Effect of Free and Combined Available Residual Chlorine upon Bacteria in Swimming Pools*†

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ONE purpose of sanitary control of swimming pools is the maintenance of some fixed amount of residual chlorine, which will kill bacteria as they are washed into the water from the bathers' bodies rapidly enough to prevent conditions of gross pollution. In an attempt to standardize the magnitude of this fixed amount of residual chlorine, inconsistent bactericidal results were frequently obtained when a standard quantity of residual chlorine was used. These incongruous bacteriological findings were probably caused by the alternate presence of the two components of total residual chlorine, namely, free available residual chlorine and combined available residual chlorine. In 1942, the report for the design, equipment, and operation of swimming pools by the joint committee of the Conference of State Sanitary Engineers and the American Public Health Association[1] recommended a residual chlorine concentration of 0.4 p.p.m. to 0.6 p.p.m. when the pool was in use, and 0.7 p.p.m. to 1.0 p.p.m. for residual chloramine. This latter figure was modified in 1948 to suggest the desirability of operating with higher chloramine residuals up to 2.0 p.p.m., based upon recent studies. The terminology, chloramine residual, has been changed to combined available chlorine residual, by the American Water Works Association committee which prepared the chapter on "Chlorination and Other Disinfection Practices" for the forthcoming *Manual of Water Quality and Treatment*.[2]

The development of the orthotolidin-arsenite colorimetric test[3] and the amperometric titration method[4] for determining residual chlorine gave the sanitary engineer quick, efficient and accurate methods for measuring in the field the quantities of free and combined available residual chlorine present in water. With these resources available a study was undertaken to measure the relative effectiveness of free and combined available residual chlorine as bactericides in swimming pool water. To obtain this measurement it was decided to use tests for the number of bacteria which grow on agar at 35–37° C., coliform bacteria and streptococci. The first two tests are in common usage in sanitary bacteriology, but the latter test is not widely used in this country. There has been some disagreement in regard to the usefulness of the streptococcal

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density in evaluating the sanitary quality of swimming pool water. Mallmann[5] and France and Fuller[6] claimed that streptococci are a good measure of contamination in swimming pool water, but Ritter and Treace[7] concluded that the presence of these organisms in swimming pool water has no public health significance.

For this study, four pools in Connecticut were selected. Each pool was operated in accordance with the requirements of the Joint Committee Report and were of the continuous recirculation type. All pools used fresh water and three were located indoors. Although combined residual chlorination through the use of ammonia gas was not the practice by intention, one pool applied aluminum ammonium sulphate and actually obtained this method of disinfection and a second pool changed to it accidentally during the test period by changing coagulants.

Each of the four pools was tested on three occasions making a series of twelve tests within this study. Before each test, the residual chlorine content was measured to insure reasonable adherence to the minimum and maximum standard for disinfection as set forth in the Joint Committee Report (see Table 1). The amperometric titration was used in preference to the orthotolidin-arsenite test to measure free and combined available residual chlorine because the high temperatures of swimming pool waters cause the color reaction of chlorine and orthotolidin to occur too quickly for accurate results.

TABLE 1

Residual Chlorine Content of Swimming Pools Included in This Study

Free Available Residual Chlorine p.p.m.	*Combined Available Residual Chlorine p.p.m.*	*Total Available Residual Chlorine p.p.m.*
0.40	0.00	0.40
0.50	0.00	0.50
0.40	0.10	0.50
0.40	0.05	0.45
0.35	0.05	0.40
0.80	0.00	0.80
0.60	0.30	0.90
0.00	0.50	0.50
0.00	0.60	0.60
0.00	0.35	0.35
0.10	0.60	0.70
0.00	0.90	0.90

The sampling procedure for the twelve tests was identical for each occasion. The pool was closed to swimmers at least one hour before the start of the test to permit the purification processes to reduce the bacterial content to a practical minimum. Just before the start of the test, samples were collected and titrated with the amperometric titrator to determine the quantity of free and combined available residual chlorine present. Three sampling points were used; one at the midpoint of the shallow end and two on the sides, one-third the distance from the shallow end toward the deep end. At the start of the test period the bathers lined up around the edge of the pool at equal intervals. The initial bacteriological samples were collected at the three sampling points, and then at a given signal all bathers dove into the pool and swam for 5 minutes. Additional samples for bacteriological analyses were collected simultaneously from the three sample stations at the following intervals, timed from the moment the bathers entered the water: ½, 1, 1½, 2, 2½, 3, 4, and 5 minutes. At the end of the 5 minute swim period another signal was given to the bathers to withdraw quickly from the water, and, following an interval of 5 minutes more during which no bathers were allowed to go into the water, a final set of samples was collected.

The bacteriological samples were analyzed promptly after collection. The procedures used were those recommended in *Standard Methods of Water Analysis*, Eighth Edition[8] except that the tryptone glucose extract agar plates were incubated at 35–37° C. for 48 hours, instead of 24 hours, to permit easier counting of the colonies. Duplicate plates of 0.1 ml. and 1.0 ml. dilutions were poured for each sample. Two per cent brilliant green bile broth was used as the confirmatory medium for coliform bacteria.

The results from the series of twelve tests were placed in one of two categories, those collected from swimming pool water containing free available residual chlorine equal to or in excess of 0.35 p.p.m., and those collected from water with combined available residual chlorine predominating and having 0.10 p.p.m. or less free available residual chlorine. The former group will be referred to as the free available residual chlorine group and the latter as the combined available residual chlorine group. Seven of the twelve sample series were classified as belonging to the free available residual chlorine group.

The first measurement of the relative germicidal properties of free available residual chlorine as compared to combined available residual chlorine used the total agar plate count. A geometric mean of the number of bacteria growing on tryptone glucose extract agar at 35–37° C. for 48 hours for 1.0 ml. for each time interval during the sampling series for each of the two residual chlorine categories was determined (see Table 2). Geometric means were used in preference to arithmetic means to limit the undue influence caused by one exceptionally high or low value. By plotting these values against time, very interesting and noteworthy results are obtained (see Chart 1). The trends of the two graphs are similar and have the characteristics of a parabola with a maximum value at the 4 minute point. No interpretation of the trend of the graph beyond the 4 minute point was made because of the limitations imposed by the 5 minute swimming period. Actually, the maximum values for both free available residual chlorine and combined available residual chlorine, occur at the

TABLE 2

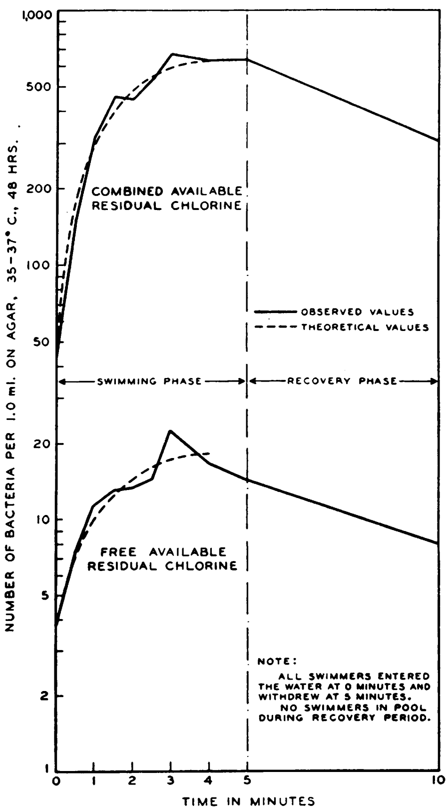
Geometric Means of the Number of Bacteria on Agar per 1.0 ml., 35–37° C., 48 Hr. Incubation

Time (in minutes)	*Geometric Mean of Number of Bacteria on Agar/1.0 ml., 35–37° C., 48 hrs.* — *Pools with free available residual chlorination*	*Pools with combined available residual chlorination*
0	3.8	42
0.5	7.4	148
1.0	11.2	315
1.5	12.9	453
2.0	13.2	445
2.5	14.3	539
3.0	22.1	672
4.0	16.5	630
5.0	14.2	634
10.0	7.9	304

Note: All swimmers entered water at 0 minutes and left at 5.0 minutes. No swimmers in water at 5–10.0 minutes.

CHART 1

GEOMETRIC MEAN OF BACTERIA IN SWIMMING POOLS WHEN IN USE



3 minute value but the deviation from the theoretical parabolic curve with a maximum value at the 4 minute point is within the limits of experimental error. Statistically, these theoretical curves fit the data best.

The difference in the magnitude of the values for free available residual chlorine and combined available residual chlorine is striking. The initial value for swimming pool water with free available residual chlorination is but 3.8 bacteria per 1.0 ml. as compared to 42 bacteria per 1.0 ml. These values represent the number of bacteria resistant to residual chlorine as they are obtained from samples collected after the passage of at least 1 hour since the last swimmer was in the water. The ratio of the magnitude of these values is 1:11.1.

Comparing the actual maximum values in a similar manner it is found that the pools with free available residual chlorine have a density of only 22.1 bacteria per ml. contrasted with 672 bacteria for pools with combined available residual chlorine. In other words the latter type pools have 30.4 times as many more bacteria present at the maximum concentration than the former. The theoretical values are 18.0 bacteria per ml. for free residual chlorine and 630 for combined available residual chlorine with a ratio of magnitudes of 1:35.0. The values for the total number of bacteria per ml. after a 5 minute recovery period during which no bathers were present in the pool are 7.9 and 304 for pools with free and combined available residual chlorine, respectively. The difference in the maximum values for free available residual chlorine and combined available

TABLE 3

Relationship of Free Available Residual Chlorine and Combined Available Residual Chlorine to the Number of Samples Positive for Coliform and Streptococcal Bacteria When Bathers Were in Swimming Pools

	Number of Samples Positive for Coliform Bacteria	*Number of Samples Negative for Coliform Bacteria*	*Total Number of Samples Analyzed for Coliform Bacteria*
Pools Practising Free Available Residual Chlorination (Free available residual chlorine equal to or in excess of 0.35 p.p.m.)	5	163	168
Pools Practising Combined Available Residual Chlorination (Combined available residual chlorine equal to or in excess of 0.35 p.p.m. and free available residual chlorine equal to or less than 0.10 p.p.m.)	84	36	120
Total	89	199	288

	Number of Samples Positive for Streptococcal Bacteria	*Number of Samples Negative for Streptococcal Bacteria*	*Total Number of Samples Analyzed for Streptococcal Bacteria*
Pools Practising Free Available Residual Chlorination (Free available residual chlorine equal to or in excess of 0.35 p.p.m.)	103	65	168
Pools Practising Combined Available Residual Chlorination (Combined available residual chlorine equal to or in excess of 0.35 p.p.m. and free available residual chlorine equal to or less than 0.10 p.p.m.)	106	14	120
Total	209	79	288

residual chlorine compares favorably with the findings of Butterfield[9] who found that to obtain 100 per cent kills in the same exposure time from 15 to 60 times (average of 30) as much combined available residual chlorine was required as compared with free available residual chlorine.

A graphic demonstration of the respective behavior of coliform and streptococcal bacteria in the presence of free and combined available residual chlorine cannot be made because these values are most probable numbers (M.P.N.) and, as such, are limited in their minimum and maximum values by the range of the dilution of the samples tested. In these tests the minimum value is less than 2.2 organisms per 100 ml. and the maximum greater than 240 per 100 ml. Results occurring at either extremity are represented by indeterminate values and cannot be used in obtaining means. To measure the relative bactericidal properties of free available residual chlorine and combined available residual chlorine the results of analyses for coliform and streptococcal bacteria were compiled into fourfold tables (see Table 3). These tables are a breakdown of the samples collected while swimmers were in the pool water to show the number of samples positive for coliform and streptococcal bacteria as compared to the alternate presence of free and combined available residual chlorine. The tabulation of the results for coliform bacteria indicates that only 5 samples out of 168, or 3 per cent, showed the presence of coliform bacteria in any dilution in the water with free available residual chlorine while 84 samples out of 120, or 70 per cent, were positive for coliform bacteria in pools having combined available residual chlorine. When the chi square test is applied to these data a very highly significant result is obtained, which means that within the range of this study free available residual chlorine is more effective than combined available residual chlorine in swimming pools in killing coliform bac-

TABLE 4

Relationship of the Number of Samples Positive for Coliform and Streptococcal Bacteria to Free Available Residual Chlorine and Combined Available Residual Chlorine.
(Samples Collected When Bathers Were in Pools)

Pools Practising Free Available Residual Chlorination (Free Available Residual Chlorine Equal to or in Excess of 0.35 p.p.m.)

	Number of Samples Positive	*Number of Samples Negative*	*Total Number of Samples Analyzed*
Samples tested for presence of coliform bacteria	5	163	168
Samples tested for presence of streptococcal bacteria	103	65	168
Total	108	228	336

Pools Practising Combined Available Residual Chlorination (Combined Available Residual Chlorine Equal to or in Excess of 0.35 p.p.m. and Free Available Residual Chlorine Equal to or Less than 0.10 p.p.m.)

	Number of Samples Positive	*Number of Samples Negative*	*Total Number of Samples Analyzed*
Samples tested for presence of coliform bacteria	84	36	120
Samples tested for presence of streptococcal bacteria	106	14	120
Total	190	50	240

teria. The tabulation of the results for streptococcal bacteria indicates that 103 samples out of 168, or 61 per cent were positive for streptococci in swimming pool water with free available residual chlorine, and that 106 samples out of 120, or 88 per cent, contained streptococcal bacteria in the presence of combined available residual chlorine. The chi square test gives a highly significant value to this relationship, indicating that within the limits of this study free available residual chlorine is a more effective bactericide against streptococcal bacteria than combined available residual chlorine.

If these same data are regrouped (see Table 4) to measure the relative resistance of coliform and streptococcal bacteria to free and combined available residual chlorine, it is found that streptococci are more resistant to both free and combined available residual chlorine in the concentrations encountered in this study than coliform bacteria, but the greatest difference in resistance was ob-

TABLE 5

Relationship of Number and Per Cent of Samples Showing Coliform Indices Greater than and Less than Streptococcal Indices as Affected by the Presence of Free Available Residual Chlorine and Combined Available Residual Chlorine

Samples from Pools with Free Available Residual Chlorination (free available residual chlorine equal to or in excess of 0.35 p.p.m.)

Time In Minutes	Number of Samples	Coliform Index Greater than Streptococcal Index		Coliform Index Less than Streptococcal Index	
		No.	%	No.	%
0.0	21	0	0	1	4.8
0.5	21	2	9.5	11	52.4
1.0	21	0	0	14	66.7
1.5	21	0	0	11	52.4
2.0	21	1	4.8	14	66.7
2.5	21	0	0	14	66.7
3.0	21	0	0	14	66.7
4.0	21	0	0	14	66.7
5.0	21	0	0	12	57.2
10.0	20	0	0	12	60.0
Total	209	3	1.4	117	56.0

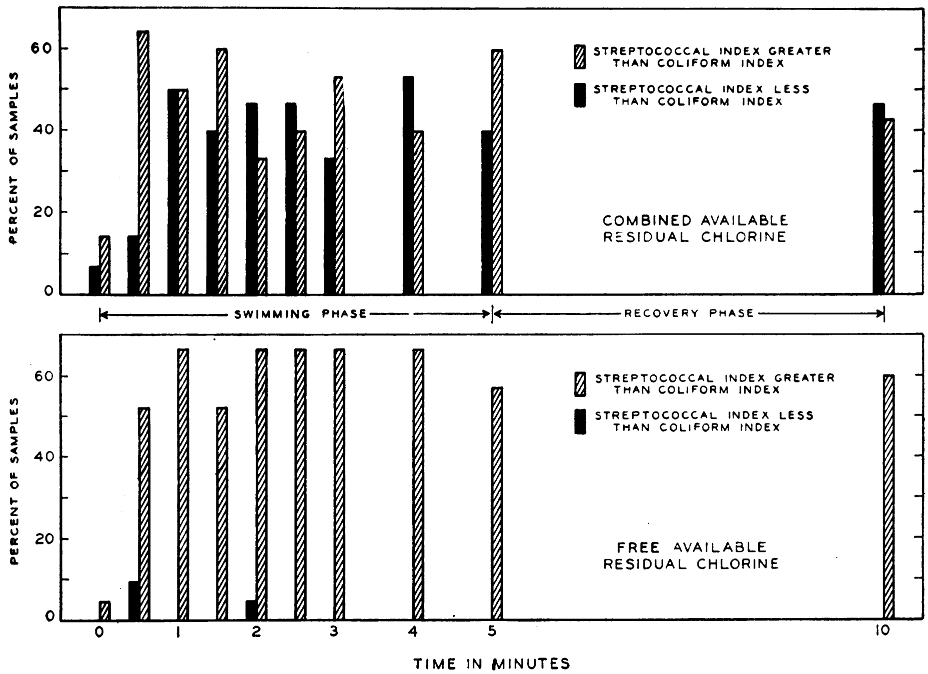
Samples from Pools with Combined Available Residual Chlorination (combined available residual chlorine equal to or in excess of 0.35 p.p.m. and free available residual chlorine equal to or less than 0.10 p.p.m.)

Time In Minutes	Number of Samples	Coliform Index Greater than Streptococcal Index		Coliform Index Less than Streptococcal Index	
		No.	%	No.	%
0.0	14	1	7.1	2	14.3
0.5	14	2	14.3	9	64.3
1.0	14	7	50.0	7	50.0
1.5	15	6	40.0	9	60.0
2.0	15	7	46.7	5	33.3
2.5	15	7	46.7	6	40.0
3.0	15	5	33.3	8	53.3
4.0	15	8	53.3	6	40.0
5.0	15	6	40.0	9	60.0
10.0	14	7	46.7	6	42.8
Total	146	56	38.4	67	45.9

Note: Swimmers entered water at 0 minutes and left at 10.0 minutes. No swimmers in pool at 5–10 minutes.

CHART 2

RELATIONSHIP OF STREPTOCOCCAL AND COLIFORM INDICES



served in the samples collected from swimming pool water containing free available residual chlorine.

Another demonstration of the resistance of streptococci and coliform bacteria to free available residual chlorine and combined available residual chlorine may be made by determining the per cent of samples having a streptococcal density greater than coliform and vice versa (Table 5 and Chart 2). Pools practising free residual chlorination show a definitely higher percentage of samples with a streptococcal index greater than coliform. This relationship cannot be shown for combined available residual chlorination practices, probably because the lethal concentration of combined available residual chlorine for coliform bacteria in swimming pool water was not reached in this study under the bathing loads encountered.

From the above data it is readily understandable why incongruous bacteriological results have been obtained from swimming pools having the same bathing loads and the same total residual chlorine content but with varying quantities of free available residual chlorine. Free available residual chlorine is the bactericide of choice to obtain acceptable bacterial densities in swimming pools while in use and at the same time maintain a low concentration of total residual chlorine.

This study failed to clarify the role of streptococci as indicators of pollution in swimming pool water, but has pointed out that streptococci are recovered readily in swimming pools when in use even in the presence of concentrations of free available chlorine that are bactericidal to coliform bacteria.

CONCLUSIONS

From this study on chlorination of

swimming pools the following general conclusions are reached:

1. Free available residual chlorine is a more effective bactericide than combined available residual chlorine for the treatment of swimming pool water.
2. The values of 0.4 to 0.6 p.p.m. of residual chlorine as recommended by the Joint Committee Report, if interpreted to mean free available residual chlorine, will produce bacteriological results that will comply with the Joint Committee Report's recommended standards for total number of bacteria and coliform bacteria in swimming pools when in use.
3. The minimum value of 0.7 p.p.m. which has been suggested in the past for swimming pools using chloramine (combined available residual chlorine) treatment is insufficient to produce bacteriological results under conditions of this study which will meet the recommended standards of the Joint Committee Reports.
4. Streptococcal bacteria in swimming pools are more resistant to chlorine than coliform bacteria.

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Waksman Foundation in France

The Trustees of Rutgers University Research and Endowment Foundation, holder of patents on streptomycin and other antibiotics developed by Dr. Selman A. Waksman in the university's department of microbiology, have approved the establishment of a foundation to encourage microbiologic investigations in France, to be known as the Waksman Foundation.

This foundation was launched with an initial contribution of 3,000,000 francs from Rhone-Poulenc, French chemical concern, which is licensed by the Rutgers Research and Endowment Foundation to manufacture streptomycin in France. The new French foundation will be supported by royalties due to the Rutgers Foundation from Rhone-Poulenc. The funds will be used for the support in France of research on antibiotics and on other problems in the field of microbiology. Four grants, totaling 2,140,000 francs, were proposed by the committee of the French foundation at its first meeting in December. Three will go to workers at the Pasteur Institute in collaboration with various hospitals in Paris, and the fourth to a staff member in the biochemical laboratories of the Ministry of the Colonies. The executive committee of the French foundation consists of a representative of the French Academy of Medicine; Professor Jacques Trefouel, member of the French Institute and director of the Pasteur Institute, and Professor R. Paul, scientific director of Rhone-Poulenc.