PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 207–214 (1975)

Magnesium Movement in Hypothyroid Sheep[1] (38774)

F. C. MADSEN, G. E. SPALDING, J. K. MILLER, S. L. HANSARD, AND W. A. LYKE

Comparative Animal Research Laboratory, Oak Ridge, Tennessee 37830 and Department of Animal Science, University of Tennessee, Knoxville, Tennessee 37901

Magnesium metabolism has been linked to thyroid activity (1), and changes in thyroid status are possibly associated with hypomagnesemia in ruminant animals (2). Thyroxine involvement in cellular transport of Mg (3) and in maintenance of normal total and ultrafilterable plasma Mg (4, 5) has also been reported.

Kinetic studies with hypothyroid humans (3) showed (a) reduced total- and cellular-exchangeable Mg and (b) negative Mg balances with reduced urinary Mg and increased fecal Mg. In contrast, Mg balances were positive in hypothyroid cattle (6) and rats (7). Hypothyroidism elevated plasma Mg concentrations in man, sheep, and the rat (3, 7, 8) but did not affect cattle (6).

Care (9) proposed a three-compartment catenated system for studying Mg distribution in sheep; later, he (10) compared a two-compartment mammillary model with a one-compartment system. Similar mathematical procedures have been used by others (11–13) to evaluate factors affecting Mg metabolism. We have used a multicompartmental kinetic model to compare Mg metabolism of severely thyroid-damaged with apparently normal sheep.

Materials and Methods. We conducted two 5-day balance studies using 10 Suffolk-Hampshire crossbred sheep fed daily 0.45 kg concentrate and 0.90 kg red clover/grass hay. Sheep, nine ewes and one wether, were paired at approximately 7 mo of age on the basis of initial body weight and thyroxine secretion rate (14). One randomly chosen sheep from each pair was given 10 mCi ^{131}I orally to alter thyroid function. Blood plasma thyroxine concentrations (T-4 diagnostic kit, Abbot Laboratories, North Chicago, IL) and thyroxine secretion rates (14) were measured immediately before and 4 mo after ^{131}I administration. Plasma thyroxine concentrations were used to quantitate thyroxine secretion rates.

At 11 mo of age, each sheep was dosed orally with 50 μCi ^{28}Mg (1 mCi/mg) as the chloride (Brookhaven National Laboratory, Upton, NY); 29 days later, 80 μCi ^{28}Mg were given intravenously. Radioisotopes were administered intravenously via jugular catheterization and orally in gelatin capsules by balling gun. For radioisotope administration, sheep were confined in metabolism stalls in a room maintained at 15.5–°. A 10-day adjustment period was allowed each time before dosing.

Blood samples were taken by jugular vein puncture at intervals from 10 min to 106 hr postdosing, were centrifuged, and plasma was removed. Urine was collected in plastic bags attached to Foley catheters (Aloe Medical, St. Louis, MO), and fecal pellets were collected in paper-lined pans, weighed, and homogenized with water (100 g + 200 ml) in a commercial blender.

After the balance studies, sheep were anesthetized with sodium pentobarbital (Diamond Laboratory Sales Corporation, Memphis, TN) and were exsanguinated. Meat was removed; entire skeletons were dryed at 150–° for 2–4 days, ashed in stainless-steel pans at 600–°, dissolved in concentrated HCl and distilled H_2O, and adjusted to a known volume.

$^{28}Magnesium$ was measured in 1–3 ml urine (or plasma) or 3 g of fecal homogenates in an automatic gamma counter (Nuclear Instrument and Chemical Corporation, Chicago, IL). Dilutions of representative dosing solutions were counted concurrently with samples, and results were expressed as percentages of administered doses. Magnesium and Ca were measured by atomic absorption spectrophotometry (Perkin–Elmer Corporation, Norwalk, CT).

[1] Research sponsored by the U. S. Atomic Energy Commission under Contract No. AT-40-1-GEN-242 with the University of Tennessee.

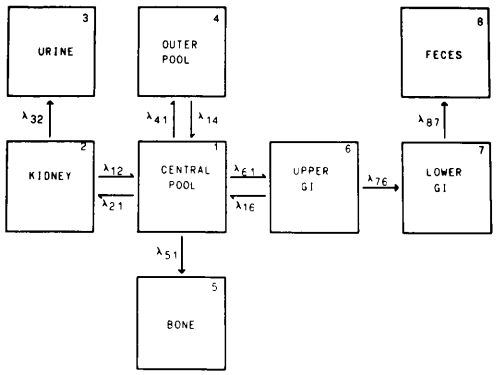

FIG. 1. Model of magnesium transport in sheep. The exchangeable Mg pool is distributed between central compartment (1) and outer compartment (4). Magnesium is lost to the feces (8), to urine (3), and to a compartment designated bone (5). ^{28}Mg was injected into the central compartment via plasma and introduced into the upper GI tract via transcellular fluids. The model was fitted simultaneously to data from orally and intravenously administered ^{28}Mg.

Linear Compartment Analysis. The six separate data sets from control and thyroid-damaged sheep included serial measurements of feces, urine, and plasma content for orally and intravenously administered ^{28}Mg. A computer program was developed to determine a set of transfer coefficients which would yield the best least-squares fit of the model to the six data sets. The model (Fig. 1) was developed with three criteria in mind: it should (a) describe the essential features of the data, (b) be biologically meaningful, and (c) be as simple as possible after meeting criteria (a) and (b). The model may appear to be unnecessarily complex since the GI tract is split into upper GI and lower GI, even though we have no direct measurements of GI tract contents; however, this division is biologically meaningful and was required in order to meet criteria (a).

In this analysis, ^{28}Mg is considered to be distributed between a rapidly exchanging central pool and an outer pool considered to be representative of slowly exchanging intracellular Mg components similar to that previously described (10, 12). Plasma ^{28}Mg contents were calculated from plasma ^{28}Mg concentrations by assuming that blood volume was 6.5% of the live body weight (15) and by estimating plasma volume from packed cell volume. Since plasma concentrations rather than central pool concentrations of ^{28}Mg were used, it was necessary to determine a "pool size" parameter to convert ^{28}Mg content of plasma to ^{28}Mg content of the central pool.

TABLE I. THYROXINE CONCENTRATION[a] AND SECRETION RATE[b] IN THYROID-DAMAGED AND CONTROL SHEEP.

	Thyroid damaged	Control
T_4 Concentration[c]	0.11 ± 0.00	7.82 ± 0.32
T_4 Secretion[c]	0.00 ± 0.00	0.39 ± 0.02

[a] T_4 concentration expressed as μg/100 ml ± SEM.

[b] T_4 secretion rates expressed as mg/day ± SEM.

[c] Treatment means differ ($P < 0.01$).

To analyze data from several compartments simultaneously and find the least-squares fit, we found it necessary to normalize data from all compartments. The computer program accepted data input in units of percent of dose. Since data expressed in this manner generally have standard deviations which are proportional to the means, the program performs a log-transform before analyzing the data, thereby providing correct weights for the data points.

Results and Discussion. Thyroid destruction in ^{131}I-treated sheep was nearly complete as indicated by reduced secretion rates and plasma levels of thyroxine ($P < 0.01$) (Table I). Although calcitonin was not measured, damage to parafollicular cells in thyroid-irradiated sheep could not be precluded. Blood plasma Mg concentrations averaged 2.87 ± 0.04 and 2.40 ± 0.06 mg/100 ml ($P < 0.01$) for thyroid-damaged and control sheep. Increased plasma Mg accompanying decreased calcitonin is in harmony with reduced plasma Mg in poultry injected with ultimobranchial gland extracts (16). Plasma calcium concentrations of thyroid-damaged sheep (11.46 ± 0.22 mg/100 ml as compared with 11.54 ± 0.19 for controls; $P > 0.05$), however, did not reveal changes in production of either parathyroid hormone or calcitonin. Therefore, the terms "hypothyroid"

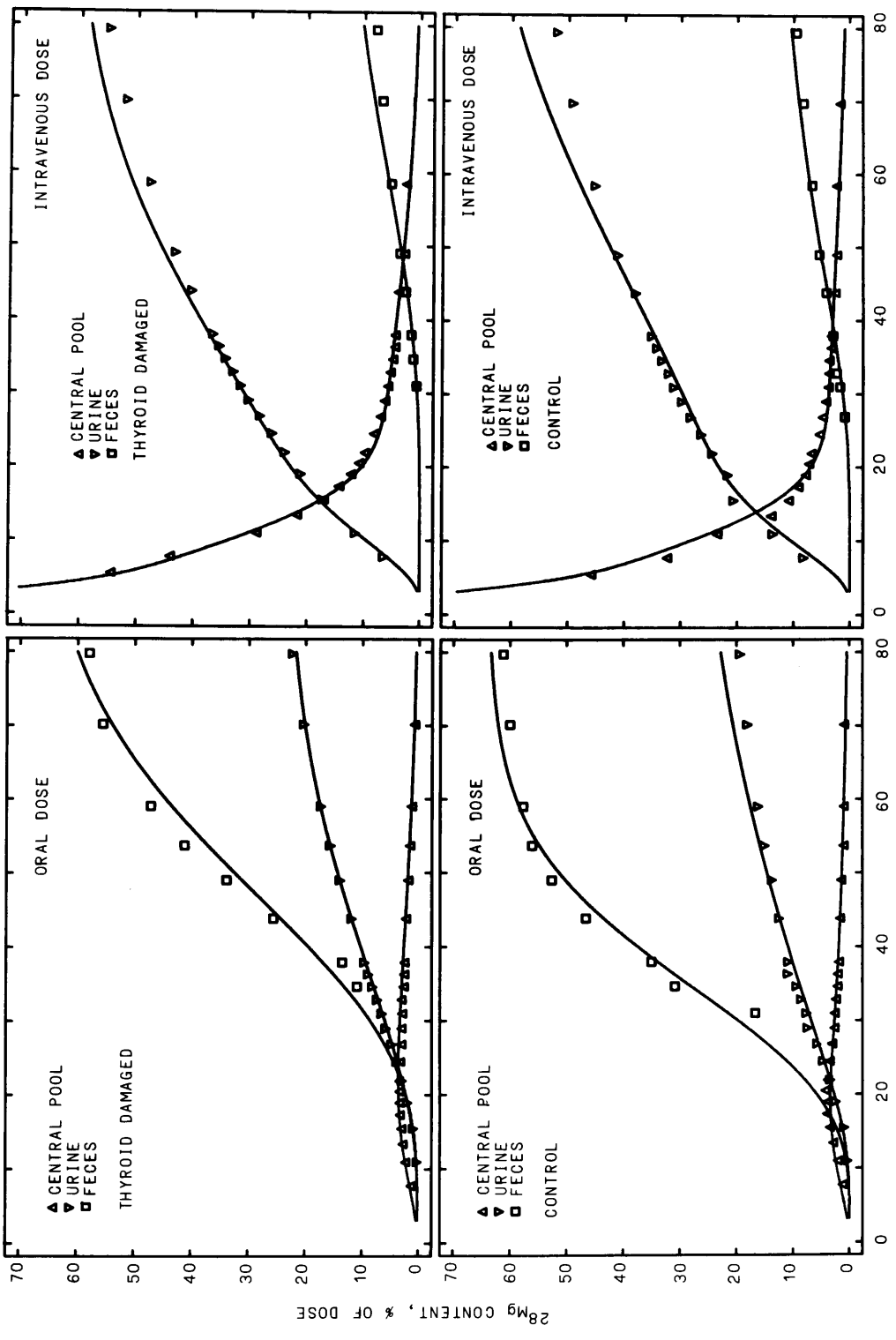

FIG. 2. Content of ^{28}Mg in central pool and in cumulative excretions in urine and feces of thyroid-damaged and control sheep during 6400 min after an oral or intravenous dose. The regression curves were calculated from the kinetic model and are shown in relation to the collected data.

TABLE II. TRANSFER COEFFICIENTS FOR ^{28}Mg TRANSPORT IN THYROID-DAMAGED AND CONTROL SHEEP, $MIN^{-1} \times 10^3$.

Compartments and direction	Thyroid damaged	Control
λ_{61} Central pool to upper GI	0.805 ± 0.04[b]	0.881 ± 0.06
λ_{16}[a] Upper GI to central pool	0.434 ± 0.01	0.544 ± 0.03
λ_{76} Upper GI to lower GI	0.705 ± 0.04	0.802 ± 0.07
λ_{87}[a] Lower GI to feces	0.459 ± 0.03	1.259 ± 0.16
λ_{21} Central pool to kidney	32.450 ± 0.79	35.300 ± 0.97
λ_{12} Kidney to central pool	50.920 ± 1.07	46.280 ± 0.63
λ_{32} Kidney to urine	3.747 ± 0.17	3.813 ± 0.14
λ_{41}[a] Central pool to outer pool	5.691 ± 0.38	7.238 ± 0.36
λ_{14}[a] Outer pool to central pool	1.266 ± 0.10	0.781 ± 0.07
λ_{51} Central pool to bone	0.895 ± 0.13	0.717 ± 0.21

[a] Treatment means differ ($P < 0.01$).
[b] Means ± SEM.

and "thyroid-damaged" can be used synonymously.

The six data sets, comprising central pool, urine, and feces for thyroid-damaged and control sheep, are plotted (Fig. 2) along with corresponding curves calculated from the model (Fig. 1). Excellent fits for the six data curves of both control and thyroid-damaged sheep are evident (Fig. 2); coefficients of multiple determination (R^2) were 0.997 for control and 0.998 for the thyroid-damaged sheep.

The regression curves of ^{28}Mg content (Fig. 2) illustrate that fecal ^{28}Mg excretion from orally administered ^{28}Mg was the only obvious difference between thyroid-damaged and normal sheep. The ^{28}Mg dose was excreted in feces at a much slower rate by thryoid-damaged than by normal sheep, so that large differences were evident during initial and intermediate portions of the collection period. As cumulative ^{28}Mg excretion curves became asymptotic, treatment differences were negligible. Effect of thyroid status on central pool or urinary ^{28}Mg content was not evident for either intravenous or oral ^{28}Mg doses (Fig. 2). Our initial conclusion from these six data sets was that little difference in Mg metabolism existed between thyroid-damaged and normal sheep, except for a slower rate of fecal ^{28}Mg excretion by hypothyroid sheep. However, kinetic analysis permitted examination of compartments of the model for which no data were obtained by experimental measurements to account for differences in fecal ^{28}Mg excretions.

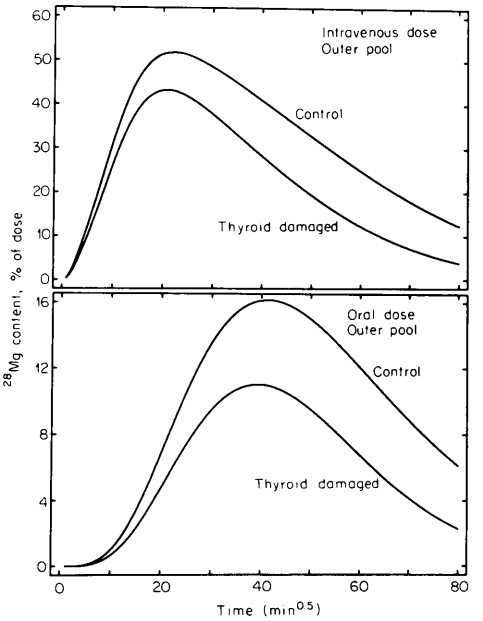

FIG. 3. Predicted ^{28}Mg content in outer pool of thyroid-damaged and control sheep during 6400 min after oral or intravenous administration.

The set of transfer coefficients giving the best least-squares fit of the model to the data is presented in Table II. Although these coefficients were obtained from a transient-state model and transient-state conditions, they are applicable to steady-state conditions as well. They may not, however, be identical with transfer coefficients describing bulk (as

opposed to tracer) Mg transport since isotopic equilibrium was not achieved during the experimental period.

Soon after oral ^{28}Mg dosing, more ^{28}Mg was contained in the outer pool (Fig. 3) than in the central pool (Fig. 2) in both thyroid-damaged and control sheep. Thyroid-damaged sheep had less ^{28}Mg in the outer pool than controls (Fig. 3), due to both a smaller ($P < 0.01$) transfer coefficient from the central to outer pool (λ_{41}) and a larger ($P < 0.01$) reverse coefficient (λ_{14}) (Table II). Since outer pool, in this analysis, is considered to be representative of slowly exchanging intracellular Mg components, reduced cellular ^{28}Mg transport appears reasonable in these hypothyroid sheep.

Delayed fecal ^{28}Mg excretion by thyroid-damaged sheep (Fig. 2) probably resulted from greater retention in both the upper and lower GI tract (Fig. 4). Predicted ^{28}Mg content of the upper GI tract was observed to decrease continuously after oral dosing, but at a slower rate in thyroid-damaged than in control sheep (Fig. 4). Percentages of intravenously administered ^{28}Mg in the upper GI tract peaked higher but slightly later in thyroid-damaged sheep, and predicted ^{28}Mg contents of the lower GI tract peaked at much higher levels and at later times in thyroid-damaged than in control sheep after both oral and intravenous doses (Fig. 4).

The coefficient describing ^{28}Mg absorption from the digestive tract was lower ($P < 0.01$) for thyroid-damaged than for control sheep despite the greater predicted ^{28}Mg content in the upper GI tract of thyroid-damaged sheep. The coefficient for transfer of ^{28}Mg from the lower GI tract to feces was also smaller ($P < 0.01$) in thyroid-damaged than in the control sheep. These results imply a slower rate of passage of digesta in hypothyroid sheep, which agrees with previous observations in cattle (17) and laboratory animals (18). There was no significant ($P > 0.05$) change in body weight in either group; however, hypothyroid sheep voluntarily consumed less feed ($P < 0.05$) than controls.

Both total true absorption of ^{28}Mg (68.6 ± 6.7 vs. $50.1 \pm 4.1\%$) calculated by the method of Hansard *et al.* (19) and Mg retention values calculated from nutritional balance data were greater ($P < 0.01$) for hypothyroid sheep. Smith (20) described Mg absorption to be a function of GI tract retention time in calves. Therefore, a slower rate of passage in hypothyroid sheep evidently increased absorption of ^{28}Mg, despite a decreased coefficient representative of absorption (Table II). Increased Mg retention by our hypothyroid sheep could have at least two possible causes: (a) increased skeletal retention, and (b) greater retention in a nondiscrete compartment. Calculations from the model showed that hypothyroid sheep retained more ^{28}Mg in the compartment designated bone (Fig. 5) than controls, although the transfer coefficients were not significantly different ($P > 0.05$) (Table II). Identical stable Mg contents of entire skeletons (0.2 g/kg body weight) in both hypothyroid and control sheep suggest no change in accretion by bone. A nondiscrete body pool most likely accounted for the increased Mg retention by hypothyroid sheep. Increased protein binding of plasma Mg has been reported in hypothyroid rats (21) and humans (22) and agrees with increased plasma concentrations in these hypothyroid sheep. Augmented protein binding of Mg in intracellular and extracellular fluids could account for the elevated Mg retention.

In contrast to results with hypothyroid humans (1) and cattle (6), ^{28}Mg loss in urine was not affected ($P > 0.05$) by the treatment. Fecal ^{28}Mg endogenous losses, whether computed from the model (λ_{61}) or isotope dilution (23) (7.0 ± 0.5 vs. $7.6 \pm 0.7\%$) were not affected ($P > 0.05$) by hypothyroidism.

Predictions for compartments for which data were not available must be used with some caution; predicted results from nondata compartments do not have the same reliability as for data compartments.

Summary. We described magnesium transport in hypothyroid and normal sheep with an eight-compartment biomodel fitted by a least-squares technique to data from multiple compartments and routes of entry for ^{28}Mg. Isotopic equilibrium was not attained during this period of observation in hypothyroid or control sheep. Hypothyroidism reduced transfer coefficients for absorption ($P < 0.01$) of ^{28}Mg from the GI tract but did not change the rate coefficient for endogenous ^{28}Mg losses to feces ($P > 0.05$). Nutritional

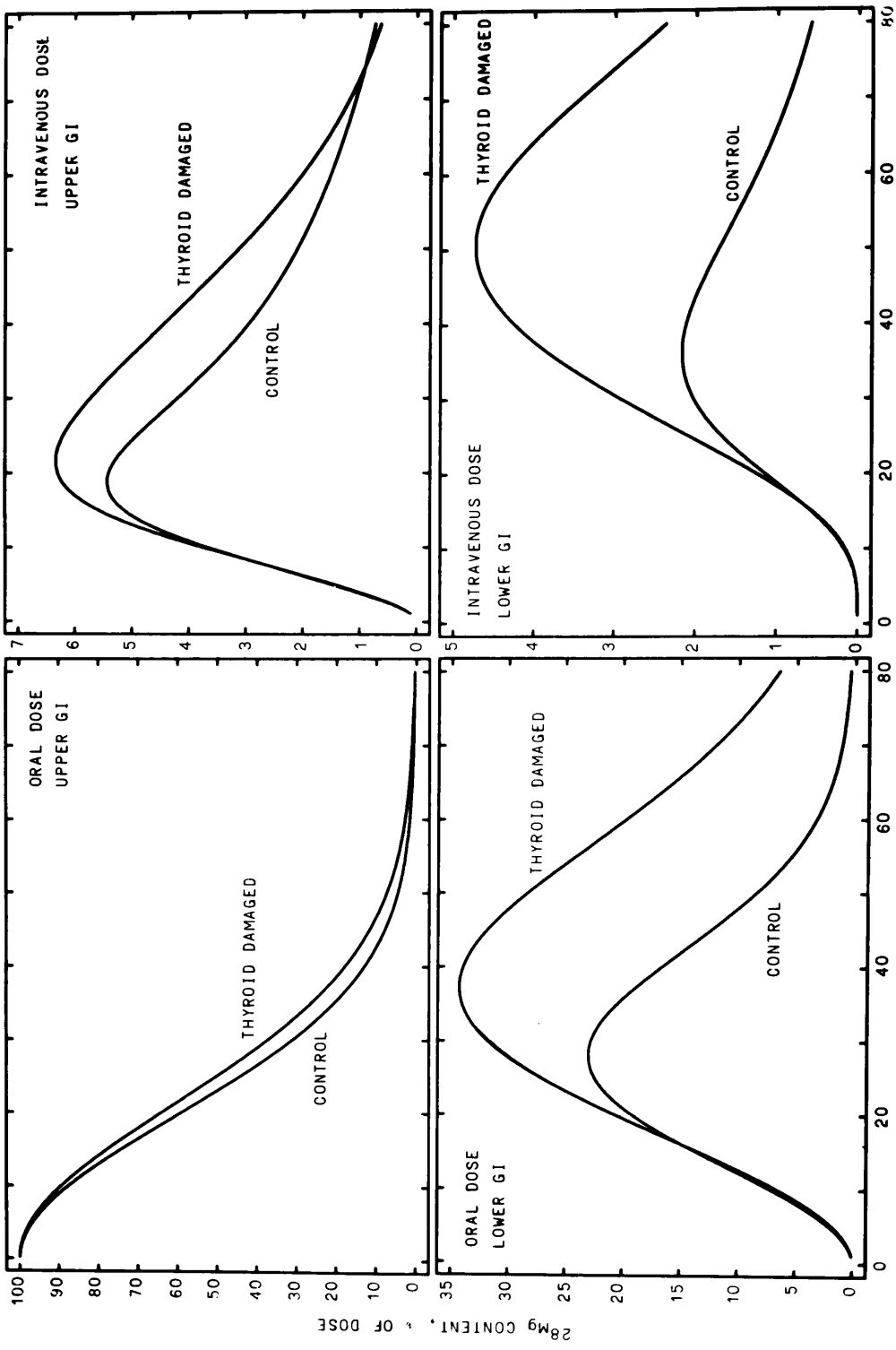

FIG. 4. Predicted ^{28}Mg content in upper and lower GI tract compartments of thyroid-damaged and control sheep during 6400 min after oral or intravenous dosing.

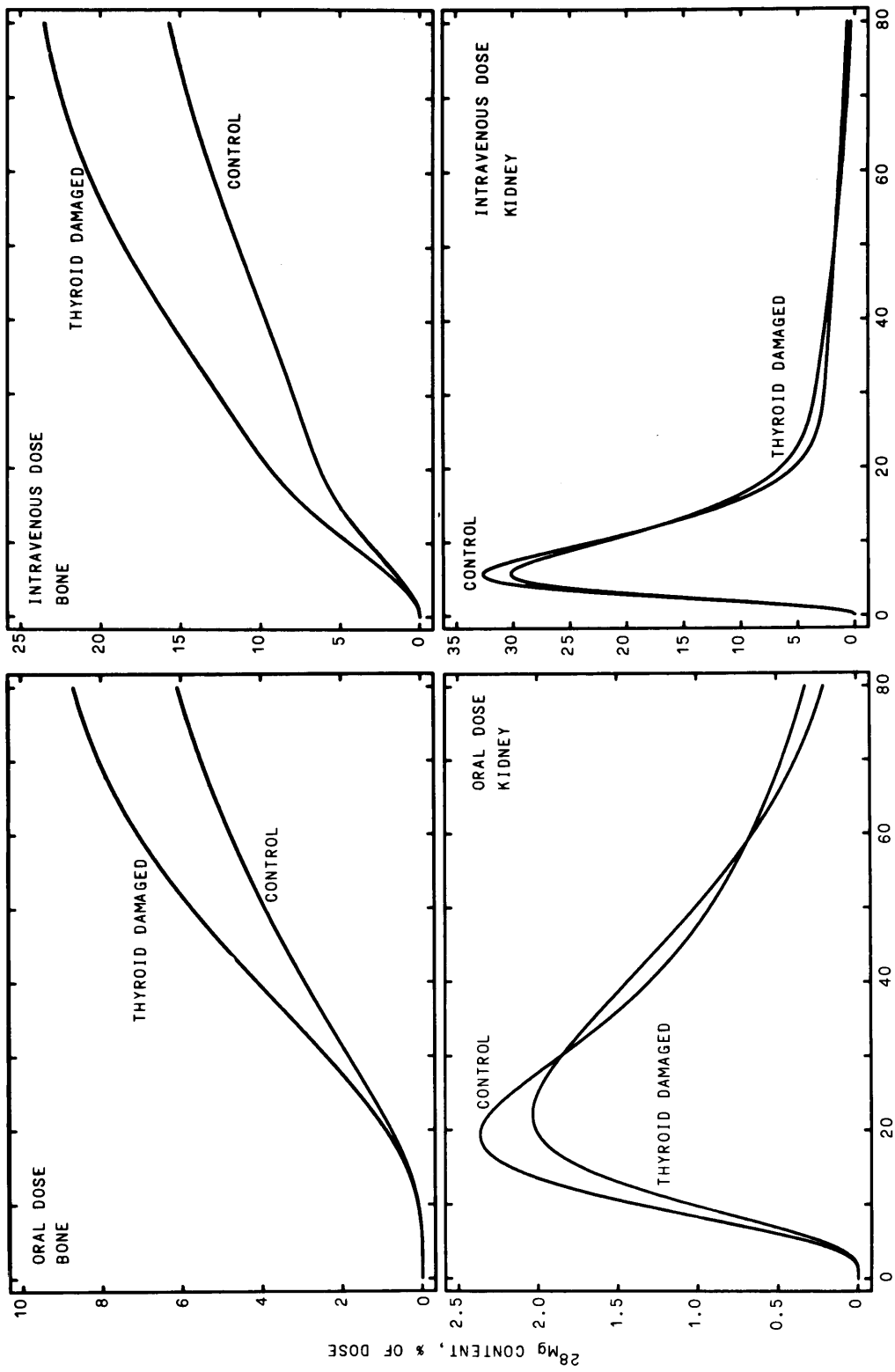

FIG. 5. Predicted ^{28}Mg content curves in compartments designated as bone or kidney after oral or intravenous administration of ^{28}Mg in thyroid-damaged and control sheep.

balance data indicated higher absorption and retention of Mg in hypothyroid sheep, and the observed decrease in rate of passage of digestive residues suggested that mean retention time of GI tract contents had a definite effect on the availability of Mg.

1. Hegsted, D. M., Vitale, J. J., and McGrath, J. H., J. Nutr. **58,** 186 (1956).
2. Grunes, D. L., Stout, P. R., and Brownell, J. R., Advan. Agron. **22,** 331 (1970).
3. Jones, J. E., Desper, P. C., Shane, S. R., and Flink, E. B., J. Clin. Invest. **45,** 891 (1966).
4. Doe, R. P., Flink, E. B., and Prasad, A. S., J. Lab. Clin. Med. **54,** 805 (1959).
5. Meyer, D. L., and Forbes, R. M., Proc. Soc. Exp. Biol. Med. **128,** 157 (1968).
6. Byrne, W. F., Ph.D. dissertation, University of Tennessee, 1971.
7. Szelenyi, I., Nam, L. B., Rigo, J., Nemesanszky, E., Simon, G., and Posch, E., Acta Physiol. Hung. **33,** 83 (1968).
8. Care, A. D., Vowles, L. E., Mann, S. O., and Ross, D. B., J. Agr. Sci. **68,** 195 (1967).
9. Care, A. D., Res. Vet. Sci. **1,** 338 (1960).
10. Care, A. D., Ross, D. B., Wilson, A. A., J. Physiol. **176,** 284 (1965).
11. Newton, G. L., Fontenot, J. P., Tucker, R. E., and Polan, C. E., J. Anim. Sci. **35,** 440 (1972).
12. House, W. A., and Van Campen, D., J. Nutr. **101,** 1483 (1971).
13. Hjerpe, C. A., Amer. J. Vet. Res. **29,** 2307 (1968).
14. Post, T. B., and Mixner, J. P., J. Dairy Sci. **44,** 2265 (1961).
15. Hansard, S. L., Butler, W. O., Comer, C. L., and Hobbs, C. S., J. Anim. Sci. **12,** 402 (1953).
16. Lloyd, J. W., and Collins, W. E., Poultry Sci. **49,** 446 (1970).
17. Miller, J. K., Moss, B. R., Swanson, E. W., and Byrne, W. F., J. Dairy Sci. **57,** 193 (1974).
18. Levin, R. J., J. Endocrinol. **45,** 315 (1969).
19. Hansard, S. L., Crowder, H. M., and Lyke, W. A., J. Anim. Sci. **16,** 437 (1957).
40. Smith, R. H., J. Biol. Chem. **71,** 306 (1959).
21. Doe, R. P., Flink, E. B., and Prasad, A. S., Endocrinology **88,** 747 (1959).
22. Ito, H., Tohoku J. Exp. Med. **96,** 1 (1968).
23. Simeson, M. G., Lunas, T., Rogers, T. G., and Luick, R. J., Acta Vet. Scand. **3,** 175 (1962).

Received Nov. 5, 1974. P.S.E.B.M., 1975, Vol. 149.