DESTRUCTION OF BACTERIA IN SEWAGE AND OTHER LIQUIDS BY CHLORINE AND BY CYANOGEN CHLORIDE

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(With 7 figures in the Text)

Treatment of sewage or sewage effluent with chlorine may be advocated for several reasons. These include disinfection of sewage in cases of emergency, destruction of pathogens or avoidance of unsightly growths where an effluent is discharged to a river or near bathing beaches, prevention of septicity in sewage before it reaches a treatment works, and the relief of ponding in trickling filters. Chlorination is much less generally advocated in this country than in America, where sewage is usually weaker and the dilution which it receives on discharge to rivers much greater than in Great Britain.

Emergency chlorination was sometimes rendered necessary during the war and the chlorination of effluents discharged to British rivers is sometimes advocated in the interests of public health. It was considered advisable for these reasons to obtain data for the effect of chlorine on samples of English sewage and to study the factors affecting destruction and aftergrowth of bacteria. Since it had been found (Allen, Blezard & Wheatland, 1946, 1948) that chlorinated effluents from sewage to which gas liquor had been admitted were particularly toxic to fish, special attention was devoted to a study of the bactericidal properties of such effluents.

PREVIOUS WORK

Houston (1910), as a result of experiments carried out for the Royal Commission on Sewage Disposal, concluded that for an effluent of good chemical quality the dose of chlorine required to ensure that no coliform organisms were detectable in 1 ml., after a period of contact of 10 hr., was between 1 and 10 p.p.m., and that the average doses required for periods of contact of 1 hr. and of 6 min. would be about 10 and 40 p.p.m. respectively.

The conception of chlorine demand and the use of the *o*-tolidine test developed by Ellms & Hauser (1913) placed chlorination on a much sounder basis. Disinfection was found by various workers to be more effective when sufficient chlorine had been added to react with all substances capable of absorbing chlorine, and to leave residual chlorine detectable by its reaction with *o*-tolidine.

Lea (1934) pointed out that chlorination of sewage may result in the formation of a wide range of derivatives, including chloramines and chlorinated proteins, different concentrations of which are required to give a colour with *o*-tolidine. For results to be significant the tests applied to sewage should, therefore, be rigidly standardized. Lea recommended the use of a more acidic reagent to ensure that the pH value of the alkaline sewage was reduced to the zone required for correct yellow colour, and a larger proportion of reagent to sewage in order to avoid the diffuse and transient colour which sometimes appeared with the weaker reagent.

Tiedemann (1927) found, in studies on sewage chlorination at Huntington (U.S.A.), that doses of chlorine sufficient to give a colour with starch iodide, but not sufficient to give a colour with *o*-tolidine, reduced both the total count and the count of coliform bacteria by 98–99 % in 10 min., and by 99·9 % in 1 hr. When there was 0·2–0·6 p.p.m. residual chlorine by the *o*-tolidine test 99·9 % of the bacteria were destroyed in 10 min.

Scott & Kleek (1934) showed that, even with large amounts of residual chlorine, periods of contact of 5 min. or less were often ineffective, whereas 0·5 p.p.m. residual chlorine acting for 15 min, or 0·2 p.p.m. acting for 30 min., gave good results.

Symons, Simpson & Torrey (1938), using the spot-plate test introduced by Symons (1937), found that doses of chlorine sufficient to give 0·1 p.p.m. residual chlorine reduced the bacterial count of sewage by 99 % in 15 min. and by 99·6–99·8 % in 30–40 min. In cold weather the bactericidal efficiency was lower than in warm weather. White (1939) found that sewage effluent at Johannesburg could not be sterilized by doses of chlorine equivalent to the 10 min. chlorine demand. It required a dose equivalent to the 4 hr. chlorine demand acting for 4 hr. to destroy coliform bacteria, and an even longer period of contact to effect complete sterility.

Free chlorine is a more effective germicide than chloramines and both are greatly affected by pH value and by temperature. Thus Streeter (1943), using the *p*-aminodimethylaniline test (developed by Moore (1943) to distinguish free chlorine from

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chloramine), found that 0·1–0·2 p.p.m. of free chlorine destroyed coliform bacteria in 2 min. at pH 6–7, in 4 min. at pH 8, and in 6 min. at pH 9. About twenty to thirty times as much chloramine was required to effect the same destruction.

Rudolfs & Ziemba (1934) found that sewage treated with a dose of chlorine below the demand produced an appreciable reduction in count on mixing with fresh sewage, suggesting the bactericidal effect of reaction products of chlorine. The percentage of bacteria destroyed in chlorinated sewage appeared to depend on the initial concentration of chlorine rather than on the percentage of the demand satisfied, so that with sewage with a high chlorine demand a dose of chlorine equal to a given proportion of the demand destroyed a relatively high percentage of bacteria.

Marks, Joiner & Strandskov (1948) found that amperometric titration or the use of *p*-aminodimethylaniline in the presence of iodide gave much higher values for 'residual chlorine' in samples of chlorinated sewage than were recorded by the *o*-tolidine method. Samples to which a dose of chlorine lower than the chlorine demand had been added, and which therefore gave no colour with *o*-tolidine, frequently showed appreciable quantities of 'residual chlorine' by these two methods. These values were assumed to be due to reaction products of chlorine which were not detected by *o*-tolidine, but which, nevertheless, exerted an appreciable bactericidal action.

The lethal effect of chlorine increases with rise in temperature. Rudolph & Levine (1941), using a suspension of *B. metiens* spores, found that between 20 and 50° C. the time required to destroy bacteria with a given dose of chlorine was reduced by 46–66 % for every 10° rise in temperature.

It has been generally agreed that the behaviour of *Bacterium coli* is a satisfactory criterion by which to assess the effect of chlorination on intestinal pathogens. Results obtained by Butterfield, Wattie, Megregian & Chambers (1943) suggest that over the pH range 8·5–10·7 *Bact. typhosum* is more sensitive than *Bact. coli* or *Ps. pyocyanea*, but that between pH 6·5 and 7·8 the reverse holds good.

EXPERIMENTAL

The chlorine demand of each sample of liquid was determined by adding carefully graduated doses of chlorine to measured quantities of the sample, and noting the lowest concentration which gave a distinct yellow colour with the modified *o*-tolidine reagent (Lea, 1934) after a period of contact of 10 min. In tests to find the effect of different doses of chlorine on the bacterial count the calculated quantities of chlorine were added to quantities of 250 or 500 ml. of the sample in sterile stoppered bottles, the liquid first being warmed to 20° C. The bottles were placed for the first hour in a water-bath at 20° C. and subsequently in an incubator at the same temperature. At intervals during incubation a sample was abstracted aseptically from each bottle and treated with a small quantity of sodium thiosulphate to destroy residual chlorine. Bacterial counts were determined on the dechlorinated sample. At different times tests were carried out with 'Stabochlor' (a stable brand of bleaching powder), with 'Chloros' (a solution of sodium hypochlorite), and with chlorine water, but in each case the chlorine demand was determined with the same chlorinating agent as was used for the subsequent bacteriological tests.

Unless otherwise stated, total counts of bacteria were determined on plates of nutrient agar incubated at 20° C. for 6 days. Presumptive counts of coliform bacteria were determined in tubes of MacConkey broth incubated at 37° C., three parallel tubes being inoculated with each dilution. It was found that the normal period of incubation (2 days) was often insufficient for development of surviving coliforms in chlorinated samples, and in these cases maximum counts were obtained only after incubation for periods up to 6 days.

Reduction in plate count

In Table 1 are shown results of tests with ten samples of settled domestic sewage, all taken from the same works at intervals over a period of several months. The pH values of samples of sewage used in this investigation were not determined but those of fifty samples, taken from the same works over a period of 5 months since the investigation was finished, have ranged from pH 6·8 to 8·7, with an average of pH 7·6. The results lead to the following conclusions:

(1) In general, for relatively short periods of contact (for example, up to 1 hr.), the reduction in bacterial count becomes greater as the initial concentration of chlorine increases. An apparent exception may occur, as in Test no. 7, where the doses are all relatively large and do not differ greatly from each other. A possible reason for this is discussed later.

(2) For doses lower than the chlorine demand, reduction in count depends not only on the proportion of the chlorine demand satisfied, but on the absolute size of the dose (cf. Rudolfs & Ziemba, 1934). This is illustrated in Table 2 which shows the percentage of bacteria destroyed after contact for 15 min. by various doses, grouped according to the proportion of the chlorine demand which they represent. It is evident that within any one group (10, 25, or 50 %) the destructive effect rises with the absolute value of the dose. With doses of chlorine just sufficiently large to satisfy the demand and to leave residual chlorine detectable by the *o*-tolidine test (100 % group) the reduction in count after

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Table 1. *Effect of different doses of chlorine, after different periods of contact, on plate counts* of bacteria in settled sewage*

Samples of domestic sewage, all taken from the same works, at intervals over a period of several months

Test no.	10-min. chlorine demand (p.p.m.)	Period of contact	Plate count of chlorinated sewage (dose of chlorine added (p.p.m.) shown in brackets at the head of each column)				
			(14)	(18)	(22)		
1	16–18	Nil	5,400,000	5,400,000	5,400,000		
		15 min.	20,600	10,500	3,500		
		30 min.	2,740	1,840	670		
			(2)	(5)	(10)	(20)	
2	18–20	Nil	9,000,000	9,000,000	9,000,000	9,000,000	
		15 min.	2,550,000	1,220,000	209,000	113,000	
		24 hr.	18,600,000	5,200,000	6,340	85	
			(7)	(14)			
3	12–14	Nil	6,500,000	6,500,000			
		15 min.	230,000	137,000			
		30 min.	132,000	51,000			
			(1)	(2·5)	(5)	(10)	
4	8–10	Nil	8,700,000	8,700,000	8,700,000	8,700,000	
		15 min.	7,100,000	6,200,000	557,000	123,000	
			(10)	(12)	(14)		
5	10–12	Nil	1,880,000	1,880,000	1,880,000		
		15 min.	105,000	71,000	57,000		
		24 hr.	1,000	900	900		
			(6)	(8)	(10)		
6	7–8	Nil	2,930,000	2,930,000	2,930,000		
		18 hr.	258,000	132,000	8,100		
			(14)	(16)	(18)		
7	15–16	Nil	3,100,000	3,100,000	3,100,000		
		45 min.	5,600	6,500	9,200		
		18 hr.	70	50	270		
			(10)	(14)	(18)		
8	9–10	Nil	3,480,000	3,480,000	3,480,000		
		45 min.	25,500	12,100	9,570		
		18 hr.	182	252	72		
			(10)	(15)	(20)	(25)	
9	14–15	Nil	11,900,000	11,900,000	11,900,000	11,900,000	
		1 hr.	7,200	6,300	4,510	2,470	
		24 hr.	1,600	70	290	15	
			(6)	(11)	(16)	(21)	(26)
10	15–16	Nil	2,300,000	2,300,000	2,300,000	2,300,000	2,300,000
		20 min.	253,000	13,620	12,800	7,610	4,360
		1 hr.	74,000	8,300	4,710	1,990	400
		6 hr.	21,600	460	110	35	45
		22 hr.	124,000	290	55	400	18

* The medium used was nutrient agar, except in Test no. 9 for which sodium caseinate agar was used.

Table 2. *Percentage reduction in plate count resulting from different doses of chlorine after a period of contact of* 15 *min.*

Dose of chlorine (as percentage of chlorine demand ...	10		25		50			100			
Dose of chlorine (p.p.m.)	1	2	2·5	5	5	7	10	10	12	14	20
Percentage reduction in plate count	18	72	29	86	93·6	96·5	97·7	98·6	96·2	97·9	98·7

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contact for 15 min. ranged from 96·2 to 98·7 %. Owing to the 'break-point' effect the bactericidal action of doses of chlorine greater than the demand is not always proportional to the size of the dose or to the concentration of residual chlorine.

(3) The proportion of bacteria destroyed increases as the period of contact is prolonged, but after reaching a minimum the count begins to increase again, as shown in Test no. 10 (Table 1). The period of contact over which bacteria continue to be destroyed depends on the size of dose and on the chlorine demand of the liquid.

Table 3. *Effect of different doses of chlorine, after different periods of contact and at different temperatures, on the presumptive count of coliform bacteria in sewage*

Test no.	10 min. chlorine demand (p.p.m.)	Period of contact	Presumptive count of coliform bacteria at 37° C. (dose of chlorine added (p.p.m.) and temperature during period of contact, shown at head of each column)					
1	12–14	Nil	7 p.p.m. (20° C.) 10^5–10^6			14 p.p.m. (20° C.) 10^5–10^6		
		15 min.	10^3–10^4			< 10		
		30 min.	10			< 10		
		2 days	10^2–10^3			< 1		
		4 days	10^4			< 1		
			10 p.p.m.			20 p.p.m.		
			1° C.	20° C.		1° C.	20° C.	
2	18–20	Nil	10^5–10^6	10^5–10^6		10^5–10^6	10^5–10^6	
		15 min.	—	10^3–10^4		—	1	
		30 min.	$> 10^3$	10–10^2		1–10	1/10–1	
			7 p.p.m.		10·5 p.p.m.		14 p.p.m.	
			5° C.	20° C.	5° C.	20° C.	5° C.	20° C.
3	12–14	Nil	10^5–10^6	10^5–10^6	10^5–10^6	10^5–10^6	10^5–10^6	10^5–10^6
		15 min.	10^4–10^5	10^3–10^4	10^3–10^4	10^2	10^2–10^3	10

Reduction in count of coliform bacteria

Results of experiments summarized in Table 3 show that at 20° C. a dose of chlorine slightly greater than the chlorine demand reduces the coliform bacteria to a very small fraction of the original number after contact for 15 min. With lower doses at the same temperature or with the same dose at lower temperatures the proportion of bacteria destroyed in the same time is much less.

Aftergrowth of bacteria in chlorinated sewage

The aftergrowth of bacteria evident in some of the tests recorded in Tables 1 and 3 was studied in a further series of experiments. When chlorine is added to sewage the rapid absorption of chlorine which takes place in the first few minutes is followed by a much slower reaction which persists for several days. The chlorine demand therefore increases appreciably as the period of contact is prolonged, an effect which is illustrated by the results in Table 4.

Table 4. *Effect of period of contact (at 20° C.) on chlorine demand of settled sewage*

Chlorine demand expressed as smallest dose, in parts per million, which gave a positive test for residual chlorine

Sample 1		Sample 2		
			Chlorine demand	
Period of contact	Chlorine demand with *o*-tolidine	Period of contact	With *o*-tolidine	With starch-iodide
15 min.	16	15 min.	15	13
1 hr.	16	1 hr.	16	13
2 hr.	18	2 hr.	16	13
19 hr.	28	4 hr.	18	13
27 hr.	> 28	6 hr.	20	14
		22½ hr.	25	21
		28 hr.	25	21
		30½ hr.	26	21
		46½ hr.	30	22

These results also show that the apparent chlorine demand may be appreciably lower when determined by the starch-iodide test than by the *o*-tolidine test.

With this slow absorption taking place it might be expected that sewage which initially contained a lethal concentration of chlorine would become non-toxic to bacteria after a certain period of incubation. Any bacteria which had survived the inhibitory period uninjured would then be expected to grow. The larger the initial dose of chlorine the longer the interval tends to be before it is absorbed and aftergrowth begins, though the relation is not so simple as this in the case of a liquid exhibiting a break point.

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If the dose were sufficiently large to effect sterilization the lag period would be infinitely long.

Results of a number of experiments in which bacterial counts of samples of sewage, treated with different doses of chlorine, were followed during a prolonged period of incubation, are expressed graphically on a logarithmic scale in Fig. 1. Curves A, B, C, D and E correspond respectively to Tests 2, 7, 8, 9 and 10 in Table 1, but they include observations recorded over a longer period. With higher initial concentrations of chlorine not only is the proportion of bacteria destroyed after a short period of contact increased, but the period before after-growth becomes appreciable tends to be prolonged. Thus for doses of 6, 11, 16, 21 and 26 p.p.m. in curves shown in Fig. 1E the periods before after-growth brought the bacterial population back to its original level were respectively 40, 61, 68, 63 and 142 hr. For many purposes the effectiveness of chlorination should be judged by this double criterion. The percentage of bacteria destroyed in a given time may by itself give a false impression of security. For example, Fig. 1A shows that although a dose of 5 p.p.m. destroyed 86 % in 15 min. there were still more than a million survivors per ml., and after 24 hr. there was a large and actively growing population.

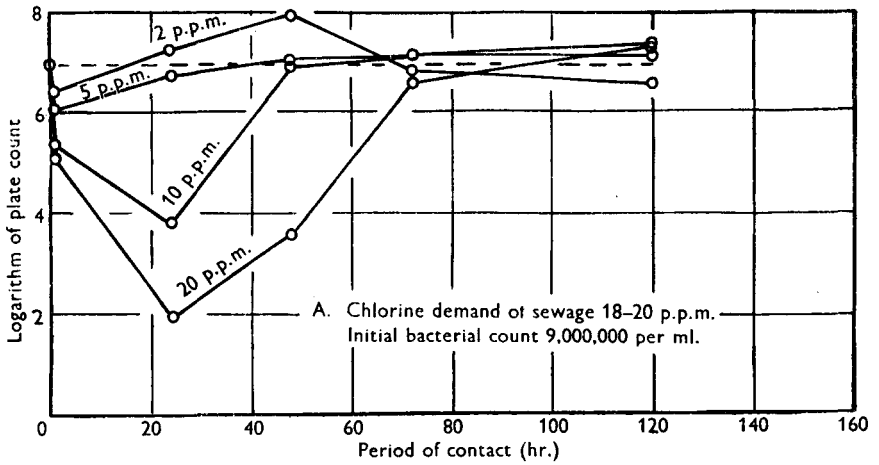
Neither the percentage of bacteria destroyed nor the lag period before aftergrowth occurs are always directly related to the size of dose. The rate at which bacteria are destroyed depends on the nature of the bactericidal agent, and the relative concentrations of free chlorine, chloramine, or other chlorine compound may vary considerably with the size of the dose. For doses near the break-point a larger addition of chlorine may result in a smaller residual concentration and a less effective germicidal action. Rapidity of aftergrowth also depends on the character of the organisms surviving. These differ with different doses of chlorine, as is shown later.

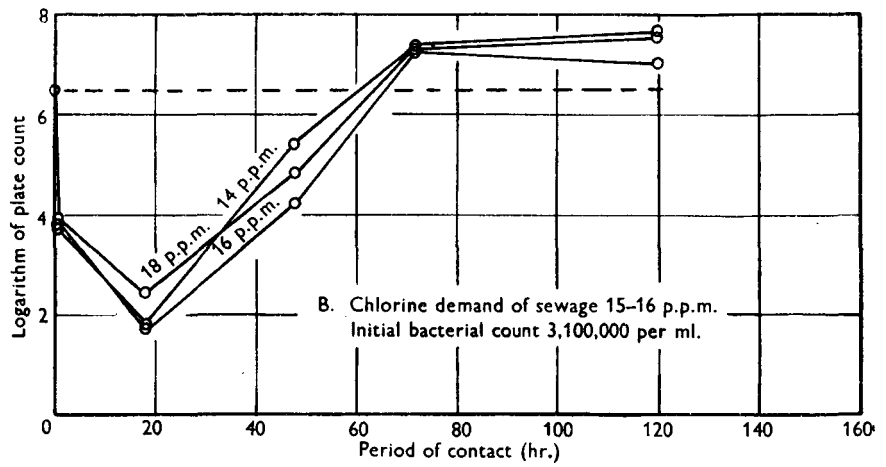
Number and character of surviving bacteria

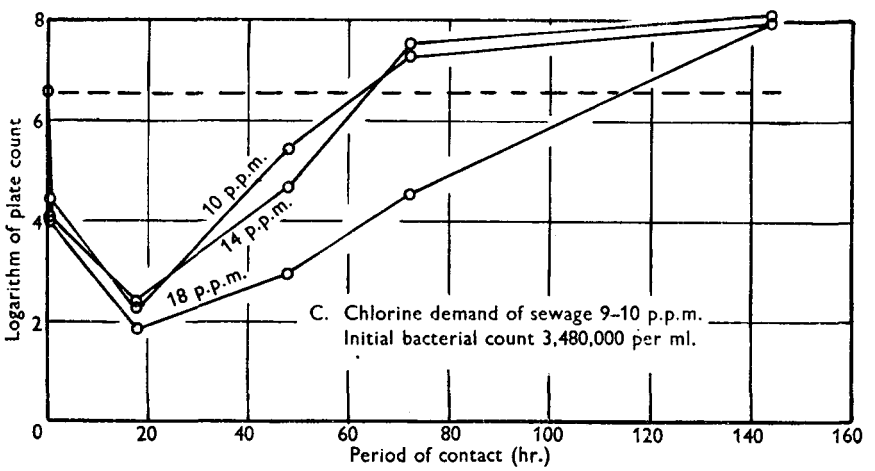
It was noticed that when the dose of chlorine was comparatively small, surviving bacteria attained only a modest level of population, but with larger doses, although the lag period was longer, the survivors eventually grew to much larger numbers. In Fig. 2 are recorded results of two experiments showing the numbers of bacteria in samples of sewage receiving different doses of chlorine. In Exp. A all the doses were comparatively small and were much below the chlorine demand. The level of population reached by the aftergrowth was in no case very high, and after reaching a peak the numbers fell rather rapidly. In Exp. B the doses of chlorine covered a wide range and included two doses smaller and three greater than the chlorine demand. Counts of bacteria in unchlorinated sewage (shown by the broken line) and in the sample treated with 6 parts of chlorine per million remained throughout the period of 16 days at a low level, usually at appreciably below 20 million per ml. In samples receiving higher doses of chlorine the numbers of bacteria reached much higher levels. In the unchlorinated sewage and in the sample receiving 6 p.p.m. in Exp. B, and in all samples in Exp. A, microscopic examination revealed the presence of Protozoa which had attained large numbers after the samples had been incubated for several days. In samples receiving the higher doses in Exp. B no Protozoa were visible. It seemed likely, therefore, that, as suggested by previous workers (cf. Rudolfs & Gehm, 1935), destruction of the Protozoa by the chlorine had removed the factor largely responsible for restricting the population. The level actually attained differed appreciably in samples receiving different doses.

Thirty-two pure cultures of surviving bacteria were isolated from plates poured from chlorinated samples used in Exp. B (Fig. 2) at various stages during incubation. These cultures were tested for their ability to ferment dextrose, lactose, sucrose and maltose, to reduce nitrate, to produce indol and to liquefy gelatin. The majority (25 cultures) were Gram-negative rods, 5 were Gram-positive rods, and 2 were Gram-positive cocci. Five cultures appeared to be species of *Proteus*, 4 were strains of *Ps. pyocyanea*, 3 were strains of *Achromobacterium*, the characters of 5 cultures corresponded with those of *Bacterium metalkaligenes* (Levine & Soppeland, 1926), and 8 were non-lactose-fermenting strains of the genus *Bacterium* (Topley & Wilson, 1946) which formed acid, but no gas in dextrose, or of *Shigella* (Bergey, 1948). All strains isolated from samples receiving the higher doses (16, 21, and 26 p.p.m.) of chlorine and showing a high level of population during aftergrowth were Gram-negative rods. One sample (which had received 16 p.p.m.) showed a virtually pure culture of *Ps. pyocyanea*, but the two remaining samples contained mixed cultures. This difference in species, no doubt, accounts for differences in the maximum numbers attained in the various samples, these being an expression of the natural levels of population of the surviving organisms.

An experiment to find the effect of chlorination on destruction and aftergrowth in a pure culture was carried out with a strain of *Proteus* isolated from chlorinated sewage. Several bottles containing settled sewage which had been sterilized by heat were inoculated with this bacterium and incubated at 20° C.; after 3 days the chlorine demand of the culture was found to be 3 to 4 p.p.m. Six bottles were then each treated with 5 parts of chlorine per million, one bottle was reserved as control, and all bottles were incubated at 20° C. At intervals during a period of 13 days plate counts were made of the control sample and of one of the chlorinated samples.







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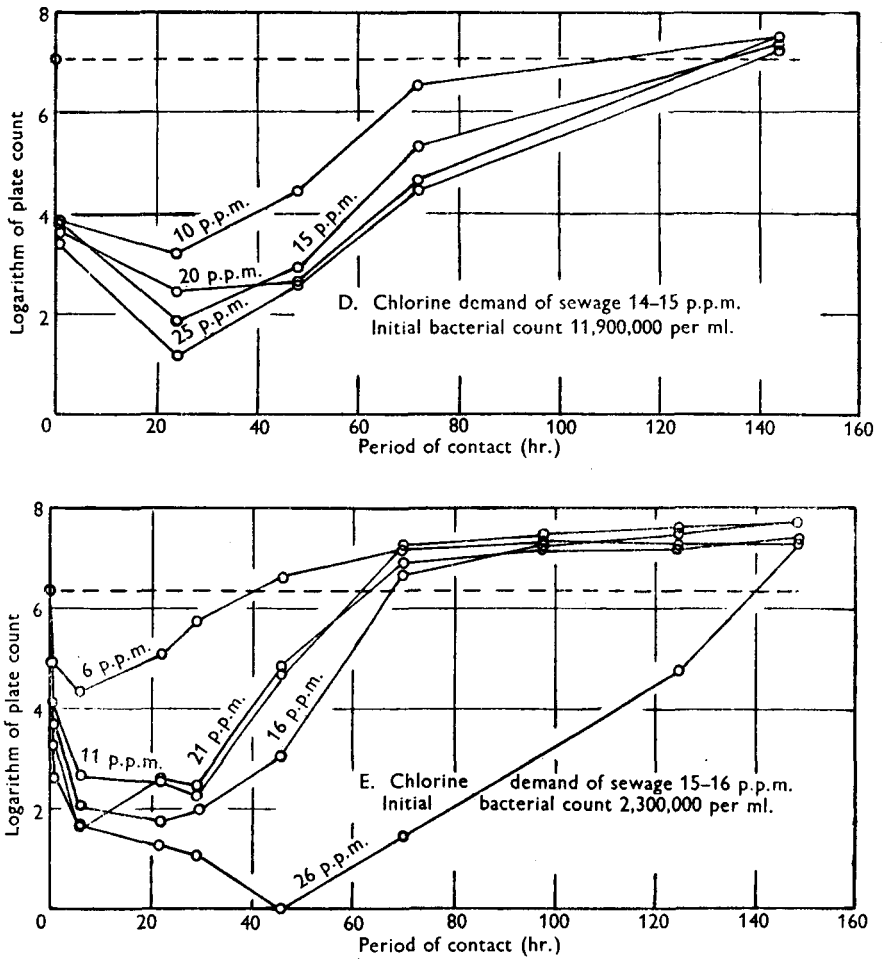

Fig. 1. Effect of size of dose and period of contact on destruction and aftergrowth of bacteria in chlorinated settled sewage (at 20° C.). (Original level of bacterial population shown by dotted line in each graph.)

Each bottle of chlorinated sewage was thus opened and used only once. Results (Table 5) show that, although nearly all the cells of this organism were killed by the chlorine, the few survivors quickly grew and eventually attained about the same level of population as those in unchlorinated sewage. It is evident, too, that aftergrowth in chlorinated sewage is due to organisms which have survived treatment rather than to contaminants introduced after chlorination, and that organisms thus surviving are not intrinsically capable of reaching a higher population than the cells in the parent culture.

Effect on bacterial count of diluting chlorinated sewage with river water

In order to see to what extent discharge of chlorinated sewage might affect the number of bacteria in a river, an experiment was conducted in the laboratory with mixtures of sewage diluted with different proportions of river water. Settled sewage was treated with sufficient chlorine (12 p.p.m.) to satisfy the 10-min. chlorine demand and to leave a slight residual value. After 15 min. at 20° C. the bacterial count had been reduced from 8,100,000 to

Table 5. *Effect of chlorination on destruction and aftergrowth of pure culture of* Proteus *sp. in sterilized settled sewage*

Period of contact	Plate counts (per ml.) Unchlorinated sample	Sample treated with 5 p.p.m. chlorine
0	166,000,000	(166,000,000)
18 hr.	109,000,000	4
3 days	112,000,000	208,000
5 days	166,000,000	67,000,000
7 days	168,000,000	88,000,000
10 days	181,000,000	107,000,000
13 days	209,000,000	205,000,000

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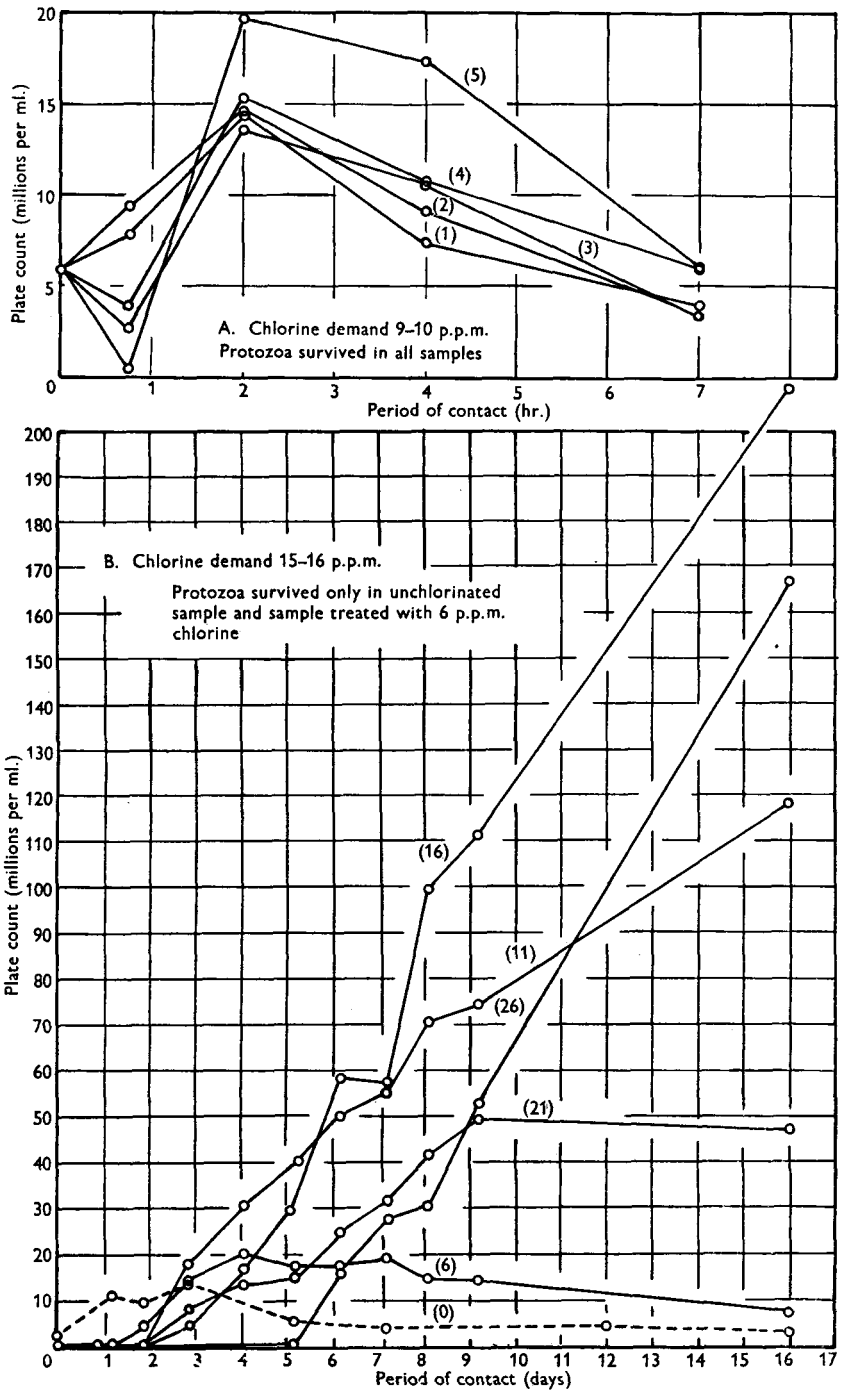

Fig. 2. Relation between size of dose, survival of Protozoa, and level of population attained by surviving bacteria in chlorinated settled sewage. (Dose of chlorine shown in brackets on each curve.)

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104,000, and at this stage the chlorinated sewage was mixed with river water (with a bacterial count of 34,500) in proportions ranging from 1:1 to 1:8, the total volume of the mixed liquids being 1 l. in each case. The mixtures were maintained at 20° C. in separate beakers, each fitted with a revolving paddle which kept the contents continually stirred. At intervals over a period of 7 days the plate count of each mixture was determined. Results (Table 6) show that admixture with the chlorinated sewage reduced the bacterial count of the river water considerably and kept it at a low level for at least 2 days. Subsequent aftergrowth, however, raised the number of bacteria considerably above the initial population of either the sewage or the river water. The initial bacterial counts of the mixtures of river water and chlorinated sewage (shown in brackets) are calculated from the known counts of the constituent liquids.

Table 6. *Effect on bacterial count of diluting chlorinated sewage with river water*

Period of contact	Plate counts of sewage mixed with river water in proportions			
	1 : 1	1 : 2	1 : 4	1 : 8
Nil	(70,000)	(59,000)	(50,000)	(44,000)
1 hr.	2,500	4,400	3,900	4,900
24 hr.	300	500	< 100	< 100
2 days	200	—	—	850
4 days	477,000	—	85,500	> 1,000,000
7 days	72,000,000	—	19,000,000	36,000,000

Effect of chlorination on bacterial counts of liquids containing gas liquor or thiocyanates

During an investigation of the effects of chlorination on sewage effluents it was noticed that, with samples of effluent from an industrial area, doses of chlorine frequently exerted a greater bactericidal and bacteriostatic effect than similar doses applied to purely domestic sewage or effluent. Such industrial effluents were also found to be highly toxic to fish when treated with a dose of chlorine much lower than the demand. Investigation showed that the substance mainly responsible for the toxicity was cyanogen chloride, formed by reaction between the chlorine and the thiocyanate (Allen *et al.* 1946, 1948). Thiocyanate is originally introduced into the sewage as a constituent of gas liquor and small quantities may survive treatment at the works. Some experiments were therefore carried out to see to what extent the presence of gas liquor or its constituents affected the death and aftergrowth of bacteria when sewage was chlorinated.

Thiocyanate has a high chlorine demand and conditions would not be comparable if tests were made, for example, with one sample of effluent and with a similar sample to which thiocyanate had been added; the same dose of chlorine would then represent a different proportion of the chlorine demand in the two samples. The tests were therefore made with peptone water. The chlorine demand of a 0·1 % solution of peptone was determined and two dilutions of the solution were prepared—one with a chlorine demand of 10 p.p.m., and the other with a chlorine demand of 15 p.p.m. The two batches were distributed in quantities of 500 ml. in sterile 1-l. narrow-necked bottles. To each bottle of the more dilute peptone water was added 0·22 ml. of a sample of spent gas liquor, preliminary tests having shown that this was the amount required to raise the chlorine demand from 10 to 15 p.p.m. One medium therefore consisted of a solution of peptone water alone, the other medium of peptone water containing 0·044 % spent gas liquor, and both media had a chlorine demand of 15 p.p.m. Five bottles of each batch were each inoculated with one loopful of sewage to provide a mixed flora and the ten bottles were incubated at 20° C. overnight to allow moderate growth of bacteria to take place. Doses of chlorine equivalent to 0, 5, 10, 15 and 20 p.p.m. were then added to the different bottles in each batch of medium, and the bottles were re-incubated. Plate counts of the contents of each bottle were determined immediately before the addition of chlorine and also at intervals for a period of 6 days.

Results (Table 7) show that the bactericidal action of chlorine was greatly enhanced by the presence of the gas liquor. Doses of 10 parts of chlorine per million and above resulted in almost complete destruction of bacteria in solutions containing gas liquor and the few survivors were unable to proliferate during the test period of 6 days. In the absence of gas liquor doses of chlorine of this order destroyed almost all the bacteria, but the survivors were able to grow after about 48 hr. and they attained large numbers after 6 days.

Experiments in which a solution of ammonium or potassium thiocyanate was substituted for gas liquor (Table 8) showed similar results. The concentration of thiocyanate found to be necessary to raise the chlorine demand of peptone water from 10 to 15 p.p.m. was about 0·8 p.p.m. (as CNS). The concentration of thiocyanate in the bottles containing gas liquor (Table 7) was approximately 0·6 p.p.m. In the two series of experiments, therefore, the concentrations were comparable.

That the bactericidal effect was not always exactly reproducible under apparently similar conditions is shown by differences in the results in Table 8. In Exp. I doses of chlorine of 10 or 15 p.p.m. resulted, in the presence of thiocyanate, in virtual destruction of the bacteria with no sign of aftergrowth for at least 6 days. In Exp. II, with doses of the same order, appreciable aftergrowth had taken place at the end of 7 days. Results of a similar series of tests with different samples of sewage sometimes revealed even

greater inconsistency. Reasons for this are discussed later.

It is possible that the enhanced bactericidal effect of chlorine in the presence of thiocyanate may have some industrial applications—for example in condenser cooling systems, where the recirculated cooling water is often chlorinated to prevent slimy growths on cooling towers. It is intended to investigate this possibility.

Comparative bactericidal effects of cyanogen chloride and chlorine

The bactericidal properties of cyanogen chloride and chlorine were compared by adding different concentrations of these substances to a culture of *Bact. coli* type I growing in peptone water. Cyanogen chloride is conveniently obtained by chlorinating a solution of thiocyanate:

$NH_4CNS + 4Cl_2 + 4H_2O \rightarrow CNCl + NH_4Cl + H_2SO_4 + 6HCl$,
$KCNS + 4Cl_2 + 4H_2O \rightarrow CNCl + KCl + H_2SO_4 + 6HCl$.

By displacing the cyanogen chloride so formed by a current of air into a flask of ice-cold distilled water a pure solution was obtained, in which the concentration of cyanogen chloride was determined by Aldridge's method (1945), using a Spekker absorptiometer. To a number of sterile samples bottles were then added measured quantities of this solution to give concentrations of cyanogen chloride ranging from nil to about 20 p.p.m. (as CNCl) when diluted. The volume of the solution in each bottle was made up to 50 ml. with sterile distilled water and to each bottle was then added 50 ml. of a 24 hr. culture of *Bact. coli* type I in 0·05 % peptone water. After mixing by inversion, sufficient of the mixture was abstracted to determine the exact concentration of cyanogen chloride in each bottle, and the bottles were then incubated at 30° C. A similar series of bottles was then prepared in which different concentrations of chlorine (as chlorine water) were substituted for cyanogen chloride.

At intervals during incubation of both sets of bottles plate counts were determined on nutrient agar, the plates being incubated at 30° C. and counted after 3 days. No thiosulphate was added to samples from either set before preparing dilutions and plating. Any residual chlorine in samples of the chlorinated series was, no doubt, absorbed rapidly by the organic matter in the nutrient medium. Cyanogen chloride, however, reacts less readily with organic matter. Any present in the sample and not removed mechanically by the process of manipulation would therefore be diluted and distributed throughout the medium in the Petri dishes and would, if present in sufficient concentration, exert a bacteriostatic effect during incubation. To this extent it might be said that the plate count was an index not only of the degree to which cyanogen chloride had destroyed bacteria in the culture, but also of the presence of residual concentrations sufficiently large to prevent growth of those surviving. It should be realized, however, that each sample was considerably diluted (at least 100 times) in the process of plating.

Table 7. *Effect of presence of gas liquor on destruction and aftergrowth of bacteria in peptone water treated with different doses of chlorine*

Plate counts per ml. of medium

Period of contact (hr.)	Dose of chlorine added (p.p.m.) 5		10		15		20	
	Without gas liquor	With gas liquor	Without gas liquor	With gas liquor	Without gas liquor	With gas liquor	Without gas liquor	With gas liquor
0	1,150,000	750,000	1,150,000	750,000	1,150,000	750,000	1,150,000	750,000
1	90,000	23,000	1,500	145	380	30	50	30
6	550,000	11,800	720	75	150	10	40	—
24	20,720,000	13,500	500	30	40	10	—	10
48	28,600,000	> 300,000	1,600	20	1,100	10	3,000	10
72	32,500,000	> 1,000,000	100,000	10	85,000	10	18,000	—
96	40,000,000	—	5,500,000	10	250,000	20	100,000	10
144	50,000,000	—	30,000,000	20	1,900,000	20	9,500,000	20

Results of the first series of experiments are shown graphically in Fig. 3.* As in previous experiments with sewage the immediate bactericidal effect of chlorine was considerable, so that at the end of a period of contact of 1 hr. the percentage of bacteria destroyed ranged from 49 to more than 99 % with different doses. With doses up to 8·6 p.p.m., however, subsequent growth of survivors brought the bacterial population back to its original level in periods varying from 4–90 hr. according to the dose. A dose of 10·7 p.p.m. (considerably above the chlorine demand of 4·75–5·0 p.p.m.) was the only one tested which kept the numbers of bacteria at a very low level throughout the period of the experiment (7 days). The immediate effect of cyanogen chloride, on the other hand, was much less

* In Figs. 3–6 the growth of bacteria in untreated sewage is shown by a broken line.

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marked than that of chlorine. With doses up to 9·3 p.p.m. the percentage of bacteria destroyed after 1 hr. ranged from 0 to 39 %. The bactericidal effect was, however, much more persistent than that of chlorine, and, with the exception of the sample to which the lowest dose had been added, the population chlorine initially added was absorbed by the organic matter. Some of the conditions under which cyanogen chloride may be lost (by hydrolysis, by aeration, or by the action of other substances) have been discussed elsewhere (Allen *et al.* 1948). Its stability in peptone solutions was tested as follows:

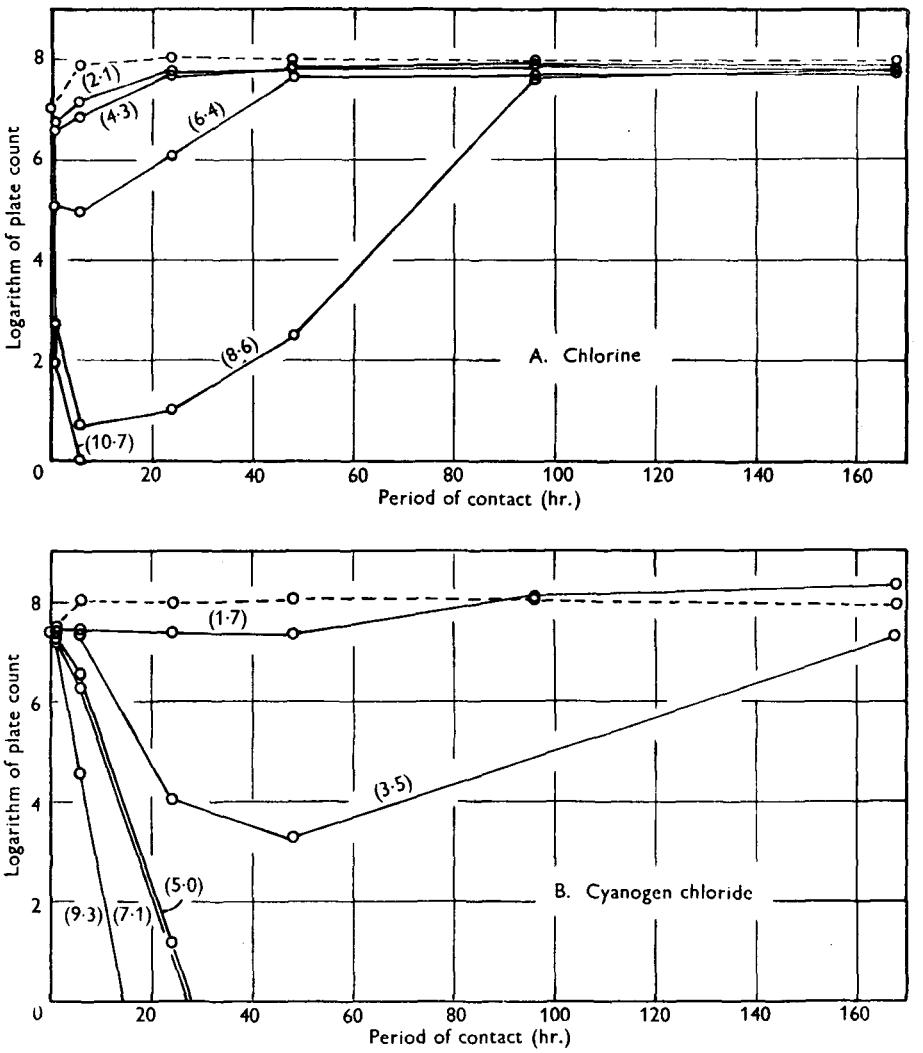

Fig. 3. Destruction and aftergrowth of *Bacterium coli* in 0·025 % peptone water (chlorine demand 4·75–5·0 p.p.m.) treated with different doses of (*a*) chlorine, and (*b*) cyanogen chloride (at pH 6·5 and 30° C.). (Dose of chlorine (p.p.m. Cl) or of cyanogen chloride (p.p.m. CNCl) shown on each curve.)

never returned to its original level. With the three higher doses, moreover, the bacteria were almost eliminated in 10–20 hr. and there was no sign of aftergrowth during the remainder of the period of test.

It seemed likely that the advantage of cyanogen chloride over chlorine as a bactericide was probably due to its persistence under conditions when the

Measured quantities of a solution of cyanogen chloride, prepared by chlorinating a solution of ammonium thiocyanate, were each diluted to 50 ml. with distilled water and were then mixed with an equal volume of a 0·05 % peptone water culture of *Bact. coli*, the pH value of the mixture being adjusted to 7·2–7·4. These preparations were incubated at 30° C. Similar preparations in peptone water without

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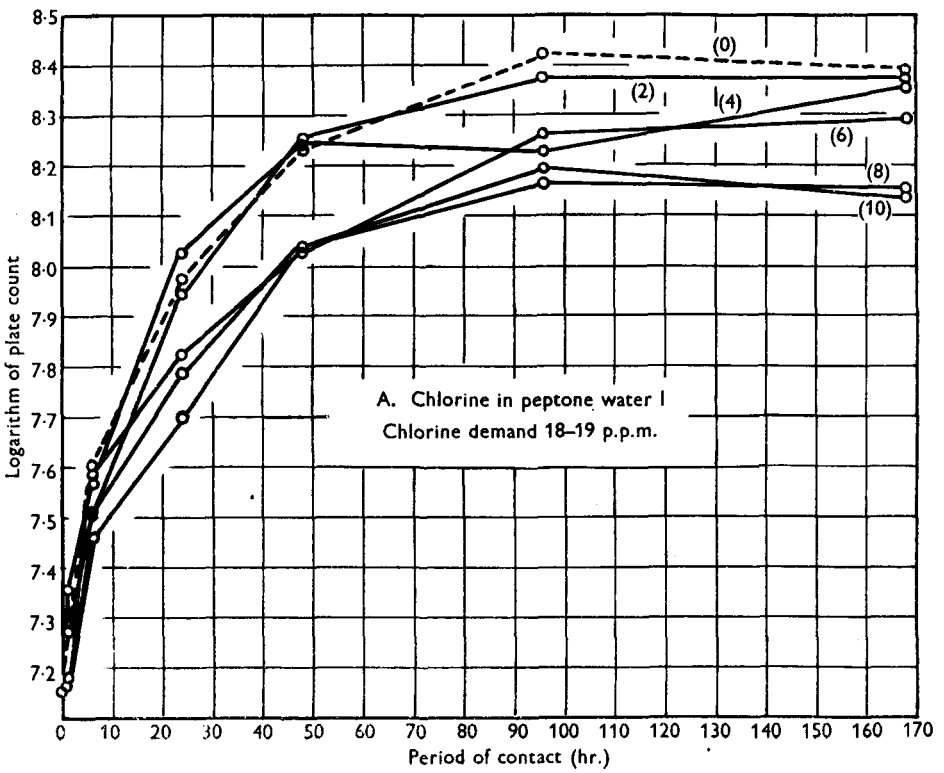
Bact. coli, adjusted in one experiment to pH 6·3–6·5 and in another experiment to 7·2–7·8, were incubated at 20° C. The concentration of cyanogen chloride in each bottle was determined at the beginning and at intervals during incubation. Results (Table 9) show that at pH values above 7·0 cyanogen chloride largely disappeared from peptone solutions in 24 hr., even under quiescent conditions. Under slightly acidic conditions, however, cyanogen chloride was much more stable and an appreciable proportion remained after nearly 3 days.

These findings suggested that the superior bactericidal effect of cyanogen chloride over chlorine would be more pronounced with liquids of comparatively

Table 8. *Effect of presence of thiocyanate on destruction and aftergrowth of bacteria in peptone water treated with different doses of chlorine*

		Plate counts per ml. of medium							
		Dose of chlorine added (p.p.m.)							
	Period of contact	5		10		15		20	
Exp. no.	(hours)	Without thiocyanate	With thiocyanate	Without thiocyanate	With thiocyanate	Without thiocyanate	With thiocyanate	Without thiocyanate	With thiocyanate
I. Thiocyanate added as NH_4CNS	0	38,000	33,000	38,000	33,000	38,000	33,000	38,000	33,000
	1	2,150	120	2,850	90	400	40	20	20
	6	5,500	70	1,730	10	80	10	30	10
	24	>500,000	30	380,000	20	13,300	30	10	10
	48	20,000,000	?100	23,000,000	40	8,500,000	—	?160	80
	72	28,300,000	170	32,400,000	10	12,100,000	30	30	20
	96	33,400,000	1,120	40,400,000	—	21,700,000	<10	40	90
	144	320,000,000	2,040,000	57,700,000	—	65,300,000	20	—	10
II. Thiocyanate added as KCNS	0	360,000	1,460,000	360,000	1,460,000	360,000	1,460,000	360,000	1,460,000
	1	875,000	34,000	74,000	930	810	10	320	<10
	6	1,400,000	6,300	129,000	<10	150	<10	<10	<10
	24	—	1,630,000	4,290,000	10	6,300	25	<10	<10
	48	65,000,000	15,000,000	18,000,000	6,900	2,480,000	570	235	<10
	72	106,000,000	21,100,000	58,000,000	380,000	22,800,000	1,080	12,700	<10
	144	164,000,000	104,000,000	111,000,000	25,000,000,	135,000,000	1,310,000	620,000	<10



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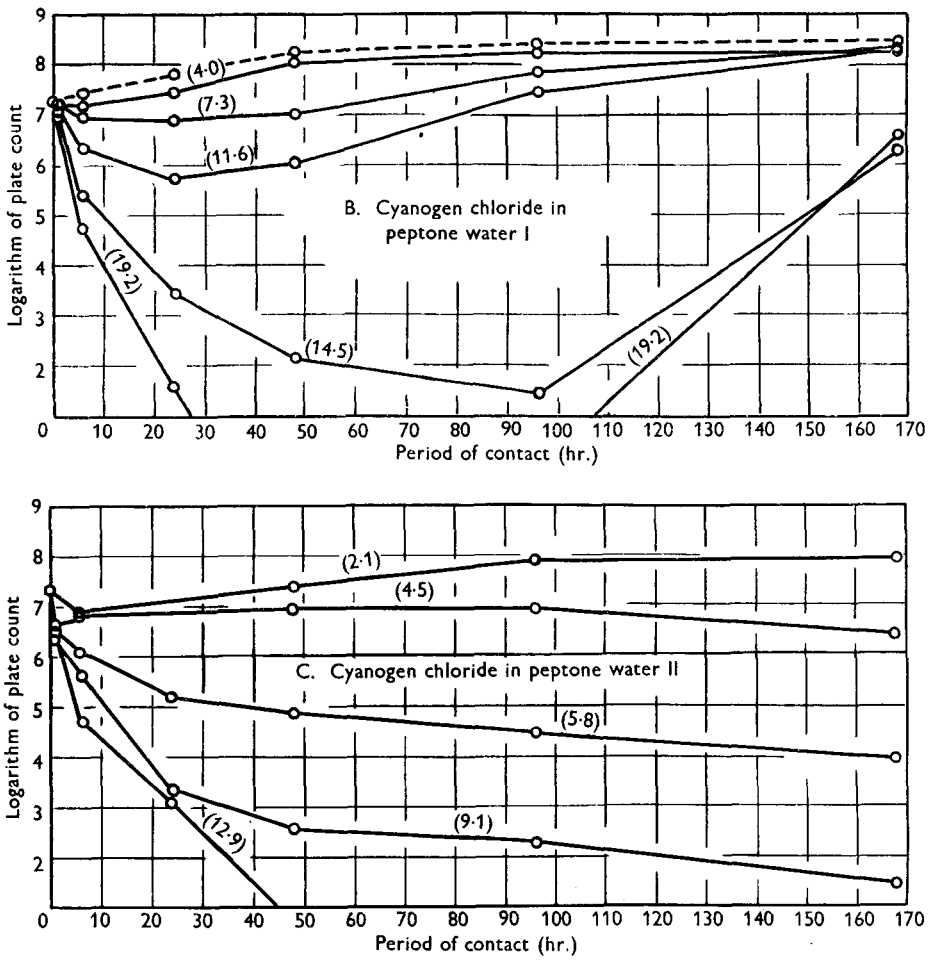

Fig. 4. Destruction and aftergrowth of *Bacterium coli* in 0·1 % peptone water treated with different doses of (A) chlorine and (B) cyanogen chloride (at pH 6·3 and 30° C.). (Dose of chlorine (p.p.m. Cl) or of cyanogen chloride (p.p.m. CNCl) shown on each curve.)

high chlorine demand since the chlorine would then be rapidly absorbed. In Fig. 4 (A and B) the effects of chlorine and of cyanogen chloride on the numbers of *Bact. coli* in 0·1 % peptone water (chlorine demand 18–19 p.p.m.) are shown, the ordinate scales being different in A and B to accommodate the different ranges of bacterial counts.

Doses of chlorine up to 10 p.p.m. exerted very little retarding influence on bacterial growth. Comparable amounts of chlorine as cyanogen chloride, however, showed a marked lethal action. Concentrations of cyanogen chloride ranging from 4·0 to 19·2 p.p.m. (containing chlorine equivalent to 2·3, 4·2, 6·7, 8·4 and 11·1 p.p.m.) reduced the bacterial count after contact for 6 hr. by 16, 53, 89, 98·6 and 99·7 %, respectively. With the two highest doses the population had not returned to its original level at the end of the experiment.

In a similar experiment with a solution of a different brand of peptone (Fig. 4C) comparable bactericidal effects were obtained with appreciably lower concentrations of cyanogen chloride.

Results of a series of comparative tests with different samples of settled sewage (Figs. 5, 6 and 7) showed the same general trend as had been found with peptone water—that the bactericidal effect of cyanogen chloride was more lasting than that of chlorine although the immediate effect of the chlorine was often considerable. The tests were conducted at 30° C. and at a pH value of 6·5.

The results of this investigation may explain why with sewage or sewage effluents from certain districts a given dose of chlorine produces a more lasting bactericidal effect than is found with domestic sewage. They do not explain the temporary though pronounced lethal action of comparatively small

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doses of chlorine which is common to sewage in general and clearly applies to the sample of domestic sewage referred to in Fig. 7.

SUMMARY AND CONCLUSIONS

When samples of domestic sewage are treated with doses of chlorine lower than the chlorine demand with domestic sewage reduction in bacterial count after addition of chlorine is followed, after an interval, by aftergrowth of survivors which rapidly brings the population to its original level or higher. For this interval to be prolonged the initial concentration of chlorine must be considerably greater than the chlorine demand. This accords with the fact that the rapid reaction of chlorine with constituents of the sewage, which occurs in the first few minutes, is followed by a slower absorption which takes place over a prolonged period, so that the initial bactericidal action of residual chlorine tends to be lost after a time. For many purposes the effectiveness of chlorination should be judged not only by the percentage of bacteria destroyed after a short period of bactericidal action depends not only on the proportion of the demand satisfied but also on the absolute size of the dose. With doses just sufficiently large to leave residual chlorine detectable by *o*-tolidine, reduction in the plate count of bacteria after contact for 15 min. ranged in various tests from 96·2 to 98·7 %.

Table 9. *Persistence of cyanogen chloride in peptone solutions*

Test no.	Nature of solution	pH value	Concentration of cyanogen chloride (as p.p.m. Cl) after following periods of contact (hr.)			
			0	6		24
1 (at 30° C.)	Culture of *Bact. coli* in 0·025 % peptone water	7·2–7·4	1·6	0·3		Nil
			2·7	0·8		Nil
			3·9	1·3		Nil
			5·4	1·9		0·2
			6·8	1·5		0·5
			0	1½	19	67
2 (at 20° C.)	0·025 % peptone water	6·3–6·5	1·6	1·6	1·1	0·4
			3·0	2·9	2·0	0·9
			4·1	4·1	2·9	1·4
			5·6	5·3	4·0	1·9
			7·1	6·2	4·8	2·1
3 (at 20° C.)	0·025 % peptone water	7·2–7·8	1·5	0·8	Nil	Nil
			2·8	1·6	0·1	Nil
			4·0	3·0	0·7	Nil
			5·2	3·5	0·5	Nil
			6·1	3·6	0·5	Nil

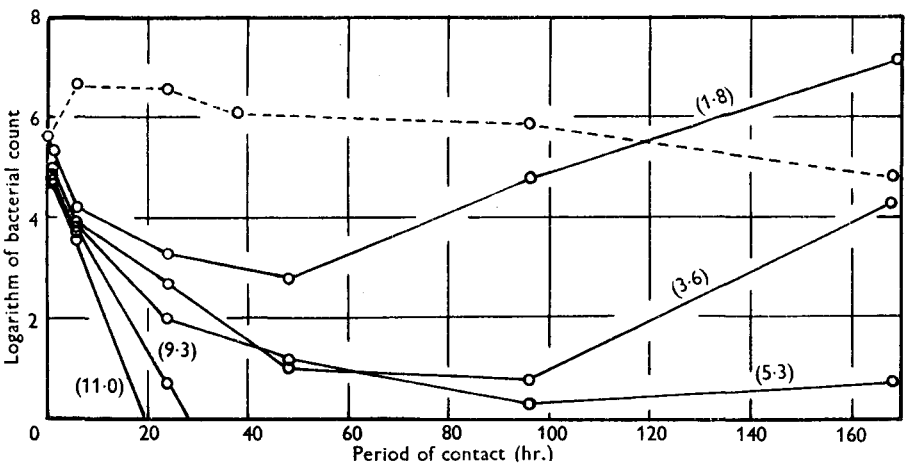

Fig. 5. Destruction and aftergrowth of bacteria in sewage (chlorine demand 1·5–1·75 p.p.m.) treated with different doses of cyanogen chloride (at pH 6·5 and 30° C.). (Dose of cyanogen chloride (p.p.m. CNCl) shown on each curve.)

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contact but also by the interval which elapses before aftergrowth becomes appreciable.

In samples of sewage treated with doses of chlorine sufficient to destroy the protozoa, surviving bacteria eventually reached a level of population much higher than that of the untreated sewage. The majority of such bacteria were found to be Gram-negative, non-sporing rods, which included strains of *Proteus* and of *Pseudomonas pyocyanea*. Tests with a pure culture of a species of *Proteus* showed that the organisms surviving chlorination are not intrinsically capable of reaching a higher population than the parent culture.

When sewage contains small quantities of gas liquor or thiocyanate the bactericidal action of doses of chlorine lower than the demand is much more prolonged than the action of comparable doses in domestic sewage, owing to the formation of cyanogen chloride.

Comparative tests of the action of chlorine and of cyanogen chloride on pure cultures of *Bacterium coli* in peptone water and on the mixed flora of sewage showed that the bactericidal effect of cyanogen chloride was more persistent than that of a comparable dose of chlorine. With sewage and with some brands of peptone the immediate effect of the chlorine, on the other hand, was often considerable.

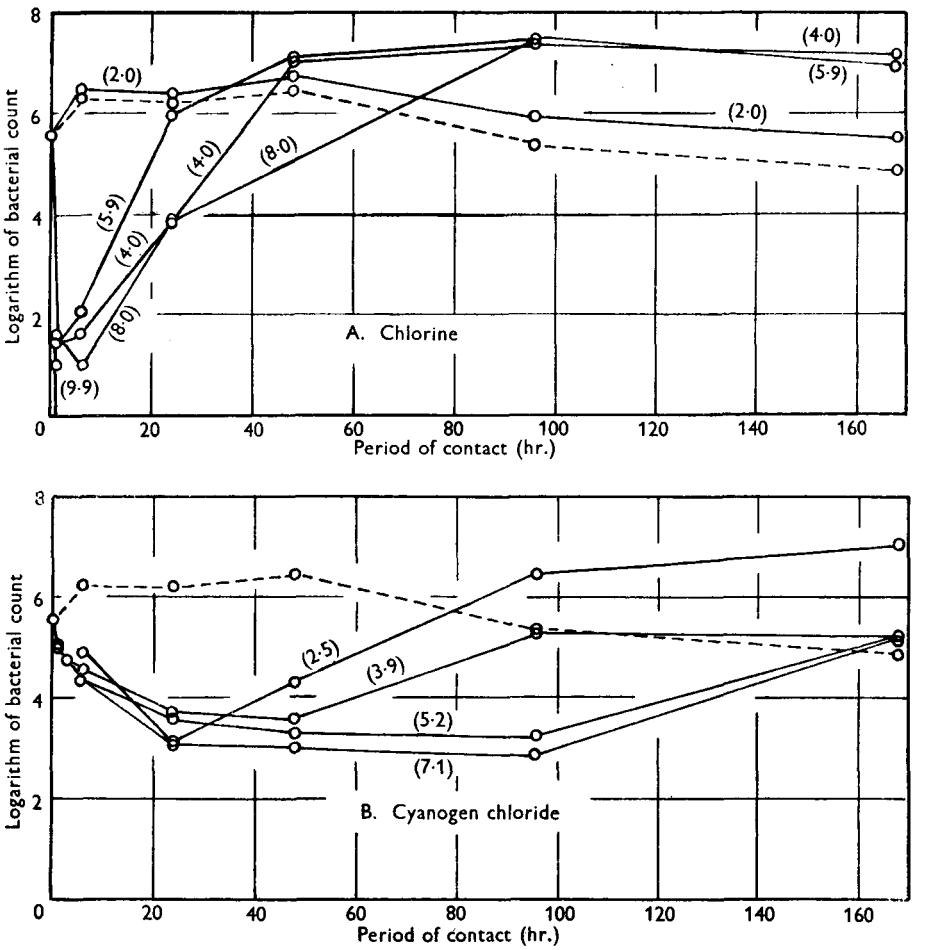

Fig. 6. Destruction and aftergrowth of bacteria in sewage (chlorine demand 3·5 p.p.m.) treated with different doses of (*a*) chlorine and (*b*) cyanogen chloride at pH 6·5 and 30° C. (Dose of chlorine (p.p.m. Cl) or of cyanogen chloride (p.p.m. CNCl) shown on each curve.)

The investigation described formed part of the programme of the Water Pollution Research Board and this paper is published by permission of the Department of Scientific and Industrial Research.

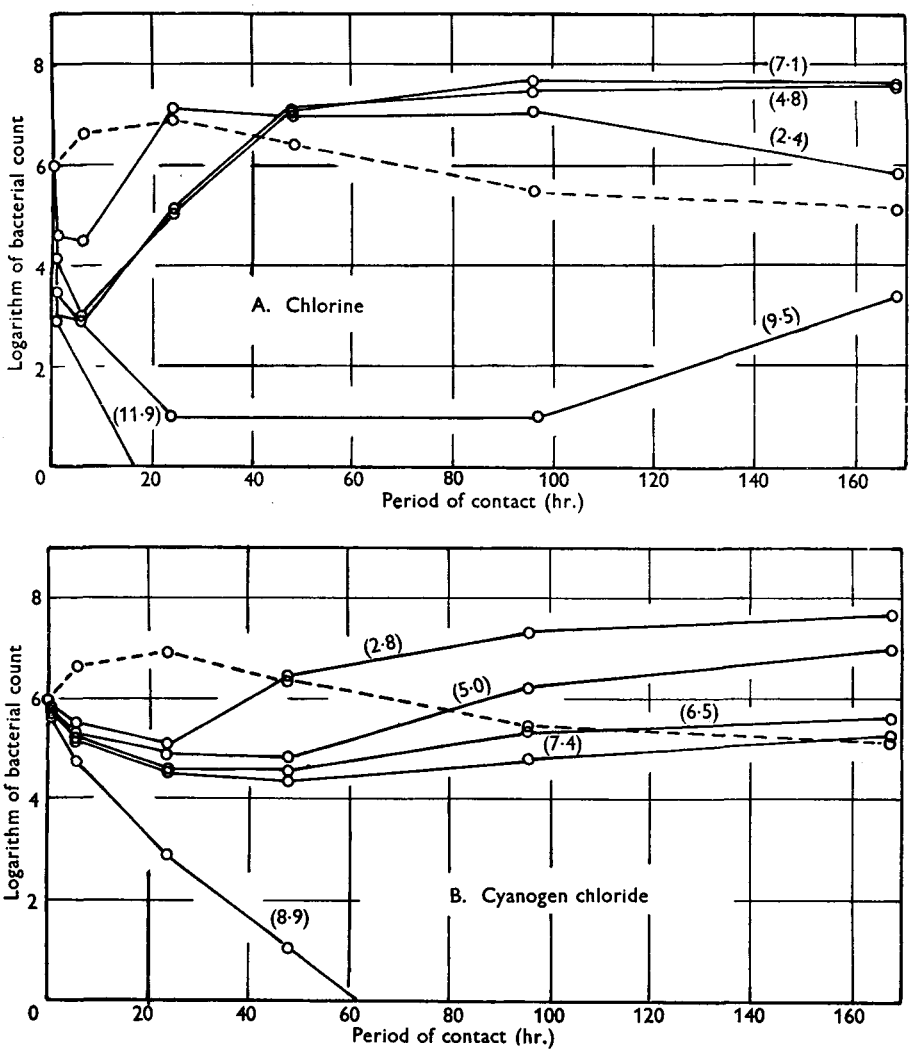

Fig. 7. Destruction and aftergrowth of bacteria in sewage (chlorine demand 6·0–6·25 p.p.m.) treated with different doses of (*a*) chlorine and (*b*) cyanogen chloride (at pH 6·5 and 30° C.). (Dose of chlorine (p.p.m. Cl) or of cyanogen chloride (p.p.m. CNCl) shown on each curve.)

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