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Effect of Magnesium Deficiency on Location of the Intestinal Absorption of Magnesium in Rats. (31618)

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The absorption of magnesium is increased in young rats fed a diet low in magnesium(1). In the following studies, Mg^{28} was used to determine whether this alteration is primarily associated with a local or general enhancement in the uptake of magnesium from the alimentary tract.

Methods. Non-fasting male albino rats (Sprague-Dawley strain) weighing 90 to 110 g at the beginning of the experiments were housed in individual metabolism cages that provided separation of the urine and the feces. The animals were fed either a low magnesium or a magnesium supplemented (control) diet (1) and distilled water *ad libitum*.

Magnesium-28 as $Mg^{28}Cl_2$ with a specific activity of 16 to 20 μC of Mg^{28}-Al^{28} per mg of stable magnesium at the time of administration was obtained from Brookhaven National Laboratory. Detailed descriptions of the equipment and methods have been presented elsewhere(1,2). In all the experiments, each animal received approximately 2 μC of Mg^{28} in 0.5 ml of 5% dextrose in water containing an additional 3 mg of magnesium as $MgCl_2 \cdot 6H_2O$. The extra magnesium was added to compensate, in part, for the much greater dilution of specific activity of the Mg^{28} which would otherwise occur in the bowel of the control rats due to ingested dietary magnesium. Except for net plasma counts lower that 100 cpm, the per cent probable error of the net counting rate was never greater than 3% and usually 2% or less.

Unless otherwise noted, the animals were anesthetized with ether during the administration of Mg^{28} and operative procedures. All blood samples were obtained by aortic exsanguination using heparin as an anticoagulant. Plasma magnesium levels were determined in duplicate on a Beckman B spectrophotometer by the titan yellow method (3,4).

Except for the multiple comparison analysis of Scheffe(5), statistical methods were taken from Snedecor(6).

Experiment 1. Absorption of Mg^{28} after direct injection through the bowel wall into the gastric or colonic lumen. Two studies of identical design were performed on a total of 51 animals, and the results were combined.

The rats were divided into 4 groups and placed on the magnesium supplemented diet. After an initial period of adjustment, 2 of the groups were transferred to the low magnesium diet. Four days later, 0.5 ml of the solution containing the Mg^{28} was injected directly into either the gastric or cecal lumen of each animal using a No. 28 needle through an abdominal incision. (Preliminary studies in which carmine red dye was used as an indicator showed that there was no loss through the puncture site.) During injection into the cecum, the ileum was gently compressed at the ileocecal junction to prevent any initial large reflux of the isotope. The abdomen was closed with sutures and metal clips, and feces were collected over the next 44 hours.

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TABLE I. Absorption of Mg^{28} Plus 3 mg of Carrier Magnesium Injected Directly into the Lumen of Bowel of Normal and Magnesium-Deficient Rats.*

	Control		Magnesium-deficient	
Site of injection	No. of animals	Initial dose absorbed (%)†	No. of animals	Initial dose absorbed (%)†
Stomach	11	57.5 ± 3.39	13	82.5 ± 1.35
Caecum	14	42.3 ± 2.19	13	66.5 ± 2.52

* Based on the 0-44 hr recovery of Mg^{28} in the feces, uncorrected for endogenous excretion.
† Mean ± S.E.M.

Although plasma magnesium levels were not measured in this part of the study, animals in many previous experiments(1) fed the same magnesium supplemented ration remained clinically and, in terms of plasma electrolyte levels, chemically normal. When normal rats were transferred to the low magnesium diet, hypomagnesemia developed within 16 hours; by 33 to 38 hours at the latest, the absorption of Mg^{28} plus 0.2 to 5.2 mg of carrier rose to levels comparable to those observed after 14 days of magnesium deprivation; and peripheral vasodilatation—a diagnostic feature of hypomagnesemia if it develops during the initial phase of magnesium deficiency—appeared regularly on the fourth to fifth day. It may be assumed, therefore, that these changes also occurred in the present experiment, especially since most of the rats on the low magnesium diet developed mild vasodilatation by the fourth day.

Experiment 2. Absorption of Mg^{28} in control and deficient rats after administration of the radioisotope by gavage. Thirty-eight rats were divided into 2 groups; one group was placed on the control, and the other on the low magnesium diet. Peripheral vasodilatation appeared in all the deficient animals by the fifth day. On the sixth day, each rat received 0.5 ml of the solution containing the Mg^{28} plus 3.1 mg of carrier. Under light nembutal anesthesia, single control and hypomagnesemic rats were killed by aortic exsanguination at the following approximate times after injection: 3/4, 1, 1-1/2, 2, 2-1/2, 3-1/4, 3-3/4, 4-1/4, 4-3/4, 5-1/4, 6, 7, 8-1/4, 9-1/4, and 10-1/2 hours. In addition, 2 rats from each group were killed at 24 hours; and the feces of the remaining animals were collected for a total period of 94 hours. A 2.0 ml aliquot of heparinized plasma was saved for counting and, in some cases, for magnesium determinations. The entire bowel of an animal was removed and divided into the following sections: colon, distal 30 cm of ileum, and remaining proximal bowel including the stomach. The intestinal segments and the feces passed per anum were placed in individual Erlenmeyer flasks containing about 10 ml of water. Because the low magnesium diet is constipating, the formed fecal pellets in the distal colon from the 24-hour deficient rats were included with the stool collections. The contents of the urinary bladder were added to previously voided urine, and the remaining carcass saved in thin cellophane bags.

In addition to 4 standards (equal in volume to those administered to the rats) in Erlenmeyer flasks, a fifth standard was placed in a lucite container with an internal volume approximately equal to that of the carcass of a 120 g rat. After an initial assay of the radioactivity in each standard, 2 were diluted to 10 ml to serve as standards for the bowel sections and feces; and one to 50 ml for the urines. The carcass standard in the lucite holder was diluted to about 120 ml. To minimize any differences in counting geometry, the tissue, fecal and urinary samples along with their respective standards were counted inside the previously described 6 × 6 × 6 foot iron cell about one foot beneath the center of a square formed by four 5 by 5 inch NaI crystals. The activity in each carcass was assayed by a method used for total-body counts of live animals(2). The plasma samples were counted in a separate well-crystal. The per cent of administered dose recovered in each specimen = (net cpm in the sample at T_N ÷ net cpm in the standard T_N) (net cpm in the standard T_O ÷ mean net

TABLE II. Absorption of Mg^{28} Plus 3 mg of Carrier Magnesium Administered by Gavage to Young Rats Fed the Control Diet.

			% of recovered radioactivity found in:						
Rat No.	Time in hr after inj	Radio-activity absorbed* (%)	Urine	Carcass	Proximal gut†§	Terminal 30 cm of small bowel§	Colon§	Feces passed per anum‡	Plasma radio-activity (cpm/2 ml)
1	¾	1.8	.8	1.0	98.2	.0	.0	.0	80
2	1½	4.6	.6	4.0	85.4	10.3	.1	.0	955
3	1	4.8	.0	4.8	85.5	10.3	.0	.0	1048
4	2	10.1	1.8	8.3	46.5	43.2	.2	.0	1479
5	2½	14.9	2.2	12.7	11.5	60.8	12.8	.0	1770
6	3¼	15.4	2.8	12.6	4.7	28.4	51.5	.0	1370
7	4¼	21.4	5.4	16.0	3.1	10.5	65.1	.0	1500
8	8¼	22.5	3.3	19.2	1.6	.5	75.1	.3	1044
9	3¾	22.9	4.3	18.6	3.4	31.6	41.5	.0	1974
10	4¾	24.7	6.5	18.2	13.2	7.2	54.9	.0	1849
11	7	27.5	8.7	18.8	1.6	.7	71.0	.0	1044
12	5¼	27.8	6.2	21.6	6.1	8.2	58.7	.0	1744
13	9¼	28.3	9.0	19.3	2.1	1.9	67.8	.0	1528
14	6	28.5	6.7	21.8	3.3	3.0	65.2	.0	1398
15	10½	30.0	10.3	19.7	1.3	1.0	67.2	.0	1343
16	24	42.0	18.2	23.8	‖	‖	16.6	41.4	¶
17	24	46.7	18.4	28.3	‖	‖	12.5	40.7	¶

* Equal to per cent of recovered Mg^{28} in carcass plus the urine.
† Entire intestinal tract from esophagus to terminal 30 cm of small bowel.
§ Includes intestinal wall and luminal contents.
‡ .0 indicates that no feces were passed during the indicated time interval or that the activity was $<0.1\%$.
‖ Included with recovered activity in carcass.
¶ Count not taken.

TABLE III. Absorption of Mg^{28} Plus 3 mg of Carrier Magnesium Administered by Gavage to Young Magnesium-Deficient Rats.

			% of recovered radioactivity found in:						
Rat No.	Time in hr after inj	Radio-activity absorbed* (%)	Urine	Carcass	Proximal gut†§	Terminal 30 cm of small bowel§	Colon§	Feces passed per anum‡	Plasma radio-activity (cpm/2 ml)
1	1	1.6	.0	1.6	95.8	2.7	.0	.0	361
2	¾	1.9	.0	1.9	98.1	.0	.0	.0	45
3	1½	9.6	.0	9.6	42.5	47.5	.2	.0	1403
4	2½	10.9	.0	10.9	47.0	41.8	.3	.0	1366
5	2	15.5	.0	15.5	40.4	43.9	.5	.0	1674
6	3¼	20.7	.8	19.9	15.2	63.6	.5	.0	2618
7	3¾	22.5	1.9	20.6	6.0	7.8	63.6	.0	**
8	4¼	30.0	2.8	27.2	13.8	49.5	6.6	.0	1885
9	4¾	32.0	2.5	29.5	4.9	13.3	49.6	.0	2148
10	5¼	34.1	1.3	32.8	5.2	11.9	48.8	.0	1855
11	7	37.9	1.1	36.8	4.5	3.4	53.2	.0	1836
12	6	38.5	3.3	35.2	(4.6)‖	2.2	54.7	.0	1661
13	8¼	42.2	2.9	39.3	4.9	1.6	51.3	.0	1317
14	10½	43.7	3.4	40.3	4.4	2.1	49.8	.0	1081
15	9¼	53.1	6.5	46.5	5.1	1.7	40.1	.0	1158
16	24	69.1	5.8	63.3	¶	¶	4.4	26.5††	**
17	24	72.2	5.3	66.9	¶	¶	2.8	24.9††	**

* Equal to per cent of recovered Mg^{28} in carcass plus the urine.
† Entire intestinal tract from esophagus to terminal 30 cm of small bowel.
§ Includes intestinal wall and luminal contents.
‡ .0 indicates that no feces were passed during the indicated time interval or that the activity was $<0.1\%$.
‖ Specimen lost. Activity based on an assumed recovery of 100% of initial dose.
¶ Included in recovered activity in carcass.
** Count not taken.
†† See text for details.

cpm in all standards T_0) $\times$ 100.

Results. Absorption of Mg^{28} after direct injection into the bowel. As shown in Table I, magnesium was absorbed in the colon. In both dietary groups, 15 to 16% more of the initial dose was taken up after direct injection into the stomach than into the colon ($P<0.01$ by Scheffe analysis); and, regardless of site of administration, the magnesium deficient animals absorbed 24 to 25% more Mg^{28} than the controls ($P<0.01$).

Absorption of Mg^{28} administered by gavage. The average recovery of administered radioactivity per rat was 100.1 $\pm$ 0.45% (mean $\pm$ S.E.M.). For comparison, each set of data was expressed as per cent of the total activity recovered in the animal from which it was taken. The amount of isotope absorbed was calculated to be equal to that found in the carcass and urine, and the results in Tables II and III are arranged in order of increasing absorption. The correlation of Mg^{28} absorption with the location of radioactivity in the contents of the various segments of bowel from individual rats killed at different times after administration of the isotope is assumed to represent the intestinal uptake of magnesium that would normally occur in a single animal as the bolus of Mg^{28} passed through the gut.

In the normal rats (Table II), the amount of radioisotope absorbed appeared to increase abruptly as the radioactivity in the distal ileum rose (rats 4 through 6) and, again, after large amounts of Mg^{28} had moved into the colon (rat 7 through 10). Based on a mean uptake of 46.6 $\pm$ 1.90% (mean $\pm$ S.E.M.) for the group,‡ at least 10% of the initial dose was absorbed before the isotope reached the colon (rats 1 through 4), and 19% was absorbed in the colon (rats 11 through 17). In rats 2 through 5, the plasma magnesium was 1.84 $\pm$ 0.08 mEq per liter (mean $\pm$ S.D.).

In the magnesium deficient rats, the mean plasma magnesium was 0.69 $\pm$ 0.10 mEq per liter (rats 1, 3, 4 and 5). As shown in Table III, at least 21% of the radioisotope was absorbed before reaching the colon (rats 1 through 6); and, based on a mean absorption for the group of 72.8 $\pm$ 1.38% (mean $\pm$ S.E.M.),‡ 35% was absorbed in the colon (rat 11). It is doubtful that any significant difference in the relative distribution of absorption of magnesium occurred as the result of magnesium deficiency.

Discussion. Magnesium is absorbed throughout the bowel of young rats fed a stock laboratory diet. Quantitatively, the most important site of absorption *in vivo* appears to be the colon(7). These results are confirmed in the present studies in both the control and magnesium deficient animals. Previous reports suggest that the small intestine is the major site of uptake for dietary magnesium (8-12), although there is evidence substantiating absorption in the large bowel(13-17). Species differences in the rate at which food traverses the alimentary tract make comparisons and interpretations of these data difficult. Such variations alter the duration of contact between luminal magnesium and the absorptive surfaces in any given segment of the bowel, and, assuming that the mineral enters the mucosal cells in an ionic form, modify the interaction of magnesium with those intraluminal factors that may change the chemical state of magnesium, rendering it unavailable for absorption. In the rat, food moves relatively rapidly through the small intestine and then pools in the proximal colon for several hours, and this may represent a partial explanation for the distribution of absorption of Mg^{28} which was observed in these animals.

The enhanced uptake of magnesium in the hypomagnesemic rat appears to be distributed throughout the gut so that the normal pattern of absorption is basically unchanged. At the present time the mechanisms underlying this specific alteration are not clear.

Summary. The intestinal absorption of Mg^{28} plus 3 mg of carrier Mg was studied in normal and magnesium deficient rats. Ab-

‡ The mean absorption for the group was calculated from the results in animals 16 and 17 and the recovery of isotope from the feces of the two animals which were followed for 94 hours. The latter values were corrected for intestinal excretion of the absorbed Mg^{28} by a previously described method(1). This correction amounted to 3.4% of the initial dose in the control and 5.2% in the deficient rats.

sorption was distributed throughout the bowel in both groups, was quantitatively the greatest in the colon, and was increased in the deficient animals. The relative distribution of absorption did not appear to be significantly altered, indicating a generalized enhancement in the uptake of Mg from the alimentary tract in magnesium deficiency.

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Production of Interferon in Embryonated Eggs and in Cell Cultures Infected with Simian Virus 5.* (31619)

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Simian virus 5 (SV5), a strain of parainfluenza 5, is a common myxovirus contaminant in uninoculated monkey kidney tissue cultures(1). Previous studies showed that SV5 was incapable of interfering with the multiplication of poliovirus in the monkey kidney cell system(2,3). In addition, Choppin found that SV5 infected monkey cell cultures did not interfere with the growth of several other viruses including Coxsackie, ECHO, vaccinia, and vesicular stomatitis virus(2).

Embryonated eggs have been commonly used for the propagation of most myxovirus types but the avian host system has not been generally applied to the cultivation of SV5. In our laboratory, attempts were made to adapt SV5 to growth in avian cells both in embryonated eggs and in tissue culture. After serial passages of SV5 in the avian host system it was noted that an inhibitor, subsequently identified as an interferon, was obtained. The results of these studies are reported here.

Materials and methods. Virus strains and infectivity assay. A newly isolated strain of SV5 was obtained from an uninoculated rhesus kidney tissue culture. Infectivity titrations were made by the hemadsorption technique, essentially as originally described by Shelokov *et al*(4).

Sindbis virus, strain AR339 M1318 Egg 2, was obtained through the courtesy of Dr. J. R. Henderson of Yale University School of Medicine. Infectivity of the Sindbis virus was determined by the plaque technique using chick fibroblast cultures in 3 oz prescription bottles as previously described(5).

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