AN INVESTIGATION OF THE TOXIC ACTIONS OF DILUTE SOLUTIONS OF THE SALTS OF CERTAIN HEAVY METALS (VIZ.: COPPER, IRON, NICKEL, COBALT, MANGANESE, ZINC, SILVER, AND LEAD) UPON THE *BACILLUS TYPHOSUS*, WITH A VIEW TO PRACTICAL APPLICATION IN THE PURIFICATION OF SHELL-FISH

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(Received June 17th, 1908)

Some years after the death of Nägeli (1), in 1891, there was published by Schwendener, a paper which had been found among Nägeli's manuscripts, entitled 'Ueber oligodynamische erscheinungen in lebenden Zellen.' In this paper Nägeli demonstrated the extreme sensitiveness of certain living plants to very minute traces of various metals.

Some years prior to his death Nägeli had observed, during some experiments with Algae, that the latter were killed when placed in distilled water, and although at first he attributed this action to various other causes, he eventually found that it was due to minute traces of copper, which had become dissolved by the water in its passage through the copper still.

Having arrived at this conclusion, Nägeli proceeded to carry out a large number of experiments, using copper coins (two-pfennig pieces, consisting of ninety-five parts of copper, four of tin, and one of zinc) in distilled water, and he even calculated approximately the amount of copper dissolved by the water, viz., 1.3 parts of copper to 100 million parts of water.

He found that this solution was toxic to *Spirogyra* and that, even when diluted ten times, it would still kill them.

There is no doubt that Nägeli considered the copper to be in a state of solution, although at first he believed the toxic action of the copper to be due to a new force, 'Isagität,' and in his original manuscript he uses the word 'isagische' in describing it, but later he substituted the word 'oligodynamische.'

During his investigations which were confined to the action on *Spirogyra*, Nägeli discovered that minute quantities of other metals, viz., silver, lead, tin, iron and mercury, showed oligodynamic properties similar to copper.

Many investigators have conducted experiments with copper and its salts, since that time, and one of the most important researches is that of Israel and Klingmann (2), which was published in 1897.

Israel and Klingmann carried out a large number of experiments on certain bacteria, including *Bacillus typhosus* and *Bacillus coli*, many animal organisms and *Spirogyra*; they used copper foil and found that it had a marked toxic effect on all the bacteria they worked with, *B. typhosus* being the most easily killed.

When the copper solutions containing the organisms were incubated at about 40° C., it was found that they were destroyed in half the time necessary to kill them at laboratory temperature.

It was also found that different organisms were differently affected by the copper solution, and although most organisms were killed in a few minutes, some, e.g., *Stylonychia*, resisted for twenty-four hours, and later experiments by other investigators have shown that some plants not only resist the action of the copper, but even appear to benefit by its presence, under certain conditions.

Locke (3) found that the traces of copper contained in water distilled from or through copper vessels were sufficient to kill *Tubifex*, a freshwater Annelid.

The fact that the degree of this toxic action is not constant but varies from species to species in plants and animals naturally suggested the practical application of this effect in the purification of water from deleterious organisms.

In 1905 an important paper was published by Moore and Kellerman (4) of the Bureau of Plant Industry of the United States

Department of Agriculture, on a method for destroying or preventing the growth of Algae and certain pathogenic bacteria in water supplies.

These authors claim that with very high dilutions of the salts of copper they were able to kill Algae and certain pathogenic organisms; one part in 50,000,000 killed Algae in watercress beds, 1 in 4,000,000 foul-smelling *Anabaena*, and 1 in 100,000 killed both cholera and typhoid in four or five hours at laboratory temperature.

Rideal and Baines carried out experiments to control these results, but were not so successful as Moore and Kellerman in disinfecting with such high dilutions, and they could not confirm their results. They came to the conclusion that 1 in 1000 of copper salts was the only safe dilution, but that twenty-four hours in a copper vessel freed the water from *Bacillus typhosus* and *Bacillus coli*.

Kraemer (5) did some very accurate work with a view to testing the efficiency of metallic copper and sulphate of copper in killing *Bacillus typhosus* and *Bacillus coli* in water. He used pure cultures of typhoid and colon bacilli, developed in bouillon for about twenty-four hours, and his results may be summarised for the purposes of the present paper.

1. Filtered tap water prepared by means of a Berkefeld filter attached to a copper spigot, contained a sufficient amount of metallic copper (dissolved from the spigot) to kill *Bacillus typhosus* in less than four hours, and although *Bacillus coli* was not actually killed, its growth was very markedly inhibited, owing to the presence of these minute traces of copper.

2. When strips of copper foil about 15 mm. wide and 18 cms. long were added to two separate series of three different flasks, (a) containing triple distilled water, (b) filtered water, etc., (c) tap water, and to each of which had been added a fresh bouillon culture of typhoid and colon bacilli, respectively, it was found that in four hours all the flasks were quite free from organisms and the solutions remained sterile.

3. When copper sulphate was added to tap water, so that there

was 1 part to 100,000 of water, 97 per cent. of the organisms were destroyed in eight hours. When the strength was reduced so that there was one part of copper sulphate to 1,000,000 parts of water, there was a reduction of 86 per cent.

Owing to the sensitiveness of typhoid and colon bacilli to the influence of copper, as previously shown, it may be inferred that they would have been included in the organisms destroyed.

In 1905 there was published by Fleet Surgeon Bassett-Smith (6) a paper entitled 'Experiments to demonstrate the Germicidal Power of Copper and Copper Salts on Pathogenic and Non-Pathogenic Organisms.'

From this paper only the results of experiments with typhoid bacilli will be quoted.

Bassett-Smith first conducted a series of experiments with different strengths of copper sulphate (viz., 1 in 1,000, 1 in 10,000 and 1 in 100,000) in tap water and distilled water.

It is not apparent from the context of the paper, what is meant by the above dilutions, nor is it possible to determine whether they were prepared from 'gram-molecular' solutions or not. The method of procedure was as follows :—

Ten cubic centimetres of each dilution was inoculated with thirty cubic millimetres of a well-grown broth culture of the organisms; these were kept at room temperature in the dark and sub-cultured at regular intervals.

The result of this series was that in all dilutions with distilled water *B. typhosus* was killed under one hour, but with solutions made with tap water, the bacilli were present at the end of one hour in the 1 in 10,000 solution, but were all dead in three hours; in the 1 in 100,000 solution, the reaction was positive after fifteen hours, but negative in twenty-four hours.

Bassett-Smith comes to the following conclusions, as a result of his experiments to purify contaminated water by means of copper sulphate :—

'The only safe dilution is the low one of 1 in 1000, the solution is best freshly prepared, and at least twenty-four hours' action is

generally necessary. The salt is most efficient when made up with distilled water ; and *Bacillus typhosus* is more easily killed than others of the coli group, being destroyed in twelve hours with the 1 in 10,000 dilution.'

In the second part of the paper the results of experiments conducted to discover whether other metals might have destructive or inhibitory power on typhoid and other organisms, are tabulated. Stoppered tubes of iron, copper, zinc, lead, tin and glass were first used, and later on metal boxes capable of containing a much larger quantity of infected water but giving a relatively smaller proportional area of metal to the cubic contents.

Sterilized tap water inoculated with broth cultures of typhoid bacilli was used, and the boxes were kept in the dark at room temperature.

From these experiments it appeared that iron and copper were the most powerfully bactericidal, no growth being obtained twelve hours after treatment ; zinc was negative in eighteen hours, lead in forty-eight hours, and the tin and glass remained positive at the end of three days.

It was found, on examination of the boxes, that the surface of the copper and zinc was coated with a thick layer of deposit, and when similar experiments were conducted with the boxes in this state, their bactericidal power was greatly decreased, the solution in the copper box still containing bacilli at the end of three days. When the insides of the boxes were thoroughly cleaned, the action again became normal.

Finally, solutions of granulated sulphate of iron, 1 in 1,000, 1 in 10,000 and 1 in 100,000, were prepared and their germicidal action tested.

Solution 1 in 100,000 was practically non-effective ; 1 in 1,000 and 1 in 10,000 were fatal to *B. typhosus* in under seven hours. 'Practically therefore, sulphate of iron added to water in the proportion of 1 in 10,000 would in less than seven hours free it from typhoid bacilli, but not from other members of the coli group, being almost as effective as the more dangerous copper sulphate solution.'

PRESENT EXPERIMENTS

In January, 1906, the present series of experiments with salts of several of the heavy metals was commenced in order to ascertain their effect on the *Bacillus typhosus* and then, if feasible, that the results obtained might be applied to the purification of shell-fish from this organism.

In the papers quoted previously none of the writers has exactly stated what is meant when speaking of dilutions of '1 in 1,000,' etc., and so the first step was the preparation of a number of gram-molecular solutions from which the dilutions subsequently used were prepared as required (the ferrous sulphate solution was prepared immediately before being used, as it rapidly becomes oxidised), and thus the exact amount of the salt employed in any particular experiment could be accurately determined. The typhoid cultures used were grown on agar, generally for forty-eight hours, some of the growth obtained being collected on a sterilized loop of platinum wire, and an emulsion of typhoid bacilli was made in 100 c.c. of distilled water.

The different solutions were made with (1) distilled water, (2) tap water, collected after being allowed to run for five minutes, and (3) sea water.

In all the experiments the water had been sterilised in an autoclave at 110° C. for thirty minutes; and all the Erlenmeyer flasks, Petri dishes and pipettes used were sterilized in the same way.

For determining the number of organisms, 1 c.c. of the respective solutions was taken at intervals with a sterile pipette and added to a sterile test-tube, containing litmus red taurocholate agar which had been previously melted by means of a steam bath, and the tubes cooled down to about 40° C. in a beaker containing water at that temperature.

The contents of the tube when inoculated, were immediately placed in a sterile Petri dish and the latter incubated at about 35° C. When the number of colonies was quite uncountable the result is described in the tables as 'infinite'; when, although there was a large number of colonies the number could have been counted, the result is indicated by the word 'positive' (all 'positive' results below

about 1,000 colonies per c.c. have the number of colonies indicated), and when the plates showed no colonies at all the result is expressed by the word 'negative.'

SERIES I.—EXPERIMENTS WITH COPPER SALTS

The first series of experiments was done with solutions of copper sulphate, in (a) distilled water, (b) tap water, which had been allowed to run for five minutes, and (c) sea water, and the results are tabulated below :—

I (a).—1 cubic centimetre of the typhoid emulsion prepared as above was added to each of the following five flasks :—

(1)	Containing 100 c.c. of distilled water.						
(2)	"	"	$\frac{1}{1,000}$	M	Copper sulphate solution made with distilled water. ¹		
(3)	"	"	$\frac{1}{10,000}$	M	"	"	"
(4)	"	"	$\frac{1}{100,000}$	M	"	"	"
(5)	"	"	$\frac{1}{1,000,000}$	M	"	"	"

The flasks were incubated at 35° C., and sub-cultures taken at regular intervals.

DISTILLED WATER

		COPPER SULPHATE SOLUTIONS				
Sub-cultures taken		Control	$\frac{M}{1,000}$	$\frac{M}{10,000}$	$\frac{M}{100,000}$	$\frac{M}{1,000,000}$
At once	...	Infinite	Infinite	Infinite	Infinite	Infinite
15 minutes after	..	"	Negative	Positive	Positive	"
1 hour after	...	"	"	Negative	Negative	Positive (5 colonies)
2 hours after	...	"	"	"	"	Negative
6 hours after	...	"	"	"	"	"
24 hours after	...	"	"	"	"	"

1. The extreme dilution of these solutions is seen when it is remembered that M strength means 63 parts of copper per 1,000, and therefore, $\frac{M}{1,000}$ means 63 parts in 1 million, and $\frac{M}{1,000,000}$ means 63 parts in 1,000 millions. It may be pointed out that the solutions used throughout are much more dilute than the $\frac{1}{1,000}$, etc., of previous authors, since these most probably mean parts by weight of the crystalline salts and not $\frac{1}{1,000}$, etc., gram-molecular solutions.

It will be seen by the above table that in the copper sulphate solutions all the bacilli were destroyed in one hour with the exception of the 1,000,000th dilution, in which five colonies per c.c. persisted, but even this solution was quite sterile at the end of two hours and remained so.

The number of bacilli on the control plate at the end of twenty-four hours was still 'infinite.'

I (b).—This was in all respects similar to I (a), with the difference that the solutions were made with sterilised tap water instead of distilled water.

It was noticed that the contents of flasks 2 and 3, *i.e.*, the $\frac{M}{1,000}$ and $\frac{M}{10,000}$ dilutions, had a distinctly blue colour and there was a thick sediment at the bottom of these two flasks.

TAP WATER

Sub-cultures taken		Control	COPPER SULPHATE SOLUTIONS			
			$\frac{M}{1,000}$	$\frac{M}{10,000}$	$\frac{M}{100,000}$	$\frac{M}{1,000,000}$
At once	...	Infinite	Infinite	Infinite	Infinite	Infinite
15 minutes after	...	"	Negative	Positive	"	"
1 hour after	...	"	"	" (14 colonies)	Positive	"
2 hours after	...	"	"	Negative	"	Positive
4 hours after	...	"	"	"	" (173 colonies)	"
8 hours after	...	"	"	"	Negative	"
24 hours after	...	"	"	"	"	" (193 colonies)
48 hours after	...	"	"	"	"	Positive (147 colonies)

I (c).—This was similar to I (a) and I (b), excepting that the solutions were made with sterilised sea water, instead of distilled or tap water. A thick precipitate was thrown down in all the flasks, but especially in No. 2. The first two dilutions were distinctly blue.

SEA WATER

			COPPER SULPHATE SOLUTIONS			
Sub-cultures taken	Control		M	M	M	M
			1,000	10,000	100,000	1,000,000
At once	Infinite		Infinite	Infinite	Infinite	Infinite
2 hours after ...	"		Positive	Positive	"	"
6 hours after ...	"		Negative	"	Positive	"
24 hours after ...	"		"	Negative	Negative	Positive
48 hours after ...	"		"	"	"	"
72 hours after ...	"		"	"	"	"

The most striking fact in the above series of experiments is the exceedingly small quantity of copper sulphate which was sufficient to kill all the typhoid bacilli in a few hours.

This is especially noticeable in the results obtained with solutions made with distilled water, when a dilution of one in a million of gram-molecular was sufficient to kill all the bacilli in less than two hours. The actual amount of copper in this dilution was 63 parts in 1,000 million parts of water, or roughly one of copper in sixteen million parts of water.

In the solutions made with tap water and sea water the action was still very marked, but less so than in the case of the distilled water. This is probably due to the fact that the tap and sea water both contained other substances, which in some way modified the action of the copper sulphate, thus weakening the solutions.

In this connection it is of interest to remember that Moore and Kellerman (4) have shown, in their paper, the relative decrease of toxicity of copper sulphate solutions, depending on the amount of organic matter present, the amount of carbon dioxide in solution, or the temporary hardness of the water.

Nägeli showed that the oligodynamic action of copper solutions was lessened by the introduction of many insoluble substances, and True and Oglevee have studied the influence of insoluble substances on the toxic action of poisons and have confirmed many of Nägeli's observations.

The manner in which the presence of such adventitious substances, inorganic or organic, in the water to which the copper salt is added, produce their effects in diminishing the toxic action, is not discussed

by previous authors, with the exception of Kraemer, who concluded that the toxicity is due to some salt of copper, which 'is probably in the form of a crystalloid rather than that of a colloid.'

It is, however, fairly obvious that the toxic action depends upon the concentration in the solution of the copper ion, because all salts of copper possess the toxicity, which must, therefore, depend upon the presence of the free kation in the solution and not upon the non-ionized molecule of the copper salts, which is different in each case.

When once it is considered that the toxicity is dependent in all cases upon the concentration of the copper ion in the solution, the varying toxicity, when the copper is added to (*a*) distilled water, (*b*) tap water, (*c*) sea water or (*d*) water containing other added salts or organic matter, becomes easy of explanation. The salts present in the water cause the degree of ionization of the copper to vary. For instance, when distilled water only is present and then copper sulphate is added, at the degree of dilution here concerned, the copper sulphate practically completely ionizes into copper ions and sulphion ions, and therefore the concentration in copper ions is proportional to the total amount of copper sulphate added. But when salts such as the phosphates are present, as in tap water or sea water, the ionization in the solution becomes that of the much more feebly ionized copper phosphate and consequently the concentration in copper ions is no longer indicated by the amount of copper sulphate added, but is reduced correspondingly to the low degree of ionization.

Similarly the addition of apparently inert organic substances, such as cellulose, silk, wool, glue, etc., will produce a like effect by forming feeble combinations or adsorptions with the copper ion and thus reduce its concentration.

Since the toxicity, in the case of salts of the heavy metals, depends on the metallic kation entirely and not on the anion of the particular acid in combination in the salt employed, it was considered sufficient in most cases to test only one salt of each metal. Also, since the amount of added salt is so small, any low degree of toxic action of the anion can be disregarded, and the most convenient and easily accessible salt of the heavy metal can be employed.

Secondly, the results of this series tend to strengthen the theory of those observers who contend that in the case of the copper foil in water, the metal itself is not actually in a state of solution as a colloid in the water, but that it forms salts and that it is the action of these salts which determines the toxicity of the solution ; for these concentrations of copper as salt are less than the amount of copper in colloidal solutions.

These experiments with such excessively dilute solutions of copper sulphate appear to show very clearly that copper in the ordinary condition of solution as a salt (that is to say in the ionized condition and not in colloidal solution) is capable of exhibiting all the so-called 'oligodynamic' properties, produced when metallic copper is immersed in water in which living organisms are present.

Accordingly, no foundation is left for the term oligodynamic as applied to copper brought into solution by the latter method, and the results obtained are merely an index of the high toxicity of copper ions upon some forms of living cells.

Attention may here be drawn to the fact that the solutions, containing the typhoid bacilli were incubated at a temperature of 35° to 40° C., and this applies to all other experiments with the exception of the ferrous sulphate and ferric chloride series, in which the flasks were kept at room temperature, *i.e.*, about 15° C. Attention is drawn to this matter, because it was proved by Israel and Klingmann that when the solutions of copper containing the organisms were placed in an incubator at 35° to 40° C. the toxic effects were manifested in one hour, but if the solutions were kept at ordinary temperature the toxic effects were not produced until two hours had elapsed ; so that, according to this statement, these experiments were conducted under the most favourable conditions.

SERIES II.—EXPERIMENTS WITH IRON SALTS

Experiments with dilutions of gram-molecular solutions of ferrous sulphate and ferric chloride made with (*a*) distilled water, (*b*) tap water, after being allowed to run for five minutes, and (*c*) sea water.

In this series seven flasks were used, and 1 c.c. of the typhoid emulsion was added to each of them :—

(1) Containing 100 c.c. of distilled water.

(2) " " $\frac{1}{1,000}$ M Ferrous sulphate solution made with distilled water.

(3) " " $\frac{1}{10,000}$ M " " " "

(4) " " $\frac{1}{100,000}$ M " " " "

(5) " " $\frac{1}{1,000}$ M Ferric chloride " "

(6) " " $\frac{1}{10,000}$ M " " " "

(7) " " $\frac{1}{100,000}$ M " " " "

The flasks were kept at room temperature, about 15° C. Nos. 2, 3, 5, and 6 had a distinct colour, 2 and 3 being at first a pale green, and 5 and 6 a rusty brown, but in twenty-four hours all the solutions were brownish in colour, due to the oxidation of the ferrous sulphate.

DISTILLED WATER

Sub-cultures taken	Control	FERROUS SULPHATE SOLUTIONS			FERRIC CHLORIDE SOLUTIONS		
		$\frac{1}{1,000}$ M	$\frac{1}{10,000}$ M	$\frac{1}{100,000}$ M	$\frac{1}{1,000}$ M	$\frac{1}{10,000}$ M	$\frac{1}{100,000}$ M
At once ...	Infinite	Infinite	Infinite	Infinite	Infinite	Infinite	Infinite
2 hours after ...	"	Positive	Positive	"	"	Positive	"
6 hours after ...	"	(314 colonies)			Negative	Negative	"
24 hours after ...	"	"	"	Positive	Negative	"	Positive
48 hours after ...	"	"	"	Negative	Negative	"	Negative

The above table shows that both with the ferrous sulphate and ferric chloride solutions, the 1 in 1,000 and 1 in 10,000 gram-molecular dilutions completely destroyed the bacilli in less than six hours, with the flasks at laboratory temperature.

In both cases the 1 in 100,000 solutions still contained over 1,000 bacilli per cubic centimetre at the end of twenty-four hours, but were quite free in less than forty-eight hours.

II (b).—This was similar to II (a), with the difference that the solutions were prepared with tap water instead of distilled water. Flasks Nos. 2, 3, 5, and 6 have a more distinct colour than in the

previous series, and the ferrous sulphate solutions have turned yellow. There is a precipitate thrown down to a slight degree in all flasks, but more marked in 2 and 3.

TAP WATER

Sub-cultures taken	Control	FERROUS SULPHATE SOLUTIONS			FERRIC CHLORIDE SOLUTIONS		
		M 1,000	M 10,000	M 100,000	M 1,000	M 10,000	M 100,000
At once ...	Infinite	Infinite	Infinite	Infinite	Infinite	Infinite	Infinite
2 hours after ...	"	Positive	Positive	"	Positive	Positive	"
4 hours after ...	"	"	"	"	"	"	"
8 hours after ...	"	Negative	Negative	Positive	Negative	Negative	Positive
24 hours after...	"	"	"	"	"	"	"
48 hours after...	"	"	"	"	"	"	"
				(227 colonies)			
							(335 colonies)

It will be noticed on comparing the above table with II (*a*) that the action of the solutions made with tap water was slower than that made with distilled water, and the 1 in 100,000 solutions were still positive at the end of forty-eight hours.

II (*c*).—Similar in details to II (*a*) and II (*b*) except that the solutions were made with sea water instead of distilled or tap water. There was a thick brownish deposit in all the flasks, after they had been incubated, with the exception of the control, in which the deposit was less marked and not coloured to any extent.

SEA WATER

Subcultures taken	Control	FERROUS SULPHATE SOLUTIONS			FERRIC CHLORIDE SOLUTIONS		
		M 1,000	M 10,000	M 100,000	M 1,000	M 10,000	M 100,000
At once ...	Infinite	Infinite	Infinite	Infinite	Infinite	Infinite	Infinite
2 hours after ...	"	Positive	Positive	Positive	Positive	Positive	Positive
5½ hours after	"	Negative	"	"	"	"	"
				(17 colonies)			
24 hours after...	"	"	Negative	"	Negative	Negative	"
48 hours after...	"	"	"	"	"	"	"

It is seen from this table that the toxicity of the solutions made with sea water is distinctly less than was the case with the distilled water, and slightly less than that of the solutions made with tap water. This is probably explained in the same way as in the case of the copper sulphate solutions, and is no doubt due to salts, etc., in the

water which modify the action of the solutions—in the latter two cases by lowering the ionization as previously described.

In this series the marked toxicity of the solutions of salts of iron is strikingly demonstrated. This is all the more remarkable, firstly, because this series was conducted at laboratory temperature, *i.e.*, 15° C. instead of about 40° C. as was the case with Series I (it has been previously shown that the incubation of the solutions greatly increases their killing power), and secondly, because salts of iron are not poisonous in the ordinary sense of the word.

It will further be seen that the action of the two salts was very similar in each series, the ferrous sulphate solutions being slightly more toxic than the ferric chloride.

This is rather remarkable, as the ferrous sulphate always became converted into a ferric salt after the lapse of a few hours, but the result may be due to increased ionization of the ferric sulphate formed.

It is interesting to compare these results with those obtained by Bassett-Smith in experiments with water infected with typhoid bacilli and contained in (*a*) an iron tube, and (*b*) an iron box of much larger capacity.

In the iron tube all the bacilli were killed in less than twenty-four hours, and in the iron box in less than 18 hours; but in the latter case the solution was not so heavily infected with bacilli as in the former. In both cases the water, at the end of twenty-four hours, was quite a rusty colour, due to the formation of oxide of iron.

The results of this series are important for several reasons. In the first place, although the toxicity of the salts of iron is less than that of the copper sulphate solutions, it is still sufficient to be of great practical importance, and whereas the safe dose for human beings has still to be determined in the case of copper salts, it is well known that not only are salts of iron not deleterious to man, but that many of them are even beneficial and are used medicinally. This fact is also of importance in regard to the purification of drinking water by addition of salts of iron or by storage in iron tanks, and also in the purification of suspected shell-fish.

It is, moreover, possible to employ iron in the manufacture of

tanks for water purification when the use of copper could not be entertained owing to its great cost.

The results of the experiments made with these salts of iron in attempting to purify Anodons infected with *B. typhosus* are tabulated in Part II of this paper.

SERIES III.—EXPERIMENTS WITH NICKEL AND COBALT SALTS

Seven flasks were used to contain the different solutions, which were made with distilled water.

The 1 in 1,000 solutions were coloured green and red respectively, but the others were colourless.

One c.c. of the typhoid emulsion was added to each of the flasks and they were then incubated at 40° C.

Sub-cultures taken	Control	COBALT CHLORIDE SOLUTIONS			NICKEL CHLORIDE SOLUTIONS		
		M 1,000	M 10,000	M 100,000	M 1,000	M 10,000	M 100,000
At once ...	Infinite	Infinite	Infinite	Infinite	Infinite	Infinite	Infinite
1 hour after ...	"	"	"	"	"	"	"
3 hours after ...	"	Positive	"	"	Positive	"	"
8 hours after ...	"	"	Positive	"	"	Positive	"
24 hours after...	"	Negative	"	Positive	Negative	"	Positive
			(107 colonies)			(203 colonies)	
48 hours after...	"	"	Negative	"	"	Negative	"

It will be seen from this table that although cobalt and nickel chlorides have a toxic action on typhoid bacilli, it is much less marked than in the case of the copper or iron salts and takes a much longer time to manifest itself.

This is interesting because these metals belong to the group of heavy metals, and yet possess a comparatively low toxicity.

Taking the 'distilled water' series in each case and comparing the results it is seen that whereas the copper sulphate solutions, including the 1 in 100,000, were clear at the end of an hour, and the first two dilutions of the ferrous sulphate and ferric chloride experiments were clear in less than six hours, *at room temperature* the corresponding cobalt and nickel solutions still contained over 1,000 organisms per c.c. in each case at the end of eight hours, and the 1 in 10,000 solutions were not clear at the end of twenty-four hours.

As the toxic effects would have taken still longer to manifest

themselves in solutions made with tap or sea water, this series was not continued, as it was considered that the results would be of no practical value, even under the most favourable conditions.

SERIES IV.—EXPERIMENT WITH MANGANESE SALTS

The solutions were made with distilled water and were incubated at about 40° C.

To each of four flasks was added 1 c.c. of the typhoid emulsion, prepared as in the previous experiments.

				MANGANESE CHLORIDE SOLUTIONS		
				M 1,000	M 10,000	M 100,000
Sub-cultures taken		Control				
At once ...	...	Infinite		Infinite	Infinite	Infinite
1 hour after ...	...	"		"	"	"
4 hours after ...	...	"		"	"	"
8 hours after ...	...	"		Positive	"	"
24 hours after ...	...	"		Negative	Positive (79 colonies)	Positive
48 hours after ...	...	"		"	Negative	" (27 colonies)

It is seen that although manganese chloride has bactericidal power, it is much feebler than in the case of the copper and iron salts.

SERIES V.—EXPERIMENTS WITH ZINC SALTS

Four flasks were used as in the previous experiment and the solutions were all made with distilled water and incubated at about 40° C. One c.c. of the emulsion of typhoid bacilli was added to the contents of each flask.

				ZINC SULPHATE SOLUTIONS		
				M 1,000	M 10,000	M 100,000
Sub-cultures taken		Control				
At once ...	...	Infinite		Infinite	Infinite	Infinite
1 hour after ...	...	"		Positive	"	"
4 hours after ...	...	"		"	Positive	"
8 " " ...	...	"		"	"	Positive
				(507 colonies)		
24 " " ...	...	"		Negative	"	"
					(43 colonies)	
48 " " ...	...	"		"	Negative	"
						(403 colonies)

These results were not exactly what were expected, having regard to those obtained by Bassett-Smith with water infected with typhoid bacilli and placed in (*a*) a zinc tube, and (*b*) a zinc box.

In the former case the water was cleared of bacilli in eighteen hours, and in the latter in less than twenty-four hours, the water in this case being more heavily infected than was the case with the zinc tube.

As against this, however, the same observer conducted a special experiment with strips of zinc foil, to see if they would be able to clear water infected with typhoid bacilli, as the copper foil had done. Strips of zinc foil, having a superficial area of 50 square centimetres were added to a flask containing 100 c.c. of sterilized tap water, and the latter was infected with a broth culture of typhoid bacilli and kept at room temperature in the dark.

‘Up to forty-eight hours this did not seem to be effective against any of the tested organisms.’

Furthermore, it must not be forgotten that the emulsion of typhoid bacilli used in all these experiments contained very many more bacilli per cubic centimetre than did the broth cultures used in the experiments cited above.

(*b*) The above series was repeated with solutions made with tap water instead of distilled water, and the flasks were kept at room temperature.

				ZINC SULPHATE SOLUTIONS		
				M	M	M
				1,000	10,000	100,000
Sub-cultures taken						
Control						
At once	...	...	Infinite	Infinite	Infinite	Infinite
2 hours after	...	...	"	"	"	"
4 "	"	...	"	Positive	"	"
8 "	"	...	"	"	"	"
24 "	"	...	"	Negative	Positive	"
48 "	"	...	"	"	"	Positive

It will be seen from the above table that with the solutions made with tap water and kept at room temperature (about 15° C.) the toxic action was even less marked.

SERIES VI.—EXPERIMENTS WITH SILVER SALTS

Experiments were carried out with different strengths of silver nitrate made with (a) distilled water and (b) tap water, after being allowed to run for five minutes.

(a) Four flasks were used, and the solutions were made with distilled water and incubated at 40° C.

One cubic centimetre of the typhoid emulsion was added to each of the flasks.

				SILVER NITRATE SOLUTIONS		
				M 1,000	M 10,000	M 100,000
Sub-cultures taken		Control				
At once		Infinite		Infinite	Infinite	Infinite
2 hours after		"		Negative	Negative	Negative
4 hours after		"		"	"	"
6 " " " " " "		"		"	"	"
24 " " " " " "		"		"	"	"
48 " " " " " "		"		"	"	"

The results of this series were very definite and proved the silver nitrate to have a very decided germicidal action on the *B. typhosus*. Comparing the above table with that showing the results of the copper sulphate solutions made with distilled water, it is seen that the action of the silver nitrate is practically as great as that of the copper sulphate.

(b) This series was the same as (a), except that the solutions were made with sterilised tap water collected as described before.

It was immediately noticed that the solutions were inclined to be milky, the 1 in 1,000 and the 1 in 10,000 decidedly so, and the 1 in 100,000 faintly milky.

This was of course due to the precipitation of the silver salt by the chlorides present in the water; the result being the formation of silver chloride which is practically insoluble in water.

				SILVER NITRATE SOLUTIONS		
				M 1,000	M 10,000	M 100,000
Sub-cultures taken		Control				
At once		Infinite		Infinite	Infinite	Infinite
2 hours after		"		Negative	Positive	"
4 " " " " " "		"		"	"	"
6 " " " " " "		"		"	Negative	"
24 " " " " " "		"		"	"	Positive
48 " " " " " "		"		"	"	"

It is noticed that in the 1 in 1,000 solution there was enough silver nitrate left after all the chlorides had been precipitated¹ to still make the solution strongly germicidal.

1. The Liverpool Water Supply is almost free from chlorides.

In the case of the 1 in 10,000 solution the result either indicated (i) that there was still enough silver nitrate left after the precipitation of the chlorides to make the solution sufficiently toxic to kill the typhoid bacilli in less than six hours, or (ii) that if all the silver nitrate was precipitated as chloride, there must have been a sufficient quantity of this so-called insoluble salt dissolved in the water to produce the toxic effect.

In the 1 in 100,000 solution, the toxicity was very greatly diminished by making it with tap water instead of with distilled water.

SERIES VII.—EXPERIMENTS WITH LEAD SALTS

This series was done with solutions of lead nitrate made with (a) distilled water and (b) tap water, collected after being allowed to run for five minutes.

Four flasks were used in each case, and were incubated at about 40° C. One cubic centimetre of the emulsion of typhoid bacilli was added to each of the flasks.

(a) Solutions made with distilled water :

Sub-cultures taken			LEAD NITRATE SOLUTIONS		
			M 1,000	M 10,000	M 100,000
At once	...	...	Infinite	Infinite	Infinite
2 hours after	...	...	Negative	Positive	"
4 hours after	...	...	"	Negative	Positive
6 "	"	...	"	"	"
24 "	"	...	"	"	"
					(879 colonies)
48 "	"	...	"	"	"
					(53 colonies)

(b) Solutions made with tap water, collected as previously :—

Sub-cultures taken			LEAD NITRATE SOLUTIONS		
			M 1,000	M 10,000	M 100,000
At once	...	...	Infinite	Infinite	Infinite
2 hours after	...	...	Negative	"	"
4 "	"	...	"	Positive	"
6 "	"	...	"	"	"
24 "	"	...	"	"	Positive
48 "	"	...	"	"	"

It is seen that the lead nitrate solutions both with distilled and tap water were decidedly toxic to the bacilli, but that the solutions made with distilled water were much stronger and quicker in their action than those made with tap water.

Here it is interesting to note the result of experiments by Bassett-Smith, done with metallic lead, to see whether solutions containing *B. typhosus* could be rendered sterile by being placed in (a) a lead tube, and (b) a lead box.

In both cases the solutions still contained many organisms at the end of forty-eight hours, and it could not be said that the lead had any germicidal action.

On the other hand, Nägeli did experiments with lead, and was satisfied that it possessed 'oligodynamic' properties similar to copper.

CONCLUSIONS TO PART I

The following conclusions were arrived at as the result of the foregoing experiments :—

(1) All the salts tested manifest a decided toxic action to typhoid bacilli, but there is a great difference in degree, shown in the case of the stronger solutions by the time taken by the corresponding solutions of the various salts in clearing the water from bacilli; and in the failure of the more dilute solutions of the less toxic salts to clear within the limit of forty-eight hours.

(2) The toxic action is most marked when the solutions are prepared with distilled water, and are incubated at a temperature of 35° to 40° C.

(3) The toxic action of the solutions made with tap water and sea water is much less than in the case of those made with distilled water, and this diminution is due to the fact that the tap and sea water both contain other substances which modify the action of the salts employed—probably by lessening the ionization.

(4) The copper sulphate and silver nitrate solutions possess the greatest toxicity; the lead nitrate, ferrous sulphate, and ferric chloride solutions being next in order of toxicity; whilst the zinc sulphate, nickel and cobalt chlorides and manganese chloride solutions take a

much longer time before the corresponding solutions are free from bacilli.

(5) The toxicity of water to which either copper foil or certain other metals (*e.g.*, silver, iron, tin, lead, etc.) have been added is probably due to a solution of some salt of that metal, and the so-called oligodynamic action of the solution is due to the presence of the ions of this salt, and not to the metal itself.

PART II

EXPERIMENTS ON PURIFICATION OF SHELL-FISH

Having determined by means of the experiments detailed in Part I the salts most likely to prove successful in purifying infected shell-fish, an endeavour was made to purify Anodons (freshwater mussels) which had previously been strongly infected with typhoid bacilli.

It was first necessary to discover the actions, if any, of the solutions it was proposed to use on the Anodons themselves, and a series of experiments was first carried out in each case with this end in view.

In these experiments a number of glass dishes was used, in which the Anodons were placed in known quantities and strengths of various solutions.

The Anodons used were of medium size and good vitality, and all the glass dishes were very thoroughly cleaned after each series of experiments.

When it was required to infect the Anodons with typhoid bacilli the process was carried out as follows :—

An emulsion of typhoid bacilli was first made in 100 c.c. of sterilized tap water, exactly as in the experiments in Part I, with the exception that tap water was used instead of distilled water.

This emulsion was made up to two litres with tap water (collected after being allowed to run for five minutes) in one of the glass dishes, and into this solution twelve Anodons were placed and left for at least twenty-four hours.

At the end of this time they were taken out as required, and put, without any preliminary cleansing, into the various solutions.

The solutions containing the Anodons were kept at laboratory temperature, and in the case of the silver nitrate experiments they were kept in the dark, as it was found that, unless this precaution was taken, the silver became reduced and the solutions turned dark brown.

The determination of the number of organisms in different parts of the Anodons before and after treatment with the solutions was made by passing a sterile capillary tube (*a*) into the stomach, (*b*) into the rectum, and (*c*) over and among the gills.

In each case the water in the capillary tube was added to a sterile test-tube, containing litmus red taurocholate agar which had previously been melted and kept at 40° C. by means of hot water in a beaker, and the contents were then placed at once in a sterile Petri dish and incubated at about 40° C.

The average capacity of the capillary tubes was 0.05 c.c., but they varied slightly in size.

Before these cultures could be made it was necessary to open the shell of the Anodon, and to do this the anterior and posterior adductor muscles had first to be cut through with a sharp knife.

When it was possible to slightly open the shell this could be done by passing the knife along inside, keeping close to the shell, and first cutting the anterior and then the posterior adductor or *vice versa*.

When, however, it was impossible to open the shell even a little, it was first necessary to cut the edge with a sharp pair of scissors, when the knife could be used as before.

The knife and scissors used were carefully sterilized after each experiment, as were also the hands of the operator.

METALLIC AND COLLOIDAL COPPER

I. Experiments with (1) Anodons and (2) Anodons infected with typhoid bacilli, in (*a*) tap water containing a piece of bright copper foil 15 cms. by 12 cms., (*b*) a solution of colloidal copper sulphate.

As a preliminary to this series, a solution of colloidal copper was prepared by passing an electric current through distilled water, using

copper electrodes, which were so arranged as to form an electric arc under the water.

The current was cut off at frequent intervals to prevent the solution from getting hot, as when this occurs the copper becomes coagulated and precipitated, and then appears as flaky masses in the solution. Altogether nearly three litres of this solution, which is of a slightly greenish colour, were prepared.

The next step was to estimate the amount of copper in the colloidal solution, and this was done as follows :—

Five hundred cubic centimetres of the solution were evaporated to dryness in a clean glass evaporating dish, the residue being dissolved in about twelve drops of strong nitric acid, diluted with a little water, and this solution was again evaporated to dryness.

This residue was dissolved in distilled water containing a drop of hydrochloric acid.

The solution was transferred to a clean glass beaker (the evaporating dish being twice washed out with a little distilled water to dissolve any residue), heated to boiling, precipitated as hydrate, and filtered and weighed in a Gooch crucible.

Weight of Gooch crucible alone ...	...	...	...	6.0960 grammes
" " containing residue ...	...	...	...	6.1098 grammes
" " weight of CuO ...	...	...	...	0.0138 "
" " " Cu ...	...	...	...	0.0110 "

This represented the amount of copper in 500 c.c. of the colloidal copper solution, and $\therefore$ the amount in one litre was 0.0220 grammes.

This represents 1.1 grammes of copper in 50,000 c.c.

A series of experiments was next done to discover the degree of toxicity of this solution.

Four Erlenmeyer flasks were taken, as under, and to each of them was added 1 c.c. of typhoid emulsion, prepared as in the experiments in Part I.

The flasks were left at laboratory temperature, and sub-cultures made at regular intervals.

- (1) Containing 100 c.c. of distilled water.
 (2) „ 100 c.c. of the colloidal copper solution.
 (3) „ 10 c.c. „ „ made up to 100 c.c. with distilled water.
 (4) „ 1 c.c. „ „ „ „

				COLLOIDAL COPPER SOLUTIONS		
Sub-cultures taken				Control	Original	1 in 10 1 in 100
At once				...	Infinite	Infinite Infinite Infinite
2 hours after				...	„	Negative Negative Negative
4 „	„	...	...	„	„	„ „
8 „	„	...	...	„	„	„ „
24 „	„	...	...	„	„	„ „

It will be seen from the above table of results that the colloidal copper solution had a marked toxic action on typhoid bacilli, and even when diluted 100 times it killed them in less than two hours at laboratory temperature.

The experiments with dilutions exceeding 1 in 1,000,000 of (a) copper in the ionic form, as present in dilute solution of copper sulphate, and (b) of colloidal copper, as in the above experiment, agree in giving complete removal of the bacilli within the two hours time.

It is almost impossible to follow with accuracy the toxic properties beyond these limits, and hence we may for the present regard the two forms of copper as being approximately equally toxic, and both as lying at the upper limit of high toxicity.

It is not at all easy to determine the exact state of the copper in this colloidal solution, for although, since the classical experiments of Graham, it has been customary to apply the name colloidal to those substances which will not pass through animal membranes, more recent researches have shown that there are two sub-classes of colloidal mixtures—the one having the characteristic properties of true solutions, *i.e.*, possessing osmotic pressure, diffusibility, and usually a limited solubility at some temperature: the other being without these properties, and being in the nature of macroscopic or microscopic suspensions.

The fact that this colloidal copper solution possesses the property

of permeating colloids, as the cell wall and the organized contents of the cell, thereby producing marked disturbances in the cell and exerting its toxic action, tends to show that it in some ways resembles the crystalloids.

Whatever may be the true nature of the solution, it is certain that its toxicity is not merely due to this 'colloidal' state, for Roaf and Whitley (7) have shown that whereas colloidal silver prepared in the above manner was exceedingly toxic to tadpoles, a solution of colloidal platinum had absolutely no effect upon them.

It must also be borne in mind that the bacilli present in the solution are producing carbonic acid and weak organic acids which are capable of acting upon the colloidal copper and causing it to pass from the colloidal form to true solution in the ionic condition. When regard is paid to the excessively minute quantity of copper present in the solution, it is obvious that the bacterial products would be quite sufficient to cause the copper to form a salt, become ionized, and act in the same way as the excessively dilute solutions of salts of copper used in the former experiment in Part I with copper sulphate.

Further, when once the colloidal copper entered the cells it would meet there with substances capable of converting it into a copper salt; so that whether the copper be present in the solution as a colloid or as an ion it would, in the end, produce its effect as copper ion; and the results obtained with copper sulphate, when present in so high a dilution as one part of copper in 10,000,000, show that copper, qua copper ion, is capable of producing all the effects obtained with colloidal solutions, so that no peculiar 'oligodynamic properties' or 'colloidal condition' need be assumed in order to explain the results obtained.

Having determined the amount of toxicity of the colloidal copper solution, the experiments with Anodons were now proceeded with in solutions containing different forms of copper.

Two Anodons were placed in each of the following dishes to

determine the action of the different solutions on them. The dishes were kept at laboratory temperature.

- (1) Containing 2 litres of tap water, collected after being allowed to run for 5 minutes.
- (2) " 2 " " with a piece of bright copper foil.
- (3) " 1 litre colloidal copper solution.
- (4) " 2 litres $\frac{M}{2,000}$ copper sulphate solution, made with tap water.
- (5) " 2 " $\frac{M}{5,000}$ " " "
- (6) " 2 " $\frac{M}{10,000}$ " " "

It was noticed that there was soon a good deal of mucus in the solutions.

	24 hours	48 hours	72 hours	1 week	2 weeks	4 weeks
1st dish	2 alive	2 alive	2 alive	2 alive	2 alive	2 alive
2nd "	2 "	2 "	2 "	2 "	2 "	2 "
3rd "	2 "	2 "	2 "	2 "	2 "	2 "
4th "	1 dead	1 dead	2 dead	—	—	—
5th "	2 alive	2 alive	2 alive	1 alive	2 dead	—
6th "	2 "	2 "	2 "	2 "	2 dead	—

It will be seen from this table that the copper foil and colloidal copper solutions did not visibly affect the Anodons in four weeks. This is probably due to the fact that the Anodons secreted a good deal of mucus, which in some way precipitated the copper in the solutions and thus stopped or greatly modified its action.

It was noticed that the copper foil rapidly lost its lustre and became dull; and in the colloidal copper solution the mucus at the bottom of the dish was tinged green, whilst the water itself was quite clear.

In the case of the copper sulphate solutions, although the Anodons secreted mucus on account probably of the irritation from the solutions, and thus, no doubt, modified their toxicity somewhat, the solutions still remained too powerful for them, and even in the 1 in 10,000 dilution both the Anodons were dead in less than two weeks.

The dead Anodons from the two strongest copper sulphate solutions were distinctly blue in colour, due to the staining with the salt.

It is interesting to remember in connection with the above experiments that Herdman and Boyce (8) found in their experiments with oysters that salts of copper always had a deleterious effect on the oysters themselves.

A series of experiments was next done with Anodons which had been strongly infected by being placed in a solution containing typhoid bacilli (prepared as previously described) for twenty-four hours.

Three of these infected Anodons were placed in each of the following dishes :—

- (1) Containing 2 litres of tap water, collected as usual.
- (2) „ a piece of bright copper foil, 15 cm. $\times$ 12 cm., in tap water collected as usual
- (3) „ 1 litre of colloidal copper solution.
- (4) „ 2 litres of $\frac{M}{10,000}$ copper sulphate solution made with tap water.
- (5) „ 2 „ $\frac{M}{100,000}$ „ „ „ „

Cultures were made at intervals from the stomach, gills, and rectum (indicated in the following tables, S., G., and R. respectively), and the results are shown below :—

Cultures taken	Control			Water with copper foil			Colloidal copper solution		
	S.	G.	R.	S.	G.	R.	S.	G.	R.
At once	312	563	233	—	—	—	—	—	—
24 hours after	—	—	—	211	294	178	53	107	21
48 „ „	243	471	187	147	207	99	47	127	34
Cultures taken	Control			$\frac{M}{10,000}$ Copper Sulphate			$\frac{M}{100,000}$ Copper Sulphate		
	S.	G.	R.	S.	G.	R.	S.	G.	R.
At once	312	563	233	—	—	—	—	—	—
24 hours after	—	—	—	43	89	52	163	419	137
48 „ „	243	471	187	47	111	23	143	352	103

It will be seen from the above table of results that only the colloidal copper solution and the 1 in 10,000 copper sulphate showed any marked power in dealing with the infected Anodons, that the maximum effect of these solutions was manifested in twenty-four hours, and that the next twenty-four hours' treatment did not seem to have any further action in diminishing the number of the bacilli.

This seems to show that the different solutions practically lost their toxicity in twenty-four hours, as far as the bacilli were concerned,

and this was probably due to the secretion of mucus by the Anodons and the consequent precipitation of the copper salts.

As regards the Anodons themselves, in the 1 in 10,000 solutions, this explanation is hardly sufficient, for if this solution lost all its toxicity in twenty-four hours, the reason for the death of the Anodons in less than fourteen days is not apparent.

It is possible that the Anodons were so injured by the solution in the first twenty-four hours as to be unable to recover from the effects, and it is certain that the toxicity of this solution to typhoid bacilli was much diminished when the Anodons were present in it.

SERIES II.—EXPERIMENTS WITH IRON SALTS

Experiments to discover the action of ferrous sulphate and ferric chloride solutions on (1) Anodons, and (2) Anodons infected with typhoid bacilli.

(a) A series of dishes was again used containing different solutions, and in each were placed two Anodons.

(1) Containing 2 litres of tap water collected as usual.

(2)	"	2	"	$\frac{M}{1,000}$	Ferrous sulphate solution made with tap water.
(3)	"	2	"	$\frac{M}{10,000}$	" " " "
(4)	"	2	"	$\frac{M}{1,000}$	Ferric chloride " "
(5)	"	2	"	$\frac{M}{10,000}$	" " " "

The result of the above series was that at the end of five weeks all the Anodons were still alive, and apparently quite healthy; and whereas at the time of starting the series one or two seemed a little sickly, these revived in the solutions and seemed to benefit by the presence of iron salts in the water.

These results also coincide with the observations of Herdman and Boyce, who found that salts of iron seemed to have a favourable action on oysters.

(b) A series was now done with infected Anodons, three of

which were placed in each of five dishes containing solutions similar to the above, and the results are tabulated below :—

Sub-cultures	Control			$\frac{M}{1,000}$ Ferric Chloride			$\frac{M}{10,000}$ Ferric Chloride		
	S.	G.	R.	S.	G.	R.	S.	G.	R.
At once	—	—	—	0	54	0	70	93	49
24 hours after	—	—	—	0	53	0	17	127	54
48 „ „	251	263	153	0	53	0	17	127	54

Sub-cultures	Control			$\frac{M}{1,000}$ Ferrous Sulphate			$\frac{M}{10,000}$ Ferrous Sulphate		
	S.	G.	R.	S.	G.	R.	S.	G.	R.
At once	—	—	—	0	7	0	61	104	53
24 hours after	—	—	—	0	7	0	61	104	53
48 „ „	251	263	153	0	0	0	10	57	11

It is seen from the above table that ferrous sulphate and ferric chloride solutions both had a decided action in clearing the Anodons from the bacilli, and this was especially noticeable in the case of the ferrous sulphate solutions which were freshly prepared. It is remarkable that these salts should have such a decided action on the Anodons infected with typhoid bacilli, but the explanation is probably to be found in the fact that the iron solutions were not only not irritating to the Anodons but were even beneficial, and so they did not attempt by secreting mucus or in other ways to nullify the toxic action of the solutions.

There was a certain amount of mucus secreted by the Anodons, however, but not nearly so much as in the copper solutions, and it was of a 'rusty' colour, due to the iron.

These results were considered satisfactory, as these salts could easily be used on a large scale for purifying oysters; for it has already been shown by Herdman and Boyce that they are likewise benefited by the presence of iron salts in the water. Again, it was noteworthy that the chief action took place in the first twenty-four hours, and this fact was the basis of another series of experiments.

(c) Another batch of infected oysters was taken and three of them placed in each of a series of glass dishes :—

(1) Containing tap water collected as usual.

(2) „ $\frac{M}{1,000}$ Ferric chloride solution made with tap water.

(3) „ $\frac{M}{1,000}$ Ferrous sulphate „ „ „

An Anodon from (1) was opened immediately, and the cultures showed stomach 314, gills 517, and rectum 271 colonies respectively.

At the end of fifteen hours the Anodons from each dish were taken and rapidly rinsed in clean water, and placed respectively in three other dishes containing fresh solutions of the same strength, and were left for ten hours longer.

Cultures were then taken from one Anodon out of each dish, and the result was as follows :—

Cultures	Control			$\frac{M}{1,000}$ Ferric Chloride Solution			$\frac{M}{1,000}$ Ferrous Sulphate Solution		
	S.	G.	R.	S.	G.	R.	S.	G.	R.
At once ...	314	517	271	—	—	—	—	—	—
25 hours after	207	384	159	0	0	0	0	0	0

This change into fresh solutions made the action much more marked, and both the Anodons tested from the iron solutions at the end of twenty-five hours were free from typhoid bacilli.

This action of ferrous sulphate and ferric chloride may possibly prove of great practical value in the treatment of suspected oysters, and a series of experiments will be carried out, shortly, using sea-water solutions of iron salts and oysters infected with typhoid bacilli, with this end in view.

SERIES III.—EXPERIMENT WITH SILVER SALTS

III. This series was started to find out whether it would be possible to use silver nitrate solutions to purify infected Anodons.

A series of three dishes was used, and they were kept at laboratory temperature in the dark.

It was noticed that in dishes 2 and 3 the solutions were milky, due to the precipitation of the chlorides in the water as silver chloride.

Two Anodons were placed in each of the following :—

- (1) Containing tap water, collected as usual.
- (2) " $\frac{M}{1,000}$ silver nitrate solution made with tap water.
- (3) " $\frac{M}{10,000}$ " " " "

In four days it was noticed that the Anodons were sickly in

dishes 2 and 3, and they were all dead in these two dishes six days after being placed in the solutions.

The Anodons in dish 1 remained healthy.

No attempt was made to purify infected Anodons with these solutions, which had proved so harmful to the Anodons themselves.

SERIES IV.—EXPERIMENTS WITH ZINC AND LEAD SALTS

IV. This series was conducted to discover the action of zinc sulphate and lead nitrate on Anodons, and, if feasible, to try and purify infected Anodons with solutions of these salts.

A series of five glass dishes was used, and into each of them two Anodons were put.

(1) contained 2 litres of tap water collected as before.

(2) " 2 " $\frac{M}{1,000}$ zinc sulphate solution made with tap water.

(3) " 2 " $\frac{M}{10,000}$ " " " "

(4) " 2 " $\frac{M}{1,000}$ lead nitrate solution made with tap water.

(5) " 2 " $\frac{M}{10,000}$ " " " "

The Anodons in dishes 2 and 4 were all dead in less than one week, and so it was concluded that these solutions could not have any practical application.

The Anodons in dishes 1, 3, and 5 were still living, and apparently healthy after three weeks.

There was a fair amount of mucus in all the dishes, but less in the control.

Another batch of Anodons was now infected with typhoid bacilli, and three of them were placed in each of the following dishes :—

(1) Containing 2 litres of tap water, collected as before.

(2) " 2 " $\frac{M}{10,000}$ zinc sulphate solution, made with tap water.

(3) " 2 " $\frac{M}{10,000}$ lead nitrate " " "

Sub-cultures were taken from the stomach, gills, and rectum of Anodons from each of the above dishes at certain intervals, as shown in the following table :—

Sub-cultures	Control			Zinc sulphate solution			Lead nitrate solution		
	S.	G.	R.	$\frac{M}{10,000}$ S.	G.	R.	$\frac{M}{10,000}$ S.	G.	R.
At once ...	453	719	327						
24 hours after ...				179	435	192	74	109	31

Both the zinc sulphate and lead nitrate showed a decided amount of toxicity, but the Anodons still contained many bacilli at the end of forty-eight hours.

The lead nitrate proved itself to be more toxic than the zinc sulphate solution.

To briefly sum up :—

Of all the different salts experimented with, it would seem that only those of iron are likely to be of use in the purification of suspected infected shell-fish.

Salts of all the other metals used, either acted detrimentally on the Anodons themselves or were not able to free them from the bacilli in a reasonable time.

The conclusions to be drawn from Part II of this paper may be tabulated as follows :—

(1) A solution of colloidal copper made by passing an electric current through distilled water, using copper electrodes so placed as to give an electric arc in the water, is exceedingly toxic to typhoid bacilli, and even when diluted 100 times will kill them in less than two hours at ordinary temperature.

(2) Salts of copper have a harmful effect on Anodons, and the stronger solutions rapidly kill them, whilst the weaker solutions are not able to purify Anodons infected with typhoid bacilli.

It is, therefore, impracticable to attempt to use salts of copper in the purification of shell-fish.

(3) Ferrous sulphate and ferric chloride solutions exert a beneficial action on Anodons placed in them, and the 1 in 1,000 solutions are able to practically free infected Anodons from typhoid bacilli in twenty-five hours.

(4) It is probable that a practicable method of purifying oysters contaminated with typhoid may be discovered by employing some salt of iron for the purpose.

(5) Salts of silver are very harmful to Anodons, and even 1 in 10,000 of M silver nitrate will kill them in less than a week.

(6) Sulphate of zinc and lead nitrate in the stronger solutions are harmful to Anodons, and in weaker solutions, although they exert a decidedly toxic action on typhoid bacilli, are unable to entirely free infected Anodons.

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