



THE SOURCE OF STREPTOCOCCI
FOUND IN
CHLORINATED SWIMMING POOLS

Thesis for the Degree of M. S.

William B. Ardrey

1936

Swimming pools
Streptococcus
Tittle

Bacteriology

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THESIS

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INTRODUCTION

With the introduction of the sodium thiosulphate treated sample bottle, the presence of bacteria has been noted in chlorinated swimming pools that were formerly believed to be bacteria free. Not only was the colon group noted, but also the presence of streptococci. These latter organisms may or may not have added significance over the colon group index, depending upon their source. If they are entirely of intestinal origin, then they have no sanitary value, as the colon group of organisms is ample evidence of intestinal pollution, but if they are of mouth and nose origin, they may be of value as indication of respiratory diseases. Certainly their absence in a pool would indicate the freedom of the pool water of respiratory disease organisms.

Since streptococci, as demonstrated by Mallmann (1), do not tend to increase in number after being introduced into swimming pool water, whereas colon bacilli do, it has been thought that they might lend valuable aid in determining the true contamination of the pools. However, most of this work originated in the analysis of drinking water where pollution would invariably come from sewage pollution and where, therefore, streptococci might be expected to present a true picture of the fecal contamination. At first it was not realized that this same picture might not hold good for swimming pools.

The possibility of large numbers of fecal streptococci entering the pool would be very slight, as the chance of the water in the pool becoming polluted by the fecal discharges of the bathers would be very rare. The only other method by which fecal streptococci might gain entrance to the water would be along with small particles of fecal material which were adhering to the surface of the body. Even this possibility would, in some measure, be done away with by the fact that bathers are required to take a shower before entering the pool. It is known that the nose and mouth are the source of large numbers of streptococci, which would have ready access to the pool. During the period of bathing the mouths and noses of the majority of bathers would be washed out with water several times, each time carrying large numbers of streptococci into the pool. It would seem, therefore, that the chance of the pool becoming contaminated with streptococci from the nose and mouth would be greater than the chance of contamination from fecal material.

Manheimer (2), in a review of the literature on enteric and respiratory diseases obtained through bathing, points out the fact that while there have been a great many enteric diseases transmitted in this manner, they have all resulted from bathing in rivers or in pools where the water is untreated. On the other hand, those cases of respiratory

diseases which have been obtained while bathing were mostly of the type caused by pyogenic cocci and were obtained from swimming pools which were apparently free from intestinal pollution. Grierson (3), in his review of the literature came to much the same conclusion as Manheimer, namely, that though the transmission of enteric diseases may be of importance in the case of sewage polluted waters, it is the respiratory diseases which are of prime importance in swimming pools. Hasty (4) states that ear and respiratory diseases are often transmitted by swimming pool water, and demonstrates the fact that water gets into the nasal chambers of the majority of swimmers, carrying with it whatever sort of contamination happens to be present.

Although investigators seem to disagree as to the source and importance of these streptococci found in swimming pools, no reference is made of anyone trying to determine from which source the greatest number are derived. Since many different diseases such as conjunctivitis, otitis media, sinusitis, and many other sinus and respiratory diseases are sometimes caused by certain pyogenic cocci, it is of some importance to try to discover the actual source of these streptococci found in swimming pools. It is, therefore, the purpose of this paper to demonstrate whether these streptococci have as their source the nose and mouth or fecal material.

HISTORICAL

Since our entire present method of swimming pool analysis is based upon the method used for the analysis of drinking water, it is not surprising that most of our ideas concerning streptococci found in swimming pools comes from the same source. Prescott and Winslow (5) report that Houston in England, in 1900, was the first to emphasize the importance of streptococci found in water. He pointed out that the streptococci were characteristic of animal discharges and were, therefore, quite indicative of dangerous pollution. The first work in this country was carried out by Winslow and Hummewell (6), in 1902, when they isolated streptococci along with Escherichia coli from the hands of school children. Later they found them to be present in sewage, feces, and in water polluted by sewage. In 1904, Prescott and Baker (7) showed these organisms to be present in each of 50 samples of polluted water, whereas they could not be demonstrated in nonpolluted waters. Savage and Read (8), in 1916, report that the estimation of streptococci in water is of undoubted value as evidence either for or against excretal contamination. Following this a great deal more work was done along the same line by other investigators, in each case the streptococci being found along with Esch. coli in the sewage polluted water. It is not surprising that when streptococci were first demonstrated to be present

in swimming pool water they were assumed to be of fecal origin.

Hasty (4), in 1927, called attention to the fact that the water in a swimming pool during the periods of its use would be subjected to the washings from both the nose and mouth of the bathers, thus receiving large numbers of streptococci which might be important in the transmission of disease. Mallmann (1), in 1923, was the first to use streptococci as an indicator of swimming pool pollution. He states, "Streptococci are constant indicators for intestinal pollution and the number found in the pool parallels the amount of pollution as indicated by the number of bathers." Grierson (3), in speaking of the methods used for determining the pollution of swimming pools states, "It is customary, therefore, to access results on the presence or absence of Bacillus coli in various quantities of water, taking into consideration the total bacterial count and the presence or absence of faecal streptococci and other organisms." Horwood, Gould, and Schwachman (9) were of the opinion that the streptococci found in swimming pools are of little importance in the determination of fecal pollution, but may be a very important factor in the transmission of diseases of the nose and throat. In all the cases where streptococci have been used as a confirmatory test for

intestinal pollution of swimming pools it has been assumed that they were of fecal origin, but there is no reference in the literature where experiments were performed to demonstrate this. In the cases where investigators believed the streptococci to have their origin, at least in part, in the nose and mouth, there has been no work done to confirm this belief.

PREPARATION OF MATERIALS

In determining the presence of streptococci from different parts of the body standard beef infusion broth was used. Its reaction was adjusted to between a pH of 7.4 and 7.6. To this broth was added 5 per cent of sterile bovine blood. In order that dilution determinations might be obtained, in the case of fecal material and material taken from the mouth, this broth was then tubed aseptically in 9 c.c. amounts. These tubes were then incubated for a period of twenty-four hours at 37°C. in order to determine if there was any contamination.

In the isolation of pure cultures of streptococci and in the making of quantitative counts blood agar was used. A base medium of beef liver infusion agar was used, as recommended by Stafseth (10). The reaction was adjusted to a pH of 7.4 to 7.6. The agar was then placed in flasks

in 200 c.c. amounts. In the preparation of the plates the agar was melted and after being cooled to between 47 and 50° C. 5 per cent of sterile blood was added and the agar then poured into plates. This same blood agar was used in the preparation of slants for the carrying of pure cultures. The tubes, before being inoculated, were incubated at 37°C. for 24 hours to determine the presence of contamination.

In the isolation of pure streptococci colonies from different parts of the body, an attempt was made to use gentian-violet, as recommended by Bryan (11), as an inhibiting agent for the other organisms present. Streptococci are, for the most part, fairly resistant to the action of germicides, especially that of certain dyes. Norton and Davis (12), in their work on the bacteriostatic action of dyes found that most streptococci are inhibited in their growth by gentian-violet in a dilution of 1 to 40,000. However, in dilutions of 1 to 150,000 and 1 to 200,000 streptococci are not inhibited whereas a great many other organisms are. Therefore, in the preparation of plates for the isolation of streptococci colonies, one per cent aqueous gentian-violet was added in amounts of about 0.1 c.c. per 200 c.c. of agar. Five per cent of blood was then added and the plates poured. In many cases, however, such small amounts of agar and dye were used that it was rather difficult to obtain the right dilution. As a result of this the streptococci and other

organisms were either both inhibited or the streptococci colonies were completely overgrown by the other organisms present. Because of this variability in inhibitory action, gentian-violet was added only to those plates where streptococci were being isolated from feces and which in the absence of an inhibitory agent, were overgrown in almost every case.

Sample bottles for the collection of water from swimming pools were prepared as recommended by Mallmann and Cary (13), with the following exceptions. Instead of the addition of a small crystal to a moist bottle or 0.5 cc. to a dry bottle and sterilization by moist heat, powdered sodium thiosulphate was added to dry bottles and these were sterilized in the hot air sterilizer instead of in the autoclave, (Devereux and Mallmann (14)).

The carbohydrate media used in the fermentation tests were prepared by adding one per cent of the carbohydrate to peptone water. The peptone water was prepared by adding 10 grams of peptone and 5 grams of salt per liter of water and the reaction adjusted to a pH of 7.4. To this was then added 2 c.c. of Andrade's indicator per liter of solution.

EXPERIMENTAL

Experiment I

Since the bathers themselves are the only conceivable source by which streptococci might gain entrance to swimming pools, a study was made of different surfaces of the body, the mouth and fecal material in order to determine which of these sources would be the greatest contributing factor

for these streptococci. Along with this, pure cultures were isolated from these different sources for comparison with pure strains isolated from swimming pools. For this work liver infusion blood agar was used. The organisms were classified into the three types of Smith and Brown (15).

In the determination of streptococci on the surface of different regions of the body sterile cotton swabs were used. These were prepared and placed in tubes containing 1 c.c. of sterile distilled water. These swabs were rubbed over different surfaces of the body. In order to obtain comparable results an area of about 1 1/2 to 2 inches square was swabbed. The swabs were then placed in tubes of broth containing 5 per cent blood and were incubated at 37°C. for a period of 24 hours. At the end of this time a hanging drop was made of each culture, and if the presence of streptococci was noted, smears were made and stained by Gram's method in order to confirm their presence. The regions from which these different swabs were taken are as follows: Mouth, anus, abdomen, arm (upper), arm (lower), ear, nose (internal), hands, chest, thigh, leg (lower), face, back, ankle, and fecal material. The results of this experiment are shown in Table I.

In cultures made from the mouth and from fecal material, as might well be expected, the presence of streptococci was noted in almost every instance. The only exceptions to this

Table I
Distribution of Streptococci on Body

Source	Trials																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Mouth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Feces	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anus	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-	+	-	+	-	+	+	+
Abdomen	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arm(upper)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arm(lower)	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Ear	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Nose	-	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+
Hand	+	-	-	+	+	+	-	-	+	-	-	+	+	+	+	-	+	+	-	-	-	-	-
Chest	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thigh	+	+	+	-	+	+	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-
Leg(lower)	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Face	-	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-
Back	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ankle	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-

were samples No.3 and No.13, obtained from fecal material. Even in the case of No. 3 there were a number of chain-like organisms present which had the appearance of very short rods. If these had been transferred a number of times and a more detailed study made of them they might have been proved to be streptococci. Sixteen cultures out of 23 from the nose proved to have streptococci present in them. This was also to be expected as the mucous membranes of the nose like those of the mouth and throat are considered as one of the chief sources for streptococci. Samples from the anus showed sixteen out of the twenty-three cases to have streptococci present. This large number of cases is beyond doubt due to contamination with fecal material. Samples from the thigh show eight of the cases to have streptococci present whereas there were only two cases where streptococci could be demonstrated on the lower section of the leg. Here again those cases found on the thigh may have been due, at least in part, to contamination with fecal material. Samples from the hands showed streptococci in ten out of twenty-three cases. This number is probably due to the fact that the hands are very often brought in contact with the nose and mouth and would thus tend to become contaminated with streptococci from these sources. For the most part the rest of the regions studied tended to show none or a very few cases with streptococci. The ear and face each showed five cases to have streptococci present, but all the other regions examined were positive in no more than three cases

and in several instances in no case. From these results it would appear that the two chief sources from which streptococci might gain entrance to swimming pools would be from the nose and mouth of the bathers and possibly from small particles of fecal material adhering to the body. The other body surfaces would seem to be of very little significance in raising the number of streptococci in the pool. In the first place streptococci are present in such few cases that even if they were introduced into the pool they would have little effect in increasing the total number present. Then there is the fact that in all well controlled pools the bathers are required to take a bath before entering the pool. This would tend to wash off a goodly number of those present so that in most cases there would be little danger of the pool becoming contaminated from this source.

In attempting to isolate pure cultures of streptococci, sterile swabs were first tried. These were dipped into the tubes of blood broth; which had previously been inoculated with fecal material, material taken from the mouth, and from swimming pools; and were then streaked on blood agar plates. This method, however, did not prove to be very successful, as the other organisms usually outgrew and overgrew the colonies of streptococci. An attempt was then made using dilutions of the inoculated broth and plating in blood agar.

This method proved to be more successful except that in some of the cases, where fecal material was used, the other colonies would still overgrow the streptococci colonies in the dilutions where the streptococci were present. It was found, however, that by the addition of gentian-violet in a dilution of about 1 to 150,000 that these organisms could be inhibited to such an extent that colonies of streptococci might be obtained. When typical streptococci colonies were noted they were fished with a platinum needle and streaked on blood agar plates, where they were incubated at 37°C. for 24 hours. At the end of this period hanging drops were made of the colonies, and, if the presence of streptococci was noted, it was confirmed by stained preparations. The colonies were then transferred to blood agar slants where they were transplanted every three weeks to insure good growth.

Experiment II

Since most of the streptococci in the swimming pools seem to be of either mouth or fecal origin an attempt was made to determine the number of streptococci present in certain given amounts of material from each of these sources. In making these quantitative tests for the number of streptococci in the mouth, 10 c.c. of sterile water was taken into the mouth and washed about and gargled for a period of about half a minute. This was then diluted out into tubes containing 9 c.c. of blood broth in dilutions

of 1 to 10, 1 to 100, 1 to 1,000, 1 to 10,000, and 1 to 100,000. These tubes were then incubated for a period of 24 hours at 37°C. At the end of this time the presence of streptococci was determined by making smears of each dilution and staining by Gram's method. The results of this work are shown in Table II.

In only two of the cases Nos 9 and 21, were there no streptococci present. In case No.17 there were only a hundred streptococci obtained in the one washing. With the exception of these three cases all showed streptococci to be present in numbers between 1,000 and 1,000,000. The average number obtained with one washing was about 100,000. This, however, does not mean that this would be anywhere near the total number of streptococci entering the pool from the mouth of any one bather. In cases where the mouth was washed a second time immediately following the first washing it was found that relatively high counts could still be obtained.

In order to check on this dilution method as a means for determining the number of streptococci, samples 1 to 7 and 18 to 20 inclusive were also determined by the plate method. In this work dilutions of the samples were made the same as before; 1 c.c. being placed in sterile petri dishes. Liver infusion blood agar was then added and the plates incubated for 24 hours. At the end of this time the number

TABLE II
Streptococci in Mouth

No.	Dilutions					Number Obtained in One Washing
	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000	
1	+	+	+	+	-	100,000
2	+	+	-	-	-	1,000
3	+	+	+	-	-	10,000
4	+	+	+	+	-	100,000
5	+	+	+	+	+	1,000,000
6	+	+	+	+	+	1,000,000
7	+	+	+	-	-	10,000
8	+	+	-	-	-	1,000
9	-	-	-	-	-	0
10	+	+	+	-	-	10,000
11	+	+	+	-	-	10,000
12	+	+	-	-	-	1,000
13	+	+	+	+	+	1,000,000
14	+	+	+	+	-	100,000
15	+	+	+	-	+	10,000
16	+	+	+	+	-	1,000,000
17	+	-	-	-	-	100
18	+	+	+	+	-	100,000
19	+	+	-	-	-	1,000
20	+	+	+	+	-	10,000
21	-	-	-	-	-	0
22	+	+	+	+	-	100,000
23	+	+	+	+	-	100,000
24	+	+	+	+	+	1,000,000
25	+	+	+	-	-	10,000

of streptococci colonies were determined by their action on the blood agar. The results of this method are shown in Table III.

In almost every case the results were similar to those of the dilution method, except that the number obtained by plate method, as might be expected, was slightly higher than by the dilution method. Samples Nos. 2 and 3, as determined by the plate method, showed no streptococci to be present, whereas the same samples tested by the dilution method showed sample No. 2 to have 1,000 streptococci present and sample No. 3 to have 10,000 streptococci present. In the plate method the lack of streptococci in these two instances was probably due to the fact that they occurred only in dilutions which would be overgrown by the other types of bacteria present.

In determining the number of streptococci present in fecal material much the same method was used as in the determination for those in the mouth. Samples of feces were obtained from different individuals and were weighed out in one gram amounts into large sterile test tubes. Ten c.c. of sterile water was then added and the material well mixed. This was then diluted in tubes of blood broth in dilutions of 1 to 10, 1 to 100, 1 to 1,000, 1 to 10,000, and 1 to 100,000. These were incubated as before and the determination for streptococci made by making smears and staining with Gram's stain. The results of this examination are shown in Table IV.

TABLE III
Streptococci in Mouth
(Plate Method)

No.	Dilutions					Number Obtained In One Washing
	1:10	1:100	1:1000	1:10,000	1:100,000	
1	overgrown	overgrown	overgrown	3	-	300,000
2	-	-	-	-	-	0
3	-	-	-	-	-	0
4	overgrown	overgrown	overgrown	2	-	200,000
5	"	"	"	9	-	900,000
6	"	"	"	overgrown	2	2,000,000
7	"	"	8	-	-	80,000
18	"	"	6	-	-	60,000
19	"	"	4	-	-	40,000
20	"	"	2	-	-	20,000

TABLE IV
Streptococci in Feces

No.	Dilutions					Number Obtained In One Gram
	1:100	1:1000	1:10,000	1:100,000	1:1,000,000	
1	+	+	+	-	-	10,000
2	+	+	+	-	-	10,000
3	+	+	+	+	-	100,000
4	+	+	+	-	-	10,000
5	+	+	+	-	-	10,000
6	+	+	-	-	-	1,000
7	+	+	+	-	-	10,000
8	+	+	+	+	-	100,000
9	+	+	-	-	-	1,000
10	-	-	-	-	-	0
11	+	+	+	+	+	1,000,000
12	+	+	+	+	-	100,000
13	+	+	+	-	-	10,000
14	+	+	+	+	-	100,000
15	+	+	-	-	-	1,000
16	+	+	+	-	-	10,000
17	+	+	+	-	-	10,000
18	+	+	+	-	-	10,000
19	+	+	+	+	+	1,000,000
20	+	+	+	+	+	1,000,000
21	+	+	-	-	-	1,000
22	+	-	-	-	-	100
23	+	+	+	+	-	100,000
24	+	+	+	-	-	10,000
25	+	+	+	-	-	10,000

In this work there was only one case, No.10, which showed a complete absence of streptococci. Case No.22 showed only 100 streptococci to be present per gram of feces whereas in all the rest of the cases the numbers ranged between 1,000 and 1,000,000. The average number per gram of fecal material was about 10,000.

Although no direct comparisons can be made between the number of streptococci obtained from a washing of the mouth and from a gram of fecal material, these experiments at least show that either one of these sources would present a very plentiful supply of streptococci if brought into contact with the water of a swimming pool. On the whole, it would seem that the chance of swimming pool water becoming polluted by fecal discharges of the bathers would be very rare. At least it seems safe to say that it would be of such rare occurrence that it would play no part in the day to day high streptococci indices which may be obtained from almost any swimming pool. Even small particles of fecal material adhering to the body would be removed in large measure due to the fact that bathers are required to take a bath before entering the pool. On the other hand it is easy to see how the large numbers of streptococci contained in the nose and mouth would have ready access to the water of the pool. Even though spitting is prohibited in the pool, there is little doubt but that the nose and mouth of each bather is well washed out several times during the course of swimming; each washing carrying large numbers of streptococci into the pool.

Experiment III

In this series of experiments an attempt was made to determine whether the streptococci found in swimming pools were of oral or fecal origin. These experiments were conducted with a class of forty students in one of the Junior high school pools. The residual chlorine in the pool was first determined by means of the ortho-tolidine test and control samples of the water in the pool were taken in sodium thiosulphate treated sample bottles before the class was allowed to enter the pool. All the samples taken, including the controls, were taken in triplicate; one at each side of the pool and one at the shallow end. The three samples were taken simultaneously. After these three control samples had been taken the class was requested to enter the pool at the shallow end, and, in so doing, to be careful that their heads were continually above the water. They were then asked to move about, meanwhile keeping their heads well out of the water in order that there might be no chance of streptococci from their mouths entering the pool. At stated intervals samples were taken of the pool water from the three different positions. After several samples had been taken in this manner, the class was given some breathing exercises which entailed repeated total submersion. In a great many of the cases this had the effect of introducing water into the nose and mouth, from

which it was shortly returned to the pool. As before samples were taken at regular intervals.

These samples were then carried to the laboratory where 10 c.c. of each sample was pipetted into each of five tubes of double strength lactose broth and 1 c.c. into each of five tubes of single strength lactose broth. These were incubated for a period of 48 hours. One c.c. portions of the water were plated out in plain agar and counts of the number of bacteria present were made at the end of 24 hours. At the end of 48 hours the tubes of lactose broth were examined and those showing gas were streaked on eosin-methylene blue agar to confirm for Esch. coli. The presence of streptococci was first determined macroscopically by the method suggested by Mallmann and Cary (13). This consists of holding the tubes up to the light and looking for a granular precipitate. If this precipitate was present after the tubes had stood for 72 hours, the supernatant liquid was drawn off and smears were made of the precipitate. These were then stained by Gram's method and examined microscopically to confirm for the presence of streptococci. From these results indices for the Esch. coli and the streptococci present in the pool were obtained. The results of this experiment are found in Tables V to IX inclusive. Each Table is divided up into three parts A, B, and C; representing the different positions about the pool from which the samples were taken. The number of bathers and the parts

TABLE X
Streptococcus and Colon Index

P.P.M. of Chlorine -- 1
Number of Bathers -- 40

A
North Side of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial count	
1	0	-	-	19	Control
2	1 min.	-	-	36	
3	2 min.	-	-	36	
4	3 min.	4	-	60	Body
5	4 min.	4	-	19	

B
South Side of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial count	
1	0	-	-	48	Control
2	1 min.	-	-	8	
3	2 min.	-	-	363	
4	3 min.	2	2	91	Body
5	4 min.	2	2	166	

TABLE X (continued)
Streptococcus and Colon Index

C
End of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial count	
1	0	-	-	24	Control
2	1 min.	-	-	97	
3	2 min.	2	6	90	Body
4	3 min.	4	-	33	
5	4 min.	2	-	37	

TABLE XI
Streptococcus and Colon Index

P.P.M. of Chlorine -- .6
Number of Bathers -- 40

A
North Side of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial count	
1	0	-	-	7	Control
2	1 min.	-	-	13	
3	2 min.	-	-	29	
4	3 min.	-	-	482	Body
5	4 min.	-	-	33	
6	5 min.	-	-	26	

B
South Side of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial count	
1	0	-	-	6	Control
2	1 min.	4	4	11	
3	2 min.	-	-	51	
4	3 min.	-	4	28	Body
5	4 min.	-	-	96	
6	5 min.	-	-	20	

TABLE VI (continued)
Streptococcus and Colon Index

C
End of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial count	
1	0	4	-	5778	Control
2	1 min.	-	-	4540	
3	2 min.	-	-	3650	Body
4	3 min.	-	-	4509	
5	5 min.	40	6	4794	
6	6 min.	10	-	1705	Mouth

TABLE VIII
Streptococcus and Colon Index

P.P.M. of Chlorine -- .75
Number of Bathers -- 28

A
North Side of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial count	
1	0	-	-	15	Control
2	1 min.	-	-	16	Body
3	2 min.	-	-	40	
4	4 min.	-	-	42	
5	5 min.	2	-	57	Mouth
6	6 min.	-	-	47	

B
South Side of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial count	
1	0	-	-	26	Control
2	1 min.	-	-	51	Body
3	2 min.	-	-	37	
4	4 min.	4	2	81	
5	5 min.	6	-	76	Mouth
6	6 min.	2	-	49	

TABLE VIII (continued)
Streptococcus and Colon Index

C
End of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial count	
1	0	-	-	25	Control
2	1 min.	-	-	65	Body
3	2 min.	-	-	57	
4	4 min.	4	-	109	
5	5 min.	-	-	56	Mouth
6	6 min.	4	-	67	

TABLE IX
Streptococcus and Colon Index

P.P.M. of Chlorine -- .5
Number of Bathers -- 38

A
North Side of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial Count	
1	0	-	-	29	Control
2	1/2 min.	-	-	33	Body
3	1 min.	2	-	119	
4	2 min.	20	-	96	
5	2 1/2 min.	-	-	72	Mouth
6	3 min.	20	-	96	

B
South Side of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial count	
1	0	-	-	16	Control
2	1/2 min.	-	-	20	Body
3	1 min.	-	-	44	
4	2 min.	6	-	57	
5	2 1/2 min.	8	-	77	Mouth
6	3 min.	4	-	91	

TABLE IX (continued)
Streptococcus and Colon Index

C
End of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial count	
1	0	-	-	22	Control
2	1/2 min.	2	-	68	Body
3	1 min.	-	-	77	
4	2 min.	4	-	58	
5	2 1/2 min.	6	-	44	Mouth
6	3 min.	8	-	96	

per million of chlorine is recorded in each case at the top of the table.

These tables are rather significant in that in every case there is a rise in the streptococcus index immediately or soon after the heads of the bathers had been submerged in the water. What is still more important is the fact that in a great many of the cases the index increases anywhere from five to thirty times over that obtained when only the body was in contact with the water. This in itself would tend to show that some new plentiful source of streptococci had been introduced. That this new supply of streptococci can not be attributed to fecal discharges of the bathers is shown by the fact that in no case was there a like increase in the colon index as might be expected if the fecal material was responsible. Furthermore the presence of Esch. coli was noted in only five out of the fifteen series of samples taken, and in only two samples did the index reach six. This would tend to show that no large amounts of fecal material had entered the pool.

In order to show that this sudden rise in the streptococcus index following the submersion of the bathers' heads was not due to the gradual release of the organisms from the body, although the sudden and in most cases rather large increase would in itself tend to disprove it, another set of experiments was run. In these experiments the same

procedure was used as before, except that all of the samples were collected with only the bodies of the bathers exposed to the water and without their heads being submerged at any time. The results of these experiments are shown in Tables X to XII inclusive. In none of these cases, where the mouths of the bathers were not allowed to come in contact with the water, did the streptococcus index rise above 4, whereas in the previous cases, Tables V to IX inclusive, where the mouths of the bathers had been in contact with the water the index often rose as high as 20 and in one case, Table VII-C, as high as 60.

In Tables X to XII inclusive, the streptococcus index seems to parallel roughly the colon index. This might well be expected due to the fact that here the two organisms are assumed to come from the same source. However, if this were true it would be expected that somewhat the same results would show up in Tables V to IX inclusive, in those samples which were taken during the period when only the body was exposed to the water. This, however, does not seem to be the case with the possible exception of Table V-A and C. In no case does there seem to be any relationship between the streptococcus or colon indices and the total bacterial count.

Experiment IV

Although all of the above results seem to point to the fact that the large majority of the streptococci found in swimming pools have the nose and mouth as their source, an

TABLE XII (continued)
Streptococcus and Colon Index

C
End of Pool

Sample number	Time in pool	Streptococcus index	Colon index	Bacterial count	
1	0	-	-	25	Control
2	1/2 min.	-	-	119	
3	1 min.	-	-	135	
4	1 1/2 min.	-	2	894	Body
5	2 min.	-	-	312	
6	2 1/2 min.	4	-	153	

attempt was made confirm these observations. This was done by taking a number of pure cultures of streptococci, which had previously been isolated from the mouth, fecal material, and swimming pools, and testing them with certain carbohydrates, to determine their ability to ferment. It was hoped that in so doing those streptococci which had been isolated from swimming pools might fall into certain definite groups which would correspond with certain groups of reactions as produced by either those streptococci isolated from the mouth or from fecal material. In this work the carbohydrates used were lactose, salicin, mannite, and inulin. The action on blood agar and in litmus milk was also determined. In naming the different groups formed, the classification of Holman was used.

Many attempts have been made to classify streptococci according to their ability to ferment different carbohydrates. As a result, a great many different test substances have been used, and a great many different results have been obtained, thus causing some workers to doubt the value of such a procedure. Bergey (16) came to the conclusion that the action of streptococci on different carbohydrates was of little value in the determination of their classification, due to the fact that they were so prone to die out in the media, thus making it difficult to retain their vigor. Thro (17, 18) demonstrated a number of different factors

which would tend to produce variation in the results of fermentation reactions of streptococci. Some of the factors are as follow: Dry media, faulty preparation of media, variability of growing power of different strains, length of time grown on artificial media, complexity of the substances used and the lack of pure ones, titration, alkali from glass, and the exceptional variability of this particular group of organisms. Holman (19), although reporting favorable results, states that confusion may easily arise from the fact that certain strains may lose their vigor when grown upon artificial media.

Aside from the fact that many investigators disagree as to the importance of various carbohydrates in the classification of streptococci there is another factor which enters in to make it a rather doubtful procedure to use in determining the source of streptococci found in swimming pools. According to the present methods of classification, the great majority of those types of streptococci which are found in the mouth may also be found in fecal material, and those types found in fecal material may also be found in the mouth. The only two varieties which would seem to have any value at all in a determination of this kind would be Streptococcus salivarius, which is found largely in the mouth, and Streptococcus fecalis, which is found for the most part in the contents of the intestine. Even here there would seem to be nothing which would hinder an interchange

of sources, thus causing a great deal of confusion in the kind of determination attempted.

The chief work and that of most value along the line of using carbohydrates in the classification of streptococci seems to have been done by Gordon, Andrews and Horder, and Holman. Gordon (20) was the first to make use of carbohydrates as a means of classifying streptococci. In this work he studied thirty-five different compounds and finally selected as those having the most value; lactose, saccharose, raffinose, inulin, salicin, coniferin, and mannite, along with the clotting of litmus milk and the reduction of neutral red. This same method was further developed by Andrews and Horder (21), and used as the basis for the classification developed by Holman (20). This classification as developed by Holman is given in Table XIII. It was this classification which was used in naming the different groups as determined by their fermentation reactions.

In determining the types of colonies on blood agar the classification as worked out by Smith and Brown was used. Schottmuller (23), in 1903, was the first to make use of blood agar in the demonstration of streptococci in regards to the effect of their growth on the red blood cells. By this method he divided streptococci into two groups. Those which dissolved the red blood cells he called

TABLE XIII

+Hemolysis..... -	+Lactose..... -	+Mannit- +Salicin- +Salicin- +Salicin- +Salicin- +Salicin- +Salicin- +Salicin- +Salicin- +Salicin- +Salicin- +Salicin- +Salicin- +Salicin- +Salicin-	Str. ignarus
			Str. equinus
			Str. non-hemolyticus iii
			Str. non-hemolyticus ii
			Str. salivarius
			Str. mitis
			Str. non-hemolyticus i
			Str. fecalis
			Str. subacidus
			Str. equi
			Str. hemolyticus iii
			Str. hemolyticus ii
			Str. anginosus
			Str. pyogenes
			Str. hemolyticus i
			Str. infrequens

hemolytic streptococci or "Streptococcus pyogenes var. ersipelatis," and those which caused a greenish discoloration of the blood with only slight or no hemolysis of the blood cells be called "Streptococcus mitior var. viridans." Smith and Brown (15), in 1915, and Brown (24), in 1919, in their description of streptococci colonies on blood described three types which they called "alpha," "beta," and "gamma." Their descriptions of these three types in brief is as follows:

"Type alpha. As observed after forty-eight hours incubation the change produced by streptococci of this type may be described as a somewhat greenish discoloration and partial hemolysis of the blood corpuscles immediately surrounding the colony forming a rather indefinitely bounded zone 1-2 m.m. in diameter and surrounded by a second, narrow, clearer, not discolored, partly hemolyzed zone.

"Type beta. This type of appearance in blood agar may be described as a colony surrounded by a perfectly clear, colorless zone of hemolysis. There is no trace of discoloration and when viewed microscopically no corpuscles are seen in the medium surrounding the colony. The colony itself is grayish by transmitted light.

"Type gamma. By the gamma type is meant the growth of the streptococcus colonies within and on the blood agar plate without the production of any perceptible hemolysis or discoloration of the surrounding medium during incubation or refrigeration."

The results of this experiment are given in Tables XIV, XV, and XVI. Table XVII shows the different groups into which the strains studied fell along with their classification as worked out according to Holman. From the results obtained it appears, as might be expected, that this method of determining the origin of streptococci in swimming pool water has little value. The two groups, Table XVII-I and V, which might be expected to be of some aid in such a determination are not outstanding enough to be of much value. As pointed out before these two strains, Str. salivarius and Str. fecalis, are fairly characteristic of the mouth and of fecal material respectively as their names would imply. However, like all the other strains it is not impossible for them to be found in either place. This is fairly well demonstrated by the results obtained. Str. salivarius was found in eight cases from the mouth and in three cases from fecal material. Str. fecalis was found in no case from the mouth and in ten cases from fecal material. Str. salivarius was found to be present in the swimming pool water in five cases and Str. fecalis in two cases. This is more or less the relationship which might be expected judging from the previous experiments, but there is not nearly enough material from which to draw any definite conclusions. The other eight groups into which the streptococci from these three different sources fell are

TABLE XIV
Fermentation Reactions

SOURCE--Mouth

Culture	Type	Lactose	Salicin	Mannite	Inulin	Milk Acid	Clot
101	Alpha	+	-	-	-	+	-
102	Alpha	+	-	+	-	+	+
103	Beta	+	+	-	+	+	+
104	Alpha	+	-	-	-	+	+
105	Alpha	+	-	-	+	+	+
106	Alpha	+	-	-	-	+	+
107	Alpha	+	-	-	-	+	+
108	Alpha	+	+	-	+	+	+
109	Alpha	+	-	-	+	+	+
110	Alpha	+	+	-	-	+	+
111	Alpha	+	+	-	-	+	+
112	Alpha	+	-	+	-	+	+
113	Alpha	+	-	-	-	+	+
114	Alpha	+	-	-	-	+	+
115	Alpha	+	-	+	-	+	+
116	Alpha	+	+	-	-	+	+
117	Alpha	+	-	-	-	+	+
118	Beta	+	+	-	-	+	-
119	Beta	+	+	-	-	+	+
120	Beta	+	+	-	+	+	+
121	Alpha	+	+	-	-	+	+
122	Beta	+	-	+	-	+	+
123	Alpha	+	-	+	-	+	+
124	Alpha	+	+	-	-	+	+
125	Alpha	+	-	-	-	+	+

TABLE XV
Fermentation Reactions

SOURCE--Feces

Culture	Type	Lactose	Salicin	Mannite	Inulin	Milk Acid	Clot
1	Alpha	+	+	+	-	+	+
2	Alpha	+	-	-	+	+	+
3	Alpha	+	-	+	+	+	+
4	Alpha	+	+	-	-	+	+
5	Alpha	+	-	+	+	+	+
6	Alpha	+	+	+	-	+	+
7	Alpha	+	+	+	-	+	+
8	Alpha	+	+	+	-	+	+
9	Alpha	+	+	-	+	+	+
10	Alpha	+	+	-	-	+	+
11	Alpha	+	+	+	-	+	+
12	Alpha	+	-	+	+	+	+
13	Alpha	+	-	-	-	+	+
14	Alpha	+	+	+	-	+	+
15	Alpha	+	+	-	-	+	+
16	Alpha	+	-	+	+	+	+
17	Alpha	+	-	-	-	+	+
18	Alpha	+	+	+	-	+	+
19	Alpha	+	-	-	-	+	+
20	Alpha	+	+	+	-	+	+
21	Alpha	+	+	+	-	+	+
22	Alpha	+	-	-	+	+	+
23	Alpha	+	+	-	-	-	-
24	Alpha	+	-	+	+	+	+
25	Alpha	+	+	+	-	+	+

TABLE XVI
Fermentation Reactions

Source -- Swimming Pool

Culture	Type	Lactose	Salicin	Mannite	Inulin	Milk Acid	Clot
201	Alpha	+	+	+	-	+	+
202	Alpha	+	+	-	-	+	+
203	Alpha	+	+	-	+	+	+
204	Alpha	+	+	-	-	+	+
205	Gamma	+	-	+	-	+	+
206	Alpha	+	-	-	-	+	+
207	Alpha	+	+	-	-	+	+
208	Alpha	+	+	-	+	+	+
209	Alpha	+	+	+	-	+	+
210	Alpha	+	+	-	-	+	+
211	Alpha	+	-	-	-	+	+
212	Alpha	+	-	+	-	+	+
213	Alpha	+	+	-	-	+	+
214	Alpha	+	-	-	-	+	+
215	Alpha	+	+	-	+	+	+
216	Gamma	+	-	-	-	+	+
217	Gamma	+	-	-	-	+	+
218	Gamma	+	+	-	+	+	+
219	Gamma	+	+	-	+	+	+
220	Alpha	+	+	-	-	+	+
221	Alpha	+	+	-	-	+	+
222	Alpha	+	-	+	-	+	+
223	Alpha	+	+	-	-	+	+
224	Alpha	+	+	+	-	+	+
225	Alpha	+	+	-	-	+	+

TABLE XVII
Fermentation Reactions

	Test Substance	Source	Number Reacting	Holman's Classification
I	No hemolysis	Mouth	8	Str. salivarius
	Lactose	Feces	3	
		Swimming Pool	5	
II	No hemolysis	Mouth	5	Str. mitis
	Lactose	Feces	4	
	Salicin	Swimming Pool	9	
III	No hemolysis	Mouth	4	Str. non-Hemolyticus 1
	Lactose	Feces	0	
	Mannite	Swimming Pool	3	
IV	No hemolysis	Mouth	2	
	Lactose	Feces	2	
	Inulin	Swimming Pool	0	
V	No hemolysis	Mouth	0	Str. fecalis
	Lactose	Feces	10	
	Salicin	Swimming Pool	3	
VI	No hemolysis	Mouth	1	
	Lactose	Feces	1	
	Salicin	Swimming Pool	5	
	Inulin			
VII	No hemolysis	Mouth	0	
	Lactose	Feces	5	
	Mannite	Swimming Pool	0	
	Inulin			
VIII	Hemolysis	Mouth	2	Str. pyogenes
	Lactose	Feces	0	
	Salicin	Swimming Pool	0	
IX	Hemolysis	Mouth	1	Str. hemolyticus i
	Lactose	Feces	0	
	Mannite	Swimming Pool	0	
X	Hemolysis	Mouth	2	
	Lactose	Feces	0	
	Salicin	Swimming Pool	0	
	Inulin			

of no value in determining the source of the streptococci from the pool water. According to Holman, Groups IV, VI, VII, and X are merely members of variations of the other six groups, as he does not consider inulin as a valuable test substance. Quinit (25), in 1926, did some work along this same line using the classification and test substances as recommended by Andrews and Horder. He came to the conclusion that the type predominate in feces is Str. fecalis, whereas that which is predominate in the mouth is Str. mitis. As in the work presented here he concluded that the results obtained were of little or no value, due to the fact that both classes are found both in the mouth and in the feces of normal individuals.

SUMMARY

From all the results obtained, it would appear that beyond a doubt the great majority of those streptococci found in swimming pools have the nose and mouth as their source. Following from this it would seem that the present use of streptococci as a confirmatory test for intestinal pollution is of little or no value. This would not mean, however, that the test should be given up, but rather that a different interpretation should be placed upon it. It has been demonstrated time and again that certain respiratory diseases caused by pyogenic cocci may be transmitted through swimming pool water. Although no hemolytic streptococci were found in the pools examined, it was probably due in large measure

to some defect in the method of isolation. At the present time there seems to be no practical method in use for controlling these streptococci in swimming pools. Hallmann, in an investigation of the bacterial pollution of swimming pools, has drawn up the following procedure and standards for streptococci in swimming pools.

"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 bathing water very undesirable. Yet to eliminate them from swimming pools would mean decidedly smaller bathing loads and decided increases in chlorine residuals, either or both of which would hamper the usefulness of the pool. The committee calls attention to the fact that streptococci tests are of value in passing on the condition of the swimming pool water. A tentative procedure and standard follows:

"1. Test for the presence of streptococci may be made from the fermentation tubes used for the determination of the colon-aerogenes group.

"2. Decant off the supernatant liquid in the fermentation tubes after 48 hours incubation at 37°C. Smear the sediment on glass slides, stain and examine for the presence of streptococci.

"3. Standards. Not more than 5 out of 10 consecutive samples taken on different dates should be positive for streptococci. The average streptococci index should not be greater than 2 per 100 c.c. of water for any considerable period of time."

CONCLUSIONS

Streptococci are not plentiful on the body surfaces except where contamination from the mouth, nose and fecal material has taken place.

Streptococci are found in large numbers in the nose and mouth and in fecal material.

The streptococcus index roughly parallels the colon index only when the bodies and not the mouths of the bathers have been in contact with the water.

There is no relationship between the streptococcus or colon indices and the total bacterial count.

Fermentation reactions are of no value in determining the source of streptococci found in swimming pools.

Streptococci, as found in swimming pools, are not indicators of the amount of intestinal pollution.

Streptococci indicate the amount of pollution which has taken place from the noses and mouths of the bathers and are therefore important in determining the safety of the water in regard to transmission of sinus and respiratory diseases.

It is therefore recommended that a streptococci test be made to measure the safety of a pool as far as respiratory diseases are concerned.

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