ALTERED PLASMA MEMBRANE ION PERMEABILITY IN MERCURY-INDUCED INJURY TO ISOLATED HEPATOCYTES FROM RAJA ERINACEA

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It has long been appreciated that mercury can influence ion, water, and nonelectrolyte transport in a variety of cells and tissues (Rothstein, 1970; Kinter and Pritchard, 1977). The plasma membrane is believed to be the target organelle for these effects, although mercury is also a potent inhibitor of intracellular enzymes and metabolic processes (Webb, 1966; Clarkson, 1972). Because of these diverse effects of mercury, it is often difficult to distinguish a primary effect of the metal on a given protein or metabolic process, from a secondary or indirect effect.

In the present study, we assessed the effects of 10-500 uM $HgCl_2$ CH_3HgCl, and p-chloromercuribenzene sulfonate (PCMBS), on plasma membrane and cell metabolic functions of skate (Raja erinacea) hepatocytes in suspension culture by measuring a) the rates of Na^+-dependent and indepenent ^{14}C-L-alanine uptake, b) Na^+-dependent and independent $^{86}Rb^+$ uptake, a measure of Na-K-ATPase activity, c) $^{86}Rb^+$ efflux, a measure of K^+ permeability, d) the difference between the ^3H-H_2O and ^{14}C-inulin distribution spaces, a measure of cell volume, e) cellular ATP concentrations, and f) glutathione (GSH) and glutathione disulfide (GSSG) levels.

Initial rates of L-alanine and $^{86}Rb^+$ uptake were inhibited in a similar fashion by each mercury compound ($HgCl_2$>CH_3HgCl>PCMBS). $HgCl_2$ produced a significant inhibition of the initial rates of Na^+-dependent uptake of L-alanine and ^{86}Rb at 10 uM and nearly complete inhibition at 100 uM. This effect was dose-dependent, immediate (observed after <5 min of incubation with the metal), and long lasting. Mercury also impaired volume regulatory mechanisms in skate hepatocytes: cells treated with 50 uM $HgCl_2$ swelled slowly over a 60 min interval to volumes nearly double those of control cells. In addition, mercury prevented the normal volume regulatory decrease observed after swelling the hepatocytes in hypotonic media. Efflux of $^{86}Rb^+$ from the hepatocytes was markedly increased by relatively low concentrations of $HgCl_2$ (5-50 uM). Mercury's effect on ^{86}Rb efflux was prevented if the metal was added to the hepatocytes as a mercaptide of GSH, but not if GSH was added to the cells after exposure to $HgCl_2$ (Fig. 1). In contrast, dithiothreitol, a more permeable thiol, prevented $HgCl_2$ toxicity when administered simultaneously and partially reversed the effects of mercury when added following $HgCl_2$ exposure (Fig. 1).

To examine whether the toxic effects of mercury were related to its ability to bind to sulfhydryl groups, we tested the effects of two other sulfhydryl reagents, N-ethylmaleimide (1mM) and diamide (20 mM), on ^{86}Rb efflux. As illustrated in figure 1, neither agent had any effect on rubidium efflux, suggesting that the specific chemical groups with which mercury is interacting are either different from those that are affected by N-ethylmaleimide and diamide, or are inaccessible to these agents. Other studies revealed that mercury had no effect on hepatocyte ATP, GSH or GSSG levels, at doses that markedly impaired membrane transport processes (Table 1). The

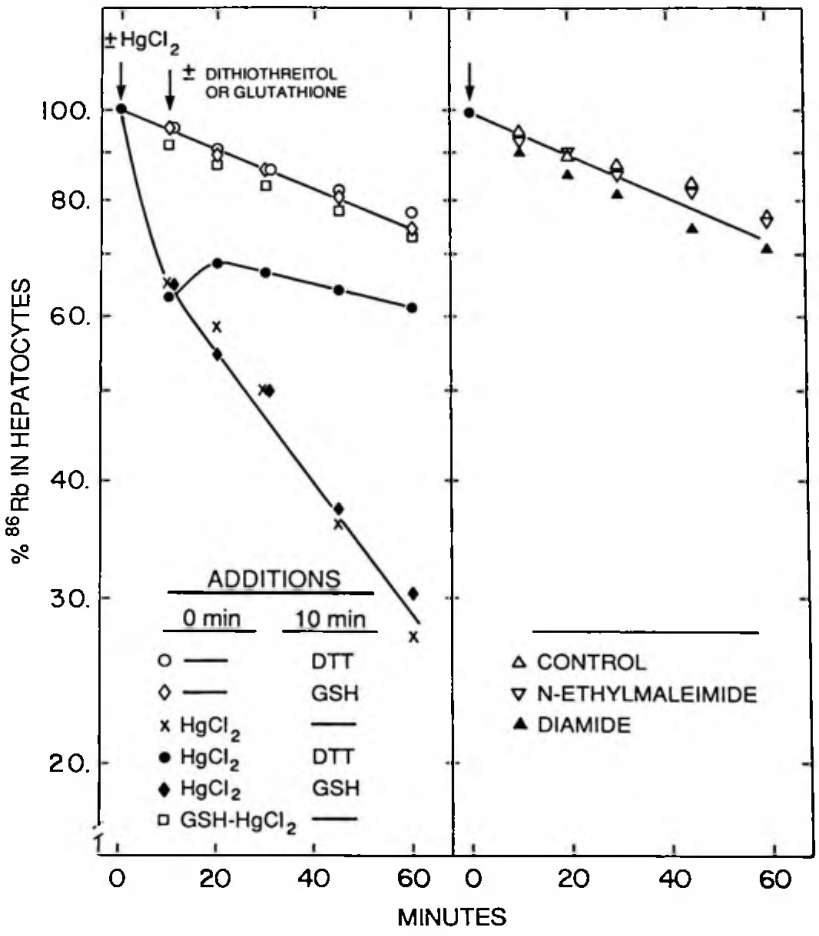

FIGURE LEGEND (Fig. 1)

Time course of ^{86}Rb efflux - reversibility of mercury's effects. Hepatocytes were preloaded with ^{86}Rb, centrifuged, and resuspended in elasmobranch Ringers with and without 25 uM $HgCl_2$ (left panel). One aliquot of the cells received mercury as a glutathione complex: GSH, 250 uM, and $HgCl_2$, 25 uM, were mixed prior to adding to the cells. Ten minutes later some of the cells received either dithiothreitol or GSH (250 uM), and ^{86}Rb content measured for a total of 60 min. The panel on the right illustrates the effects of N-ethylmaleimide (1 mM) and diamide (20 mM) on ^{86}Rb efflux. Values are means of 3 cell preparations, each performed in duplicate.

findings indicate that the $^{86}Rb^{+}$ (K^{+}) permeability of the plasma membrane of skate hepatocytes is highly sensitive to the toxic effects of mercury. Alteration in K^{+} permeability may contribute to the inhibition of transport processes energized by these gradients, such as electrogenic Na^{+}-alanine cotransport, and volume regulatory mechanisms without effecting cellular metabolic processes. The results support the hypothesis that the plasma membrane is a target organelle for mercury's toxic effects.

TABLE 1. ATP and Glutathione (GSH) levels in Skate Hepatocytes 60 min After Exposure to $HgCl_2$

	ATP	GSH	GSSG
	umole g^{-1}protein		
Control (n=6)	7.0±0.9	3.0±1.3	0.8±0.9
$HgCl_2$ (10 uM) (n=5)	8.4±2.2	2.9±0.7	0.8±0.7
$HgCl_2$ (100 uM) (n=5)	5.8±2.5	--------	-------

Values = X ± S.D. No significant differences from control values were observed. ATP and glutathione levels were measured according to Jaworek and Welsch, and Tietze respectively.

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