. The table shows a highly significant ($P < 0.01$) increase in the frequency of isolation of group C₁ from

TABLE 5. Total number of meat-and-bone meal samples showing different somatic groups^a

Somatic group	Selenite 30 and 3 g		Tetrathionate 30 and 3 g		Selenite and tetrathionate 30 and 3 g		1 and 2 days 30 and 3 g		1 and 2 days with selenite and tetrathionate	
	1 Day	2 Days	1 Day	2 Days	1 Day	2 Days	Selenite	Tetrathionate	30 g	3 g
Group B	3	4	5	9	8	13	7	14	14	7
C ₁	11	19 ^b	18	20	29	39 ^c	30	38	50	26
C ₂	3	2	0	1	3	3	5	1	6	0
E ₁	4	3	3	5	7	8	7	8	11	4
E ₂	3	4	6	6	9	10	7	12	11	8
E ₃	6	7	5	5	11	12	13	10	17	6
E ₄	11	19	9	10	20	19	20	19	22	17
E ₄ ^d	2	1	3	2	5	3	3	5	6	2
G	5	5	5	4	10	9	10	9	14	5 ^e
H	2	0	0	1	2	1	2	1	3	0
35	4	3	1	2	5	5	7	3	5	5
18	2	2	3	2	5	4	4	5	5	4
30	0	1	0	0	0	1	1	0	1	0
21	1	0	1	1	2	1	1	2	3	0
Arizona	4	3	2	4	6	7	7	6	7	6
Poly A	3	3	0	1	3	4	6	1	3	4
Poly A-1	1	1	1	1	2	2	2	2	4	0
Poly D	3	4	2	2	5	6	7	4	9	2
Poly B	0	0	0	1	0	1	0	1	0	0
Total Positive ^f	28	28	27	29	56	57	56	56	73	39 ^e

^a Critical χ^2 .975 (1df) = 5.02.

^b χ^2 = 3.50.

^c χ^2 = 2.59.

^d E₄, positive H₂S.

^e χ^2 = 3.86.

^f These were used as marginal totals for chi-square.

^g Difference was very significant ($P < 0.01$).

tetrathionate as compared to selenite. Highly significant increases in the frequency of isolation of groups G, 35, and poly D were found with selenite. It should be noted that group 35 was very infrequently isolated (only two samples were positive); thus, the chi-square difference might be viewed as biased when all 22 samples were considered. The chi-square for 2 days versus 1 day tetrathionate using 30- and 3-g was significant at the 5% level (favor of 2 days).

DISCUSSION

The use of lysine-iron agar slants alone for identification of salmonellae worked very well in these studies. Triple-sugar iron agar, although providing a good preliminary separation of lactose and nonlactose fermenters, would have required the extra step into lysine-iron agar for preliminary salmonella grouping. The lysine decarboxylase reaction seems to be a better first-selection criterion than the nonability to ferment sucrose or lactose; indeed, apparently lactose-positive salmonellae, as isolated in this study, would have been missed if

triple-sugar iron had been the first differential medium.

The inability of either selenite-cystine or tetrathionate alone to pick up all positive samples was another confirmation of the results or opinions of other workers using a number of different raw materials (5, 6, 9, 15, 8). This study showed a significant difference in the individual somatic groups depending on whether selenite-cystine or tetrathionate was used.

This study also showed the relatively high contamination rate of meat-and-bone meal samples. Disallowing six samples which were suspected of being positive and one previously found to be negative and retested here, there were 16 positive samples out of the 18 picked at random. This study was, however, more thorough than the usual analysis where only one selective enrichment medium is incubated for 1 day. Leistner et al. (5) found 37% of meat-and-bone meal samples to be positive for salmonellae, whereas Leistner et al. (6) found 61% positive. Both of these studies were conducted using tetrathionate and selenite-cystine media.

TABLE 6. Numbers of somatic group positive colonies

Somatic group	Selenite 30 and 3 g		Tetrathionate 30 and 3 g		Selenite and tetra- thionate 30 and 3 g		1 and 2 days 30 and 3 g	
	1 Day	2 Days	1 Day	2 Days	1 Day	2 Days	Selenite	Tetrathionate
Group B	5	11	10	17	15	28	16	27
C ₁	118	138	135	136	253	294	256	291
C ₂	3	3	0	1	3	4	6	1
E ₁	13	11	14	16	27	27	24	30
E ₂	14	16	14	11	28	27	30	25
E ₃	24	27	23	24	47	51	51	47
E ₄	28	19	28	25	56	44	47	53
E ₄ ^b	3	2	6	4	9	6	5	101
G	27	26	10	11	37	37	53	21 ^a
H	3	0	0	1	3	1	3	1
35	13	20	1	5	14	25	33	6 ^a
18	8	4	6	4	14	18	12	10
30	0	2	0	0	0	2	2	0
21	1	0	2	3	3	3	1	5
Arizona	8	7	9	10	17	17	15	19
Poly A	5	4	0	1	5	5	9	1
Poly A-1	5	4	4	4	9	8	9	8
Poly D	11	16	5	5	16	21	27	10 ^c
Poly B	0	0	0	1	0	1	0	1
Total somatic-posi- tive ^d	289	310	267	299 ^c	556	609	599	566
Total presumptive- positive ^e	399	424	408	407	807	831	823	815

^a Significantly different at 1% level.

^b E₄, H₂S positive.

^c Significantly different at 5% level.

^d Marginal totals for χ^2 for serogroups.

^e Marginal totals for χ^2 for somatic-positive totals.

The number of serotypes isolated (an underestimate in this study since no H agglutination was used) ranged from 1 to 11 with an average of almost 4. Leistner et al. (6) found that 70% of their positive samples had more than one serotype; one of 47 of their samples had six serotypes. The study reported here showed that 16 of 22 positive samples (73%) contained more than one serogroup; 1 had 10 and another had 11. The average number of serogroups was nearly 4. A common practice is to pick two suspect colonies for further identification. Therefore, if the figure of four serotypes is typical of the entire meat-and-bone meal population, it would be impossible to determine the entire gamut of serotypes with only two colonies. On the other hand, to be fairly certain that a sample contains salmonellae of any serotype, the two colonies would appear to be adequate.

The significantly higher isolation rates of the C₁ from tetrathionate and of G, 35, and poly D from selenite underscores the importance of using both these selective media in salmonella isolations. Leistner et al. (5) also showed that

certain serotypes preferred one or the other of these two enrichment media.

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