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ENRICHMENT MEDIA IN THE ISOLATION OF PATHOGENIC ORGANISMS BELONGING TO THE COLON-TYPHOID GROUP

Thesis for the Degree of M. S.
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Grace Annabelle Teninga

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THESIS

This is to certify that the

thesis entitled

Enrichment Media in the Isolation of Pathogenic Organisms Belonging to the Colon-Typhoid Group

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ENRICHMENT MEDIA IN THE ISOLATION OF PATHOGENIC ORGANISMS BELONGING TO THE COLON-TYPHOID GROUP

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INTRODUCTION

Often negative results have been obtained in bacteriological examination of fecal specimens containing pathogenic bacteria, because the size of the inoculum was too small. By increasing the size of the inoculum, positive results were increased, as was evidenced by the use of Wilson and Blair medium where growth was found on the plate containing 5 ml. of a saline suspension of 1 g. of feces and not on the plate containing 1 drop. This method, however, could not be applied to a streak plate.

Attempts were made to obtain a liquid enrichment medium in which a large inoculum could be placed. This medium should support the growth of the few pathogens present while inhibiting or delaying the growth of the non-pathogens during an incubation period of 12 to 24 hours. Then, from this culture, streaked inoculation could be made on solid agar for colonial isolation.

Müller in 1923, used a broth medium containing sodium thiosulfate to which was added an iodine solution. This resulting tetrathionate medium was kept basic with chalk, and increased the positive results when used with routine media.

Schaeffer employed the above medium with less chalk. He was able to isolate four times as many fecal pathogens of the paratyphoid and thyphoid group as were found by ordinary methods.

Kauffman found that Müller's and Schaeffer's modification of Müller's tetrathionate medium allowed many lactose fermenters

and Proteus to grow. In an attempt to limit this he added ox bile and brilliant green to Müller's broth. Later workers, Knox, Gell and Pollack, found that this medium of Kauffman's also inhibited Salmonella typhi.

Jones also added dyes to the tetrathionate broth in an attempt to control the lactose fermenters. He added a combination of brilliant green and eosin which he titrated to a point where it would grow S. typhi but not Escherichia coli. This he added to a tetrathionate broth titrated in the same way. He claimed very good results with this medium.

Knox, Gell, and Pollack published many articles on their work with tetrathionate broth when used for growing Salmonella organisms. They found that the Salmonellae reduced tetrathionate rapidly to thiosulfate. They advocated the use of a balanced medium. ($2Na_2S_2O_3.5H_2O + I_2 \longrightarrow Na_2S_4O_6 + 2NaI + 5H_2O$) They claimed that an excess of iodine kills all bacteria and an excess of thiosulfate inhibits S. typhi. Müller, in his original research work, used a balanced medium but recommended an increase of thiosulfate five times greater than iodine for routine work.

In a comparative study by Knox, Gell and Pollack, a combination of tetrathionate broth and brilliant green, methylene blue agar gave better results than Wilson and Blair's medium or Eosin methylene blue agar alone.

Tetrathionate broth was used successfully in several epidemics by Jones, Gell and Knox and by Holt, Vaughan and Wright.

There were workers, however, who were not satisfied with tetrathionate broth. Mallov, Winter and Steinberg found that although tetrathionate broth increased the total number of positives, more were picked up by SS alone than by tetrathionate and MacConkey's. Invanics found that six strains of Aerogenes inhibited the growth of S. typhi in tetrathionate broth more than in plain broth. But Wynn and Williams found the opposite to be true.

In Holland, Pot (1943) used "Muller's tetrathionate successfully but had to abandon it in Curacoa, Netherlands, West Indies because of the spoilage by Proteus.

Another liquid medium which has been used extensively in this country, and which has been recommended by Maryland State Health Department, is a Selenite broth as suggested by Einar Leifson (1936). In his research work, he found that typhoid bacilli, cholera organisms, Proteus, and most parathyphoid organisms grew in a solution of 1% sodium selenite. Enterococci, Aerogenes, Shigella sonnei and Salmonella gallinarum grew in a 0.5% but not a 1% sodium selenite solution. E. coli grew in a .2% but not a .5% sodium selenite solution while Brucella grew only in a 1% solution.

In 1943, Cohen also worked with a Selenite broth medium. He was not aware of Leifson's work. He found that Salmonella parathyphi A and B, Salmonella enteritidis and Streptococcus fecalis were more resistant to selenite than E. coli. Salmonella was slightly more so.

He recommended a 0.2% Sodium Selenite broth or peptone water. This solution was weaker than the medium recommended by Leifson.

EXPERIMENTAL WORK

In order to find which was the best enrichment medium for the isolation of the pathogens found in fecal specimens, a comparative study of the efficiency of the following four media has been made:

Selenite - a dehydrated product produced by the Digestive Ferments Company

Mandelic Acid - also a dehydrated product from the Digestive Ferments Company

Tetrathionate broth - Müller's formulae

Tetrathionate broth - suggested by Knox, Gell and Pollack

This research was divided into two parts:

1. A comparative study of human feces inoculated artificially with:
   a. Salmonella typhi
   b. Salmonella enteritidis
   c. Salmonella typhimirium
   d. Salmonella oranienberg
   e. Shigella paradysenteriae Flexnerz
2. A comparative study of fecal specimens from carriers, carrier suspects, feces from patients suffering from Salmonella (food poisoning and typhoid fever.

Human feces artifically inoculated with pathogens.

Method: 1. A suspension of the pathogen was made from an agar slant by rubbing the surface gently with a sterile cotton swab and immersing it in sterile saline solution. The resulting saline suspension was diluted to match the Kaolin standard of 250 p.p.m. or 1000 million organisms per ml.

2. Serial dilutions were made from this suspension: 1-50, 1-100, 1-200, 1-400, 1-800 and 1600.

3. One tenth ml. quantities of the above dilutions were inoculated into 10g. of pooled human feces found negative on routine examination for enteric organisms in the Michigan Department of Health Laboratory.

4. Approximately 0.1 gram of the seeded feces was inoculated into 10 ml. enrichment medium and into 10 ml. saline.

5. One tenth ml. of the saline was streaked onto an SS plate. The next day colonial counts were made. This gave an approximation of the number of organisms present per 0.1 ml. of enrichment medium.

6. The enrichment medium were incubated for 12-24 hours. Then 0.1 ml. of the enrichment broth was streaked onto SS and MacConkey's agar. Wilson and Blair poured plates were made from 0.1 ml. and 5 ml. of the broth.

7. The SS and MacConkey plates were incubated 24 hours. Then colonial counts were made and compared with the original counts.

8. Wilson and Blair poured plates were incubated for 48 hours and counted.

9. The organism was identified by biochemical and rapid slide agglutination tests.

Method of Making Serial Dilutions

	Am't. of Saline	Dilution	0.1 ml. in 10 g. feces	0.1 ml. in 10 ml. broth	theoretical organisms in 0.1 ml.broth
1=1 ml of B	9 ml	1-50	1-5000	1-500,000	200
2=5 ml of 1	5 ml	1-100	1-10,000	1-100,000	100
3=5 ml of 2	5 ml	1-200	1-20,000	1-200,000	50
4=5 ml of 5	5 ml	1-400	1-40,000	1-4,000,000	25
5=5 ml of 4	5 ml	1-800	1-80,000	1-8,000,000	12
6=5 ml of 5	5 ml	1-1600	1-160,000	1-16,000,000	6

A=organisms diluted to standard 250 ppm or 1000 million organisms per milliliter

B=1 ml of A and 4 ml saline

Experiments and Results. At first attempts were made to use pooled feces, negative to pathogens, but showing bacterial growth on MacConkey's or SS plates. These feces were seeded with S. typhi and put into the tetrathionate broth (Müller's; and Knox, Pollack and Bell) incubated and plated on MacConkey's, SS and Wilson and Blair media. SS and MacConkey plates were all overgrown with lactose fermenters or Pseudomonas. Wilson and Blair medium gave negative results.

Use of feces showing no growth. Because of this overgrowth, feces showing no growth on routine examination by the Michigan Department of Health were used. These were pooled and mixed. Many of the specimens were several days to several weeks old and many contained bile.

Tetrathionate Broths. Again serial dilutions of S typhi were made and 0.1 ml. quantities were seeded into 10 g. feces. One tenth gram samples of these seeded feces were inoculated into Müller's and Knox' tetrathionate broth. These broths were then incubated for twenty hours. Then 0.1 ml. was plated onto SS and MacConkey's solid media. Poured plates of Wilson and Blair medium were made with 0.1 and 5 ml. amounts of the broths. All SS and MacConkey plates showed a marked increase in the number of organisms, the colonies being too numerous to count. Wilson and Blair plates had counts of 100 to too many to count on the plates containing 0.1 ml. of broth and one to ten colonies on plates having 5 ml. tetrathionate broth. These plates were discolored, therefore, it is probable that there were too many organisms for the medium to support a good growth.

SELENITE F. AND MANDELIC ACID BROTHS:

The above experiment was repeated using Selenite F (Difco) and Mandelic Acid broth (Difco) as the enrichment media. The control counts showed that the suspension contained clumps. The counts ranged from 200 to no colonies. Plated Selenite F medium showed a marked increase in organisms, the colonies being too numerous to count. This was not the case with Mandelic Acid broth; the increase was great but countable as seen by the Table. There was no growth on Wilson and Blair medium at any dilution.

Mandelic Acid & Selenite F inoculated with feces seeded with S.Typhosa.
December 5, 1946

Plate Counts. Organism/0.1 ml broth	43	200	120	105	-	-
0.1 ml Mandelic Acid plated on SS after 20 hrs.incubation	Many	Many	64	13	200/	55
0.1 ml Mandelic acid plated on Wilson and Blair	-	-	-	-	-	-
0.1 ml Selenite broth on SS after 20 hrs.incubation	Many	Many	Many	Many	Many	Many
0.1 ml Selenite F broth on Wilson and Blair	-	-	-	-	-	-

It was noted that there was a zone of inhibition on the SS plate where Selenite F and Mandelic acid broths diffused into the SS medium. There was a slight change in color at this point from red to orange. It is possible that there was a reaction between the SS medium and the Selenite and Mandelic acid broths making it more inibitive to the organisms, or that the double action of the Selenite--SS and Mandelic acid--SS kept the organism from growing.

A second test was run using the Selenite F and Mandelic acid enrichment media. The inoculum was smaller than that used in the first experiment on Selenite F and Mandelic acid broths. The broths were incubated for six, twenty and forty-four hours to find if the period of incubation had any significance on the growth of the organism. The inoculum seemed to be too small for the Mandelic acid broth. No organisms could be isolated on SS plates in 20 hours. Selenite plates on SS showed a decrease in six hours but an increase in twenty and forty-four hours.

Feces showing no growth with routine examination seeded with S. typhi and inoculated into Selenite F and Mandelic acid enrichment broth.
January 7, 1947

Plate counts colonies in one tenth ml broth	41	13	6	2	2	2
Mandelic acid 0_1/ml on SS	-	-	-	-	-	-
after 6 hrs. incubation	-	4	-	1	-	-
after 20 hrs. incubation	-	-	-	-	-	-
after 44 hrs. incubation	-	-	-	-	-	-
Selenite F 0.1 ml on SS						
after 6 hrs. incubation	-	5	-	2	-	-
after 20 hrs. incubation	-	many	1	300+	37	19
after 44 hrs. incubation	77	many	125	many	many	many

THE ZONE OF INHIBITION

Because Mandelic acid broth and Selenite F broth showed this zone of inhibition of all organisms where the broth media diffused into the SS plate, a special test was made. One tenth ml of sterile Selenite medium was pipetted onto one-half plate of two lots of SS agar and two lots of MacConkey's agar. The plates were allowed to stand until the

broth had thoroughly diffused into the solid media. Then two strains of S.typhi, one of an unidentified Salmonella and one of Shigella, Flexner strain, were streaked across the plate. On the SS medium there was complete inhibition of all organisms on the experimental side but good growth on the control side of the plate. MacConkey's medium showed a slight inhibition of the organisms where the Selenite diffused into the solid medium and good growth on the rest of the plate. Those colonies present were smaller and there was a reddening of the center of the colony. A new lot of Selenite F medium showed the same results.

The Mandelic acid medium was tested as was Selenite F and the results were the same.

Since it was possible that the organisms were present in the Selenite F and Mandelic acid broths but were inhibited by the double action of the SS -- Selenite or SS -- Mandelic acid media, attempts to dilute out the enrichment media were made. Three organisms were used with this experiment: a Salmonella, an S. typhi, and a Flexner organism.

Serial dilutions of these were seeded into pooled human feces showing no growth on routine examination, and these feces were inoculated into Selenite F and Mandelic Acid broths. After twenty hours, one tenth milliliter of the broth was transferred into 10 ml. sterile saline. One tenth ml of the saline was transferred onto SS and MacConkey plates and streaked. Colonial counts were made the next day. The Flexner and typhoid bacilli showed no growth. The original counts

showed that the inoculum was very small. Twenty-three organisms were 0.1 ml of broth was the heaviest inoculum of Flexner organisms, and seventy-two was the heaviest of the typhoid bacelli. It is possible that growth occurred but was small and was diluted out.

In the case of the Salmonella the inoculum was heavier throughout. The results show that the Selenite F broth supported the growth of the pathoges to a greater extent than the Mandelic acid broth. No zone of inhibition was noted.

Salmonella seeded into feces showing no growth with routine examination and inoculated into Mandelic Acid and Selenite F Broth

Original Colonial counts organisms /0.1 ml broth	125	48	20	16	8	2
Counts after 20 hrs.incubation						
Mandelic acid broth 0.1 ml in 10 ml saline 0.1 saline on SS	120	-	-	-	-	-
Mandelic acid 0.1 ml in 10 ml saline 0.1 saline on MacConkeys	40	78	-	-	-	-
Selenite F 0.1 in 10 ml Saline 0.1 ml Saline on SS	92	249	300+	-	-	-
Selenite F 0.1 ml in 10 ml Saline 0.1 ml saline on MacConkeys	134	203	86	-	-	-

Use of Negative Feces. Feces negative for pathogens but showing growth on MacConkey's or SS plates with routine examinations were then used. These were pooled and ten gram samples were seeded with the test organism. Three attempts to isolate S. typhi from seeded negative feces inoculated into the tetrathionate broths resulted in overgrowth on the SS plates and MacConkey plates by Pseudomonas or lactose fermenters. Wilson and

Blair poured plates were negative.

Selenite F, and Mandelic acid broths were inoculated with negative feces seeded with S. typhi, a Salmonella and a Shigella Flexner strain. No pathogens were isolated from any plates inoculated with 0.1 ml of the broths. All non-lactose fermenters proved to be spore formers. There were not many lactose fermenters present. The Salmonellae; enteritidis, typhimurium and oranienberg were then seeded into feces negative to pathogens. These feces were inoculated into all four broth media; Knox tetrathionate, Müller's tetrathionate, Selenite F and Mandelic acid. Only S. oranienberg could be isolated from the tetrathionate broths due to the extreme overgrowth of lactose fermenters. One colony of S. oranienberg was isolated from Mandelic acid broth plated on MacConkey's medium. All other plates were negative. All Selenite plates were negative. There were very few lactose fermenters on plates inoculated with Mandelic acid and Selenite F media.

Beef extract broth was inoculated at the same time from the same seeded feces, to see if any liquid media would increase positive results. As a whole, the broth inoculated plates were grossly overgrown with lactose fermenters. Swarming was much more noticeable than was noticeable with tetrathionate broths. An occasional oranienberg could be isolated.

S. enteritidis was again seeded into negative feces and these in turn were inoculated into all four enrichment media and into

tetrathionate broth prepared by the Michigan Department of Health. All plates inoculated with the tetrathionate broths were overgrown with lactose fermenters. The Wilson and Blair medium was negative with all enrichment media. There were very few lactose fermenters on the SS plates inoculated with Selenite F and Mandelic acid broth. Eight to ten uncolored translucent colonies appeared on each SS plate inoculated with Selenite and Mandelic acid broths. These proved to be very slow lactose fermenters and not S. enteritidis, seeded into the feces.

DISCUSSION

From the evidence gathered thus far it appears that normal feces seeded with pathogenic organisms was not a good test for the comparison of the efficiency of enrichment media. Feces showing no growth during routine examination often contained antibiotics such as bile and possibly penicillin which further inhibited or counteracted the inhibitory substances already present in the enrichment media. Feces negative for pathogens but showing growth on routine media, often contained rapidly growing non-pathogens, such as lactose fermenters, spore formers, and coliform organisms, in proportions much greater than found in the freshly passed specimen. The consistency and possibly the chemical composition were not the same as the pathogenic stool. The isolated organisms inoculated into these specimens were not as protected by protein and fats and by other

organisms, as were the non-pathogens present in the stool.

The second part of this research problem was therefore in order.

RESULTS OF SPECIMENS FROM PATIENTS

Carriers and Carrier Suspects. Fecal specimens from carriers of Salmonella and thyphoid bacilli were then tested. About one gram of feces was inoculated into each broth. These were incubated for 18 to 24 hours. One drop was placed on an SS plate and spread with a glass spreader. This spreader was then rubbed over the surface of a plate of MacConkey's agar. One drop was placed in a Petri dish and a poured Wilson and Blair bismuth sulfite plate was made.

The results of sixteen specimens were tabulated. Selenite and tetrathionate broths proved to be superior to Mandelic acid broth for these sixteen specimens. Selenite showed 33%, Mandelic acid 16.%, Knox tetrathionate 28.4% and Müller's tetrathionate 28.5% more positive than routine media.

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SPECIMENS POSITIVE TO SALMONELLA

	Enrichment Media Positive				Positive on routine analysis	Total positive feces	% increase over routine
	on SS	on MacConkeys	on Bi.S.	on any of the media			
Selenite	16	15	4	16	10	16	33%
Mandelic acid	12	9	1	13	10	16	16.5%
Knox tetrathionate	12	13	9	14	10	14*	28.5%
Müller's tetrathionate	12	12	4	14	10	14	28.5%

Shigella para dysenteriae carriers. Fecal specimens from carriers of Shigella paradysenteriae, Flexner were tested by these four enrichment media. The results for all the media were poorer than the results found with routine media. This is understandable when it is realized that these media were made for the isolation of the Salmonella group.

Shigella Paradysenteriae Flexner Carriers

	Positives found on SS	MacConkeys	either	Percent Decrease	Total Specimens
Selenite	1	0	1	33.3	15
Mandelic acid	3	1	3	40	15
Knox tetrathionate	2	0	2	46	15
Müller's tetrathionate	2	1	2	46	15
Routine media			9		15

KNOX TETRATHIONATE AND SELENITE MEDIA USED FOR SIXTEEN SPECIMENS

Approximately a gram of feces from sixteen selected routine specimens were inoculated into Knox tetrathionate and Selenite media. These media were incubated for 20 hours and then one drop was placed on SS medium and spread with a glass spreader. This spreader was then rubbed over the surface of the agar of a second SS plate. A poured Wilson and Blair plate was made with one drop of the enrichment media.

Both enrichment media showed more positives for Salmonella than were found on routine media. Three more positives were found with Knox tetrathionate broth plated on SS and Bismuth sulfite and two more were found with Selenite. One specimen was found positive with routine media but not with Selenite media. A Boyd 103 strain of dysentery bacilli was found on routine media but not with the enrich-

ment media. There were many more lactose fermenters on plates inoculated with the tetrathionate broth than those inoculated with Selenite broth. Selenite was found to be inhibitory enough to find isolated colonies on the first SS plate making the second one unnecessary.

	Positives On			Total Specimens	Percent Increase
	SS	Wilson & Blair	Lither		
Selenite	3	2	3	16	6
Knox tetrathionate	5	4	5	16	9
Routine			2	16	

Routine specimens tested with Knox tetrathionate and Mandelic acid enrichment media. A comparative study of Mandelic acid and Knox tetrathionate was made on routine specimens. There was no increase in positive results for either of the media but all specimens found positive for Salmonella with routine culture were positive with Knox tetrathionate plated with Wilson and Blair medium. None of these were found positive with Mandelic acid or with Knox tetrathionate plated on SS medium. Two paradysenteriae Boyd 103 and one paradysenteriae Flexner were found in specimens on routine examination but not with the enrichment media. Twenty-two specimens (five of which were pathogenic) showed no growth on SS plates inoculated from the Mandelic acid broths. Only six of these gave no growth on SS plates inoculated with tetrathionate.

Routine Specimens Tested for Salmonella

	Positive for Salmonella on SS	Bi S	either	Total Specimens	Percent Decrease
Knox tetrathionate	1	6	6	43	0
Mandelic acid	0	0	0	43	14
Routine examination			6	43	

Müller's tetrathionate, Knox tetrathionate, and Selenite F used for Routine Fecal Specimens. Eighty-three routine specimens sent into the Michigan Department of Health were inoculated into Knox tetrathionate broth, Müller's tetrathionate broth and Selenite F media. Salmonella was found in four and S. typhi in six of these eighty-three specimens when cultured with routine media. Two more Salmonellae were found on Wilson and Blair media plated with both tetrathionate broths. There was no increase in the number of positives with Selenite broth culture. Five specimens were positive with routine and not with Selenite F; two were positive with routine and not with Knox tetrathionate. One was positive with routine and not with Müller's tetrathionate. All specimens were one day to a week older than when examined with routine culture. One specimen positive in routine examination was overgrown with Pseudomonas on the SS plates of the tetrathionate broths but was positive on Wilson and Blair medium plated from Müller's tetrathionate broth and on SS medium streaked with Selenite broth. A Flexner strain of dysentery bacilli which was isolated with routine media was not found with any of the enrichment media.

Eighty-three Specimens Tested with Enrichment Media

	Positives On			Relative %	Total %
	SS	Bi S	Either	Increase	Increase
Knox tetrathionate	5	10	10	0	2.4
Muller's tetrathionate	8	11	11	1.2	2.4
Selenite F (Difco)	5		5	- .6	- .6
Routine			10		
Total Positives			12		

DISCUSSION OF PART II

Appearance of SS plates. Mandelic acid is very inhibitory; only a few colonies appear on the plate. There were very few lactose fermenters. Many plates showed no growth. There is a zone of inhibition where the broth medium diffused into the SS plate if there was growth on the plate.

Selenite F was less inhibitive than Mandelic acid but much more inhibitive than was the tetrathionate broths. Most of the plates contained isolated colonies. Except in rare instances there were very few lactose fermenters present. Sometimes there was a pure culture of the pathogens. There was a zone of inhibition where the Selenite diffused into the SS plate. Many plates contained only lactose fermenters with tetrathionate broth but plates from the same specimen had no growth with Selenite broth.

Müller's tetrathionate supports the growth of the pathogens very well but it allowed the non-pathogenic lactose fermenters to grow. Often these overgrow the pathogens. Sometimes it is hard to find an isolated colony on the second plate containing a very small inoculum.

Knox tetrathionate supports the growth of the pathogens equally

as well as Müller's but there is not quite as many lactose fermenters. Some plates show no growth with Knox tetrathionate when there are lactose fermenters on plates inoculated with Müller's containing the same specimen. The colonies do not swarm quite as much.

SUMMARY

1. Feces showing no growth on routine examination, seeded with a pathogen and inoculated into four enrichment media proved that Mandelic acid medium was more inhibitive to the pathogen than were Knox, Gell and Pollack's tetrathionate, Müller's tetrathionate and Selenite F broths.

2. Most pathogens were overgrown by the non-pathogens when artifically seeded negative feces were used.

3. Selenite F medium was more inhibitive to the non-pathogens, especially lactose fermenters, than were the tetrathionate broths.

4. A zone of inhibition to all organisms was noted where Mandelic acid and Selenite F media diffused into the SS solid medium.

5. Mandelic acid broth inhibited many pathogens as well as the non-pathogens from routine stool specimens.

6. There was little difference in the results in Knox, Gell and Pollack tetrathionate broth and Müller's tetrathionate broth. Both produced more positive results than the other two media.

7. Selenite F medium increased the total number of positive specimens. However, it failed to give positive results with several specimens that were positive with routine media and the tetrathionate broths. It has an advantage of giving more isolated colonies on SS medium than are found with tetrathionate broths on SS medium.

FORMULAE

MULLER'S TETRATHIONATE

To 180 ml sterile nutrient broth with 9 grams chalk was added 20 ml sodium thiosulphate solution. (50 g sodium thiosulphate in 100 ml water, sterilized by steaming)
4 ml Lugols solution (25 g. iodine, 20 g. potassium Iodide in 100 ml water

KNOX, GELL AND POLLACK BALANCED TETRATHIONATE

To 80 ml broth containing 4½ g. chalk was added 5.5 ml thiosulphate (24.8g. Sodium thiosulfate to 100 ml water) and 5.5 ml Iodine solution (20g. Potassium Iodide and 12.7 g. Iodine to 100 ml water)

DIFCO SELENITE F

2.3 g. dehydrated product made to 100 ml was not sterilized
This media was very unstable even in the dehydrated state

Ingredients per liter:		
	Sodium and Selenite	5 g.
	Bacto-Tryptone	4 g.
	Bacto-Lactose	4 g.
	Disodium Phosphate	10 g.

DIFCO MALONIC ACID

3.3 g. dehydrated product made to 100 ml. This was not sterilized. (Formulae not known)

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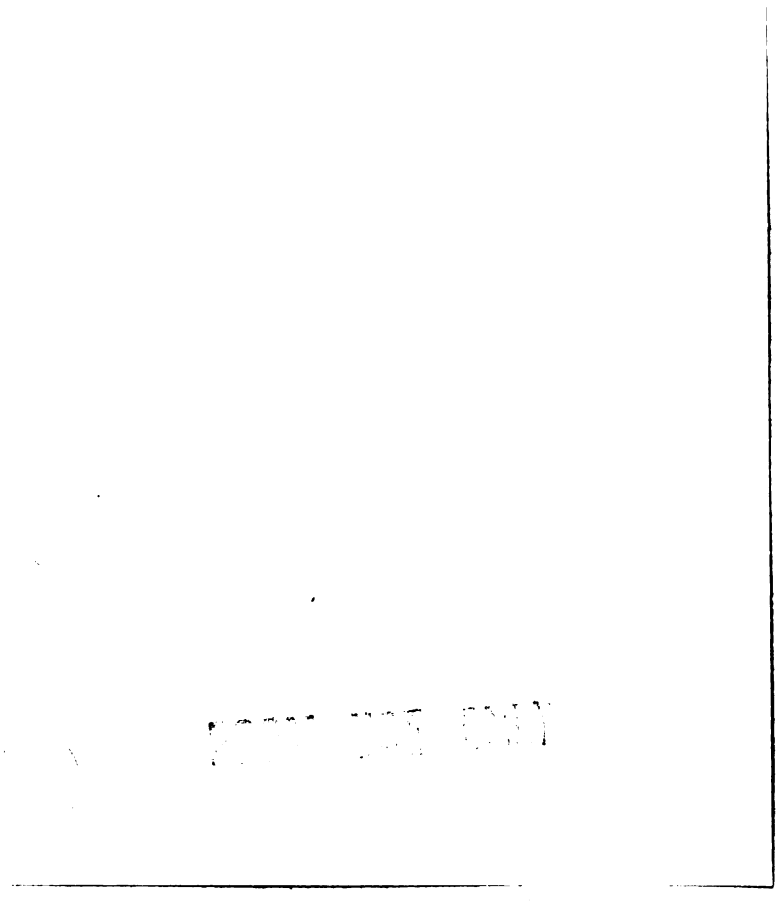

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