Study of Bacteriological Methods of Testing and Means of Disinfecting Water with Chlorine

With Particular Reference to Swimming Pool Water*

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SINCE the adoption of continuous feed chlorination of swimming pool water whereby residual chlorine contents of 0.2 to 0.5 p.p.m. may be maintained irrespective of the bathing load, bacterial quality comparable to drinking water has been obtained. In 1926, Stovall, Nichols, and Vincent [1] reported that the relationship between the bacterial purity of the water and the residual chlorine content as measured by the orthotolidine test was so satisfactory that this test could be depended upon as a measurement of sanitary quality. In the same year Mallmann [2] observed such a close agreement in the bacteriological and the orthotolidine tests that he recommended the latter as the sole measurement of the sanitary quality of swimming pool water. Since then numerous reports have appeared showing the absence of *Escherichia coli* in 50 c.c. of water in swimming pools maintaining a residual chlorine content of 0.2 to 0.5 p.p.m. There has been no occasion to question the reliability of these data, until recently in a series of studies on swimming pools, the writers had occasion to test swimming pool water immediately after it was withdrawn from the pool. In 1930, Schoepfle [3] reported that pool side bacteriological tests and tests on the same stored samples tested later at the laboratory were practically the same. He concluded that

> . . . the increment of pollution to the pool water due to swimmers seemed to be handled effectively by maintaining the residual chlorine within proper limits;

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this sterilizing action on the added pollution by bathers seemed to be practically immediate, at least within the time required for making a rapid plating and tubing, that is approximately one minute.

In a recent series of studies Mallmann and Schalm[4] have demonstrated that increased alkalinities in water cause a marked retardation of germicidal action of chlorine. Gerstein[5] found that chloramine could not be used as a disinfectant in Chicago water because of delayed germicidal action. The fact that this latter treatment, because of its lessened taste and odor nuisances, is rapidly finding favor in swimming pool disinfection, and the establishment of delayed killing action of chloramine and of chlorine in alkaline waters, raised the question of their sanitary value for swimming pools. The sterilization of a swimming pool water is quite different from that of a drinking water. In swimming pool water disinfection the residual chlorine must destroy large numbers of bacteria as they are removed from the bodies of the bathers; otherwise during periods of heavy bathing loads, an accumulation of bacteria would occur and, at least, a temporary period of pollution would ensue.

To study the influence of chlorine and chloramine treatment without the interference of such other factors as filtration and dilution, a fill and draw pool of 45,000 gallons capacity was selected. An attempt to use a circulating system pool gave unsatisfactory results as the circulation introduces factors that are difficult to control. Furthermore, chloramine had not been used in a pool with a circulating system prior to the experiment and the possibility of the filters removing the ammonia lends another unknown factor. There is also some question as to whether or not the ammonia, when once introduced into a closed system such as a circulating system pool, would remain available for further combination with chlorine after it had released its initial load for sterilization purposes. Available data indicate that in such a closed system after the initial treatment with an ammonia-chlorine ratio of 1 to 2 or 1 to 3 subsequent treatments can be reduced to 1 to 10.

To determine the necessity of pool-side testing a bacteriological laboratory was set up in the school readily accessible to the pool. For each test two samples were collected simultaneously, one of which was tested immediately while the other was kept and tested several hours later at the city laboratory. The latter was handled in a manner typical of the customary routine. In Table I are presented results representative of a mass of data collected to measure the comparative

TABLE I

VALUE OF POOL-SIDE TESTING VS. REGULAR LABORATORY TESTING

Pool—45,000 gal. capacity—fill and draw type

Treatment—Chloramine

Bathing— 9:30– 9:50 30 men
10:15–10:35 37 men
1:15– 1:35 12 women

		Analyses at pool side		Analyses at laboratory	
Time of sampling	*Resid. Cl.*	*Gas in five 10 c.c. samples*	*Count*	*Gas in five 10 c.c. samples*	*Count*
9:25	0.15	0 out of 5	0	0 out of 5	0
10:25	0.18	5 out of 5	65	0 out of 5	0
10:50	0.05	5 out of 5	34	0 out of 5	0
1:00	0.25	0 out of 5	19	0 out of 5	0
1:30	0.25	4 out of 5	22	0 out of 5	0
2:30	0.25	0 out of 5	0	0 out of 5	0

value of pool-side and the customary laboratory examinations. In this particular series of tests, the pool received 3 oz. of chlorine and 1.5 oz. of ammonia, a ratio of 2 to 1, prior to the tests. The pool had been previously emptied and cleaned so it was free of organic matter at the start of the experiment. It will be observed that in each case during the period when the pool was in use, marked pollution, as measured by gas production in lactose broth, was obtained in the pool-side samples. The same samples tested at the laboratory in every instance showed no evidence of pollution. In cases of minimum bathing loads and of high residual chlorine contents (0.5 to 1 p.p.m.) pool-side tested samples failed to show pollution. Thus it would seem that under proper conditions, as pertain to bathing load and amount of residual chlorine, a pool can be maintained according to the standard set by the Joint Committee on Swimming Pools and Bathing places of the American Public Health Association and the Conference of State Sanitary Engineers when examined by pool-side testing.

That such a method of testing is logical, is attested by the fact that the presence of even a minimum chlorine residual indicates the presence of germicidal chlorine. If germicidal chlorine is present, it necessarily follows that any bacteria present are going to be affected accordingly. If a period of time elapses between sampling and testing, a reduction of the bacteria must result. Granted a sufficient period and a sufficient amount of residual chlorine, an otherwise polluted sample would be sterile when tested.

In the following studies only the results of pool-side testing will be reported as, in practically all cases, the samples tested at the laboratory were sterile. Also the presence of streptococci will be reported. The regular Durham lactose broth tubes were examined for the presence of these organisms. The method of detecting streptococci consisted of a macroscopic examination followed by a confirmatory microscopic examination. Macroscopically, a presumptive test for streptococci may be made by holding the suspected tube to the light and examining for a granular precipitate having a close resemblance to the precipitate obtained in an agglutination test. Frequently, a granular floc adheres to the outer sides of the tube and on the outer surfaces of the inverted vial. Although the appearance of the granular precipitate is not always confirmed microscopically, the absence of such granular precipitates does not demand microscopical examination.

Mallmann and Schalm[4] demonstrated in a series of laboratory experiments that increasing alkalinities caused a decreased activity or delayed killing action of chlorine. It will be observed that at the same pH values, a marked similarity exists between chlorine and chloramine in the germicidal activity. That increasing pH values cause a decrease in the germ killing speed of chloramine, as well as chlorine, is demonstrated in Figure I. It will be noted that at pH 6, the killing rate is much more marked than at pH 7.7.

To determine the practical value of chloramine treatment, a fill and draw pool of 45,000 gal. capacity was treated with chloramine using calcium hypochlorite and ammonium sulphate as sources of chlorine and ammonia respectively. Prior to the experiment the pool was emptied and cleaned. One ft. of water was admitted, followed by 12 oz. of ammonium sulphate. After another ft. of water was added, 6 qt. of calcium hypochlorite solution (20 lb. to 20 gal. of water) was introduced. The pool was then filled. The applied chlorine was in the proportion of 1 p.p.m. and the ammonia-chlorine ratio was 1 to 2. Frequent duplicate samples were taken throughout the day during periods of use and rest to obtain a complete picture of the bacterial pollution. One set of

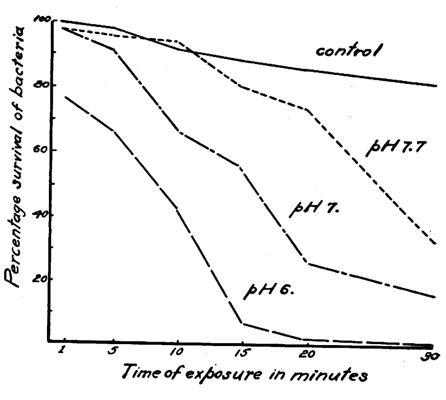

FIGURE I—Influence of varying the pH on a constant chloramine content in killing *E. coli* samples was tested immediately after collection. The results are presented in Figure II. It will be observed that the colon bacilli indices rise during the period of use and fall during the intervening rest periods; that the streptococci indices follow the same trend as the colon bacilli indices, except that streptococci are less frequent; that the residual chlorine content remains fairly constant although no additions of chlorine were made during the period of the test. During the rest periods between bathing loads, sterilization was not effected although a fall in colon bacilli and streptococci indices is evident. In considering these data it should be remembered that the other set of samples collected in the usual manner and tested later in the city laboratory without exception failed to show either streptococci or colon bacilli in all tubes. It may be concluded from these data that 0.5 p.p.m. available chlorine as chloramine failed to control pollution as measured by colon bacilli and streptococci indices.

The following day, after emptying and cleaning the pool, chlorine treatment was tested under as nearly identical conditions as possible. The pool was treated with 6 qt. of calcium hypochlorite solution (20 lb. to 20 gal. of water) to give an applied dosage of 1 p.p.m. At the start of the experiment the pool had a residual chlorine content of 0.45 p.p.m. The data are presented in Figure III. A similar set of data was obtained during periods of rest, except that greater and sharper falls in pollution indices were obtained. The streptococci and colon bacilli indices follow the same trend; the streptococci, how-

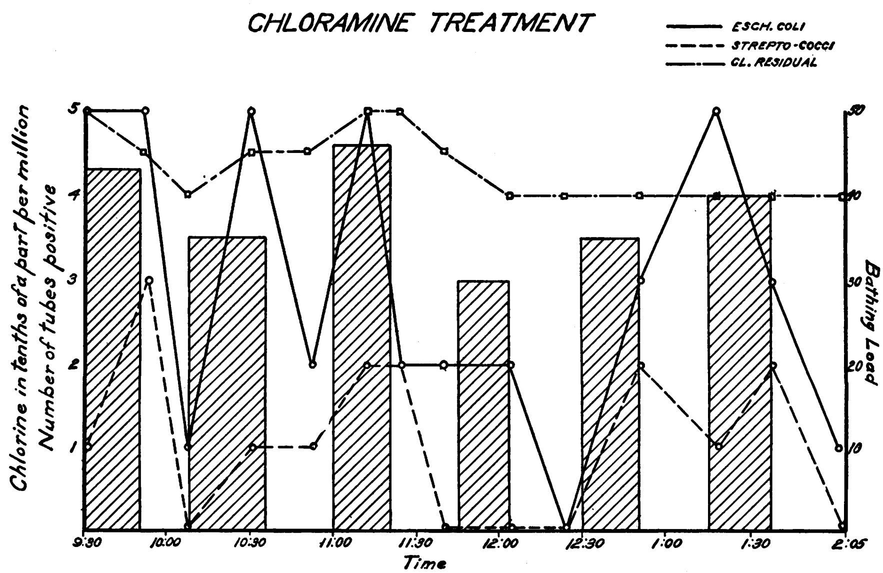

FIGURE II—Relationship of pollution, chlorine residual contents, and bathing load in a chloramine treated swimming pool ever, having a lower incidence. The residual chlorine content fell rapidly and it was necessary to make additions to keep it above 0.2 p.p.m.

It will be observed that as the residual chlorine content falls a marked increase in colon bacilli and streptococci indices occurs. Recovery after pollution was always very rapid. In other tests not presented, under conditions as above, sterility generally occurred within 10 minutes after the bathers had left. This was not true with chloramine treated pools or chlorine treated pools with alkalinities of pH 8.

In the studies cited, the incidence of colon bacilli was considerably higher than that of streptococci. That this relationship is not always true is shown in Tables II and III. These samples were obtained

TABLE II

COMPARISON OF CHLORINE AND CHLORAMINE TREATMENT OF SWIMMING POOL WATER

Treatment—Chloramine—0.1 p.p.m.
Reaction—pH 7.8
Bathers—247

Sample No.	*Gas in lactose broth*						*Streptococci*						*Count*	*Organism*
	10	10	10	10	10	1	10	10	10	10	10	1		
1	+	+	+	+	−	−								
	+	+	+	+	−	−	+	+	+	+	+	+	Inn.	*A. aerogenes*
2	+	+	+	−	−	+								
	+	+	+	−	−	+	+	+	+	+	+	−	Inn.	*A. aerogenes*
3	+	+	−	−	−	−								
	+	+	+	−	−	−	+	+	+	+	+	−	Inn.	*A. aerogenes*

Treatment—chlorine—0.1 p.p.m.
Reaction—pH 7.3
Bathers—283

Sample No.	*Gas in lactose broth*						*Streptococci*						*Count*	*Organism*
	10	10	10	10	10	1	10	10	10	10	10	1		
1	+	−	−	−	−	−								
	+	+	+	−	−	−	+	+	+	+	+	−	40	*E. coli*
2	−	−	−	−	−	−								
	−	−	−	−	−	−	+	+	+	+	+	+	25	
3	−	−	−	−	−	−								
	−	−	−	−	−	−	+	+	+	+	+	+	350	

from a 260,000 gal. out-door pool with a recirculation system. In Table V, are shown the results obtained on successive days, using chloramine treatment and chlorine treatment with residual chlorine contents of 0.1 p.p.m. in both cases. All samples were collected during periods when the pool was in use. Although the numbers of colon bacilli were far less in the case of chlorine treatment there was no change in the incidence of streptococci. Table III gives

TABLE III

COMPARISON OF CHLORINE AND CHLORAMINE TREATMENT OF SWIMMING POOL WATER

Treatment—chloramine—0.6 p.p.m.
Reaction—pH 7.6
Bathers—740

Sample No.	*Gas in lactose broth*					*Streptococci*					*Count*	*Organism*
	10	10	10	10	10	10	10	10	10	10		
1	—	—	—	—	—							
	—	—	—	—	—	+	+	—	+	+	32	
2	+	+	—	—	+							
	+	+	—	—	+	+	+	+	+	+	226	*A. aerogenes*
3	—	—	—	—	—							
	—	—	—	—	—	+	+	+	+	+	41	

Treatment—chlorine—0.6 p.p.m.
Reaction—pH 6.9
Number of bathers—273

Sample No.	*Gas in lactose broth*						*Streptococci*						*Count*
	10	10	10	10	10	1	10	10	10	10	10	1	
1	—	—	—	—	—	—							
	—	—	—	—	—	—	+	+	—	+	+	—	0
2	—	—	—	—	—	—							
	—	—	—	—	—	—	+	—	+	+	—	—	0
3	—	—	—	—	—	—							
	—	—	—	—	—	—	+	—	—	—	—	—	0

the data obtained from the same pool using 0.6 p.p.m. available chlorine in the case of both chlorine and chloramine.

In this case, colon bacilli were absent from the chlorine treated water and a marked reduction in the number of streptococci occurred. It may be stated, however, that both colon bacilli and streptococci follow the same trend as a measurement of pollution in a chlorinated pool. Their relative relationship as to numbers apparently varies. Insufficient data on this particular point make any explanations impossible.

The writers are not prepared to weigh the significance of the colon bacilli and streptococci as found by means of the pool-side testing during periods of use of the swimming pool. Whether or not the pollution found has any sanitary significance remains to be seen. It is true, however, that the usual method of testing, whereby samples are held for a period of time between collecting and testing, does not give a true picture of the sanitary quality of a swimming pool. Such methods of testing as are now in general use are to be condemned as giving a false sense of security if we are to consider the presence of the colon bacilli and streptococci as a criterion.

The disappearance or loss of residual chlorine varies with the conditions under which the chlorine is introduced. A number of investigators have reported that chloramine treatment stabilizes the residual chlorine content. To obtain confirmatory data upon this point, residual chlorine contents were observed under 3 conditions, namely, high alkalinity (pH 7.3–7.9), neutral reaction (pH 6.8–7.2), and chloramine treatment (pH 7.2). The data are presented in Table IV. In the case of the chloramine treatment, although only an initial dose of calcium hypochlorite was made, the residual chlorine content remained practically constant. With chlorine treatment at pH 6.8 to 7.2, 33 per cent more chlorine was necessary to retain a minimum dose in excess of 0.2 p.p.m. available chlorine with a considerably smaller bathing load. With chlorine treatment pH 7.3 to 7.9, the chlorine loss was not as great as pH 6.8 to 7.2 but more so than with chloramine treatment. However, although delayed killing action occurred, the extent of pollution during periods of use was far less than with chloramine treatment. On the other hand, the chloramine treated pool with an average residual chlorine content of 0.45 p.p.m. showed marked pollution during periods of use as measured by pool-side testing. The stability of chlorine residuals in chloramine treated pools has little value if the sanitary quality of the pool is sacrificed. Examination of 3 chloramine treated pools with chlorine residuals of 0.1 to 0.6 p.p.m. has failed to show the desired sanitary quality as measured by pool-side testing.

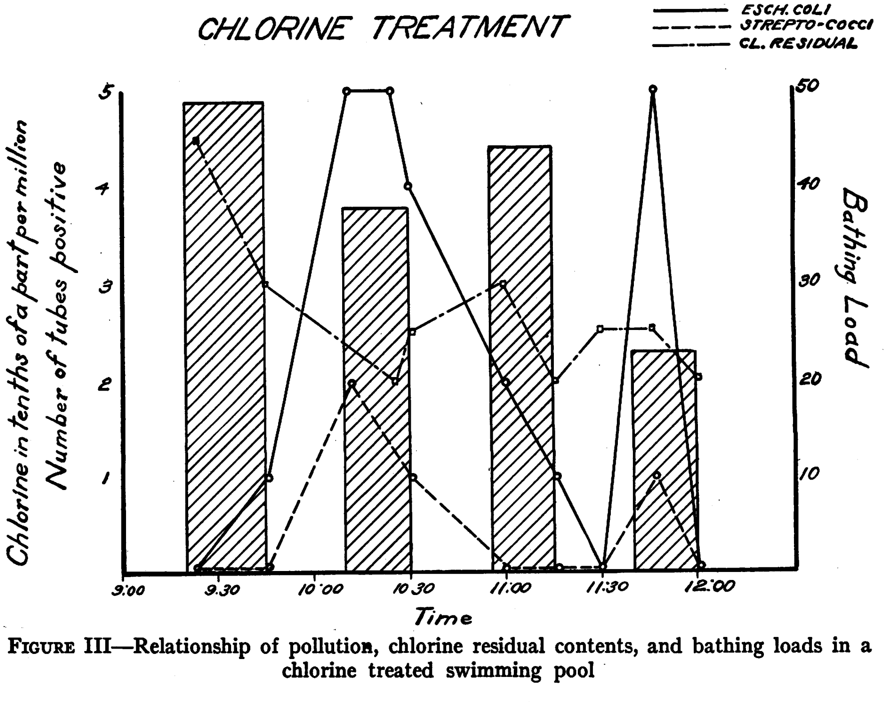

FIGURE III—Relationship of pollution, chlorine residual contents, and bathing loads in a chlorine treated swimming pool

TABLE IV

STABILITY OF CHLORINE IN ALKALINE CHLORINE, NEUTRAL CHLORINE AND CHLORAMINE TREATED POOLS

Alkaline chlorine			*Neutral chlorine*			*Chloramine*		
Amnt. cl. added	*Res. cl.*	*Bathing load*	*Amnt. cl. added*	*Res. cl.*	*Bathing load*	*Amnt. cl. added*	*Res. cl.*	*Bathing load*
10 oz.	0.7	20	6 oz.	0.45	49	6 oz.	0.5	
	0.6	48		0.3			0.5	43
	0.7	40		0.2	38		0.45	
	0.6		2 oz.	0.25			0.4	35
	0.5	28		0.3	44		0.45	
	0.4			0.2			0.45	
	0.5	41		0.25	23		0.5	46
	0.5			0.25			0.5	
	0.5			0.2			0.45	30
	0.5	35					0.4	
	0.25						0.4	34
	0.25						0.4	40
							0.4	
							0.4	
							0.4	
10 oz.		232	8 oz.		154	6 oz.		237

The writers feel that the present method of sampling should be supplanted by a method of pool-side testing. Pool-side testing is, of course, out of the question as a routine procedure, but a method of collecting samples in sodium thiosulphate treated bottles makes possible the elimination of the residual chlorine so that the sample can be transported to the laboratory without destruction of the bacteria.

The bottle is prepared by adding a small crystal of sodium thiosulphate to a moist bottle or 0.5 c.c. of tenth normal sodium thiosulphate to a dry bottle subsequent to sterilization by moist heat. Care must be taken not to rinse the bottle in collecting the sample. The sodium thiosulphate added is sufficient to neutralize a residual chlorine content of at least 1 p.p.m. The results obtained from sodium thiosulphate treated bottles and from pool-side tested samples are similar.

Samples collected from chlorinated swimming pools should be taken during use to determine the presence of transient pollution. Samples taken during periods of rest will, if collected 15 to 30 minutes after use, generally fail to show pollution. All samples should be collected in sodium thiosulphate treated bottles.

The building up of pollution in the swimming pool in the presence of residual chlorine would seem to indicate that bathing loads should be regulated to avoid excessive pollution. The view that the presence of residual chlorine indicates the absence of bacteria must be revised.

Further work on this phase of the problem is in progress in a number of swimming pools in Detroit and Lansing.

This report is very largely of a preliminary nature and will be followed by reports on more complete studies.

SUMMARY

1. Samples of swimming pool water collected during periods of use and tested immediately showed more pollution than duplicate samples handled in the usual manner by storing and testing later.

2. During periods of use, swimming pool water showed marked pollution as measured by colon bacilli and streptococci indices in the presence of residual chlorine contents of 0.2 to 0.5 p.p.m.

3. During periods of rest, the pollution evidenced during use, disappeared. The rate of disappearance depended upon the type of treatment used.

4. With chloramine treatment, a delayed germicidal action occurred. This was also true of chlorine treatment in alkaline water, but to a lesser extent.

5. The colon bacilli and streptococci indices roughly parallel each other. The preponderance of the colon bacilli and streptococci incidences varies.

6. A sodium thiosulphate treated sample bottle is recommended for collecting pool samples.

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