Magnesium metabolism in rabbits using Mg^{28} as a tracer[1]

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AIKAWA, JERRY K., ELOISE L. RHOADES, DALE R. HARMS AND JACQUELINE Z. REARDON. *Magnesium metabolism in rabbits using Mg^{28} as a tracer.* Am. J. Physiol. 197(1): 99–101. 1959.— Tracer doses of Mg^{28} were used to study the metabolism of parenterally administered magnesium. Plasma clearance was rapid, and accumulation of radioactivity in bone started within 2 hours. Of the tissues studied, skin and muscle showed the lowest concentrations of Mg^{28}. In most tissues the ratio of tissue to serum radioactivity became fairly constant after 18 hours. The values for the exchangeable body pool of magnesium, calculated from the specific activities of urine specimens obtained between 18 and 24 hours, approximated the carcass content of magnesium. During starvation the renal excretion of endogenous magnesium amounted to 61.7 mEq/kg of weight loss.

MAGNESIUM IS, NEXT TO POTASSIUM, the most abundant intracellular cation present in the body. Although much has been learned within the past decade about the metabolism of sodium, potassium and calcium, information about magnesium is still very meager. The production of a new synthetic radioactive isotope of magnesium, Mg^{28}, has made available a versatile new tool for re-evaluating the metabolism of this element in animals and man. Preliminary to a study of the role of magnesium in immunophysiology, we explored the behavior of Mg^{28},[3] in normal rabbits. This report summarizes our observations on serum magnesium concentration and on the plasma clearance, urinary excretion, tissue distribution and exchangeable body content of magnesium. The effect of starvation on the urinary excretion of magnesium was also studied.

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[2] Established Investigator of the American Heart Association.
[3] Mg^{28} was obtained from the Brookhaven National Laboratory, Upton, Long Island, N. Y., on allocation from the U. S. Atomic Energy Commission.

MATERIAL AND METHODS

Normal domestic adult rabbits of both sexes, with initial body weights between 2 and 4 kg, were kept in individual stainless steel metabolism cages and were fed a stock diet of compressed pellets. Water was given without restriction.

Mg^{28} was received as $MgCl_2$ in concentrated hydrochloric acid. Two hundred microcuries was contained in 25–30 mEq of stable magnesium. The material was neutralized with 1 N sodium hydroxide and was then diluted in distilled water. Urine was collected in chemically clean glassware; spot urine specimens were obtained by catheterization. Blood was obtained from the marginal ear vein or by cardiac puncture and was collected in heparinized test tubes. All animals used for tissue analyses were killed by air embolism.

Serum and urine magnesium determinations were performed by the molybdivanadate method for phosphate (1).

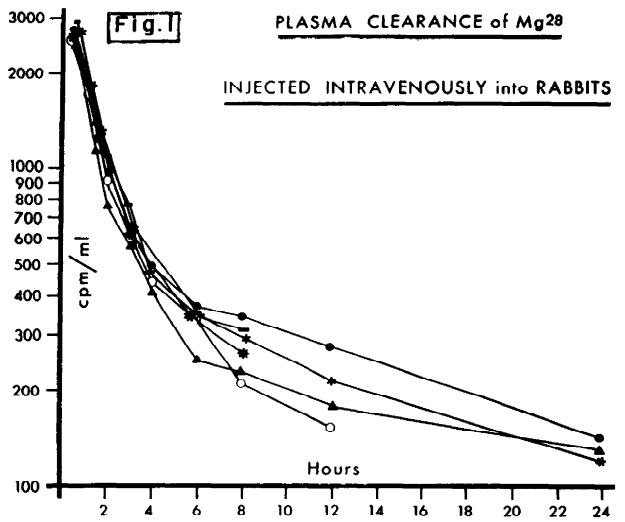
Radioactivity assay. Samples of serum, urine and tissues were assayed for gamma ray activity with a Nuclear-Chicago model DS-3 well scintillation counter which was connected to a Tracerlab Superscaler. A total of 10,000 counts was made on each sample. All determinations were corrected for physical decay.

Calculation of tissue relative activity. The counting rate per gram (wet weight) of tissue was compared with that per milliliter of plasma, and the result expressed as the ratio, cpm/gm tissue/cpm/ml plasma.

Exchangeable magnesium content. The following formula was used to calculate the value for the exchangeable magnesium content of the body:

$$Mg_e = \frac{Mg_i^{28} - Mg_o^{28}}{Mg_u^{28}/Mg_u^{26}}$$

Mg_i^{28} = quantity of radiomagnesium administered (arbitrary units).
Mg_o^{28} = quantity of radiomagnesium excreted in the urine up to the time the spot specimens were obtained.
Mg_u^{28} = concentration of radiomagnesium in the spot specimen.



Mg_u^{26} = concentration of stable magnesium in the spot specimen.
Mg_u^{28}/Mg_u^{26} = specific activity of the spot specimen.
Mg_e = quantity of exchangeable magnesium in milliequivalents.

TABLE 1. *Relative Radioactivity* of Tissues in Rabbits Following an Intravenous Injection of Mg^{28}*

Tissue		Time after injection (Hours) 2	4	6	8	12	16	20	24
Bone	M″	8.71	14.90	21.20	17.85	26.51	28.02	31.97	33.73
	R′	5.0-11.1	11.5-16.9	6.4-43.1	15.6-18.8	22.0-32.1	15.4-49.1	27.9-35.3	27.9-39.9
Kidney	M	7.88	11.74	12.08	10.64	10.15	7.76	9.13	8.16
	R	4.1- 9.9	10.6-12.8	10.1-14.5	9.0-12.2	8.5-12.1	6.1- 9.2	7.8- 9.8	7.1- 8.6
Lymph node	M	4.52	9.56	9.72	11.54	11.01*	11.73	14.69	12.09
	R	2.4- 6.1	6.2-11.9	5.9-12.2	10.1-13.2		9.0-14.8	13.2-16.2	9.3-14.1
Appendix	M	3.90	7.31	8.93	8.07	11.92	9.30	14.06	12.26
	R	2.5- 4.4	6.7- 7.9	7.8- 9.6	3.6-11.2	10.0-14.4	8.1-10.6	12.1-15.5	10.4-13.4
Heart	M	3.89	9.21	10.07	11.30	11.02	9.09	11.48	9.46
	R	1.5- 6.4	9.1- 9.3	8.2-11.2	9.6-13.1	9.2-13.1	7.5-10.4	10.1-12.9	8.0-10.5
Liver	M	3.06	5.07	6.32	8.40	11.14	8.03	10.44	10.02
	R	2.1- 4.5	3.7- 6.9	3.9- 8.0	6.0-11.5	7.7-15.3	6.1- 9.9	9.7-11.6	8.6-11.4
Adrenal	M	2.63	4.94	6.60	6.13	5.12	5.99	9.38	9.71
	R	1.8- 3.4	4.6- 5.2	5.0-8.8	4.6- 7.8	3.7- 7.1	4.4- 8.9	7.0-11.6	9.4-10.3
Skin	M	0.93	1.18	1.56	1.57	2.05	2.06	3.57	3.28
	R	0.7- 1.1	0.8- 1.6	1.4- 1.7	1.2- 2.1	1.5- 3.6	1.7- 3.4	3.0- 4.3	2.9- 3.5
Muscle	M	0.29	0.82	0.74	0.83	1.17	1.37	1.95	2.06
	R	0.2- 0.4	0.3- 1.3	0.5- 1.1	0.4- 1.4	1.0- 1.5	0.9- 1.6	1.6- 2.2	1.7- 2.5

M'' = mean of duplicate specimens in each of 4 rabbits. R' = range. * = single determination.

* Relative radioactivity = $\frac{\text{cpm/gm tissue}}{\text{cpm/ml serum}}$ at time of death.

RESULTS

Toxicity studies. Sudden death followed the rapid intravenous administration of more than 2.0 mEq of magnesium/kg of body weight. Rapid intraperitoneal injection of 2.0 mEq/kg resulted in transient peripheral vasodilatation and weakness of the skeletal muscles, with recovery within a few minutes. Up to 5 mEq/kg has been given slowly by vein without permanent ill effects; all the animals so treated recovered within 30 minutes.

Plasma magnesium concentration in normal rabbits. The mean serum or plasma magnesium concentration in 31 normal rabbits was 2.00 ± 0.36 mEq/l.

Plasma clearance of Mg^{28}. Six rabbits weighing between 2.2 and 2.9 kg were given 1 mEq of magnesium tagged with Mg^{28} by vein, and serial blood specimens were obtained at ½, 1, 1½, 2, 3, 4, 8, 12 and 24 hours. Mg^{28} disappeared from the blood stream rapidly during the first 6 hours, and more slowly thereafter over a period of 24 hours (fig. 1).

The apparent volume of distribution of Mg^{28}, as calculated from the plasma concentrations, exceeded the thiocyanate space of rabbits (2) in less than 1 hour, and exceeded the volume of total body water (70% of body weight) between the 3rd and 4th hour

Urinary excretion. The urinary excretion of tracer-tagged magnesium over a period of 18–24 hours has been measured in 88 instances after the intravenous or intraperitoneal injection of doses ranging from 0.25 to 3.20 mEq/kg. The percentage of the administered dose which was recovered varied between 0.2 and 67.1; the mean value was 29.3%. There was no relationship between the dose of magnesium administered and the urinary excretion of radioactivity.

Tissue distribution of Mg^{28}. A tracer dose of tagged magnesium (0.5 mEq/kg) was injected intravenously into 32 rabbits in order to determine the relative rate of its uptake by different tissues. The animals were killed by air embolism between 2 and 24 hours after the injection, and samples of plasma, liver, kidney, bone, heart, appendix, skin, adrenal, lymph node and skeletal muscle were weighed and assayed for radioactivity content. The radioactivity content of the tissues was then compared with that of plasma obtained at the time of death, and ratio, cpm/gm of tissue to cpm/ml of plasma, was calculated.

The relative radioactivity was considerably higher in bone than in any of the other tissues studied (table 1). This concentration in bone began within 2 hours, at which time the ratio was 8.7. This ratio increased steeply to a value of 26.5 at 12 hours, and continued to rise during the next 12 hours. At 4–6 hours the relative activities of the other tissues were in the following order of decreasing ratios: kidney (12), heart (10), appendix (9), liver (6), skin (1.6) and muscle (1). The ratios in the heart, appendix, liver and kidney had become stable by 12 hours after injection, and showed little variation during the next 12 hours.

Exchangeable magnesium content. The data obtained in studying the tissue distribution of Mg^{28} suggested that equilibration of the injected radioactive atoms with most of the body content of magnesium may have occurred within 16–24 hours. An attempt was made to measure the exchangeable magnesium content of the intact rabbit by isotope dilution principle. Plasma, because of its low concentration of magnesium and the technical difficulties involved in repeated collections of sufficient amounts for study, was not suitable for this purpose; urine was therefore used for the determination of specific activities. Pooled specimens of urine collected from 0 to 18 hours after the injection of Mg^{28} were assayed for radioactivity content in order to determine the amount

of radioactivity remaining in the animals after this time. Between 18 and 24 hours rabbits were catheterized in order to obtain serial urine specimens, and the specific activity of magnesium in each of these spot specimens was determined.

The initial determinations of exchangeable magnesium content were made following the injection of more than 0.5 mEq of Mg tagged with Mg^{28}/kg of body weight, and the results were erratic. When 0.5 mEq/kg or less was injected, the specific activities of the catheterized urine specimens obtained between 18 and 24 hours became reproducible. Four catheterized specimens obtained from each of 12 rabbits during this time interval showed an average variation of 7.2% from the mean specific activity in each animal. The mean exchangeable magnesium content in 15 determinations was 36.5 ± 7.7 mEq/kg. This mean value closely approximates the amount of magnesium found by direct analysis of two whole carcasses—36.8 and 29.7 mEq/kg. Subsequent attempts to reproduce these Mg_e values have been unsuccessful, however, and the results tended to be lower. The various factors involved in the over-all exchangeability of intravenously administered Mg^{28} are currently being studied.

Effect of starvation on urinary excretion of magnesium. Preparatory to a study of the effects of various experimental conditions on magnesium metabolism, the effect of acute starvation on the urinary excretion of magnesium and the relationship between urinary magnesium and the resultant decrease in body weight were investigated. Six rabbits with a mean initial weight of 2.13 kg excreted an average of 7.44 mEq of magnesium daily while being fed a stock diet of compressed pellets. After 1 week of starvation, body weight had decreased by an average of 0.300 kg (14% of the initial body weight), and during this week the mean urinary excretion of magnesium per animal was 18.5 mEq. Thus, the urinary loss of endogenous magnesium during acute starvation amounted to 61.7 mEq/kg of weight loss.

COMMENT

The low specific activity and the short physical half-life of Mg^{28} made impossible the injection of a truly tracer dose of radiomagnesium; however, the dose usually used (0.5 mEq/kg) was considerably lower than the toxic dose.

The normal serum or plasma magnesium concentration in rabbits is slightly higher than that in man. The plasma clearance of intravenously administered Mg^{28} in rabbits is rapid, and its rate is comparable to that observed in human subjects (unpublished observations). The apparent volume of distribution of Mg^{28} rapidly exceeds that of the extracellular fluid volume and the total water content of the body. Concentration of Mg^{28} in tissues, especially in bone, was evident within a few hours. The relative concentrations in the various tissues were characteristic and reproducible. Although muscle contains one of the body's largest stores of magnesium, exchange with this pool appeared to be slow, whereas exchange with the magnesium contained in bone appeared to be more rapid than that in any other tissue studied. The concentration in bone, however, was variable—a fact which may account in part for the variability observed in the exchangeable magnesium values calculated from urine specimens obtained between 18 and 24 hours. Further studies are necessary to determine the various factors involved in the tissue uptake of Mg^{28}.

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