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REPORT ON A SERIES OF TESTS PERFORMED TO ASCERTAIN THE EFFICIENCY OF THE AMMONIA CHLORINE TREATMENT OF WATER APPLIED BY A STANDARDIZED METHOD.

BY MAJOR E. F. W. MACKENZIE, O.B.E., M.C.,
Royal Army Medical Corps.

THE ammonia chlorine method of water purification has for many years been used successfully in civil practice. Chlorine gas or its compounds and ammonia or its various salts have been employed in different combinations, the materials and the method of administration varying in accordance with local conditions, cost and convenience. The method has recently been adapted for Army use for purification of water in the water cart tank (Regimental Water Cart) after preliminary clarification brought about in the clarifying cylinders by filtration through cloths, assisted by the prior addition of water-clarifying powder.

Briefly, the method consists in the addition to 100 gallons of water in the cart of 0·7 gramme of ammonium chloride in solution, followed by 4 grammes of chlorosene previously made into a paste and mixed with water [1].

Before finally adopting chloramine, administered by this standard method, for use in India, it was considered desirable to carry out further tests of the method as applied to the different conditions existing in that country, particularly as regards the absence of filtering apparatus and the existence of many waters having a considerable degree of hardness and a

high pH value. A preliminary survey of some sixty-two waters drawn from various sources throughout the Punjab showed that in twenty-nine the pH value of the water was 8·0 or more.

At the commencement it appeared that the following important points required investigation :—

A. The behaviour of chloramine in the presence of hard waters of high pH value.

B. The effect of bright sunlight upon the stability of chloramine.

C. The behaviour of chloramine in the presence of waters heavily polluted with organic ammonia.

Water used for a public supply has been found with a free and saline ammonia content as high as 0·0256 per 100,000. In civil practice the added ammonia can be adjusted to the native ammonia content in such a manner as to preserve the correct ammonia chlorine ratio. The optimum dose can be decided in each case, accurately administered, and constantly controlled thereafter. Under Service conditions this cannot be done, and it was considered possible that the variations in the ratio might upset the formation of chloramine.

During the course of preliminary tests the results obtained raised doubts regarding the constant efficiency of the standard method. This led to further tests in the course of which observations were made which gave rise to doubts whether the chemical substance formed by the standard method under all conditions is chloramine, and, moreover, whether any constant compound of ammonia and chlorine results from the dosage laid down. It appeared possible that different combinations of chlorine arose at different times and under varying conditions, and that certain of these combinations were unstable and acted very slowly.

It also appeared possible that the group of substances which it has become customary to include under the omnibus term "chloramine" constitutes an undetermined quantity, since the combinations formed varied over a wide range in their bactericidal effects. This was not demonstrable to the same extent when the chloramines were manufactured from chlorine gas instead of chlorosene [2]. It was further found that the variations noted were brought about by very slight differences in the method of production or by application to waters of different chemical constitution.

The influence of temperature on the ultimate product was not considered in this investigation but reduced temperature is known to affect profoundly the bactericidal velocity.

From the literature available there appeared to be great variation in the findings of different investigators. The trend of opinion pointed to the fact that increased pH value caused a slowing up of bactericidal velocity which was more pronounced in the case of chloramine than of chlorine [3, 4]. Further work on this subject recently published shows that, under the conditions then obtaining, the velocity and the amount of disinfection of water with both high and low organic loads are increased

by the use of ammonia with chlorine [5]. As regards velocity this is contrary to the findings of the investigation under report.

Reports to the effect that chloramine had been found unstable in the presence of iron oxide in small quantities or when exposed to bright sunlight were received later. The investigation therefore threatened to become very much more extensive than was originally anticipated, and, since the time which could be devoted to it was limited, it appeared desirable to confine the field to somewhat extreme cases, which, though each might not arise in such degree in practice, would be of value as indicating tendencies and as a starting point for subsequent investigation throughout the complete range of each adverse factor. Moreover a combination of several adverse conditions, each in a comparatively small degree, might be found to bring about failure and would be encountered frequently in practice.

For the reasons set forth above it appeared unlikely that any one series of tests would furnish results exactly comparable with another series carried out under different conditions. The investigation was therefore resolved into comparative tests of the efficiency, under various conditions, of the new method as compared with the old, which consists of simple chlorination after estimation of the dose required by means of the Horrocks box.

It should be pointed out that the standard required is the provision of a safe drinking water within one hour from any source of supply likely to be required for use under active service conditions. Long contact with prolonged action of residual, which is the rule in civil practice, is therefore impossible.

All tests were approximated as closely as possible to field service conditions and the following method was adopted as a routine.

A. Three galvanized iron tanks were employed and were numbered I, II, III.

B. Each tank held 25 gallons of water. One quarter of the standard dose (0·7 gramme of ammonium chloride and 4 grammes chlorosene) required for 100 gallons was placed in each tank.

The method of dosing laid down was adhered to throughout. The ammonium chloride was added in solution, thoroughly mixed with water, and the solution of chlorine derived from choloresene was added subsequently.

Measuring by a levelled scoop, even with a skilled operator, of necessity gives rise to slight variations in the quantity administered. In each test the same solution of chlorine derived from chlorosene was used for each tank and the doses of chlorine and chloramine in any one test are therefore exactly comparable. The doses employed in different tests are not exactly comparable for the above reason.

The available chlorine content of the chlorosene supplied has ranged between 27·4 and 24·7 per cent and no sample which has been tested gave

the reputed 30·0 per cent. This variation in chlorine content upsets the ammonia chlorine ratio and might in itself cause varying results from this method. The necessity for adjusting the dose is drawn attention to by Elliott [6].

It should also be noted here that the scoops provided with the tins of chlorosene as received from the makers held, when levelled off as directed, an average of 3·5 grammes of chlorosene and not 2 grammes as stated. This is of importance since there is a possibility that other investigators have accepted this as a 2-gramme scoop and have consequently given a large overdose of free chlorine. This is the more probable since, in the majority of trials carried out to date, no titration figures are recorded and the error in the size of the measure has not been brought to light. A scoop was obtained which held an average of 2 grammes and this measure was employed throughout.

A *Bact. coli* of intestinal type conforming to Thresh's requirements [7] was furnished by the Officer in charge of the Enteric Laboratory, Kasauli, and the saline emulsions of this bacterium employed in all tests were standardized at 100 million per millilitre by that officer, who was also kind enough to provide the large quantities of media necessary.

In the majority of tests the presence of acid and gas in MacConkey's media was considered presumptive evidence of the presence of *Bact. coli* but in certain tests in which the result differed from the findings of other workers the culture tubes were subcultured and the possibility of the presence of sporing organisms which might lead to confusion excluded. In these cases the organisms recovered gave the same biochemical reactions as the one originally employed.

In the majority of tests tanks No. I and No. II were used as test tanks, No. III was used as a positive control to which no sterilizant was added. This tank remained positive on all occasions and in all dilutions and the results are therefore not recorded in this report.

The method employed throughout was as follows:—

(1) Each tank was filled with twenty-five gallons of water.

(2) Each was inoculated with 100 million *Bact. coli* and thoroughly mixed.

(3) Each was subcultured in quantities of 25 millilitres and 1·0 millilitre.

(*Note.*—In the earlier tests quantities of 25·0, —10·0, —5·0, —1·0, —0·5, —0·1, and 0·01 millilitre were employed but this range was found unnecessary.)

(4) The sterilizant was added to tanks Nos. I and II.

(5) Tanks Nos. I and II were titrated for free chlorine or chloramine immediately after the addition of the sterilizant. Titrations were carried out as follows:—

A. To 355 millilitres of the water under test was added freshly prepared potassium iodide and starch indicator; this was titrated with freshly prepared N/100 sodium thiosulphate. The titrations were carried out with a

N.P.L. B burette with graduations permitting a reading of 0·05 millilitre.

B. The number of millilitres of thiosulphate required was recorded as "first fraction." After acidification with 20 per cent. sulphuric acid the titration was repeated and the number of millilitres required to discharge the colour a second time was recorded as "second fraction."

(6) Each tank was titrated for residual and cultured in quantities of 25 millilitres and 1 millilitre at half hour, one hour, two hours, twenty-four hours, etc., after the addition of the sterilizant. The titration figures are recorded as 0 + 0 to represent Harold's first and second fractions which will subsequently be referred to as such.

(7) Before each subculture or titration each tank was thoroughly agitated by a "standard stir" carried out by means of iron paddles well coated with hard varnish to prevent rusting.

(8) Culture tubes were read after incubation at 37° C. for twenty-four hours, the reading being confirmed after forty-eight hours. A.G. represents acid and gas after forty-eight hours. Neg. represents absence of acid or gas after forty-eight hours. In all cases in which reinoculation tests at the end of twenty-four hours were carried out the reading at that time was confirmed after forty-eight hours.

Chlorine concentrations are expressed in parts per million.

Unless otherwise stated the water used was the normal supply to the laboratory which is obtained from a hillside spring. The analysis of this water was as follows :—

Physical characters	Clear, colourless and odourless
Reaction	Alkaline, pH 8·1
Sediment	Nil

PARTS PER 100,000.

Free and saline ammonia	0·0004
Albuminoid ammonia	0·0052
Oxygen absorbed from permanganate in ½ hour at 27° C.	Nil
Oxygen absorbed from permanganate in 4 hours at 27° C.	0·166
Chlorine	0·35
Nitrates	Nil
Nitrites	Nil
Total solids	10·8
Total hardness	6·8
(*a*) Temporary	0·5
(*b*) Permanent	6·3

The water was clear and free from any form of particulate matter.

A large number of tests was performed. For the sake of brevity only those typical of each series carried out in confirmation of preliminary tests have been recorded.

Although doubts have arisen with regard to the chemical nature of the substances formed by the standard method the term chloramine has been adhered to in order to conform with previous reports and is used in this somewhat loose sense throughout this report.

TEST OF THE COMPARATIVE VALUE OF CHLORINE AND CHLORAMINE IN EQUAL CONCENTRATIONS IN A RELATIVELY PURE WATER.

TEST NO. 1.

Type of Water	Chloramine 0·2 p.p.m.			Chlorine 0.2 p.p.m.		
Laboratory supply	Concentration	Culture		Concentration	Culture	
		25 ml.	1 ml.		25 ml.	1 ml.
Immediate ..	0·2 + 0	A G	A G	0·1 + 0	A G	A G
½ hour	0·15 + 0	A G	A G	0·1 + 0	Neg.	Neg.
1 hour	0·15 + 0	A G	Neg.	0 + 0	Neg.	Neg.
2 hours	0·1 + 0	Neg.	Neg.	0 + 0	Neg.	Neg.

Standard chloramine added to pure water in sufficient quantity to produce a concentration of 0·2 part per million failed to kill *Bact. coli* in one hour but did so in two hours.

Chlorine, added in the same amount, killed *Bact. coli* in one half hour.

This test brings out the following point :—

That the *Bact. coli* employed is not unduly resistant to the action of chlorine or chloramine and is, in fact, rapidly killed by either in low concentrations.

It confirms the following facts observed by other workers :—

(1) That the action of chloramine is considerably slower than that of chlorine.

(2) That the ammonia chlorine treatment provides a titratable chlorine residual which persists longer than is the case with chlorine alone.

The smaller residual of chlorine on immediate titration after administration of the same dose is doubtless due to deviation during the necessary interval for thorough mixing.

TEST NO. 2.

Type of Water	Standard chloramine			Chlorine 2·0 p.p.m.		
Laboratory supply	Concentration	Culture		Concentration	Culture	
		25 ml.	1 ml.		25 ml.	1 ml.
Immediate ..	1·5 + 0	A G	A G	1·5 + 0	A G	A G
½ hour	1·5 + 0	Neg.	Neg.	1·5 + 0	Neg.	Neg.
1 hour	1·45 + 0	,,	,,	1·3 + 0	,,	,,
2 hours	1·4 + 0	,,	,,	1·0 + 0	,,	,,
24 hours	1·0 + 0	,,	,,	0·2 + 0	,,	,,

Both were equally efficient in bringing about the rapid death of *Bact. coli*. The persistence of the chloramine residual is further confirmed.

It has been stated that, as a result of this residual, chloramine has the

power of disposing of infection added after primary treatment, as, for example, during distribution of the water.

With a view to testing this each tank was reinoculated at twenty-four hours with 100 million *Bact. coli* with the following result:—

TEST No. 3.

Type of Water	Standard Chloramine			Chlorine Horrocks Test 2·0 p.p.m.		
Laboratory supply	Concentration	Culture		Concentration	Culture	
		25 ml.	1 ml.		25 ml.	1 ml.
24 + ½ hour ..	1·0 + 0	A G	A G	0 + 0	A G	A G
24 + 24 hours ..	0·6 + 0	A G	A G	0 + 0	A G	A G
24 + 48 hours ..	0·3 + 0	A G	A G	0 + 0	A G	A G

This result first cast doubt upon the efficiency of the residual as a sterilizing agent and considerable doubt was felt regarding its accuracy. The test was carried out at a time when the water temperature was approaching 0° C. This possibly accounts for the complete failure of post-sterilization which was not confirmed subsequently when the water temperature was higher. As a result further tests were carried out at a later date of which the following is typical.

TEST No. 4.

Type of Water	Standard Chloramine			Chlorine Horrocks Test 1·0 p.p.m.		
Laboratory supply	Concentration	Culture		Concentration	Culture	
		25 ml.	1 ml.		25 ml.	1 ml.
Immediate ..	1·9 + 0	A G	A G	0·9 + 0	A G	A G
½ hour	1·8 + 0	Neg.	Neg.	0·8 + 0	Neg.	Neg.
1 hour	1·6 + 0	Neg.	Neg.	0·5 + 0	Neg.	Neg.
2 hours	1·5 + 0	Neg.	Neg.	0·4 + 0	Neg.	Neg.
24 hours	1·3 + 0	Neg.	Neg.	0·1 + 0	Neg.	Neg.

This result accords with expectations. At twenty-four hours each tank was reinoculated with 100 million *Bact. coli* and the following results were recorded.

TEST No. 5.

Type of Water	Standard Chloramine			Chlorine Horrocks Test 1·0 p.p.m.		
Laboratory supply	Concentration	Culture		Concentration	Culture	
		25 ml.	1 ml.		25 ml.	1 ml.
24 + ½ hour ..	——	A G	A G	——	A G	A G
24 + 1 hour ..	1·2 + 0	A G	A G	0 + 0·1	Neg.	Neg.

The residual of over 1·2 parts per million chloramine again failed to kill *Bact. coli* in one hour. The result from the chlorine tank was somewhat puzzling. For some reason the residual of 0·1 part per million first fraction changed to second fraction and the tank became negative to *Bact. coli* in one hour.

In order to test further what may perhaps be termed the post-sterilizing action of chloramine three tanks were put up. All were inoculated with 100 million *Bact. coli*. Nos. I and II were each dosed with standard chloramine. No. III was kept as the usual positive control and showed AG—AG throughout.

Nos. I and II each gave AG—AG on immediate culture, became negative at half hour and remained so to twenty-four hours.

At twenty-four hours No. I was reinoculated with 100 million *Bact. coli*. Tank No. II was not reinoculated and was kept as a negative control.

The results of titration and culture are shown in Test No. 6.

TEST No. 6.

Type of Water	Standard Chloramine (reinoculated at 24 hours)			Standard Chloramine (not reinoculated)		
Laboratory supply	Concentration	Culture		Concentration	Culture	
		25 ml.	1 ml.		25 ml.	1 ml.
24 + ½ hour..	1·2	A G	A G	1·3	Neg.	Neg.
24 + 1 hour..	1·2	A G	A G	1·3	Neg.	Neg.
24 + 2 hours	—	A G	A G	—	Neg.	Neg.
24 + 24 hours	1·1	Neg.	Neg.	1·2	Neg.	Neg.

Tank No. III, the positive control remained positive.

Tank No. II, in which a previous dose of *Bact. coli* had been killed by the standard dose of chloramine, remained negative throughout.

Tank No. I, with a residual of 1·2 parts per million, failed to dispose of *Bact. coli* in two hours.

This series appears to confirm definitely the finding of Test No. 3 to the effect that, although there may be a titratable residual of over 1·0 part per million efficient sterilization may not take place for two hours under what appear to be optimum conditions.

In order to dispose of the possibility of the presence of lactose fermenting sporing organisms a broth tube inoculated at twenty-four + two hours in the particular test recorded above was subcultured by the Officer in charge of the Enteric Laboratory.

Examination did not reveal the presence of any sporing organisms and an organism fulfilling Thresh's definition of a *Bact. coli* and identical biochemically with the one inoculated was recovered.

Twenty-four hours after reinoculation the tank was negative to *Bact. coli* whereas the positive control remained positive. The high residual therefore acted only very slowly.

At a later date further tests were designed with a view to ascertaining more exactly at what period the death of *Bact. coli* took place in the presence of both freshly prepared standard chloramine and chloramine residual.

The result is shown in Test No. 7.

TEST No. 7.

A tank containing twenty-five gallons of water from the laboratory supply was inoculated with 100 million *Bact. coli* and mixed.

Culture showed *Bact. coli* present in 25·0 millilitres and 1·0 millilitre.

At *X hours* a standard dose of chloramine was administered.

Immediate concentration 1·3+0.

Cultured at	Reading at 48 hours	
	25 ml.	1 ml.
X + 5 minutes	A G	A G
X + 10 ,, ...	A G	Neg.
X + 15 ,, ...	Neg.	Neg.
X + 20 ,, ...	Neg.	Neg.
X + 25 ,, ...	Neg.	Neg.

At *Y hours* (*X* + 35 minutes) the tank was reinoculated with 100 million *Bact. coli*.

Cultured at	Reading at 48 hours	
	25 ml.	1 ml.
Y + 5 minutes	A G	A G
Y + 10 ,, ...	A G	A G
Y + 15 ,, ...	A G	A G
Y + 20 ,, ...	A G	A G
Y + 25 ,, ...	A G	A G
Y + 30 ,, ...	A G	A G
Y + 60 ,, ...	Neg.	Neg.

It will be seen that freshly-prepared chloramine killed *Bact. coli* in fifteen minutes. The same chloramine, after standing for thirty-five minutes, failed to kill the same organisms in thirty minutes but did so in one hour. The residual at the end of two hours was over 1·0 part per million. The action of residual was somewhat more rapid than in previous tests in which twenty-four hours elapsed before reinoculation, possibly because the more active substance had not entirely disappeared, or perhaps on account of an increase in water temperature which had risen to over 70° F.

The phenomenon of delayed action in the residual has been observed constantly throughout the tests. It is of considerable interest and perhaps of some practical importance, and rather distracted attention from the

original objective. Although an explanation might have been sought it was considered advisable to proceed with comparative tests.

Up to the present only a relatively pure water had been employed. As was to be expected the standard dose of 2·0 parts per million of chloramine had effected purification within the desired period of one hour.

THE NEXT SERIES OF TESTS WAS DESIGNED TO COMPARE THE EFFICIENCY OF CHLORAMINE AND CHLORINE IN THE PRESENCE OF URINE AND EXCREMENTAL MATTER.

A standard decoction of cow dung was prepared and to each of three tanks was added 100 millilitres of this decoction + 100 millilitres of stale urine. Analysis of the water gave the following :—

Ammonia free and saline	.. ..	0·17 part per 100,000
Ammonia albuminoid	.. ..	0·08 ,, ,, ,,

Finely divided particulate matter was visible to the naked eye.

Horrocks test gave 1·0 part per million as the effective dose of chlorine and this was employed.

The results of these experiments were as follows :—

TEST NO. 8.

Type of Water	Standard Chloramine			Chlorine Horrocks Test 1·0 p.p.m.		
Laboratory supply + 100 ml. decoction of dung + 100 ml. stale urine	Concentration	Culture		Concentration	Culture	
		25 ml.	1 ml.		25 ml.	1 ml.
Immediate ..	1·5 + 0	A G	A G	0·6 + 0	A G	A G
½ hour	1·4 + 0	A G	A G	0·6 + 0	A G	A G
1 hour	1·3 + 0·1	A G	A G	0·6 + 0	A G	A G
2 hours	1·3 + faint trace	A G	A G	0·6 + 0	A G	A G
24 hours ..	1·0 + 0	Neg.	Neg.	0·3 + 0	Neg.	Neg.

The persistence of a residual in the chlorine tank was evidently due to the high native ammonia present in the water.

A faint second fraction appeared in the chloramine tank.

At twenty-four hours both tanks were reinoculated with 100 million *Bact. coli* with the following results :—

TEST NO. 9.

Type of Water	Standard Chloramine			Chlorine Horrocks Test 1·0 p.p.m.		
Laboratory supply + 100 ml. decoction of dung + 100 ml. stale urine.	Concentration	Culture		Concentration	Culture	
		25 ml.	1 ml.		25 ml.	1 ml.
24 + ½ hour ..	0·9 + 0	A G	A G	0·3 + 0	A G	A G
24 + 1 hour ..	0·9 + 0	A G	A G	0·3 + 0	A G	A G
24 + 2 hours ..	0·9 + 0	A G	A G	0·3 + 0	A G	A G

Both chloramine in the standard dose of 2 parts per million and chlorine in the Horrocks dose of 1·0 part per million failed to kill *Bact. coli* in two hours initially, but did so in twenty-four hours. Residuals of 0·9 and 0·3 part per million also failed to kill in two hours.

The second fraction noted earlier in the chloramine tank disappeared later. In view of subsequent findings this is not without interest.

It was thought possible that the failure to carry out rapid sterilization in this case might be due to the presence of particulate matter.

The following series was therefore performed :—

Each of three tanks of perfectly clear water was contaminated with 100 millilitres of urine.

On this occasion Horrocks test indicated 2·0 parts per million chlorine, and this dose was administered.

The higher deviation is accounted for by the analysis of the water which showed :—

Ammonia free and saline	..	0·84 part per 100,000
Ammonia albuminoid ..	..	0·24 ,, ,, ,,

The results were as follows :—

Test No. 10.

Type of Water	Standard Chloramine			Chlorine Horrocks Test 2·0 p.p.m.		
Laboratory supply + 100 ml. of urine	Concentration	Culture		Concentration	Culture	
		25 ml.	1 ml.		25 ml.	1 ml.
Immediate ..	0·7 + 0	A G	A G	0 9 + 0	A G	A G
½ hour	0·7 + 0	A G	A G	0·6 + 0·1	A G	A G
1 hour	0·55 + 0·15	A G	A G	0·65 + 0·05	A G	A G
2 hours	0·55 + 0·15	A G	A G	0·65 + 0·05	A G	A G
24 hours	0·1 + 0	Neg.	Neg.	0·05 + faint trace	Neg.	Neg.

Again, chloramine failed equally with chlorine to kill *Bact. coli* in two hours, but both did so in twenty-four hours.

The rapid initial loss indicates that this was a severe test. It nevertheless indicates that the presence of urine in water seriously interferes with the action of chloramine, since the only difference between this test and Test No. 1 was the addition of 100 millilitres of urine per 25 gallons. In Test No. 1 0·2 part per million of chloramine killed *Bact. coli* in less than two hours. In the test under consideration a residual of 0·7 part per million failed in two hours.

In both tanks a reaction which had not been noted previously was observed. On the addition of potassium iodide and starch no blue colour appeared during an interval of approximately five seconds. It then appeared slowly, reaching a maximum in some seconds. This has been stated by Harold to be characteristic of chloramine.

(To be continued.)