A COMPARATIVE STUDY OF COLIFORM ORGANISMS FOUND IN CHLORINATED AND IN NON-CHLORINATED SWIMMING BATH WATER

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(With 3 Figures in the Text)

In a former series of water examinations (Bardsley, 1934) it was noted that a larger number of *Bact. lactis aerogenes* was obtained from swimming baths which had been chlorinated than from upland surface supplies or from wells and springs. It was suggested that the higher *aerogenes* content might be due to resistance of this organism to the action of chlorine.

A further series of examinations has since been carried out on 386 samples of which 339 were collected from chlorinated and forty-seven from non-chlorinated baths in Manchester, and a number of experiments have been made to test the effect of chlorine on certain bacilli of the coliform group.

Purification of the swimming baths

The open-air pools were not filtered or treated chemically, but about once a month, or oftener, if the bacteriological results indicated it, the baths were emptied and scrubbed out with chloride of lime.

The general principle of purification in the indoor baths was continuous filtration and chlorination similar to the system recommended by the Ministry of Health (1929). The water from the deep end of the bath was pumped to an aerating sprinkler suspended over a large iron collecting tank on the roof of the building. After aeration alumino-ferric was added to precipitate the solids and soda ash to maintain alkalinity. The coarse sand filters were of the "Bell" type. They were washed at least twice a week and opened daily for inspection. The filtered water was steam-heated and treated with chlorine gas or with chlorine in the form of "Chloros" (sodium hypochlorite) which was introduced by a continuous dropping method into the pipes leading from the filters. The purified water entered on the floor of the bath at the shallow end and caused constant bubbling on the surface.

The whole contents of the bath should pass through the purification system about once every 4 hours. The tiles at the bottom were scrubbed daily and any surface scum removed with a hose pipe and a skimming pole. The pH of the water should lie between 7 and 7·6, and the temperature should be about 72° F.

https://doi.org/10.1017/S0022172400011578 Published online by Cambridge University Press

Chlorine content

The free chlorine content in the bath was estimated every hour by the orthotolidine method (Race, 1918). It is not easy to keep this within the prescribed limits of 0·2–0·5 part per million, particularly when the load of bathers is high and the chlorine is absorbed by organic matter almost as soon as it is added. Periods elapse when the chlorine content is insufficient to meet the chlorine demand, and then some bacteria are either unaffected or are not killed and capable of multiplying later. It is the practice in many baths to throw "Chloros" into the plunge in an attempt to remedy the chlorine deficiency. The "Chloros" cannot, however, be properly distributed in the water, and pockets occur where the chlorine content is excessive while at other places there may be scarcely a trace of chlorine present.

Collection of samples

Hicks and his co-workers (1933) pointed out the difficulty of obtaining representative samples of water from swimming baths where the bacterial content varies with depth. The examination of a few paired samples at the beginning of this investigation revealed a discrepancy in chlorine content in samples from the deep and from the shallow ends of the same bath, the deeper end often having a higher chlorine figure and consequently fewer organisms than the shallower part. This difficulty was aggravated in the present instance because "Chloros" had often been added. To eliminate sampling errors as far as possible it was decided finally to take all samples from the outlet. The water was collected in sterile bottles with the aid of a long rod fitted with a clamp to hold the bottle.

Technique of examination

For the first 208 samples, each of ten tubes of double strength MacConkey's bile salt medium was inoculated with 10 c.c. of the sample. Tubes of ordinary strength broth were inoculated with 1 c.c. as follows: three with the undiluted sample, three with 1 : 10, three with 1 : 100 and one with 1 : 1000. After 48 hr. incubation at 37° C. spread plates were made from all tubes which showed acid and gas and three colonies picked from each plate. The strains which were all Gram-negative bacilli were tested in lactose P.W., gelatin, litmus milk, peptone water for indole, for growth in citrate and for the methyl red and Voges-Proskauer reactions.

The last 178 samples were examined by the method recommended by the Ministry of Health (1934), and growth in MacConkey broth at 44° C. was added to the confirmatory tests.

The free chlorine content was estimated in 247 samples by the orthotolidine method and the *p*H determined for eighty of the samples.

Results of the coliform test

(*a*) *On the basis of samples.*

Among the 339 samples from chlorinated baths 160 gave a positive presumptive result in MacConkey broth at 37° C., and among the forty-seven samples from outdoor baths thirty were positive. Table I shows the incidence of *coli*, *aerogenes* and intermediate type on the basis of samples from which they were isolated.

Table I. *The incidence of the types of coliform organisms in chlorinated and non-chlorinated baths*

			Samples yielding							
			coli		Intermediate		*aerogenes*		Other coliform types	
Source of sample	No. examined	No. positive	No.	%	No.	%	No.	%	No.	%
Chlorinated baths	339	160	116	72·50	78	48·75	112	70·00	18	11·25
Non-chlorinated baths	47	30	22	73·33	12	40·00	13	43·33	3	10·00
Totals	386	190	138	72·63	90	47·37	125	65·79	21	11·05

The percentage of samples yielding *coli* was approximately the same in both chlorinated and non-chlorinated waters and was higher than for any other coliform bacillus.

The open-air pools were subject to soil contamination and might, therefore, be expected to furnish more samples containing *aerogenes* and intermediate type than the indoor pools which were protected. Table I shows that on the contrary these organisms were much more frequently found in chlorinated samples. This applied particularly to *aerogenes* which occurred in 70% of chlorinated compared with 43% of non-chlorinated samples.

The twenty-one samples from which other coliform bacilli were isolated comprised two with *coli* II, two with *cloacae*, one with irregular IV, two with irregular V, one with irregular VI, one with irregular VII, and twelve in which the organisms gave anomalous reactions and could not be classified according to Wilson's method (1935).

(*b*) *Results considered on the basis of strains.*

The total number of strains isolated was 2400, of which 2130 were from chlorinated and 270 from non-chlorinated bath water. The classification of these organisms is given in Table II. Only 421 strains were tested in MacConkey broth at 44° C., but there can be little doubt from the other reactions that the organisms belong to the groups indicated (Wilson, 1935).

The *aerogenes* and intermediate types were found much more frequently in chlorinated than in non-chlorinated baths. It is, of course, impossible to determine whether *aerogenes* was a soil contaminant in the open-air pools or was

Table II. *The classification of* 2400 *strains of coliform bacilli isolated from chlorinated and non-chlorinated bath water*

Type of organism	No. isolated from: Chlorinated baths No.	%	Non-chlorinated baths No.	%
Bact. coli I	911	42·77	151	55·93
Bact. coli II	2	0·09	4	1·48
Intermediate I	395	18·54	34	12·59
Intermediate II	10	0·47	8	2·96
Bact. lactis aerogenes I	666	31·27	51	18·89
Bact. lactis aerogenes II	95	4·46	16	5·93
Bact. cloacae	14	0·66	—	—
Irregular I	8	0·38	—	—
Irregular IV	3	0·14	—	—
Irregular V	8	0·38	—	—
Irregular VI	2	0·09	—	—
Irregular VII	1	0·05	—	—
Unclassified by Wilson's method	15	0·70	6	2·22
Totals	2130	100·00	270	100·00

introduced by the bathers themselves. There can be little doubt, however, that in indoor baths the presence of *aerogenes* in large numbers was the result of human contamination, although it remains to be proved whether, being more resistant than *coli*, it was to some extent selected by the chlorine treatment. These points are discussed more fully later on.

Free chlorine and the coliform content

The correlation of the *Bact. coli* results and the free chlorine content in 247 samples is given in Table III.

Table III. *The correlation of the coliform content with the free chlorine present in* 247 *samples of bath water*

No. of samples	Free chlorine content in parts per million	Absent in 100 c.c. No.	%	Coliform organisms found in 100 c.c. No.	%	10 c.c. No.	%	1 c.c. No.	%
81	Nil	27	33·33	19	23·5	29	35·8	6	7·40
106	0·01–0·1	47	44·30	27	25·5	24	22·6	8	7·50
60	More than 0·1	51	85·00	4	6·7	5	8·3	0	—
247		125	50·61	50	20·24	58	23·48	14	5·67

The results of the *coli* test were on the whole very similar for water that contained no chlorine or when it was present in less than 0·1 part per million. Coliform organisms were absent in 100 c.c. in 33 % of samples where there was no free chlorine, and in 44 % of those with the low chlorine content. Their absence may be explained by assuming that in alkaline waters a certain amount of residual chloramine may remain and continue the germicidal action after the free chlorine has been used in the oxidation of organic matter (Race, 1929). Among the samples with the prescribed chlorine content 85 % showed no *coli* in 100 c.c., but 6·7 % had *Bact. coli* present in 100 c.c.

Free chlorine content, alkalinity and total bacterial counts

The effect of alkalinity on the efficacy of chlorine treatment is demonstrated in eighty samples of bath water in Table IV, where the number of bacteria present is given in relation to the *p*H value and the free chlorine content.

Table IV. *Alkalinity and free chlorine content correlated with total bacterial counts in* 80 *samples of swimming bath water*

		No. of samples in which the total counts per c.c. at 22° C. and at 37° C. were	
*p*H	Chlorine content in parts per million	Less than 500	More than 500
7–8·0	More than 0·1	35	4
7–8·0	Less than 0·1	16	16
Less than 7·0	Less than 0·1	2	7

All samples with a high free chlorine content had a *p*H of at least 7·0. Among those with more than 0·1 part per million of chlorine and *p*H of 7·0–8·0, nearly 90% had counts below 500 per c.c. and contained no *coli* in 100 c.c. Samples with less than 1 part per million of chlorine and alkaline reaction were equally divided into those with counts above and those with counts below 500 per c.c. Most of the samples with a low chlorine content and *p*H below 7·0 had high counts.

It is doubtful whether alkalinity in itself affects the bacterial content of bath water apart from the chlorine content, but it is evident that the presence of free chlorine in appreciable quantities was almost always associated with an alkaline reaction.

The effect of chlorination on coli, aerogenes *and intermediate type*

Experiments were carried out to test the effect of chlorine on pure cultures of *coli, aerogenes* and intermediate type isolated from swimming bath water. The organisms were grown on agar for 16–18 hr. at 37° C. and made into suspensions containing approximately 200 million bacilli per c.c.

The chlorine was added in the form of "Chloros" (sodium hypochlorite) as used in certain of the baths. It was stored in the ice-chest where it could be kept for several months without deterioration.

In the first experiments tap water was used for making the suspensions and for preparing chlorine water, but it contained so much oxidizable matter that the chlorine was rapidly absorbed without reducing the bacterial content appreciably. Distilled water was then substituted. This eliminated the variations in chlorine demand and made it easy to calculate the amount of "Chloros" required to give known concentrations of free chlorine. Free chlorine was detected by the orthotolidine method. The standard colour tubes for comparative purposes were prepared according to Race (1918) and were made afresh for each experiment. It was found that 1·25 c.c. of a 0·05% solution of "Chloros"

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in 100 c.c. of distilled water gave a chlorine concentration of 0·5 part per million. To chlorinate the organisms 100 c.c. of water containing 2·5 c.c. of a 0·05% solution of "Chloros" was mixed with 100 c.c. of bacterial suspension. This provided 0·5 part per million of chlorine in the whole and any diminution was due to absorption of chlorine by the organisms. Larger amounts of chlorine destroyed all the organisms unless the bacterial suspension contained considerably more than 200 million per c.c.

The best way of mixing the bacteria and chlorine solution proved to be by pouring them simultaneously into a wide-mouthed sterile flask large enough to

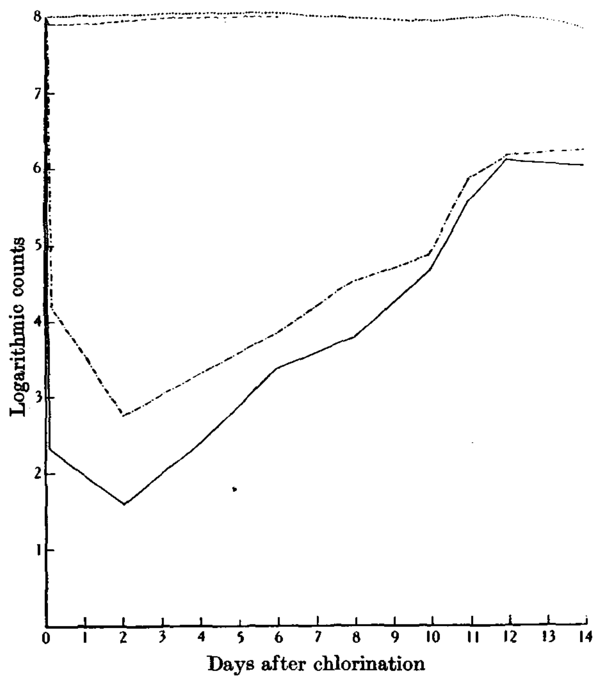

Fig. 1. The effect of chlorine on *Bact. coli*.

——— Control. —·—·— 0·3 parts per million.
– – – – 0·1 parts per million. ——— 0·5 parts per million.

allow thorough shaking. To obtain initial counts the bacterial suspension was mixed with an equal volume of sterile distilled water. Sterile distilled water was used in making dilutions for the plate counts. The temperature was controlled as far as possible by keeping the suspensions in the incubator at 22° C.

Six experiments were carried out with each organism varying the dose of chlorine and to some extent the number of bacteria. Figs. 1–3 show the results obtained with doses of 0·1, 0·3 and 0·5 part of chlorine per million. The experiments represented by these figures were put up on the same day under identical conditions; the figures obtained from them (experiments A) and from the other experiments are to be found in Table V.

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Table V. *The effect of chlorination on strains of* coli, aerogenes *and intermediate type*

Experiment	Organism	Initial counts per c.c. of suspension	Amounts of chlorine in parts per million	Counts per c.c. within 4 hr. of chlorination	Counts per c.c. 48 hr. after chlorination	No. of days required for recovery	Counts per c.c. after recovery
A	*coli*	100,000,000	0·1	80,000,000	85,000,000	—	—
		100,000,000	0·3	16,000	570	12	1,400,000
		100,000,000	0·5	200	40	12	1,200,000
B		150,000,000	0·5	115	32	12	800,000
C		200,000,000	0·5	610	83	15	2,500,000
D		50,000	0·3	None	None	—	—
A	Intermediate	60,000,000	0·1	55,000,000	53,000,000	—	—
		60,000,000	0·3	9,500	1,400	9	730,000
		60,000,000	0·5	315	70	8	168,000
B		80,000,000	0·5	2,000	106	9	2,700,000
C		81,000,000	0·5	3,600	1,580	7	2,000,000
D		3,200,000	0·3	11,000	370	9	165,000
A	*aerogenes*	80,800,000	0·1	75,000,000	77,000,000	—	—
		80,800,000	0·3	12,000	3,400	5	5,000,000
		80,800,000	0·5	1,400	92	5	8,100,000
B		72,000,000	0·5	87,000	250	6	1,350,000
C		79,000,000	0·5	52,500	870	4	2,200,000
D		12,000,000	0·3	46,000	1,330	5	510,000

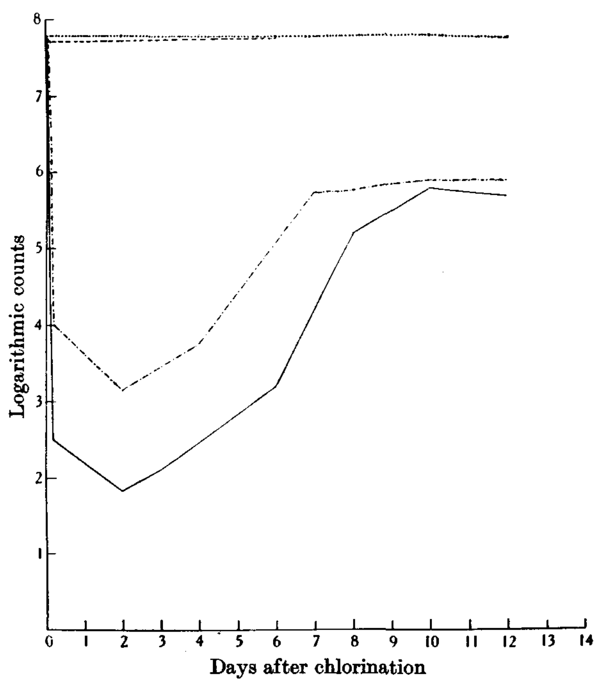

Fig 2. The effect of chlorine on intermediate type.

············ Control. — — — — 0·3 parts per million.
- - - - - 0·1 parts per million. ——— 0·5 parts per million.

There was very little change in bacterial content with 0·1 part of chlorine per million and the experiment was not repeated. Hicks *et al.* (1933) showed that when the concentration of chlorine was below 0·3 part per million the organisms were not killed quickly enough to purify bath water.

Treatment with 0·3 and 0·5 part of chlorine caused a marked reduction in the number of viable organisms. This reduction was rapid, was greater with the larger dose of chlorine and was associated with an immediate reduction in free chlorine from 0·3 to 0·1 and from 0·5 to rather less than 0·2 part per million. The extent of the initial fall was on the whole somewhat less with *aerogenes*

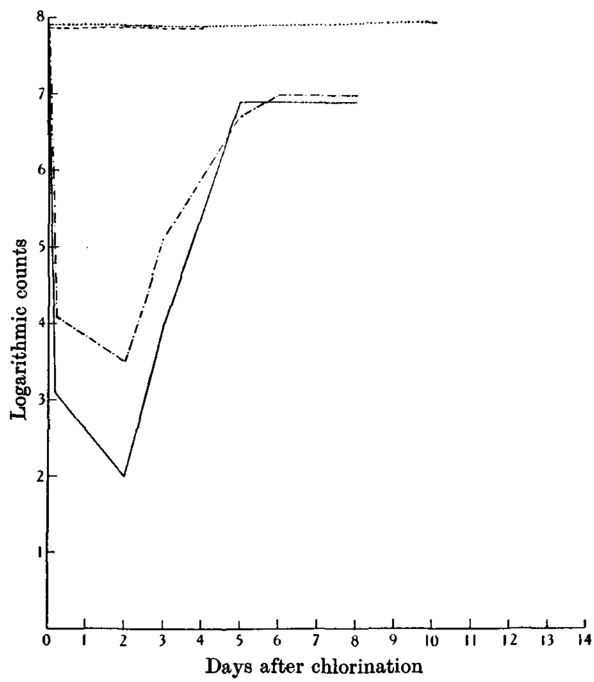

Fig. 3. The effect of chlorine on *aerogenes*.

——— Control. —·—·— 0·3 parts per million.
------ 0·1 parts per million. ——— 0·5 parts per million.

than with the other organisms, but in each case the counts continued to fall gradually for about 48 hr. when all trace of chlorine had disappeared. A gradual rise in numbers then took place but the multiplication of bacteria did not go on indefinitely and never reached the numbers contained in the original suspensions. This may have been due to the fact that distilled water had been used and the organic matter present was limited to the amount provided by the dead bacteria themselves. The highest proportion of organisms found after recovery when working with *aerogenes* was about 1/9th of the original number, with intermediate type it was about 1/30th and with *coli* about 1/100th.

When recovery appeared to be complete the strains were cultured and submitted to the usual confirmatory tests. None of them showed any change in the reactions given, neither were the fermentative processes retarded or altered in any way.

Jordan (1915) and Race (1918) noted the "aftergrowth" of *coli* following chlorination. Race rejected the theory that multiplication may be due to the survival of a resistant minority or that some of the organisms may be merely forced into a state of suspended animation. He pointed out that many factors are involved in the consideration of the phenomenon which is influenced by the dosage of chlorine, by temperature, by the mass of organisms present, and by the chemical nature of the water.

In the experiments described above it would appear that when an amount of chlorine was added which was bactericidal but insufficient to kill all the bacteria, the reduction in numbers took place in two phases. In the first phase the fall was extremely rapid but as soon as the chlorine was reduced to less than 0·2 part per million the destroying action became very slow and was only effective after a prolonged period of contact. Regrowth seems to have started as soon as all the chlorine was removed and as far as could be determined depended largely on the amount of organic matter available, though it is probable that the temperature of 22° C. at which the suspensions were kept also had its effect. It was not considered necessary to try variations from 22° C. as this was approximately the temperature of water in the swimming baths.

DISCUSSION

It has already been pointed out that most of the coliform organisms in swimming bath water are of excretal origin and consist chiefly of bacilli of the *coli* I type although a certain number of intermediate type and *aerogenes* may also occur.

Skinner & Brudnoy (1932) isolated nearly 11% of citrate utilizers among 585 strains of lactose-fermenting bacilli from human faeces, Cruickshank (1930) found that ninety-eight out of 135 samples of human stools yielded *aerogenes* and Gray (1932) isolated this organism from thirty-seven of forty similar specimens by preliminary incubation in citrate medium followed by plating on MacConkey agar. Hill and his co-workers (1929) isolated more than 39% *aerogenes* among 200 strains of Gram-negative bacilli from genito-urinary infections and Burke-Gaffney (1933) found that 8% of 500 faecal and 52% of 1000 urinary strains were *aerogenes*. These facts help to account for the presence of *aerogenes* and intermediate type in swimming baths, but they do not explain why these organisms should be found much more often in chlorinated than in non-chlorinated baths.

The exposure of suspensions of coliform bacilli to chlorine has not revealed any great difference in sensitivity although *aerogenes* does, on the whole, seem to be less rapidly killed by chlorination than intermediate or *coli*, and it cer-

https://doi.org/10.1017/S0022172400011578 Published online by Cambridge University Press

tainly shows the most vigorous recovery. Recovery is, however, very slow even with *aerogenes* and is hardly likely to be a factor in determining the flora of baths which are efficiently chlorinated. Unfortunately it is extremely difficult in practice to carry out effective chlorination, not only because of the constantly changing organic content of the bath, but also because there is only one point at which chlorine can be introduced, so that there are bound to be portions where chlorination is feeble and where the difference in sensitivity between *coli* and *aerogenes* would have its effect. The fact that this difference is so slight explains why *aerogenes* does not necessarily become dominant in chlorinated swimming baths, although the proportion of *aerogenes* to *coli* certainly increases in waters which have been chlorinated. This has been noted in two previous series of examinations. *Aerogenes* was found in 35 % of thirty-seven polluted bath waters compared with 20 % of 286 polluted waters from wells and 8 % of 750 polluted waters from upland surface areas (Bardsley, 1934). In the second series of examinations (Bardsley, 1938) McCrady figures were available and numerical estimations were made. The *aerogenes* count was here included with the other citrate utilizers and grouped under the heading I.A.C. (intermediate, *aerogenes*, *cloacae*). Among 225 samples of non-chlorinated town water the ratio of *coli* I : I.A.C. was 100 : 28 by the Ministry's method and 100 : 64 by Wilson's method; among 149 samples of chlorinated town water these ratios were 100 : 50 and 100 : 98. Among nineteen samples from non-chlorinated baths the ratio of *coli* I : I.A.C. was 100 : 68 by the plating method and 100 : 79 by Wilson's method, while among thirty-nine samples of chlorinated bath water the ratios were 88 : 100 by the plating method and 47 : 100 by Wilson's method. In the latter series the I.A.C. group were actually shown by the numerical method of estimation to be dominant among the comparatively few bath samples tested.

It is possible that chlorination may be superseded by some other method of disinfection, particularly the use of ozone or ozonized air. It would be interesting to compare ozonized bath water with chlorinated bath water if such samples were available for bacteriological examination.

Summary

The investigation deals with the bacteriological examination of 386 samples of water from Manchester swimming baths where the method of purification could be studied.

There were 339 samples from chlorinated pools of which 160 gave acid and gas in MacConkey broth at 37° C. and 2130 strains were isolated. The remaining forty-seven samples were from untreated open-air pools. Thirty of these samples were positive and 270 strains were isolated. The strains were classified according to Wilson's method into the *coli*, intermediate, *aerogenes* and irregular groups, and only 1·78 % of the total were found to be irregular. Both series of samples included more than 72 % which contained *coli*, and among the chlori-

nated samples no less than 70% yielded *aerogenes* and nearly 49% intermediate type, while among the non-chlorinated baths there were 43% with *aerogenes* and 40% with intermediate type. There was a similar difference in the proportions of *coli*, *aerogenes* and intermediate type in chlorinated and in non-chlorinated baths when the results were considered on the basis of strains (Table II).

Since these differences in proportion were considered significant, and since the chief variation in conditions between outdoor and indoor baths consists of chlorination, a series of experiments was devised to test the effect of chlorine on pure cultures of *coli*, *aerogenes*, and intermediate type. There was very little change after adding 0·1 part of chlorine per million, but the addition of 0·3 and 0·5 part was followed by very rapid reduction in numbers with diminution in free chlorine. This reduction was on the whole least with *aerogenes*. The numbers continued to fall for about 48 hr. until all trace of chlorine had disappeared and then regrowth occurred. This regrowth was most vigorous and most rapid with *aerogenes* (see figures) but was very slow in all cases.

ACKNOWLEDGEMENTS. I am greatly indebted to Mr Teasdale, the Manchester Baths Superintendent, and to Inspectors J. E. Lord and A. Moss for their kindness in collecting samples.

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(*MS. received for publication* 30. VII. 38—Ed.)