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The Fate of Mercury Species Injected into Coelomic Fluid of Starfish *Leptasterias polaris*

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Mature starfish *Leptasterias polaris*, collected in the St Lawrence Estuary (eastern Canada), were exposed to two mercury species ($HgCl_2$ and CH_3HgCl) via injections into the coelomic fluid. *In vivo* effects of some complexing agents (glutathione, mercaptoethanol and EDTA) on the distribution of ^{203}Hg-labelled species in starfish organs and tissues and their possible role in mercury transport through membranes were studied over a 24 h period. The excretion of ammonia and mercury was also measured. When injected alone, inorganic mercury and methylmercury [$CH_3Hg(II)$] were distributed in all organs, with a preferential adsorption in gonads, pyloric caeca and stomach. Mercury excretion was very low under all conditions studied. Mercaptoethanol, a small thiol ligand, was very efficient in reducing both mercury species in the coelomic fluid and seems to have promoted translocation towards most organs of the starfish. Its action is attributed to the formation of small and neutral complexes, HgL_2 and CH_3HgL, which can diffuse through membranes preserving their integrity. Glutathione increased the translocation of $CH_3Hg(II)$ towards surrounding organs, but had no apparent effect on inorganic mercury. EDTA promoted the transport of inorganic mercury only. These results highlight (1) the particular interest of starfish to workers studying *in vivo* chemical complexation of mercury species, and (2) the potential role of complexing molecules in the biotransport of mercury species through living membranes.

Keywords: mercury; methylmercury; glutathione; mercaptoethanol; thiols; EDTA; complexation; starfish; translocation; invertebrates

INTRODUCTION

The environmental chemistry and the toxicology of heavy metals in aquatic organisms have received continuous interest in the last two decades.[1,2] Among non-essential trace metals, mercury is of particular interest, being present in at least two chemical forms in the aquatic environment and being highly toxic to most living organisms.[3] In marine coastal ecosystems, starfish occupy a high trophic level and appear to be important in the trace metals balance between benthic organisms.[4] Bioaccumulation of methylmercury in the starfish *Leptasterias polaris* feeding on contaminated mussels was examined in our laboratory a few years ago,[5] and results showed an autopurging process taking place after a few weeks of continuous uptake. These preliminary observations stimulated a more detailed investigation of mechanisms involved in the translocation of mercury species towards organs and tissues.[6] The general cavity (or coelom) of a starfish is filled with a coelomic fluid, similar in composition to seawater surrounding the animal, as starfish and other echinoderms do not have any osmoregulation capability.[7] The mouth and the stomach are located in the central disk, and from the stomach rise pairs of pyloric caeca, each pair being located in the cavity of one arm. Each arm contains also a pair of gonads and a rather complex water–vascular system which functions as a means of locomotion (Fig. 1). The inner surface of the body wall is covered with a coelomic peritoneum composed of ciliated cells responsible for a continual circulation of the coelomic fluid. The fluid-filled coelom surrounding the internal organs provides the principal means of internal transport.[8] This very particular physiology of starfish offers the opportunity to observe and describe the distribution and the translocation of exogenous compounds in various organs and tissues from the coelomic fluid, by-passing the usual uptake routes (i.e. diffusion through respiratory organs or digestive tracts). It was possible to inject saline solutions containing substances of interest into the coelomic fluid without disturbing the animal and then to observe the behaviour of these chemicals (mainly translocation and excretion processes) as a function of experimental

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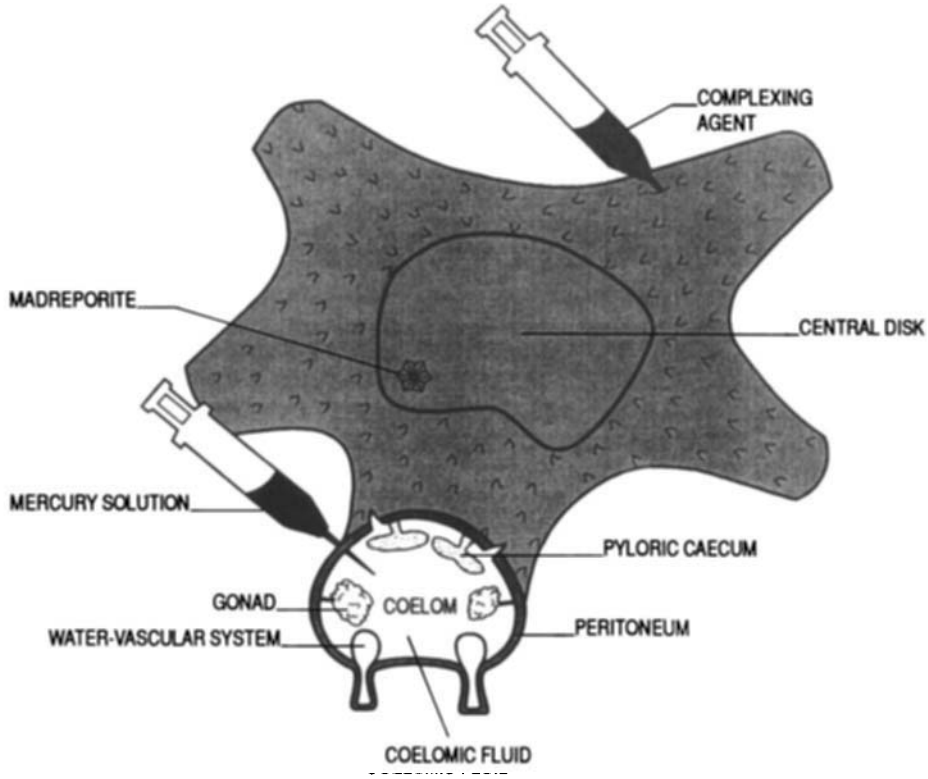

Fig. 1 The general anatomy of the starfish and the injection procedure used to introduce mercury species and complexing agents.

parameters, such as complexing agents which could modify the chemistry of studied chemicals.

General transport processes of mercuric cation (Hg^{2+}) and methylmercuric cation (CH_3Hg^+) through artificial membranes are well documented and seem related to the passive diffusion of neutral chloro complexes.[9,10] However, the transport of mercury species through living epithelium and membranes as well as the fundamental role and effects of small complexing agents, such as amino acids and small peptides, in mercury transport and subsequent mercury uptake by cells and tissues are still unexplored.

In an attempt to use a living benethic invertebrate to study absorption and transport mechanisms of mercury through biological tissues, this paper describes the results of a series of experiments involving the injection of labelled $^{203}Hg^+$ and $CH_3\,^{203}Hg^+$ species directly into the coelomic fluid of *Leptasterias polaris* in the presence of various complexing agents: glutathione (a natural small peptide), mercaptoethanol (a small and powerful mercaptan), and EDTA (a general-use chelating agent). The effects of these ligands on the translocation of mercury species through various organs of the starfish are tentatively discussed as a function of some physical-chemical properties of the expected complexes.

MATERIALS AND METHODS

Mature starfish *Leptasterias polaris* (70–220 g wet wt) were collected at Pointe-au-Père, Québec, Canada, by scuba diving and were kept in a 540 l standing flow-through aquarium receiving filtered seawater with salinity ranging from 20 to 30. Three days before each experiment, animals were acclimatized in a smaller recirculating aquarium maintained at a water temperature of 6 ± 1 °C. Animals were fed with cultured blue mussels *ad libitum*.

Contamination procedure

Mercury and complexing-agent solutions were prepared in filtered (45 μm) seawater to minimize physiological stress induced by the injection in the general cavity of the starfish. Concentrations of stock solutions of unlabelled mercury species were 5×10^{-5} M. Radiotracer ^{203}Hg was added at a

nominal activity of 79 kBq ml^{-1} for $^{203}HgCl_2$ (purchased from Amersham). Labelled methylmercury was synthesized[11] from inorganic ^{203}Hg and was added at the nominal activity 285 kBq ml^{-1} of $CH_3{}^{203}HgCl$ (0.005 M in Na_2CO_3). Complexing agents used in this series of experiments [glutathione (GSH), mercaptoethanol (MerOH) and ethylenediamine tetra-acetic acid (EDTA)] were obtained from Aldrich and the concentration of stock solutions in filtered seawater was 5×10^{-3} M. Two starfish were used for each experimental condition involving a combination between one complexing agent and one mercury species.

Mercury solutions (0.5 ml containing 25 nmol Hg) were injected through the calcareous skeleton in the last one-third of one arm of the starfish (Fig. 1). When a complexing agent was used, its seawater solution (0.5 ml containing 2500 nmol) was injected in the opposite arm immediately following the mercury injection. As the coelomic fluid is free to flow through the whole coelom of the animal, the starfish was gently rotated for 1 min to initiate a good mixing of solutions inside the general cavity. After injections, each starfish was placed in a large glass beaker containing 3 l of seawater kept at 6 °C and air was bubbled through to ensure the mixing and the aeration of the medium. During the 24 h exposure period, water was periodically sampled at 0, 0.5, 1, 2, 4, 6, 8 and 24 h for ammonia and mercury analysis.

Animals were sacrificed after 24 h; coelomic fluid was collected first, weighed and sub-sampled for ^{203}Hg gamma counting. The entire cardiac and pyloric stomachs, gonads and pyloric caeca from the four arms which were not directly injected with the mercury solution or the complexing agent, as well as sub-samples of calcareous skeleton, were dissected. All samples were weighed and counted for ^{203}Hg.

Octanol–seawater partition coefficient (K_{osw})

An aliquot (1 ml) of a mercury solution [1 mM of the metal species dissolved in filtered (0.45 μm) seawater and containing 135 kBq ml^{-1} of $^{203}HgCl_2$ or $Ch_3{}^{203}HgCl$ as radiotracers] was mixed with 1 ml of a complexing agent solution (100 mM prepared in filtered seawater). Solutions of mercury and complexing agent (2 ml) were added to 2 ml of octanol (HPLC grade), the mixture was vigorously shaken in a separatory funnel for 10 min, and then it was allowed to equilibrate in darkness for 96 h. Organic and aqueous phases were separated and both counted for ^{203}Hg. The octanol–seawater partition coefficient was calculated as the ratio between counts measured in each phase. Each K_{osw} measurement was repeated five times and the standard deviation calculated.

Radioactivity detection and chemical analysis

The presence of ^{203}Hg was detected by gamma counting on a 1272 Clinigamma, LKB counter using a counting time of 5 min for each sample at an energy of 279.19 keV. Radioactivity counts (dpm) were corrected for background level and the natural decay of the isotope (half-life: 47 d), and converted to pmol Hg g^{-1} (wet wt). The mean coefficient of variation of 50 successive readings of the same sample was 5%. The detection limit was about 50 pmol g^{-1}. As the concentration of Hg(II) excreted in seawater during the 24 h exposure period was usually very low, it was necessary to freeze-dry samples to concentrate solutes. Precipiated salts were dissolved in 3 ml of 3 M hydrochloric acid (HCl) and acidic samples were then counted, following conditions used for biological tissues. Seawater samples collected from beakers with methylmercury were solvent-extracted.[12] Briefly, methylmercury was complexed by adding a large excess of urea (8 M), and $CuSO_4$ was added to prevent further complexation with sulphydryl-bearing compounds. The aqueous solution was acidified (5 M HCl) and extracted twice with benzene. Organic fractions were combined and then used for gamma counting under the conditions described above. Ammonia was analysed in seawater samples using the phenol–hypochlorite method.[13] Tissue subsamples (pyloric caeca, gonads and calcareous skeleton) were freeze-dried and then treated twice with dichloromethane (CH_2Cl_2) in order to extract lipids. These extracts were dried and weighed accurately to determine percentage lipid content.

RESULTS

Seawater-octanol partition coefficient and lipid content

In the absence of any complexing agent, inorganic mercury is assumed to be present as $HgCl_2$ and was found to be about 14 times less soluble in

Table 1 Octanol–seawater partition coefficient (K_{osw}) of Hg(II) and $CH_3Hg(II)$ species determined at 25 °C and in the presence of various water-soluble complexing agents (mean ± SD, $n = 5$)

Conditions	K_{osw}	
	HgL_2	CH_3HgL
Hg species alone (in chloride forms)	0.17 ± 0.017	2.40 ± 0.07
Hg + glutathione (GSH)	≤0.01	≤0.01
Hg + mercaptoethanol (MerOH)	1.19 ± 0.02	3.30 ± 0.10
Hg + EDTA	0.16 ± 0.03[a]	2.30 ± 0.10[a]

[a] K_{osw} not significantly different from K_{osw} determined for the mercury species alone (based on the Student *t*-test).

octanol than the corresponding methylmercury chloride (Table 1). The K_{osw} of $HgCl_2$ observed here is almost four times lower than a previously reported value of 0.62 for salt waters.[14] However, the value for K_{osw} of CH_3HgCl was close to the value of 1.7 reported in the same study.[14]

Complexing agents had different effects on the organic solubility of both metal species in octanol Glutathion (GSH) significantly decreased the octanol solubility of mercury species while EDTA had no effect. Only mercaptoethanol (MerOH) ($K_{osw} = 1.19$) significantly increased mercury solubility in octanol and thus could increase the liposolubility of inorganic mercury.

Methylmercury alone (as CH_3HgCl) was quite soluble in the octanol phase, and EDTA and MerOH did not modify that solubility very much. However, the complex formed with GSH was almost insoluble in the octanol phase ($K_{osw} \leq 0.01$). Pioneer work by Westöö[15] mentioned that the addition of a water-soluble thiol enhanced the extraction of methylmercury from an organic matrix to an aqueous solution. The very low K_{osw} observed in the presence of GSH confirms that previous observation. Lipid content in gonads and pyloric caeca was quite low, averaging 4.2%, and only 0.5% lipid was found in calcareous skeleton.

Concentration of Hg(II) in organs and tissues

Mercury concentrations (pmol g^{-1} wet wt) observed in different organs and tissues of the starfish *L. polaris* 24 h after injection of inorganic mercury solutions into the coelomic fluid are shown in Table 2. The mean coefficient of variation calculated for all pairs of data reported as means ± S.D. in Table 2 was 27%. The highest variability between two starfish submitted to the same treatment was usually observed for stomach, a small organ difficult to dissect and easy to contaminate by accidental contact with surrounding organs. One day after the injection of mercury chloride into the coelomic fluid without the addition of any complexing agent, most of the mercury escaped from the coelomic fluid and was recovered in organs and tissues at concentrations varying from 374 pmol g^{-1} in gonads to 94 pmol g^{-1} in calcareous skeleton. The initial concentration of Hg(II) in the coelomic fluid, estimated to be 1675 pmol g^{-1} (5.00 µg of Hg in 14.7 g of coelomic fluid for a 100 g starfish) decreased to 89 pmol g^{-1} due to strong absorption of mercury into organs in direct contact with the coelomic fluid.

The injection of complexing agents into the coelomic fluid simultaneously with the injection of the mercury solution induced some changes in mercury concentrations in organs. While EDTA induced little change in the retention of mercury in the coelomic fluid, GSH increased by about twice the retention of mercury in the coelomic fluid. MerOH was the only complexing agent which reduced significantly the concentration of Hg(II) in the coelomic fluid. The Hg(II) concen-

Table 2 Mercury concentration (pmol g^{-1} wet wt) in different organs and tissues of starfish *L. polaris* 24 h after injection of inorganic mercuric solutions into the coelomic fluid[a]

Conditions	Coelomic fluid	Pyloric caeca	Stomach	Gonads	Calcareous skeleton
$HgCl_2$ untreated	89 ± 4	330 ± 50	305 ± 50	374 ± 50	94 ± 50
$HgCl_2$ + glutathione (GSH)	227 ± 98	261 ± 34	394 ± 50	493 ± 5	103 ± 34
$HgCl_2$ + mercaptoethanol (MerOH)	≤50	1034 ± 345	1857 ± 157	79 ± 83	84 ± 10
$HgCl_2$ + EDTA	123 ± 25	966 ± 325	1039 ± 354	1034[b]	133 ± 44

[a] Values are means ± SD for two starfish. The initial concentration of Hg(II) in the coelomic fluid was about 1675 pmol g^{-1}.

[b] Result from one specimen only.

Table 3 Methylmercury concentration (pmol g^{-1} wet wt) in different organs and tissues of starfish *L. polaris* 24 h after injection of methylmercuric solutions into the coelomic fluid[a]

Conditions	Coelomic fluid	Pyloric caeca	Stomach	Gonads	Calcareous skeleton
CH_3HgCl untreated	256 ± 5	488 ± 64	734 ± 768	650 ± 163	79 ± 30
CH_3HgCl + glutathione (GSH)	≤ 50	1305 ± 54	980 ± 744	916 ± 680	217 ± 54
CH_3HgCl + mercaptoethanol (MerOH)	≤ 50	551 ± 59	759 ± 355	586 ± 365	236 ± 20
CH_3HgCl + EDTA	409 ± 108	197 ± 79	305 ± 103	493 ± 340	≤ 50

[a] Values are means ± SD for two starfish. The initial concentration of $CH_3Hg(II)$ in the coelomic fluid was about 1675 pmol g^{-1}.

tration was increased in the pyloric caecum and in the stomach by most complexing agents in the following order: MerOH ≥ EDTA ≫ GSH. The concentration of Hg(II) in the calcareous skeleton was much lower than in soft tissues and was usually very close to the mercury concentration remaining in the coelomic fluid.

Concentration of $CH_3Hg(II)$ in organs and tissues

Concentrations of methylmercury (pmol g^{-1} wet wt) observed in different organs and tissues of *L. polaris* are presented in Table 3. The mean coefficient of variation of data pairs in Table 3 was 39%, with the highest variability observed in stomach and also in gonads. Methylmercury injected alone was found in all organs and tissues at concentrations ranging from 79 pmol g^{-1} in calcareous skeleton to over 700 pmol g^{-1} in stomach. $CH_3Hg(II)$ remained in the coelomic fluid at a concentration almost three times higher than inorganic mercury left in the coelomic fluid under similar conditions. Only EDTA induced a high retention of the organometal in the coelomic fluid, while the action of MerOH and GSH is remarkable in reducing the remaining concentration of $CH_3Hg(II)$ in coelomic fluid to values below 50 pmol g^{-1}. EDTA, a bulky chelating agent, increased the retention of $CH_3Hg(II)$ in the coelomic fluid and, as a result of its action, mercury concentration was decreased in all organs and tissues including the calcareous skeleton. Methylmercury concentrations in the calcareous skeleton seem to have been increased by both GSH and MerOH. The most remarkable increase was induced by GSH in pyloric caeca.

Excretion of ammonia, mercury and methylmercury

Results of ammonia and mercury excretions are presented in Table 4. The excretion rate of ammonia was estimated from the slope of ammo-

Table 4 Excretion rate of ammonia and amount of mercury recovered in seawater 24 h after injection of mercuric solutions in the coelomic fluid of starfish *L. polaris*[a]

Conditions	$HgCl_2$		CH_3HgCl	
	Ammonia (pmol g^{-1} h^{-1})	Hg^{2+} (pmol)[b]	Ammonia (pmol g^{-1} h^{-1})	CH_3Hg^+ (pmol)[b]
Hg species untreated	26.9 ± 0.8	236 ± 118 (0.96)	3.9 ± 1.1	187 ± 25 (0.76)
Hg + glutathione (GSH)	3.6 ± 0.3	305 ± 83 (1.2)	3.6 ± 0.8	167 ± 10 (0.68)
Hg + mercaptoethanol (MerOH)	8.1 ± 0.8	261 ± 25 (1.1)	8.9 ± 0.6	212 ± 10 (0.86)
Hg + EDTA	9.2 ± 0.8	591 ± 246 (2.4)	7.2 ± 1.9	167 ± 10 (0.68)

[a] Values are means ± SD of two starfish.
[b] Percentage of excreted mercuric species relative to the initial injected dose is given in parentheses.
[c] Mercury detected in one case only.

Table 5 Molecular formulae of complexing agents and molar volumes of mercury (HgL_2) and methylmercury (CH_3HgL) complexes

Complexing agent L	Molecular formula	Molar volume ($cm^3\ mol^{-1}$)[a]		
		L	HgL_2	CH_3HgL
Glutathione	**HOOC—CH(NH$_2$)(CH$_2$)$_2$CONHCH(CH$_2$—SH)CONHCH$_2$)—COOH**	316	639	353
Mercaptoethanol	**HS—CH$_2$—CH$_2$—OH**	85	177	122
EDTA	**(HOOCCH$_2$)$_2$N—CH$_2$—CH$_2$—N(CH$_2$COOH)$_2$**	290	302[b]	327

[a] Molar volumes are estimated by the LeBas method.[19]

[b] Assuming the formation of mono-substituted complex Hg–EDTA.

nia concentration measured in seawater as a function of the elapsed time, while the excreted mercury was calculated from ^{203}Hg counts in seawater at the end of the 24 h exposure period and reported for a 100 g standard starfish. The excretion of ammonia was very low under all conditions studied, corresponding to the expected low metabolic activity of *L. polaris* at a seawater temperature of 6 °C.

Amounts of Hg(II) and $CH_3Hg(II)$ found in seawater were also quite small, representing less than 1% of the total amount of methylmercury injected and less than 3% of the inorganic mercury. None of the complexing agents tested modified the diffusion of methylmercury species towards the seawater medium.

DISCUSSION

The general cavity of a mature *L. polaris* (containing about 15 ml of coelomic fluid) is somewhat similar to a large test-tube with biological walls and filled with seawater. The injection of mercury compounds into this biological test-tube resulted in a distribution of mercury species among organs and tissues according to their location in the cavity, their size and their adsorption capacity. The injection of a complexing agent into the coelomic fluid immediately after the injection of a mercury solution is quite similar to the addition of an excess of a complexing ligand (L) to a mercury solution with the expectations of formation of the complexes HgL_2 and CH_3HgL. The aim of this procedure was to mimic toxicological studies involving the use of complexing agents as chemotherapeutic agents for mercury poisoning[16] and to study the chemical action of these agents in a strong ionic medium.

The effectiveness of complexing agents for detoxification purposes in methylmercury poisoning of mammals is confirmed by previous studies.[17] In the case of thiol ligands, the mechanism of their action involves a competition between the endogenous sulphydryl groups and those of the incoming ligand. The complex formed seems to be free to diffuse and the rate of efflux of methylmercury from cells and organisms has been enhanced in the presence of some thiols.[18] A similar mechanism could be expected in starfish, where the comlexing agents present in excess in the coelomic fluid would influence the adsorption and the diffusion of mercury complexes towards organs and tissues as a function of the chemical nature of the complexing agents used. Epithelium and membranes surrounding caeca, gonads and calcareous skeleton can be considered as primary sites of interactions and as a barrier to mercury transport from the coelomic fluid towards organs and tissues.

Complexing ligands used in our experiment were selected to represent a range of chemical properties which could interact with the mercury distribution in organs. The computation of molar volumes for the expected complexes HgL_2 and CH_3HgL (Table 5) provides a new parameter useful in an attempt to establish a relationship, if any, between the size of the ligand and the corresponding mercury complex and its ability to enhance mercury uptake. In spite of the limited number of starfish used for each chemical combination and the impossibility of using statistical methods to compare distribution of species among organs and tissues, some characteristic patterns of mercury species distribution can be highlighted.

Behaviour of inorganic mercury

Complexation of inorganic mercury resulted in a general increase of mercury concentration in all organs and tissues for most agents. A similar

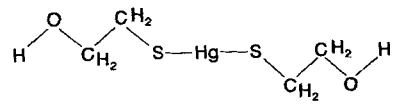

Fig. 2 Molecular structure of $Hg(MerOH)_2$.

uptake increase has been observed in previous studies for other species,[20,21] and may be an indication of a facilitated transfer of complexed species across epithelium and membranes.[22,23]

Mercaptoethanol was the smallest molecule tested. Assuming the formation of an HgL_2 complex, its molar volume would be only 177 cm^3 mol^{-1} and the resulting molecule would have the structure of a linear, symmetrical and neutral complex with hydroxyl groups at both ends (Fig. 2). The relatively high liposolubility of this complex is indicated by its K_{osw} being about seven times higher than K_{osw} for untreated Hg(II) (Table 1).

Interestingly, MerOH decreased slightly the Hg(II) content in the skeleton. The inner surface of the skeleton is covered by a coelomic epithelium which is in contact with muscular and connective tissues that house the skeletal system.[8] These tissues have a very low lipid content (0.5%) and probably offer little opportunity for a neutral, relatively liposoluble complex such as $Hg(MerOH)_2$ to be fixed and then incorporated.

In contrast with $Hg(MerOH)_2$, the complex $Hg(GSH)_2$ is quite bulky, non-linear and bears many carboxylic acid groups (Fig. 3). This structural feature accounts for its high water solubility and its subsequent retention in the coelomic fluid.

Complexing agents increasing the uptake into the pyloric caecum (MerOH and EDTA) also increased mercury transfer to the stomach. As the stomach is not in direct contact with the coelomic fluid, this is an indication that a particular process is involved in the translocation of mercury. Passive diffusion of mercury species across membranes is not the unique route for mercury translocation towards tissues, and mercury mayu also follow the nutrient route. Among the possible systems for nutrient translocation in starfish,[24] the haemal system would have the main function of transporting the complex nutritive substances from stomach and caeca to the gonads and the ambulacral area. After its absorption by pyloric caeca, inorganic mercury travelled towards the stomach quite rapidly as the concentrations observed seem to reflect an equilibrium between the two organs. The particularly high mercury concentration measured in the stomach in the presence of MerOH supports the hypothesis that the integrity of $Hg(MerOH)_2$ would be preserved across the caecum epithelium, and continues to favour the translocation of mercury to the stomach.

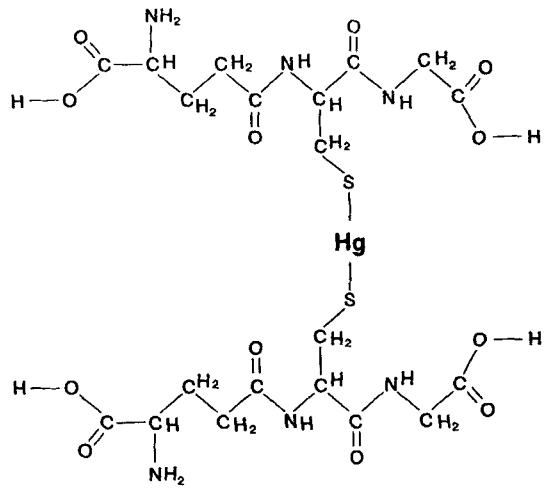

Fig. 3 Molecular structure of $Hg(GSH)_2$.

Behavior of methylmercury

The bahaviour of methylmercury in the presence of ligands appears more complex than inorganic mercury and the variability of concentrations between replicates was generally higher. However, it seems clear that MerOH, GSH and EDTA had major effects on MeHg retention in the coelomic fluid and its transfer to organs. As for Hg(II), MerOH forms the smallest (molar volume = 122 cm^3 mol^{-1}) and the simplest linear molecule with $CH_3Hg(II)$ (Fig. 4). This molecule exhibits the highest K_{osw} measured for all mercury species (Table 1) and the transport mechanism suggested for $Hg(MerOH)_2$ seems to hold also for $CH_3HgMerOH$.

Glutathione presented quite surprising and interesting behavior. The expected complex with CH_3Hg is the biggest of the group (with almost three times the molar volume of $CH_3HgMerOH$) and its K_{osw} is the lowest of the group. In spite of these apparently unfavourable properties, GSH seems to have been quite efficient in transporting methylmercury towards caeca and gonads, and into the skeleton. It should be noted that the structure of the CH_3HgGSH complex is much less

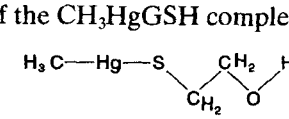

Fig. 4 Molecular structure of $CH_3HgMerOH$.

bulky than the $Hg(GSH)_2$ structure shown above, where one bulky GSH group is replaced by a small CH_3 group.

GSH is a naturally occurring tripeptide often considered as a metal carrier across living membranes. Numerous authors have mentioned the particular role of GSH in mechanisms of metal transport, storage and excretion.[17,25] It was recently proposed[26] that the predominant mechanism of mercury transport across the canalicular membrane of cell liver was coupled to the transport of GSH, and mercury apparently crossed the membrane as a GSH–mercaptide complex via the glutathione transport system, evidence for this hypothesis being stronger for CH_3Hg. The coelomic fluid is rich in chloride ions and relatively poor in thiol groups[7] and is quite different from plasma in composition. However, the addition of a large amount of GSH to the coelomic fluid increased the amount of SH groups and re-established conditions for favourable transport via the GSH route.

EDTA also had a strong effect by retaining MeHg in the coelomic fluid and decreasing transfer towards pyloric caeca and skeleton. Following the Free Ion Activity Model,[27] EDTA is a good chelating agent which usually reduces the availability of metals to complexation with surface membranes and subsequently reduces toxicity. EDTA is recognized as diminishing heavy metal accumulation in marine organisms.[28] In the present experiment, we expected a reduction of the absorption of both inorganic and methylated mercury species in the presence of EDTA. The expected reduction in mercury translocation was observed only for $CH_3Hg(II)$, whereas EDTA faciliated the inorganic mercury uptake. The reasons for this particular behaviour are still unclear.

Finally, it should be mentioned that the low excretion rates of mercury and methylmercury observed in all cases are most probably related to the low metabolic rate of starfish at 6 °C. Reabsorption of excreted mercury on the external surface of the starfish could also happen, and would lower the apparent amount of free mercury excreted in the surrounding seawater.

In summary, these results indicate that the effects of complexing agents on the uptake and the transport of mercury species in living organs and tissues cannot be ascertained using simple models or concepts such as liposolubility (K_{osw}), formation constants of complexes or molecular structures. We found that one small thiol ligand, mercaptoethanol, was very efficient in reducing both mercury species in the coelomic fluid. Its action is attributed to the formation of small, neutral and linear complexes with Hg^{2+} and CH_3Hg^+ which can diffuse through membranes while preserving their integrity. These results also support recently published work[6] where MerOH was found to be an efficient carrier for methylmercury transport through the digestive system of starfish.

In addition to these preliminary results on the ability of some molecules, chosen for their particular chemical properties, to modify the behaviour of mercury species dissolved in a strong ionic medium, we also illustrated the particular interest of starfish to workers studying the *in vivo* chemical behaviour of mercury and other trace metals, and particularly their transport through epithelium and membranes and their subsequent uptake by tissues and organs of benthic invertebrates. The next step in this work would be to compare mercury fluxes across model membranes[10] with those obtainable from living membranes.

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