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THE MODES OF ACTION OF TOXIC AGENTS
III. MERCURIC CHLORIDE AND *N*-AMYLMERCURIC CHLORIDE ON CRUSTACEANS

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(Text-figs. 1–3)

In a recent study (Corner & Sparrow, 1957) it was found that differences between the toxicities of various mercury compounds to certain Crustacea were related to the resistances of the test animals to mercury poisoning, the differences being greater when a more resistant species was used. In addition, the toxicities of these poisons to a highly resistant animal were found to be closely related to the corresponding lipoid solubilities. These results were consistent with the view that Hg poisons act by penetrating the test animals, the enhanced toxicity of a highly lipoid-soluble poison such as *n*-amylmercuric chloride being the result of this compound's ability to penetrate rapidly. In the present work, tracer isotopes have been used in experiments with larvae of the brine shrimp *Artemia salina* (L.), larvae of the barnacle *Elminius modestus* Darwin and adults of the prawn *Leander serratus* (Pennant), in order to investigate further the modes of action of mercuric chloride ($HgCl_2$) and *n*-amylmercuric chloride (n-$C_5H_{11}HgCl$) as poisons to crustaceans. The results have provided evidence more direct than that obtained previously in favour of the view that penetration of the test animal is an important factor in mercury poisoning.

GENERAL METHODS

^{203}Hg-*labelled mercuric chloride*. Radioactive mercuric oxide was obtained from A.E.R.E., Harwell, and a stock solution containing 10 mg Hg/ml. was prepared by dissolving 1 g of this material in 93 ml. 50% HCl. Appropriate quantities of this solution were then added to sea water and the pH values of the solutions were adjusted to 8·1 with 2N-NaOH. To estimate the amounts of radioactivity in these sea-water solutions, samples (0·05–0·10 ml.) were first mixed with an excess of sodium sulphide to prevent volatilization of the Hg, and then evaporated to dryness on planchettes.

^{203}Hg-*labelled* n-*amylmercuric chloride*. A pure sample of this material (m.p. 121–122° C) was obtained from the Radiochemical Centre, Amersham, and a stock solution containing 0·5 mg Hg/ml. was prepared in absolute ethanol. Measured amounts of this solution were added to sea water and estimates of the radioactivity in these sea-water solutions were made on samples (0·05–0·10 ml.) slowly evaporated to dryness after treatment with an excess of reduced glutathione to prevent volatilization of the Hg compound.

Animals. For experiments in which *Artemia* and *Elminius* larvae were used the animals were obtained by methods described earlier (Corner & Sparrow, 1956). Studies were also made with the prawn *Leander serratus*, and the specimens used in these experiments were from a stock maintained at the Plymouth Laboratory.

EXPERIMENTS

Uptakes of mercuric chloride and *N*-amylmercuric chloride by *Elminius* and *Artemia*

All the experiments were carried out at 18° C. Solutions of the poisons in sea water were prepared immediately before the experiments were started because an earlier study (Corner & Rigler, 1957) had shown that significant amounts of Hg are lost from sea-water solutions of mercuric chloride on standing. *Elminius* larvae (200–300 animals) were added to samples (50 ml.) of sea water containing 5·0, 1·0 and 0·2 mg Hg/l. as ^{203}Hg-labelled $HgCl_2$, and a second series of samples containing 0·25, 0·05 and 0·01 mg Hg/l. as ^{203}Hg-labelled n-$C_5H_{11}HgCl$. At suitable time intervals the animals were removed from the solutions by filtration on a disc of bolting silk (200 m.p.i.), suspended in plain sea water (5 ml.) and removed from the washing medium by filtration on a new disc of bolting silk. The animals were then dried by gentle suction, the sample, together with the silk, was transferred to a planchette, and its radioactivity was estimated. Control experiments showed that the bolting silk used in the first filtration was always contaminated with mercury and that this contamination was considerable on silks used to collect animals during the later stages of the experiments. It had to be taken into account in calculations. However, it was found that if the animals were washed quickly with sea water immediately after they had been removed from the toxic media they did not lose any of the Hg which they had taken up, and the disc of bolting silk used for the second filtration bore no trace of radioactivity. Accordingly, this washing procedure was used in all the experiments. After the animals in each sample had been examined for radioactivity, they were transferred to acidified sea water containing a trace of sodium azide and, as soon as all were motionless, they were counted and the quantity of Hg which they had taken up was determined as μg Hg/animal. At the end of the experiment, which was usually continued until approximately 75% of the test animals had died, the animals from all the samples were collected on a

previously weighed disc of bolting silk and were dried to constant weight at 105° C. The dry weight of the animals so determined was then used to calculate the amount of Hg taken up as mg Hg/g dry wt. of larvae.

Uptake experiments were also carried out with *Artemia* larvae immersed in sea-water solutions containing 1 g and 0·5 g Hg/l., as $HgCl_2$ (10% of the mercury as ^{203}Hg) and 1·0 and 0·5 mg Hg/l. as ^{203}Hg-labelled *n*-$C_5H_{11}HgCl$. The method used in these experiments with *Artemia* was identical with that just described, except that after they had been removed from the toxic media the animals were washed twice with sea water because it was found that a single washing (as used in the procedure with *Elminius*) did not remove all traces of radioactivity from the bolting silk.

Reduced glutathione

Corner & Sparrow (1957) have shown that certain thiol compounds greatly reduce the toxicities of mercury poisons to *Elminius* and *Artemia*, and have reported that very marked effects are observed when reduced glutathione is used to protect *Elminius* from poisoning by $HgCl_2$. These workers have also demonstrated that reduced glutathione lowers the death rate of *Elminius* returned to plain sea water after a preliminary immersion in either $HgCl_2$ or *n*-$C_5H_{11}HgCl$.

Protection experiments. In the present work, by means of the methods described earlier, the rates of uptake of Hg by *Elminius* from sea water containing ^{203}Hg-labelled $HgCl_2$ (1 mg Hg/l.) and *n*-$C_5H_{11}HgCl$ (0·05 mg Hg/l.) were compared with those from similar solutions to which a tenfold excess of reduced glutathione had been added.

Recovery experiments. A study was made of the effect of reduced glutathione on the rates at which mercury was lost from poisoned *Elminius* and *Artemia* when the animals were returned to plain sea water. The procedure used was as follows. A large number of *Elminius* larvae were immersed for 20 min in sea water containing 1 mg Hg/l., and similarly *Artemia* larvae in sea water with 1 g Hg/l. Representative samples were then removed and examined immediately for radioactivity in the usual way. Simultaneously, a second series of samples were transferred to plain sea water and a third series to sea water containing reduced glutathione (10 mg/l.). The radioactivity of these animals was then estimated after they had been immersed in the two media for 1 and 5 h (*Elminius*) and 2 h (*Artemia*). Attempts were also made to repeat these experiments using ^{203}Hg-labelled *n*-$C_5H_{11}HgCl$, but these were unsuccessful because the mercury compound was insufficiently radioactive.

Experiments with *Leander serratus*

Studies were made with prawns to determine the distribution of radioactivity on and inside the test animals after they had been poisoned with ^{203}Hg-labelled $HgCl_2$ and n-$C_5H_{11}HgCl$.

Preliminary experiments showed that prawns immersed in sea water containing 50 mg Hg/l. as $HgCl_2$ and 1 mg Hg/l. as n-$C_5H_{11}HgCl$ died in approximately the same time (3 h). The animals were, therefore, immersed in the equitoxic media for 2 h, after which time they were washed with plain sea water and various tissues, both internal (e.g. the hepatopancreas) and external (e.g. body chitin) were removed by dissection and examined for radioactivity. With chitin, pieces (*ca.* 1 cm^2) were cut from various parts of the body, dried and immediately placed under the Geiger counter. Soft body tissues, however, were first worked into a paste with ethanol and small quantities of the mixture (*ca.* 2 mg) were smeared on planchettes. The samples were then dried by slow heating and their radioactivity was estimated. As fairly large amounts of tissue were used and as ^{203}Hg is a weak emitter of β radiation it is probable that these determinations of radioactivity gave low results because of errors introduced by self-absorption. However, as exactly the same procedure was used to examine animals which had been treated either with $HgCl_2$ or with n-$C_5H_{11}HgCl$, an adequate comparison of the distributions of the two poisons was possible. After the radioactivity had been estimated the samples were dried to constant weight at 105° C and the mercury content was expressed as mg Hg/g dry wt. of tissue.

Because these experiments showed high concentrations of Hg on the gills and on the tissue under the branchiostegites of prawns poisoned with the two Hg compounds, tests were made to show whether the poison was merely attached to the surfaces of these tissues or had penetrated into them. In these experiments prawns were immersed for 2 h in sea water containing 50 mg ^{203}Hg/l. as $HgCl_2$. The animals were then removed from the solution, quickly washed with plain sea water and dissected, and the gills and branchiostegites of one of them were then examined at once for radioactivity. Gills and branchiostegites taken from one side of each of the remaining animals were immersed for 30 min in *Homarus* Ringer solution (Cole, 1941), and those dissected from the other side were immersed for the same time in *Homarus* Ringer solution containing 100 mg reduced glutathione/l. After this washing, the tissues were examined for radioactivity in the usual way.

Further experiments were carried out with prawns to show whether injecting the animals with a small quantity of a mercury poison led to a distribution of radioactivity throughout the tissues similar to that observed after the animals had been immersed in a toxic solution of the same poison. ^{203}Hg-labelled $HgCl_2$ and n-$C_5H_{11}HgCl$ were used in these experiments, and care was taken to see that the animals received the same dose of each com-

pound. The fact that the n-$C_5H_{11}HgCl$ had to be injected in ethanolic solution restricted the size of the dose which could be used, for in control experiments it was found that the test animals (approximately 10 g body wt.) showed signs of distress when injected with more than 0·02 ml. of ethanol. Accordingly, the quantity of each Hg compound injected was equivalent to 10 μg Hg. The needle was inserted laterally between the second and third abdominal segments and the solution was injected dorsally into the lacuna at the first segment. Immediately after the injection had been made the animals were placed in fresh sea water from which specimens were then removed at suitable intervals for dissection followed by examination of various tissues for radioactivity in the usual way.

RESULTS

Experiments with *Elminius* and *Artemia*

Corner & Sparrow (1957) have shown that n-$C_5H_{11}HgCl$ is 20 times as toxic as $HgCl_2$ to *Elminius* and 1000 times as toxic as this compound to *Artemia*. Interest therefore attaches to our experiments, which have shown that the rates at which *Elminius* removes mercury from sea water containing various concentrations of n-$C_5H_{11}HgCl$ are roughly the same as those at which the

TABLE 1. QUANTITIES OF MERCURY AS $HgCl_2$ AND AS n-$C_5H_{11}HgCl$ TAKEN UP BY *ELMINIUS* AND *ARTEMIA* FROM EQUITOXIC CONCENTRATIONS OF THE POISONS IN SEA WATER

(Concentrations of poisons gave TD_{50} of approximately 3 h with each species.)

	$HgCl_2$		n-$C_5H_{11}HgCl$	
Animal	Concn. in toxic medium (mg Hg/l)	Concn. taken up by animals (mg Hg/g dry wt.)	Concn. in toxic medium (mg Hg/l)	Concn. taken up by animals (mg Hg/g dry wt.)
Elminius	0·2	0·92	0·01	0·70
Artemia	1000	0·47	1·0	0·28

TABLE 2. INFLUENCE OF REDUCED GLUTATHIONE ON THE LOSS OF MERCURY BY *ELMINIUS* AND *ARTEMIA* POISONED WITH $HgCl_2$

(*Elminius* immersed in sea-water solution of $HgCl_2$ (1 mg Hg/l.) for 20 min. *Artemia* immersed in sea-water solution of $HgCl_2$ (1 g Hg/l.) for 90 min. GSH = reduced glutathione (10 mg/l.).)

	Washing medium			
	Sea water. Concn. of Hg in animals		GSH in sea water. Concn. of Hg in animals	
Time (h)	mg Hg/g dry wt.	%	mg Hg/g dry wt.	%
	Elminius			
0	0·54	100	0·54	100
1	0·35	65	0·35	65
5	0·16	29	0·18	33
	Artemia			
0	0·52	100	0·52	100
2	0·33	63	0·27	52

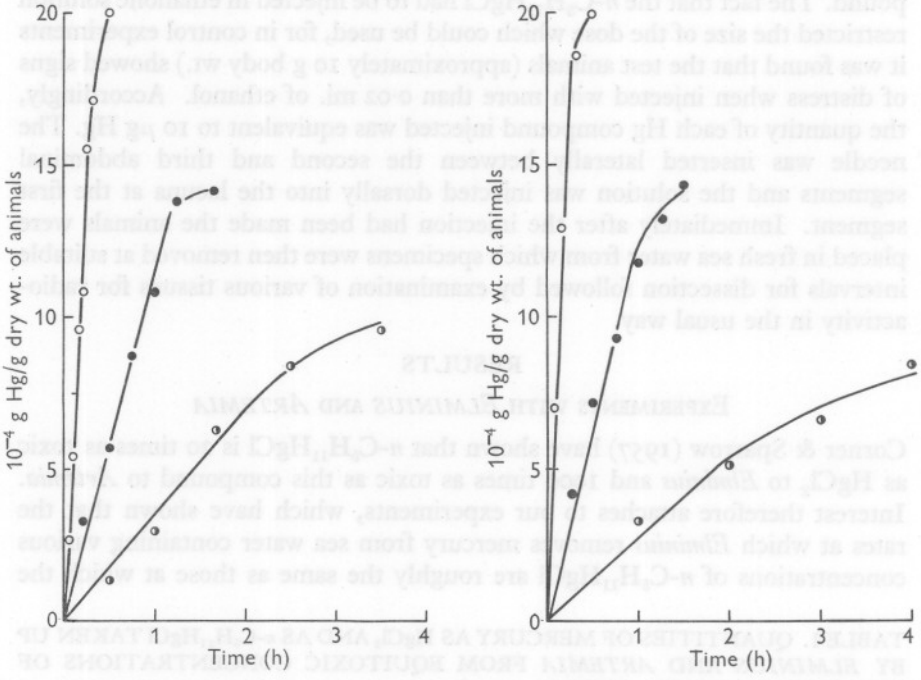

Fig. 1. Left: uptake of mercury as mercuric chloride by *Elminius* larvae. ○—○, 5 mg Hg/l. (TD_{50} = 0·55 h); ●—●, 1 mg Hg/l. (TD_{50} = 1·1 h); ◑—◑, 0·2 mg Hg/l. (TD_{50} = 2·9 h). Right: uptake of mercury as *n*-amylmercuric chloride. ○—○, 0·25 mg Hg/l. (TD_{50} = 0·6 h); ●—●, 0·05 mg Hg/l. (TD_{50} = 0·9 h); ◑—◑, 0·01 mg Hg/l. (TD_{50} = 3·0 h).

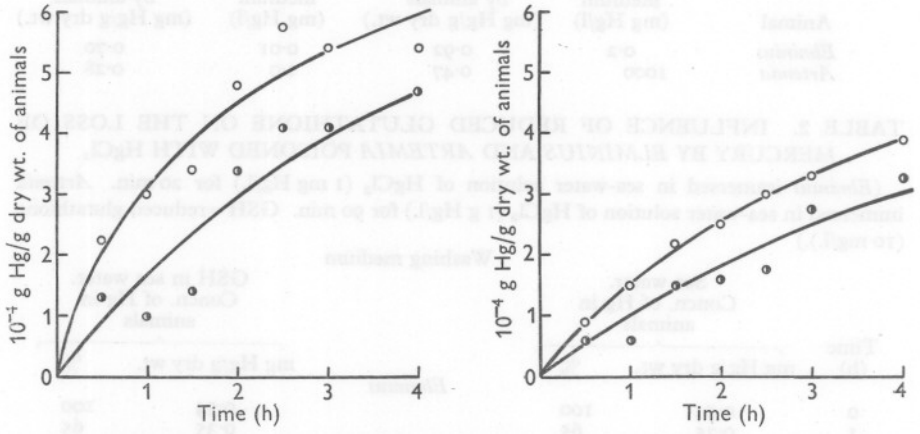

Fig. 2. Left: uptake of mercury as mercuric chloride by *Artemia* larvae. ○—○, 1 g Hg/l. (TD_{50} = 2·3 h); ◑—◑, 0·5 g Hg/l. (TD_{50} = 3·6 h). Right: uptake of mercury as *n*-$C_5H_{11}HgCl$. ○—○, 1 mg Hg/l. (TD_{50} = 2·5 h); ◑—◑, 0·5 mg Hg/l. (TD_{50} = 3·5 h).

animal takes up Hg from sea water containing 20 times each concentration as $HgCl_2$ (Fig. 1). Similar experiments with *Artemia* (see Fig. 2) have shown that the rates at which this animal concentrates mercury as n-$C_5H_{11}HgCl$ are approximately the same as those at which it takes up the poison from sea water containing 500 times each concentration as $HgCl_2$. The correlation between toxicity and uptake data is, therefore, less exact for *Artemia*. Nevertheless, it may be concluded that differences between the toxicities of the two poisons reflect to a considerable extent differences between the rates at which the compounds are taken up by both species of test animal.

The results shown in Table 1 draw attention to the vast difference between the concentrations of each Hg compound which must be added to the surrounding medium in order to kill *Artemia* and *Elminius* at the same rate.

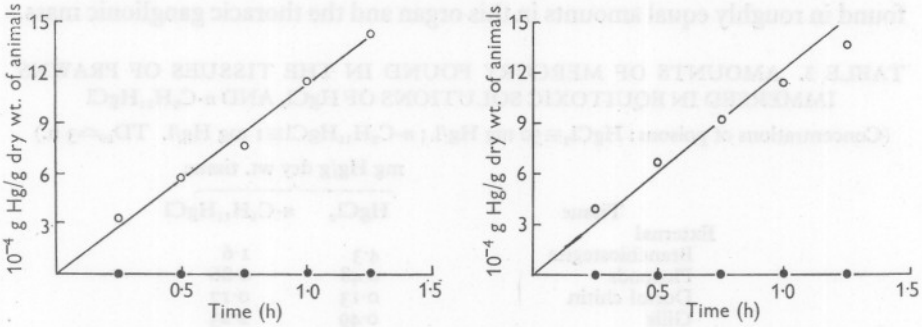

Fig. 3. Left: uptake by *Elminius* larvae of mercuric chloride (1 mg Hg/l.), alone (○—○) and in the presence of a tenfold excess of reduced glutathione (●—●). Right: uptake of n-$C_5H_{11}HgCl$ (0·05 mg Hg/l.) alone (○—○) and in the presence of a tenfold excess of reduced glutathione (●—●).

Thus, the concentrations of $HgCl_2$ and n-$C_5H_{11}HgCl$ which are equitoxic to *Artemia* and *Elminius* are in the ratio 5000:1 and 100:1 respectively. Of greater interest, however, is the finding that although much higher external concentrations are needed to kill *Artemia*, the actual quantities of mercury which these animals accumulate by the time 50% have died are significantly less than the corresponding amounts taken up by *Elminius*.

The results of experiments in which reduced glutathione was used to protect *Elminius* from the toxic effects of $HgCl_2$ and n-$C_5H_{11}HgCl$ (see Fig. 3) showed that the addition of a tenfold excess of the thiol compound to the toxic media completely prevented any uptake of mercury by the test animals during the time in which more than 75% of the unprotected animals died (3 h). When further experiments were carried out to examine the influence of reduced glutathione on the rates of loss of mercury by *Elminius* and *Artemia* previously poisoned with $HgCl_2$ and n-$C_5H_{11}HgCl$ it was found that the thiol compound had little or no effect on these very slow processes (see Table 2).

Experiments with *Leander*

Findings made in experiments with prawns were of considerable interest because they demonstrated that animals killed by immersion in toxic solutions of $HgCl_2$ and n-$C_5H_{11}HgCl$ contained significant amounts of Hg in several internal organs (see Table 3). Moreover, after the animals had been killed by immersion for 2 h in equitoxic solutions of the two poisons the patterns of distribution of the Hg throughout the animals were markedly different. Thus, when tissues in contact with the surrounding medium were examined it was found that whereas $HgCl_2$ concentrated in the tissue beneath the branchiostegites, n-$C_5H_{11}HgCl$ was detected primarily in the gills. The distributions of the poisons inside the test animals also differed in that whereas $HgCl_2$ concentrated almost exclusively in the antennary gland, n-$C_5H_{11}HgCl$ was found in roughly equal amounts in this organ and the thoracic ganglionic mass.

TABLE 3. AMOUNTS OF MERCURY FOUND IN THE TISSUES OF PRAWNS IMMERSED IN EQUITOXIC SOLUTIONS OF $HgCl_2$ AND n-$C_5H_{11}HgCl$

(Concentrations of poisons: $HgCl_2 \equiv 50$ mg Hg/l.; n-$C_5H_{11}HgCl \equiv 1$ mg Hg/l. $TD_{50} \simeq 3$ h.)

	mg Hg/g dry wt. tissue	
Tissue	$HgCl_2$	n-$C_5H_{11}HgCl$
External		
Branchiostegite	4·3	1·6
Pleopods	0·48	0·86
Dorsal chitin	0·13	0·17
Gills	0·49	2·23
Internal		
Antennary gland	0·32	0·46
Hepatopancreas	0·02	0·05
Central nervous system	0·04	0·38
Muscle	0·00	0·04

By contrast, it was found that after the animals had been injected with sublethal doses of $HgCl_2$ and n-$C_5H_{11}HgCl$ the patterns of distribution of the two poisons were similar in that both compounds were found to concentrate in the gills and antennary glands of the test animals (see Table 4).

Further experiments using reduced glutathione gave results consistent with those obtained in similar studies with *Elminius* and *Artemia*. Thus, no significant amount of mercury was removed when the gills of prawns previously treated with mercuric chloride were washed either with *Homarus* Ringer alone or fortified with reduced glutathione. In addition, the branchiostegites of these animals were found to lose less than half their Hg content when washed with either medium (see Table 5). Because these tissues are in continuous contact with the surrounding medium and were found to accumulate far more Hg than any other tissue, it seemed reasonable to expect that if Hg acted by simply becoming attached to the surfaces of the test animals these would be the most likely sites of attachment. However, it appeared

from these findings that all the mercury accumulated by the gills and most of that taken up by the tissue under the branchiostegite was not attached to the surfaces of these tissues, but had penetrated into them.

TABLE 4. AMOUNTS OF MERCURY DETECTED IN THE TISSUES OF PRAWNS INJECTED WITH MERCURIC CHLORIDE AND *n*-AMYLMERCURIC CHLORIDE

(Concentrations used: 10 μg Hg/l animal injected in 0·01 ml. sea water ($HgCl_2$) and 0·02 ml. ethanol (n-$C_5H_{11}HgCl$).)

Tissue	μg Hg/g dry wt. tissue	
	$HgCl_2$	n-$C_5H_{11}HgCl$
External		
Branchiostegite	13·0	4·3
Pleopods	2·2	1·0
Dorsal chitin	3·4	0·2
Gills	29·3	22·0
Internal		
Antennary gland	13·3	6·7
Hepatopancreas	4·4	4·2
Central nervous system	3·5	3·0
Muscle	2·7	0·0

TABLE 5. THE REMOVAL OF MERCURIC CHLORIDE FROM THE GILLS AND BRANCHIOSTEGITES OF PRAWNS POISONED WITH THIS COMPOUND

Washing medium	Counts/min/mg dry wt. of tissue	
	Gills	Branchiostegite
None	100	1795
Homarus Ringer	118	1160
Homarus Ringer (plus 100 mg reduced glutathione/l.)	102	1150

DISCUSSION

In earlier studies, Corner & Sparrow (1956, 1957) showed that great differences are found between the susceptibilities of *Artemia* and *Elminius* to mercury poisons; that differences between the toxicities of certain organomercury poisons and that of mercuric chloride are far greater when a highly resistant test animal like *Artemia* is used; that, in general, poisons which are very toxic are also highly lipoid-soluble; and that no correlation exists between the toxicities of these compounds and their abilities to inactivate enzymes. Corner & Sparrow considered these observations to be consistent with the view that Hg poisons act by penetrating the test animal and assumed that the extreme resistance of *Artemia* was a direct result of this animal's impermeability. Their findings did not, however, exclude the possibility that the large differences between the susceptibilities of *Artemia* and *Elminius* to Hg poisons were not because *Artemia* accumulated the compounds at a much slower rate, but because this animal was able to tolerate a much higher concentration of mercury in its tissues. This possibility has therefore been examined in the present work, and it has been found that both $HgCl_2$ and n-$C_5H_{11}HgCl$ are, in

fact, accumulated much more rapidly by *Elminius* than by *Artemia*. Moreover, the amount of Hg, as either compound, taken up by *Artemia* at a time when 50% of the test animals die, is significantly less than the corresponding amount accumulated by *Elminius*. It therefore seems clear that *Artemia* is very resistant to Hg compounds, not because the tissues of this animal are able to withstand large concentrations of mercury, but because this animal accumulates the poisons at a very slow rate.

Further results of the present work were that both *Artemia* and *Elminius* take up *n*-$C_5H_{11}HgCl$, which is very toxic and very lipoid-soluble, at a rate much faster than that at which they accumulate $HgCl_2$; and that the relative rates of uptake of the two poisons are related to their relative toxicities. However, although these findings emphasized that high toxicity attends a high rate of accumulation by the test animal, they did not demonstrate how this accumulation took place, and the possibility could not be ignored that the organomercury compound might be taken up more rapidly than $HgCl_2$ because of preferential adsorption on the animal's surface. It is true that Corner & Sparrow (1957) have provided evidence in favour of the view that as far as interaction with proteins present on the surface of the test animal is concerned, *n*-$C_5H_{11}HgCl$ is likely to be less effective than $HgCl_2$, but in the present work it was thought desirable to seek more direct evidence that penetration of the test animal took place. Accordingly, semi-quantitative studies were made with *Leander*, an animal large enough to permit excision of various internal organs which could be examined for their Hg content. In these experiments it has been found that when the animals are immersed in equitoxic solutions of the two poisons, significant amounts of Hg can, in fact, be detected in their internal tissues. Moreover, these amounts are slightly greater for *n*-$C_5H_{11}HgCl$, although the concentration of this poison used in the external medium is equivalent to only one-fiftieth of the quantity of Hg used as $HgCl_2$. These experiments, therefore, provide direct evidence that Hg compounds can penetrate a crustacean and that an organomercury poison enters the test animal at a rate much faster than that of $HgCl_2$. The poisons have also been found in considerable quantity on external sites such as the gills and branchiostegites, on which they might be in a position to exert at least part of their toxic action. Further experiments, however, have provided evidence in favour of the view that attachment to the surface is not of primary importance for the toxic action of either poison. Thus, if the compounds act simply by becoming attached to these surfaces it seems likely that their toxic effects would be readily reversed by substances such as reduced glutathione, because the marked affinities of thiol compounds for heavy metals (cf. Gurd, 1954) have often been exploited as a means of removing these poisons from biological surfaces. During toxicity studies with *Elminius* and *Artemia*, Corner & Sparrow (1957) found that thiol compounds effectively protect the test animals against mercury poisons, and in the present work it has been

found that reduced glutathione can prevent *Elminius* from accumulating mercury either as $HgCl_2$ or as n-$C_5H_{11}HgCl$. These results support the view that the affinity of the thiol compound is greater than that of the surface of the test animal for either poison. Because of this it might be expected that reduced glutathione would readily remove these poisons from the surface of *Elminius*, and some evidence of this was obtained from further experiments by Corner & Sparrow in which it was found that reduced glutathione lowers the death rates of *Elminius* transferred to plain sea water after preliminary treatments with mercury poisons. However, these experiments lasted a long time and it is possible that the thiol compound did not remove the poisons from the surfaces of the test animals but penetrated into them and inactivated a proportion of the mercury poison after it had reached the tissues. The results of shorter experiments carried out in the present work have shown that reduced glutathione has little or no influence on the very slow rates of loss of mercury by either *Elminius* or *Artemia* previously poisoned with mercuric chloride, nor does it influence the loss of mercury from the surface tissues (e.g. gills and branchiostegites) of prawns poisoned with this compound. These findings therefore lend support to the view that the Hg compound was not simply attached to the surfaces of the test animals, but penetrated into them.

Examination of the patterns of distribution of n-$C_5H_{11}HgCl$ and $HgCl_2$ injected into prawns has given further information concerning the origin of the mercury detected on the gills and branchiostegites, for it has been found that these tissues also contain large quantities of Hg after the animals have been injected with either poison. This finding suggests that the Hg detected on these surfaces after the animals have been immersed in toxic solutions of the poisons might not arise exclusively as the result of direct accumulation from the surrounding medium, but might to some extent represent an attempt by the animal to excrete through these tissues some of the assimilated Hg. On this point it is interesting that Hg poisons have been found to concentrate in the antennary gland of the prawn after the animals are immersed in toxic solutions, and after they have been given a sublethal dose of each compound by injection, for the decapod antennary gland is known to be associated with excretory mechanisms. A further point of interest is that $HgCl_2$ has been found to affect osmoregulation in *Daphnia magna* (Holm-Jensen, 1948).

There are obvious dangers in attempting to base a theory of Hg poisoning on the results of studies made with several different test species, but from the findings just discussed it appears that penetration of the animal and subsequent interference with excretory mechanisms is a useful working hypothesis on which to base future studies of the modes of action of Hg compounds as poisons to crustaceans.

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SUMMARY

Experiments have been carried out with ^{203}Hg-labelled *n*-amylmercuric chloride (n-$C_5H_{11}HgCl$) and mercuric chloride ($HgCl_2$) to study the modes of action of these compounds as poisons to some crustaceans.

Differences between the susceptibilities of *Artemia salina* and *Elminius modestus* to the poisons do not reflect differences between the quantities of these compounds which the animals can tolerate in their tissues, but are directly related to the rates at which the poisons are accumulated. Thus, experiments with either species have shown that Hg is taken up at approximately the same rate from equitoxic solutions of the two poisons; and the rates at which Hg is accumulated by the two species from equitoxic solutions of either poison are of the same order.

Experiments with reduced glutathione have given results consistent with the view that most of the Hg taken up by either species penetrates into the tissues of the test animals and does not act simply by becoming attached to their surfaces.

Direct evidence of the penetration of Hg compounds into a crustacean has been obtained from experiments with *Leander serratus*. Considerable amounts of Hg have been detected in the antennary glands.

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