FUNDAMENTAL ROLES OF BIOLOGICAL BARRIERS IN MERCURY ACCUMULATION AND TRANSFER IN FRESHWATER ECOSYSTEMS (analysis at organism, organ, cell and molecular levels)

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ABSTRACT. In the framework of an ecotoxicological approach to the processes of bioaccumulation and transfer of Hg in freshwater systems, we present a synthesis of our experimental studies concerning the interactions between inorganic Hg and MeHg and biological barriers :

- at organism and organ levels : three biological models are selected : fish (*Salmo gairdneri*), burrowing mayfly nymphs (*Hexagenia rigida*) and rooted macrophytes (*Elodea densa, Ludwigia natans*). Results show strong specificities of the biological barriers (gills, intestine, roots, ...) towards metal fixation and absorption, closely related to the chemical form of the metal, the contamination sources (water, sediments or food) and the physico-chemical characteristics of the medium ;
- at cell and molecular levels : biophysical study of Hg fixation on membrane reveals a new binding site on the phospholipids, the primary amine group on serine and ethanolamine polar heads, jointly with the SH groups of proteins ; Hg(II) induces a strong rigidification of the phospholipidic bilayers. Inorganic Hg and MeHg transports through model membranes (BLM) are essentially due to diffusion of neutral chloride species. These interactions between Hg compounds and membranes are strongly dependent on Hg chemical speciation (pH and pCl effects).

1. Introduction

In any contamination process, whatever the biological complexity of the organisms, accumulation of toxic products is necessarily based on interactions with biological barriers which separate the surrounding environment from the internal medium. The structural and functional characteristics of these barriers are extremely varied, depending on the species considered, the organs or even the stages of development. This "biological barrier" concept could be also extended to the different contamination phases inside the organism : transport via the circulatory system, storage in the target organs and tissues, and excretion.

In aquatic systems, from a general and conceptual point of view, interactions between Hg compounds and biological barriers are based on three fundamental groups of ecotoxicological factors, which are closely interconnected (Boudou and Ribeyre, 1989) :

- the physicochemical characteristics of the medium : temperature, pH, chloride concentration, nature and abundance of inorganic and organic ligands in the dissolved and particulate phases, etc. ;

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- the contamination factors, which correspond to the contamination sources, Hg chemical forms and species in the biotopes (water column and sediments), their partitioning and bioavailability, their concentrations, etc. ;

- the structural and functional properties of the biological barriers : exchange surface, abundance and accessibility of the fixation sites, transport processes and absorption capacity, etc..

In natural conditions, the majority of these factors are changing more or less constantly, in space and in time. Moreover, their effects on Hg bioavailability and bioaccumulation are based on isolated actions but also on interactions, inducing additive, synergistic or antagonistic effects.

Whatever the organism or organ considered, each biological barrier, at the cell level, is based on a unitary structure : the plasmic membrane, "a two-dimensional solution of a mosaic of integral membrane proteins embedded in a fluid lipid bilayer with peripheral proteins bound loosely to either surface" (Singer and Nicholson, 1972). Thus, the contamination of cells by Hg may be described as a sequence of processes starting with interactions between the metal and some ligands of the cell membrane, followed by the transport across this barrier and reactions with different cytoplasmic and nuclear components.

In this paper, we present a synthesis of our different approaches concerning the interactions between Hg compounds and biological barriers, successively at organism, organ, cell and molecular levels.

2. Interactions between mercury compounds and biological barriers : analysis at organism and organ levels

In freshwater ecosystems, for heterotrophic organisms, Hg contamination results from the superposition of two routes : the direct route, from the metal present in the water, with the biological barriers providing adsorption or absorption being the skin and the respiratory organs ; the indirect or trophic route, based on metal transfers from the consumed prey, through the digestive tract.

In order to illustrate these processes, we select first the "fish model" and our experimental approach to the bioaccumulation of two Hg compounds -$HgCl_2$ and CH_3HgCl- by the rainbow trout -*Salmo gairdneri*. We have studied separately the four components of the bioaccumulation process :

- direct contamination, by using automatized modules which produce the same concentration in the water for the two compounds (cyclical renewal of the contaminated medium in the experimental units - duration of each cycle : 6 hr), during the exposure period (30 days), and also identical abiotic conditions (temperature, photoperiod, pH, etc.) (Ribeyre and Boudou, 1981) ;

- trophic contamination, based on daily ingestion of living prey (alevins) previously exposed by direct route to the two Hg compounds, metal burdens introduced via the food during the 30 days' exposure being the same for the two chemical forms ;

- decontamination after direct contamination, fish being placed in large tanks with no exogenous source of Hg, except natural levels in water and food, for 250 days ;

- decontamination after trophic contamination, with similar conditions as those for the preceding experiment, for 60 days.

These four approaches are based on a chronological study, in order to define the tendencies of the phenomena ; thus, for example, four exposure durations were selected for direct contamination (5, 10, 20 and 30 days) and five for the corresponding decontamination phase (17, 31, 56, 120 and 250 days). Total Hg accumulation in *Salmo gairdneri* is quantified by two complementary

criteria : "concentration" (ng $Hg.g^{-1}$, fresh weight) and "burden" (mg Hg). Two analysis levels are used : whole fish and organ or tissue (liver, brain, gills, skeletal muscle, posterior intestine, kidneys, spleen, blood).

After direct contamination by the two chemical forms of Hg, the gill barrier represents both a compartment for the accumulation of the metal and a more or less permeable structure for its absorption :

- after 30 days'contamination by $HgCl_2$, average Hg burden in the gills corresponds to 28% of the quantity measured in the whole organism ; for the organic compound, this relative burden is close to 10% (Ribeyre and Boudou, 1984a) ;

- the efficiency of Hg absorption through the gill epithelium varies considerably according to the chemical form of the metal added to the water. Indeed, for the same exposure conditions (1mg $Hg.L^{-1}$, $HgCl_2$ or CH_3HgCl), a factor of 6.2 separates the average concentrations measured in the whole fish, after 30 days'exposure, in favor of MeHg (Figure 1A).

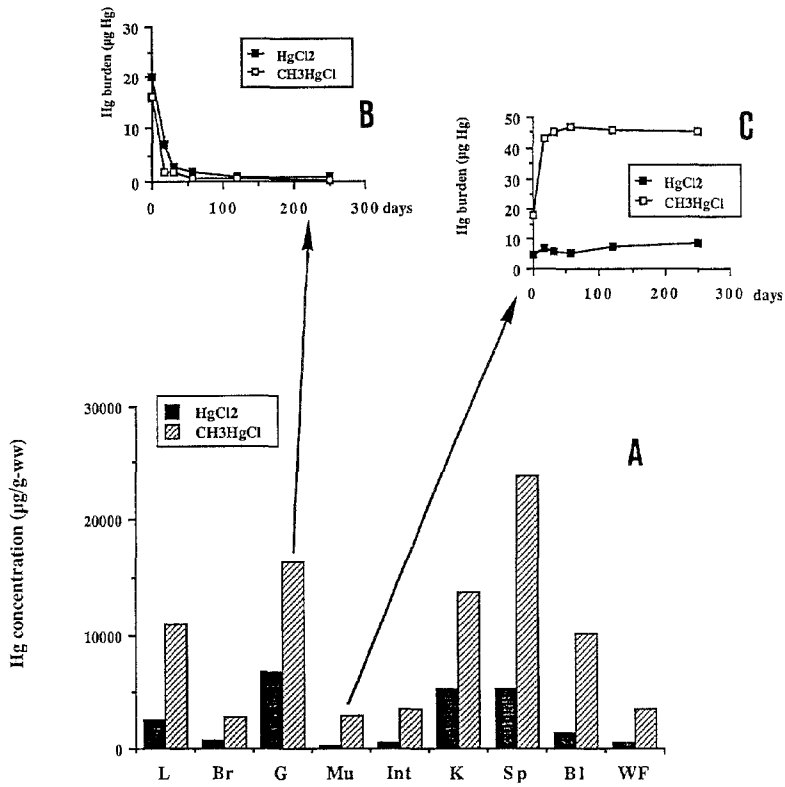

Figure 1 : (**1A**) - Mercury organotropism (total Hg concentrations) in *Salmo gairdneri* after 30 days'direct contamination by inorganic ($HgCl_2$) or organic (CH_3HgCl) Hg (1ug $Hg.L^{-1}$). L : liver ; Br : brain ; G : gills ; Mu : skeletal muscle ; Int : posterior intestine ; K : kidneys ; Sp : spleen ; Bl : blood ; WF : whole fish. (**1B**) - Variation in Hg burden in the gills with time, during decontamination phase (250 days), after direct contamination. (**1C**) - Variation in Hg burden in the skeletal muscle with time, during decontamination phase (250 days), after direct contamination.

During the decontamination phase, the Hg burden accumulated in the gills at the end of the direct contamination period shows a very rapid and efficient decrease : for the two compounds, 90% of the metal disappeared after 31 days (Figure 1B). Gills are a perfect illustration of "donor" organs during the decontamination phase, the metal being transferred directly to the surrounding medium, but especially, via the blood, to other tissues, that we call "receiver" organs. Thus, after direct contamination by MeHg, Hg burden in the skeletal muscle strongly increases during the first period of the decontamination phase, the rate of increase being higher than 170% after 31 days, compared to Hg content at the beginning of decontamination (Figure 1C). During the second phase - 31 to 250 days -, Hg burden in the muscle compartment remains constant , while the average concentration progressively decreases, owing to growth dilution effect (Ribeyre and Boudou, 1984b).

The experimental approach to the trophic contamination of *Salmo gairdneri* by inorganic and organic Hg reveals very specific properties of the intestinal barrier regarding Hg fixation and absorption, in relation to the chemical form used for the contamination of the living prey :

- after 30 days'exposure to food enriched by inorganic Hg, the Hg burden in the posterior intestine, below the pyloric caeca, corresponds to 36% of the amount accumulated in the whole fish. Taking into account the relative fresh weight of this organ - 0.8% on average -, the corresponding concentration is very high, 42 times higher than that measured at the organism level (Figure 2A). In contrast with this very high capacity for Hg accumulation, the intestine is not very permeable to inorganic Hg : average Hg transfer rates estimated between food ingested on the one hand and blood and other organs or tissues of the fish on the other, are between 5 and 10% (Boudou and Ribeyre, 1985) ;
- after 30 days'exposure to food enriched by MeHg, accumulation of the metal in the intestine is very low, the relative burden measured in this organ being lower than 3%. In contrast, transfer levels of organic Hg through this barrier are very high, estimated transfer rates being between 76 and 86%.

Similar results were obtained after "in vivo" perfusion of the posterior intestine of *Salmo gairdneri* with aqueous solutions of Hg ($HgCl_2$ and CH_3HgCl) (Boudou, 1982).

During the decontamination phase after trophic contamination, the most important question concerns the evolution of the metal accumulated in the posterior intestine after ingestion of food enriched with inorganic Hg. Results indicate a very rapid and efficient decontamination, with 80% of the metal disappearing after only 15 days (Figure 2B). However, Hg burdens measured in the other organs do not increase during this period, even decrease, as they do for the whole fish, showing that the metal accumulated in the intestine during the contamination phase is not transferred within the fish but is "excreted", via the faeces (Boudou and Ribeyre, 1983).

Some experiments were done using X microanalysis and electron microscopy (Boudou, 1982), to try to determine the metal distribution in the intestinal wall, at the sub-cellular scale. It was not possible to detect Hg with the microprobe, probably owing to the physicochemical treatments undergone by the samples during the different stages of their preparation. Indeed, the very high accumulation levels of inorganic Hg in the posterior intestine during the trophic contamination phase, the very low absorption capacity of this chemical form through this barrier and also the very rapid and efficient decontamination rate when the metal uptake is suppressed, lead to the hypothesis that most of the metal is adsorbed at the interface between intestine lumen and intestine wall. The presence of an abundant and thick cell-coat on the apical part of enterocytes, with many negative charges (e.g. sialic acid ; Gas and Noaillac-Depeyre, 1981), supports this interpretation.

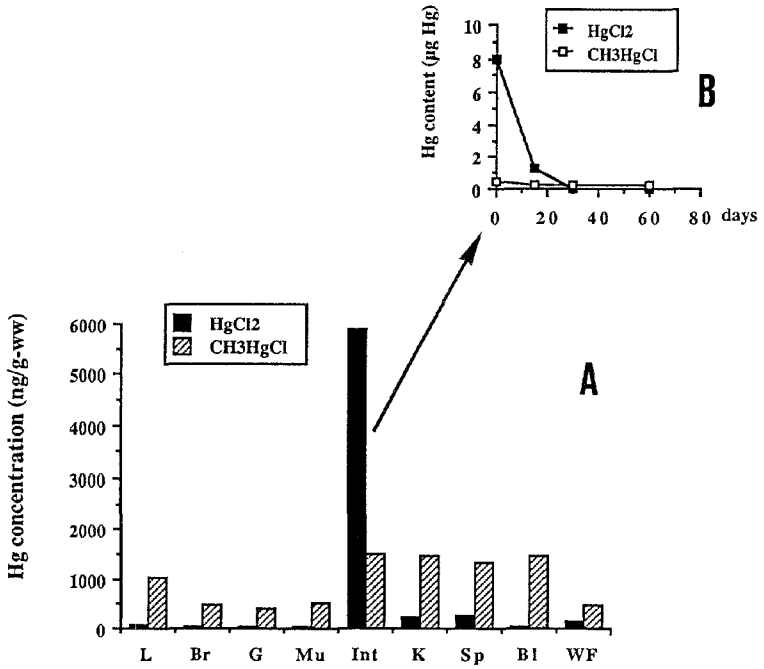

Figure 2 : (**2A**) - Mercury organotropism (total Hg concentrations) in *Salmo gairdneri* after 30 days'trophic contamination by living prey previously exposed by direct route to inorganic ($HgCl_2$) or organic (CH_3HgCl) Hg. L : liver ; Br : brain ; G : gills ; Mu : skeletal muscle ; Int : posterior intestine ; K : kidneys ; Sp : spleen ; Bl : blood ; WF : whole fish. (**2B**) : Hg burden evolution in the posterior intestine during the decontamination phase (60 days), after trophic contamination.

This experimental approach to the properties of gill and posterior intestine barriers in fish in relation to Hg fixation and absorption reveals strong specificities, closely related to the contamination source -water column or food- and the chemical form of the metal.

If other "biological" models are used, in the same experimental framework, similar processes appear or, at the opposite, important differences, with regard to the interactions between Hg compounds and biological barriers. We shall select two examples to illustrate these phenomena :

- 1/. We performed experiments to investigate bioaccumulation processes in *Hexagenia rigida*, a burrowing mayfly, at the organism and organ levels, when Hg compounds - $HgCl_2$ and CH_3HgCl - were initially added to the water column or to the sediment compartment. This ephemeroptera species is detritivorous and lives in burrows which the nymphs construct in the upper layers of freshwater lentic sediments (silty substrates) (Craven and Brown, 1969 ; Friesen, 1981). Mass culture of nymphs was initiated in the laboratory from large quantities of eggs collected each summer (Freshwater Institute, Winnipeg, Canada).

When contamination of the experimental systems is based on the sediment source (natural sediment from the Garonne river ; artificial contamination with identical concentrations for $HgCl_2$ and CH_3HgCl), the amount of total Hg accumulated in the nymphs after 28 days'exposure is 60 times greater when the organic form of the metal is initially added to the sediment (Saouter et al., 1989). Mercury organotropism in *Hexagenia rigida* nymphs shows major differences between these two contamination conditions (Figure 3). Nymphs in their burrows live in contact with both dissolved and particulate Hg. Thus, metal accumulation may derive both from direct uptake from the interstitial water and the water column, owing to Hg release from the sediment, and also from trophic transfer, via the metal associated with ingested sediment (Hg bound to sediment particles and dissolved in the interstitial water) (Saouter et al., 1990).

When experimental systems are contaminated by the water source -identical amounts of $HgCl_2$ or CH_3HgCl added twice a day to the experimental units-, total Hg accumulation in the nymphs after 28 days'exposure is very similar for the inorganic and organic compounds, concentration ratio being equal to 1.2 to 1.3, always in favor of MeHg, but the organotropism is very different (Figure 3).

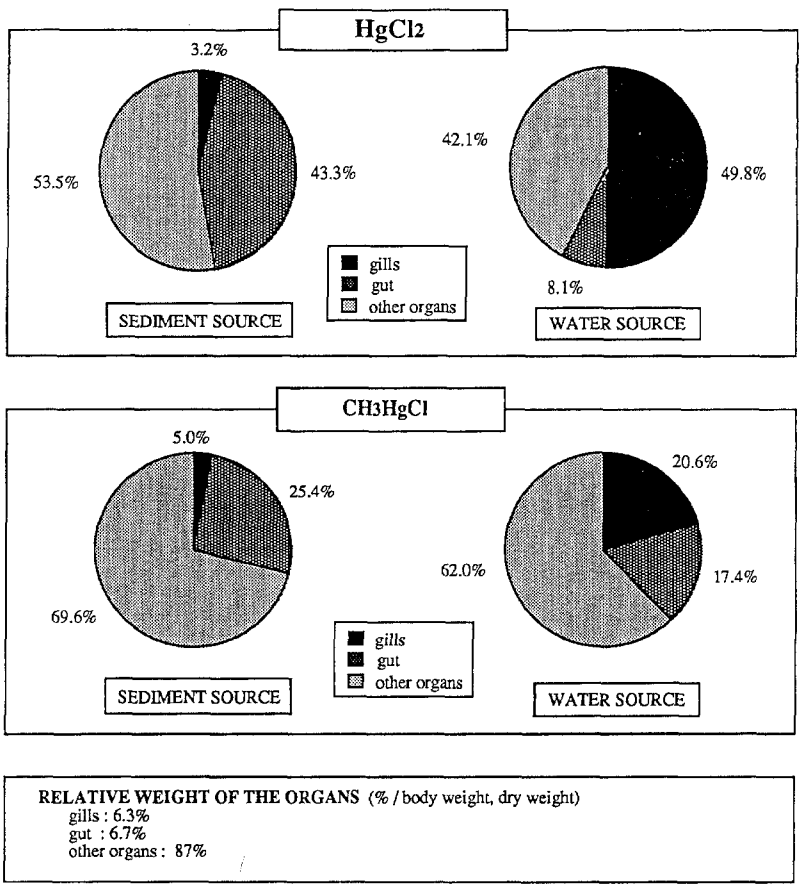

Figure 3 : Total relative Hg burdens (%) in gills, gut and the rest of the body of *Hexagenia rigida* nymphs, after 28 days'contamination by the sediment source ($HgCl_2$ or CH_3HgCl) or the water source ($HgCl_2$ or CH_3HgCl).

When experimental systems are contaminated by inorganic Hg, biological barriers -gills (water source) and gut (sediment source)-, in spite of their very low weight, represent, respectively, 50 and 43% of the average quantity accumulated in the nymphs, after 28 days'exposure ; for MeHg, the relative burdens in these organs are lower, respectively, 20 and 25% (Saouter et al., 1989).

- 2/. Experimental study of Hg transfers between contaminated sediment ($HgCl_2$, 10 mg $Hg.kg^{-1}$; CH_3HgCl, 1 mg $Hg.kg^{-1}$) and rooted macrophytes (*Elodea densa, Ludwigia natans*) shows that MeHg absorption occurs through the root barrier, metal accumulation being essentially in the leaves (Hg leaves / Hg whole plant = 0.78, after 28 days'exposure - Figure 4B) ; on the other hand, very small amounts of the inorganic Hg initially added to the sediment are accumulated by this route, metal found in stems and leaves originating essentially from indirect water source, via metal released from the upper layers of sediment (Maury et al., 1988). Thus, the ratio of Hg accumulation capacity of rooted macrophytes exposed to the two chemical forms of the metal in identical concentrations in the sediment, is higher than 40 for *Elodea densa*, after 28 days'exposure, in favor of MeHg (Maury and Engrand, 1986 ; Ribeyre, 1988).

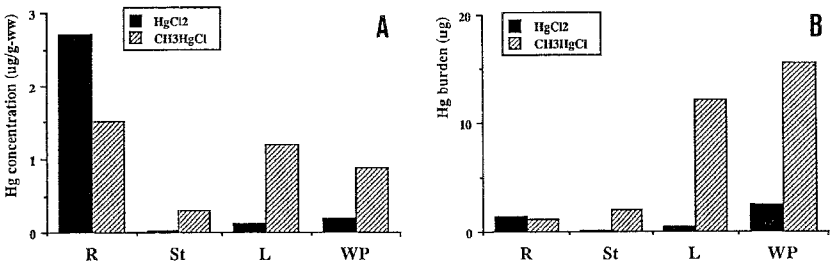

Figure 4 : Hg accumulation in *Elodea densa* -average concentrations (**4A**) and burdens (**4B**)-, after 28 days'exposure from the sediment source ($HgCl_2$, 10 mg $Hg.kg^{-1}$; CH_3HgCl, 1 mg $Hg.kg^{-1}$) - Metal distribution in roots (R), stems (St), leaves (L) and whole plant (WP).

Mercury accumulation in the roots, after they were thoroughly cleansed to remove sediment particles before Hg determination, shows very high concentrations, especially when the sediment was initially contaminated by inorganic Hg (Figure 4A) ; we should point out that the differences between the two contamination conditions are in fact even more marked, as the concentration of inorganic Hg in the sediment was ten times greater than that of MeHg. The corresponding relative burdens (Hg roots/Hg whole plant x 100) are very different according to the chemical form of the metal initially added to the sediment : 57% for $HgCl_2$ and only 7% for CH_3HgCl (Maury and Engrand, 1986).

Contamination of the water column by $HgCl_2$ or CH_3HgCl, in similar experimental conditions, leads to very low differences between Hg concentrations in the whole plants. For example, after 28 days'exposure, the ratio is close to 1.5, in favor of MeHg (Ribeyre, 1988 ; Ribeyre and Boudou, 1990).

Based on factorial experimental designs and specific equipment, our experimental protocols can take into account simultaneously more than one hundred different ecotoxicological conditions,

resulting from the combination of two or three levels for the controlled abiotic factors and contamination factors. Data treatment by multilinear regression reveals significant actions and interactions of these factors in relation to Hg bioaccumulation and transfers (Ribeyre, 1985 ; Maury-Brachet et al., 1990 ; Ribeyre and Boudou, 1990). Results obtained with rooted macrophytes, for example, show very strong effects of temperature, pH, photoperiod and light intensity on mercury accumulation in the whole plants, but these effects differ according to the contamination source and the chemical form of the metal ; significant interactions between "temperature and pH" or "pH and Hg chemical form" emerge (Ribeyre and Boudou, 1989, 1990).

In these experimental conditions, as in natural conditions, Hg bioavailability in the water column and/or in the sediment is more or less modified by the actions and interactions of the ecotoxicological factors, in close relation with its chemical speciation. At the same time, the structural and functionnal properties of the living organisms can also be changed. Mercury adsorption and absorption efficiencies are thus directly or indirectly linked to these processes, with biological barriers at the interface between biotope and organisms playing a fundamental role.

3. Interactions between mercury compounds and biological barriers : analysis at cell and molecular levels

3.1. MERCURY FIXATION ON CELL MEMBRANES :

At the cellular level, the plasmic membrane may be considered primarily as a complex system of potential binding sites for Hg chemical species (Boudou et al., 1983).

Mercury is well known to be highly specific for sulfhydryl groups, this property being responsible for its fixation on membrane proteins. Many biochemical studies have shown metal fixation on structural proteins or enzymes : spectrin, actin, cholinesterase, adenyl-cyclase, ATP-ases (Carty and Malone, 1979 ; Berg and Miles, 1978 ; Weed et al., 1962). However, Hg binding to SH groups or S-S bridges is closely related to their accessibility, which depends on their location in the membrane and on the physico-chemical properties of the metal (Hg chemical forms, neutral or ionic species, hydro- and liposolobility, etc.). For example, distribution of sulfhydryl groups within the erythrocyte membrane is not homogeneous : only 3% are associated with the outer surface ; 57% are located on the inner surface and 40% in the hydrophobic core of the plasmic membrane (Rothstein, 1981). Inorganic Hg can reach and titrate virtually all SH groups of red cell ghosts ; organic Hg compounds, such as p-chloromercuribenzene sulfonic acid, can interact with only about 20% of these groups (Rothstein, 1981). Mercury-sulfur bonds are thermodynamically stable but they are known to undergo rapid exchange reactions, especially for MeHg (Rabenstein, 1978).

The role of the lipidic bilayer of biological membranes in the binding of Hg has been little investigated. Reichert and Malins (1974) demonstrated that inorganic Hg is able to form reversible complexes with some phospholipids in chloroform (phosphatidylcholine, PC ; phosphatidylserine, PS ; phosphatidylethanolamine, PE) ; no interactions were detected with cholesterol. Phospholipid titration in the presence of MeHg also reveals interactions with acidic headgroups (PS, PI : phosphatidylinositol) (Leblanc et al., 1984).

Recently, we have studied effects induced by inorganic Hg on model membranes composed of natural or synthetic phospholipids bearing different headgroups, the thermotropic properties being followed by fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) (Delnomdedieu et

al., 1989). The results suggest a specific interaction between Hg and the primary amine groups, which are common to both serine and ethanolamine headgroups of phospholipids. PS and PE are abundant in biological membranes ; they represent 45% of the total phospholipids in erythrocyte membrane and correspond to approximately $0.5x10^8$ NH_2 sites/red blood cell, of which 80% are in the inner layer of the membrane (Ansell et al., 1973 ; Verkleij et al., 1973).

This new kind of metal-lipid membrane interaction is not electrostatic in nature and hence different from the extensively studied interactions between divalent or trivalent cations (Ca^{2+}, Zn^{2+}, Cd^{2+}, Al^{3+}) and negatively charged phospholipids (Jacobson and Papahadjopoulos, 1975 ; McLaughlin, 1982). The effects of Hg on the thermotropic properties of the lipids are concentration and pH dependent and thus related essentially to the chemical speciation of the metal. Structural data, by Nuclear Magnetic Resonance spectroscopy (NMR - ^{199}Hg), are under current work in our laboratory in order to better analyze and characterize interactions between Hg chemical species and phospholipid polar heads (Delnomdedieu et al., 1990).

This Hg fixation on NH_2 groups of phospholipids is supported by other NMR studies which have stressed that amino groups of the base moiety of the nucleic acids (adenine, guanine, cytosine) are potential binding sites for Hg(II) and MeHg (Taylor et al., 1981) ; this fixation may explain the chromosomal damage and the genotoxicity induced by this heavy metal (Zoll et al., 1988). A similar NMR approach indicates the involvement of N in amino acids as a binding site for Hg compounds (Reid and Podanyi, 1988).

3.2. MERCURY TRANSPORT THROUGH CELL MEMBRANES :

As well as its Hg binding capacity, the cell membrane acts as a biological barrier towards metal transfers between the external medium and the cytoplasm. Many transport processes can theoretically be responsible for the crossing of the membrane by an exogenous product : passive transport (diffusion due to a concentration gradient, displacement in an electric field, presence of a solvent, etc.) ; active transport, with energy consumption ; absorption by endocytosis.

According to Rothstein (1981), absorption of Hg compounds through cell membranes is essentially based on three transport processes : neutral chemical species crossing the membrane by partitioning into the lipid phase ; anionic species passing via the anion transport systems ; a small fraction probably useing other protein channels (cation permeation channels).

For MeHg, there is overwhelming evidence to support the notion that membrane transport is diffusion controlled (Wood, 1989). For many authors, the high bioaccumulation capacity for this compound, by direct or trophic route, is due to its liposolubility. In fact, the n-octanol/water partition coefficient for MeHg is close to 2.54 (Halbach, 1985) ; similar results were obtained for its partition coefficient in a water-lipid mixture (Lakowicz and Anderson, 1980).

Indirect transports could also take place via the metal bound to amino acids (arginine, lysine, histidine,.. ; Carty and Malone, 1979).

Two complementary approaches on model membranes provide detailed information on the role of lipidic bilayer of biological membranes in the transport of Hg :

- 1/. Using the fluorescence quenching method (N-alkyl carbazole derivatives embedded in lipid bilayers), Lakowicz and Anderson (1980) concluded that lipid membranes are highly permeable to MeHg, permeability being about 30% of that of an equivalent thickness of water or ethanol. However, its partitioning into lipid bilayers is very limited : "rapid diffusion across membranes rather than lipoid affinity is responsible for the transport of MeHg".

With the same technique - fluorescence quenching of pyrene-, we have demonstrated that the accessibility of inorganic ($HgCl_2$) and organic (CH_3HgCl) Hg to the hydrophobic core of model membranes is highly influenced by chemical forms and species of the metal, pH of the medium and the different phospholipid constituents of the bilayers (zwitterionic, PC, or negatively charged, PS, phospholipids)(Figure 5) (Boudou et al., 1982).

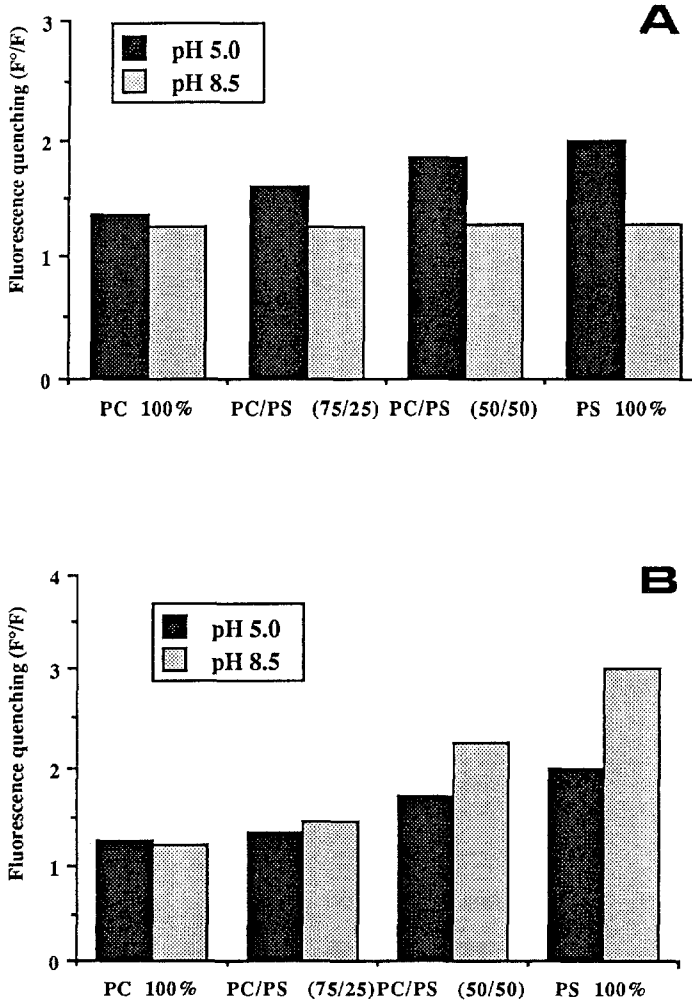

Figure 5 : Comparative analysis of pyrene quenching (F°/F) by $HgCl_2$ (**5A**) and CH_3HgCl (**5B**), as a function of the pH and the nature of the phospholipids used for the model membranes (PC : phosphatidylcholine ; PS : phosphatidylserine).

- 2/. Comparative analysis of both transmembrane fluxes of total mercury and negatively or positively charged species through planar lipid bilayers or "optically black membranes" (BLM). Results obtained by Gutknecht (1981) show that inorganic Hg, and more particularly its neutral chloride complex $HgCl_2$, is a highly permeable species with a membrane permeability coefficient 20-fold higher than the permeability to water and a million times higher than the permeabilities to Na^+, K^+ and Cl^-. The other major Hg(II) chemical species -Hg^{2+}, $HgCl^+$, $HgCl_3^-$, $HgCl_4^{2-}$- do not cross the membrane at a significant rate under physiological conditions but they contribute to diffusion through the aqueous unstirred layers adjacent to the membrane.

With the same BLM technique, we have studied comparatively transfers of inorganic Hg and MeHg, as a function of membrane lipid composition (PC, PS and Cholesterol), pH and chloride concentration (Bienvenue et al., 1984). Results of total Hg flux measurements show that both chemical forms of the metal easily cross the BLM , but the overall permeabilities are strongly dependent on pH and chloride concentration (Tables I and II).

Hg chemical form	$HgCl_2$		CH_3HgCl	
pH	5.0	8.5	5.0	8.5
J_{Hg}	0.75	0.08	1.12	0.72

Table I : Hg diffusion fluxes (J_{Hg} x 10^{10} mol.cm^{-2}.s^{-1}) through planar lipid bilayers (BLM), as a function of the chemical form of the metal and pH. Experimental system : Buffer (pH 5.0, acetate buffer ; pH 8.5, phosphate buffer) + $HgCl_2$ or CH_3HgCl (5.10^{-4} M) / BLM (PC + Cholesterol) / Buffer (pH 5.0, acetate buffer ; pH 8.5, phosphate buffer).

NaCl (mM)	0	1	10	100	500
J_{Hg}	0.20	0.22	0.22	0.14	0.08

Table II : Variation of the unidirectional flux of Hg (J_{Hg} x 10^9 mol.cm^{-2}.s^{-1}), as a function of NaCl concentration. Experimental system : Acetate buffer (pH 5.0) + NaCl (0 to 500 mM) + $HgCl_2$ (1 mM) / BLM (PC + Cholesterol) / Acetate buffer (pH 5.0) + NaCl (0 to 500 mM) + $HgCl_2$ (1 mM).

On the other hand, electrical measurements reveal that, depending on the chemical speciation, the transport is essentially due to diffusion of neutral chloride species ($HgCl_2$ and CH_3HgCl) ; this is in agreement with the conclusions put forward by Gutknecht (1981) for inorganic Hg. For the two chemical forms, BLM permeabilities are not apparently influenced by the different phospholipid constituents of the bilayers, but are affected by diffusion through the aqueous unstirred layers.

3.3. MERCURY EFFECTS ON STRUCTURAL AND FUNCTIONAL PROPERTIES OF CELL MEMBRANES :

Fixation of Hg on SH groups is able to affect several membrane functions :

- many enzymes are inhibited by Hg compounds : cholinesterase, adenyl-cyclase, ATP-ases, etc. (Carty and Malone, 1979 ; Rothstein, 1981). Biochemical studies show that these inhibiting effects are linked to the chemical forms of the metal, which govern its accessibility to S groups. Although fixation of Hg usually disturbs enzymatic functions, in some cases it may cause favorable conformational changes (Hatefi et al., 1969) ;
- several transport systems are disturbed by Hg, such as sugar, lactate, glycerol, cations, etc. ; however, transport of anions and non-electrolytes are not affected by the metal (Rothstein, 1981). These effects are directly connected to Hg actions on electrophysiological properties of excitable membranes (Shrivastav et al., 1976) or on osmoregulation, at gill level, for example (Lock and Overbeeke, 1981) ;
- Hg also interacts with structural proteins, which play an important role in stability and deformability of the cell membrane. For example, it may increase the osmotic fragility of red blood cells, inducing hemolysis ; two peripheral proteins - spectrin and actin - may be detached from the membrane by organic Hg compounds, without proteolysis (Weed et al., 1962).

If the effects of Hg on membrane proteins are currently better understood, few data are available concerning the actions of this metal on the properties of the phospholipid matrix.

Nakada et al. (1978) demonstrated that the permeability of lipid bilayers (liposomes) to glucose increased in response to exposure to $HgCl_2$ and CH_3HgCl. Increasing the ratio of cholesterol in model membrane decreased the release of glucose ; opposite effects were observed when the ratio of unsaturated fatty acids in membranes increased. Consequently, membrane fluidity seemed to play a significant role. The presence of negatively or positively charged lipids in the membrane modified the effects of the two compounds. Other divalent cations - Cd^{2+}, Co^{2+},Ca^{2+}, Mn^{2+}, Pb^{2+}, Zn^{2+} - did not significantly influence the permeability of liposomes to glucose.

Using phospholipid monolayers, Suzuki and Matsushita (1969) showed that inorganic Hg increased the surface pressure of PE+PS films ; this rigidification indicated phospholipid-Hg(II) complexes with a large polymeric arrangement.

More recently, using fluorescence polarization measurements, Bevan et al. (1983) showed that, in the presence of inorganic Hg and at neutral pH, the phase transition temperature (Tm) of vesicles composed of a mixture of a zwitterionic phospholipid (dipalmitoylphosphatidylcholine : DPPC) and a negatively charged one (PS) was slightly but significantly increased to about 2°C, while the Tm of pure DPPC vesicles was unaffected. These results were interpreted as revealing an electrostatic interaction between divalent Hg and the acidic headgroups of PS. However, these data do not agree with the very weak effect of Hg(II), in contrast with the strong effects of Ca^{2+}, Zn^{2+} and Cd^{2+} ions, on the surface pressure and potential of stearic acid monolayers, at pH 5.5 and with 154 mM NaCl (Gordziel et al., 1982). This lack of effect on stearic acid monolayers had been ascribed to the extremely low concentration of cationic Hg species in the aqueous sub-phase, for the physicochemical conditions retained. Indeed, considering the theoretical speciation diagrams of Hg(II) in aqueous phase (Hahne and Kroontje, 1973 ; Shin and Krenkel, 1976 ; Dyrssen and Wedborg, 1980), for pH between 5 and 8 and pCl between 0.8 and 3, the cationic species - Hg^{2+}, $HgCl^{+}$, $HgOH^{+}$ - are practically absent, the only species apparently available being neutral, or negatively charged ($HgCl_2$, HgOHCl, $Hg(OH)_2$, $HgCl_3^{-}$, $HgCl_4^{2-}$) and hence no significant

effect may be expected on the acidic groups of fatty acids or phospholipids.

Our proposal that the primary amine of serine and ethanolamine headgroups are special binding sites for inorganic Hg enables us to interpret the increase of Tm observed by Bevan et al. (1983) as a binding of the metal on NH_2 groups of PS and not as a classical cation-negative phospholipid charge interaction. From fluorescence polarization measurements, we found that inorganic Hg induces drastic changes in the thermotropic behaviour of lipids bearing serine and ethanolamine headgroups, the transition of these lipids being totally abolished at Hg concentrations of about 0.5 mM (Figure 6). It must be stressed that this effect occurs without any significant Tm shift concomitantly with the dose-dependent amplitude decrease of the phase transition ; moreover, the metal induces a strong increase in the fluorescence polarization parameter above Tm, producing a strong rigidification of model membranes. These interactions may have an important physiological and toxicological significance (Delnomdedieu et al., 1989).

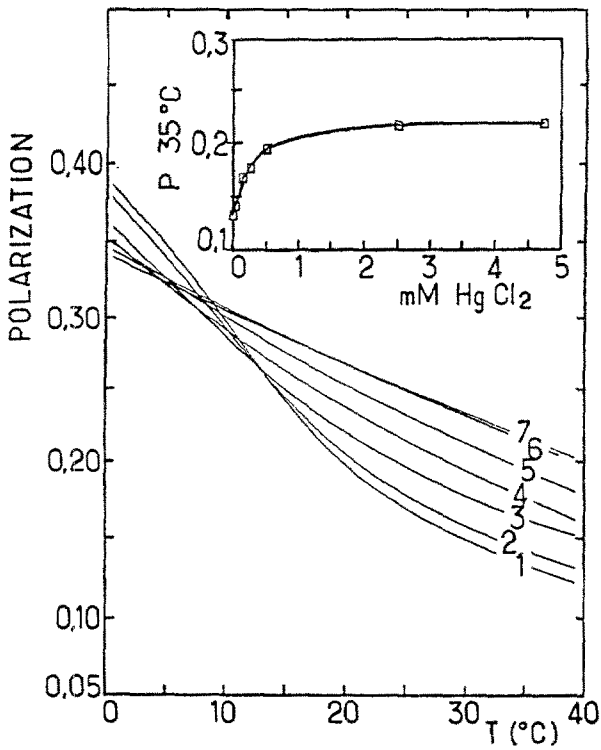

Figure 6 : Effect of temperature on the degree of fluorescence polarization P of DPH embedded in unsonicated dispersions of PS (Phosphatidylserine, 0.2 mM), in the presence of increasing concentrations of $HgCl_2$, at pH 5.8 (acetate buffer). Curves : 1, control ; 2-7, $HgCl_2$: 2, 0.05 mM ; 3, 0.15 mM ; 4, 0.25 mM ; 5, 0.5 mM ; 6, 2.5 mM ; 7, 4.75 mM. Inset : Isotherm fluorescence polarization ratio (35°C) of PS in function of increased $HgCl_2$ concentrations.

From an ecotoxicological point of view, the current state of our knowledge on the interactions between biological barriers and Hg compounds shows that these processes are very complex and strongly dependent on a very large number of factors, both abiotic (physicochemical characteristics of the medium and their variations), biotic (structural and functional properties of the barriers) and also contamination factors (mercury chemical forms and species, contamination routes, partitioning in the biotopes and in the food, bioavailability).

Mechanistic approaches are necessary for a more detailed understanding of these processes, at the different biological levels, from organism to molecule, even atom level. They must be developed in close complementarity with the other researches in Ecotoxicology, results from field studies being considered as reference systems with regard to the dualism between reductionism and representativity.

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