J. mar. biol. Ass. U.K. (1957) 36, 459–472
Printed in Great Britain

THE MODES OF ACTION OF TOXIC AGENTS

II. FACTORS INFLUENCING THE TOXICITIES OF MERCURY COMPOUNDS TO CERTAIN CRUSTACEA

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(Text-figs. 1–3)

It is well known that certain alkylmercuric halides are more toxic than inorganic mercury to a number of widely different test organisms, such as fungi (Yoshiyuki & Shintani, 1942), zooplankton (Hoffman, 1950), bacteria (Okamoto & Nagayama, 1952) and molluscs (Bond & Nolan, 1954). However, although the considerable toxicities of the organomercury compounds have often been observed, their modes of action seem to have attracted little attention.

In a recent investigation (Corner & Sparrow, 1956) the toxicities of ethylmercuric chloride and mercuric chloride were compared using two crustacean species as test animals. It was found that whereas the alkylmercuric compound was far more toxic than mercuric chloride to *Artemia salina* (L.), an animal highly resistant to mercury poisoning, the difference between the toxicities of the two compounds to *Elminius modestus* Darwin, a much less resistant species, was far smaller. These findings led to the view that rates of penetration are important factors influencing the toxicities of mercury compounds, and further evidence in support of this concept has now been obtained from the present work. The relative toxicities of a number of organomercury compounds to *Artemia* and to *Elminius* have been measured, and a study made of the extent to which these values are related to the lipoid solubilities of the compounds and their abilities to interact with proteins.

EXPERIMENTS

GENERAL METHODS

Animals

Artemia salina was reared in the laboratory. Larvae were collected 24 h after they had hatched, concentrated in a small volume of filtered sea water, and samples of the concentrate were added to the various toxic media under

test. Adults of *Elminius modestus* were obtained from rocks at Tinside, below the Plymouth Laboratory. They were placed in filtered sea water and the larvae liberated were collected after 1 h and added to the toxic solutions.

Poisons

The organomercury compounds used were the first five members of an homologous series of primary alkylmercuric chlorides and, in addition, *iso*-propyl-, *iso*-amyl- and phenylmercuric chlorides. Inorganic mercury was

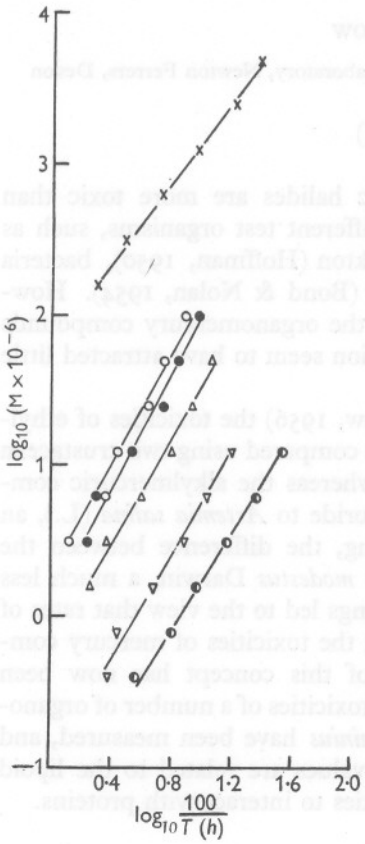

Fig. 1

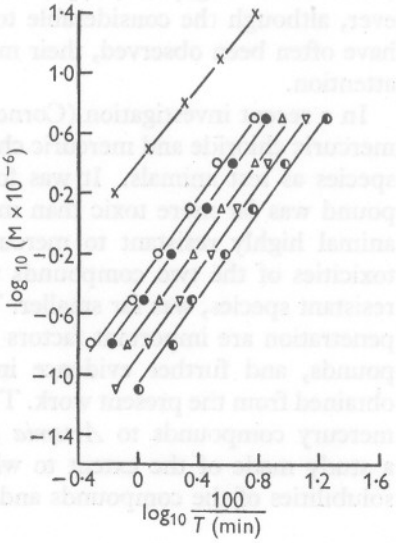

Fig. 2

Fig. 1. Toxicities of mercuric chloride (×—×), methylmercuric chloride (○—○), ethylmercuric chloride (●—●), *n*-propylmercuric chloride (△—△), *n*-butylmercuric chloride (▽—▽) and *n*-amylmercuric chloride (◐—◐) to *Artemia* larvae in sea water, pH 8·1.

Fig. 2. Toxicities of mercuric chloride (×—×), methylmercuric chloride (○—○), ethylmercuric chloride (●—●), *n*-propylmercuric chloride (△—△), *n*-butylmercuric chloride (▽—▽) and *n*-amylmercuric chloride (◐—◐) to *Elminius* larvae in sea water, pH 8·1.

used as mercuric chloride and mercuric iodide. It was found that the organo-mercury compounds were only slightly soluble in sea water, and that as the homologous series was ascended, solubility decreased. In order to obtain a known concentration of each compound in sea water a concentrated solution in warm ethanol (*o-cyclo*hexanone in the case of phenylmercuric chloride) was first prepared, and a measured volume of the solution was then added to the sea water. In this way, sea-water solutions of methyl-, ethyl-, *n*-propyl- and *iso*-propylmercuric chlorides equivalent to 10 mg Hg/l. (5×10^{-5} M) were prepared: the other mercury compounds were used at half this concentration. In all experiments with both species of test animals control tests showed that the quantities of ethanol and *o-cyclo*hexanone present in the toxic solutions had no significant toxic effect.

RELATIVE TOXICITIES

The methods used in toxicity experiments with *Artemia* and *Elminius* were those described in an earlier paper (Corner & Sparrow, 1956). The data from experiments using mercuric chloride and the homologous series of organo-mercury compounds are shown in Fig. 1 (*Artemia*) and Fig. 2 (*Elminius*). By plotting the logarithm of the molar concentration against the logarithm of the reciprocal of the time at which 50% of the animals died (TD_{50}) a linear relationship was found for each poison with each test animal. From these data

TABLE 1. RELATIVE TOXICITIES OF VARIOUS MERCURY COMPOUNDS TO *ARTEMIA*

Standard mercuric chloride solution equivalent to 200 mg Hg/l. (10^{-3} M). TD_{50} values were 12·5 h (Expt. 1) and 16 h (Expt. 2).

Mercury compound	No. of times as toxic as mercuric chloride	
	Expt. 1	Expt. 2
CH_3HgCl	15	15
C_2H_5HgCl	18	17
n-C_3H_7HgCl	47	54
n-C_4H_9HgCl	300	294
n-$C_5H_{11}HgCl$	930	980
i-C_3H_7HgCl	—	25
i-$C_5H_{11}HgCl$	—	450
C_6H_5HgCl	17	23
HgI_2	29	24

TABLE 2. RELATIVE TOXICITIES OF VARIOUS MERCURY COMPOUNDS TO *ELMINIUS*

Standard mercuric chloride solution equivalent to 1 mg Hg/l. (5×10^{-6} M). $TD_{50} = 1$ h.

Mercury compound	No. of times as toxic as mercuric chloride	Mercury compound	No. of times as toxic as mercuric chloride
CH_3HgCl	4·7	i-C_3H_7HgCl	2·4
C_2H_5HgCl	6·8	i-$C_5H_{11}HgCl$	16
n-C_3H_7HgCl	9·7	C_6H_5HgCl	4·3
n-C_4H_9HgCl	16	HgI_2	4·6
n-$C_5H_{11}HgCl$	20		

the concentration of each poison which killed 50% of the test animals in the same time was calculated and compared with that of mercuric chloride as a standard. The ratios of these equitoxic concentrations were then used to determine the relative toxicities of the poisons to each species, and the results are shown in Table 1 (*Artemia*) and Table 2 (*Elminius*). In the course of experiments with *Artemia*, determinations of relative toxicities were made on several occasions over a period of about 6 months, using different batches of test animals at different room temperatures. It was found, however, that the relative toxicities of the poisons, using mercuric chloride as a standard, were fairly consistent (see Table 1).

STUDIES WITH CYSTEINE AND REDUCED GLUTATHIONE

Studies of the nature of heavy metal poisoning have shown that mercury compounds are usually far less toxic to the test material when they are used in the presence of substances which contain a sulphydryl group. Thus, in studies of the inactivation of papain (Hellerman & Perkins, 1934), urease (Hellerman, Chinard & Deitz, 1943; Evert, 1952), succinoxidase (Barron & Kalnitsky, 1947), invertase (Gemmill & Bowman, 1950), amylase and phosphorylase (Ewart, Siminovitch & Briggs, 1953) and yeast carboxylase (Stoppani, Actis, Deferrari & Gonzalez, 1953) it has been found that the inhibitory powers of mercury compounds are much less when the enzyme is protected by such compounds as BAL, cysteine, reduced glutathione and thioglycollic acid. Further, even when the enzyme has been inhibited, the addition of a sulphydryl compound to the system can restore enzymatic activity. Analogous studies carried out with other test material have shown that cysteine and reduced glutathione counteract the inhibitory effects of mercury compounds on the growth of bacteria (Jude, Nordmann, Girard, Nordmann, Servant & Gauchery, 1952) and partially prevent the toxic effects of mercury and copper on *Daphnia magna* (see Holm-Jensen, 1948).

Because of these findings, it seemed of interest to examine the possibility that the toxic effects of some of the mercury compounds used in the present work might be diminished when cysteine or reduced glutathione was added to the toxic solutions: further, that after the test animals had been poisoned, subsequent treatment with thiol compounds might facilitate their recovery. The following experiments were, therefore, carried out.

Protection studies

Using *Elminius* as the test animal, each of six mercury compounds was examined for toxicity when used alone, and when used with a tenfold excess of reduced glutathione. It was found that the thiol compound completely abolished the toxicity of mercuric chloride, and considerably reduced those of methyl-, ethyl- and *n*-propylmercuric chlorides. By comparison, however, its

influence on the toxicity of *n*-butylmercuric chloride was much less, and still less with *n*-amylmercuric chloride (see Table 3).

When a similar study was made with *Artemia*, mercuric chloride could not be used because of the large amounts of glutathione needed to provide a tenfold excess of a lethal concentration of the poison. The results of experiments with the other mercury compounds, however, showed that the toxicities of methyl-, ethyl-, *n*-propyl- and *n*-butylmercuric chlorides were considerably reduced by both cysteine and reduced glutathione, the latter compound being

TABLE 3. THE EFFECT OF REDUCED GLUTATHIONE ON DEATH-RATES OF *ELMINIUS* IN SOLUTIONS OF VARIOUS MERCURY COMPOUNDS

Each poison used at a concentration giving a TD_{50} of 1 h. Reduced glutathione used in tenfold excess of each mercury compound.

Mercury compound	Percentage increase in TD_{50}	Mercury compound	Percentage increase in TD_{50}
$HgCl_2$	∞*	*n*-C_3H_7HgCl	800
CH_3HgCl	900	*n*-C_4H_9HgCl	420
C_2H_5HgCl	870	*n*-$C_5H_{11}HgCl$	410

* Animals died no faster than controls.

TABLE 4. EFFECTS OF CYSTEINE AND REDUCED GLUTATHIONE ON THE TOXICITIES OF MERCURY COMPOUNDS TO *ARTEMIA*

Each poison used in a concentration giving TD_{50} of 10 h. Thiol compounds used in tenfold excess of each poison.

	Percentage increase in TD_{50}	
Mercury compound	Cysteine	Reduced glutathione
CH_3HgCl	84	240
C_2H_5HgCl	87	260
n-C_3H_7HgCl	89	280
n-C_4H_9HgCl	92	270
n-$C_5H_{11}HgCl$	20	96

the more effective of the two. Moreover, as in the experiments with *Elminius*, it was found that the protection which either thiol compound provided against poisoning by *n*-amylmercuric chloride was noticeably less than that observed when any of the other four mercury compounds was used (see Table 4).

Recovery studies

Elminius larvae were placed in sea-water solutions of mercuric chloride (1 mg Hg/l.) and *n*-amylmercuric chloride (0·05 mg Hg/l.) for measured times. The toxic solutions were then centrifuged, the supernatants were decanted off and the larvae were suspended in fresh sea water. After a second centrifuging, half the animals were transferred to plain sea water and the remainder to a sea-water solution of reduced glutathione (5×10^{-5} M). The rates of death of the two sets of animals were then compared. It was found that animals which had 'died' (i.e. become completely quiescent) in the toxic

media did not recover after they had been transferred either to plain sea water or to sea water containing glutathione. It was also observed that, of the animals which had lost their ability to move freely in the toxic solutions, a few completely recovered their mobility when they were transferred to the new media, and the remainder continued to die at a very slow rate. However, of the latter animals, those placed in sea water containing glutathione died much more slowly than the larvae transferred to plain sea water (Table 5).

TABLE 5. EFFECT OF REDUCED GLUTATHIONE ON *ELMINIUS* PREVIOUSLY TREATED WITH MERCURY

GSH = reduced glutathione (5×10^{-5} M) in sea water (SW).

Mercury compound	Time of immersion (min)	Second medium	TD_{50} in second medium (h)
$HgCl_2$	15	SW	29
	15	GSH	42
	30	SW	14
	30	GSH	21
n-$C_5H_{11}HgCl$	15	SW	17
	15	GSH	26
	30	SW	9
	30	GSH	21

TABLE 6. EFFECT OF REDUCED GLUTATHIONE ON *ARTEMIA* PREVIOUSLY TREATED WITH MERCURY

GSH = reduced glutathione (5×10^{-4} M) in sea water (SW).

Mercury compound	Time of immersion (h)	Second medium	TD_{50} in second medium (h)
$HgCl_2$	4	SW	108
		GSH	115
n-$C_5H_{11}HgCl$	4	SW	45
		GSH	57

An experiment identical with the one just described was carried out with *Artemia*. It was found that the influence of reduced glutathione on the death-rate of the animals was negligible (see Table 6).

INHIBITION OF UREASE BY VARIOUS MERCURY COMPOUNDS

Mention was made earlier of studies of the effects of heavy metals on enzymes. These investigations were primarily concerned with establishing whether or not a certain enzyme possessed sulphydryl groups, and the extent to which such groups influenced catalytic activity. More recently, however, Okamoto & Nagayama (1952) have compared the relative abilities of a number of mercury compounds as inhibitors of catalase and as bactericides in order to test the possibility that there was a correlation between their abilities to react with proteins and their potencies as antibacterial agents. No correlation was found. In the present work, similar experiments were carried out to see

whether or not the affinities of mercury compounds for protein, estimated from their abilities to inhibit urease, might be correlated with the toxicities of these compounds to *Artemia* and *Elminius*.

Because the purpose of this study was simply to compare the toxicities of a number of substances to urease, it was not thought necessary to prepare the enzyme in pure crystalline form. Commercial preparations, however, were found to be much too inactive and, accordingly, the enzyme was obtained by aqueous-acetone extraction of Jack-bean meal in the manner described by Sumner (1926). This procedure usually gave a preparation with an activity of 0·20 Sumner units/mg. Various concentrations of each mercury compound in ethanol were added to solutions (5 ml.) of the enzyme (10 Sumner units) in 0·01 M phosphate buffer, pH 6·7 (the amounts of ethanol used had no influence on enzyme activity). Each mixture was allowed to stand for 15 min at room temperature and its ureolytic activity was then compared with that of a control solution, using the colorimetric method of Van Slyke & Archibald (1944). In this way, the extent of the inactivation caused by each concentration of each mercury compound was measured, and the quantity of each poison required to cause a 50% loss of ureolytic activity was estimated. The potencies of the various compounds studied as inhibitors of urease, expressed in terms of that of mercuric chloride as a standard, are shown in Table 7.

TABLE 7. RELATIVE POTENCIES OF VARIOUS MERCURY COMPOUNDS AS INACTIVATORS OF UREASE

Mercury compound	No. of times as effective as mercuric chloride	Mercury compound	No. of times as effective as mercuric chloride
CH_3HgCl	0·76	i-C_3H_7HgCl	0·63
C_2H_5HgCl	0·66	i-$C_5H_{11}HgCl$	0·95
n-C_3H_7HgCl	0·70	C_6H_5HgCl	0·95
n-C_4H_9HgCl	0·76	HgI_2	1·09
n-$C_5H_{11}HgCl$	0·88		

ETHER: SEA-WATER PARTITION COEFFICIENTS OF MERCURY COMPOUNDS

From a study of the permeability of rabbit skin to ethyl-iodide, methanol, ethanol, thiourea, glycerol, urea and glucose, Treherne (1956) found that the rates of penetration of these compounds were closely related to the corresponding ether: water partition coefficients. This result provided quantitative demonstration of an earlier view put forward by Calvery, Draize & Laug (1946) and by Rothman (1943) that the lipoid solubility of the penetrating substance was an important factor in the mechanism of skin permeability.

Because of the possibility that penetration of a lipoid barrier might be an important factor influencing the toxic actions of mercury compounds, it was considered worth while to examine whether or not a close parallel could be found between the ethyl ether: sea-water partition coefficients of these poisons

and their toxicities. Accordingly, the following experiments were carried out using *Elminius* as the test animal.

Each mercury compound was dissolved in sea water to give a concentration corresponding to 1 mg Hg/l. (5×10^{-6} M). A volume of this solution (50 ml.) was then shaken with an equal volume of ethyl ether for 1 min in a separating funnel. After the two phases had separated the lower one was run off and a stream of air was passed through it for 20 min to remove the ether. The quantity of the mercury compound left in this ether-free solution was then determined by comparing its toxicity to *Elminius* with that of a series of sea-water solutions containing known amounts of the mercury compound. The quantity so determined, when subtracted from the amount originally added to the sea water (1 mg Hg/l.), gave the amount extracted by the ether. The ratio of this latter quantity to that remaining in the sea water was then estimated as the partition coefficient.

Because the toxicity data for the mercury compounds had been expressed in terms of mercuric chloride as a standard poison, it was thought necessary to express their lipoid solubilities in a similar way. For this reason it was important that an accurate value should be obtained for the ethyl ether:sea-water partition coefficient of mercuric chloride. It was found, however, that the accuracy of the bio-assay method proved inadequate in the case of mercuric chloride because only a relatively small quantity of this compound was extracted into the ether phase. Accordingly, the value for mercuric chloride was obtained from the use of tracer isotopes. As details concerning the use of radioactive mercury are given elsewhere (Corner & Rigler, 1957), only a brief description of the experimental procedure is included in the present account. ^{203}Hg-labelled mercuric chloride was added to sea water to give a concentration equivalent to 1 mg/l., and estimates of radioactivity in samples (0·1 ml.) were immediately made. After the sea water (50 ml.) had been extracted with an equal volume of ether for 1 min it was again examined for radioactivity in order to determine the quantity of mercury which had been extracted by the ether. From these data the partition coefficient was estimated in the usual way and was found to be 0·26.

Further experiments were carried out with ^{203}Hg-labelled mercuric chloride and *n*-amylmercuric chloride in order to examine the possibility that the aeration of sea-water solutions of mercury compounds in order to remove traces of ether might cause volatilization of the compounds and lead to further errors in the bio-assay method. In these experiments, small quantities of ether were added to sea-water solutions of the two mercury compounds (each equivalent to 1 mg ^{203}Hg/l.) and the media were aerated until the amounts of ether in them were sufficiently small to have no influence on the viability of the test animals (20 min). Determinations of radioactivity in these solutions after aeration had taken place showed that no loss of mercury had occurred and this procedure, therefore, introduced no errors into the bio-assay method.

The partition coefficient for each mercury compound, expressed in terms of that for mercuric chloride as a standard, is shown in Table 8.

TABLE 8. RELATIVE VALUES OF ETHYL ETHER:SEA-WATER PARTITION COEFFICIENTS OF VARIOUS MERCURY COMPOUNDS

Experimental conditions as given in text. Value for standard $HgCl_2$ solution (5×10^{-6} M) = 0·26 at 15° C.

Mercury compound	No. of times partition coefficient greater than that of mercuric chloride	Mercury compound	No. of times partition coefficient greater than that of mercuric chloride
CH_3HgCl	15·9	*i*-C_3H_7HgCl	42
C_2H_5HgCl	63	*i*-$C_5H_{11}HgCl$	1641
n-C_3H_7HgCl	255	C_6H_5HgCl	360
n-C_4H_9HgCl	879	HgI_2	18·9
n-$C_5H_{11}HgCl$	2490		

RESULTS

Ferguson (1939) has drawn attention to the fact that the logarithms of equitoxic concentrations of members of an homologous series decrease linearly as the series is ascended, and that a similar relationship holds when certain physical constants (e.g. water solubility, vapour pressure, etc.) of these compounds are expressed in the same way. In the present work it has been found that if the toxicity data for ethyl-, *n*-propyl-, *n*-butyl- and *n*-amylmercuric chlorides are plotted in the manner shown in Fig. 3, linear relationships exist when the compounds are tested with either *Artemia* or *Elminius*. Differences between toxicities to *Artemia*, however, are far greater than those observed when *Elminius*, which is much more readily poisoned, is used as the test species. These observations confirm and extend the results of some earlier experiments (Corner & Sparrow, 1956) which have shown that orders of difference between the toxicities of certain mercury compounds to a test animal are much greater when the animal used is one which is highly resistant to mercury poisoning. It will also be seen from Fig. 3 that stepwise increments in toxicity to *Artemia*, but not to *Elminius*, approximate to corresponding increments in lipoid solubility, consistent with the view that toxicity to *Artemia* is much more influenced by lipoid solubility than is toxicity to *Elminius*.

Two secondary alkylmercuric halides have been studied in the present work, and these poisons have been found to be significantly less toxic than the corresponding primary compounds to both the test species. Nevertheless, as with the primary compounds, the lipoid solubilities of *iso*-propyl- and *iso*-amylmercuric chlorides are more closely related to their toxicities to *Artemia* than to their toxicities to *Elminius*. Similar results have also been obtained in experiments with methylmercuric chloride and mercuric iodide. An exception, however, is phenylmercuric chloride, which possesses a much greater lipoid solubility than might be expected from its toxicity to either species.

It has been found that thiol compounds protect both *Elminius* and *Artemia* from poisoning by mercuric chloride and primary alkylmercuric chlorides. However, further experiments have shown that when the lipoid solubility of the poison is very high (e.g. *n*-amylmercuric chloride) thiol compounds provide less protection than when the poison used is one of low lipoid solubility (e.g. mercuric chloride). Interest also attaches to the additional finding that when lipoid solubilities greatly influence the toxicities of organomercury

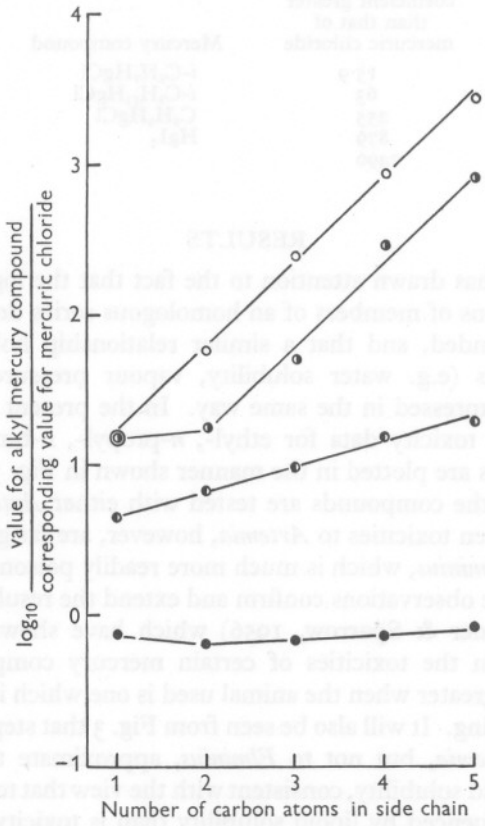

Fig. 3. Toxicity and other data for primary alkylmercuric chlorides. Lipoid solubility, ○—○; toxicity to *Artemia*, ◑—◑; toxicity to *Elminius*, ◐—◐; potency as urease inhibitor, ●—●.

compounds (experiments with *Artemia*) protection of the test animal by thiols is much smaller than under conditions where lipoid solubilities are of less importance (experiments with *Elminius*). Further, when in complementary studies the test animals have been given preliminary treatments with mercury compounds and measurements subsequently made of their rates of death in plain sea water, it has been found that adding reduced glutathione to the sea water has little effect on the viability of *Artemia*, but a marked influence on

that of *Elminius*. A possible explanation of this finding is that the ability of a given poison to interact with proteins on the surface of the test animal may play a more important part in determining its toxicity to *Elminius* than its toxicity to *Artemia*. A further possibility is that reduced glutathione may be able to penetrate *Elminius* but not *Artemia* and inactivate some of the mercury poison after it has reached tissues inside the test animal.

Finally, it is obvious from Fig. 3 that differences between the toxicities of mercury compounds to either *Artemia* or to *Elminius* are significantly greater than corresponding differences between the potencies of the poisons as inhibitors of urease, a finding which lends further support to the view that, in general, the toxicities of these compounds are influenced by their lipoid solubilities far more than by their abilities to combine with proteins.

DISCUSSION

Of the possible mechanisms of action of organomercury poisons, one is that the compounds may interact with the surface of the test animal, and another is that these poisons may penetrate into the tissues and inhibit metabolism. If the poisons exerted their toxic action solely by acting on the surface of the animal it might be expected that a reasonable correlation would be found between the toxicities of the poisons to the test animal and their abilities to combine with proteins. However, neither in the present work, nor in studies made by other workers (Okamoto & Nagayama, 1952) has any correlation been found. Moreover, if surface effects alone are responsible for the toxicities of organomercury compounds it would seem unlikely that great differences would be found between the resistances of different test species to the poisons. In this, and a previous study (Corner & Sparrow, 1956), however, the resistance of *Artemia* to mercury poisons has been found to be of a much higher order than that of *Elminius*.

If, however, in order to penetrate the test animal organomercury compounds had to pass through a lipoid barrier, it would be expected that the toxicities of these compounds would largely depend on their lipoid solubilities: and, further, that the importance of lipoid solubility in determining toxicity would vary with different animals according to whether or not a lipoid barrier was dominant among the factors involved in resistance. In general, the findings made in the present study have shown that the toxicities of organomercury compounds are, in fact, related to the lipoid solubilities of these poisons: and, in addition, that whereas this correlation is close in the case of *Artemia*, it is less so in experiments with *Elminius*. These findings are, therefore, consistent with the view that the mechanism of action of an organomercury compound involves penetration of the test animal.

It is necessary, however, to consider the possibility that in order to act on the animal, the poison may not have to penetrate into the tissues and inhibit metabolic changes, but may exert its toxic effects by some physical mechanism

as soon as it has entered the lipoid barrier. Although the results of the present work provide no definite information on this point it is possible that the anomalous findings made with phenylmercuric chloride are best explained by assuming that interference with metabolism in the tissues of the animal does, in fact, occur. Thus, if phenylmercuric chloride exerted its toxic action once it had entered the lipoid barrier, it is difficult to see why the toxicity of this poison should be so much smaller than one might expect from its considerable lipoid solubility. On the other hand, if this compound became involved in metabolic processes, then it would be possible to account for its reduced toxicity by assuming that it underwent changes in the tissues which resulted in its detoxication. Although mechanisms of detoxication in *Artemia* and *Elminius* are completely unknown, it is possible that these animals may be able to carry out some of the reactions whereby other species are known to detoxicate administered organic compounds (cf. Young, 1939; Williams, 1949). Thus, it is possible that phenylmercuric chloride, like certain other aromatic compounds, might be conjugated *in vivo* with cysteine and so rendered less toxic. In addition, hydroxylation of the aromatic ring might occur, followed by conjugation of the product so formed with sulphuric or glucuronic acids.

Many of the conclusions drawn from the results of the present study are based on the assumption that *Artemia* is able to survive in the presence of large concentrations of mercury compounds simply because these poisons penetrate the species very slowly. It is possible, however, that mercury compounds may enter *Artemia* at rates commensurate with those at which they penetrate *Elminius*, a species which is much more readily poisoned, and that *Artemia* is highly resistant to the poisons either because it possesses a very efficient means of detoxicating them, or because it can tolerate far higher concentrations of the poisons in its tissues. In order to examine these possibilities further, a study is at present being made in which ^{203}Hg-labelled mercuric and *n*-amylmercuric chlorides are being used to measure the rates of uptake of these compounds by *Artemia* and *Elminius*.

We wish to thank Mr O. D. Hunt, Mr T. W. Robinson and Mr R. Robinson of the Research Staff of International Paints Ltd. for their valuable advice and criticism. We are also grateful to Dr H. W. Harvey, F.R.S., for his many helpful suggestions and continuous interest. One of us (E.D.S.C.) is indebted to International Paints Ltd. for a Research Fellowship and for assisting financially in defraying part of the expenses of the present work. In addition, both of us wish to acknowledge the many facilities provided by the Plymouth Laboratory of the Marine Biological Association. Finally, we are most grateful to Lunevale Products Ltd., and to L. Light and Co. Ltd. for kindly supplying free samples of all the organomercury compounds used.

SUMMARY

A study has been made of the toxicities of mercuric chloride, mercuric iodide and methyl-, ethyl-, *n*-propyl-, *n*-butyl-, *n*-amyl-, *iso*-propyl-, *iso*-amyl- and phenylmercuric chlorides to larvae of the crustaceans *Artemia salina* and *Elminius modestus*. With both species it has been found that all the mercury compounds are more toxic than mercuric chloride, that primary alkylmercuric chlorides are more toxic than the corresponding secondary compounds, and that as the homologous series of primary compounds is ascended, toxicities increase. In addition, it has been found that *Elminius* is much more readily poisoned than *Artemia* by each mercury compound, and that differences between the toxicities of the poisons to *Elminius* are much smaller than corresponding differences observed in experiments with *Artemia*.

It has been found that the toxicities of primary alkylmercuric chlorides to both species are considerably less when cysteine and reduced glutathione are added to the toxic media. However, these thiol compounds give less protection against poisoning by the higher homologues. Other experiments have shown that when the animals are given a preliminary immersion in toxic solutions of mercuric chloride and *n*-amylmercuric chloride, and are then transferred to fresh sea water containing reduced glutathione, the influence of the thiol compound on the subsequent death-rate of *Elminius* is significant, but negligible in the case of *Artemia*.

Measurements have been made of the potencies of the compounds as inhibitors of urease in order to estimate their abilities to combine with proteins. No correlation has been found between these abilities and the toxicities of the compounds to either test species.

The lipoid solubilities of the compounds have been estimated from measurements of their ethyl ether : sea-water partition coefficients. In general, a good measure of agreement has been found between the relative lipoid solubilities of both primary and secondary alkylmercuric chlorides and their respective toxicities to *Artemia*. In addition, it has been found that although this correlation is not so exact for *Elminius*, nevertheless, compounds with high lipoid solubilities have usually been found to be more toxic. However, phenylmercuric chloride is an exception in that its lipoid solubility is much higher than would be expected from its toxicity to either test species.

The results of this study, which in general are consistent with the view that organomercury compounds act by penetrating the test animals, have been discussed.

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