damage of the pancreatic islet cells(6). Ni^{++}, but not Zn^{++}, acts likewise in the rabbit(7). It is also known that Co^{++}, Ni^{++} and Zn^{++}, but not other ions tested, react with insulin(8). However, it is not clear how these mechanisms would lead to increased corticoid excretion.

Morphine caused a marked reduction in adrenal response to Co^{++} suggesting that it acts prior to morphine sensitive area in the brain. Because of possibility of complex formation between Co^{++} and morphine, interpretation of the finding must be accepted with caution.

Summary. Several divalent ions were administered intraperitoneally to guinea pigs, at a dose of 4 μM/100 g body weight. Urinary corticoid excretion was doubled by Co^{++}, Ni^{++} and Zn^{++}. Fe^{++}, Cu^{++}, Hg^{++}, and Pb^{++}, produced little or no effect upon corticoid excretion.

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Received January 25, 1960. P.S.E.B.M., 1960, v104.

Accumulation of Strontium-90 and Calcium-45 by Fresh Water Fishes.* (25737)

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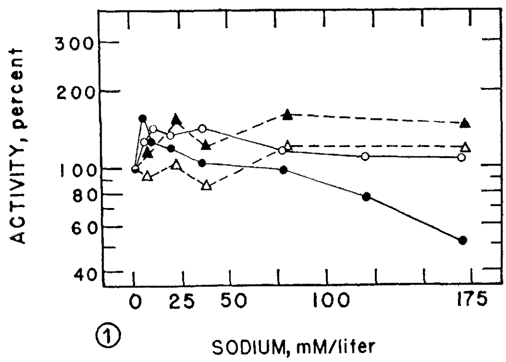
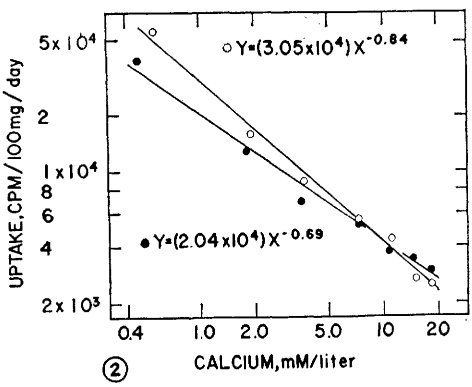
Our previous studies suggested that fresh water fishes do not discriminate against strontium relative to calcium when the isotopes are taken up from water in which they swim(1,2). Unlike fresh water fishes, Boroughs *et al.*(3) found that marine *Tilapia* discriminate against strontium relative to calcium. Discrimination against strontium also appears to be well documented in small laboratory mammals and in man(4-6). However, Wasserman *et al.*(7) found that strontium discrimination in rats is not affected by dietary calcium levels. The present results, using double tracer technics, indicate that fresh water fishes display only a small degree of discrimination against strontium relative to calcium and essentially substantiate our previous data.

Methods and materials. Adult fresh water fishes, obtained from commercial sources, were of mixed sexes for zebra fish (*Danio*) and white cloud mountain fish (*Tanicthys*) but guppies (*Lebistes*) were sexually mature males. Fish were placed in artificial pond water(8) containing both strontium-90 and calcium-45. The basic solution, adjusted to pH 7.0, contained 50 mg/liter of each of the following analytical reagent grade salts: sodium nitrate, potassium sulfate and magnesium sulfate. Solutions were modified by additions of calcium chloride, strontium chloride and sodium chloride as shown in results. Addition of strontium-90 and calcium-45 contributed approximately 0.25 mM/liter calcium(2). Small amounts of these ions in the basic solution are not included in the calculation. After 5 days in various solutions, fish were sacrificed and prepared for radioactive assay as previously described(9). Samples were held for 21 days to attain secular equilibrium of strontium-90 and its yttrium-90 daughter nuclide. Total beta activity (strontium-90 plus calcium-45) was determined with windowless gas flow counter. The

* This study was supported by grant in aid from U. S. Atomic Energy Comm. I am grateful to Paul R. Myers for technical assistance.





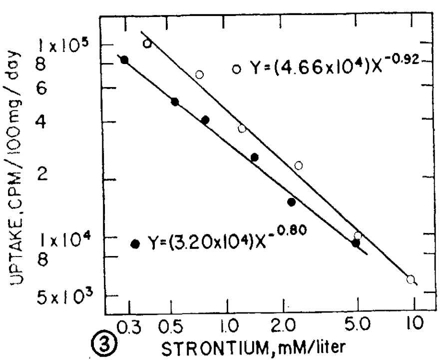


FIG. 1. Effect of sodium chloride on uptake of strontium-90 (▲, ●) and calcium-45 (△, ○) by male *Lebistes* in 2 separate experiments. Water contained approximately 10^5 cpm/ml of each isotope.

FIG. 2. Effect of calcium chloride on uptake of strontium-90 (●) and calcium-45 (○) by male *Lebistes*. Water contained approximately 10^5 cpm/ml of each isotope.

FIG. 3. Effect of strontium chloride on uptake of strontium-90 (○) and calcium-45 (●) by male *Lebistes*. Water contained approximately 10^5 cpm/ml of each isotope.

yttrium-90 component was determined with mica-end window Geiger counter with aluminum absorber (65 mg/cm^2) to absorb the weak beta emissions of calcium-45 and strontium-90. Samples of water were assayed in identical manner. Sample counts were corrected for the absorber, decay and self absorption with previously determined factors.

Results. Two typical experiments obtained with *Lebistes,* demonstrating variable effect of increasing sodium chloride concentration on accumulation of strontium-90 and calcium-45 are shown in Fig. 1. In these and other experiments, an increase in osmotic pressure resulted in either an increase or decrease in accumulation of both strontium-90 and calcium-45. In all instances however, the effect of sodium chloride on strontium-90 accumulation was essentially parallel to that of calcium-45. Similar results were also obtained for other fresh water fishes, *Tanicthys* and *Danio.*

Addition of non-radioactive strontium or calcium chlorides to the basic salt solution depresses accumulation of strontium-90 or calcium-45 in *Lebistes* (Fig. 2,3). Decrease in accumulation of both nuclides with increasing alkaline earth elements is adequately described by a function of the form $Y = aX^b$, and is similar to data previously described for calcium(9). However, the regression lines are not parallel but tend to cross at higher concentrations of salts indicating that strontium ion depresses accumulation of strontium-90 more than calcium-45 and, conversely, calcium ion depresses accumulation of calcium-45 more than it does strontium-90.

We previously showed(1) that accumulation of strontium-90 and calcium-45 by fresh water fish is a function of activity of the water in which they swim. This function, which we called the "concentration factor" (2), is adequately described by the equation,†

$$\text{Concentration Factor} = \frac{\log \text{uptake in cpm/100 mg/day}}{\log \text{water activity in cpm/ml}}.$$

This relationship has recently been confirmed for salt water *Tilapia* by Townsley and colleagues(10). A relationship between concentration factors of one isotope (strontium-

† The "concentration factor" in ref. 2 is erroneously given as $\log \frac{\text{uptake in cpm/100 mg/day}}{\text{water activity in cpm/ml}}$ although the text discussion is accurate.

TABLE I. Effect of Sodium Chloride on Rate of Uptake of Ca^{45} and Sr^{90} and Discrimination of Sr^{90} by Fresh Water Fishes.

Species	Sodium conc., mM/l	Rate of uptake ± S.E. Ca^{45}	Sr^{90}	Discrimination factor ± S.E.	"t"†	P
		10^4 cpm/100 mg/day				
Lebistes		9.55 ± .74	16.4 ± 1.76	.949 ± .003 (9)*	16.12	<<.01
Tanicthys	Basic	4.09 ± .34	12.9 ± .75	.921 ± .011 (8)	15.72	"
Danio		.59 ± .07	2.05 ± .20	.964 ± .013 (8)	9.88	<.01
Lebistes		8.89 ± .98	19.3 ± 4.00	.966 ± .005 (8)	7.36	"
Tanicthys	5.75	3.43 ± .21	10.8 ± .64	.920 ± .002 (10)	43.91	<<.01
Danio		5.57 ± .50	2.18 ± .16	.984 ± .010 (7)	1.81	>.05
Lebistes		9.68 ± .90	25.4 ± 2.24	.987 ± .010 (9)	1.41	"
Tanicthys	19.5	3.25 ± .15	12.3 ± .81	.936 ± .004 (9)	8.34	<.01
Danio		.50 ± .02	2.22 ± .09	.995 ± .006 (6)	1.05	>.05
Lebistes	172	10.8 ± .68	24.1 ± 2.04	.970 ± .002 (8)	16.81	<<.01
Tanicthys	172	2.19 ± .35	11.8 ± .23	.976 ± .008 (9)	2.98	.02
Danio	120	.25 ± .04	1.17 ± .18	1.034 ± .010 (6)	3.38	"

Water activities: *Lebistes*—Ca^{45} = 8.71 × 10^4 cpm/ml; Sr^{90} = 2.77 × 10^5 cpm/ml. *Tanicthys*—Ca^{45} = 4.02 × 10^4 cpm/ml; Sr^{90} = 3.54 × 10^5 cpm/ml. *Danio*—Ca^{45} = 4.85 × 10^3 cpm/ml; Sr^{90} = 3.99 × 10^4 cpm/ml.

* Values in parentheses indicate No. of fishes analyzed.

† "t" values calculated from differences of concentration factors for Ca^{45} and Sr^{90}. P values indicate probability of discrimination factor being significantly different from 1.0.

90) to another (calcium-45) may be expressed as a "discrimination factor," Discrimination

$$\text{Factor} = \frac{Sr^{90}\text{ conc. factor}}{Ca^{45}\text{ conc. factor}}.$$

In absence of discrimination of one isotope relative to that of another the discrimination factor would be unity.

These calculations have been made for 3 species of fresh water fishes (*Danio, Tanicthys,* and *Lebistes*) over a wide range of sodium ion concentrations (Table I). At low concentrations of sodium ion, all 3 species of fresh water fishes discriminate against strontium-90 as compared to that of calcium-45 by factors ranging from 0.921 to 0.964. However, as sodium ion concentration increases, discrimination of strontium-90 becomes less and is essentially absent for all 3 species of fishes at sodium ion concentrations greater than 20 mM/liter. However, in no instance does the discrimination of strontium-90 occur to a greater extent than 0.920 for our experiments.

Similar calculations have been made for *Lebistes* over a wide range of strontium and calcium chlorides (Table II). At low concentrations of alkaline earth elements, *Lebistes* discriminate against strontium-90 relative to that of calcium-45 by factors ranging from 0.937 to 0.949. Discrimination is no longer apparent when water contains more than 3.6 mM calcium/liter or 9.6 mM strontium/liter. The lowest discrimination factor observed (0.937) again demonstrates that fresh water fishes have little ability to distinguish between strontium and calcium.

Discussion. In previous experiments, performed with tap water and individual isotopes (1,2), we obtained discrimination factors for strontium-90 relative to calcium-45 that ranged between 0.93 and 1.03. These results, with factors ranging around 1 are substantiated in the present report and indicate that fresh water fishes similarly metabolize alkaline earth elements. The present experiments, using double tracer technics, eliminate variables such as age, physiological state of animals and variations of water composition which are difficult, if not impossible to control. With double tracer technics, each animal acts as its own control. These experiments, however, demonstrate that fresh water fish may distinguish between strontium and calcium when water contains very low concentrations of salts. Since addition of sodium ion tends to eliminate discrimination in a manner similar to that of strontium or calcium, discrimination appears to be a function of osmoregulatory mechanism. That ionic composition of water plays an important role in uptake of ions by fresh water fishes has recently been

TABLE II. Effect of Calcium and Strontium Chlorides on Rate of Uptake of Ca^{45} and Sr^{90} and Discrimination of Sr^{90} by Male *Lebistes*.

Salt	Conc., mM/l	Rate of uptake ± S.E. Ca^{45} 10³ cpm/100 mg/day	Rate of uptake ± S.E. Sr^{90} 10³ cpm/100 mg/day	Discrimination factor ± S.E.	"t"†	P
Basic		91.2 ±27.4	104.1 ±30.7	.937 ± .003 (6)*	17.19	<<.01
Calcium	.45	10.5 ± 1.36	11.7 ± 1.59	.939 ± .016 (8)	5.39	<.01
	3.60	2.24 ± .13	3.52 ± .27	.977 ± .010 (7)	2.29	>.05
	14.5	1.18 ± .18	1.88 ± .29	.974 ± .006 (3)	1.49	"
Strontium	.30	18.9 ± 2.34	24.4 ± 3.32	.949 ± .003 (8)	14.41	<<.01
	9.6	1.53 ± .13	1.92 ± .14	.958 ± .019 (8)	2.14	>.05
	14.6	1.38 ± .17	1.86 ± .19	.968 ± .023 (8)	1.54	"

* Values in parentheses indicate No. of fishes analyzed.

† "t" values calculated from differences of concentration factors for Ca^{45} and Sr^{90}. P values indicate probability of discrimination factor being significantly different from 1.0.

Water activities: $Ca^{45} = 6.19 \times 10^4$ cpm/ml; $Sr^{90} = 1.51 \times 10^5$ cpm/ml.

shown by Phillips *et al.*(11). These investigators found that small amounts of calcium, magnesium or sodium added to a variety of different types of fresh water markedly alters incorporation of cobalt-60 in the gills of trout. It is well known that the gills and integument of fishes play a major role in absorption and excretion of ions, active processes accompanied by corresponding expenditure of energy to maintain osmoregulation in a steady state(12). On the other hand, we previously showed that the spine:body ratio of accumulated strontium-90 in *Lebistes* is not altered by concentration of strontium or calcium in water in which the fish swim, while spine: body ratio of accumulated calcium-45 increases with increasing calcium concentration of water(13). This suggests that bone discriminates against calcium rather than strontium. The divergent results obtained in fish and mammals require further study, and comparisons between animals with such diverse physiology may be hazardous.

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