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Inorganic Mercury (Hg^{2+}) Transport through Lipid Bilayer Membranes

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Summary. Diffusion of inorganic mercury (Hg^{2+}) through planar lipid bilayer membranes was studied as a function of chloride concentration and pH. Membranes were made from egg lecithin plus cholesterol in tetradecane. Tracer (^{203}Hg) flux and conductance measurements were used to estimate the permeabilities to ionic and nonionic forms of Hg. At pH 7.0 and [Cl^-] ranging from 10–1000 mM, only the dichloride complex of mercury ($HgCl_2$) crosses the membrane at a significant rate. However, several other Hg complexes (HgOHCl, $HgCl_3^-$ and $HgCl_4^{2-}$) contribute to diffusion through the aqueous unstirred layer adjacent to the membrane. The relation between the total mercury flux (J_{Hg}), Hg concentrations, and permeabilities is: $1/J_{Hg} = 1/P^{ul}[Hg^t] + 1/P^m[HgCl_2]$, where [$Hg^t$] is the total concentration of all forms of Hg, P^{ul} is the unstirred layer permeability, and P^m is the membrane permeability to $HgCl_2$. By fitting this equation to the data we find that $P^m = 1.3 \times 10^{-2}$ cm sec^{-1}. At Cl^- concentrations ranging from 1–100 mM, diffusion of Hg^t through the unstirred layer is rate limiting. At Cl^- concentrations ranging from 500–1000 mM, the membrane permeability to $HgCl_2$ becomes rate limiting because $HgCl_2$ comprises only about 1% of the total Hg. Under all conditions, chemical reactions among Hg^{2+}, Cl^- and/or OH^- near the membrane surface play an important role in the transport process. Other important metals, e.g., Zn^{2+}, Cd^{2+}, Ag^+ and CH_3Hg^+, form neutral chloride complexes under physiological conditions. Thus, it is likely that chloride can "facilitate" the diffusion of a variety of metals through lipid bilayer and biological membranes.

Key words. Mercury; chloride; membrane permeability; lipid bilayer; facilitated diffusion.

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The biological transport of heavy metals is important in both physiology and toxicology. Although mercury is one of the most toxic metals in the environment, little is known about the mechanisms of Hg absorption, accumulation, and excretion. Determination of membrane permeabilities and transport mechanisms is complicated by the binding of Hg and other heavy metals to a variety of organic and inorganic ligands. Several investigators [1, 12] have noted that methylmercury crosses cell membranes and tissues more rapidly than inorganic (mercuric) mercury. However, observations of rapid Hg^{2+} uptake by yeast cells led Passow and Rothstein [7] to suggest that the neutral dichloride complex, $HgCl_2$, is also a permeant species. No quantitative data are available, however, on the membrane permeabilities to the various Hg(II) species which exist under physiological conditions.

In this study I used lipid bilayer (lecithin-cholesterol-tetradecane) membranes and ^{203}Hg to determine permeabilities of the various mercuric complexes which exist in inorganic salt solutions. The results show that $HgCl_2$ is a highly permeant species with a membrane permeability coefficient of about 10^{-2} cm sec^{-1}. The other major mercuric complexes ($Hg(OH)_2$, HgOHCl, $HgCl_3^-$ and $HgCl_4^{2-}$) do not cross the membrane at a significant rate under physiological conditions. However, chemical reactions among Cl^-, OH^- and Hg^{2+} play an important role in transport through the unstirred layers adjacent to the membrane.

Theory

Mercuric ion forms complexes with a variety of inorganic and organic ligands. In this study I used only inorganic salt solutions and the species which were present in significant amounts (>0.1% of the total Hg) are: $HgCl_2$, $HgCl_3^-$ $HgCl_4^{2-}$, HgOHCl and

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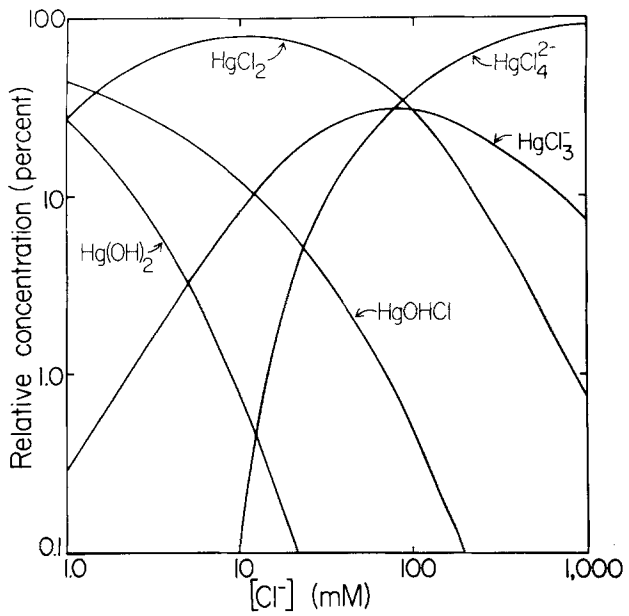

Fig. 1. Relative concentrations of five Hg(II) complexes as a function of $[Cl^-]$ at pH 7.0. The relative concentrations were calculated from association constants tabulated by Smith and Martell [11]

$Hg(OH)_2$. Figure 1 shows the relative concentrations of these five species as a function of $[Cl^-]$ at pH 7.0. Under physiological conditions (100 mM Cl^-, pH 7.0), $HgCl_2$, $HgCl_3^-$ and $HgCl_4^{2-}$ exist in roughly equal concentrations. At lower $[Cl^-]$, HgOHCl and $Hg(OH)_2$ become important. At high $[Cl^-]$, $HgCl_3^-$ and $HgCl_4^{2-}$ are the predominant species. Note that three of the five complexes are nonionic, i.e., $HgCl_2$, HgOHCl, and $Hg(OH)_2$, and would thus be suspected to show significant permeabilities through lipid bilayer and biological membranes.

A membrane and its associated aqueous unstirred layers are analogous to conductances in series. All the mercuric complexes can diffuse through the unstirred layer, which is analogous to several conductance pathways in parallel. If only one species, i.e., $HgCl_2$, crosses the membrane, then the total Hg flux (J_{Hg}) is given by the following equation [2, 3]:

$$\frac{1}{J_{Hg}} = \frac{1}{P^{ul}[Hg^t]} + \frac{1}{P^m[HgCl_2]} \tag{1}$$

where $[Hg^t]$ is the total Hg concentration, P^{ul} is the unstirred layer permeability coefficient, and P^m is the membrane permeability to $HgCl_2$. We assume that chemical reactions between Hg^{2+}, Cl^- and OH^- are fast compared to diffusion through the membrane and unstirred layer, i.e., that the reactions are in equilibrium throughout the unstirred layer. For simplicity, we assume also that the unstirred layer permeability coefficient is similar for all forms of Hg. I will justify later the assumption that only $HgCl_2$ crosses the membrane at a significant rate.

Multiplying both sides of Eq. (1) by $[Hg^t]$ gives the relation

$$\frac{1}{P^t} = \frac{[Hg^t]}{P^m[HgCl_2]} + \frac{1}{P^{ul}} \tag{2}$$

where P^t is the total Hg permeability coefficient, i.e., $J_{Hg}/[Hg^t]$. If the assumptions used in Eq. (1) are correct, then a plot of $1/P^t$ *vs.* $[Hg^t]/[HgCl_2]$ will give a straight line with a slope of $1/P^m$ and an intercept of $1/P^{ul}$. Thus, Eq. (2) provides a way of estimating statistically P^m and P^{ul}, and Eq. (2) also allows us to pool the data obtained with different total Hg concentrations, provided that P^m is not affected by $[Hg^t]$.

Materials and Methods

Lipid bilayer (optically black) membranes were formed by the brush technique of Mueller and Rudin [6]. The membranes were formed from a mixture of egg lecithin and cholesterol (1 : 1 mol ratio) in tetradecane. Tetradecane was used because capacitance measurements have shown that lecithin-cholesterol tetradecane bilayers contain very little hydrocarbon solvent [4]. The egg lecithin concentration ranged from 30 to 50 mg/ml, and the cholesterol concentration ranged from 15 to 25 mg/ml. In a few experiments, membranes were formed from bacterial phosphatidylethanolamine in decane (25 mg/ml). Membranes were formed on a 1.8 mm^2 hole in a polyethylene partition which separated two magnetically stirred solutions of 1.1 ml each. The temperature was 24 ± 1 °C.

In most experiments I measured net Hg fluxes from an Hg-containing "*cis*" solution into a Hg-free "*trans*" solution. The *cis* solution obtained $NaNO_3$ plus NaCl ranging from 0 to 1,000 mM. The *trans* solution usually contained $NaNO_3$ plus EDTA (1 mM). The association constant for HgEDTA is about 10^{22} [8]; thus all the Hg entering the *trans* solution was converted into the impermeant EDTA complex. Unless otherwise specified, both *cis* and *trans* solutions were buffered at pH 7.0 with HEPES (3–5 mM). The total Hg concentration in the *cis* solution ranged from 40 to 180 μM. In all experiments the ionic strengths of the *cis* and *trans* solutions were identical and ranged from 0.1 to 1.0.

After a stable black film was formed, ca. 20 μCi of $^{203}Hg(NO_3)_2$ was injected into the *cis* solution. The rate of appearance of radioactivity in the *trans* compartment was measured by continuous perfusion (1.3 ml/min) and collection of samples at 2 or 3 min intervals. The samples were collected by aspiration into a vacuum trap. During the flux experiment the *cis* solution was sampled periodically with a microsyringe. Samples were counted by liquid scintillation.

The one-way flux of Hg was calculated by the equation:

$$H_{Hg} = \frac{^{203}Hg^{trans}}{tASA^{cis}} \tag{3}$$

where J_{Hg} is the flux (mol cm^{-2} sec^{-1}), $^{203}Hg^{trans}$ is the total amount of tracer (cpm) entering the *trans* compartment during the time interval t (sec), A is the surface area of the membrane (cm^2) and SA^{cis} is the specific activity of tracer in the *cis* compartment (cpm/mol).

The membrane resistance was measured at approximately 3-

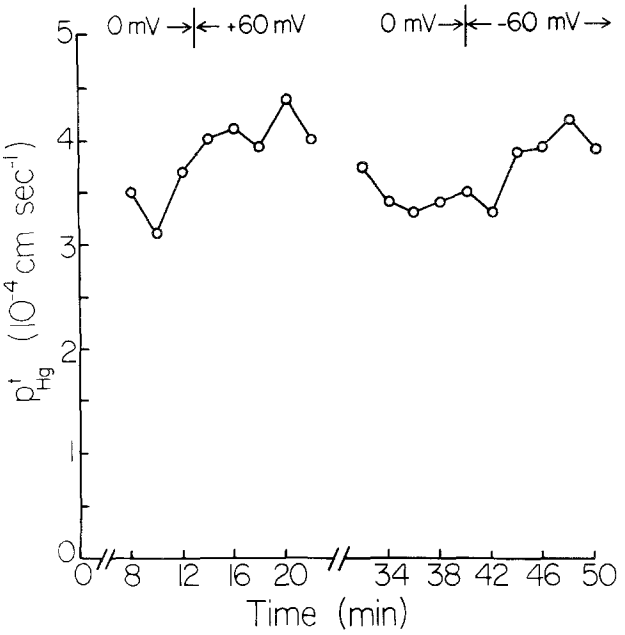

Fig. 2. Noneffect of membrane voltage (±60 mV) on Hg net flux through a lipid bilayer membrane. Both *cis* and *trans* solutions contain NaCl (0.1 M) and TES buffer (3 mM), pH 7.0. Total Hg concentration (*cis* solution only) is 90 μM. Membrane is bacterial phosphatidylethanolamine in decane (25 mg/ml). Sign of the voltage is that of *cis* solution relative to *trans*. Time indicates time after membrane formation. Data are from two different membranes

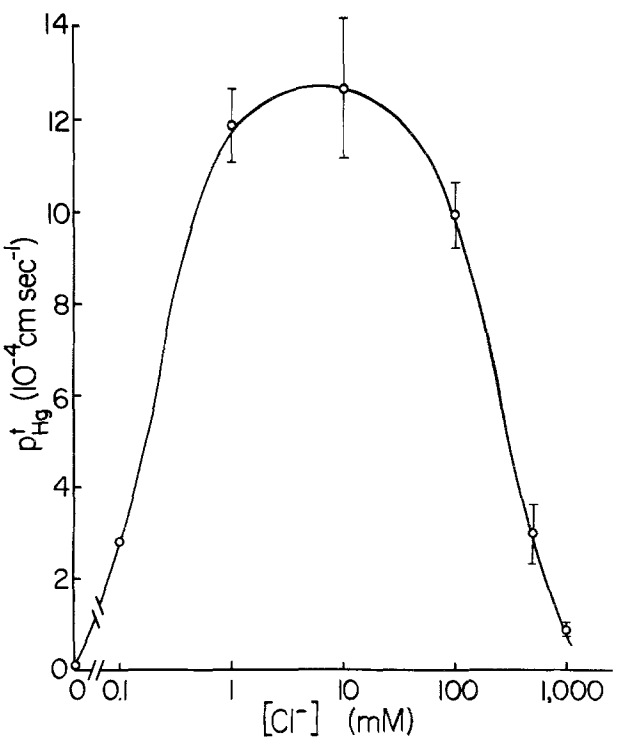

Fig. 3. Total Hg permeability coefficient as a function of Cl^- concentration at pH 7.0. The *cis* solution contained $Hg(NO_3)_2$ at concentrations ranging from 40 to 180 μM and NaCl concentrations ranging from 0 to 1.0 M. The *trans* solution was $NaNO_3$ (0.1 to 1.0 M) plus EDTA (1 mM). Both solutions were buffered with HEPES (3–5 mM), pH 7.0. At Cl^- concentrations less than 0.1 M, sufficient $NaNO_3$ was added to raise the ionic strength to 0.1. The membrane forming solution was egg lecithin plus cholesterol (1 : 1 mol ratio) in tetradecane. Error bars are standard deviations of 2–4 membranes

min intervals by applying a known voltage pulse across the membrane in series with a known resistance (voltage divider circuit). The membrane potential was recorded as the potential difference between two calomel-KCl electrodes which made contact with the *cis* and *trans* solutions.

Egg lecithin was obtained from Lipid Products (Surrey, England). Cholesterol, tetradecane, and pH buffers were from Sigma Chemical Company (St. Louis, Mo.). $^{203}Hg(NO_3)_2$ was obtained from New England Nuclear (Boston, Mass.).

Results

Preliminary experiments in 0.1 M NaCl showed a very high Hg permeability ($J_{Hg}/[Hg^t]$) of about 4×10^{-4} cm sec^{-1} (Fig. 2). This permeability is similar to that expected for diffusion through the aqueous unstirred layers which have a combined thickness of about 100 μm [3]. Furthermore, membrane conductance was affected only slightly by Hg^{2+}. The membrane conductance at ionic strength of 0.1 (NaCl or $NaNO_3$, pH 7.0) was $(7.2 \pm 5.0) \times 10^{-8}$ S cm^{-2} in the absence of Hg and $(4.0 \pm 1.9) \times 10^{-7}$ S cm^{-2} in the presence of Hg^{2+} (100–180 μM). Finally, the Hg flux was not affected by clamping the membrane voltage at ±60 mV (Fig. 2). These results suggest that Hg crosses the membrane in a nonionic form, i.e., $HgCl_2$, HgOHCl and/or $Hg(OH)_2$. I have not studied in further detail the effects of Hg^{2+} on membrane conductance because of the relatively small conductance change at mercury concentrations substantially higher than those occurring under physiological conditions.

Figure 3 shows the Hg permeability as a function of Cl^- concentration at pH 7.0. At physiological Cl^- concentrations, the total permeability (P^t) is very high and is apparently rate limited by diffusion through the unstirred layer. At both higher and lower [Cl^-], P^t decreases by 1–2 orders of magnitude. In Cl^- "free" solutions, P^t was $(11 \pm 7) \times 10^{-6}$ cm sec^{-1}, about 100-fold less than P^t in 1–100 mM Cl^-. In $NaNO_3$ solutions at pH 7.0 most of the Hg exists as the neutral complex, $Hg(OH)_2$ [11]. Thus the Hg permeability in Cl^- "free" solutions may represent permeation of $Hg(OH)_2$. However, the Cl^- "free" solutions contain unknown amounts of Cl^- contamination from three sources, i.e., Cl^- contamination from the calomel electrode and combination pH electrode used for titrating solutions, as well as micromolar amounts of Cl^- in the $NaNO_3$ salt. Furthermore, lowering the pH of the *cis* solution from pH 7 to pH 3 did not reduce P^t in Cl^- "free"

solutions, despite the fact that at pH 3 most of the $Hg(OH)_2$ is converted to Hg^{2+}. Thus, I suspect that the residual Hg flux in Cl^- "free" solutions at pH 7 is probably due to permeation of $HgCl_2$ or HgOHCl derived from micromolar concentrations of Cl^- in the $NaNO_3$ solutions.

To find out whether HgOHCl contributes to the total Hg permeability, I compared P^t at pH 3 and 7. At 1 mM Cl^- and pH 7.0, HgOHCl comprises about 44% of the total Hg (*see* Fig. 1). At pH 3 the HgOHCl concentration is reduced by about 4 orders of magnitude to less than 0.002% of $[Hg^t]$. However, the ratio $P^t(pH=3)/P^t(pH=7)$ was 0.90 ± 0.08, which indicates that HgOHCl makes, at most, a small contribution to P^t at pH 7.0.

At Cl^- concentrations >100 mM, $HgCl_2$ is the only nonionic species present in significant amounts (Fig. 1). The relative concentration of $HgCl_2$ decreases from about 30% at 100 mM Cl^- to 0.7% at 1,000 mM Cl^-. The decrease in P^t with increasing $[Cl^-]$ suggests that $HgCl_2$ is the permeant species. For example, the ratio of P^t at 500 mM compared to 1000 mM Cl^- is 3.35, similar to the ratio of $HgCl_2$ concentrations, i.e., 3.65. At lower $[Cl^-]$, P^t is not proportional to $[HgCl_2]$ because the unstirred layer is rate limiting.

If $HgCl_2$ is the only permeant species, then a plot of $1/P^t$ *vs.* $[Hg^t]/[HgCl_2]$ will give a straight line with a slope of $1/P^m$ and an intercept of $1/P^{ul}$ (Eq. 2). Linear regression analysis of the data yields values of $P^m = 1.28 \times 10^{-2}$ cm sec^{-1} and $P^{ul} = 1.56 \times 10^{-3}$ cm sec^{-1} (Fig. 4). The value of P^{ul} reflects the permeability of only the *cis* unstirred layer, because the presence of EDTA prevents any back diffusion of $HgCl_2$ from the *trans* solution. When the *trans* solution contains Cl^- and no EDTA, the total permeability is substantially lower due to the back diffusion of $HgCl_2$ from the *trans* unstirred layer (*cf.* Fig. 2). The slope of the line in Fig. 4 is determined primarily by the total Hg permeability at $[Cl^-] \geqq 100$ mM. Thus, a small contribution of HgOHCl to the total permeability would not be evident in this analysis.

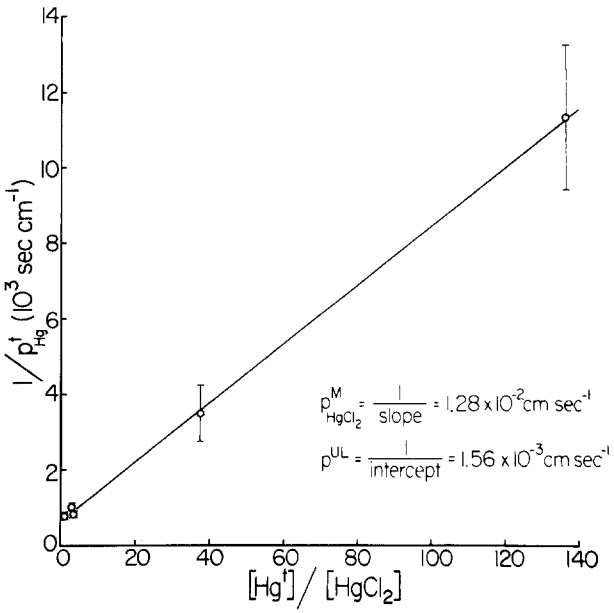

Fig. 4. Total Hg permeability as a function of $[Hg^t]/[HgCl_2]$, plotted according to Eq. (2). Data are the same as those in Fig. 3 for $[Cl^-]$ ranging from 1 to 1,000 mM. Error bars are standard deviations

Discussion

My results indicate that the high permeability of Hg(II) through lipid bilayer membranes is due primarily to permeation of the neutral dichloride complex, $HgCl_2$. The membrane permeability to $HgCl_2$ is about 1.3×10^{-2} cm sec^{-1}, about 20-fold higher than the permeability to water and more than a million times higher than the permeabilities to Na^+, K^+ and Cl^-. Because P^m is much higher than P^{ul}, diffusion through the unstirred layer is rate limiting at Cl^- concentrations ranging from 1–100 mM. Only at higher $[Cl^-]$ does the membrane permeability to $HgCl_2$ become rate limiting. For example, when $[Cl^-] = 1.0$ M the ratio $[Hg^t]/[HgCl_2] > 100$, and thus >99% of Hg diffusion through the unstirred layer occurs as $HgCl_3^-$ and $HgCl_4^{2-}$. Thus, these impermeant anionic complexes act as Hg "carriers" which facilitate the diffusion of Hg through the unstirred layer. As $HgCl_2$ crosses the membrane, its concentration is replenished by conversion of $HgCl_3^-$ and $HgCl_4^{2-}$ to $HgCl_2$ at the *cis* membrane surface.

Figures 5 and 6 show schematic concentration profiles for the major Hg complexes at low and high $[Cl^-]$. At 10 mM Cl^- (Fig. 5), most of the Hg exists as $HgCl_2$ (Fig. 1). From a knowledge of J_{Hg} and P^{ul}, we can calculate the concentration gradient of Hg-EDTA across the *trans* unstirred layer. Then from a knowledge of J_{Hg} and P^m we calculate the $HgCl_2$ gradient across the membrane, assuming that $[HgCl_2] = 0$ at the *trans* membrane surface. Then from a knowledge of $[HgCl_2]$ at the *cis* membrane surface and the assumption of chemical equilibrium we calculate the concentrations of HgOHCl and $HgCl_3^-$ at the *cis* membrane surface. As shown in Fig. 5, large concentration gradients of all the Hg complexes occur in the unstirred layers under these conditions.

Figure 6 shows schematic concentration profiles for the major Hg species at 1.0 M Cl^-. Under these conditions, $HgCl_2$ is only 0.7% of the total Hg. Thus, $HgCl_3^-$ plus $HgCl_4^{2-}$ can diffuse through the

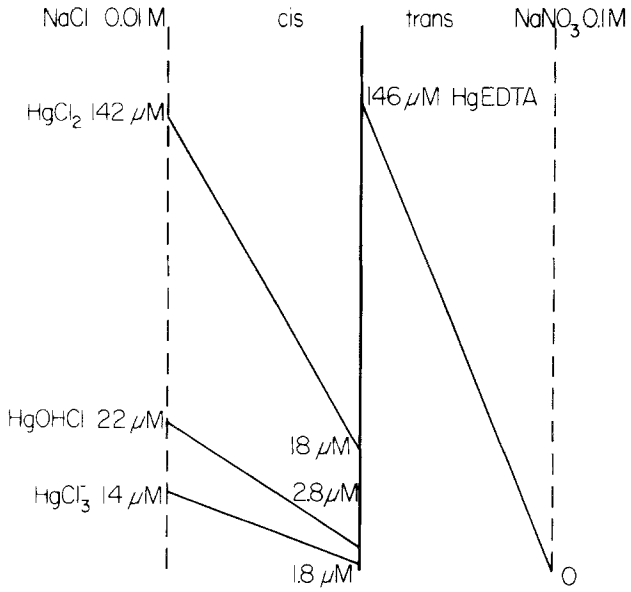

Fig. 5. Concentration profiles for various mercuric complexes across a membrane and unstirred layers at 10 mM Cl^-, [Hg^t] = 180 μM, pH 7.0. Concentration profiles are not drawn exactly to scale

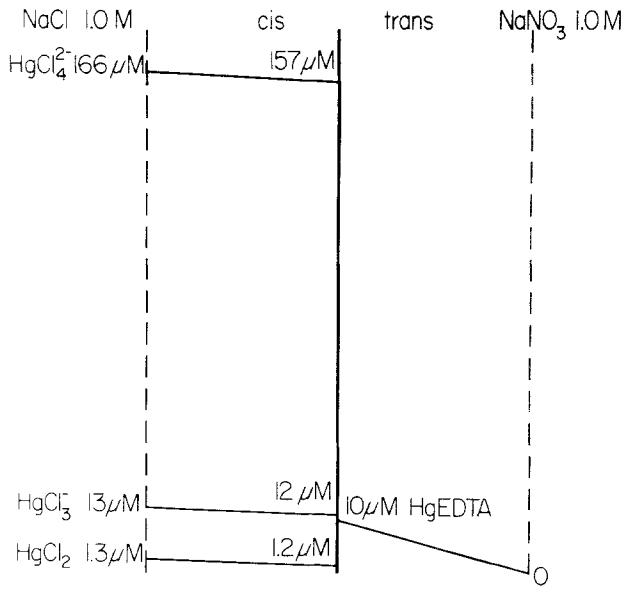

Fig. 6. Concentration profiles for various mercuric complexes across a membrane and unstirred layers at 1.0 M Cl^-, [Hg^t] = 180 μM, pH 7.0. Concentration profiles are not drawn exactly to scale

unstirred layer faster than $HgCl_2$ can diffuse through the membrane. Consequently, the membrane permeability to $HgCl_2$ is rate limiting and the concentrations of $HgCl_2$, $HgCl_3^-$ and $HgCl_4^{2-}$ at the *cis* surface are similar to the concentrations in the bulk solution.

My results support the hypothesis of Passow and Rothstein [7, 9] that $HgCl_2$ is a permeant species in biological membranes. In erythrocytes both the neutral and anionic chloride complexes have been suggested to be permeant species [16]. The Cl^- permeability of the erythrocyte membrane is about 10^{-4} cm sec^{-1} [13], roughly 100-fold less than $P^m_{HgCl_2}$ in lipid bilayers. If a lecithin-cholesterol bilayer is a reasonable model for the lipid barrier in the erythrocyte membrane, then my results suggest that $HgCl_2$ is more permeant than $HgCl_3^-$, assuming that the erythrocyte permeability to $HgCl_3^-$ is similar to or less than the Cl^- permeability.

In addition to Hg^{2+}, a variety of other metal ions form neutral chloride complexes under physiological conditions, e.g., Cu^+, Ag^+, Zn^{2+}, Cd^{2+}, Sn^{2+} and Cu^{2+} [11]. Organometal ions, e.g., CH_3Hg^+, also form neutral Cl^- complexes. Recently Lakowicz and Anderson [5] used fluorescence quenching to show that lipid bilayer (liposome) membranes "do not pose a significant permeability barrier" to the diffusion of CH_3HgCl. My results are qualitatively consistent with theirs, since the permeability to CH_3HgCl is expected to be even higher than the permeability to $HgCl_2$, i.e., $>1.3 \times 10^{-2}$ cm sec^{-1}.

In some cases the association constants for metal-OH complexes are also very high. For example, tributyltin and phenylmercury facilitate the diffusion of both halide and OH^- through lipid bilayer and biological membranes [10, 14, 15, 17]. In biological systems, a facilitated diffusion of both the metal and the inorganic ligand, e.g., OH^-, may be physiologically or toxicologically important. It seems likely that the biological transport of a variety of metals and organometals may involve chemical reactions with Cl^- and/or OH^- and subsequent diffusion of the neutral complexes through the membrane.

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