A Study of Bacterial Flora in Swimming Pool Water Treated with High-Free Residual Chlorine

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High-free chlorine residuals in swimming pool water have proved so satisfactory in maintaining the sanitary quality of the water that less emphasis will need to be placed on bacterial standards if accurate titrations of the residuals are routinely carried out, say the reporters of this research.

Standards of swimming pool operation have been developed to prevent the spread of communicable diseases and to insure the safety and well-being of persons frequenting swimming pools. Of principal importance are the standards related to the bacterial quality of the water and the procedures for purification. Because of its simplicity of application, its economy of use, and its continuing bactericidal action through the maintenance of residual levels, chlorine has been and is being used extensively to control the quality of swimming pool water. Many studies have been conducted to measure the effectiveness of chlorination of swimming pool water under varying conditions and to develop improved methods of application, and much consideration has been given to the amount and type of chlorine residual of the swimming pool water that should be maintained and the choice of organism or group of organisms that should be used as an indication of pollution.[1-7]

The Joint Committee on Bathing Places of the American Public Health Association and the Conference of State Sanitary Engineers in its 1949 report [1] recommended that the amount of available or excess chlorine in the water, at all times when the pool was in use, should not be less than 0.4 ppm or greater than 0.6 ppm. In 1952 this standard was modified by a statement that data were available to indicate that for most effective results these values should be present as free available chlorine.[2] The 1956 report of this committee proposes the acceptance of high-free residual chlorination as the maintenance of relatively high concentrations of free available chlorine (1.0 ppm and above) with accompanying high alkalinities (usually pH 8.0–8.9). High-free residual chlorination, while being relatively new in swimming pool water treatment in the United States, has been used in Great Britain for some years with excellent results [8-10] and has been accepted as a standard recommended procedure in The Purification of the Water of Swimming Baths [7] but under the title of breakpoint chlorination.

To measure the effectiveness of swim-

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ming pool water treatment procedures and the relative freedom of the water from potential disease producing organisms, bacteriologic examinations for the presence of indicator organisms of pollution are recommended. Recent Joint Committee on Bathing Places reports recommend that "all chemical and bacterial analyses should be made in accordance with the procedures recommended in the Standard Methods for the Examination of Water, Sewage, and Industrial Wastes of the American Public Health Association in so far as these methods are applicable to swimming pool waters." [1-3, 11] This statement gives rise to questioning the meaning of the bacteriologic methods that "are applicable to swimming pool waters." Basically, Standard Methods for the Examination of Water, Sewage, and Industrial Wastes [11] uses the coliform index to measure the presence of bacterial pollution. Mallmann,[5] France and Fuller,[6] Ritter and Treace,[12] Mood,[13] and others have investigated the use of streptococci as an index of pollution of swimming pool water with differing results and conclusions.

As early as 1934 Winslow [14] made a strong plea to those using standard methods for the examination of water samples to remember that there was a great divergence of opinion with regard to levels of standards, that the tests were devised by mere human beings like ourselves, and that basically what was of the greatest interest was merely a test—any test which would show the greatest possible difference between good and bad waters. During the following years the path of research in this field has not deviated widely from that which had been criticized by Winslow. Gilcreas [15] in discussing the 10th edition of Standard Methods for the Examination of Water, Sewage, and Industrial Wastes pointed out that the foundation for the bacteriologic examination of water, namely the detection and enumeration of intestinal bacteria, had not changed since the first edition was published. He specifically commented on the inadequacy of the coliform group of bacteria as an index of pollution in swimming pool waters because the most significant bacterial contamination of such water was not of intestinal origin. He further pointed out that the available data on the presence of streptococci in swimming pool waters was insufficient to permit the inclusion of any method for their detection in Standard Methods. This he believed was due to the lack of knowledge concerning the quantitative significance of this group of organisms in such water.

No changes have been made in the reports of the Joint Committee on Bathing Places since 1949 concerning the bacterial quality of swimming pool waters and now with the trend toward the maintenance of higher chlorine residuals reevaluation of this problem seems essential. The stage was set for this reappraisal when in 1950 the Smith College swimming pool was converted from marginal to high-free residual chlorination as a means of correcting some operational problems. It is believed that this was the first intentional use of high-free residual chlorination of swimming pool water in the United States. Since September, 1950, this pool has been operated continuously by maintaining the free residual chlorine concentrations well above 1.0 ppm. The clarity and color have been markedly improved and the density of bacterial flora reduced drastically. The greatest improvement, however, came in the reactions of the swimmers who no longer complained of eye irritation and bothersome chlorinous odors.[16-18]

Preliminary bacteriologic studies of swimming pool water treated with high-free residual chlorination indicated the complete inadequacies of the presently accepted standardized procedures, for repeated samples taken under any con-

Table 1—Operational Data on Swimming Pools Studied

Pool	Type	Capacity in Gallons	Turnover Ratio per 24 Hours	Type of Chlorination	Range of Hydrogen-ion Concentrations (pH)	Range of Chlorine Residuals (ppm)	
						Free	Total
Smith	College indoor	81,000	3	Continuous hypochlorite	8.0–8.2	1.39–3.58	2.36–4.80
Colt Park	Public outdoor	231,000	2	Continuous gas	8.0–8.4	0.24–2.32	0.52–2.68
A	College indoor	177,500	3	Continuous gas	7.6–7.7	0.13–0.39	0.31–0.58
B	Club indoor	28,500	2	Intermittent hypochlorite	8.2–8.3	0.00–0.08	0.00–0.55

dition of pool operation yielded negative results.* A preliminary investigation demonstrated the very low density of bacteria of any type, and particularly of body origin that might survive high-free residual chlorination. By using highly selective media and testing with large quantities of sample it was possible under certain conditions to isolate strains of Micrococcus and Streptococcus, but it was evident that they were not surviving in any appreciable numbers.

Additional studies were conducted but little or no progress was made in studying the types and numbers of bacteria surviving high-free residual chlorination until the membrane filter technic became available for general laboratory use.[23, 24] Exploratory studies seemed to indicate that here was a simple procedure for both qualitative and quantitative bacterial studies of waters having high-free chlorine residuals. Rather than attempting to develop a new medium or to improve one in current use for the isolation of potential pathogens, it was decided to use the membrane filter to make direct bacterial counts of those organisms that survived the treatment of high-free residual chlorination, and to isolate and classify as many of them as was possible. No special circumstances of pool operation were provided and the results reported here are representative of findings on typical days of routine use of swimming pools.

Data from four swimming pools have been included in this report for comparative purposes. The first is at Smith College and the second an outdoor one at Colt Park in Hartford, Conn., which was converted to high-free chlorination in 1952. Preliminary results of bacterial data from these two pools have previously been reported.[19] A third designated as pool A is a representative of the conventional type of indoor college pools maintaining marginal chlorination. The fourth, designated as pool B, is an indoor type operated by a club and is representative of pools treated with intermittent hand applications of hypochlorites, with resultant low values of marginal residual chlorine. Pertinent data, including the pH and the minimum and maximum residual chlorine values obtained during the testing periods on these four pools, are summarized in Table 1.

All samples for bacteriologic exam-

* Unpublished results obtained in the Smith College Bacteriology Laboratory.

ination were collected and tested within a 30-minute period according to recommended procedures of Standard Methods for the Examination of Water, Sewage, and Industrial Wastes [12] for the total plate counts and coliform determinations. Membrane filter studies were carried out in principle according to the tentative procedures of Standard Methods—the amount of sample filtered varied with the residual chlorine level of the water at the time of collection and three media were used, two of which were selective in their action. These were Endo broth * and Slanetz' medium for the isolation of enterococci.[25] The third medium, enrichment broth,* was chosen after preliminary laboratory bacterial studies using pure cultures had shown that typical body organisms, such as various cocci and coliforms grew readily and consistently upon it following filtration through a membrane filter. All samples were incubated at 35° C and were read after 20 hours of incubation using a binocular wide-field dissecting microscope having a magnification of 10 diameters. Total colony counts were made and, when possible, all colonies or representative types of colonies were transferred to trypticase soy agar slants * and, following an 18-hour incubation period at 35° C were stored in a refrigerator at 4° C until Gram stains and biochemical studies for identification could be made.

At the time the samples were collected for bacteriologic examination, separate samples were obtained for immediate pH and residual chlorine determinations. All pH determinations were carried out potentiometrically using the glass electrode method. All residual chlorine values were determined by amperometric titrations using the method of Marks, Williams, and Glasgow.[23]

Standard agar plate counts and coliform determinations were done on all samples collected except on those from Colt Park pool. Only pool B failed to comply with the standards for bacterial densities and the confirmed test for coliform bacteria as set forth in the Joint Committee Reports on Bathing Places.[3] It should be stated that only once during the period of testing was any free available residual chlorine found in this pool and that but 0.08 ppm.

To determine the presence or absence of bacteria of the coliform group by the membrane filter method 200 ml samples were taken from the Smith College pool and pool A, and 100 ml samples from Colt Park pool and pool B. All samples were filtered through membrane filters which were then incubated on pads saturated with Endo broth. No typical coliform colonies as determined by the presence of metallic sheen were observed from any samples except those obtained from pool B. These colonies all proved to be Escherichia coli. Atypical colonies, i.e., those developing no sheen, developed on one sample taken from Colt Park pool and all were identified as yeast organisms. Seven differing types of atypical colonies appeared from samples obtained from pool B, three of which on typing proved to be of the coliform group of organisms. Thus, it appears that not all members of the coliform group of organisms that may be found to have been present in swimming pool waters will produce the typical metallic sheen that is characteristic of this group of bacteria when Endo broth is used. However, all sheen-producing colonies found in this study proved to be E. coli. The results obtained on the samples from the Smith College pool, the Colt Park pool, and pool A indicate that the coliform densities of these swimming pool waters are either extremely low or nonexistent.

An attempt to determine the presence on the membrane filter of enterococcal bacteria in these swimming pool waters by the use of a selective medium was

* From the Baltimore Biological Laboratory, Baltimore, Md.

carried out on all samples except those from Colt Park pool. Samples for enterococcal determination were filtered in 100 ml amounts and Slanetz' modification of the Chapman mitis-salivarius medium was used. Positive results were found only from seven samples of pool B and these were obtained in water that had swimmers in it at the time the samples were collected. Typical enterococcal colonies were isolated from these samples and both Streptococcus fecalis and Streptococcus salivarius were identified. No evidence was obtained which would indicate that streptococcal bacteria could survive in waters which have free chlorine residuals.

The use of enrichment broth proved to be most rewarding of all the membrane filter work. The flora of the Smith College pool water is made up primarily of members of the genus Bacillus as 78.7 per cent of the colonies developing proved to be members of this group, whereas 13.6 per cent were species of the Micrococcus and 7.7 per cent were identified as either actinomycetes or molds. When swimmers were in the pool the Micrococcus flora increased and represented 26.9 per cent of the total isolated. These results are in direct contrast to those obtained from the samples taken from pool A where marginal chlorination is carried out. Here (Table 2) the normal flora as determined in this study was made up of 10.2 per cent Bacillus strains and 85.4 Micrococcus strains. Samples obtained while swimmers were in this pool showed that of the total bacterial populations 92.1 per cent of the organisms were cocci. Thus it would appear evident that levels of free residual chlorine must be kept sufficiently high in order to destroy the body type of organisms, as represented by the genus Micrococcus. A further study was made to identify as many of these Micrococcus strains as possible and the results indicate that the majority of those surviving a high-free residual chlorination were M. epidermidis, a normal parasite on the human skin, while M. pyogenes var. aureus and albus, potential pathogens, were predominant in the samples taken from water with low values of or no residual free chlorine.

Examination of Table 3 will show that the water in the Smith College pool appears to have attained stability in its

Table 2—Membrane Filter Studies Using Enrichment Broth: Types of Bacteria Surviving Chlorination of Swimming Pool Waters (100 ml Samples)

Pool	Use of Pool	Number Samples Tested	Total Colonies Isolated	No. of Bacteria by Type			Per cent of Bacteria by Type		
				Bacillus	Micro-coccus	Molds, Actino-mycetes	Bacillus	Micro-coccus	Molds, Actino-mycetes
Smith	Swimmers present	11	123	92	20	11	74.7	26.9	8.1
	None	5	46	41	3	2	89.1	6.5	4.3
	Total	16	169	133	23	13	78.7	13.6	7.7
A	Swimmers present	6	166	10	153	3	6.0	92.1	1.8
	None	7	88	17	64	7	19.3	71.5	7.7
	Total	13	254	27	217	10	10.2	85.4	3.9

Table 3—Membrane Filter Studies Using Enrichment Broth on Sixteen Samples of Smith College Swimming Pool Water

Numbers of Swimmers in Pool	Chlorine Residual ppm		Total Bacterial Colonies per 100.0 ml	Types of Bacteria		
	Free	Total		Bacillus	Micro-coccus	Molds Actino-mycetes
0	1.45	2.58	11	11		
0	1.94	3.05	13	12	1	
0	1.75	2.92	8	6		2
0	2.59	3.76	6	6		
0	2.85	3.81	8	6	2	
10	1.40	2.36	15	12	2	1
12	1.73	2.74	7	6		1
5	1.39	2.60	14	14		
13	2.05	3.22	22	18	4	
23	3.58	4.80	10	6	4	
15	1.74	2.76	7	6	1	
3	1.77	2.92	9	8		1
37	1.87	2.90	18	9	4	5
40	1.73	2.88	7	4	2	1
23	2.26	3.55	10	6	2	2
23	2.36	3.25	4	3	1	

bacterial flora and total microbial population. The numerically low bacterial population as determined by the membrane filter and enrichment broth method varied from four to 22 colonies per 100 ml of sample and was primarily spore-forming rods with a coccal count never exceeding four colonies per 100 ml of water. As long as the free residual chlorine in value is at least 1.0 ppm, neither fluctuations of the free residual level nor the magnitude of the swimming load appears to change this flora. It appears evident that the continuous maintenance of a sufficiently high-free chlorine residual affords complete protection against the survival of any normal body bacteria in swimming pool water.

The bacteriologic studies on the samples from Colt Park pool when enrichment broth was used show clearly in Table 4 the bactericidal action of the high-free residual chlorine. These samples were obtained from three different areas of the pool: the shallow end, the middle portion, and the deep end. In each of these areas differences in the residual chlorine, free and total, were found. The over-all effect of the free residual chlorine upon the total bacterial counts as determined on the membrane filters is marked and is shown in Figure 1 where it is seen that the number of bacteria dropped from 357 colonies to six colonies per 100 ml of sample with an increase in free residual chlorine from 0.24 to 2.32 ppm (Table 4). The first sample was collected immediately following the entrance of 200 to 250 swimmers into the pool waters. These swimmers remained in the pool during the entire sampling period. The difference in time between samples No. 1 and No. 2 was only 40 minutes yet with an increase in free chlorine from 0.67 ppm to 2.32 ppm (caused by an increase in the rate of chlorine application) the total bacterial colony count dropped from 298 to six per 100 ml of sample. Representative colonies were isolated from the mem-

Table 4—Membrane Filter Studies Using Enrichment Broth on Six Samples Obtained from Colt Park Swimming Pool *

Sample	Time of Collection	Location of Sampling Point	Residual Chlorine Values as ppm		Colony Count per 100 ml	Bacterial Types Identified
			Free	Total		
1 †	1:35 p.m.	Shallow end	0.67	0.96	298	Bacillus Micrococcus Actinomycetes
2	2:15 p.m.	Shallow	2.32	2.68	6	Micrococcus
3	2:50 p.m.	Middle	1.47	1.79	10	Bacillus Micrococcus
4	3:15 p.m.	Middle	1.64	1.96	8	Micrococcus
5	2:00 p.m.	Deep end	0.41	0.67	19	Bacillus Gaffkya Sarcina Actinomycetes
6	2:35 p.m.	Deep end	0.24	0.52	357	Sarcina Micrococcus

* Swimming load at all times 200–250.
† Sample 1 was taken five minutes after bathers first entered the pool.

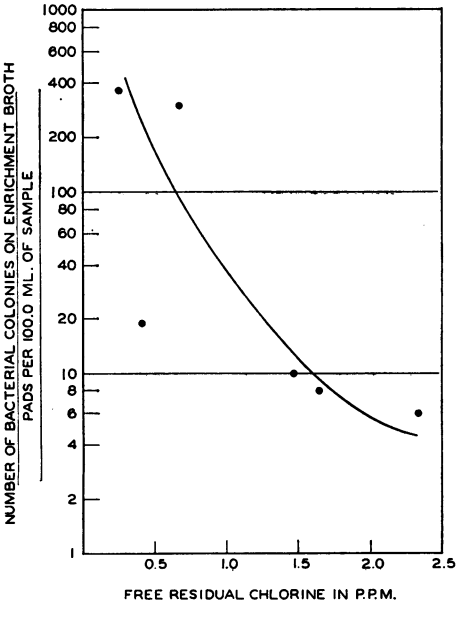

Figure 1—Relationship of the Number of Bacterial Colonies on Membrane Filters to Free Residual Chlorine Levels in Six Samples from the Colt Park Swimming Pool.

brane filters with the higher counts (samples No. 1 and No. 6) and the majority of them were identified as M. epidermidis with some strains of Bacillus and actinomycetes also present. The six colonies obtained from sample No. 2 all proved to be M epidermidis. The second and third samples had counts of 10 and eight colonies, respectively, and from each of which were isolated M. epidermidis, M. flavus, and Bacillus strains. The deep end of the pool having the lowest free chlorine residuals (0.41 ppm and 0.24 ppm) showed an increase in the total bacterial counts within a 35 minute testing period. The numbers of colonies increased from 19 to 357 colonies per 100 ml of water during this time interval. From the lower count was isolated strains of Gaffkya tetragena, Bacillus, Sarcina lutea, M. epidermidis, M. flavus, and occasional actinomycetes, while Sarcina lutea and M. epidermidis, and M. flavus predominated in the higher count. The main-

tenance of a residual of free chlorine is again shown to be highly effective in keeping the total bacterial population at low levels even during periods of peak swimming loads.

The membrane filter studies using enrichment broth on samples from pool B were complicated by the high bacterial density. One (1.0) ml portions of the water were filtered and the results are expressed in terms of colonies per 1 ml of sample. No attempt was made to determine the percentage of the various types of organisms present, but the total counts were made and various representative colonial types were isolated for identification. The number of colonies which developed from each sample varied in numbers from three to too many to count. Among the bacteria classified from the types isolated were: M. pyogenes aureus and albus (predominant types), M. flavus, M. candidus, Neisseria catarrhalis, Gaffkya tetragena, Sarcina lutea, Streptococcus fecalis, Streptococcus salivarius, Escherichia coli, and Aerobacter aerogenes. A few molds and actinomycetes were also isolated.

It has been stated [18] that in dealing with drinking waters the real application of chemistry begins where that of bacteriology ends, for when pollution is so gross that its existence is obvious and only the amount needs to be determined bacteriologic tests are not of value because of their excessive delicacy. In the present study of swimming pool waters having high-free chlorine residuals the same principle may be applied, but in reverse order. The accepted bacterial tests are not sufficiently delicate or precise to determine the relatively few organisms that survive high-free residual chlorination. It becomes necessary to evaluate newer procedures and to determine the actual need for using biologic examinations of water having low bacterial populations through this new method of water treatment.

The membrane filter procedures are not difficult to carry out. A variety of culture media may be used, any group of organisms may be searched for, isolated, and classified, and the exact bacterial flora determined with reasonable accuracy. Yet in view of the efficiency of high-free chlorine in destroying organisms as rapidly as they enter the water it appears that such a procedure is unnecessary and that the chemical titration of the chlorine fractions is enough to give evidence of a safe or unsafe pool water with regard to bacterial densities. Here then is a type of test which would meet Winslow's criterion for determining a safe water.

There has been no attempt in this present study to define or obtain a critical limit of free residual chlorine with regard to its action on any group of bacteria that might enter the water from the swimmers. It does seem clear, however, that when the chlorine residual is maintained sufficiently high as is done in high-free residual chlorination practices there is no danger of resistant organisms developing or accumulating in large numbers as residual bacterial flora. The most resistant organisms encountered in this study all proved to be strains of M. epidermidis. This confirms the results of Seligman [24] who proposed a "coccus" index of 15 per ml of sample which he claimed was easy to maintain in waters having a residual of 0.4 ppm or more. This index does not appear to offer any particular degree of safety in classifying "good" or "bad" waters. The results of the present study show that although M. epidermidis was the most resistant of the body type of organisms isolated, it never was found in numbers above four colonies per 100 ml of water having a high-free residual.

Conclusions

Swimming pool water which has a high-free residual chlorine has been

demonstrated as having a low bacterial density when the membrane filter technic is used to demonstrate surviving organisms. Coliform and enterococcus organisms were consistently absent from these waters when portions up to 200 ml were tested. When the free residual chlorine value was 1.0 ppm or higher, the bacterial density on the membrane filter was not greater than 22 colonies per 100 ml of sample when enrichment broth was used for incubation. The numbers of swimmers in the pool does not appear to be a factor in determining the numbers of bacteria which survive high-free residual chlorination.

It is therefore concluded:

¶ Levels of high-free chlorine residuals are easy to maintain regardless of the swimming load.

¶ High-free residual chlorination of swimming pool water provides a water which has minimal bacterial density.

¶ The organisms surviving high-free chlorine residuals are those of the genus Bacillus and Micrococcus. The latter represent organisms of body origin and M. epidermidis, a normal skin parasite, was the predominating species. It, however, was never present in concentrations of more than 4 colonies per 100 ml of sample.

¶ Less emphasis need be placed on bacterial standards for swimming pool waters having a high-free chlorine residual if accurate titrations of the chlorine residuals are carried out routinely.

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