A STUDY OF DIFFERENT MEDIA FOR THE DETECTION OF MINIMAL NUMBERS OF STREPTOCOCCI IN SWIMMING POOL WATER

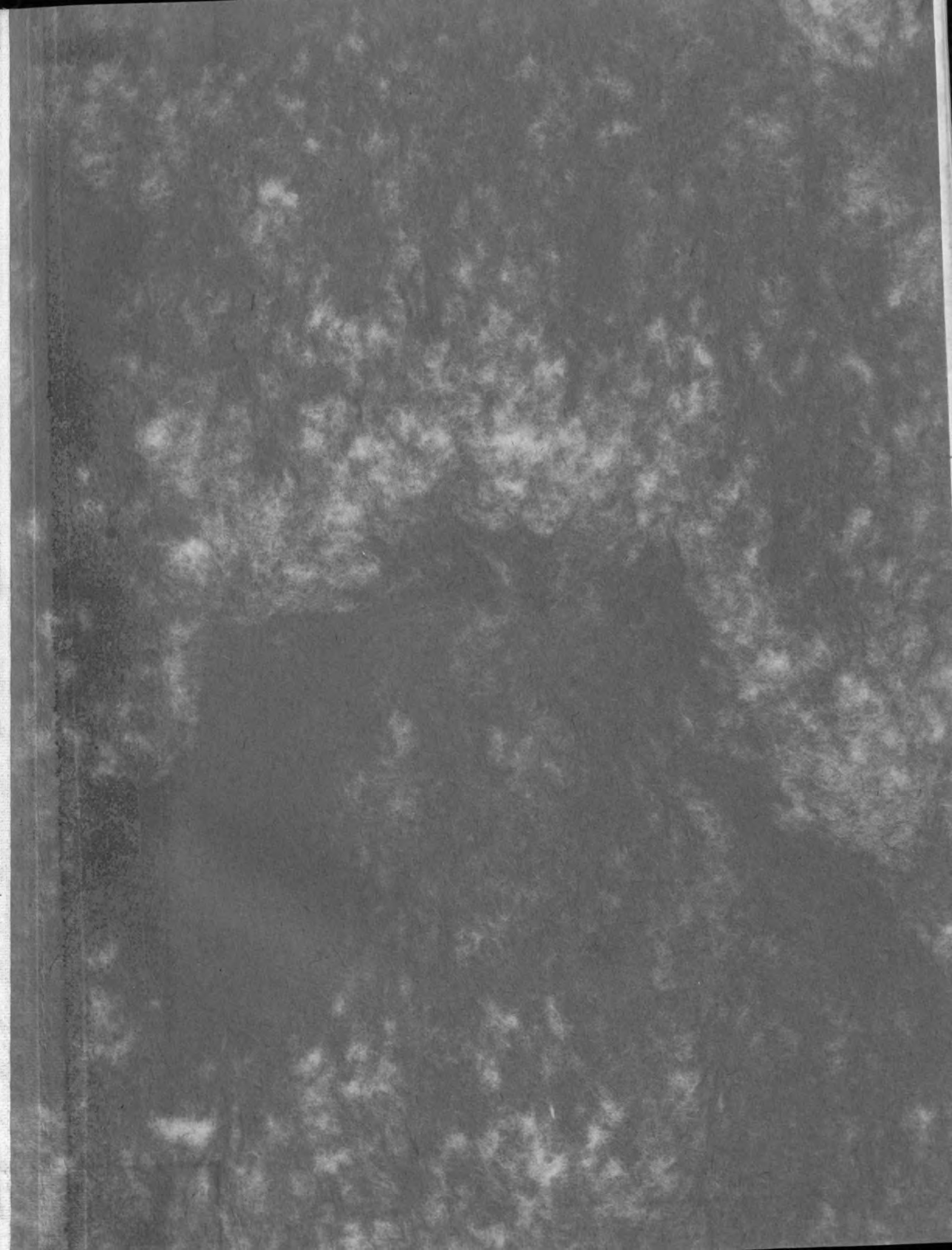
Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Wells A. Shulls

1939

THESIS



Michigan State College
of
Agriculture and Applied Science

A Study of Different Media For the Detection of Minimal Numbers of Streptococci in Swimming Pool Water

A Thesis
Submitted to the Graduate Faculty
For the Master of Science Degree

Department of Bacteriology and Hygiene

by

Wells A. Shulls
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THESIS

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Introduction

A study in this laboratory by Darby and Mallmann (1) paralleling this presentation, demonstrated that more coliform organisms would develop from minimal plantings with a tryptose lactose medium than would with lactose peptone broth made according to specifications of the American Public Health Association "Standard Methods of Water Analysis" (2).

This study was initiated on the thesis that this same medium or perhaps a modification would allow the development of a greater number of streptococci upon minimal plantings in a manner comparable to that evidenced with the coliform organisms as cited by Darby and Mallmann.

Although the basic findings of this study are more or less applicable to the cultivation of streptococci from any source, the studies presented are applied primarily to the isolation of streptococci from swimming pool waters. The use of pool water as a source was selected for three reasons, (1) that swimming pool waters contain both Escherichia coli and streptococci introduced by the bathers, (2) that samples could be obtained with ease, and (3) the degree of pollution of the swimming pool could be controlled.

The importance of streptococci has been shown by Mallmann (3) who found that the incidence of streptococci

paralleled the amount of pollution more closely than did the Esch. coli index.

Studying the pollution indices of natural bathing places Mallmann and Sypien (4) found that the streptococcus index fluctuated with the bathing load and that streptococci were not found at points free from bathing pollution. Their findings were also true in indoor swimming pools.

The importance of streptococci in swimming water is further demonstrated by the fact that these organisms represent, contrary to most opinion, a measurement of respiratory contamination. In studies by Mallmann and Ardrey (5) it was shown that the streptococci found in pool water were of respiratory origin.

If Esch. coli were more resistant to disinfection than other organisms encountered in swimming pools the present test would be satisfactory, but Klang (6) has shown this is not true. It appears that the streptococci are the more resistant to the chlorine in the pool and therefore the more important, particularly in the light of their sanitary significance.

The use of various media was studied by Habermann (7) with the result that lactose peptone broth was found to be the best of all the media tested for the growth of streptococci. As the streptococci present in a swimming pool should not be in great numbers, a medium functioning well for minimal plantings

is needed. Thus the medium containing tryptose was used in the hope that such a medium would show higher indices than lactose broth. With these ideas in mind the following experiments were conducted.

Historical

One of the first mediums mentioned for growing streptococci from water supplies as a routine method of analysis was made by Prescott and Baker (8). They planted the water sample in lactose, dextrose, sucrose, and other sugar broths and on litmus agar. The streptococci and Esch. coli were differentiated in the litmus agar by the difference in reaction.

Continuing along this line of color differentation of the streptococci, Todd (9) found that with neutral red in a one per cent solution in lactose agar the streptococci gave a typical color reaction. Several species of streptococci could be distinguished by this method.

Crowe (10) using blood for enrichment prepared a medium using Hiss serum water to which he added 10 per cent of a boiled blood filtrate. This was prepared by thoroughly boiling one part of defibrinated blood with three parts of water and autoclaving the filtrate. In 1923 Crowe (11) used a special glucose-blood-agar medium which grew streptococci well and upon which they showed different morphological charactistics.

Using a medium which contained meat infusion, peptone,

gelatine, casein, glucose, disodium hydrogen phosphate, and sodium citrate. Ayers and Johnson (12) grew streptococci well and kept them alive for over four months. The reasons they gave for this growth are: " consistency of medium, presence of casein, slight amount of sugar which serves as an easily available source of carbon, and the favorable buffering of the medium."

Crowe (13) used his special blood agar to differentiate various species of streptococci by the color of the colonies. Continuing with the principle of blood agar, Spray (14) used an agar containing 5 to 10 per cent of a fluid, which he obtained from fresh blood clot that had been digested. This medium grew streptococci abundantly.

Using a culture of streptococci grown in blood agar and a culture kept alive by passage through rabbits, Beckwith and Rose (15) found that (1) the passage strain had a more active proliferation than the stock culture, (2) that the passage strain was more virulent, and (3) the passage strain had a lower thermal death point.

Medalia, Bailey, and Atwood (16) developed a modified Loeffler's blood serum medium which they used in routine examination for streptococci. This medium called for pig serum pH 6.8 which after autoclaving gave a solid transparent medium.

The strangest medium developed is that of James' (17)

using a 20 per cent infusion of Quahaug (common American round clam) or five per cent of fish roe infusion. The infusion seems to supply the necessary growth substances. The growth on fish roe equaled blood infusion agar, but a decrease in growth took place if the concentration of the infusion exceeded five per cent. The medium consisted of the infusion, Witte's peptone, sodium chloride, agar, and 0.5 ml. of sterile defibrinated sheep blood which was added after autoclaving and cooling to 45°C.

Wright (18) found that the size of the streptococci colonies is influenced by the thickness of the layer and the concentration of the agar, the addition of blood or serum and the availability of the foodstuff.

The size of the colony is not affected by omitting glucose from the medium if peptone is present in adequate amounts. Filtration through thick filters may cause marked deterioration of the broth, and it is best filtered through glass wool or thin paper.

Going back to the earlier work on the selectivity of dyes, Chapman, Lieb, Berens, and Curcio (19)produced a medium which contained bromthymol blue in lactose agar at a pH of 8.6. With this medium it was possible to differentiate by color differences pathogenic from non-pathogenic streptococci on initial planting.

In order to grow Streptococcus salivarius, Saffor,Sherman,

and Hodge (20) used a medium made of 1.0 per cent lactose, 0.1 per cent glucose, 0.3 per cent meat extract, and 0.5 percent each of yeast extract and peptone. Sherman and Stark (21) found that gas could be produced in lactose if Streptococcus thermophilies (lactose positive) and Proteus (lactose negative) were grown together.

The rate of fermentation of Streptococcus lactis was studied by Rahn, Hegarty, and Devel (22) using a 2 per cent phosphate buffer + 2 per cent glucose + 0.3 to 0.5 per cent peptone. The optimum pH was found to be 7.0. The most rapid rate was in a buffer solution containing 4 per cent phosphate.

In considering this subject it was thought advisable to make a very short survey of the nutritional requirements and the biochemical reactions of the streptococci. Lloyd (23) found that certain accessory factors of growth were present in blood, serum, milk, and animal tissues and vegetable tissue, they were soluble in water and alcohol, but that they could be absorbed from the medium by filter paper. He also prepared a transparent blood agar.

Later Cole and Lloyd (24) concluded that the important factors necessary for growth are a suitable pH concentration, a high concentration of free amino-acids, and the presence of certain special "growth" hormones.

In the study of streptococci metabolism, Kendall, Day,

Walker, and Ryan (25) concluded that carbon was the source of energy and that it was indispensable for the structural requirements of the organisms. Ayers and Mudge (26) shed some light on the composition of the "growth" promoting factors of Lloyd in vitamines. They used an autolyzed yeast extract containing these growth promoting substances. The water soluble B did not appear to be of significant value. Fats and oils, vegetable, animal, and mineral even in very small amounts were found to stimulate the growth. They concluded: "either the growth promoting property of fats and oils is not due to fat-soluble H, or this vitamin is present in mineral oils, or the stimulation is due to different causes in the case of the vitamin-containing fats and oils and the mineral oils."

Whitehead (27) found that on tryptic digestion of caseinogen the digested mixture may be separated into three fractions with Ethyl alcohol. Thus he was able to remove all the inorganic phosphate present in the fractions by precipitation with 66 percent alcohol. There was a decided lag in media prepared from the two fractions containing no inorganic phosphate.But if phosphate is added rapid growth occurs. He concluded from this that at least three fractions appear to be concerned in the growth of streptococci; two are probably protein derivatives and the third is phosphate preferably in an inorganic form. It appears that the phosphate catalyzes the reaction.

Hucker (28) found tnat the strains of streptococci producing levo-lactic acid hydrolyze lactose faster than other strains.

Krashaw and Gies (29) developed a liver-peptone medium which was useful in storing streptococci. They were successful in keeping cultures three years on this medium. Considering the amino-acid requirements of streptococci, Hutner (30) used deproteinized skimmed milk and a casein hydrolysate demonstrating these requirements.

No historical review of work on media would be complete without some short résumé of the literature on the important subject of hydrogen-ion concentration. It is a known fact that the hydrogen-ion concentration plays a big role in the success of a medium to grow bacteria.

Using electrical pH measurements, Ayers (31) found that the final pH for streptococci from humans was 4.6 to 4.8 from broth that was pH 6.8 to 7.0 initially.

Grace and Highberger (32) found that a broth with an initial reaction of pH 6.8 will favor a more rapid growth while a broth of pH 7.6 will distinctly retard growth.

The final pH of glucose or lactose broth produced by streptococci was found by Foster (33) not to be influenced by the presence of K_2HPO_4 in concentrations up to one per cent providing sufficient glucose is present. He believed

the optimum pH for acid formation to be 7.8. He concluded that acidity is the chief factor in inhibition and death of streptococci. A table for minimum and maximum growth is also given.

In studying the variation in pH of broth media Foster and Randall (34) found that there was a change in broth of 0.2 pH upon autoclaving. In general the pH change was in the direction of increased acidity (decrease of pH); thus the greatest pH change would come in broth alkaline in reaction, less in neutral broth, and the least in acid broth.

Experimental

The supply of water used in the following experiments was obtained from five secondary school swimming pools and one out-door swimming pool. In most cases these pools were over loaded. Of course there were times when the pool load decreased to only a few bathers.

The samples were collected in sterile thiosulphate bottles, as recommended by Mallmann and Cary (35), and taken immediately to the laboratory and tested.

The lactose peptone broth and nutrient agar used in the experiments were made according to standard methods of A.P.H.A. (2) the tryptose broth and agar were made as follows: 2 percent tryptose, 0.5 per cent lactose, 0.4 per cent K_2HPO_4, 0.15 per cent KH_2PO_4, and 0.5 per cent sodium chloride per

liter. The agar contained 2 per cent tryptose, 0.1 per cent dextrose, 0.5 per cent sodium chloride, and 2 per cent agar.

In order to maintain the concentration of lactose and peptone as recommended by A.P.H.A. "Standard Methods of Water Analysis," (2) the broths were made with twice the necessary concentration of peptone and lactose; so when a 10 ml. sample of water was added to 10 ml. of broth the concentration of the required 0.3 per cent of peptone and lactose was reached. In order to determine the smallest amount of sample to give positive results various sized samples of water were used in the 10 ml. of broth. All the broth was tubed, 10 ml. per tube, with a Durham's gas insert, adjusted to pH 6.8 and sterilized at 15 pounds pressure for 20 minutes.

The culture used for comparative tests on the media was a non-hemolytic streptococcus isolated from an ulcer in the colon of a man. This organism was carried on 2 per cent tryptose and 2 per cent agar slants. A hemolytic streptococcus isolated from a dog's kidneys was tried, but it grew too scantily.

The broth used in the growth curves was the same as above, 100 ml. of single strength broth to a flask. All incubations were conducted at 37°C.

A short résumé of the procedure follows. At least four samples were obtained from each pool for each series of tests.

The samples were collected in sterilized thiosulphate bottles as aseptically as possible. The samples were transported to the laboratory immediately. Five 10 ml. portions of each sample were planted in 5 tubes containing 10 ml. of double strength tryptose lactose broth and 5 tubes of double strength lactose peptone broth. One ml. portions of the water were plated in duplicate in standard dextrose agar. The tubes and plates were incubated at 37°C. with a 24 hour and a 48 hour observation. Any gas was noted, a loopful of the broth showing gas formation was planted in 10 ml. of brilliant green lactose bile broth for confirmation of coliform organisms.

After the 48 hour observation Gram stains were prepared and examined for streptococci. The streptococci index was determined in the same manner as the Phelps colon index.

The procedure for the growth curve was carried out as follows. An appropriate amount of the organisms was scraped from an agar slant with a loop and put into a 10 ml. saline blank to give a faint turbidity. This was then diluted to 1:1000 and one ml. portions were seeded in 100 ml. flasks of lactose peptone broth and in 100 ml. flasks of tryptose lactose broth. The flasks were shaken well and 1 ml. was plated in tryptose agar. The plates and flasks were incubated at 37°C. At three hour intervals for twelve hours suitable dilutions of the broths were plated in tryptose lactose agar.

The plates were incubated at 37 C. for 48 hours and counted. Duplicate plates were made of all dilutions.

Discussion

As stated previously this problem was undertaken to see if tryptose lactose medium could be used for the isolation of streptococci from swimming pools. It is true that lactose peptone broth does grow streptococci from swimming pool waters, but it is questionable whether or not this medium allows the development of all of the streptococci present.

Portions of five 5 ml., five 1 ml., five 0.5 ml. of water were used in 10 ml. tubes of tryptose lactose and lactose peptone double strength broth respectively, in order to determine if these amounts would show any variation in the number of positive tubes. Five 10 ml. tubes of the broth were used as this number was easy to handle and was large enough to reduce most of the errors that might be introduced.

In the above series of tests it will be noted that double strength broth was used rather than single strength which is recommended by standard procedure of A.P.H.A. These concentrations were used purposely to determine the tolerance of the streptococci to varying amounts of tryptose, peptone, and salt. Double strength tryptose lactose medium contains a high concentration of tryptose (4 per cent) while the lactose peptone broth contains only 1 per cent peptone. With the portions used there would be very little if any dilution of the medium. The results of these series are very interesting.

There were only five samples tested using five 5 ml. portions. Samples tested in the tryptose lactose broth have a higher streptococci index than those tested in the lactose peptone medium. The mean Phelps index is 17.6 for tryptose lactose broth and 14.4 for lactose peptone medium.

Fifty four samples were tested using 1 ml. dilution series. Table 1 shows that the streptococci index is approximately equal for the two broths. The mean Phelps index is the same for each broth, namely 63.4.

It is evident from the above, that with the samples used the dilutions tested were not high enough. Too many streptococci were present in each tube, so that sufficient organisms were present to allow growth in both lactose peptone and tryptose lactose broth media. With the 0.5 ml. portions the dilution was such that the tubes received minimal numbers of organisms. Of the 22 samples, table 2, used in this series the tryptose lactose medium index was higher than lactose peptone broth index. The mean Phelps index was 17.12 for tryptose lactose broth, and 16.86 for the lactose peptone broth.

These results can be shown graphically by plotting the frequency of the number of times the Phelps index of the sample occurs. Graph 1 shows the 5 ml. portions and the 0.5 ml. portions. 0.5 ml. portions contained, as stated before, minimal numbers of streptococci, this curve shows a tendency

TABLE I

A comparison of standard lactose peptone broth and tryptose lactose broth using 5 ml.

Date of Sampling	Source	Cl Res. ppm.	Bathing Load	Streptococci Indices		Colon Index	Total 37° C Count
				Lactose Broth	Tryptose Broth		
7/12/38	Pattingill	0.4	27	12	16	0	18 8
7/13/38	Moore's Park	0.5	225	20	20		
				8	20		
				16	20		
				16	12		

A comparison of standard lactose peptone broth and tryptose lactose broth using 1 ml.

Date of Sampling	Source	Cl Res. ppm.	Bathing Load	Streptococci Indices		Colon Index	Total 37° C Count
				Lactose Broth	Tryptose Broth		
7/12/38	Pattingill	0.4	27	60	80	0	18 8
7/13/38	West Junior	0.5	40	40	20	0	251 151
				40	60	0	424 510
7/14/38	Pattingill	1.5	32	60	80	0	1 3
				80	20	0	6 8
				80	60	0	43 5
				20	80	0	4
7/15/38	Moore's Park	1	200	100	100	6	116 119
				100	100	0	194 32
				100	100	0	25 1800
				100	100	0	288
7/15/38	West Junior	0.75	38	60	100	0	23 21
				100	40	0	209 75
				0	60	0	46 19
				20	60	0	33 14

TABLE I (continued)

A comparison of standard lactose peptone broth and tryptose lactose broth using 1 ml.

Date of Sampling	Source	Cl Res. p.p.m.	Bathing Load	Streptococci Indices		Colon Index	Total 37°C Count
				Lactose Broth	Tryptose Broth		
7/18/38	Moore's Park	2	175	100	100	0	192 166
				80	100	0	38 8
7/20/38	West Junior	0.5	30	80	60	0	1210 1159
				20	100	0	1792 1956
				80	100	0	1816 716
				0	80	0	985 556
7/20/38	Pattingill	0.3	35	0	0	0	80 50
				20	20	0	4 93
				40	20	0	71 89
				40	40	0	63 85
7/20/38	Moore's Park	2.5	225	40		0	35 3
				80	80	4	94 73
		2.5	225	100	80	2	94 73
				100	80	0	32 79
7/21/38	Pattingill	0.4	32	0	20	0	16 9
				20	40	0	13 55
				0	20	0	22 20
				0	0	0	15 26
7/21/38	West Junior	0.5	20	20	40	0	13 3
						0	103 157
						0	367 202
				20	20	0	66 123

TABLE I (continued)

A comparison of standard lactose peptone broth and tryptone lactose broth using 1 ml.

Date of Sampling	Source	Cl Res. p.p.m.	Bathing Load	Streptococci Indices		Colon Index	Total 37°C Count
				Lactose Broth	Tryptose Broth		
7/21/38	Moore's Park	2	150	100	100	0	100 88
				100	60	0	6 3
7/22/38	Moore's Park	1	175	100	100	0	17 22
				100	100	2	
				100	100	4	
				60	20	0	
7/25/38	Moore's Park	1	200	100	100	4	
				100	60	2	23 20
				100	100	0	30 6
10/11/38	Walter French	0.6	4	0	40	0	
				60	0	0	
				60	100	0	
				80	100	0	
				20	40	0	
				100	100	0	
				0	60	0	
				80	60	0	

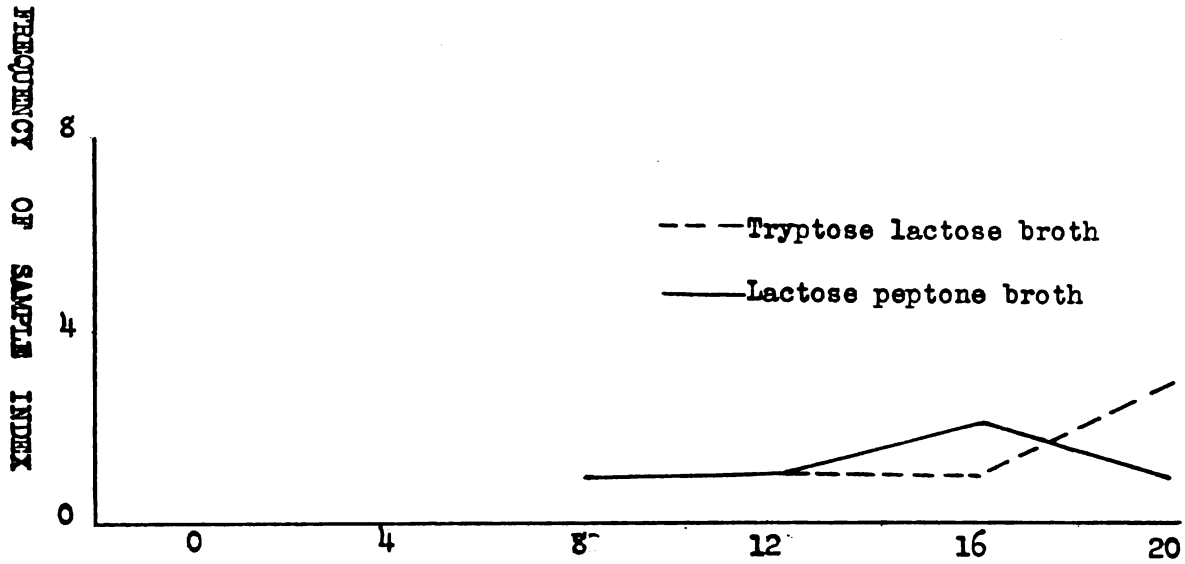
TABLE II

A comparison of standard lactose peptone broth and tryptose lactose broth using 0.5 ml. samples.

Date of Sampling	Source	Cl Res. p.p.m.	Bathing Load	Streptococci Indices		Colon Index	Total 37°C Count
				Lactose Broth	Tryptose Broth		
7/12/38	Pattingill	0.4	27	24	32	0	18 8
7/26/38	Pattingill	0.4	32	0	24	0	47 45
				24	32	0	83 21
				7	8	0	169 151
				16		0	16 38
7/26/38	West Junior	0.6	38	0	0	0	117 119
				0		0	233 189
				0	0	0	78 152
						0	102 122
7/26/38	Moore's Park	1	150	32	32	2	59 84
				32	8	6	120 195
7/28/38	West Junior	0.6	36	0		0	419 165
				0	16	0	340 476
				16	16	0	1467 568
				8	0	0	934 593
7/28/38	Pattingill	0.75	28	0	0	0	16 7
				16	8	0	14 5
				32	40	0	22 27
				40	40	0	148 208
7/28/38	Moore's Park	1	175	32	40	0	56 7
				32	24	0	104 148
				40	40	0	95 99

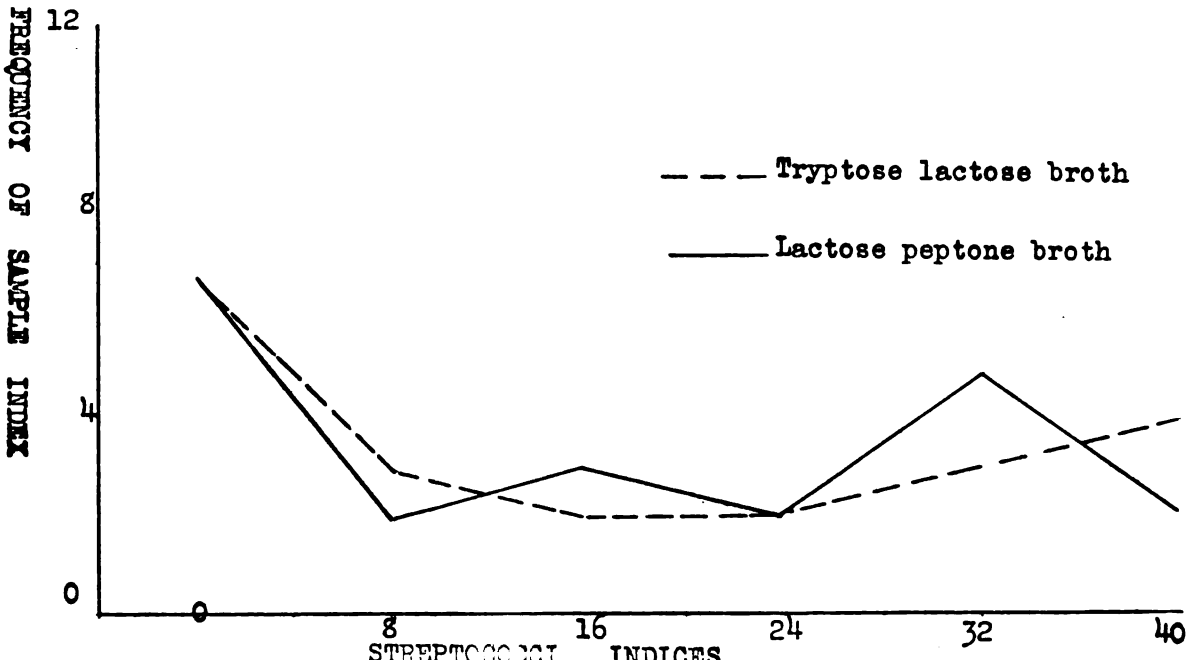
GRAPH I

STREPTOCOCCI INDICES OF LANSING SWIMMING POOLS USING A 5 ML. SAMPLE



GRAPH I

STREPTOCOCCI INDICES OF LANSING SWIMMING POOLS USING A 0.5 ML. SAMPLE



of the tryptose lactose broth to give a frequency curve that is smoother than the corresponding curve of lactose peptone broth. From the number of samples present, based on the curve and mean Phelps index, the tryptose lactose broth is better than the standard lactose medium with minimal plantings. The curves suggest that this tendency of the curve to approach the ideal frequency curve is due to the fact that this medium may be approaching the ideal in the detection of positive tubes.

Graph II shows the 1 ml. dilution series. The frequency of the number of positive tubes is clearly shown to be higher for the tryptose lactose broth. There also is a lower frequency of negative samples with the tryptose lactose medium, which would indicate that all streptococci were detected even in minimal numbers in this dilution.

The fact that streptococci were detected even in minimal numbers and in a broth with high concentration, indicates that the streptococci found in swimmingpool waters either have a tolerance for tryptose or that the tryptose is used easily by them. This action would, of course, be shown in their growth rate in the presence of tryptose. To determine if there was an increase in growth rate several growth curves were run comparing lactose peptone and tryptose lactose media. The results are shown in graphs 3, 4 and 5. They show clearly that the growth rate was increased, and that the lag phase

GRAPH II

STREPTOCOCCI INDICES OF LANSING SWIMMING POOLS USING A 1 ML. SAMPLE

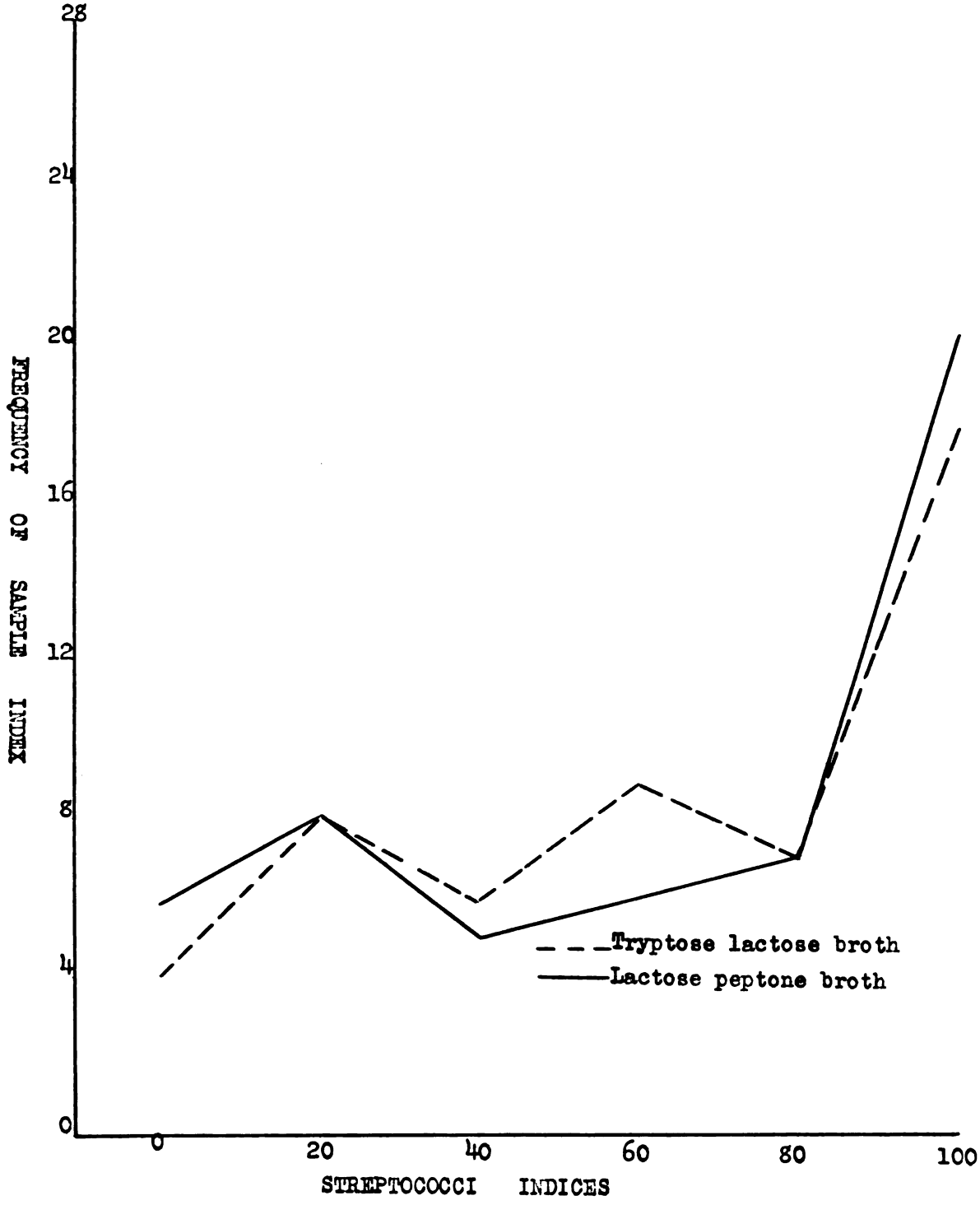


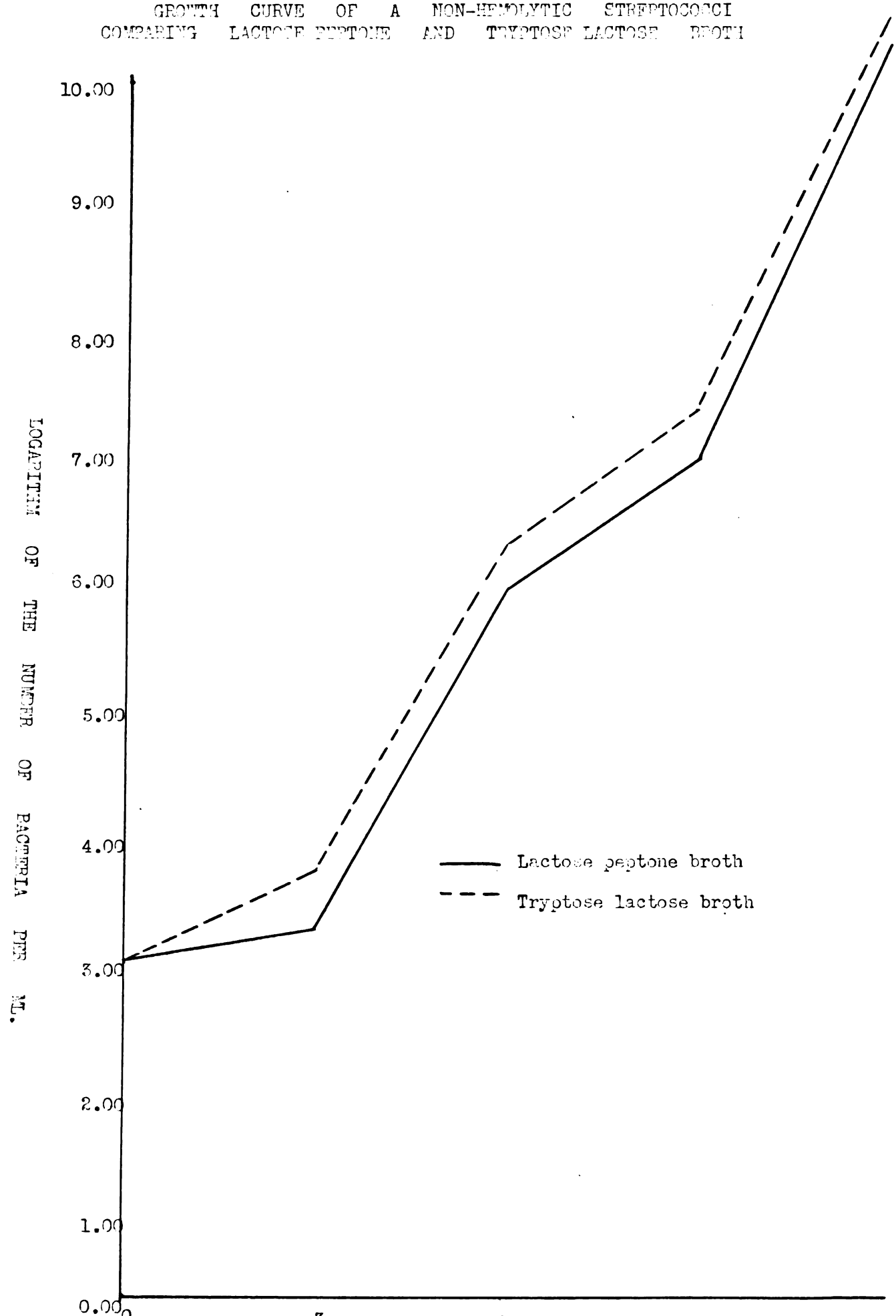
TABLE V

Growth curves of a non-hemolytic streptococci comparing lactose peptone and tryptose lactose broths.

Date of Testing	Medium	Sample No.	Number of Bacteria per ml.				
			0 hour	3 hours	6 hours	9 hours	12 hours
1/27/39	Lactose	1	987	2,000	852,000	44,500,000	39,200,000,000
		2	1,544	3,000	1,400,000	43,500,000	100,000,000
	Average		1,265	2,500	1,126,000	44,000,000	19,650,000,000
1/27/39	Tryptose	1	1,088	9,600	3,600,000	10,200,000	47,900,000,000
		2	1,655	5,300	1,444,000	45,700,000	54,300,000,000
	Average		1,371	7,450	2,522,000	27,950,000	51,100,000,000
2/3/39	Lactose	1	1,136	1,900	733,000	4,600,000	19,000,000
		2	1,047	2,400	940,000	1,100,000	2,000,000
	Average		1,091	2,150	836,500	4,350,000	15,500,000
2/3/39	Tryptose	1	1,147	6,000	2,984,000	196,215,000	341,000,000
		2	1,137	1,200	2,584,000	138,136,000	341,000,000
	Average		1,142	3,600	2,784,000	167,172,500	341,000,000
2/10/39	Lactose	1	233	1,340	104,000	17,800,000	260,000,000
		2	261	1,550	85,000	14,600,000	260,000,000
	Average		247	1,445	99,500	16,200,000	260,000,000
2/10/39	Tryptose	1	277	1,900	125,000	52,500,000	1,089,000,000
		2	255	1,550	186,000	41,700,000	1,144,000,000
	Average		216	1,725	155,500	42,100,000	1,126,500,000

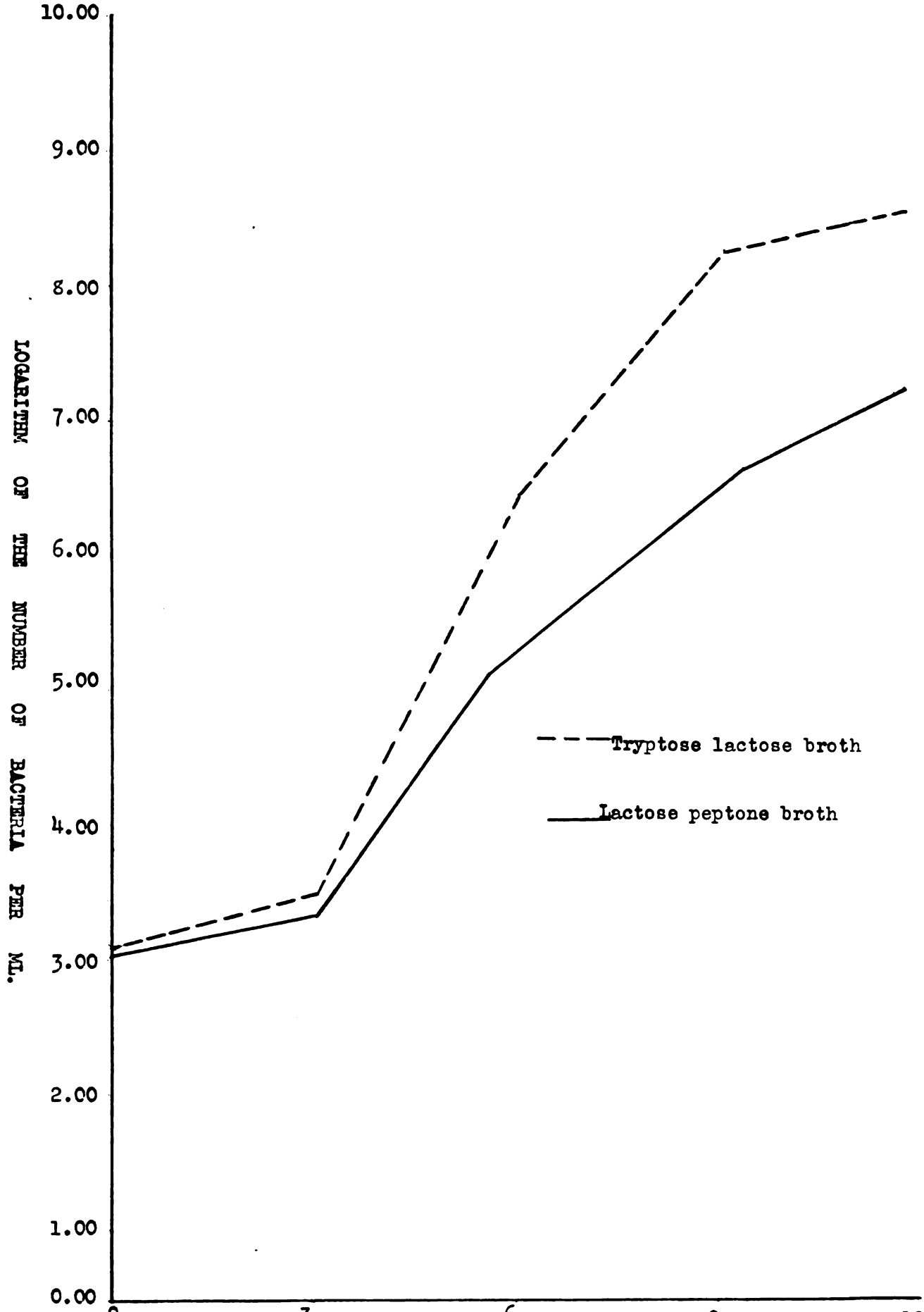
GRAPH III

GROWTH CURVE OF A NON-HEMOLYTIC STREPTOCOCCI
COMPARING LACTOSE PEPTONE AND TRYPTOSE LACTOSE BROTH



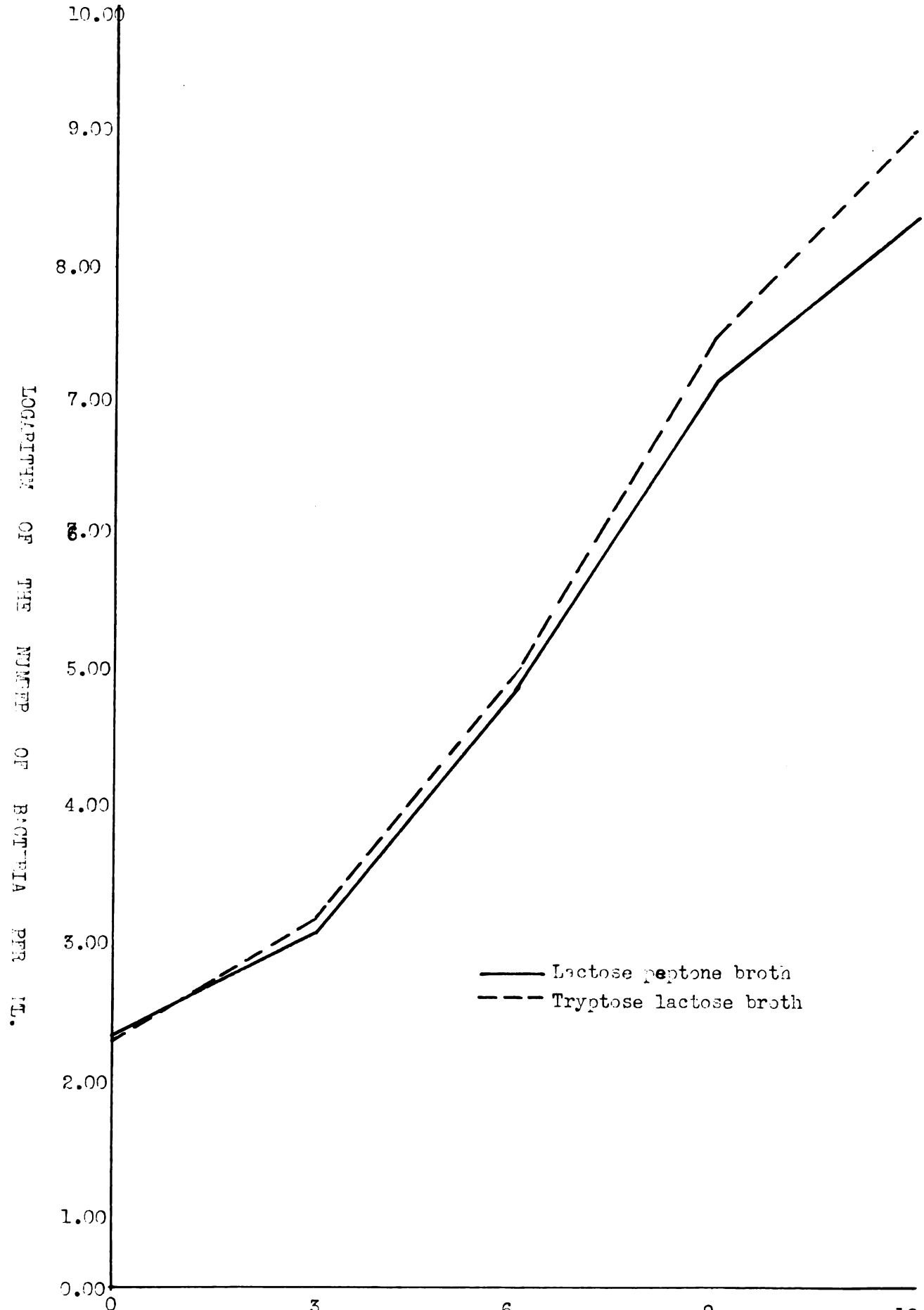
GRAPH IV

GROWTH CURVE OF A NON-HEMOLYTIC STREPTOCOCCI COMPARING LACTOSE PEPTONE AND TRYPTOSE LACTOSE BROTH



GRAPH V

GROWTH CURVE OF A NON-HEMOLYTIC STREPTOCOCCI
COMPARING LACTOSE PEPTONE AND TRYPTOSE LACTOSE BROTH



of the streptococci in tryptose lactose broth is shorter. The reproduction rate was parallel for one half to one hour at which time tryptose lactose broth increases. There was a more or less uniform growth rate up to six hours. Then the tryptose lactose medium began to accelerate more rapidly than before. This is shown especially well on graph 4. At the end of nine hours incubation the reproduction in both broths became constant and the number of organisms in the broths tend to show parallel lines on the graphs. This increase was probably due to the fact that the first few organisms are in a dormant condition and thus take longer to reproduce, but after the first few generations the organism loses this dormancy and as it was in a favorable environment starts to reproduce rapidly. The tryptose lactose broth was readily used by the streptococci and therefore they grew faster in this medium while in lactose peptone broth the organisms find themselves in an environment which they do use, but rather slowly.

As tryptose lactose broth gave favorable results with minimal numbers of streptococci, it was decided to determine how tryptose lactose broth would measure under routine conditions. A number of samples were tested using the A.P. H.A. standard methods. (2) These results are given in tables 3 and 4.

Fifty eight samples, table 3, were tested routinely and in parallel with tryptose lactose broth and lactose peptone broth. The tryptose lactose medium shows very clearly a higher streptococci index than lactose peptone broth. The mean Phelps index was 5. 73 for lactose peptone broth and 6.83 for the

TABLE III

A comparison of standard lactose peptone broth and lactose-tryptose broth using 10 ml. samples.

Date of Sampling	Source	Cl Res. p.p.m.	Bathing Load	Streptococci Indices		Colon Index	Total 37° C Count
				Lactose Broth	Tryptose Broth		
7/20/38	West Junior	0.5	33	6	8	0	1210 1153
				4	8	0	1792 1956
				6	10	0	816 716
				2	6	0	985 556
7/20/38	Pattingill	0.3	35	4	2	0	80 50
				2	6	0	4 93
				0	2	0	71 19
				4	6	0	63 85
7/20/38	Moore's Park	2.5	225	6	6	0	35 3
				10	6	4	94 73
				10	10	2	32 79
				8	10	0	16 9
7/21/38	Pattingill	0.4	32	0	4	0	13 55
				0	2	0	22 20
				0	0	0	15 26
				2	0	0	13 3
7/21/38	West Junior	0.5	20	8	10	0	203 157
				0	0	0	367 202
				10	10	0	66 123
				10	6	0	100 88
7/21/38	Moore's Park	2	150	10	10	0	6 3
				10	10	0	17 22

TABLE III (continued)

A comparison of standard lactose peptone broth and lactose-tryptose broth using 10 ml. samples.

Date of Sampling	Source	Cl Res. p.p.m.	Bathing Load	Streptococci Indices		Colon Index	Total 37° C Count
				Lactose Broth	Tryptose Broth		
7/22/38	Moore's Park	1	175	8	8	0	282 460
				8	10	2	787 489
				10	10	4	479 686
				8	10	0	131 68
7/25/38	Moore's Park		200	10	10	4	
				10	10	2	23 20
				8	10	0	30 6
7/26/38	Pattingill	0.4	32	2	6	0	47 45
				6	10	0	83 21
				2	8	0	169 151
				2	6	0	10 38
7/26/38	West Junior	0.6	38	2	4	0	112 119
				2	4	0	233 189
		00.6	38	0	0	0	78 152
				0	0	0	102 122
7/26/38	Moore's Park	1.0	150	10	10	2	59 84
				10	10	6	120 195
7/28/38	West Junior	0.6	36	0	6	0	419 165
				0	10	0	340 476
				8	10	0	1467 568
				0	2	0	934 593

TABLE III (continued)

A comparison of standard lactose peptone broth and lactose-tryptose broth using 10 ml. samples.

Date of Sampling	Source	Cl Res. p.p.m.	Bathing Load	Streptococci Indices		Colon Index	Total 37°C Count
				Lactose Broth	Tryptose Broth		
7/28/38	Pattingill	0.75	28	2	0	0	16 7
				10	10	0	14 5
				10	10	0	22 27
				10	10	0	148 208
7/28/38	Moore's Park	1.0	175	8	4	0	56 7
				6	10	0	104 148
				8	10	0	95 99
10/11/38	Walter French	0.6	40	2	2	0	
				10	0	0	
				6	10	0	
				6	10	0	
				10	10	0	
				10	10	0	
				6	8	0	
				10	6	0	

GRAPH VI

STREPTOCOCCI INDICES OF LANSING SWIMMING POOLS USING A 10 ML. SAMPLE

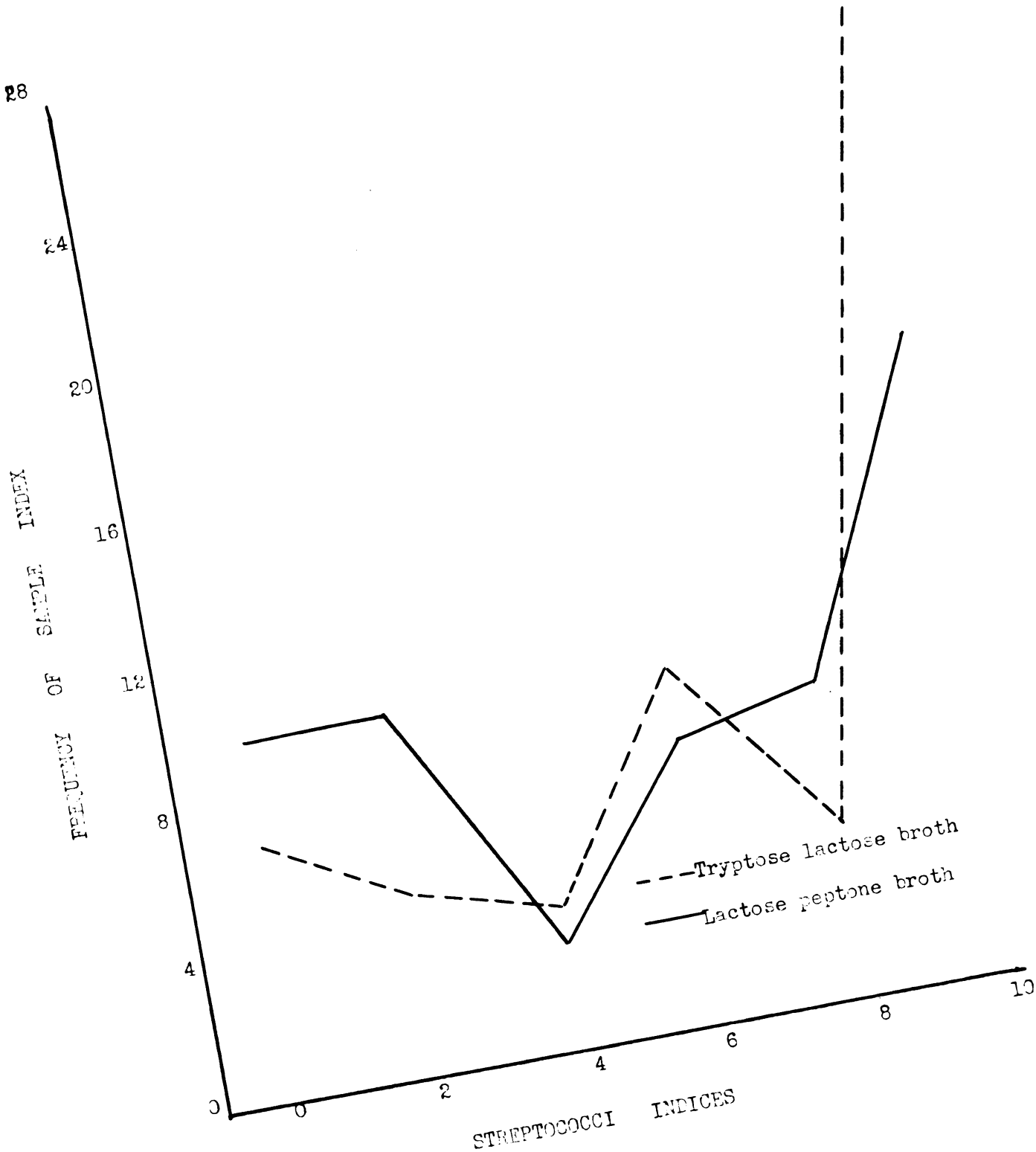


TABLE IV

A comparison of standard lactose peptone broth and tryptose lactose broth using a 10 ml. sample.

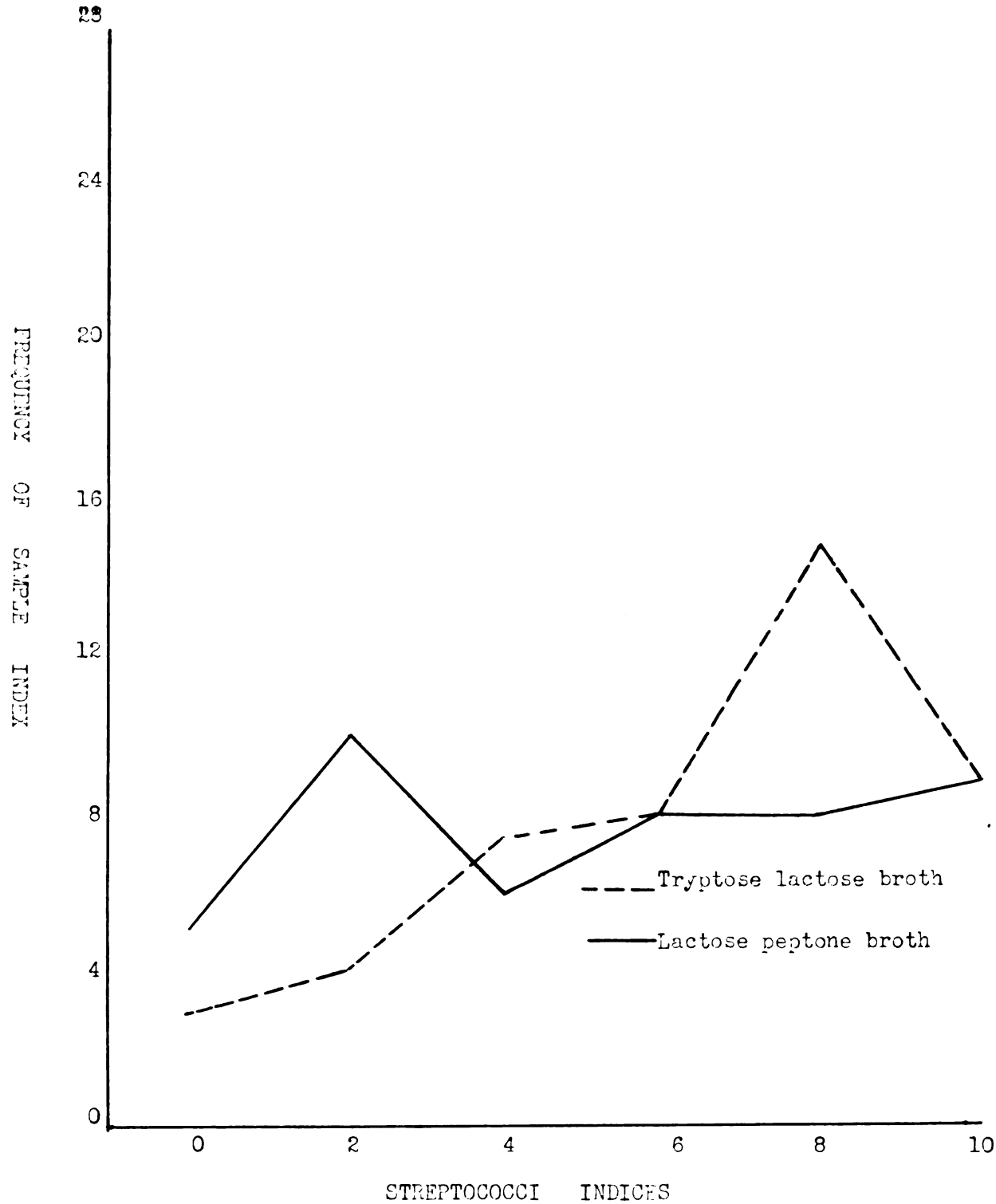
Date of Sampling	Source	Cl Res. p.p.m.	Bathing Load	Streptococci Indices		Colon Index	Total 37°C Count
				Lactose Broth	Tryptose Broth		
1/16/39	Pattingill	0.35	27	10	10	0	
				10	10	0	
				8	10	0	
				6	8	0	
1/17/39	West Junior	1.5	35	2	6	0	23
				6	6	0	
				8	6	0	
1/17/39	Eastern			2	2	0	
2/17/39	Walter French			8	10	0	
				10	10	0	
				10	8	0	
2/17/39	West Junior			6	8	0	
2/17/39	Eastern			2	10	0	
2/31/39	Pattingill	0.6	35	2	4	0	00
				8	8	0	
				0	0	0	
				2	10	0	
				0	2	0	
				0	8	0	
				2	6	0	
				10	4 (2 samples)	0	
				8	6	0	
				2	4	0	
				8	6	0	
				2	2	0	

TABLE IV (continued)

A comparison of standard lactose peptone broth and tryptose lactose broth using a 10 ml. sample.

Date of Sampling	Source	Cl Res. p.p.m.	Bathing Load	Streptococci Indices		Colon Index	Total 37° C Count
				Lactose Broth	Tryptose Broth		
2/27/39	Walter French	0.2	30	6	8	0	3 5
3/2/39	West Junior	1		6	8	0	1 5
3/2/39	Pattingill	1		6	8	0	175 247
3/2/39	Eastern	1		6	8	0	0 0
3/7/39	Pattingill	1	38	10	8	0	7 12
				10	8	0	14 10
				10	8	0	8 5
				8	10	0	1 5
				10	11	0	1 7
				6	8	0	9 4
3/6/39	West Junior		18	4	4	0	0 1
3/6/39	Walter French	0.75	10	4	6	0	1 2
3/6/39	Pattingill	1	37	2	6	0	4 3
3/6/39	Eastern	0.8	12	0	0	0	1 0
3/6/39	Central	0.4	2	0	4	0	
3/14/39	West Junior	0.3	18	8	2	0	32 46
3/14/39	Walter French	0.75	32	4	0	0	5 2
3/25/39	West Junior	0.6	31		4	0	37 67
3/25/39	Pattingill	0.5	30		6	0	1 0
3/25/39	Walter French	0.5	15		2	0	8 3
3/25/39	Central	0.75	4		0	0	13 8
3/27/39	Eastern	0.1	15	4	8	0	0 0
3/27/39	Pattingill	0.5	34	2	4	0	0 0
3/27/39	Walter French	0.75	38	4	4	0	0 0
3/27/39	West Junior	0.4	18	4	8	0	0 0

GRAPH VII
STREPTOCOCCI INDICES OF LANSING
STINTING POOLS
USING A 10 ML. SAMPLE



tryptose lactose broth.

Using the forty six samples in table 4, the tryptose lactose broth has an index that was higher in almost half of the samples. The mean Phelps index was 5.35 for lactose peptone medium and 6.39 for tryptose lactose broth.

Using the frequency of the sample index, curves for the above series of tests were drawn. Both graphs (6 and 7) showed a lower frequency of negative samples for the tryptose lactose medium. While the lactose peptone medium had the highest frequency of negative samples, and it reaches its peak frequency between an index of 0and6, but the tryptose lactose broth peak comes between 4and10. As it has been shown that tryptose lactose broth was more sensitive to minimal numbers of streptococci, it was shown in the above samples that it was equally sensitive to large numbers of streptococci, and does not show as many negative samples as lactose peptone from the same sample.

Stains made from lactose peptone broth showed streptococci that were in graceful chains, while those from tryptose lactose broth had very short chains.

Conclusions

1. Streptococci show less lag phase in tryptose lactose broth than in lactose peptone broth.
2. Higher streptococci indices were obtained in lactose tryptose

broth from minimal plantings than in lactose peptone broth.

3. Swimming pool samples tested in parallel in tryptose lactose broth and lactose peptone broth gave higher streptococci indices in the former broth.
4. The substitution of tryptose lactose broth for lactose peptone broth for isolation of streptococci from swimming pool waters is recommended.

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Shulls

A study of different media for the detection of minimal numbers of streptococci in swimming pool water.