This apparent hyperosmotic transport was verified by direct observation of the osmotic pressure of the fluid transported by bladders suspended in air (Table 2). The osmotic pressure of this reabsorbed fluid averaged about 35 mOsmoles higher than the mucosal fluid. Routine measurements of the osmolality of the mucosal fluid of bladders suspended in Forster's saline consistently showed osmotic dilution of this fluid (Table 2).

The electrical potential difference measured across the urinary bladder wall with Forster's solution on both sides of the epithelium averaged 23.4 $\pm$ 4.8 (S.E.) (n=9) mV inside negative (range 10.4 - 53.1 mV). The time course for the development of the equilibrium potential and the maximum potential developed by each bladder were variable. The expected transepithelial electrical potential difference for passive distribution of ions under the conditions stated was of course zero. The same basic pattern was shown by all bladders with respect to the development of the equilibrium potential difference. Immediately after introduction of the mucosal fluid the P.D. was a few millivolts positive inside. After a variable time period the mucosal side became more negative with respect to the serosal side. Contractions of the bladder caused the mucosal side to become electrically more positive. Addition of 0.1 gm NaCN/1 to the external bath completely abolished the P.D.

The cardiac glycoside ouabain at a concentration of 10^{-4}M, greatly reduced the net flux of water and ions across the bladder wall (Table 1). Na^+ flux appeared to be more affected than Cl flux. K^+ secretion was also reduced. The presence of 10^{-4}M ouabain in the mucosal fluid of bladders suspended in air prevented hyperosmotic transport (Table 2). A very small amount of fluid was collected from the serosal side of these bladders in the presence of ouabain and found to be isosmotic to the mucosal fluid however this serosal fluid may have appeared because of hydrostatic pressure exerted by the fluid inside the suspended bladders.

The apparent concentrations of Na^+ and Cl^- in the transported fluid of the bladders treated with ouabain were extremely high (Table 1). However because of the greatly reduced flux in the presence of ouabain the error involved in determining these concentrations may be quite large and these values may be erroneous.

In the present study no chemical gradient existed to promote the movement of Na^+, Cl^-, K^+ or water. Ouabain sensitivity of transport indicated dependence on ATPase. Measurements of electrical potential difference across the bladder wall with the same solution inside and outside showed that a substantial electrical potential, negative inside, was maintained. Sodium is thus moving against an electrochemical potential and may be presumed to be actively transported. K^+ and Cl^- were not moving against the electrochemical gradient.

1972 #41

EFFECTS OF MERCURIALS ON SODIUM METABOLISM IN *Fundulus heteroclitus*

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Ample evidence exists to indicate that mercurials can alter the osmoregulatory ability of an organism. The primary evidence for this derives from the effect of mercurial diuretics on kidney

function (Pharmacol Rev. 20(2):89-116, 1968). Meyer (Fed. Proc. 11:107-108, 1952) showed inhibition of sodium uptake by goldfish treated with mercuric chloride. Recent interest in the effects of heavy metal accumulation on aquatic organisms stems from the fact that relatively inert mercury compounds can undergo biotransformation in the bottom strata of streams, lakes, and seas producing either methyl mercury or dimethyl mercury which is readily accumulated by gill breathing organisms (Nature 223:753-754, 1969). Very little information has been obtained to indicate the effect of such accumulation.

This laboratory has undertaken several studies on a limited basis to attempt isolation of physiological manifestations of heavy metal poisoning.

Fundulus heteroclitus, a small euryhaline killifish, was studied with regard to the effect of organic and inorganic forms of mercury on sodium metabolism. Initial experiments have dealt with the ability of sodium-depleted killifish to extract sodium from a dilute solution of salts. These experiments have involved the use of mercuric chloride and methyl mercury chloride.

In connection with the study of sodium metabolism it was considered important to determine the distribution of the mercurial compounds within the animals. Clarkson (J. Occupa. Med. 10(7): 351-355, 1968) reported that the toxicity to mice and rats of organo-mercurials appeared to be related to the amount of Hg^{++} released from these compounds within the animal. In the present study the tendency of the methyl group to leave methyl mercury was evaluated. Two groups of fish in identical environments were exposed for 24 hours to methyl mercury. One tank contained ^{14}C-labelled methyl mercury, and the other contained ^{203}Hg-labelled methyl mercury. Fish were retrieved from each tank simultaneously at regular intervals up to 117 hours after initial exposure and the radioactivity in the whole body of each fish determined. The ratio of the amount of the ^{14}C label to the amount of ^{203}Hg label was determined for each time period (Table 1). The ^{14}C label in the bodies of the fish decreased with time more rapidly than did the ^{203}Hg label. This was an indication that methyl mercury is probably metabolized within the animal resulting in the production of inorganic mercury. In a few animals it was possible to compare the ratio of the two labels in gill tissue alone. At 24 hours the ratio was 2.07 however after 50 hours the ratio was only 0.56.

TABLE 1

The Tendency of the Methyl Group to Leave Methyl Mercury Chloride after Uptake by *Fundulus heteroclitus* is Shown. The Data are Expressed as the Ratio of $^{14}CH_3HgCl$ to $CH_3{}^{203}HgCl$ in the Whole Body

Time (hrs)	5	20	45	69	117
$^{14}C/^{203}Hg$	1.72	1.31	1.26	1.35	1.07

The distribution and changes in distribution with time of inorganic mercuric chloride compared to organic methyl mercury chloride within the bodies of the fish were also studied. One group of animals was exposed to 84 ppb (parts per billion) ^{203}Hg-labelled mercuric chloride (1.68 mg/20 liters) and another group was exposed to the same concentration of ^{203}Hg-labelled methyl mercury chloride for 24 hours. At intervals ranging from four to 168 hours the animals were sacrificed and the radioactivity of various tissues determined. The concentration of mercuric chloride remained highest in the gill tissue after 72 hours (Table 2). There was a gradual redistribution of mercuric chloride primarily into the liver and kidneys. Methyl mercury redistributed more rapidly into liver

TABLE 2

Distribution of $^{203}HgCl_2$ and $CH_3\ ^{203}HgCl$ in Various Tissues of *Fundulus heteroclitus* in Freshwater. The Distribution is Shown as the Ratio of the Amount of ^{203}Hg in each Tissue to the Amount in Gill Tissues

	TIME			
$HgCl_2$	5 hrs	24 hrs	48 hrs	72 hrs
Gill	1.00	1.00	1.00	1.00
Liver	0.323	0.394	0.771	0.905
Kidney	0.167	0.493	0.560	0.700
Spleen	--------	0.090	0.253	0.221
Muscle	0.015	0.021	0.038	0.038

CH_3HgCl	4 hrs	24 hrs	48 hrs	72 hrs	168 hrs
Gill	1.00	1.00	1.00	1.00	1.00
Liver	0.81	0.98	1.89	0.62	1.30
Kidney	0.45	0.17	0.85	2.93	0.70
Spleen	0.73	------	1.06	1.59	1.04
Muscle	0.07	0.20	0.15	0.07	0.18

and kidney tissues and was higher in these tissues after 50 hours than in gill tissue (Table 2).

The effect of mercuric chloride and methyl mercury chloride on the ability of sodium-depleted killifish to extract sodium from a dilute solution of salts was determined. The animals were depleted for four to six days in deionized, distilled water. Non-depleted fish had an average plasma sodium and potassium level of 178.1 ± 2.9 (S.E.) (n = 10) and 6.60 ± 0.37 (S.E.) (n = 10) mEq/1 respectively. Depleted fish averaged 126.2 ± 3.9 (S.E.) (n = 10) and 10.18 ± 1.04 (S.E.) (n = 10) mEq/1 respectively. Experimental animals underwent 24 hours exposure to 0.125 ppm of mercuric chloride or methyl mercury chloride in 700 ml of deionized water just prior to introduction into the uptake medium. Control animals were treated identically except for mercury exposure. The uptake medium consisted of 400 ml of a solution containing 0.5 mEq Na/L, 0.1 mEq K/L, 0.1 mEq Cl/L, 0.45 mEq HCO_3/L, and 0.025 mEq H_2PO_4/L. This solution was aerated and circulated past a continuously recording sodium electrode (Orion Corporation). The electrode was periodically calibrated by the use of flame-photometry (Instrumentation Laboratories, Inc. Model 343).

The ability of the animals to take up sodium was completely inhibited by mercuric chloride (Figure 1). Methyl mercury had only a transient effect on sodium uptake (Figure 1). A possible explanation for the different effects initially produced by these two mercurials may be obtained from examination of their distribution within the fish with time (Tables 1 & 2). Upon cessation of exposure to methyl mercury chloride this substance may be mobilized out of the gills and into other

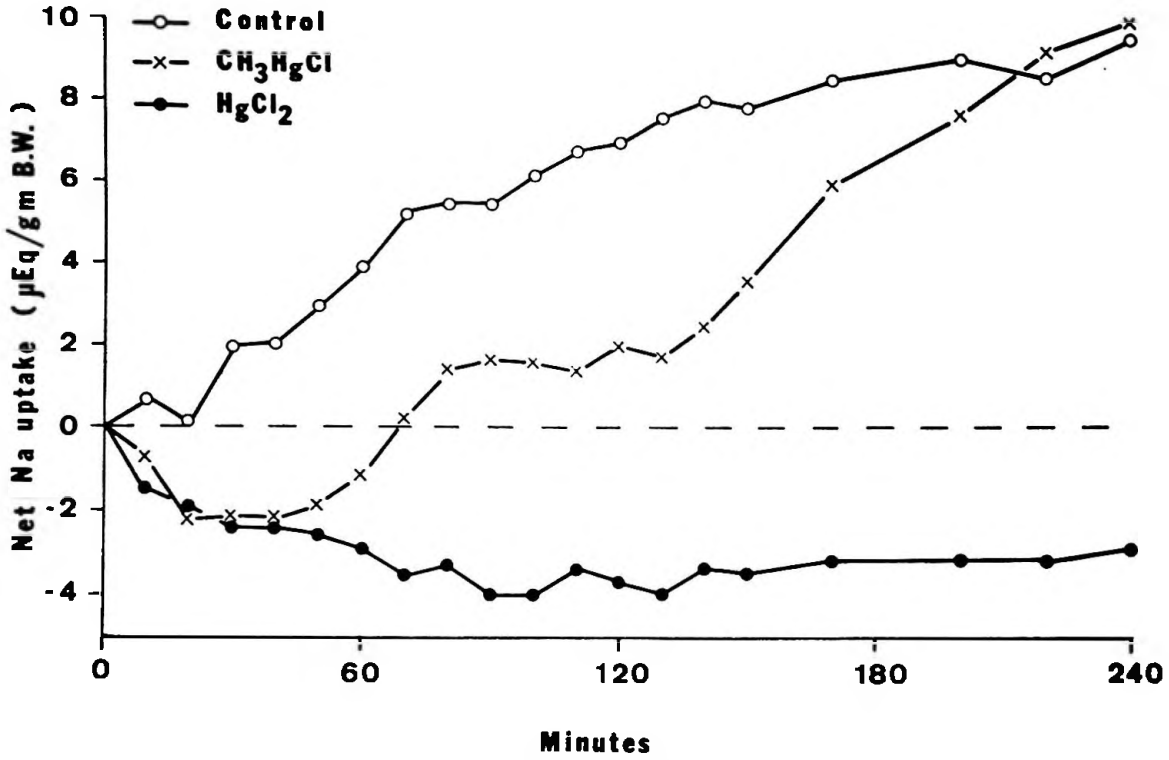

Figure 1. The effects of mercuric chloride and methyl mercury chloride on Na uptake ability are shown.

tissues thus allowing recovery of the sodium uptake mechanism. Further experiments will be necessary to make a positive statement on this point.

The second portion of our experiments dealt with determination of the effect of mercurials on the unidirectional flux of sodium in the killifish. The animals were exposed to 0.18 ppm of either mercuric chloride or methyl mercury chloride for four hours prior to flux determinations. The data obtained thus far indicate that the mercurials have little effect on either sodium distribution or efflux of sodium (Table 3). Sodium efflux was calculated on the basis of the rate constant for sodium

TABLE 3

Rate Constants for Turnover of ^{22}Na, Apparent Na Space, Plasma Na Concentration, and the Unidirectional Efflux (ϕ_{Na}) of Na in *Fundulus heteroclitus* Controls, $HgCl_2$ Treated and CH_3HgCl Treated

	Control	$HgCl_2$	CH_3HgCl
Rate constant of Na efflux (hr^{-1})	5.1 (5.0 and 5.1)	4.4 ± 0.8** (3)	4.2 ± 1.1 (3)
Apparent Na space (ml/100 gm*)	28.5 ± 1.7 (4)	31.4 ± 3.9 (4)	26.6 (1)
ϕ_{Na} (mEq/kg*hr.)	2.56 (2.59-2.54)	2.44 ± 0.47 (3)	1.99 ± 0.50 (3)

* Body wet weight
** Mean $\pm$ S.E.M. (n)

efflux times the total exchangeable sodium pool within the fish. The exchangeable sodium pool was assumed to be the apparent sodium space times the plasma sodium concentration. Since net sodium uptake was altered by the mercurials (Figure 1), unidirectional influx of sodium must be less in mercury treated animals. The influx of sodium is due in part to the expenditure of metabolic energy therefore it might be hypothesized that the mercurials decrease sodium uptake by interfering with active sodium transport rather than by structural damage to the gill epithelium.

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1972 #42

DYNAMICS OF VENTRICULAR FLUID ABSORPTION IN *Squalus acanthias*

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The existence of bulk absorption of CSF, as well as the dependence of the rate of absorption on CSF pressure, have been demonstrated in the rabbit (Brain 93: 665-678, 1970). It was the purpose of this study to explore the pressure-bulk relationship of ventricular fluid (CSF) absorption in *Squalus acanthias*.

Dogfish were anesthetized with 20 mg/kg of pentobarbital, their gills perfused with running seawater, and their bodies totally immersed in seawater in a small tank. A 20-gauge needle attached to polyethylene tubing was inserted percutaneously into the cerebellar ventricle and its intraventricular placement confirmed by testing with dilute trichloracetic acid for the presence (extradural fluid) or absence (ventricular fluid) of a protein precipitate in the fluid aspirated through the needle. If a bloody aspirate was obtained or if there was evidence of leakage around the puncture site on examination at the end of the experiment results were discarded. Four fish were judged to comply with these criteria. A pipette reservoir filled with dogfish Ringers and Evans blue which served as an indicator for leakage was arranged in such a way that it could be connected either to a calibrated pressure transducer or to the tubing leading into the cerebellar ventricle. The artificial CSF pressure was adjusted by varying the height of the pipette. The pressure transducer was used to determine either the effective artificial CSF pressure head in the reservoir or the intraventricular pressure. The pressures were recorded on a polygraph. At the beginning of each experiment all air was carefully removed from the tubing, the ventricular system was opened to atmospheric pressure to allow for equilibration, and the initial intraventricular pressure ($P_{initial}$) was measured. The artificial CSF was then allowed to run into the ventricular system at a previously determined pressure and the fluid loss from the pipette was measured by regular intervals. After an initial period of fluid flow adjustment the rate of infusion became steady and was assumed to represent the rate of bulk absorption of CSF at that pressure. Several short, preliminary measurements of the rate were made. If the flow was constant for the several preliminary tests, one long measurement of five to seven minutes duration was made and recorded as the bulk absorption rate. After the last period of measurement the infusion was stopped and the infusion pressure ($P_{terminal}$) again read. Each fish was subjected to seven to 13 of such runs. The ΔP's ($P_{terminal} - P_{initial}$) were varied in a random fashion from 4.5 to 26.5