A REPORT ON THE VIABILITY OF SWIMMING POOL ORGANISMS TO RESIDUAL CHLORINE

THESIS FOR DEGREE OF B. S.

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A Report

on

The Viability

of

Swimming Pool Organisms

to

Residual Chlorine

a

Thesis

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to

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Review of literature.

Reduction of Organisms when Exposed to Chlorine.

For practical purposes 0.2-0.3 p.p.m. of available chlorine is sufficient for the destruction of organisms indicative of fecal contamination in swimming pools when exposed for a minimum length of time. Under laboratory conditions 5 minutes is usually to remove all but the resistant minority. Engineers maintain that at least 30 minutes exposure is necessary when in the pool waters under ordinary conditions and longer when there is a possibility of organic matter being present.

In the case of a sample of the swimming pool water the reduction is around 97%, the 3% or the resistant minority are spore formers and spreaders.

After treatment with chlorine not all B. coli colonies appear to be alike. For instance on litmus-lactose agar the colonies are much smaller and fail to produce acid. This is explained by the fact that the organisms are stunned by the action of the chlorine.

Lake water emulsions containing pure cultures of B. coli showed the reduction with 0.3 p.p.m. available chlorine to be 99.6% after the first 5 minutes. The initial counts were from 3,000 to 500,000 in 1 c.c.. It is maintained that the initial reduction and not the percent reduction should be considered as the percent reduction will naturally be greater with a large number of organisms in the original suspension.

Results obtained by using B. coli formers as the test organism and using calcium hypochlorite for 15 minute contact periods. The initial count being 450/c.c.

P.p.m. Cl.	Percent reduction
25	3
50	45
75	80
100	97
200	99
300	99
400	99

(Lederer and Buchanan. Eng. Record Vol.65 p 566-568)

The Effect of Organic Materials.

The comparative cheapness of chloride of lime and its efficiency as a disinfectant, as shown by all the leading authorities on disinfection has caused a great increase in its use as an efficient means of effecting a proper control of swimming pools.

The same report stresses the need for the addition of an excess where organic matter is present. This is necessary to overcome the resistance offered by the matter and to overcome the organisms combined with the materials which would otherwise be left unharmed. The fact that a few organisms are nearly as harmful as a large number necessitates complete destruction in order to insure safety in the pool. (Report of A.P.H.A. Committee on Chloride of Lime for Swimming Pool Disinfection. Vol. 9).

Nothing could be found in literature as to the results produced on Streps. by the action of available chlorine, in the nature of percentage reductions. Several authorities, however, maintain that they are more resistant to its action that are the gas formers.

The results given regarding the gas formers are in connection with mixed cultures, therefore it is impossible to make any comparison of results.

Purpose of the problem.

The importance of fecal organisms in swimming pools has long been the interest of many investigators and especially those connected in any manner with the problems offered by the dangers of swimming pools.

It was with the desire to determine the relative resistance of the strains of Streptococci found in the pools to those of B. coli and B. aerogenes that this problem was undertaken.

The two later organisms have been used as a standard for a means of devising a method of control via the chlorine method and the presence of Streptococci has been neglected. It is without a doubt this organism is as important or even moreso than either B. coli or B. aerogenes.

With this fact in mind it has been my desire to determine the relative resistance of these organisms to each other.

Many thanks are due to Mr. W.L.Mallman and Mr. H.A.Koch. who have offered many valuable suggestions which have been very beneficial to me.

Methods

After a thorough study of various methods for the isolation and identification of the organisms to be used the following have been chosen.

For the isolation of B. coli and B. aerogenes a method devised by Prescott and Winslow has been chosen, first because of its simplicity and second because of its accuracy.

The method is as follows:

Place 10 c.c. of lactose broth in 100 c.c. of the water from which the organisms are to be isolated.

Incubate at 37°C. for 24 hours.

Place 10 c.c. of the above incubated sample in each of (5) fermentation tubes of double strength lactose broth and 1 c.c. in a tube of single strength.

Incubate these tubes at 37°C. for 12-24 hours or until gas appears.

From the tubes showing gas, streak on eosin-methylene-blue plates.

Make duplicates for 20° and 37°C. incubations and incubate for 24 and 48 hours.

Pick typical B. coli colonies which are black with a metallic sheen and B. aerogenes which are black without the metallic color and confirm with the Voges-Proskauer and the Methyl-Red tests.

The V-P test is made as follows:

Pick the typical colonies and plant in dextrose broth.

Incubate for 5 days at 37°C.

At the end of the incubation period add 5 c.c. of a 10% solution of potassium hydroxide (KOH) to 5 c.c. of the 5 day broth culture.

Hold this solution over night for the formation of the eosin-pink color, which constitutes a positive test for the B. aerogenes.

The Methyl Red test is made as follows:

Inoculate the dextrose tubes with typical colonies and incubate for 5 days.

To 5 c.c. of the culture add 5 drops of methyl red solution.

After a short period of incubation a red or yellow color will develop. The red is positive for the presence of B. coli.

The Voges-Proskauer and Methyl-Red scheme is:

B. coli	B. aerogenes
V-P -	V-P +
M-R +	M-R -

The broth used in the Methyl-Red test has a number of special ingredients whereas that used for the V-P test is plain dextrose broth. The former is made as follows:

Distilled water	1000 mils
Peptone	5 grams
Di-potassium-hydrogen phosphate	5 grams
Dextrose	5 grams

Dissolve the mixture of materials over a free flame, make up to volume, filter and sterilize for 30 minutes at 15 pounds pressure.

The Methyl-Red indicator is made by dissolving 0.1 grams of methyl red in 300 c.c. of alcohol and diluted to 500 c.c. with distilled water.

To isolate Streptococcus the water samples used to isolate B. coli and B. aerogenes are permitted to incubate for 24-48 hours or until a sediment occurs in the tube. The supernatant fluid is removed and the sediment stained and examined microscopically for the presence of the organisms. If they are present a small portion of the sediment is suspended in saline and then smeared on 1.5% liver agar plates. The colonies are visible only after 3-5 days incubation at 37°C. and are very white and usually pin point in size. The isolated colonies are examined again microscopically for their true identity, if positive liver agar slants are streaked.

As the viability of the Strepts. is more or less limited it has been found necessary that transfers of the culture be made once in 48 hours.

To determine the amount of available chlorine in the test solution a method devised by E. B. Buchanan and H. G. Perkins has been employed with satisfaction. The method is very simple as well as rapid therefore making it especially convenient for a test of this nature.

Two 2" x 6" blocks 15" in length are fastened together on the flat surfaces and holes sufficiently large to permit the insertion of Nesslers tubes are made through the six inch dimension. Fourteen of these tubes are placed in the block and numbered and the following amounts of reagents are placed in each tube:

Cl/p.p.m.	$CuSO_4$/c.c.	$K_2Cr_2O_7$
0.01	0.0	0.8
0.02	0.0	2.1
0.03	0.0	3.2
0.04	0.0	4.3
0.05	0.4	5.5
0.06	0.8	6.6
0.07	1.2	7.5
0.08	1.5	8.7
0.09	1.7	9.0
0.10	1.8	10.0
0.20	1.9	20.0
0.30	1.9	30.0
0.40	2.0	38.0
0.50	2.0	45.0

A rubber stopper is fitted with a Nesserman tube and the closed end of the tube permitted to project through the small end of the stopper. This permits the closed end of the tube to be immersed in the standard. The rack is so constructed that light is permitted to pass through the bottom of the tube, therefore when looking through the hole of the stopper through the small tube it is possible to make a comparison with great rapidity and a degree of accuracy obtainable only by means of a colorimeter.

Method for the test.

A small amount of chloride of lime is added to about three liters of sterile tap water and the mixture stirred well and permitted to stand for at least thirty minutes.

A twenty-four culture of the organism to be tested is washed from the liver agar slant with sterile saline. A loop transfer is made to a larger quantity of saline. The number of organisms in this suspension is determined by means of a haemocytometer. If the number desired is different than that in this suspension either dilution with saline or addition of the culture may be made. When the number desired is obtained in the suspension four plates are made to check the actual number. Two plates of the original suspension are made by using ½ c.c. amounts and two of a 1/100 dilution using ½ c.c. amounts.

At a convenient time a portion of the original suspension is added to the solution containing the chlorine, the amount of residual chlorine having been determined and at the end of 1, 3, 5, 10, 15 and 30 minuteintervals ½ c.c. amounts of the test solution is removedand immediately plated. The plate being poured with the liver agar.

It is imperative that the plate be poured immediately after the portion has been removed to arrest the action of the chlorine being removed with the sample, also it is desirable to have the temperature of the agar sufficiently low that it will not harm the organisms as their resistance to heat would be lowered due to the action of the chlorine.

The plates are inverted after cooling and incubated for twenty-four hours at 37°C. after which time they are counted. The counting plate of Wolfhugel's being employed for the purpose. (Giltner's Lab. Manual Page 54)

From the number of colonies growing the percent of the organisms destroyed is determined.

Results.

The results of a test using B. coli as the experimental organism is as follows:

Against .5 p.p.m.

Time of exposure	Count	Percent reduction
1 min	1,429	99.43
3 min	1,143	99.55
5 min	847	99.67
10 min	424	99.84
15 min	97	99.93
30 min	0	100.00

.1 p.p.m.

1 min	3,529	98.59
3 min	2,114	99.18
5 min	1,840	99.27
10 min	1,434	99.43
15 min	894	99.65
30 min	347	99.87

.09 p.p.m.

1 min	5,628	97.74
3 min	4,986	98.01
5 min	4,426	98.23
10 min	2,756	98.90
15 min	2,149	99.17
30 min	1,564	99.38

.07 p.p.m.

1 min	6,456	97.4
3 min	5,042	97.63
5 min	5,456	97.89
10 min	3,874	98.46
15 min	2,9[illegible]	99.21
30 min	864	99.81

.08 p.p.m.

1 min	6,728	97.21
3 min	4,840	98.45
5 min	3,868	98.00
10 min	3,421	98.67
15 min	3,268	98.69
30 min	2,648	99.17

.04 p.p.m.

1 min	6,358	97.26
3 min	4,880	98.46
5 min	3,604	98.66
10 min	3,402	98.82
15 min	3,124	98.87
30 min	2,206	99.22

The original suspension count was 250,000 organisms per cubic centimeter. The temperature was 22°C.

The results of a test using A. aerogenes as the test organism is as follows:

Against .3 p.p.m.

Time of exposure	Count	Percent reduction
1 min	32	99.99
3 min	18	99.99
5 min	6	99.99
10 min	2	99.99
15 min	0	100.00
30 min	0	100.00

.2 p.p.m.

1 min	40	99.9[illegible]
[illegible] min	18	99.9[illegible]
5 min	16	99.99
10 min	12	99.99
15 min	6	99.99
30 min	0	100.00

.09 p.p.m.

1 min	320	99.[illegible]
3 min	270	99.90
5 min	200	99.91
10 min	108	99.92
15 min	47	99.96
30 min	18	99.99

.07 p.p.m.

1 min	450	99.87
3 min	326	99.89
5 min	275	99.90
10 min	145	99.95
15 min	58	99.97
30 min	18	99.99

.05 p.p.m.

1 min	560	99.86
3 min	385	99.87
5 min	190	99.90
10 min	107	99.92
15 min	62	99.98
30 min	19	99.99

.04 p.p.m.

1 min	685	99.78
3 min	358	99.89
5 min	175	99.91
10 min	64	99.96
15 min	40	99.99
30 min	28	99.99

The original test suspension contained 310,000 organisms per cubic centimeter. The test was run at room temperature 22°C.

The results of a test using Streptococcus as the test organism is as follows:

Against .3 p.p.m.

Time of exposure	Count	Percent reduction
¼ min	183,750	15.67
½ min	74,273	66,99
¾ min	22,[illegible]94	90.02
1 min	14,900	[illegible]3.34
3 min	12,270	94.5[illegible]
5 min	8,42[illegible]	96.26
10 min	6,[illegible]92	97.21
15 min	3,218	98.57
30 min	980	[illegible].56

.1 p.p.m.

¼ min	[illegible]00,000	11,12
½ min	9[illegible],750	58.70
¾ min	[illegible]8,084	82.83
1 min	1[illegible],[illegible]54	91.44
3 min	15,764	93.00
5 min	12,[illegible]12	[illegible]4.55
10 min	8,750	96.31
15 min	4,598	97.96
30 min	1,974	99.12

.09 p.p.m.

1 min	20,700	90.77
3 min	18,552	91.77
5 min	17,978	92.01
10 min	15,[illegible]	94.11
15 min	10,094	95.26
30 min	4,214	98.13

.07 p.p.m.

1 min	22,[illegible]	90.22
3 min	21,848	90.28
5 min	10,322	91.81
10 min	14,764	93.36
15 min	13,260	94.11
30 min	------	-----

.04 p.p.m.

1 min	28,[illegible]20	87.12
3 min	27,[illegible]74	87.04
5 min	------	-----
10 min	24,8[illegible]	88.96
15 min	23,[illegible]18	89.38
30 min	20,460	90.91

The original test suspension contained 225,000 organisms per cubic centimeter. The test was run at 22°C. The first two tests included platings at intervals of less than one minute to gain some idea as to the rate of destruction during the first minute.

Conclusions.

The experimental results of this test are in keeping with those found in the review of literature. It is known to most workers that the destruction of B. coli and B. aerogenes is nearly 100 percent in solutions containing .2 p.p.m. and over of available chlorine and that any fecal strain of Streptococcus is more resistant than either of the other organisms tested. The percent of organisms remaining are called the resistant minority and are capable of withstanding .4 p.p.m. for short intervals of time.

It is to be expected to find strains more resistant to the effects of chlorine disinfection as it is often possible in many instances to find them present in the sediment of fermentation tubes that show negative results to gas formers e.g. B. coli and B. aerogenes.