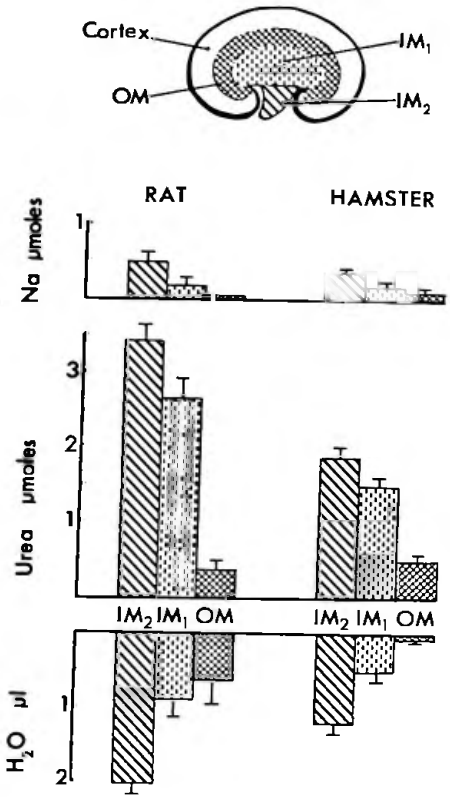

Figure 3.--Slopes for the regression lines for the changes in amounts of solutes and water in inner and outer medulla. The values thus represent the amounts in µl or µmoles that must be removed or added per mg solute free dry tissue when the papillary osmolality changes by 1000 mOs.

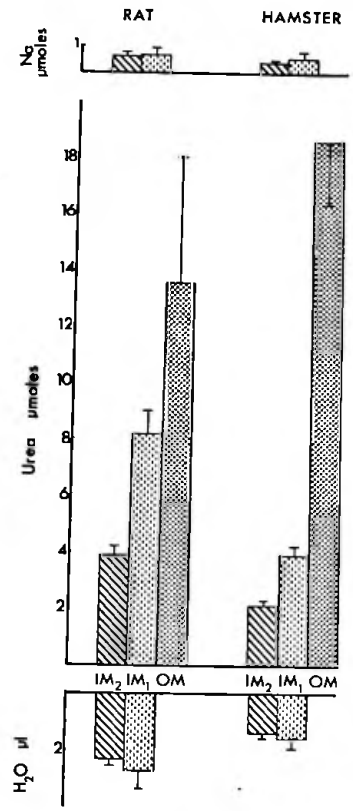

Figure 4.--Total amounts per two kidneys of solutes that must be added and water that must be removed when the papillary osmolality increases by 1000 mOs kg H_2O. These values are derived by multiplying the slopes shown in Figure 3 by the total dry weight of the three kidney zones for the two kidneys of an 80g animal.

In summary: in both rats and hamsters the major changes occurring when papillary osmolality increases are:

1. a large decrease in amount of water in inner medulla, and
2. a large increase in amount of urea. Urea increases in both inner and outer medulla.
3. Na increases slightly and primarily in the renal papilla.
4. K decreases very slightly but significantly in the renal papilla.

Changes in water and urea are significantly higher in rats than in hamsters.

The results indicate that net addition of Na to the inner medulla in antidiuresis is much less significant than hitherto assumed, and that the net addition of Na is significant in the papilla only.

EFFECTS OF $HgCl_2$ ON ELECTRICAL CHARACTERISTICS OF FUNDULUS OPERCULAR SKIN, A GILL MODEL

Kevin J. Degnan and David S. Miller, Department of Physiology, New York University Medical Center, New York, New York and Mount Desert Island Biological Laboratory, Salsbury Cove, Maine

The teleost gill is a potentially important site of environmental pollutant action, because 1) gill tissue is exposed directly to the external environment, 2) it comprises a substantial portion of the total surface area of the

fish, and 3) it performs several essential functions, e.g., osmoregulatory ion transport and respiratory gas exchange. Detailed toxicological studies of gill function have been complicated by many technical and interpretive problems, since available whole fish and isolated gill techniques are clearly inadequate. Recently, a teleost opercular skin preparation has been proposed as an in vitro model for gill osmoregulatory function (Karnaky et al., Science, 195: 203-205, 1977). Since the preparation is a flat epithelial sheet which can be mounted and studied in a lucite chamber, its use avoids problems associated with other preparations. In the present communication, we report our initial findings on the effects of $HgCl_2$ on the electrical properties of an opercular skin preparation from sea water adapted Fundulus heteroclitus.

Procedures for the dissection and mounting of the operculum and a description of the chambers used are presented in detail elsewhere (Degnan et al., J. Physiol., 27: 155-191, 1977). In the present experiments, tissues were bathed on both sides by a modified Forster's marine teleost medium with 10 mM glucose that was gassed with 95% 02/5% CO_2. Tissues were maintained under short circuit conditions, except for brief intervals when open circuit potential differences were measured to calculate transepithelial resistance. After a suitable control period (usually 30-60 min), small aliquots of a concentrated solution of $HgCl_2$ in Ringers was added to one side of the preparation. Final Hg concentrations in the bathing media ranged from 10 - 1000 μM.

Under control conditions, short circuit currents (SCC) averaged 146 $\pm$ 22 uA/cm^2 (data from 8 skins). This corresponds to a net movement of negative charge from the serosa to the mucosa and has been shown to be a direct measure of active chloride secretion across the epithelium. It is analogous to the net blood-to-sea water movement of chloride across the gills of marine teleosts. In general, SCC tended to be relatively stable, exhibiting a steady decline of less than 10% per hour. Transepithelial resistances averaged 175 $\pm$ 21 Ohm-cm^2 under control conditions; only small changes in resistance were observed during control periods. These data agree well with published values for Fundulus opercular skin (Degnan et al., op cit.). Addition of 100 μM $HgCl_2$ to either the mucosal or serosal compartments produced a rapid decline in SCC (Fig. 1); 1 hour after addition of Hg, SCC values had dropped to only

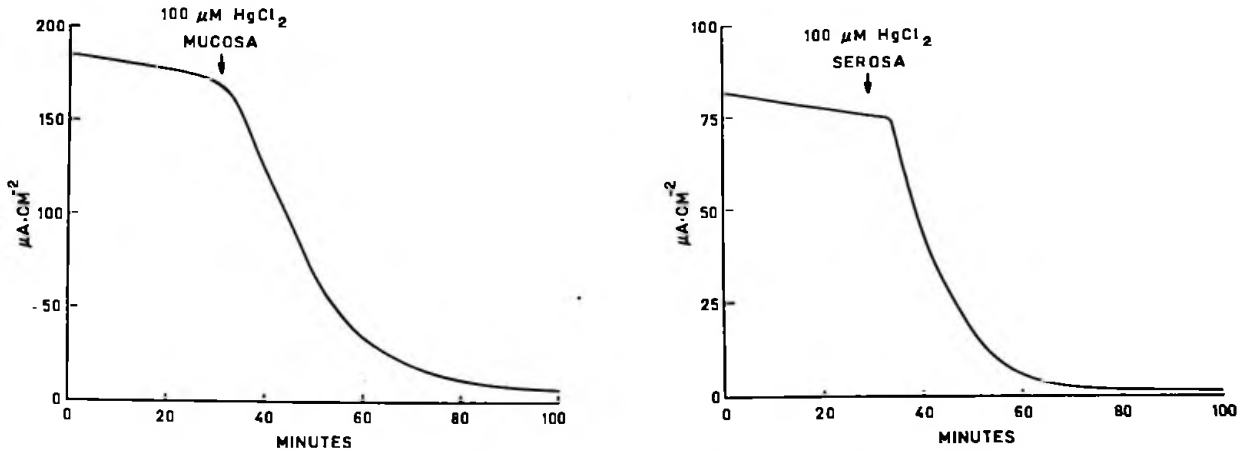

Figure 1.--Typical experiment showing the decline in SCC after addition of $HgCl_2$ to the solution bathing the mucosal or serosal surfaces of the opercular skin.

a few uA/cm^2, indicating that active Cl secretion was nearly abolished. Dose response data for the preparation are given in Fig. 2; inhibition data for mucosal and serosal exposures were indistinguishable (Fig. 2). From these data,

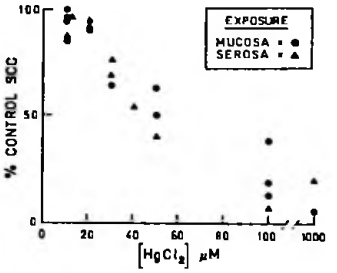

Figure 2.--Effects of $HgCl_2$ exposure on SCC across isolated opercular skin. Change in SCC based on value for initial control period (after correction for slight decline; see text) and value 20 - 40 min after addition of $HgCl_2$. Each point represents the observed change for a single skin.

we estimate I_{50} values (concentration of Hg causing 50% inhibition) to be about 50 μM; maximal inhibitions were found with 100 μM Hg. Even at the lowest concentration of Hg that caused a measureable effect, neither addition of 1 mM glutathione nor rinsing the chambers with Hg-free Ringers reversed the observed inhibitions (not shown).

In contrast to the SCC, the effects of $HgCl_2$ on tissue resistance were clearly dependent on the side of the tissue that was exposed to Hg. As shown in Table 1, serosal exposure caused an increase in resistance, while mucosal exposure had the opposite effect. In general, effects on resistance tended to lag behind effects on SCC. In addition, maximal effects were found with only 50 μM Hg (Table 1), a concentration that caused roughly 50% reduction in SCC (Fig. 2). An adequate explanation of these findings requires additional experiments.

Table 1.--Effect of $HgCl_2$ exposure on tissue resistance*

$HgCl_2$ Conen	% change in tissue resistance	
	mucosal exposure	serosal exposure
10	- 7 ± 6 (4)	+ 3 ± 4 (5)
50	-69 ± 8 (3)	+40 ± 9 (6)
100	-69 (2)	+33 ± 8 (3)

*Data given as mean ± SE (n), where n is the number of preparations.

The results of the present preliminary study show that short exposures to low concentrations of $HgCl_2$ rapidly and irreversibly reduce active chloride secretion (as measured by SCC) across Fundulus opercular skin, a model for the marine teleost gill. Available evidence indicates that the chloride cell is the cell type that is responsible for chloride secretion (Karnaky et al., Bull. MDIBL, 19: 109-111, 1979); this, this cell type appears to be a site of $HgCl_2$ action. In both gill and operculum, the enzyme, Na, K-ATPase, has been localized primarily in chloride cells and it has been implicated in the active chloride secretion process (Karnaky, A.J.P., 238: R185-R198, 1980). Since the ATPase from gill tissue is inhibited by exposure to heavy metals in vitro (Miller, unpublished data), this enzyme is one likely subcellular site of action of serosal Hg. The dramatic effects of mucosal Hg suggest that there are other sensitive sites, presumably on the apical surface of the cell. Clearly, additional experiments are required to define the inhibitory sites, to correlate effects observed in the model epithelium with those found in the whole animal, e.g., plasma osmoregulation and tissue resistance. Supported by NIH Grants GM 25002 and ES 00920.