THE TOXIC ACTION OF COPPER AND MERCURY SALTS BOTH SEPARATELY AND WHEN MIXED ON THE HARPACTICID COPEPOD, *NITOCRA SPINIPES* (BOECK)

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INTRODUCTION

In connexion with the anti-fouling investigations by the Marine Corrosion Sub-Committee of the Iron and Steel Institute (now a Committee of the British Iron and Steel Research Association) the toxicities of a large variety of substances have been determined (reported by Harris, 1946). The primary object was a comparison of the behaviour of these substances in laboratory toxicity tests using a marine animal, with their anti-fouling performance when used in paint media. Copper and mercury salts were included in these tests, since compounds of these metals are commonly used in anti-fouling compositions. In the course of this work, tests were carried out on mixtures of copper and mercury salts as well as on the salts used alone. The results with these two heavy metal salts seem worth recording since, although no experiments were made to elucidate more fully the factors involved, no reference to such striking synergic phenomena in oligodynamic effects of heavy metals could be traced in the literature.

EXPERIMENTAL

A rapid and simple technique was required to deal with the large number of substances. The animal used was the Harpacticid Copepod *Nitocra spinipes* (Boeck). Animals were collected on the day of use and all the experiments quoted were done at the same time of the year. No attempt was made to select individuals of a particular size group; good agreement between the numerous replicates indicates that no considerable error had arisen from using mixed populations.

Ten animals were transferred to 5 ml. of the poison solution contained in small flat-bottomed dishes which were then covered with a glass plate. Inspection was made after 6 and 24 hr., when the animals were classified as dead, moribund or living. There was little difficulty in deciding when the animals were dead since they then showed a characteristic dorsal flexure of the urosome. Replicates (using ten animals for each test) were carried out so that for any one concentration or combination of concentrations the figures given in Table 1 represent the mean of observations on approximately 100 animals. Controls were invariably living at the end of the test period.

Sea water was collected from outside the Plymouth Breakwater and stored in carboys. The mercuric chloride (A.R.) solutions were made up in sea water and diluted as required: the copper sulphate (A.R.) solutions were made up initially in distilled water to a concentration of 10 g./l., with subsequent dilutions in sea water.

RESULTS

Table 1 shows the percentage of animals killed by the two poisons alone and admixed. Only the results for the 24 hr. inspection are quoted, since those for the 6 hr. period showed the same trend.

Table 1

Concentration (mg. Hg/l.)

| Concentration (mg. Cu/l.)	0	0·07	0·15	0·31	0·40	0·60	0·70	1·5	3·0	4·4
0	0	0	1·4	10·0	16·7	50	72	78	84	100
0·026	1·3	9·1	14·5	12·7	50·0	61·8	76·4	87·3	100	100
0·26	11·3	11·9	20·0	45·6	93·7	100	100	100	100	100
2·6	21·2	—	78	82	98	100	100	100	100	100
26	42·5	—	—	—	—	—	—	—	—	—

DISCUSSION

Solubility considerations of copper and mercury

In any consideration of these results the state of the metals in solution in the presence of dissolved salts will be of importance. The solubility of mercuric chloride in distilled water is 72·2 g./l. at 25° C. The dissociation is said to proceed in two stages, Morse (1902):

$$HgCl_2 \rightleftharpoons HgCl^+ + Cl^-,$$

$$HgCl^+ \rightleftharpoons Hg^{++} + Cl^-.$$
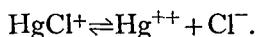
Sherrill (1903 & 1904) considers that the complex ion $HgCl_4^=$ is also present, and in addition there are indications that polymerization takes place.

$$xHgCl_2 \rightleftharpoons (HgCl_2)x.$$
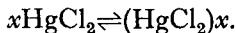
The solubility in chloride solution increases with increasing chloride content (Thomas, 1939) and in sea water is of the order of 270 g./l. at 25° C. In these chloride solutions a number of complex ions have been postulated and some evidence obtained for their existence. In saturated solutions of the salt in chloride solutions Garrett (1939) has indicated that the dominant ion is that of $HgCl_3^-$, accompanied by very small amounts of $HgCl_4^=$ and complex ions, such as $(HgCl_2)Cl^-$, formed from the polymers. In view of the lack of agreement between various workers it is not possible to calculate the quantities of the various entities present, but in very dilute solutions polymerization might be expected to be small, and calculations based on any of the data suggest that there are only traces of the mercuric ion present and that the major entities are undissociated mercuric chloride

and complex ion. For example, if Sherrill's equilibrium constants are used, the solution for median lethal dose (0·6 mg. Hg/l.) contains

$$Hg^{++} = 4 \times 10^{-5} \text{ mg. Hg/l.,}$$

$$HgCl_4^{=} = 0.46 \text{ mg. Hg/l.,}$$

$$HgCl_2 = 0.14 \text{ mg. Hg/l.}$$

It is also of interest to note that the work of Le Blanc and Noyes and Richards and Archibald (see Garrett, 1939), indicates that the complex is a highly stable strong electrolyte.

The solubility relations of the cupric ion in sea water are no less complex. When metallic copper is immersed in sea water in the presence of an adequate supply of oxygen a blue-green precipitate is formed. Bengough & May (1924) considered the product to be a mixture of basic cupric carbonate and chloride, predominantly the former. The concentration of copper in equilibrium with such a freshly precipitated 'basic copper carbonate' is of the order of 0·5 mg./l., but if the precipitate is allowed to age in contact with the solution, its solubility is reduced to the order of 0·1 mg. Cu/l., suggesting that there may be a slow conversion to a true atacamite (basic cupric chloride) (Ferry & Riley, 1946). Free (1908) has shown that the solubility of 'basic copper carbonate' decreases on contact with water containing carbon dioxide, and is increased in the presence of chlorides. The solubility product $[Cu^{++}][OH^-]^2$ in equilibrium with solid cupric hydroxide has been given as 1.6×10^{-19} (Feitknecht, 1944). At pH 8·0 this would correspond to a copper concentration of approximately 0.6×10^{-4} mg. Cu/l. (Taking the ionic strength of sea water as 0·63 and the activity of divalent cupric ion in such a solution as 0·17.) This is much less than the experimental values already given for the solubility of the 'basic copper carbonate'.* Further, when solutions of copper sulphate are added to sea water, no immediate evident precipitation takes place at concentrations far in excess of either the solubility of the hydroxide or even the experimentally determined values for the 'basic carbonate'. Even with high copper concentrations it has been found that precipitation may be delayed for many hours. This appears to have been the experience of other workers. The above figures would suggest that the copper is not present as cupric ion and other possibilities have to be considered.

The formation of complex ions is indicated but their character is not clear. Ammonia readily forms complexes with the cupric ion but, since the ammonia concentration is only of the order of 0.02×10^{-5} mg./l., it can play only a small part when this value is considered in relation to the data of Stackelberg & Freyhold (1940) for the association constant of the first two complexes of the cupric ion and ammonia. Basic compounds other than atacamite have been reported, whose solubility product may be higher, and complex double salts with alkali chlorides and sulphates are known.

It would appear that on the rapid addition of a copper sulphate solution of moderately high concentration to sea water complex ions are formed, including

* The possibility of the precipitation of copper phosphate has been neglected since it would not affect the present argument.

small concentrations of cations such as $[Cu^{++}][NH_3]$ and $[Cu^{++}][NH_3]_2$ with ammonia, and anions with the chlorides or even sulphates present; these slowly react with the carbonate and hydroxyl ions to produce extremely insoluble basic salts. The latter may exist in a colloidal form before aggregating to give a precipitate.

A further contributory factor may be the lowering in pH which takes place moderately rapidly on storing sea water and which would allow a higher concentration of cupric ions to be present.

It remains to consider the toxic action of the two metals in solution.

The toxic action of the copper and mercury solutions

It is evident that mercuric chloride is a very effective poison, the median lethal dose being of the order of 0·6 mg. Hg/l. In contrast, copper sulphate does not kill 50% of the animals at a 'concentration' of 26 mg. Cu/l., and a tenfold increase in 'concentration' from 0·26 to 2·6 mg./l. only results in an increase of from 11 to 21 % in the kill. These results suggest that the mode of action of the two salts may be different. It is possible, however, that the slow increase in effective action with increasing copper concentration may be due to the formation of colloidal basic compounds, whose poisonous action may be entirely different from the cupric or cupri-complex ion.

In investigations of fresh-water animals Jones (1937), extending the work of Carpenter (1927), has shown that heavy metal poisoning (including that by copper and mercury) of the minnow, *Phoxinus phoxinus*, is due to asphyxiation following coagulation of the gill mucus. In addition, respiration studies by the same worker (1941–2) on *Gammarus pulex* and *Polycelis nigra* gave results which indicate that both metals acted on these two animals in a similar manner. Jones (1941) has also shown that $HgCl_2$ alone, and in the presence of NaCl, does not differ in toxicity to the minnow. Comparative work, using a brackish form and investigating the effect of these metals at variable salt content, would be valuable in this respect: they might be expected to reveal that mercuric salts, due to a considerable concentration of undissociated $HgCl_2$ in either fresh or saline solutions, would show a similar behaviour, whilst that of copper solutions would vary with salinity due to the complex solubility relations in sea water. Mr K. A. Pyefinch and Mr W. Russell Hunter (personal communication), working with *Marinogammarus marinus*, have recently shown that changes in the nature of copper poisoning with variations in salinity take place which are in accordance with the above suggestion. Further, it is of interest to note that Clarke (1947) found that 'basic copper carbonate' was more toxic to barnacles than the complex citrate or tartrate, although the difference was not striking, and that Miller (1946) states that Carritt and Riley were unable to find any difference between cupric and cuprous copper poisoning. It has been established that cuprous salts exist in sea water largely as complex cupro-chloride ions.

The combined effect of copper and mercury solutions

It is clear from Table I that mixtures of these poisons are often far more effective than either poison alone at the same concentrations and, moreover, that toxicity is frequently far greater than would be expected on an additive basis.

These results strengthen the hypothesis that the two metals behave differently towards the animal, and that different systems or at least different parts of the same system are attacked. The simplest explanation of the synergy would appear to be that lowered vitality, due to one type of poisoning, does not allow the animal to deal as effectively with the second poison as when the latter is used alone. Thus impairment of the excretory system by mercury poisoning may allow sufficient accumulation of copper to interfere with the respiratory system which is thought by many workers to be involved in copper poisoning. Clarke (1947) has recently shown that barnacles and mussels can accumulate and excrete quantities of copper, and that even after considerable exposure to copper the animals can recover on return to normal conditions. He suggests that this metal acts slowly and not so much by destruction of some particular tissue or vital material as by a general retardation of vital processes. Thus the poisoning action of mercury may impair the excretory function and permit larger concentrations of copper to be built up.

Alternatively, the effect of one poison could change the permeability characters of the animal or cell membranes and so facilitate the entry of the second poison. In view of its solubility in organic solvents undissociated $HgCl_2$ might be expected to enter the cell rapidly, but its effect in coagulating any protein constituent might considerably change the permeability to ionic copper.

There is also the possibility that the effect is one external to the animal and not concerned with poisoning processes. Heavy metals, and particularly copper and mercury, are readily adsorbed on colloidal material; during the course of toxicity experiments a proportion of the poison in the external solution will be adsorbed on such material already present, and also on material secreted into solution during the course of the experiment (perhaps in increased amount in response to the poison). The amount available for poisoning might, therefore, be only a fraction of that added or estimated by analysis. In mixed poison solution preferential adsorption of one entity could allow a greater concentration of a second to be available for poisoning action.

It is clear that the problem of the toxic action of copper and mercury salts is one of great complexity. The above discussion is intended to draw attention to some of the physico-chemical equilibria involved in such toxic solutions in sea water, and also to possible variations in the mode of action upon the animal of the toxic compounds used. It is clearly very desirable to have more experimental work.

SUMMARY

1. The results are given of the poisoning of *Nitocra spinipes* (Boeck) by copper and mercury salts used together and separately.

2. The state of copper and mercuric salts in sea water is examined using the available physico-chemical data.

3. The results suggest that the two poisons act in a different manner and possible reasons for this are considered.

4. The striking synergic effects obtained when the two metals are used together are considered to support the suggestion in 3 above and various possible explanations, both biological and chemical for this synergism, are examined.

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