Effects of Digitoxin on Exchangeable and Tissue Contents of Magnesium.* (27034)

JERRY K. AIKAWA, JACQUELINE Z. REARDON AND DALE R. HARMS
Department of Medicine, University of Colorado School of Medicine, Denver

While the effects of digitalis on the myocardium are well known, its extracardiac effects are not. The similarity in chemical structure between digitalis and other steroid compounds known to alter salt and water metabolism would suggest that they might have similar effects. It has been shown, in fact, that under certain experimental conditions digitalis does act in a fashion similar to the adrenal cortical hormones(1,2). In a previous study on rabbits. administration of large doses of digitalis produced a significant reduction in the exchangeable body content of potassium and in the potassium content of many tissues(3).

It has not been known whether or not digitalis induces any alteration in metabolism of another important intracellular cation, magnesium. Since the availability of a radioactive isotope of magnesium, Mg^{28}, has made possible an exploration of this problem, the present experiment was undertaken to determine the effects of digitoxin on magnesium metabolism in the rabbit. Mg^{28} was used to measure the exchangeable body content of magnesium and its relative tissue uptake.

Material and methods. Twelve normal, male young adult rabbits were placed in individual stainless steel metabolism cages and fed a stock diet of compressed pellets. Tap water was given without restriction.

Commercial crystalline digitoxin dissolved in a 40% solution of alcohol was employed.

Mg^{28} was received as $MgCl_2$ in concentrated HCl. Magnesium was precipitated out as $Mg(OH)_2$ with an excess of 1N NaOH. The precipitate was dissolved with 1N H_2SO_4 and the Mg was made to a final concentration of 0.4 meq per ml.

To determine the exchangeable magnesium content (Mg_e) of the body, each animal was given 5 ml of this solution (a total of 2 meq of Mg and 10 microcuries of Mg^{28}) by intravenous injection. The urine was collected for 24 hours and pooled. Between the 24th and 28th hours, 2 spot specimens were collected by catheterization and their specific activities were determined. The Mg_e value was calculated using the following formula:

$$Mg_e = \frac{Mg_i^{28} - Mg_o^{28}}{Mg_u^{28}/Mg_u^{24}}$$

Mg_i^{28} = quantity of radiomagnesium administered (arbitrary units).

Mg_u^{28} = quantity of radiomagnesium excreted in the urine up to the time the spot specimens were obtained.

Mg_u^{28} = concentration of radiomagnesium in spot specimen.

Mg_u^{24} = concentration of stable magnesium in spot specimen.

Mg_u^{28}/Mg_u^{24} = specific activity of spot specimen.

Mg_e = quantity of exchangeable magnesium in milliequivalents.

Samples of plasma, urine, and tissues were assayed for gamma ray activity with a well-type scintillation counter.

Magnesium concentration in urine and serum(4) and in tissues(5) was determined by a modification of the molybdivanadate method for phosphate.

To determine the external balance of magnesium, body weight, total food consumption, and 24-hour urine volume were measured each morning before the rabbits were fed.

Experimental procedure. Baseline measurements of body weight, food intake, and urinary excretion were made daily during the first 7 days. Starting on the 7th day and continuing every day for 8 days, 0.1 mg of digitoxin per kg body weight was given intravenously to each of 8 animals. The remaining 4 rabbits were not given digitoxin, and served as controls. Two baseline Mg_e determinations were

* This work was supported by Contract between U. S. Atomic Energy Commission and Univ. of Colorado.

Mg^{28} was supplied by Brookhaven National Laboratory, Upton, L. I., on allocation from U. S. Atomic Energy Commission.

made on the 1st and 7th days of the experiment; the test measurement was made on the 16th day. Twenty-eight hours after completion of the third (test) Mg_e, the animals were killed by air embolism, and 2 samples of each tissue were obtained for radioactivity assay and determination of the magnesium content.

The 4 animals not receiving digitoxin were killed in the same fashion 24 hours after administration of Mg^{28}, and tissues were obtained for assay of radioactivity and magnesium content.

Statistical method. In the analysis of the balance data, each animal served as its own control. After the differences between the mean of the baseline values and the mean of the values obtained during the experimental period had been calculated for each animal, mean difference for the group was determined. The significance of the mean difference was obtained by the use of the "t" test(6), a "P" of less than 0.01 being considered significant.

Mean relative activity and tissue magnesium content of each type of tissue were determined for both test and control groups, and the significance of the differences between group means was tested(6).

Results. While no significant changes were found in the external balance study, the exchangeable body content of magnesium increased significantly in the animals injected with digitoxin (Table I). No significant changes were noted in the relative radioactivity of the tissues studied (Table II), although in every instance, mean values were higher in the test group than in the control group. A significant reduction in magnesium content was noted in skin of animals given digitoxin (Table III).

Comment. The results suggest that digitalis might induce some subtle changes in the dynamics of magnesium metabolism which are not reflected in the external balance of this ion. The significant increase in Mg_e, associated with an increase (even though a small one) in the mean values for the relative radioactivity of all the tissues studied, suggests that digitalis produced a generalized increase in the tissue uptake of Mg^{28}. The significant decrease in magnesium content of skin is difficult to interpret in the light of our present knowledge of the pharmacology of digitoxin.

TABLE I. Effects of Digitoxin, Given Daily for 8 Days in Doses of 0.1 mg/kg, on External Magnesium Balance, Exchangeable Magnesium, and Serum Magnesium in 8 Normal Rabbits

	Mean Baseline Values†	After Injection of Digitoxin
Weight (kg)	1.635	1.754
Serum Mg (meq/l)	2.0	1.6
Exchangeable Magnesium (meq)	51.5	82.1*
" " (meq/kg)	31.1	46.8*
Mg Intake (meq/day)	12.4	15.0
Urine Mg Excretion (meq/day)	6.3	5.9
" Volume (ml/day)	141	135

† Mean value for 7 days.
* Statistically significant difference when compared with the mean baseline value.

TABLE II. Effects of Digitoxin on Relative Radioactivity of Tissues in 8 Rabbits

Tissue	Relative Radioactivity† Control Group‡	Test Group§
Muscle	2.06 ± .16‖	2.77 ± .35
Skin	3.28 ± .13	3.38 ± .24
Kidney	8.16 ± .38	9.33 ± .65
Heart	9.46 ± .55	10.01 ± .68
Liver	10.02 ± .62	12.74 ± 1.47
Appendix	12.26 ± .67	14.82 ± .64
Bone	33.73 ± 2.47	39.72 ± 3.62

† Relative radioactivity $= \frac{\text{cpm/gm tissue}}{\text{cpm/ml plasma}}$ 24-28 hr after intravenous injection of Mg^{28}.
‡ Mean values in 4 rabbits not given digitoxin.
§ Mean values in 8 rabbits given intravenous injection of digitoxin, 0.1 mg/kg, daily for 8 days.
‖ Mean ± standard error.

TABLE III. Effects of Digitoxin on Tissue Content of Magnesium in 8 Rabbits.

Tissue	Magnesium Content (meq/kg wet wt) Control Group†	Test Group‡
Muscle	21.5 ± .15§	19.8 ± .83
Skin	6.4 ± .27	5.0 ± .32*
Kidney	12.2 ± .66	11.9 ± .41
Heart	13.0 ± .40	13.7 ± .41
Liver	12.4 ± .50	14.9 ± .98
Lung	11.8 ± .76	14.5 ± 2.69

† Mean values in 4 rabbits not given digitoxin.
‡ Mean values in 8 rabbits given intravenous injection of digitoxin, 0.1 mg/kg, daily for 8 days.
§ Mean ± standard error.
* Statistically significant difference when compared with control group.

It has been previously suggested that digitalis might have pharmacologic effects similar to those of the adrenal cortical steroids. Cortisone and digitalis both tend to deplete the body store of potassium(3,7). In rabbits cortisone

produces an increase in the Mg_e, accompanied by an accelerated uptake of Mg^{28} in muscle, appendix, and heart(8). Digitalis, in the present study, produced similar changes in magnesium metabolism. Since it is difficult to explain these findings solely on the basis of the cardiotonic effects of digitalis, the results of the present study furnish additional evidence that this drug also has an extracardiac action.

Summary. In a test group of 8 rabbits, administration of digitoxin by intravenous injections of 0.1 mg/kg, given daily for 8 days, produced no significant changes in the external balance of magnesium. On the day following the last injection the exchangeable body content of magnesium, measured with Mg^{28}, was significantly increased. The results suggest that digitoxin produces subtle changes in the dynamics of magnesium metabolism which are not reflected in the external balance data.

1. Kinsell, L. W., Zillesson, F. O., Smith, A. M., Palmer, J., *Endocrinology*, 1942, v30, 221.

2. Zwemer, R. L., Lowenstein, B. E., *Science*, 1940, v91, 75.

3. Aikawa, J. K., Rhoades, E. L., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 332.

4. ————, ————, *Am. J. Clin. Path.*, 1959, v31, 314.

5. Stutzman, F. L., *Studies in Magnesium Metabolism*, Ann Arbor, Mich., Univ. Microfilms, 1952.

6. Snedecor, G. W., *Statistical Methods*, Ames, Iowa, Iowa State Coll. Press, 1946.

7. Aikawa, J. K., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 105.

8. ————, *Am. J. Physiol.*, 1960, v199, 229.

Received September 5, 1961 P.S.E.B.M., 1961, v108

Use of Radioiodinated Antipyrine to Measure Liver Function in the Rat.* (27035)

JAY M. SULLIVAN,** WILLIAM H. STRAUB*** AND JOHN C. ROSE

Department of Physiology and Biophysics, Georgetown University Schools of Medicine and Dentistry, Washington, D. C.

Radioiodinated antipyrine (RIAP), when injected in the rat, releases significant quantities of its I^{131} tag within one hour. The I^{131} tag appears in the peripheral blood in an increasingly diffusible form. This phenomenon can be measured by the rate of dialysis of radioactivity from plasma through a cellophane membrane(1). Brodie and Axelrod(2) have shown that in man, 30% of a dose of antipyrine is hydroxylated by the liver at the 4′ position, immediately conjugated with glucuronic or sulfuric acid and excreted in the bile. It has been suggested that since the I^{131} tag of RIAP is located at the 4′ position, its separation from the antipyrine molecule was a function of the liver(1). In the experiments reported here, liver function was altered by 3 different methods in rats. RIAP was then administered to these and to control animals. A plasma sample was obtained after one hour, and the amount of radioactivity remaining in the sample after a one hour dialysis was determined. The results indicate that impairment of liver function in the rat greatly reduces the loss of I^{131} tag from RIAP.

Methods. Young adult Sprague-Dawley rats, weighing 150-200 g were used in all experiments, male rats in Exp. I and II, females in Exp. III. 20 μc of RIAP (Abbott) in 0.5 ml 0.9% saline was administered intravenously to each animal. In all animals, anesthesia was induced with 40 mg/Kg sodium pentobarbital, given intraperitoneally.

In Exp. I, male rats were divided into experimental and control groups and allowed food and water *ad libitum* until anesthetized. The experimental animals were then eviscerated after ligating and severing the celiac axis and portal vein. The liver was left *in situ*, after

* Supported by Grant from Nat. Inst. of Arthritis and Metab. Dis.

** Medical Student Research Fellow, U.S.P.H.S.

*** Medical Student Research Fellow, National Science Foundation.