THE RELATIVE IMPORTANCE OF STREPTOCOCCI, ESCHER'CHIA COLI, AND TOTAL COUNT AS INDICATORS OF P[illegible]LUTION IN CHLORINATED SWIMMING POOLS

THESIS FOR DEGREE OF M. S.

ROBERT T. HABERMANN

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of

AGRICULTURE AND APPLIED SCIENCE

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A Thesis

Submitted to the Graduate Faculty

For the Master of Science Degree

Department of Bacteriology and Hygiene

by

Robert T. Habermann
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THESIS

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The belief that the maintenance of a residual chlorine content of 0.2 to 0.5 p.p.m. in a swimming pool at all times is a guarantee that no bacteria are present and therefore, a guarantee that disease germs are also absent has been shattered by the bacteriological picture presented through the introduction of the sodium thiosulphate treated sample bottle for collecting the pool-side samples. The resulting bacteriological picture is sometimes quite startling when contrasted to that obtained by the use of the usual sample bottle. With the old method of sampling, the residual chlorine continued in its gradual but constant destruction of the bacteria present in the bottle during the period of transportation to the laboratory and during the storage period subsequent to examination. The results thus obtained generally showing sterility, were not representative of the pool water. The degree of purity depended to a large extent upon the rapidity of handling the sample. On the other hand, the sodium thiosulphate treated sample bottle gives the actual bacteriological picture of the pool at the time of collection, because the sodium thiosulphate eliminates the germicidal chlorine and frees the bacteria from any further injury from the chlorine. The sample may then be transported to the laboratory and cultured the same as any unchlorinated water.

That the resulting bacteriological picture using this method of sampling, as recommended by Mallmann and Cary (1), it totally different from that obtained with the old sample

bottle was vividly demonstrated in the studies of the above-mentioned workers. They found in a small pool in Detroit, much higher total counts and much higher Escherichia coli and streptococci indices when the samples were tested immediately after collection. Although they showed that the presence of residual chlorine even in amounts of 0.2 to 0.5 p.p.m. of available chlorine was insufficient to effect continued sterilization their data do not show the differences that might be expected under varying conditions. During the past year this laboratory has been collecting data from seven pools to determine these variations in order that new standards may be developed or the present standards changed to fit the new picture that has arisen. This thesis is largely concerned with the presentation of these data.

Swimming pool water, although different from drinking water as far as the bacterial flora is concerned, has always been tested in the same manner for purity. The significant bacteria in drinking water are the disease-producing bacteria that come from sewage pollution. On the other hand, the bacteria found in swimming pools are not only from the intestinal tract, but also from the nose, throat, and other exposed parts of the body. If Esch. coli were more resistant to disinfection than other organisms encountered in swimming pools the present test would be satisfactory, but as shown by Klang (2) this is not the case. It appears evident that any method for the bacteriological examination of swimming pools should be based on the most resistant and the most

numerous bacteria present.

The method of bacteriological examination recommended by the American Public Health Association Committee on Swimming Pools in 1926 (3) is essentially the same as that required for drinking water. This standard recognizes only the incidence of Esch. coli and the total number of colonies developing on beef extract agar at 37°C in 24 hours. The committee has assumed that the absence of Esch. coli indicates that disease-producing bacteria are also absent. The data on which these standards are based were obtained by using the old method of collection and not by using the sodium thiosulphate treated sample bottle.

The use of streptococci as an indicator of pool pollution was first suggested by Mallmann (4) when he found that the incidence of streptococci paralleled the amount of pollution more closely than did the Esch. coli content. Although considerable work has been done along this line, still no careful comparative studies of the relative incidence of these two indicators, namely Esch. coli and streptococci, have been made to determine their importance. Such a study is presented in this thesis. Before making this study it was found necessary to try various media for growing streptococci to be sure that the method finally used was giving the streptococci present an opportunity to grow. The results of this work are also presented.

Historical

In the last few years there has been much discussion as to the reliability of the colon index when applied to swimming pool pollution. The first work along this line was done by Mallmann (4) in 1928. He showed that Esch. coli did not parallel the bathing pollution. In a pool, where the only means of purification was filtration, he found that the streptococci index paralleled the number of bathers in the pool.

That the presence of streptococci in swimming pools is not unexpected is demonstrated by a review of the literature. Prescott and Winslow (5), are of the opinion that streptococci occur more commonly on the surfaces of human and animal bodies than anywhere else in nature. Gordon (6), showed that certain streptococci are present in normal mouths. In a laboratory experiment conducted during the fall, Mallmann recovered only 2 alpha streptococci from the mouths of 45 college students. In the winter, however, he found only 2 students that did not harbor streptococci. In addition to these organisms, other cocci (emanating) from the nose and other exposed parts of the body are present in swimming pools.

All types of streptococci have been found in swimming pools. Alpha and gamma streptococci have been isolated by Klang (2). He found that alpha streptococci would grow after being subjected to a chlorine residual of 0.9 p.p.m. for 90

seconds. Recently, Horwood, Gould, and Swachman (7) found beta streptococci in swimming pools in Boston.

The type of infection obtained from bathing in pools and rivers is of importance in determining the relationship between enteric and respiratory diseases. This relationship has been studied by many workers. Manheimer (8), cites examples of both of these types of infection as reported by the following: Reece reports 34 cases of enteric fever among soldiers who used a swimming pool which contained sewage polluted water; Jager reported intestinal proteus infection among soldiers who had bathed in the Danube river; Pfuhl attributes 49 cases of typhoid fever to bathing in the Elbe; and Shiga reports 413 cases of dysentery from bathing in a river. It will be noted that only cases of enteric diseases were reported from swimming in rivers or in pools where untreated waters were used, thus showing the importance of the Esch. coli test for detecting unsafe conditions from polluted water.

The following diseases, usually caused by pyogenic cocci, are also cited by Manheimer: Tehr reports 20 cases of eye infection amidst patrons of a swimming pool; Schultz reports 18 cases of trachoma in persons using a pool; and Skutch reports an epidemic of gonorrheal vulvovaginitis which spread to 236 girls using a pool in Posen. Forbes (9), cites H.N. Ogden as a source for the following: W.L. Lewis reports influenza, colds, sore throats, and occasionally pneumonia restricted to users of the swimming pool at Northwestern

University; Bunker reports nose and ear infection among members of a swimming team at Brown University, and Ornersbach reports an outbreak of conjunctivitis and catarrhalotitis among the members of a swimming club. Forbes has examined a case of meningitis contracted in a swimming pool. Hasty (10) finds that the pool organisms are directly the cause of paranasal sinus infection. It is interesting to note, in the above references, that all cases of enteric diseases were caused from swimming in rivers or in pools where untreated waters were used. Also that infection of the eye, ear, nose, and throat, generally caused by pyogenic cocci, were obtained only in swimming pools where water free from intestinal pollution was used. The evidence cited seems to warrant the conclusion that cocci are the important organisms to be looked for when testing swimming pool waters.

In 1927 Mallmann (11) suggested that the ortho-tolidine test should supplant the Esch. coli test, because Esch. coli were apparently absent from pools containing 0.2 to 0.5 p.p.m. available chlorine. Stovall, Nichols, and Vincent (12) in the same year made a similar report. In a later publication Mallmann and Cary (1) in 1932, contrary to the findings of Schoeple (13), found that pool-side testing of swimming pools yielded large numbers of Esch. coli and streptococci; whereas the same sample when carried to the laboratory and tested several hours later was found to be free from pollution.

These data demonstrate that any standard based on the usual methods of examination wherein chlorinated waters were transported from their source to the laboratory and where a marked time period intervened before testing, were incorrect. They found that, in either chlorine or chloramine treated pools, chlorine residuals used were not always sufficient to destroy all Esch. coli and streptococci present. The results show the inadequacy of the ortho-tolidine test and the necessity of a bacteriological analysis. They recommended the use of sodium thiosulphate to dechlorinate the sample at the time of collection to eliminate the difficulties of pool-side testing.

Many methods and media for the isolation of streptococci have been reported, but their application to swimming pool isolations presents many problems. Houston (14) although searching for streptococci in polluted river water summarizes some of the difficulties as follows: "In searching for streptococci many difficulties are met with. Frequently these is no growth in the broth tube, the streptococci having presumably lost their vitality. Sometimes the growth is greatly delayed, and on resorting to further sub-culture a negative growth is obtained, the organisms having been obtained in a state of feeble vitality. Again it not uncommonly happens that a growth occurs, but the organisms turn out an examination not to be streptococci." Other obstacles met with in isolating streptococci are the overgrowth of other organisms and the inability of the strepto-

cocci to grow when seeded into other media from the original broth.

No study of media has been made to determine the best medium for growing streptococci from the swimming pool water samples. In order to know when a pool is free from streptococci a medium that will allow all the viable streptococci present to grow is necessary.

The English workers suggest the use of litmus lactose agar and dextrose neutral red broth for growing streptococci from polluted waters. Mallmann (4) found that streptococci would grow in standard lactose broth when swimming pool water was added. He demonstrated the presence of streptococci by making a microscopic examination of the sediment in the bottom of the lactose broth tubes. Later Mallmann and Gelpi (15) devised a method to concentrate the streptococci by drawing off the supernatant lactose broth with negative pressure.

In as much as only two liquid media have been used, namely, standard lactose broth and dextrose neutral red broth, a comparative study of the various media was made. To determine the influence of the protein base, a comparison of beef extract and veal infusion was made. The latter infusion has been very satisfactory for growing many pathogens particularly streptococci. To determine the influence of the carbohydrate, dextrose and lactose were used. To determine the influence of soluble proteins, the

following peptones were tested; Bacto-peptone, Proteose-peptone and Neo-peptone of the Digestive Ferments Company, and a peptone manufactured by Fredrick Stearns and Company. It is well known that peptones vary markedly in their ability to grow various organisms. Gentian violet, having been used with success by Bryan (16), in isolating streptococci from cases of mastitis by inhibiting the growth of Esch. coli and other contaminants in milk, was tested in the media using dilutions ranging from 1 to 50,000 to 1 to 1,000,000.

Preparation of Media:

Gentian violet liver infusion agar. The medium was prepared according to the directions of Bryan (17). The following formula was used:

500 cc. beef liver infusion
500 cc. tap water
20 grams agar
5 grams NaCl

The medium was adjusted to pH 7.4 and filtered through non-absorbent cotton to clarify. The medium was sterilized by steam under pressure. Prior to use, defibrinated bovine blood was added to the melted agar (45°C) to obtain a 5 per cent concentration, and sufficient gentian violet (1 per cent aq. solution) to give a final dilution of 1 to 200,000 in the medium.

Veal infusion Proteose-peptone dextrose broth. This medium was prepared according to the following formula:

1000 cc. veal infusion
(The veal infusion was prepared by adding 500 cc. of tap water to each pound of ground lean veal, mixing and then steaming in the Arnold steamer for 1 hour. The infusion was then clarified by filtering through a cheese-cloth). 20 grams of Proteose Peptone was added to the infusion. The pH was then adjusted to 7.4. This was heated in the autoclave at 15 lbs., pressure for 30 minutes to "break down the precipitate," and then filtered through a filter paper. To the peptone veal infusion 10 grams of dextrose was added. The broth was then tubed and sterilized at 15 lbs. pressure for 30 minutes.)

Veal infusion Stearns-peptone dextrose broth. This medium was prepared the same as the veal infusion Proteose-peptone dextrose broth, except that Stearns-Peptone was added.

Veal infusion Neo-peptone dextrose broth. This was prepared in a similar manner, except that Neo-peptone was added in the place of Stearns-peptone.

Veal infusion Bacto-peptone dextrose broth. This was prepared the same as the above, but Bacto-peptone was added instead of Neo-peptone.

Lactose broth. Lactose broth was prepared according to the following formula which represents a doublt strength medium:

1000 cc. tap water
6 grams Liebigs meat extract
20 grams Bacto-peptone
10 grams NaCl

The broth was adjusted to a pH of 7. and filtered through non-absorbent cotton to clarify. Twenty grams of lactose was

then added. The broth was tubed and sterilized at 15 lbs. pressure for 30 minutes.

Proteose-peptone lactose broth. This was prepared in a similar manner, except that Proteose-peptone was added in the place of Bacto-peptone.

Neo-peptone lactose broth. This was prepared the same as lactose broth, but Neo-peptone was added instead of Bacto-peptone.

Dextrose broth. This was prepared by adding 10 grams of dextrose to Bacto-peptone beef extract broth before tubing.

Blood dextrose broth. Blood dextrose broth was prepared the same as the dextrose broth except that sterile defibrinated bovine blood was added to make a final concentration of 5 per cent.

Serum dextrose broth. This was prepared by adding 0.5 per cent serum to dextrose beef extract broth.

Litmus lactose agar. Difco litmus lactose agar was used for the preparation of litmus lactose agar.

Blood agar. This was prepared according to the following formula:

1000 cc. tap water
3 grams meat extract
20 grams agar
10 grams Bacto-peptone
5 grams NaCl

The medium was adjusted to a pH of 7 and filtered through non-absorbent cotton to clarify. The medium was sterilized for 30 minutes at 15 lbs. pressure. Prior to use, sterile

defibrinated bovine blood was added to make a final concentration of 5 per cent.

Experimental

The experimental work that follows is divided into two parts; the study of the growth of streptococci in different media and the incidence of streptococci and *Esch. coli* in chlorinated swimming pool waters.

All of the various methods attempted in the growing and isolating of streptococci are presented. Both direct and indirect methods of isolation were attempted. The procedures were as follows:

Direct methods. To assure the presence of streptococci, water was obtained from the Lake Lansing bathing beach. This water was used because streptococci are always present, even in diluted samples, when fifty or more people are bathing. The water was collected at the end of the bathing dock in sterile 500 cc. flasks approximately 400 feet from shore. The water was transferred to sterile centrifuge tubes under aseptic conditions and centrifuged for 40 minutes. The supernatant fluid was decanted and the sediment was streaked on three media, namely, blood agar, blood agar plus gentian violet (1 to 10,000), and the litmus lactose agar. The results obtained when streak plates were made from the sediment of centrifuged water are omitted as all three media failed to grow the streptococci. The use of blood agar, gentian violet blood agar and litmus lactose agar as media for isolating streptococci was, therefore, eliminated.

The use of a direct selective enrichment medium was tried. This medium was an adaptation of that used by Bryan (17). Bryan has successfully used gentian violet in liver infusion agar as a selective agent for the isolation of streptococci from milk from cows affected with mastitis. It was hoped that the gentian violet in the liquid media would inhibit the growth of most of the other bacteria found in the lake water and allow the unrestricted growth of the streptococci. Dye dilutions ranging from 1 to 5,000 to 1,000,000 were tried. Water known to contain streptococci was tested in amounts of 10, 1, 0.1, and 0.01 cc. The water was added in the case of the 10 cc. amount to 15 cc. of double strength lactose broth and in the smaller amounts of water to single strength broth. All tubes were incubated at 37°C for 72 hours when they were examined microscopically. The results using gentian violet broth were unsatisfactory. Other organisms were always present and the streptococci were found just as plentiful in broth tubes that had not received any gentian violet. Experiments employing gentian violet in as an inhibitory agent direct isolations were therefore discontinued.

Indirect methods. Gentian violet liver infusion blood agar was made as directed by Bryan (17). The liver infusion agar, 150 to 200 cc., was placed in 300 cc. flasks and sterilized. To the melted agar, cooled to approximately 45°C was added sufficient gentian violet in one per cent dilution to make a final dilution of 1 to 200,000 and sterile bovine blood to make a 5 per cent concentration.

The technic used for plating the streptococci consisted in adding 0.1 cc. of the sediment from 24 hour lactose broth tubes to a number of petri dishes and adding approximately 10 cc. of gentian violet liver infusion blood agar. The petri dishes were then gently rotated to distribute the organisms. The plates were incubated for 24 hours at 37°C. The appearance of all three types of streptococci on this medium is the same as on blood agar. All streptococcus colonies were confirmed by microscopic examination and by growing in veal broth in pure culture.

Although the microscopic examination of the broth tubes often showed streptococci to be present in the initial broth tubes, when the gentian violet liver agar plates were negative, still alpha and beta streptococci were isolated in many instances. In the addition of gentian violet to the blood agar, the quantity used was found to be very important. If an insufficient amount was added, the streptococci were over-grown by other organisms and if too much was added, the streptococci were inhibited. The results obtained although not perfect are encouraging. This medium makes it possible to obtain in pure culture the various streptococci present. The medium was not used in the later routine studies.

In studies by Hallmann (4) streptococci were cultured from standard lactose broth used for culturing Esch. coli. The effectiveness of this medium in the isolation of streptococci was never checked. Accordingly other media were tested in a comparative manner to determine the effective-

ness of this medium and also to discover, if necessary, a better medium. Several peptones and several types of infusions were tried. The media used are described in detail in an earlier section of this thesis.

The procedure for checking the media follows: A sample of water from the Lake Lansing bathing beach, during a period of a heavy bathing load was transferred in quantities of 100, 10, 1, 0.1, and 0.01 cc. to the different broth media using various concentrations of the broth media so the ratio of water to the food nutrients was practically the same in all instances. The tubes were incubated at 37°C for 72 hours. At the end of 24 and 48 hours incubation, the percentage of gas, an evidence of Esch. coli, was recorded. After 72 hours incubation, the supernatant fluid was removed by suction and the sediment smeared on slides. The slides were stained with aqueous methyl-violet or gram stain and examined microscopically for streptococci.

The microscopical results obtained after 72 hours incubation at 37°C are tabulated in Table I. The growth of streptococci occurred in all media, but was more abundant in Bacto-peptone dextrose veal infusion broth, Proteose-peptone dextrose veal infusion broth, and Neo-peptone dextrose veal infusion broth. Many additional tests were made on the various media listed with results very similar to those presented in Table I. Considering all of the data gathered, Proteose-peptone veal infusion broth was found the best for growing streptococci from lake waters.

Table I. The results of a microscopic examination presenting the growth of streptococci in various media after 72 hours incubation at 37°C

Medium	Amounts of water planted			
Beef Extract Media	10	1	0.1	0.01
Bacto peptone lactose broth	+*	-	-	-
Proteose peptone lactose broth	++	+	-	-
Neo-peptone lactose broth	+	++++	++	++
Bacto peptone dextrose broth	++++	++	++	+
Serum Difco-peptone dextrose broth	++	+	+	+
Blood Difco-peptone dextrose broth	+++	+	+	+
Veal infusion media				
Bacto-peptone dextrose broth	+++	+	++	++++
Proteose-peptone dextrose broth	++++	+	+	++++
Neo-peptone dextrose broth	+++	+++	+	+++

*+ to ++++ indicate degrees of macroscopic growth of streptococci in tubes

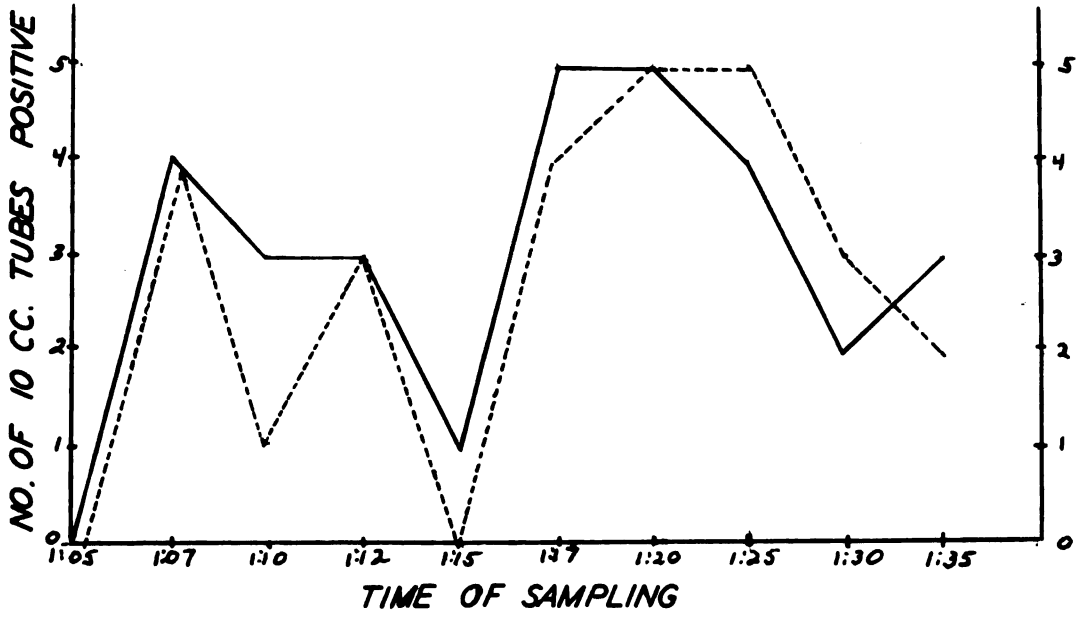
In a further study of nutrient media to determine their relative values for growing streptococci, it was decided to test their values on a chlorinated water from a swimming pool. Accordingly the above-mentioned medium, Proteose-peptone veal infusion broth was compared with standard Bacto-peptone beef extract broth made according to the directions of the Standard Methods for Water Analysis, 1933. An examination of Graph I will show that very little difference occurred between these two media. Because of the similarity of the results obtained, it was decided to use these two media in parallel over a larger number of samples. The results of these studies are presented later in this thesis.

Having determined the best media for growing streptococci, a survey of seven chlorinated pools was undertaken to determine which medium was best suited for the isolation of streptococci under routine conditions. Further, more data are needed to determine the significance of streptococci, colon bacilli, and total 37°C count in chlorinated pools when sodium thiosulphate sample bottles are used.

The work of Mallmann and Cary (1) demonstrated that pools that have been showing consistently negative colon indices and zero counts under the old method of sampling, showed at times marked evidences of pollution when sodium thiosulphate sample bottles were used for collecting the samples. Although these writers call attention to this

COMPARATIVE VALUE OF BACTO-PEPTONE LACTOSE BROTH AND PROTEOSE-PEPTONE VEAL BROTH FOR CULTURING STREPTOCOCCI. GRAPH I

---- PROTEOSE-PEPTONE VEAL BROTH
——— BACTO-PEPTONE LACTOSE BROTH



condition and suggest the use of the dechlorinated sample, they were unable, with the small amount of data they presented, to arrive at any figures pertaining to standards. Furthermore, it has been customary to collect samples at any time of the day, regardless of whether the pool was in use or not, ratherthan at peak periods of bathing load when the dangers of pollution would be greatest. For this study samples were always taken during such periods.

In this study the method of preparing the sample bottle was changed. Formerly Mallmann prepared the sample bottles by adding approximately 0.01 gm. of powdered sodium thiosulphate to the wet sample bottle. The glass stopper was then replaced, a paper cover was placed over the stopper and the bottle was sterilized by moist heat under pressure. Unless the steam was introduced into the autoclav slowly, the sudden heating frequently broke the bottles. To avoid this, Mallmann adopted the use of dry heat. In this procedure, the powdered sodium thiosulphate is added to the dry bottle. The bottles are then sterilized at a temperature not to exceed 200°C. If the temperature should exceed 220°C the sodium thiosulphate will decompose. Using this method, the bottles seldom break and sterile bottles are assured. In collecting samples, using sodium thiosulphate treated bottles, care was always exercised not to rinse the bottles, as the sodium thiosulphate would be washed out. To be sure that

this had not occurred, all samples after collecting for the bacteriological tests were tested with ortho-tolidine to be sure that free chlorine was not present. In several instances where samples were collected by inexperienced inspectors, free chlorine was found in the bottle. In all such cases the data were discarded.

All samples were tested by planting five 10 cc. portions into lactose broth (A.P.H.A.) and lactose proteose-peptone veal infusion broth respectively. Two 1 cc. portions were plated on standard plain nutrient agar. All cultures were incubated at 37°C for 48 hours. Gas production was recorded at the end of 24 and 48 hours incubation. All tubes showing gas (plus or minus 10 per cent) were checked by smearing on eosin-methylene blue agar plates. The latter step was found essential in all cases because frequently gas production was not due to Esch. coli. All plate counts were made at the end of the 24 hour incubation. After the 48 hour incubation at 37°C, the fermentation tubes were placed at room temperature for 3 to 5 days.

This was done in order that the streptococci that might be present would settle to the bottom of the tubes. The supernatant fluid was then carefully removed by suction avoiding, as much as possible, the disturbing of the precipitate in the bottom of the tubes. The sediment was then smeared on slides, stained, and examined microscopically for streptococci.

As each sample was collected, an ortho-tolidine test was made to determine the residual chlorine content of the pool. The number of bathers in the water at the time of collection and the total time that had elapsed since the group entered the water was recorded. These data were recorded on the report sheet which accompanied the sample to the laboratory. This report sheet is presented on the following page. It will be noticed that this sheet provides a place for recording the laboratory findings.

To determine the relative values of standard lactose broth and proteose peptone dextrose veal infusion broth in growing streptococci from routinely tested swimming pool samples, all samples received at the laboratory for a period of several months were examined using these two media in parallel plantings. The results are presented in Table II. The data show that with 268 samples tested, the following results were obtained: For the presence of streptococci 110 samples were positive with standard lactose broth and 113 with proteose-peptone veal broth. Considering the individual 10 cc. portions, the standard lactose broth showed 258 portions positive; whereas the proteose peptone veal broth showed 292. These data indicate only a slight advantage for the latter medium, an advantage that is likely within the experimental error in making the tests. For the determination of Esch. coli, the standard lactose broth gave 28 positive samples; whereas the proteose-

Michigan State College
Department of Bacteriology and Hygiene

Name of Pool_____Date_____

Time of sampling _____Residual chlorine at time of sampling____

Number of bathers in pool ____Length of time in pool_____

Sex of bathers _____

Bacteriological
count on plain agar at 37°C

	1 cc.	1 cc.	0.5 cc.	cc.	cc.
24 hrs.					
37 hrs.					

Percentage of gas produced in lactose broth

	10	10	10	10	10	1	0.1	0.1	0.001
24 hrs.									
48 hrs.									

Confirmation of Esch. coli on eosin-methylene blue agar

_____

Presence of streptococci

_____

Esch. coli_____Aerobacter aerogenes_____

Streptococcus index _____

Colon index _____

Remarks _____

_____

_____

peptone veal broth gave only 26, or a variation of 17.2 per cent occurred in favor of the standard lactose broth. Considering the individual 10 cc. portions, in the standard lactose broth 67 portions were positive as compared with 51 positive portions in proteose peptone veal broth, or a variation of 23.9 per cent in favor of the standard lactose broth. For the determination of Esch. coli the standard lactose broth was unquestionably the better medium. In as much as the value of standard lactose broth was nearly equal to the proteose-peptone broth in determination for streptococci and far superior for the detection of Esch. coli, the latter broth was discarded. It appears that standard lactose broth is the best medium of those tested for the detection of both streptococci and Esch. coli. The writer recommends that this medium be used for both tests.

The selection of a simple procedure for measuring the pollution of a swimming pool is of considerable concern. At present, the presence of Esch. coli together with a maximum count of 200 is in general use. Harwood, Gould, and Swachman (8) report that "the total count at 37°C after 24 hours of incubation represents the best and most simple index of the sanitary quality of water in the pool." It must be remembered, however, that their work was done using the ordinary sterile bottles for collecting samples. The residual chlorine present undoubtedly played an important part in disturbing the picture of the actual

Table II - A comparison of standard lactose broth and proteose-peptone dextrose veal broth on routine swimming pool samples

Source	No. of samples tested	Presence of Esch. coli				Presence of streptococci			
		Lactose broth		Proteose-peptone broth		Lactose broth		Proteose-peptone broth	
		Samples +	Portions +	Samples +	Portions +	Samples +	Portions +	Samples +	Portions +
Pool A	25	0	0	0	0	3	6	4	9
B	25	5	7	3	4	15	28	13	33
C	25	1	3	4	8	6	12	5	9
D	25	3	7	3	7	6	11	6	13
E	25	1	4	3	8	7	14	7	12
F									
G	20	11	34	8	18	15	38	16	44
Serial Samples									
Samples Pool B	74	6	10	5	6	29	78	34	92
A	7	1	2	0	0	3	15	4	19
F	42	0	0	0	0	26	56	24	61
Total	268	28	67	26	51	110	258	113	292

Table III - Incidence of streptococci, Esch. coli and total counts in excess of 200 bacteria per cc. in routine samples tested arranged by pools

	Pools Examined							Total
	Eastern	Pattengill	Central	West Jr.	Walter French	M.S.C.	Moore's Park	
Strept. alone	14	17	10	14	14	32	41	142
E. coli alone	1	2	1	2	1	2	5	14
Both E.coli and Strept.	0	6	3	4	4	1	54	72
Neither E.coli or strept.	38	32	40	37	38	60	21	266
Total	53	57	54	57	57	95	121	494
More than 200 bacteria per cc.	8	20	5	14	7	4	40	98
E.coli and 200 bacteria	1	8	0	3	2	1	29	44
Strept.and 200 bacteria	2	12	1	5	4	3	34	61
Strept. + E.coli + 200 bacteria per cc.	0	6	0	2	4	1	26	39
Total	11	26	1	10	10	5	89	144
No. of samples	54	56	54	57	56	95	127	499

Table IV - Incidence of streptococci, Esch. coli and total bacterial counts in serial tests arranged by pools

	Pools examined			Total
	West Junior	Pattengill	M. S. C.	
Streptococci alone	11	29	72	112
Esch. coli alone	0	1	1	2
Both E. coli and streptococci	2	4	0	6
Neither E. coli or streptococci	9	40	54	103
Total	22	74	137	210
Bact. count over 200	7	18	0	25
Bact. count between 100 and 200	5	4	0	9
Total count 100	12	22	0	34
Bact. count below 200	10	52	114	185

Table V - Incidence of streptococci, Esch. coli and total counts of all samples examined

	All samples examined				Total	
	Routine	Per cent	Serial	Per cent	Number	Per cent
Streptococci alone	142	28.74	112	53.33	254	36.07
Esch. coli alone	14	2.81	2	0.95	16	2.27
Both Esch. coli and streptococci	72	14.57	6	2.85	78	11.07
Neither Esch. coli or streptococci	266	53.82	103	49.04	369	52.41
Total showing streptococci	214	43.31	118	56.19	342	48.57
Total showing Esch. coli	86	13.64	8	38.09	94	13.35
Bact. count in excess of 200	98	19.83	25	11.90	123	17.47
Bact. counts below 200	396	80.01	185	88.09	581	82.52
Total samples	494		210		704	

pollution as it existed in the pools at the time of collection. In the light of the present studies, the conclusions of Harwood, Gould and Swachman appear unwarranted. To obtain some idea of the relationship of total counts at 37°C and colon and streptococci indices, the results of all the bacterial tests made on swimming pools has been compiled to show their relative value. In this compilation a sample is considered positive to Esch. coli or streptococci if one or more of the 5-10 cc. samples planted in double strength standard lactose broth were found positive. The data are presented in Tables III, IV, and V. In Table III are presented the findings arranged according to the pools tested. These samples represent only routine samples taken only when bathers were in the pool. In all cases, the chlorine residual of the pool exceeded 0.2 p.p.m. and in a few instances over 1 p.p.m. of available chlorine. A very marked variation in the occurrence of Esch. coli, streptococci, and total counts in excess of 200 bacteria per cc., existed in the various pools. Eastern High School pool showed only 1 sample containing Esch. coli and 14 containing streptococci out of 53 samples tested. Eight samples had counts in excess of 200 bacteria per cc. This pool never had heavy loads; in general the loads were exceedingly low. On the other hand, Moore's Park pool, an outdoor pool, showed a high incidence of Esch. coli (54 out of 121 samples). The bacterial counts exceeded 200 bacteria per cc. and the incidence of streptococci was also pre-

dominant. This pool throughout the season carried bathing loads far in excess of the capacity of the pool. Even with chlorine residuals of 0.6 to 0.8 p.p.m., pollution indices were invariably high.

In Table IV are presented the data on samples gathered in serial testing. In serial testing, during a given bathing period samples were taken (1) before the bathers entered the pool, (2) during the bathing period every 2 to 3 minutes, and (3) after the bathers had left, for a period varying from 5 to 15 minutes. In general, a series consisted of approximately 17 samples taken over a period of 20 to 45 minutes depending upon the length of the bathing period. In most cases these samples were purposely taken during periods of heavy bathing loads as the writer was interested in obtaining pollution curves for the entire bathing period.

As a result the incidence of streptococci and Esch. coli would naturally show in a larger number of samples. In Table V is presented a summary of Tables III and IV. The percentage incidence of Esch. coli alone and together with streptococci, of streptococci alone, and of samples showing bacterial counts in excess of 200 bacteria per cc. are given. A critical examination of these data fail to show any parallelism among the incidence of streptococci, Esch. coli, or excess counts. The data show that in the 704 samples tested 48.57 per cent showed streptococci, 13.35 per cent showed Esch. coli and 17.47 per cent had counts in excess

of 200 bacteria per cc. In general it had been the writer's observation that streptococci were present when Esch. coli were present. Streptococci frequently were present when Esch. coli was absent and when the counts were under 200 bacteria per cc. The arbitrary acceptance of a total count of 200 bacteria per cc. standard without consideration of Esch. coli and streptococci would be unjustified and would not be in accordance with the data presented. It would seem more reasonable to conduct further studies on the significance of Esch. coli and streptococci from a sanitary standpoint and set the maximum numbers of these organisms that would not endanger the bather. The total count represents largely organisms of no sanitary value and should not be considered unless their numbers vary directly with the streptococci and Esch. coli; and this is apparently not true. The conclusion of Harwood, Gould, and Swachman, therefore, appears untenable.

No objections were raised to the present swimming pool standards until 1933 when Mallmann and Cary (1) presented their studies on the bacterial picture of the swimming pool during periods of use. They found as stated briefly in the introduction to this thesis that, contrary to the accepted opinion, a chlorinated pool having the recommended chlorine residual (0.2 to 0.5 p.p.m.) when in use contained quite frequently large numbers of bacteria. This condition had escaped notice due to the fact that it was an accepted practice to collect the samples in the usual sterile

sample bottle, transport them to the laboratory when convenient, and test them when convenient. This meant that under the most favorable conditions of collecting and testing at least ten minutes elapsed before testing and, more frequently, one to two hours. During this period the residual chlorine continued to act and all the bacteria that might have been in the sample at the time of collection had been destroyed. The result was a sterile sample. With a chlorine residual of 0.3 parts or more, the report would always show sterile conditions irrespective of the size of the load at the time of sampling. To offset this killing action in the sample during transit, Mallmann and Cary suggested the use of the sodium thiosulphate sample bottles.

In the following presentation of the current standards for pools, it will be borne in mind that these standards were based on tests obtained on pools using the old method of examination.

The bacteriological standards recommended by the Joint Committee on Bathing Places of the American Public Health Association and the Conference of State Sanitary Engineers in 1926 (2) follows:

"B. Bacteria Count on Agar or Litmus Lactose Agar -24 hours - 37°C.: Not more than 10 per cent of samples covering any considerable period shall contain more than 100 bacteria per cc. No single sample contains more than 200 bacteria per cc.

"C. B. coli - Presumptive Test: Not more than two out

of five samples collected on the same day, not more than three out of any ten consecutive samples collected on different dates shall show a positive presumptive test."

The standard for chlorine residuals (3) is as follows: "Whenever chlorine, calcium hypochlorite or other chlorine compounds are used for swimming pool disinfection, the amount of available or excess chlorine in the water at all times when the pool is in use shall not be less than 0.2 p.p.m. or more than 0.6 p.p.m."

On the basis of these standards, let us examine some pools observed during the past year.

In Lansing there are three Junior High School pools of exactly the same dimensions with exactly the same equipment and carrying comparable bathing loads. These pools have a capacity of 65,000 gallons and a filter capacity for a six hour turnover of the water. The pools are 24 ft. wide and 60 ft. long. Inlets are located at the shallow end and outlets at the deep end. Because the pools are exactly alike and the type and number of bathers are similar the bacteriological results from these pools have been massed together for the presentation of the data that follow:

In Table VI are presented the bathing loads arranged according to the chlorine residuals of the pools at the time of sampling. It will be observed that the bathing loads are practically the same for all chlorine residuals, so that all samples collected were taken with approximately the same number of bathers. In Table VII are presented the bacterio-

logical data arranged in the same manner as in Table VI. Interpreting these data on the basis of the Standards of the Committee of the A.P.H.A., it will be noted that satisfactory results were obtained down to a minimum chlorine residual of 0.4 p.p.m. With 0.3 p.p.m. chlorine, the pools were unquestionably unsafe as measured by either total count or colon indices. Although streptococci were in evidence at all chlorine residuals, a sharp increase in their numbers occurred at the same time the colon indices increased sharply at 0.3 p.p.m. chlorine.

The data presented show that these pools with a bathing load of 31 can be kept free of pollution, provided a chlorine residual of 0.4 p.p.m. or more is maintained.

In Table VIII are presented the bathing loads for two pools, a 85,000 gallons. (a) and a 145,000 gallon pool (b). The loads are arranged according to the chlorine residuals as in the previous tables. In Pool A the load averaged 13 for all chlorine residuals and varied only from 10 to 16 bathers. In Pool B the average was 28 with extremes of 5 to 83. This means that in the latter pool the data represent very unsignificant up to extremely heavy loads. The averages on this pool mean very little but the maximums are important.

In Table IX are presented the bacteriological findings for Pools A and B. In Pool A with a load of 13 in 85,000 gallon of water, pollution should not occur even with low chlorine residuals. It is surprising to find that 0.2 p.p.m. showed decided pollution as measured by our present standards.

Table VI - Bathing Loads of three 65,000 gallon capacity pools arranged according to the residual chlorine contents of the pools at the time of sampling.

Chlorine Residual	Bathing Loads Average	Median	Minimum	Maximum	No. of samples
1+	31	28	15	40	8
0.9	37	36	28	41	12
0.8	33	35	18	40	13
0.7	32	33	19	42	13
0.6	32	30	18	40	30
0.5	30	30	13	42	37
0.4	28	26	16	47	25
0.3	26	26	21	40	12
0.2	35	-	30	40	2

Table VII - Summary of Bacteriological Data on three 65,000 gallon capacity pools arranged according to the chlorine residual contents for an average bathing load of 31.

Chlorine Residual	No. of Samples	Bacteria Count 37°C				Colon Index	Strept. Index	Quality of Water				Report
		Average	Median	Minimum	Maximum			Based on Count		Based on colon		
								Bad	Good	Bad	Good	
1+	8	50	0	0	350	0	0.23	0	8	0	8	Safe
0.9	12	250	80	0		0	0.50	0	12	0	12	Safe
0.8	13	20	10	0		0	0.30	0	13	0	13	Safe
0.7	13	250	200	0		0.46	1.50	0	13	1	12	Safe
0.6	30	75	7	0		0.26	1.13	0	30	2	28	Safe
0.5	37	500	35	0		0.30	1.65	4	33	4	33	Safe
0.4	25	200	0	0		0.40	1.12	2	23	1	24	Safe
0.3	12	1200	75	0		3.30	3.33	5	7	6	6	Unsafe
0.2	2	5000	-	7500		9.00	5.00	2	0	0	2	Unsafe

Table VIII - Bathing Loads of a 65,000 gallon capacity pool (A) and a 145,000 capacity pool (B) arranged according to the residual chlorine content of the pools at the time of sampling

Pool	Chlorine Residual	Bathing Loads Aver.	Median	Minimum	Maximum	No. of samples
A	1.	16	16	16	16	1
	0.5	10	10	10	12	4
	0.4	11	10	10	18	10
	0.3	12	12	7	18	17
	0.2	13	14	10	14	7
B	0.5	30	25	5	80	30
	0.4	27	22	8	83	44

As the loads are always small in this pool, the residual chlorine is kept between 0.3 to 0.5 p.p.m. at all times.

In Pool B with much larger loads, a much higher chlorine residual must be maintained. In this pool, as in the three pools discussed earlier in this paper, 0.4 p.p.m. shows a safe condition, but with 0.3 or 0.2 p.p.m. the colon index and total count increased rapidly. With loads ranging up to 90, the chlorine residual is maintained between 0.4 to 0.7 p.p.m. with a median of 0.6 p.p.m.

These data show what can be done on indoor pools under certain bathing loads using the total count and colon indices as measurements of pollution. Later in this paper a presentation of streptococci will be made.

In Table X are presented data on a 240,000 gallon outdoor pool showing the extent of pollution in the presence of varying amounts of chlorine. An examination of this table reveals the fact that according to our present standards this pool was unsafe at all chlorine residuals up to 1 p.p.m. when based on either the colon index or the total count alone, or both. These results are in marked contrast to those obtained with the indoor pools, but when the heavy load, lack of proper bathing prior to entering the pool, and the use of all types of unsterilized suits are considered, it can readily be understood that excessive pollution enters the pool. The results show that it is more difficult to sterilize the water under the conditions found in this outdoor pool, than with an indoor pool with similar bathing

Table IX - Summary of bacteriological data on a 65,000 gallon pool (A) and a 145,000 gallon pool (B) arranged according to the residual chlorine contents at the time of sampling

Pool	Chlorine Residual	No. of Samples	Bacteria Count 37°C			Colon Index	Strept Index	Quality of Water				Report
			Average	Minimum	Maximum			Based on count		Based on colon		
								Bad	Good	Bad	Good	
A	1	1	0	0	0	0	0	0	1	0	1	Safe
	0.5	4	1	0	5	0	0.4	0	4	0	4	Safe
	0.4	10	40	0	300	0	2.4	0	10	0	10	Safe
	0.3	18	30	0	375	0	1.6	0	18	0	18	Safe
	0.2	6	1800	150	6000	0.33	2.0	2	4	1	5	Unsafe
B	0.5	34	12	0	2600	0	1.2	0	34	0	34	Safe
	0.4	48	98	0	2500	0.12	1.6	2	46	3	45	Safe

Table X - Summary of bacteriological data on a 240,000 gallon outdoor swimming pool arranged according to the residual chlorine contents at the time of sampling

Source	Chlorine residual	No. of samples	Bacteria Count 37°C			Colon Index	Strept Index	Quality of water				Report
			Average	Minimum	Maximum			Based on count		Based on colon		
								Bad	Good	Bad	Good	
Shallow end	1.+	3	47	0	150	0.60	4.6	0	3	1	2	question
	0.9	8	350	0	1,000	1.20	6.0	1	7	1	7	Unsafe
	0.8	8	3000	0	25,000	0.25	5.5	1	7	1	7	unsafe
	0.7	25	2000	15	24,000	4.50	5.4	5	20	18	7	unsafe
	0.6	10	1000	8	10,000	4.20	8.2	1	9	9	2	unsafe
deep end	1.+	1	0	0	0	0	0	0	1	0	1	safe
	0.9	8	250	0	650	1.0	5.0	0	8	1	7	question
	0.8	5	150	0	300	0.5	6.0	1	4	2	3	unsafe
	0.7	26	1700	0	22,000	3.4	4.9	4	22	14	12	unsafe
	0.6	10	3400	6	22,000	6.8	8.4	2	8	9	1	unsafe
	0.5	5	160	14	200	5.2	9.2	0	5	4	1	unsafe

loads. If the bathing loads had been materially reduced, the number of bacteria would have decreased accordingly. Frequently the number in the pool and on the runways exceeded 400 bathers, although the number in the pool seldom exceeded 100. During the last summer when a protracted cold spell occurred and the bathing loads in this pool decreased to a decided degree, the pollution lessened. This would indicate that a reduced bathing load would result in a pool of good quality which would meet the present standards.

The studies presented so far have dealt with the total count and colon indices and no attention has been paid to the streptococci present. An examination of Tables VII, VIII, and IX shows that streptococci were present in most of the samples examined and that they were quite evident in chlorine residuals where both appreciable total counts and colon indices were absent. Particularly in the samples collected from the outdoor pool (Table X), the incidence of streptococci was extremely high. To determine their occurrence in pools, a study was made of 145,000 gallon pool during heavy bathing loads. A progressive sampling procedure was used taking samples at intervals of two minutes throughout an entire bathing period. The results of this study are presented in Graphs II, III, IV, and V.

To determine the rapidity of their occurrence in a pool, all of the swimmers (66 men) were lined up at the edge of the pool and ordered to enter simultaneously. The results are presented in Graph II. It will be observed that the pool, which previous to the massed entrance of the bath-

ers was sterile, within 30 seconds was showing a maximum index of 10. This maximum index continued for 2 to 3 minutes when a diminution of the streptococci occurred in spite of the fact that the bathers remained in the pool. Later in this period the bathers were out of the pool 5 minutes and when they returned to the water, immediately the streptococci index went up and then as they remained in the water the numbers became less. When the bathers have been out for 10 minutes the pool was again sterile. No Esch. coli or total count was obtained at any time during this experiment.

As shown in Graphs III and IV, similar experiments were conducted except that the bathers entered the water as soon as they entered the natatorium. This meant that for a period of 14 minutes each bather had entered the pool for a few minutes and then retired to the runways for instruction for the period. The actual number in the pool at any one time under these conditions seldom exceeded 20. When the entire group entered at once later in the period the streptococci were less pronounced then they were when they entered initially simultaneously.

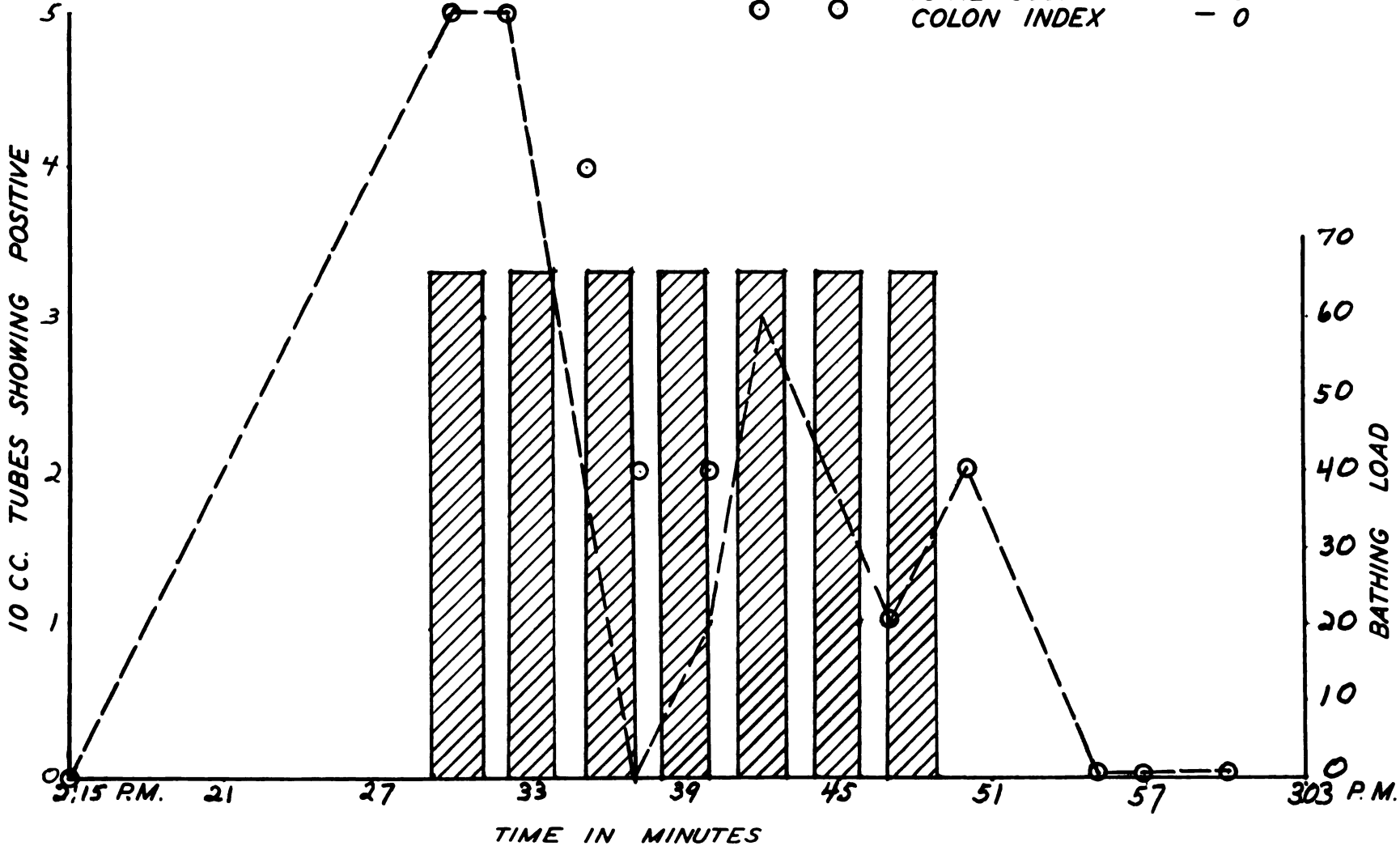
In Graph V, the loads were introduced into the pool in groups for 4 to 10 minute periods interspersed with rest periods of 5 to 10 minutes. The streptococci indices accordingly rise and fall with the shift in the bathing load. Incidently this graph shows another interesting observation. In collecting these samples, the actual number of bathers in the pool was counted at the time each sample was collected.

BACTERIOLOGICAL FINDINGS IN A POOL USING PROGRESSIVE SAMPLING DURING USE.

GRAPH II

--STREPTOCOCCI INDEX
O TOTAL COCCUS FORMS

JAN. 11, 1934
145,000 GAL. CAPACITY
CHLORINE RESIDUAL — 0.4 P.P.M.
BATHING LOAD — 66 MEN
TOTAL COUNT — 0
COLON INDEX — 0

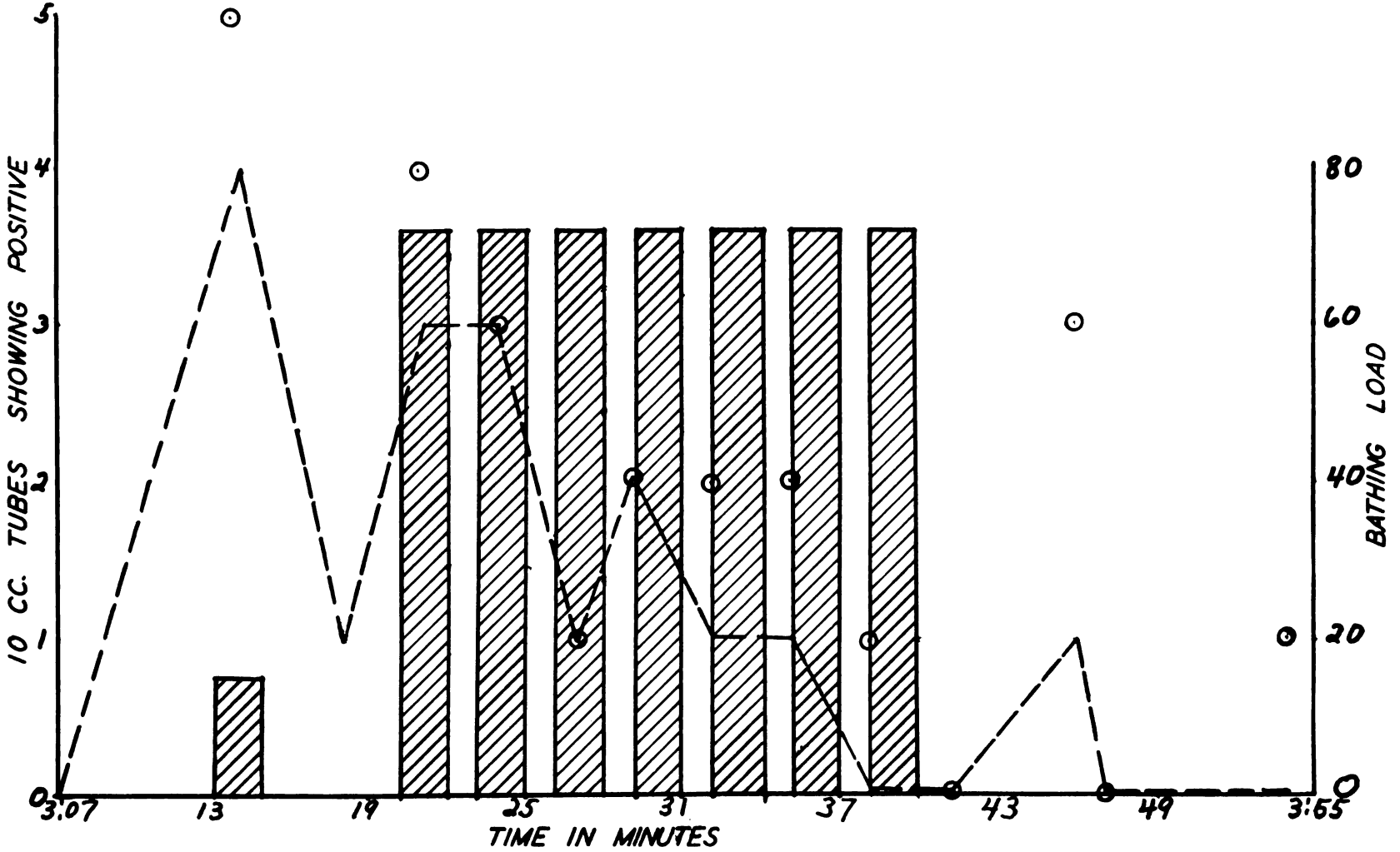


BACTERIOLOGICAL FINDINGS IN A POOL USING PROGRESSIVE SAMPLING DURING USE.

GRAPH III

FEB. 12, 1934
145,000 GALS. CAPACITY
CHLORINE RESIDUAL 0.4 P.P.M.
BATHING LOAD 72 MEN
TOTAL COUNT 0
COLON INDEX 0

– – STREPTOCOCCI INDEX
O TOTAL COCCUS FORMS

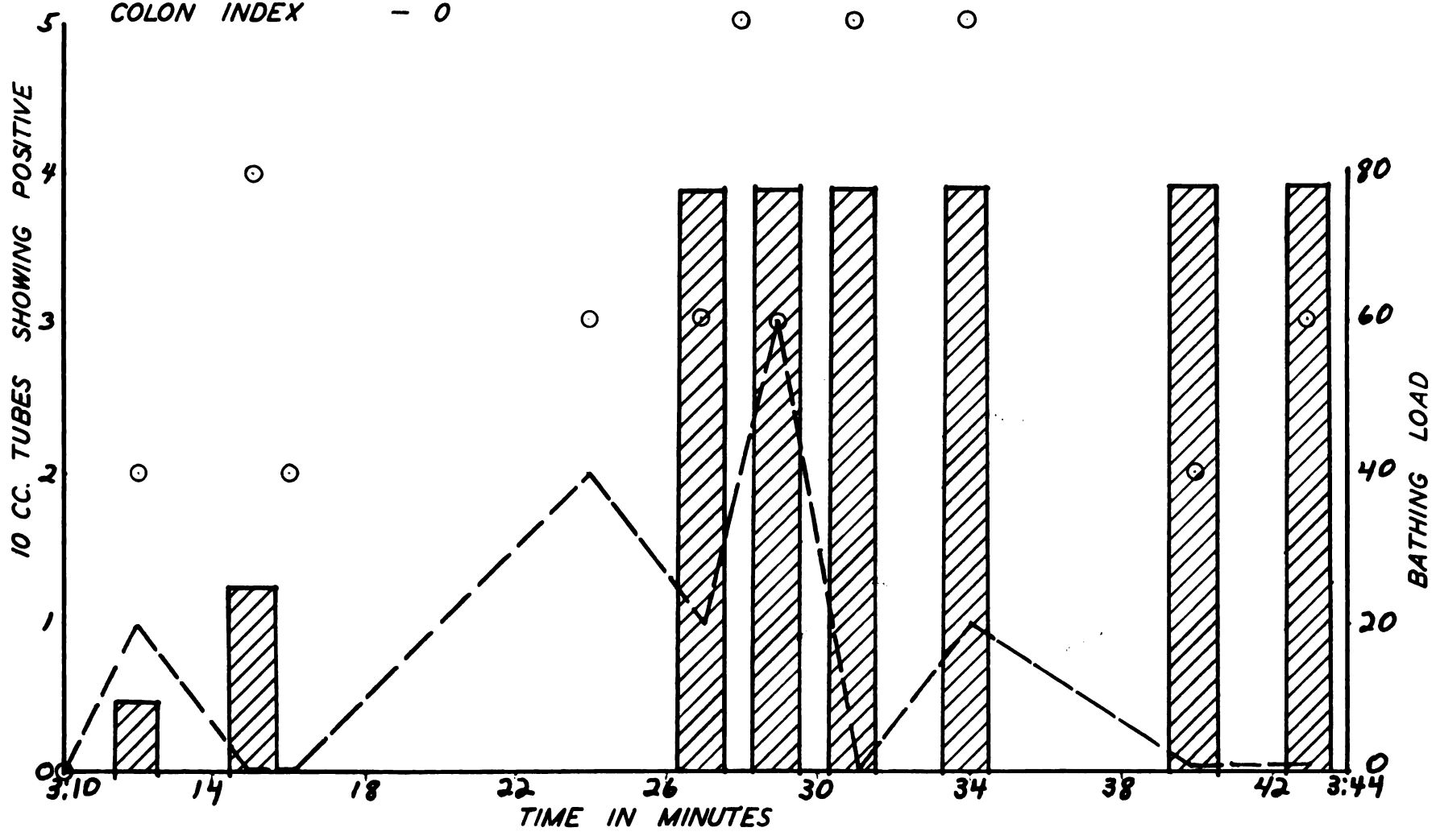


BACTERIOLOGICAL FINDINGS IN A POOL USING PROGRESSIVE SAMPLING DURING USE.

GRAPH IV

JAN. 26, 1934
145000 GALS CAPACITY
CHLORINE RESIDUAL – 0.5 P.P.M.
BATHING LOAD – 78 MEN
TOTAL COUNT – 0
COLON INDEX – 0

——STREPTOCOCCI INDEX
O TOTAL COCCUS FORMS

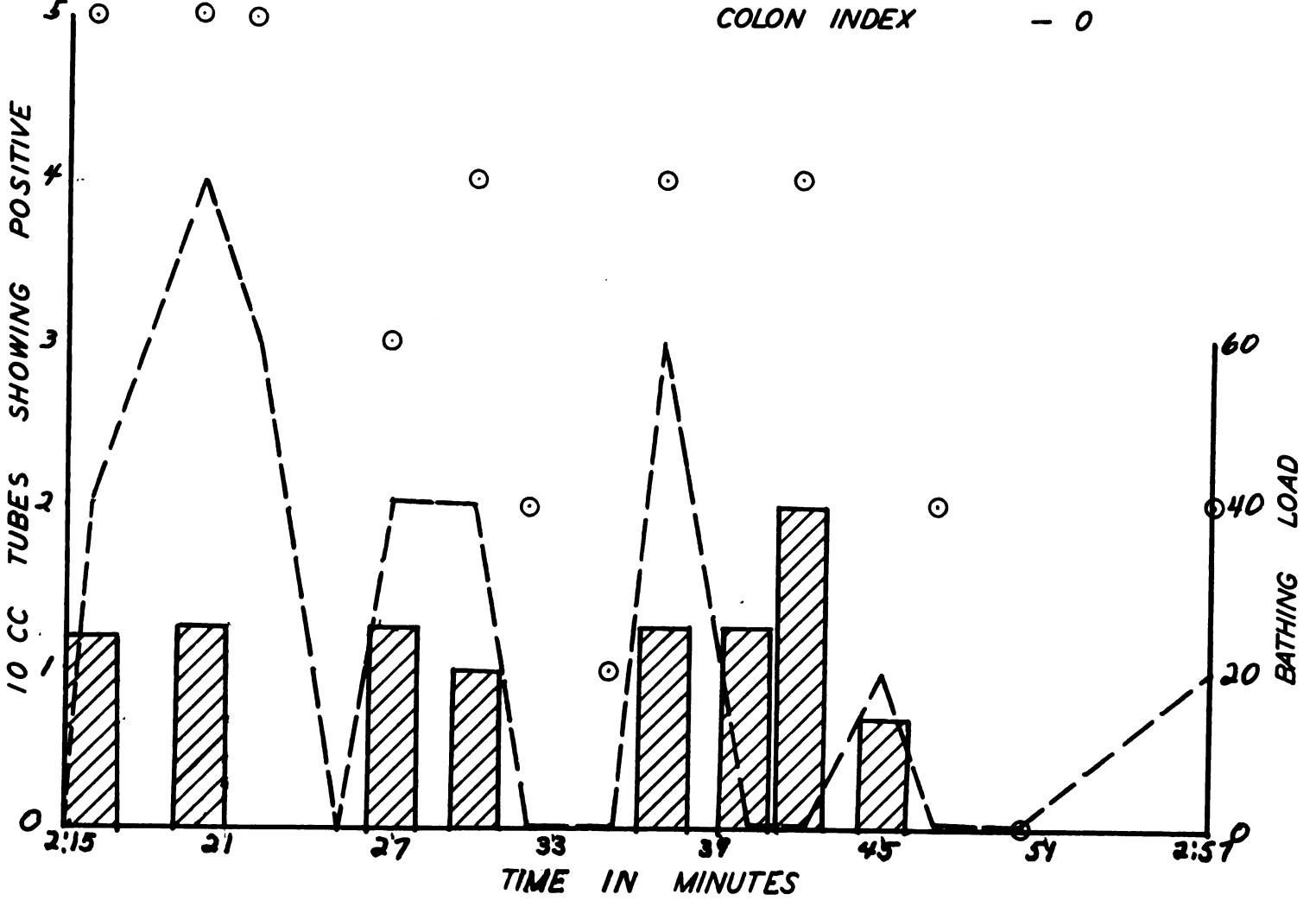


BACTERIOLOGICAL FINDINGS IN A POOL USING PROGRESSIVE SAMPLING DURING USE

GRAPH V

—— STREPTOCOCCI INDEX
○ TOTAL COCCUS FORMS

FEB. 27, 1934
145,000 GALS. CAPACITY
CHLORINE RESIDUAL — 0.3 P.P.M.
BATHING LOAD — 72 MEN
TOTAL COUNT — 0
COLON INDEX — 0



It was interesting to note that the number never exceeded 40 although the 72 present were free to enter if they cared to do so.

The part played by the various strains of streptococci in the respiratory diseases and their prevalence in the intestinal, buccal, and nasal discharges make the presence of streptococci in the pool very questionable. Frankly I would rather not see them in the pool, but to eliminate them would mean decidedly smaller bathing loads and decided increased in the chlorine residuals, either or both of which decrease the usefulness of the pool. Judging from the data available, it would seem advisable to continue the established standards, and require the use of sodium thiosulphate treated sample bottles.

Before leaving the topic of standards of pollution, it might be of interest to examine the present standards for bathing loads as recommended by the committee on Bathing Placed (3). The maximum bathing loads for a recirculation pool with continuous disinfection are as follows: "The total number of bathers using a swimming pool during any period of time shall not exceed 20 persons for each 1,000 gallons of clean water added during that period." What would the maximum load be for a pool like that at Michigan State College? This pool has a capacity of 145,000 gallon with an 8 hour turn-over and each 24 hours fresh water to the amount of 30,000 gallon is added. The amount of clean water added dairly amounts to 3 times the total capacity

(145,000 gal.) + 30,000 gallon of fresh water or 465,000 gallon total clean water added. The committee allows a maximum of 20 bathers per 1,000 gallon clean water so the pool may have a load of 9,300 per day. As the pool operates 10 hours daily this would mean 930 bathers per hour. Such standards have outlived their usefulness and should be revised.

The committee also places limits on the load based on the safety factor. They divide the pool into 3 zones, namely diving zone, swimming zone, and non-swimmer zone.

Limits for each zone are as follows:

diving zone - 10 ft. area beyond diving board
allow 3 divers
allow 9 divers on runway
total - 12 divers

swimming zone
allow 36 sq. ft. per swimmer
" 50% of swimming load on runways or
27 sq. ft. for all swimmers

non-swimming zone
allow 18 sq. ft. per bather
allow 100% of non-swimmer load on runways
or 10 sq. ft. per non-swimmer

Safety limits for pool at M.S.C. based on these standards

diving area-30 ft. by 20 ft. = 2 diving boards
allows 6 divers
" 18 divers on runway - 24 divers

swimming area - 50 ft. by 30 ft. = 1500 sq. ft.

1500 - 27 = 55 swimmers - 55 swimmers

non-swimming area - 600 sq. ft.

600 - 10 = 60 non-swimmers 60

total load 139 bathers

Or if entire pool is used for swimmers

90 ft. x 30 ft. = 2700 sq. ft.

2700 ÷ 27 = 100 bathers

These safety limits are in general far in excess of practical teaching loads and in my opinion in excess of safety loads. I have repeatedly watched in the college pool, the number of bathers in the water at any one time when diving was not permitted and I have never counted more than 40 bathers at one time in a class of 80 or 90 men. This gives each swimmer 67 sq. ft. of swimming area instead of 36 sq. ft. allowed by the committee and allows 50 per cent of the group on the runways which would permit a class of 60. The area per each member of the class would be 45 sq. ft. instead of the 27 sq. ft allowed. With this load a swimming pool of the size mentioned can be easily maintained in a safe condition as measured by total counts and coli indices. It has been our experience that the smaller pools (65,000 gallon capacity with dimensions of 60 ft. x 25 ft.) can carry heavier loads than the larger pools, so that the area per a bather may be lessened to a figure approaching that of the present standard. I would suggest 30 sq. ft. per member of the class or, a pool of these dimensions, a maximum of 43 bathers at any period.

Suggested recommendations for consideration of the committee on standards.

1. Abolish maximum limits based on clean water added to the pool.

2. Base maximum loads on surface area of the pool.

3. Adjust maximum loads on ability of the pool water to remain bacteriologically safe rather than on the safety limit. Arbitrarily the area per bather is as follows:

For pools of 2700 sq. ft. or more - 45 sq. ft.

For pools under 2700 sq. ft. - 35 sq. ft.

4. Chlorine residuals should be based on bathing loads. Light loads may operate successfully with low residuals and heavy loads with high residuals, but in general with the bathing maximum recommended in this paper the chlorine residuals should range between 0.4 to 0.6 p.p.m., depending somewhat upon the type of water.

5. The present bacteriological standards as regards total count and coli index may be retained.

6. All samples should be collected in sodium thiosulphate treated sample bottles.

7. No recognition of the streptococci in the standard should be made until further studies on the significance of the streptococci have been completed.

8. Samples should be collected at the time of greatest pollution, namely from five to ten minutes after the bathers have entered the pool. Samples for sanitary analysis should never be taken when the pool is not in use.

Conclusions

Standard lactose broth is a satisfactory medium for the growth of streptococci from swimming pools.

No direct parallelism occurs among the three indicators of swimming pool pollutions namely colon index, streptococcus index and total 37°C bacterial count.

Streptococci occur more frequently in chlorinated swimming pools than does Esch. coli.

The period of greatest pollution occurs shortly after the bathers enter the pool.

Chlorine residuals of swimming pools should be maintained between 0.4 to 0.6 p.p.m.

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