from "albumin" by ion-exchange chromatography. This nonantagonistic albumin did not become antagonistic when redialyzed through boiled or unboiled dialysis membranes. Nonantagonistic "albumin" did become antagonistic when reextracted from an aqueous solution by the Debro procedure and dialyzed through either boiled or unboiled dialysis membranes. An aqueous extract from the dialysis membranes did not exhibit significant insulin antagonism at concentrations as high as 1000 μg/ml.

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Roles of Thyroid and Parathyroid Hormones in Renal Calcification Induced by Magnesium Deficiency in the Rat (32968)

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The mechanism for maintaining plasma magnesium within narrow limits was proposed (1) to be the variation in parathyroid hormone secretion in response to plasma magnesium changes. Other investigators (2–4) have suggested that parathyroid hormone regulates magnesium metabolism either by affecting magnesium absorption from the gut or its mobilization from bone. Heaton and Anderson (5) have theorized that the increased activity of the parathyroid glands during magnesium deficiency is responsible for renal calcification in part through production of hypercalcemia and in part through a direct action of parathyroid hormone (PTH) on the kidney. The known effects of parathyroid hormone on mineral reabsorption from the kidney tubule and on bone resorption seem to support the above theories. It is probable that any condition altering blood-bone mineral balances would tend to alter parathyroid secretion rates which in turn may contribute to soft tissue calcification.

The thyroid gland has also been implicated in the metabolism of minerals. Hyperthyroidism was reported to increase calcium excretion, whereas hypothyroidism decreased calcium excretion (6). Improper function of the thyroid also affects the renal handling of phosphorus (7). Others (8,9) have reported an inhibitory effect by thyroid hormone on renal calcium deposition in magnesium deficient rats.

The present studies were designed to further define the roles of the parathyroid and the thyroid glands in the renal calcification

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accompanying magnesium deficiency in the rat.

TABLE I. Composition of Experimental Diets.

Ingredient	(%)
Casein	15.0
DL-methionine	0.5
Corn oil	8.0
Cellulose	3.0
Glucose	65.34
Cod liver oil	0.5
Mineral mixture[a]	2.66
Vitamin mixture	5.0

[a] Mineral content of diets: Calcium, 0.5%; Phosphorus, 0.5%; Magnesium, 0.001% in deficient diet, and 0.047% in adequate control diet.

Materials and Methods. Young male Sprague-Dawley rats were used in these experiments. Chemical ablation of the thyroid gland was accomplished with propylthiouracil[2] (PTU) according to the method of Gordon *et al.* (10). Because weanling animals of approximately 50 gm were used, the compound was added to the diet at only 0.15% instead of the 0.2% recommended by the above workers for 200 gm rats. This method required from 1–2 weeks for depletion of endogenous stores of thyroid hormone. Therefore, animals were fed a nutritionally complete, purified diet containing PTU for 2 weeks prior to being placed on the experimental diets (Table I). Weight gain data confirmed that PTU effectively caused hypothyroidism by 10–12 days.

Surgical ablation of thyroid and parathyroid glands was performed 1 week prior to placing the animals on the magnesium deficient diet in the second experiment. By using animals with transplanted parathyroids we were able to study the effects of thyroidectomy in animals with functional parathyroids. Animals were thyroparathyroidectomized (TPTX), parathyroidectomized (PTX), thyroidectomized (TX) with parathyroid glands transplanted to the ear, or sham-operated (SHAM). Parathyroidectomized animals were rejected if 3-day postoperative serum calcium levels rose above 7.5 mg/100 ml, and TX animals in which parathyroids had been transplanted were rejected if they were unable to maintain an 8.0 mg/100 ml serum calcium level.

In preparation for mineral analysis, tissue and diet samples were ashed with nitric acid followed by hydrogen peroxide, and tibia samples were ashed in a muffle furnace at 590°C for 12 hours. Determination of calcium and magnesium were made by standard procedures using the Perkin-Elmer model 303 atomic absorption spectrophotometer. Phosphorus was measured by the Fiske and Subbarow (11) technique.

The following procedures apply to the specific experiments:

1. *Effects of chemical thyroid ablation on serum mineral levels and kidney mineral content in normal and magnesium deficient rats.* Male Sprague-Dawley rats weighing 50 gm were randomly divided into five experimental groups. All animals received a nutritionally complete diet for 2 weeks with three of the five groups receiving PTU at 0.15% of the diet. Feeding of the nutritionally complete or magnesium deficient diets, either with or without PTU, began on the fourteenth day and continued for 3 weeks. One group of magnesium deficient, PTU-treated animals received thyroid hormone replacement therapy of 25 μg of *l*-thyroxine per animal per day administered intraperitoneally. At the end of the experimental period, blood samples were drawn by aortic puncture, the animals were killed and the kidneys were removed for analysis.

Statistical comparisons were made between the mean of the thyroxine-injected group and other appropriate group means by a *t* test, while treatment of data from magnesium deficient and control groups with and without PTU was by analysis of variance techniques for the 2 × 2 factorial design. Main and interaction effects are described in the results and levels of significance are indicated.

2. *Effects of surgical removal of thyroid and/or parathyroids on serum mineral levels, bone ash and mineral content, and kidney mineral content of magnesium deficient rats.* Male Sprague-Dawley rats weighing approximately 150 gm were divided 1 week after surgery into four experimental groups: (a)

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TABLE II. Effects of Thyroxine and Propylthiouracil on Serum Mineral Levels in Normal and Magnesium Deficient Rats (mg/100 ml).

Dietary treatment[a]	Calcium	Magnesium
Mg (—)	10.45 ± .24[b] (5)[c]	.777 ± .272 (5)
+ PTU	10.25 ± .23 (5)	.316 ± .022 (5)
+ T_4	9.42 ± .34 (4)	.846 ± .054 (3)
Mg (+)	9.67 ± .19 (5)	1.747 ± .013 (5)
+ PTU	9.43 ± .12 (4)	1.647 ± .118 (4)

[a] Mg (—) = magnesium deficient; Mg (+) = magnesium adequate control; PTU = propylthiouracil; and T_4 = thyroxine given ip at 25 μg/animal per day.

[b] Standard error of mean.

[c] Number of animals represented in mean.

sham operated (SHAM); (b) thyroparathyroidectomized (TPTX); (c) thyroidectomized with parathyroids transplanted to the ear (TX); and (d) parathyroidectomized with intact thyroids (PTX). All animals were placed on the magnesium deficient diet previously described for 14 days. At the end of this period, blood samples were collected, the animals were killed and bone and kidney samples were obtained. Mineral analyses were performed as described above, and data were compared statistically by analysis of variance techniques for the 2 × 2 factorial experiment. The main and interaction effects of parathyroidectomy and thyroidectomy are indicated in the results and levels of significance are given.

Results. 1. *Effects of chemical thyroid ablation on mineral levels and kidney mineral content in normal and magnesium deficient rats.* The serum data are summarized in Table II. At the end of 21 days, animals fed the magnesium deficient diet with or without PTU had significantly elevated serum calcium levels compared with controls receiving adequate magnesium ($p<.01$). Administration of 25 μg of *l*-thyroxine per day to PTU-treated, magnesium deficient animals resulted in serum calcium levels within the control range.

As would be expected, serum magnesium levels fell significantly in magnesium deficient animals ($p<.01$), but propylthiouracil treatment as a main effect did not alter serum magnesium concentration. On the other hand, there was a significant interaction in which PTU further depressed serum magnesium in magnesium deficient animals ($p<.05$) but not in controls.

Characteristic skin lesions such as reddening of ears, eyelids, and paws were evident by the sixth day in magnesium deficient animals not receiving PTU, and hyperirritability was evident by 14 days. Other groups failed to show any of these signs of magnesium deficiency.

The results of the analysis of kidneys for calcium are summarized in Fig. 1. Deprivation of magnesium resulted in significantly greater concentrations of calcium in kidney ($p<.01$). Kidneys of magnesium deficient animals contained approximately 0.95% calcium, while those from controls contained only 0.04% calcium on a dry tissue basis. Treatment with PTU significantly increased kidney calcium in both magnesium deficient and control animals ($p<.05$), but a much larger increase was found in the magnesium deficient group ($p<.05$) in which tissue calcium concentrations reached 3.65%. Thyroid hormone administration to PTU-treated,

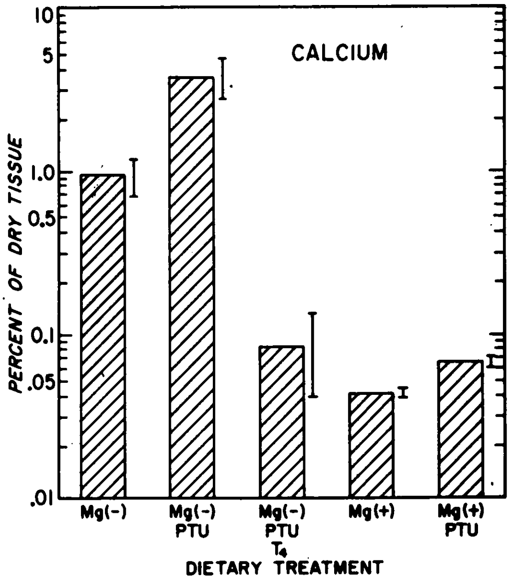

FIG. 1. Effects of dietary magnesium level and thyroxine on calcium in kidney tissue. Data are plotted on a logarithmic scale. (Brackets indicate standard error of mean.)

TABLE III. Effects of Surgical Ablation of Parathyroid and Thyroid Glands on Serum Mineral Levels in Magnesium Deficient Rats (mg/100 ml).

Surgical treatment[a]	Calcium	Phosphorus	Magnesium
SHAM	9.23 ± .50[b,c]	10.42 ± .45	.739 ± .071
TX	8.46 ± .27	11.83 ± .71	.536 ± .057
PTX	5.50 ± .35	15.84 ± .94	.565 ± .055
TPTX	5.24 ± .30	15.14 ± .71	.432 ± .026

[a] SHAM = sham-operated control; TX = thyroidectomized with parathyroids transplanted to the ear; PTX = parathyroidectomized; and TPTX = thyroparathyroidectomized.

[b] Six animals per treatment mean.

[c] Standard error of the mean.

magnesium deficient animals depressed the accumulation of calcium in the kidney ($p<.02$), and such animals exhibited renal calcium concentrations within the range of controls.

2. *Effects of surgical removal of thyroid and/or parathyroids on serum mineral level, bone ash and mineral content, and on kidney mineral content of magnesium deficient rats.* Serum mineral concentrations are shown in Table III. Surgical removal of the parathyroids resulted in a fall in serum calcium ($p<.005$), while there was a rise in serum phosphorus ($p<.005$). These effects would be expected upon the removal of parathyroid hormone from the system. Thyroidectomy produced no significant changes in serum calcium or phosphorus levels.

Serum magnesium levels were reduced in animals devoid of parathyroids ($p<.05$) and in animals in which the thyroid had been removed ($p<.01$). The interaction effect showed that the magnitude of the serum magnesium reduction due to thyroid removal was greater in animals having functional parathyroids than in parathyroidectomized animals.

The results of analysis of the tibiae are summarized in Table IV. A reduction in percent bone ash ($p<.005$) was accompanied by an increased concentration of magnesium in bone ($p<.005$) as a result of removal of the parathyroid glands. Removal of the thyroid had no effect on bone ash or magnesium concentrations. The interaction effect showed that thyroid ablation resulted in a reduction in bone calcium only when in combination with parathyroidectomy ($p<.05$).

Figure 2 shows the influence of our surgical procedures on the mineral content of kidney tissue. Removal of the parathyroids prevented the accumulation of calcium and phosphorus in kidney ($p<.005$), a condition commonly seen in magnesium deficiency, and also lowered kidney magnesium concentration ($p<.01$). Thyroid ablation did not significantly alter the kidney mineral content due to the large variation observed among animals within groups, even though there appears to be a reduction in kidney calcium and phosphorus in TX animals.

Discussion. Renal mineral accumulation in magnesium deficiency requires the presence of the parathyroid glands. This is borne out by our data showing that parathyroidectomy prevented the marked increase in calcium and phosphorus concentrations and the lesser increase in magnesium concentration in kidneys of magnesium deficient animals.

Both parathyroid and thyroid glands are necessary for the maintenance of serum magnesium at normal levels. The serum mineral data show that in magnesium deficient animals serum magnesium is maintained at higher levels in the presence of thyroid hormone than in athyroid animals having chemically ablated or surgically removed thyroids. Parathyroidectomized animals with or without thyroid glands had lower serum magnesium concentrations concurrent with higher bone content of magnesium. Bone, therefore, appears to be the source of magnesium to

TABLE IV. Effects of Surgical Ablation of Parathyroid and Thyroid Glands on Tibia Ash and Mineral Levels in Magnesium Deficient Rats.

Surgical treatment	Ash (%)	(mg/gm of dry bone) Calcium	Magnesium
SHAM	65.8[a]	268.1	3.05
TX	65.4	270.7	3.25
PTX	64.9	270.1	3.54
TPTX	64.3	262.2	3.85

[a] Six animals per treatment mean.

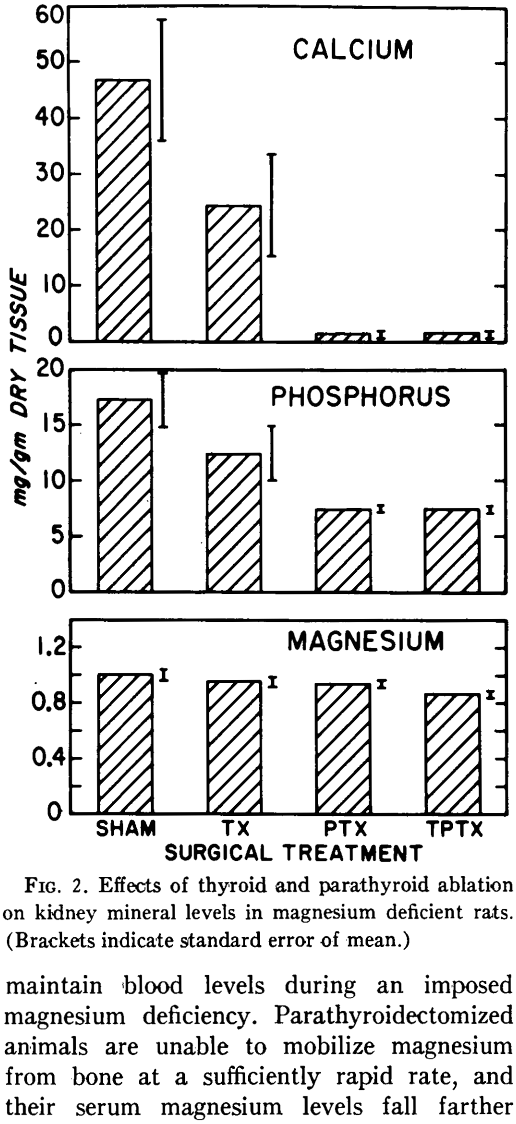

FIG. 2. Effects of thyroid and parathyroid ablation on kidney mineral levels in magnesium deficient rats. (Brackets indicate standard error of mean.)

maintain blood levels during an imposed magnesium deficiency. Parathyroidectomized animals are unable to mobilize magnesium from bone at a sufficiently rapid rate, and their serum magnesium levels fall farther than those of intact magnesium deficient animals. Similarly, it has been shown (12) that in early stages of magnesium deficiency the reduction in serum magnesium proceeded more rapidly than that in bone. Heaton (4) also found lower serum magnesium levels with higher femur magnesium in magnesium deficient rats which had been parathyroidectomized than in intact magnesium deficient animals.

It is possible that increased activity of the parathyroid glands resulting from dietary mineral imbalance is responsible for the renal calcification through production of hypercalcemia and other serum mineral changes associated with magnesium deficiency as previously proposed (5). Low levels of magnesium intake resulted in a 20-fold increase in kidney calcium concentration. Treatment with PTU elicited an additional 4-fold increase in kidney calcium concentration in magnesium deficient animals, but evoked only a 50% increase in control animals receiving adequate magnesium. Serum mineral changes resulting from low magnesium intake are characteristic of those known to be produced by parathyroid hormone administration.

The depression of serum magnesium by PTU could have resulted from the loss of endogenous thyroxine because such depression was reversed by the injection of 25 μg of *l*-thyroxine daily. This dose of thyroxine may have been more than the normal endogenous production since kidney calcium in PTU-treated animals receiving thyroxine was lower than that in untreated, magnesium deficient animals. The results indicate that the thyroid gland aids the parathyroids in maintaining normal balances of magnesium and calcium in kidney tissue and blood in addition to the well-known requirement of thyroxine for normal growth and metabolic rate. *In vitro* work (13) has shown that excess thyroxine of endogenous or exogenous origin decreased active transport of calcium. Thyroxine may play a role in transport of both magnesium and calcium across cell membranes, and tissue deposition of such minerals may be increased in athyroid animals.

The large increase in renal calcification consequent to total suppression of thyroid hormone with PTU did not occur when the thyroid was removed surgically. Rather, calcification was moderately depressed in surgically thyroidectomized animals. This apparent paradox is probably an indication that surgical removal of the thyroid did not totally deprive the animals of thyroactive compounds. It is known that mammalian tissues may produce thyroxine in the absence of the thyroid gland, and that thyroid follicles are often located in areas separate from the pharyngeal region. It is not unlikely, also, that iodinization of some proteins, followed by hydrolysis, may yield thyroxine without enzymatic intervention (14). Thyroxine formation has been reported (15) in thyroidectomized rats. Thus, the results from these two experiments are not necessarily contradictory.

Consideration must also be given to the possibility that thyrocalcitonin production was altered in conjunction with chemical thyroid ablation and was probably eliminated entirely by surgical thyroidectomy. Thyrocalcitonin is a hypocalcemic principle of thyroid origin which is released in response to a rise in blood calcium level, and which is probably active in opposing calcium liberation from bone. Elimination of hypercalcemia by thyrocalcitonin (16) is intimately related to parathyroid function and the thyroxin-dependent metabolic status of the animal. In our experiments, PTU treatment may have stimulated thyrocalcitonin release since there was a slight reduction in serum calcium levels in the animals receiving PTU.

Surgical thyroid ablation removed the source of the hypocalcemic principle, thyrocalcitonin, and higher serum calcium levels would be expected in these operated animals when compared with sham-operated controls if thyrocalcitonin was exerting a strong influence in magnesium deficiency. On the contrary, our results show slightly lower serum calcium levels in animals which underwent surgical thyroid ablation and which were consequently devoid of thyrocalcitonin. Therefore, the authors feel that thyrocalcitonin is not an important confounding factor and does not play an important role in the renal calcification herein described.

Although we cannot reach a definite conclusion regarding the action of thyroxine in renal calcification, it is apparent that an interaction exists in which both thyroid and parathyroid hormones regulate tissue mineral levels and that an imbalance in mineral intake can significantly alter this regulatory function.

Summary. Experiments were conducted to study the effects of endogenous and supplemental thyroxine on the renal deposition of calcium resulting from magnesium deficiency in rats and the role of the parathyroid glands in contributing to such calcification. Elimination of endogenous thyroxine with propylthiouracil (PTU) effectively increased the calcium concentration in renal tissue of both normal and magnesium deficient animals. Exogenous thyroxine completely reversed the effects of PTU ablation of the thyroid. In addition, endogenous thyroxine apparently plays a role in the maintenance of serum magnesium as does the parathyroid hormone which functions to promote mobilization of bone magnesium to maintain blood levels. The presence of the parathyroid glands was required for calcification of renal tissue to occur. Surgical removal of these glands resulted in the reduction to normal of renal tissue concentrations of calcium and phosphorus in magnesium deficient animals. It is possible that the dietary mineral imbalance imposed upon the animals increased the activity of the parathyroids and was in this way responsible for mineral deposition in renal tissues.

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Interaction of Respiratory Syncytial Virus with Polyions: Enhancement of Infectivity with Diethylaminoethyl Dextran* (32969)

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For working on the plaque isolation of respiratory syncytial (RS) virus, a high rate of infectivity of virus is very desirable. Polyions were examined as substances which would stimulate the interaction between RS virus and cells. Polyions, such as diethylaminoethyl (DEAE) dextran and protamine sulfate, have been used extensively to facilitate the uptake of intact virus (1,2) and infectious viral ribonucleic acid by cells in tissue culture (2–6), and thus increase the rate of infectivity. The experiments in this paper show that after exposure to DEAE dextran, the infectivity of RS virus markedly increased, whereas the opposite effect was obtained after treatment of the virus with dextran sulfate, heparin, and protamine sulfate. The experiments used HEp-2 and African green monkey kidney (AGMK) cell cultures.

Materials and Methods. Virus. The AGMK-adapted, plaque-purified stock of RS virus (21113-38) was used throughout this study.

Tissue cultures. The HEp-2 cells were grown as monolayers in tissue culture dishes (35 × 10 mm, Falcon Plastic) in Eagle's minimum essential medium containing Earle's balanced salt solution (BSS) with 10% fetal bovine serum, 2 m*M*/ml of glutamine and antibiotics. The AGMK cells were grown in tissue culture dishes in Earle's BSS containing 0.5% lactalbumin hydrolyzate with 2% fetal bovine serum, 2 m*M*/ml glutamine and antibiotics.

Polyions. Stock solutions of DEAE dextran (mol. wt. 2×10^6, Pharmacia, Uppsala, Sweden), protamine sulfate (Upjohn Co.), dextran sulfate (Pharmacia) and heparin (Calbiochem) (kindly supplied by Dr. K. K. Takemoto, NIH) were prepared in distilled water and kept under refrigeration.

Plaque assay. Plaque assays were performed on HEp-2 and AGMK cell monolayers as described by Coates, *et al.* (7) with slight modification. Plates (HEp-2,3–4 days old; AGMK, 7–9 days old) were washed and exposed to 1.0 ml of diluted virus. After incubation at 36°C in the CO_2 incubator for a minimum of 2 hours, the inoculum was removed and the cell sheet was overlaid with 4 ml of a fluid medium consisting of Cooper's (8) or L-15 medium (9), 5% heat-inactivated fetal bovine serum, 1% methylcellulose (Fischer Scientific Co., 4000 Centipoise), 2 m*M*/ml of glutamine and antibiotics. Cultures were incubated in the regular incubator without CO_2 for 3 days (HEp-2) or 8 days (AGMK) at 36°C. At that time, the overlay was replaced with 10% formaldehyde solution to fix the cell sheet, and cells were stained with Giemsa stain. The plaque count was made with the aid of a Bausch and Lomb stereozoom dissecting microscope.

Results. Effect of polyions on RS virus infectivity. The HEp-2 cell monolayers were exposed to virus containing various concentrations of DEAE dextran, protamine sulfate, dextran sulfate, or heparin. After incubation at 36°C for 2 hours, the inoculum containing polyion was removed, and the cultures were

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