THE SUSCEPTIBILITY OF STREPTOCOCCI TO CHLORINE

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THE SUSCEPTIBILITY OF STREPTOCOCCI TO CHLORINE

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Introduction:

The popularity of swimming has increased enourmously in the past decade; the result being the construction of a large number of indoor and outdoor pools. Although methods of design and means of disinfection have been greatly improved, methods of examination for hygienic conditions have always been unsatisfactory. The methods of examination of drinking water have been used although the pollution is not analogous to that of drinking water. In recent years the American Public Health Association (1) method of water analysis, with the name requirements for purity, has been used. Such a Standard would appear to be very strict and judged from the quality of drinking water, it should be a very satisfactory standard.

Escherichia coli is used as an idex organism in A.P.H.A. procedure. This organism indicates fecal or intestinal pollution which in drinking water is important. Recently, there has been a good deal of discussion on the reliability of the Esch. coli index when applied to swimming pool water. Some of the first work along this line was done by Mallmann (2) in 1925 when he pointed out that Esch. coli is not a reliable index of swimming pool pollution, and that streptococci indicate an unsafe condition. This brings up a group of questions: Are streptococci generally found in pools? What is the relationship between the amount of enteric diseases and the respiratory diseases? Are the streptococci resistant to the amounts of chlorine used in pool disinfection?

Pools contain, beside the initial pollution contained in the tap water, pollution from the nose and throat discharges and body washings. Such material would be very rich in cocous forms. Thus, the constant douching of the mouth and nasal passages while swimming, along with forms from the intestines, would account for the presence of streptococci in the pools. Streptococci were isolated from pools in 1925 by Hallmann (2). Recently Harwood (3) isolated beta strepococci from swimming pools in Boston. Hallmann and the writer (4) isolated alpha and gamma streptococci from pools within the last year.

The relationship between enteric diseases and respiratory diseases has been studied by many workers. Hamheimer (5) cites examples of both of these types of diseases as reported by the following: Reice reports 54 cases of enteric fever among the soldiers who used a swimming pool which contained some polluted water, Jager reported intestinal protozoa infection among soldiers who bathed in the Danube river, Pfuhl attributes 49 cases of typhoid fever to bathing in the Elbe, Shiga reports 413 cases of dysentery from bathing in a river, Fehr reports 20 cases of eye infection among patrons of a certain swimming pool, Schultz reports 18 cases of trachoma in persons using a pool, Skutch reports an epidemic of gonorrheal vulvovaginitis which spread to 276 girls using a swimming pool in Posen. Forbes (6) cites H. F. Ogden as a source for the following; W. L. Lewis reports influenza, colds, sore throat, and occasionally pneumonia restricted to users of the swimming pool at Northwestern University, Barker reports nose and ear infections among members of the swimming team at Brown University, Oberbach reports an outbreak of conjunctivities and catarrhal otitis among the members of a swimming club. Forbes himself studied first hand

a fatal case of meningitis contracted in a swimming pool. Hasty (7) finds that the pool organisms are directly the cause of paranasal sinus infections. The above references show that infections of the eye, ear, nose, and throat generally caused by pyogenic cocci are more liable to be transmitted by swimming pools than those of an intestinal nature. The cases of intestinal diseases cited above are due to swimming in rivers or pools where untreated river water was used.

Since the most popular germicide used in the disinfection of pools is chlorine, the susceptibility of streptococci to chlorine becomes a very important subject. Wallmann and Selpi (8) in 1930 suggested that a residual chlorine range of 0.2 - 0.5 p.p.m. should be maintained to insure safety. This paper is a bacteriological study of six swimming pools to determine the incidence of Mach. coli, streptococci and total count in relation to bathing loads and residual chlorine contents. Streptococci isolated from these pools together with streptococci obtained directly from the throats of infected individuals, were used to determine the susceptibility of streptococci to chlorine under laboratory conditions.

Experimental:

Of prime importance in the examination of swimming pools is the use of the thiosulphate bottle as recommended by Wallmann and Cary (9). The introduction of such an aid into laboratory procedure allows a very close approach to the pool side conditions even though the sample is transported many miles. The thiosulphate bottle is prepared by adding a crystal of sodium thiosulphate to an ordinary sample bottle prior to sterilization by moist heat. Thus, when the sample is placed in the bottle any chlorine which may be present is neutralized and its germicidal action is destroyed. This keeps the sample from being sterilized while in transit. Until this procedure was demonstrated by Wallmann and Cary, swimming pools all had clean bills of health because the pool sample was sterilized by the chlorine while being transported to the laboratory.

The experimental work which follows is divided into two parts, the practical examination of six college and secondary school pools and the laboratory demonstration of the susceptibility to chlorine of alpha, beta, and gamma streptococci of both pool and stock cultures.

The six pools examined are in the neighborhood of Lansing, Michigan and within short driving distance from the laboratory. A series of samples using the thiosulphate bottles, was collected from these pools and a complete A. P. H. A. water analysis made of each sample. Along with the regular colon bacillus examination, the streptococcus test as used by Wallmann and Delpi was also applied. Material from the tubes which showed a typical veily growth clinging to the insert tube and sides of the Durham fermentation tube were smeared upon slides, stained and examined microscopically and the presence of

streptococci noted.

The laboratory demonstration of the susceptibility of streptococci was carried on using culture of streptococci which were isolated from swimming pools and human throats along with stock cultures, one of which was the Dochez strain of scarlet fever. The organisms were of all three types -- alpha, beta, and gamma. The procedure of this laboratory work follows:

Five one liter flasks containing 500 c.c. each of college tap water were sterilized by autoclaving. (Tapwater was used in order to approximate pool conditions.) One of the flasks was used as a control and to each of the others was added a definite amount of chlorine. The amounts used in this experiment were 0.45, 0.52, 0.75, and 0.90 p.p.m. Then a suspension of the organisms was added in such quantity so as to give a count of 100,000 to 300,000 organisms per c.c. in the flask. At definite time periods, namely, 15, 30, 45, 60, 90, 120, and 180 seconds, transplants of 1 c.c. were made into 9 c.c. of veal protoose peptone broth (0.3% dextrose). These later were transferred serially to give dilutions of 1/100 and 1/1000. These tubes were incubated for 24 hours at 37°C and the growth recorded. The number of organisms per c.c. in the flask was determined by means of the Helber counting cell. The results are shown in tables 1 to 5. As a control, and check Esch. coli was used in this experiment in place of streptococci.

Technique:

The streptococcus test was run along with the regular water examinations for Esch. coli. The method consists of picking out the suspected tubes and staining the material. The suspicious tubes were chosen by the presence of a veily growth which clings to the sides of the tube and insert. Tubes showing this growth invariably gave a positive test. The material was concentrated by removing most of the liquor by suction. The sediment which remained was then smeared on slides and stained. These slides were then examined microscopically for streptococci and the results recorded.

The Esch. coli is the test organism used in the A. P. H. A. test. The pool water is planted in tubes of lactose broth and also plated in nutrient agar. At 24 hours the quantity of gas was recorded and a plate count made. The tubes showing 10 percent gas were smeared on eosin - methylene-blue agar and incubated 48 hours. At the end of 48 hours the data were again recorded. All tubes now showing gas were smeared on eosin - methylene-blue plates. Typical Esch. coli colonies are picked from the eosin - methylene - blue plates and planted in lactose broth to further confirm the presence of Esch. coli The same tubes are used for streptococci.

The sampling of the pools, as has been stated, is done with the aid of the thiosulphate sample bottle; two samples being taken from each pool. These samples were taken at the same time but from opposite sides of the pool. Samples were collected at times when the pool was in use.

The residual chlorine content of the pools was determined by means of a Hellige apparatus which used the colorimetric determination with

ortho tolidine. The chlorine content of the pools ranged from 0.00 to 2.00 p.p.m. of chlorine. Since the pools used the same source of water continually the pH effect upon the germicidal action of the chlorine is omitted. A strict comparative basis was maintained in all pools. The same method of determining chlorine residual was used in the laboratory experiment. The source of chlorine for the experiment was B. K., a hypochlorite solution.

The factor of pool loads could not be controlled because the pools used were in public schools and so the number in the pool while the sample was taken, was recorded. This factor is important in determining the amount of chlorine needed to sterilize a pool. The number of persons using these pools varied from 0 to 48 at the time of sampling. The effect of bathing load upon the chlorine residual and the amount of pool pollution is shown in graphs 2 to 7.

The culture used came from four sources, swimming pools, human throats, animals, and stock collections. The pool cultures were alpha, and gamma streptococci; the human cultures were alpha and beta streptococci; the animal culture was a bovine gamma streptococci; and the stock cultures were alpha, beta, and gamma streptococci. The beta streptococci of this latter group was the Dochez scarlet fever strain. The pool cultures were isolated by the writer.

Organism			15	30	45	60	90	120	180
6183	∫	p	3	3	3	3	3	3	3
301	β	s	3	3	3	3	3	3	3
lg	∫	s	3	3	3	3	3	3	3
6184	∫	p	3	3	3	3	3	3	3
17-2	α	p	3	3	3	3	3	3	3
2119	α	s	3	3	3	3	3	3	3
72v2	α	p	3	3	3	3	3	3	3
Cobb	β	h	3	3	3	3	3	3	3
Mervin	β	h	3	3	3	3	3	3	3
Ada	∫	a	3	3	3	3	3	3	3
61v3	α	p	3	3	3	3	3	3	2
20-3	α	s	1	0	0	0	0	0	0
Erma	α	h	0	0	0	0	0	0	0

1 - 10 -- 1
1 - 100 -- 2
1 - 1000 -- 3

Symbols
p - pool
h - human
s - stock
a - bovine

			0.45 p.p.m Chlorine						
			15	30	45	60	90	120	180
6184	Γ	p	3	3	3	3	3	3	3
6183	Γ	p	3	3	3	3	3	3	3
201	β	s	3	3	3	3	3	3	3
13-2	α	p	3	3	3	3	3	3	2
2119	α	s	3	3	3	3	3	2	1
1a	Γ	s	3	3	3	3	2	0	0
61v3	α	p	3	3	3	2	2	2	1
Ada	Γ	a	3	2	2	2	1	1	0
Cobb	β	h	3	2	2	1	1	1	1
Kervin	β	h	2	1	0	0	0	0	0
72w2	α	p	2	1	0	0	0	0	0
20-3	α	s	1	0	0	0	0	0	0
Ermy	α	h	0	0	0	0	0	0	0

.52 p.p.m

Organism			15	30	45	60	90	120	180
61G3	Γ	p	3	3	3	3	3	3	2
201	β	s	3	3	3	3	2	2	1
2119	γ	s	3	3	3	3	2	1	1
13-2	α	p	3	3	3	3	2	1	0
61G4	Γ	p	3	3	3	3	1	1	1
1c	Γ	s	3	3	3	1	1	0	0
61v3	α	p	2	2	2	1	1	1	1
Cobb	β	h	3	2	2	0	0	0	0
Ada	Γ	a	3	2	1	1	1	1	0
72v2	α	p	1	1	0	0	0	0	0
Herwin	β	h	2	0	0	0	0	0	0
20-3	γ	s	1	0	0	0	0	0	0
Ermn	α	h	0	0	0	0	0	0	0

.75 p.p.m.

Organism			15	30	45	60	90	120	180
61G3	Γ	p	3	3	3	3	2	1	0
1g	Γ	s	3	3	3	2	0	0	0
201	β	s	3	3	2	1	0	0	0
13-2	α	r	3	3	2	1	0	0	0
2119	α	s	3	3	2	0	0	0	0
61G4	Γ	p	3	3	0	0	0	0	0
61v3	α	p	1	1	1	1	1	0	0
Ada	Γ	a	1	1	1	1	0	0	0
Cobb	β	h	3	0	0	0	0	0	0
73v3	α	p	1	0	0	0	0	0	0
Kervin	β	h	0	0	0	0	0	0	0
20-3	α	s	0	0	0	0	0	0	0
Ernu	α	h	0	0	0	0	0	0	0

.90 p.p.m

Organism			15	30	45	60	90	120	180
61G3	⌐	p	3	3	3	2	1	0	0
201	β	s	3	2	2	0	0	0	0
1g	⌐	s	3	2	1	0	0	0	0
61v3	α	p	1	1	1	1	1	1	1
13-2	α	p	3	0	0	0	0	0	0
2119	∧	s	3	0	0	0	0	0	0
72v2	∧	p	3	0	0	0	0	0	0
Cobb	β	h	1	0	0	0	0	0	0
61G4	⌐	p	1	0	0	0	0	0	0
Kervin	β	h	1	0	0	0	0	0	0
Ada	⌐	a	1	0	0	0	0	0	0
80-3	α	s	0	0	0	0	0	0	0
Ernu	α	h	0	0	0	0	0	0	0

Discussion:

The numbers, 1 to 3, as used in the data blocks indicate the dilution in which growth took place. In this case these numbers are coincident with the log number of the dilutions used. This method of recording the data was used to avoid a more unweildy system as used in the original recording of the data.

The data from the laboratory experiment show quite clearly the range of susceptibility of streptococci to chlorine. One of the organisms, a human gamma streptococcus being killed by the tap water itself, and the other extreme, a gamma streptococcus isolated from a swimming pool which grew in a 1/10 dilution after 90 seconds exposure to 0.90 p.p.m. of chlorine. Between these extremes the remaining stock, pool, human, and bovine cultures are arranged according to their decreasing resistance to chlorine. In 0.90 p.p.m. available chlorine the two most resistant organisms are a pool gamma streptococcus and a stock beta streptococcus, the latter being the Dochez scarlet fever streptococcus. Following these are a stock gamma streptococcus and an alpha streptococcus isolated from a pool. Since the concentration of chlorine in swimming pools is generally nearer 0.45 p.p.m., an analysis of the most resistant organisms of this group follows at this concentration. The four least susceptible organisms are in descending order - 2 pool gamma streptococci, a stock beta streptococcus, and a pool alpha streptcoccus. The susceptibility of these organisms to chlorine is comparable since they grew out in 1/1000 and 1/100 dilution after three minutes exposure to 0.45 p.p.m. of chlorine. The resistance of these organisms to so great a concentration of chlorine is a good argument against the chances of pool

sterilization by lesser amounts of chlorine. Although board of health pool reports previous to the use of the tiosulphate sample bottle show complete sterility in pools wherein the chlorine residual was less than 0.2 p.p.m. and at the same time the pools were overloaded with bathers. Also since streptococci such as the Dochez scarlet organism are quite resistant to rather large quantities of chlorine, it is not at all improbable that infectious diseases especially those of the respiratory tract may be contracted in pools. The data from the pool samples were recorded and graphed according to schools. The graphs show the incidence of streptococci, Esch. coli, total count, chlorine residual, and pool load by days for each sample. The relationship between the presence of streptococci, Esch. coli, and total count and the chlorine residual is very marked. Every rise in residual chlorine being accompanied by a drop in the amount of organisms. This is to be expected, because of the germicidal action of the chlorine.

The swimming pool data as graphed illustrate very plainly the following points: (a) the need of a streptococci index rather than a Esch. coli index for standard swimming pool analysis, (b) the greater implied resistance to chlorine of streptococci as compared with Esch. coli, (c) the relation between the chlorine residual and the streptocci, and Esch. coli indices, and the total count, (d) and the effect of pool load upon the pollution in the presence of chlorine. Explanation of these point follows:

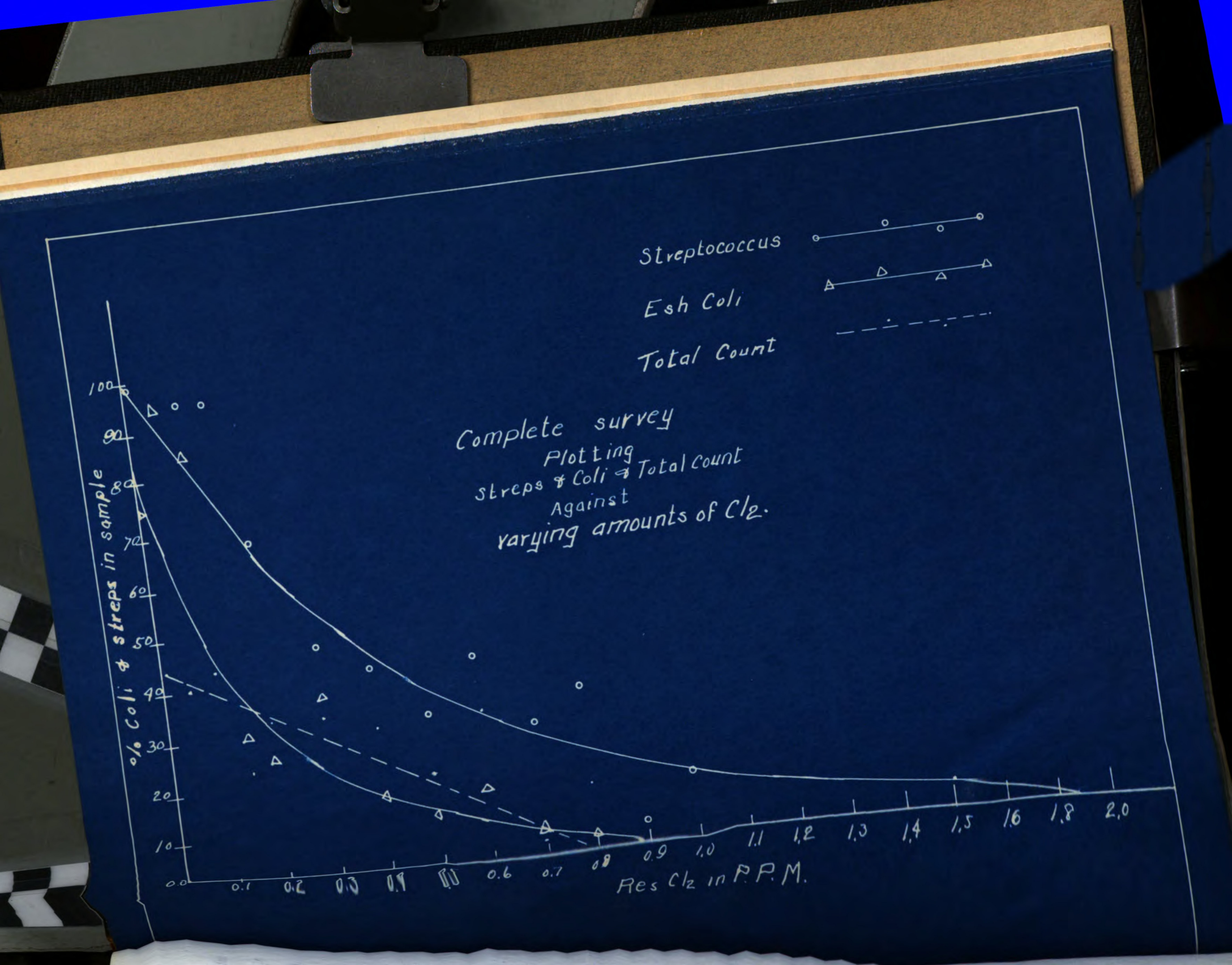
(a). The streptococci index is exceeded but once by the Esch. coli index in the entire experiment. This occurs in pool A (graph 2) upon the fourth day. Thus, this would be the one day when the A.P.H.A.

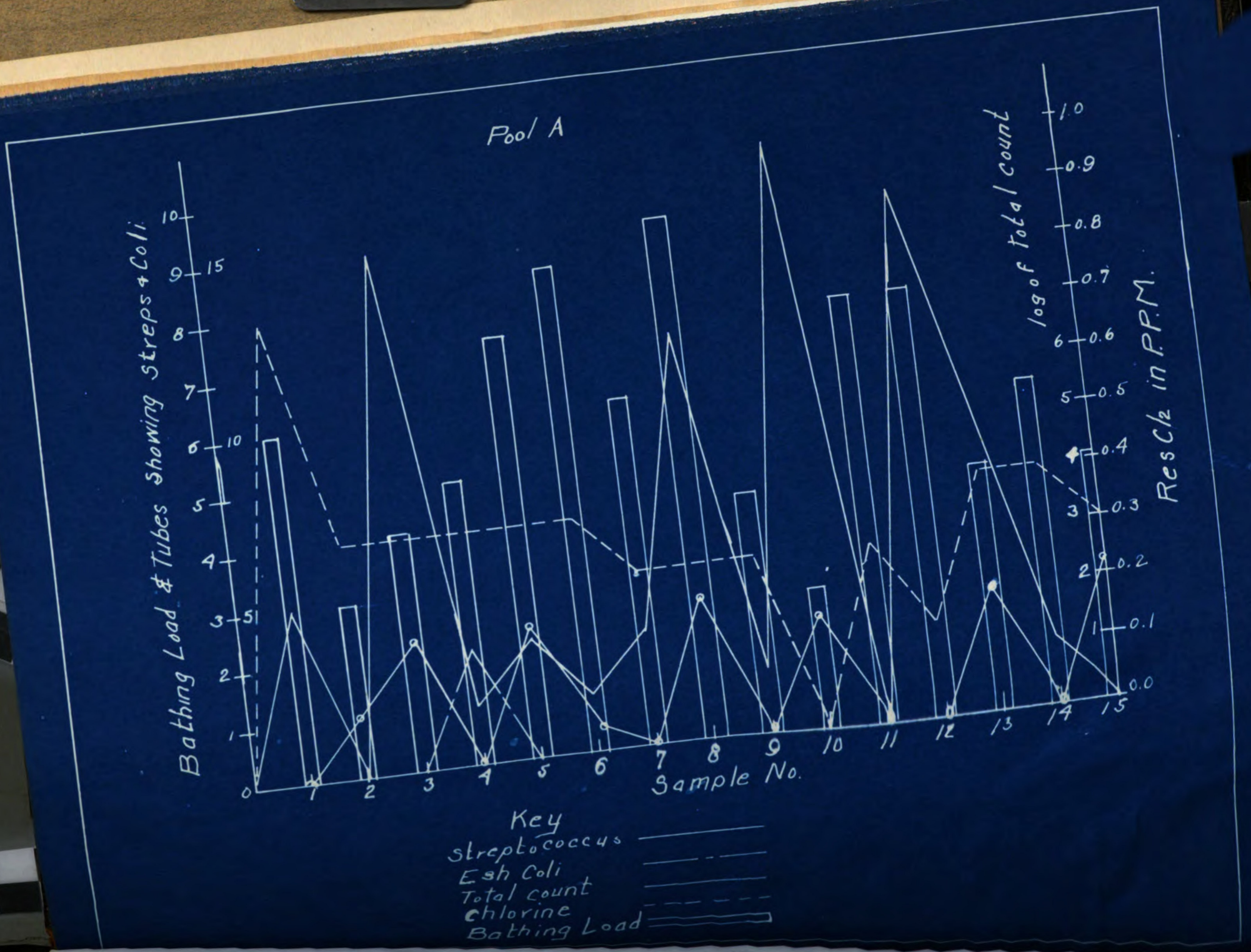
method would be a true index of pollution, and the remainder of the days which show an excess streptococci pollution would be unnoticed. The streptococci pollution would probably not be shown by the plates of the A.P.H.A. method since enrichment materials are necessary to grow streptococci on solid media.

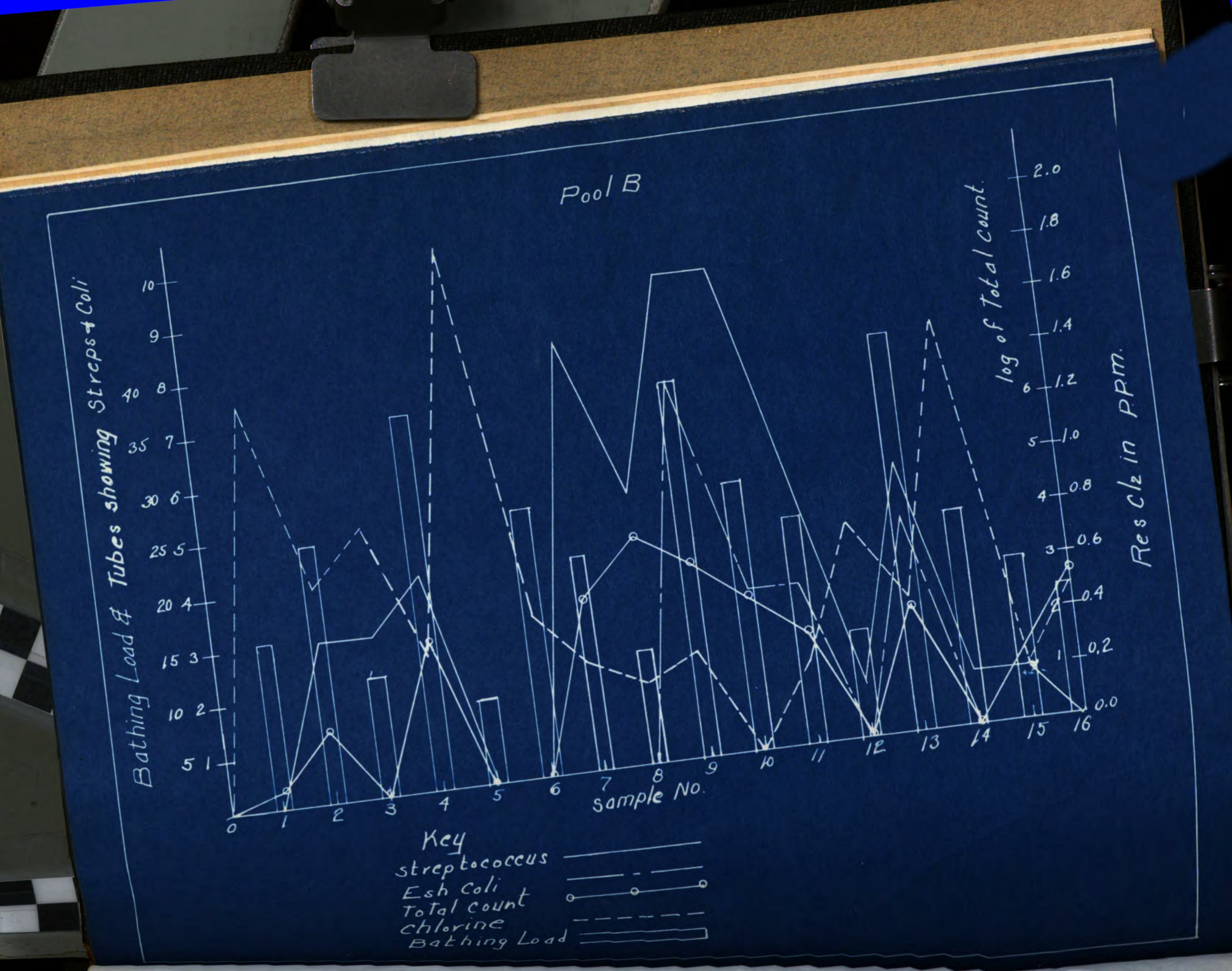
(b). Since the streptococci index is greater than the Esch. coli index in all but one sample, and since all of these samples are taken from the same pools, it is implied that the streptococci are more resistant to chlorine than Esch. coli.

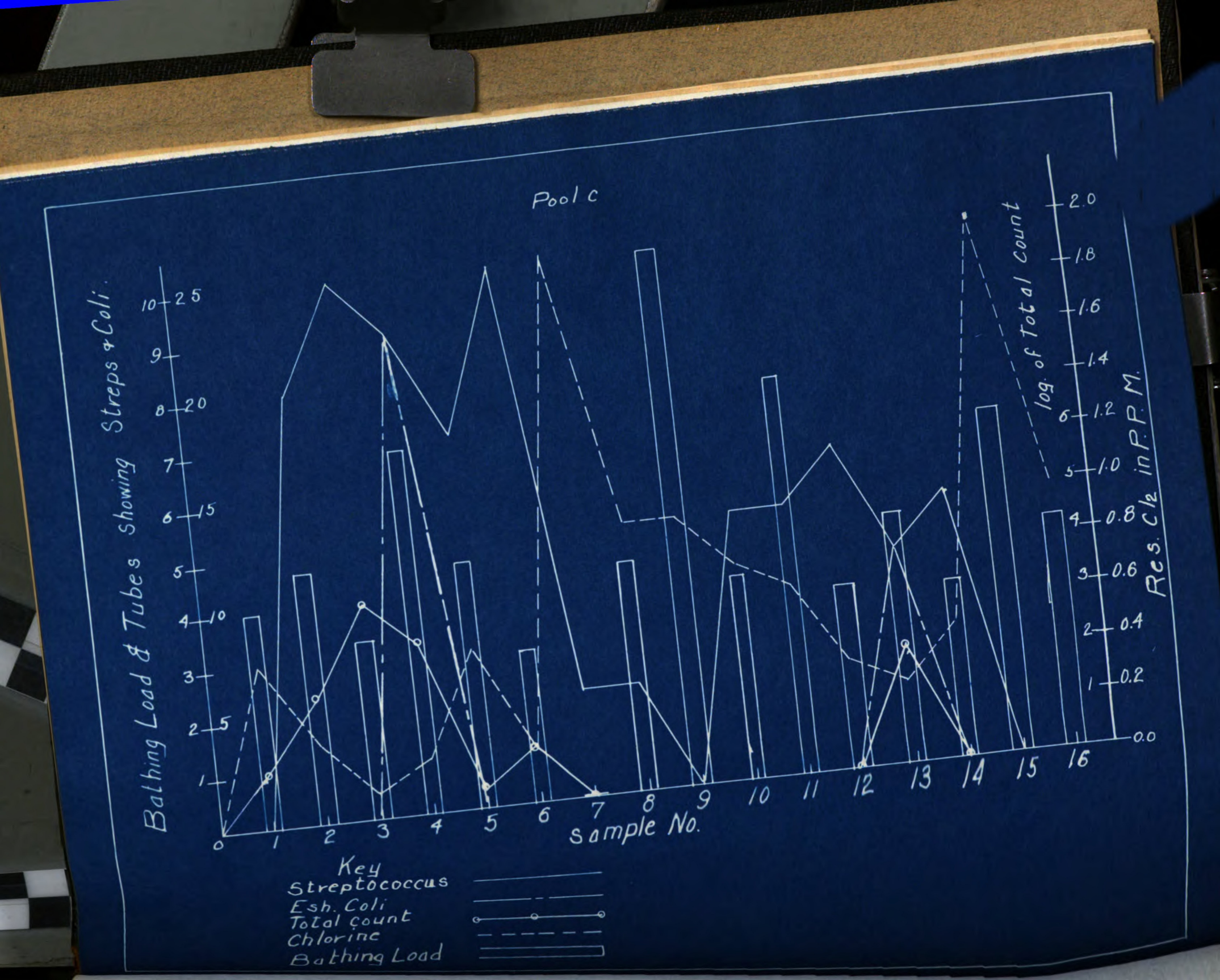
(c) Graph 1 shows the relationship among the per centum quantities of Esch. coli, streptococci, and the total count. The advantage of the streptococci as an index organism is shown by the broad range of its growth in chlorinated pools. The streptococci grow out in chlorine residuals of 0.0 to 2.0+ p.p.m, while the Esch. coli grows out in chlorine residuals of 0.0 to 0.9 p.p.m. The total count, according to the graph, coincides more nearly with the Esch. coli curve than with that of the streptococci, however, the rise above the Esch. coli curve may be due to the effect of the streptococci count. This is highly improbable since the solid media used was a plain nutrient agar.

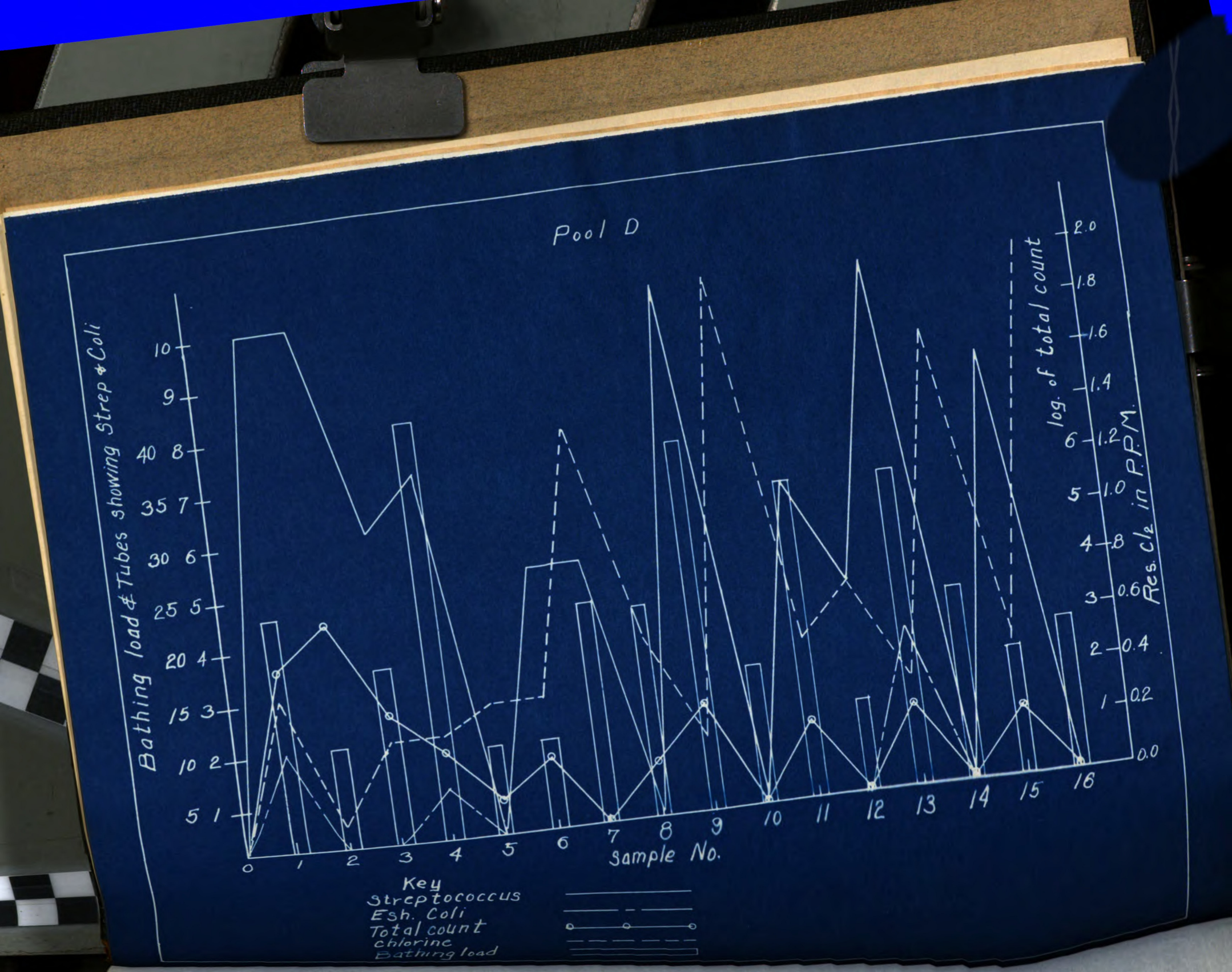
(d) The effect of the pool load upon pollution may be noticed in many places on the graphs. The best illustration of this effect is pool F (graph 7). The chlorine residual remains practically constant, yet when the pool load is high the streptococci pollution increases. The chlorine content of this pool was at no time below 0.4 p.p.m.

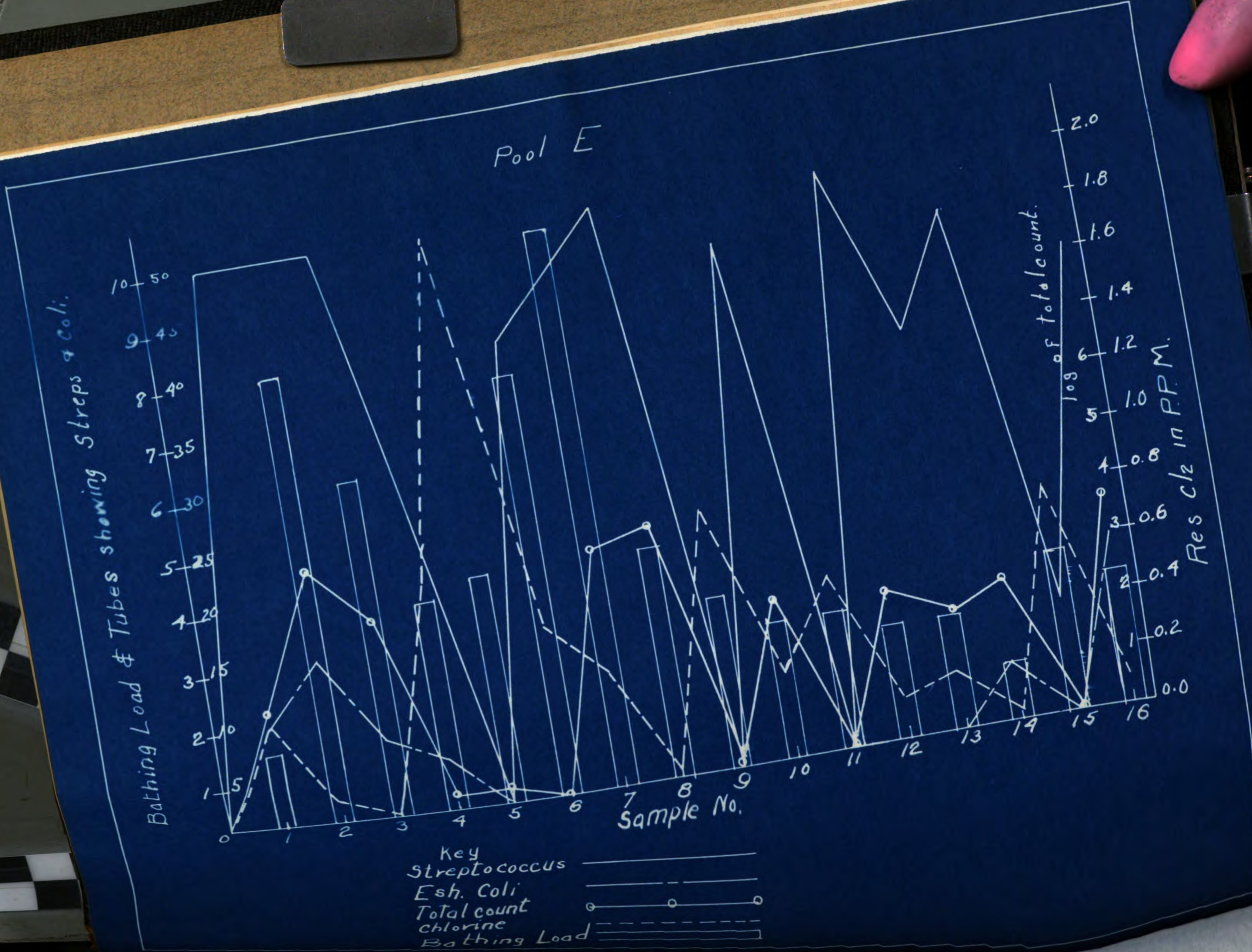

Streptococcus
Esh Coli
Total Count
Complete survey
Plotting
Streps & Coli & Total Count
Against
varying amounts of Cl2.
% Coli & streps in sample
100
90
80
70
60
50
40
30
20
10
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.8
2.0
Res Cl2 in P.P.M.

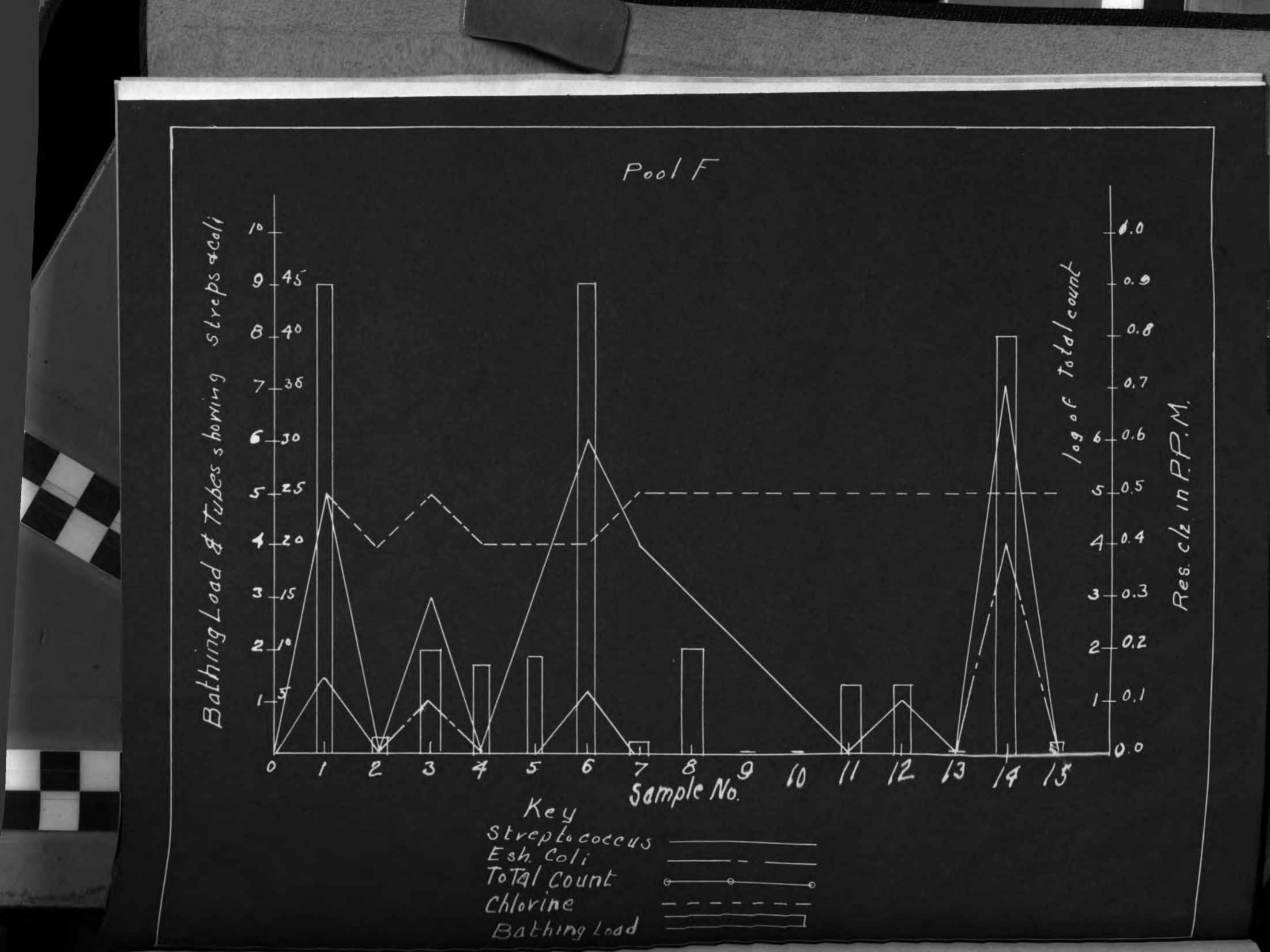

Pool A
Bathing Load & Tubes Showing Strepts + Coli
log of total count
Res Cl2 in P.P.M.
Sample No.
Key
Streptococcus
Esh Coli
Total count
Chlorine
Bathing Load


Pool B
Bathing Load & Tubes showing Strepts + Coli
log of Total count.
Res Cl2 in PPM.
Sample No.
Key
streptococcus
Esh Coli
Total count
Chlorine
Bathing Load


Pool C
Bathing Load & Tubes showing Streps & Coli.
log. of total count
Res. Cl2 in P.P.M.
Sample No.
Key
Streptococcus
Esh. Coli
Total count
Chlorine
Bathing Load


Pool D
Bathing load & Tubes showing Strep & Coli
log. of total count
Res. Cl2 in P.P.M.
Sample No.
Key
Streptococcus
Esh. Coli
Total count
Chlorine
Bathing load


Pool E
Bathing Load & Tubes showing Streps & Coli.
log of total count.
Res Cl2 in P.P.M.
Sample No.
Key
Streptococcus
Esh. Coli
Total count
chlorine
Bathing Load


Pool F
Bathing Load & Tubes showing strepts & coli
log of Total count
Res. Cl2 in P.P.M.
Sample No.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
1 5
2 10
3 15
4 20
5 25
6 30
7 35
8 40
9 45
10
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
Key
Streptococcus
Esh. Coli
Total Count
Chlorine
Bathing Load

Conclusions:

1. The total count and Esch. coli index does not parallel the amount of pool pollution, as accurately as the streptococci index.
2. Streptococci were isolated in pools containing 0.0 to 2.0+ p.p.m. chlorine residual.
3. Freshly isolated streptococci from the animal body were equally as resistant to chlorine as those freshly isolated from pools.
4. As judged by laboratory experiments, disease producing streptococci could survive in swimming pools for a period of time sufficient to cause transmission of disease.
5. In laboratory experiments there is no appreciable difference in the susceptibility to chlorine of alpha, beta, and gamma streptococci.
6. The use of streptococci as an index of swimming pool pollution is recommended.

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