steroids, 17-ketosteroids, 17-ketogenic steroids, and dehydroepiandrosterone were determined in urine collected from healthy young men who ingested various quantities of fluid. Individual coefficients of correlation between urine and ketosteroid output varied from significantly positive to negative, with the majority showing no correlation. These results suggest that in most men daily variations in urine volume do not influence excretion of ketosteroids. However, group coefficients of correlation reveal that a significant positive correlation was obtained only for 17-ketosteroids. These data suggest that 1) men who normally produce relatively large volumes of urine also excrete large quantities of this steroid, and 2) type and number of subjects must be considered when calculating group coefficients of correlation.

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Effect of Co^{++}, Ni^{++}, and Zn^{++} on Corticoid Excretion by the Guinea Pig.* (25736)

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The guinea pig is useful for study of factors which influence ACTH release(1,2,3) since urinary corticoids are readily measurable in this species. The effect of Co^{++} was investigated because it has been suggested that events leading to establishment of polycythemia by this substance follow from production of a relatively anoxic state(4). It was observed that increased corticoid excretion followed intraperitoneal (but not subcutaneous or intramuscular) administration of Co^{++}. Increased corticoid excretion was also induced by intraperitoneal injection of Ni^{++} and Zn^{++}.

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Methods. The salts employed were $FeCl_2 \cdot 4H_2O$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, $HgCl_2$, $ZnCl_2$, $PbCl_2$, $CuCl_2 \cdot 2H_2O$. 300-350 g animals of either sex were injected intraperitoneally with various divalent ions, in 5 ml of saline; control animals received 5 ml saline as well. Co^{++} was also administered along with 2.5 mg morphine sulfate/100 g according to procedure previously described(2). Urine was collected for 6 hours(3). Urinary corticoids were determined by methods of Silber and Porter(5). When Co^{++} was administered subcutaneously or intramuscularly it was administered in 0.25 ml saline. To compare toxicity of these ions Co^{++}, Ni^{++}, Fe^{++}, Cu^{++} and Zn^{++} were administered twice daily at

TABLE I. Effect of Divalent Ions on Urinary Corticoid Excretion. 4 μM/100 g. 6 hr collection period.

Group	No.	Corticoids	Group	No.	Corticoids
A. *Subcut. route*			C. *Intraper. route*		
Control	6	127 ± 19*	Control	18	103 ± 14
Co^{++}	6	112 ± 28	Co^{++}	11	204 ± 31§
B. *Intramusc. route*			Ni^{++}	18	190 ± 25§
Control	6	108 ± 17	Fe^{++}	12	109 ± 12
Co^{++}	6	121 ± 20	Fe^{++}†	18	141 ± 14
			Cu^{++}	18	112 ± 22
			Pb^{++}	18	118 ± 17
			Zn^{++}	18	199 ± 28§
			Hg^{++}‡	14	90 ± 19

* Stand. error.
† 16 μM/100 g.
‡ 2 μM/100 g, when 4 μM/100 g was administered, all animals died while in collection cages.
§ p value $<.01$.

doses of 4 μM/100 g to each of 10 animals.

Results. Co^{++} administered intraperitoneally (but not subcutaneously or intramuscularly) induced an increased urinary corticoid excretion. It was determined with use of graded doses of Co^{++}, that a dose of 4 μM/100 g consistently doubled the 6-hour excretion value. Accordingly, this dose was employed for other ions (Table I). The response to Co^{++}, Ni^{++} and Zn^{++} was similar. Cu^{++} and Pb^{++} induced a moderate, but insignificant increase in corticoid excretion (1.1-1.2 ×) Fe^{++} did not produce any effect although 40% increase was observed if dose was increased 4-fold. Hg^{++} at this dose level proved lethal. When administered at dose level of 2 μM/100 g (non-fatal) corticoid excretion was 10% below controls. Diarrhea was present in all but one of animals which received this dose of Hg^{++}. Several animals which received Cu^{++} died after collection was completed. No toxicity was manifest with other ions following a single injection. A red pigment was observed in urine of Co^{++} treated animals but not in others.

In the toxicity study Ni^{++} and Cu^{++} caused death of all within 5 days. At this time only one animal receiving Co^{++}, 2 receiving Fe^{++} and 3 receiving Zn^{++} had died. After 8 days, 5 receiving Co^{++}, and 3 receiving Fe^{++} and Zn^{++} remained. Gross inspection revealed that survivors from Co^{++} had peritonitis with ascites and obvious hepatic and splenic pathology. Fibrinous exudation was present. The peritoneal cavity of Zn^{++} and Fe^{++} treated animals appeared normal except for 2 mm zone of hepatic necrosis in one of the former, and a peritoneal adhesion to liver, in one of the latter. None of the 10 control animals, which received intraperitoneal injections of saline, exhibited any pathology.

Inspection of peritoneal cavity of guinea pigs 6 hours after a single injection of Co^{++} suggested presence of slight hyperemia.

Co^{++} was administered along with morphine to determine whether it acted at some site before or after morphine sensitive areas in the brain, in the central nervous system, hypothalamus, anterior pituitary, adrenal complex, involved in increased adrenal steroid production. There was a marked reduction in adrenal response (Table II).

Discussion. The 3 ions Co^{++}, Ni^{++} and Zn^{++} have approximately the same ability to increase corticoid excretion on an equimolecular basis. There appears to be no other property of these ions, not possessed by one or more of the other ions tested, which might explain these findings. Peritoneal reaction was not observed following Zn^{++}. The red pigment appeared only after Co^{++} administration. Ni^{++}(4) and Zn^{++} are not known to be capable of inducing erythropoiesis. Perusal of the literature in regard to the effects of various ions on enzymatic activity fails to reveal those which are common to Co^{++}, Ni^{++} and Zn^{++}, but not to other ions which failed to cause increased corticoid excretion.

The fact that Co^{++} is not effective in inducing increased corticoid excretion when administered subcutaneously and intravenously at dose of 4 μM/100 g, suggests that for Co^{++}, at least, the site of action may be found in an organ served by the portal system. For example, a relatively large dose of Co^{++} entering the pancreas might induce alpha cell

TABLE II. Effect of Morphine on Corticoid Excretion Following Co^{++} Administration.

Group	No.	Corticoids
Control	11	99 ± 7‡
M*	11	45 ± 4
Co^{++}†	12	203 ± 24
M* + Co^{++}†	12	93 ± 14

* 2.5 mg morphine sulfate/100 g was administered according to(2).
† 4 μM/100 g.
‡ Stand. error.

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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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

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