antigenicity of ubiquitous connective tissue.

Goodman(8) has recently reported that rabbits injected with human liver nucleoprotein developed antibodies not only to human nucleoprotein extracts, but also to such extracts obtained from rabbit liver. The results of the present study suggest that auto- and iso-antibodies to connective tissue may also be induced by foreign material. It is uncertain if the stunted growth of the guinea pigs is related to development of these anti-connective tissue antibodies.

Identification of the antigenic component in connective tissue is under study.

Summary. Tissue fixed globulin has been demonstrated in interstitial tissue and basement membranes of various guinea pig organs following inoculation of guinea pigs with saline extracts of rabbit tendon. These animals had a significantly diminished growth. It is likely that the fixed globulin represents "auto-antibody" induced by heterologous antigen.

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Effect of Thyroxine and Propylthiouracil on Magnesium Metabolism in the Rabbit. Study with Mg^{28}.* (25918)

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The factors regulating the metabolism of magnesium are obscure. Previous studies have shown that insulin and glucose(1), cortisone(2), pyridoxine and desoxypyridoxine (3), and alloxan (unpublished) may affect distribution of Mg^{28} in rabbits. Very little is known about the relationship between magnesium metabolism and the thyroid gland, although it has been suggested that either hyperthyroidism or hypothyroidism may alter the partition of magnesium in serum(4). The present study was designed to investigate the effects of experimentally induced hyper- and hypo-thyroid states on magnesium metabolism in the rabbit. Before and after administration of thyroxine or propylthiouracil to 2 groups of animals, the external balance of magnesium was determined and exchangeable magnesium content was measured with Mg^{28}. Mg^{28} was also used to determine relative tissue radioactivity in rabbits which had been on larger doses of thyroxine or propylthiouracil.

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Material and methods. Thirty-two normal domestic adult male rabbits were kept in individual stainless steel metabolism cages and were fed a stock diet of compressed pellets. Except in exp. 2 and 4, tap water was given without restriction. *Thyroxine sodium* was dissolved in physiologic saline solution made slightly alkaline with a few drops of 1 N NaOH, and was made up to a final concentration of 0.3 mg/ml. In preliminary observations, 7 rabbits were given 2 intravenous injections of thyroxine, 0.3 mg per kg of body weight, within 48 hours. A striking loss of body weight ensued, and 3 of the 7 rabbits died within 7 days. In the definitive experiment, therefore, only one dose of 0.3 mg/kg was administered initially. When it appeared that maximal sublethal effects were not developing, a second dose of thyroxine was administered. *Propylthiouracil*, U.S.P., was dissolved in distilled water and made up to a concentration of either 0.05% or 0.10%. *Serum and urine magnesium* determinations were performed by modification of molybdivanadate method for phosphate(5). *Radioactivity assay.* Samples of plasma and tissues were assayed for gamma ray activity with scintillation counter. A total of 10,000 counts were made on each sample, and all determinations corrected for physical decay of the isotope. *Exp. 1 and 2. External balance studies* for magnesium were performed for 15 days in 8 rabbits in *Exp. 1* and for 27 days in 8 rabbits in *Exp. 2*. Each morning before rabbits were fed, body weight, total food consumption, and 24-hour urine volume were measured. Each gram of feed was known to contain 0.1723 meq of magnesium. *Measurement of exchangeable magnesium content* (Mg_e). Mg_e determinations were made at weekly intervals in both groups. Method of preparing magnesium solution for injection has been described(6). Five ml of magnesium solution, containing/ml approximately 2 μcuries of Mg^{28} in 0.4 meq of untagged magnesium, was injected intravenously. Urine collected 20 hours after injection was pooled; between 24th and the 27th hour, 2 spot specimens were collected by catheterization, and their specific activities determined. The exchangeable magnesium content was calculated as previously described(7). *Exp. 1.* Control data were obtained during first 2 days. On second day, after completion of initial Mg_e determination, each animal received, by intravenous injection, 0.3 mg of thyroxine sodium/kg body weight. On eleventh day of experiment, 9 days after first dose of thyroxine sodium, each animal was given a second intravenous injection containing the same dose of thyroxine sodium. The third Mg_e determination was made on fourth day following this second dose of thyroxine (15th day of experiment). *Exp. 2.* Control data were obtained during first 4 days. Beginning on morning of fifth day, drinking water was replaced by 0.05% solution of propylthiouracil in distilled water. Each animal was given 250 ml of this solution to drink daily for 27 days. *Exp. 3 and 4. Tissue uptake studies. Exp. 3.* Sixteen rabbits were divided into 2 groups of 8 animals each. Each of 8 rabbits in test group was given 3 intravenous injections of thyroxine sodium (1.0 mg/kg) within 4 days. Mg^{28} was injected 24 hours after third dose of thyroxine, and animals were killed exactly 4 hours later. Tissues were then analyzed for radioactivity, as previously described(6). 8 animals in control group were not given thyroxine sodium, but were otherwise treated identically with those in the test group. *Exp. 4.* Six of surviving animals in Exp. 2 were given propylthiouracil in drinking water in concentration of 0.1% from days 28 through 32. On 32nd day, each animal received intravenous injection of Mg^{28}, and was killed 4 hours later for tissue analyses. The control group was the same as in Exp. 3. *Statistical methods. Exp. 1 and 2.* Each animal served as its own control. After differences between baseline values and values obtained at subsequent Mg_e determinations had been calculated for each animal, mean differences for the whole group were obtained. Significance of mean difference was determined by use of "t" test, a "P" of less than 0.01 being considered significant(8). *Exp. 2 and 4.* Mean relative activity of each type of tissue was determined for both test and control groups, and significance of differences between group means was then tested(7).

Results. Effects of Thyroxine. No significant changes were noted in body weight, food intake, urinary magnesium excretion, external magnesium balance, and Mg_e at 7 and 4 days after injection of first and second doses of thyroxine sodium respectively. A significant decrease in serum magnesium concentration, from 2.11 to 1.65 meq/l, was noted on second day after second injection of thyroxine.

In animals killed after receiving 3 mg of thyroxine/kg of body weight within 4 days, a significant increase was found in relative radioactivity of skin, liver, appendix, and heart (Table I). Muscle and bone showed no significant change when compared with respective mean values in control group.

TABLE I. Effects of Thyroxine and Propylthiouracil on the Relative Radioactivity of Tissues in Rabbits.

Tissue	Control group‡	Relative radioactivity* Thyroxine group§	Propylthiouracil group‖
Muscle	.50 ± .10¶	.68 ± .05	.41 ± .63
Skin	1.25 ± .14	2.13 ± .26†	1.39 ± .15
Appendix	3.90 ± .23	7.15 ± .45†	5.70 ± .71
Lung	4.25 ± .26	3.98 ± .18	3.58 ± .17
Liver	5.10 ± .42	9.92 ± .82†	3.47 ± .21†
Heart	5.90 ± .99	10.00 ± .58†	7.89 ± .51
Kidney	11.00 ± .60	11.20 ± .78	10.24 ± .54
Bone	12.90 ± 1.13	12.89 ± .91	6.24 ± .81†

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

† Statistically significant difference when compared with control group.

‡ Mean values in 8 rabbits not treated with thyroxine or propylthiouracil.

§ Mean values in 8 rabbits receiving thyroxine sodium, 3 doses of 1 mg/kg in 4 days.

‖ Mean values in 6 rabbits receiving propylthiouracil (.05% solution for 4 wk, followed by .10% solution for 4 days).

¶ Stand. error.

Effects of propylthiouracil. One of the 8 animals given propylthiouracil died during second week, and another during third week. These 2 animals ate poorly, lost weight, and had terminal Mg_e values of 3.7 and 11.0 meq. In remaining 6 rabbits, body weight was significantly increased on 13th, 20th, and 27th days of propylthiouracil administration (Table II). Mean daily food intake was decreased during first week, and the urinary excretion of magnesium was decreased during second week. Mg_e was significantly decreased at end of third week. Initial mean value for protein-bound iodine was 4.1 μg/100 ml; at end of fourth week, it was 2.5 μg/100 ml.

When the rabbits were killed after 4 days on double dose of propylthiouracil, a significant decrease was found in relative radioactivity of liver and bone. The other tissues showed no significant changes.

Comments. Although administration of thyroxine in Exp. 1 produced clinical evidences of a hypermetabolic state—tachycardia, hyperpnea, and increased irritability—the external balance study revealed no significant changes in magnesium metabolism. Two days following second injection of thyroxine, serum magnesium concentration was significantly depressed, although exchangeable body content of magnesium was not significantly altered. In Exp. 3 tissue uptake of Mg^{28} was increased in heart, appendix, skin, and liver, but not in muscle and bone. The decrease in serum magnesium concentration may have been caused by redistribution of extracellular magnesium into the intracellular compartment of tissues showing increased radioactivity.

Rabbits fed propylthiouracil were less active than controls, and values for protein-bound iodine, suggest that they were indeed hypothyroid. Tissue uptake of Mg^{28} by liver and bone was suppressed, and Mg_e was significantly depressed.

Previous studies demonstrated increase in exchangeable body content of magnesium under circumstances which produced an increase in relative radioactivity of *muscle* or *bone*. For instance, administration of cortisone to rabbits caused an increase in relative radio-

TABLE II. Effects of Propylthiouracil on Magnesium Metabolism in Rabbits.†

	Mean baseline values	Mean values at Wk I‡	Wk II§	Wk III‖	Wk IV¶
Wt (kg)	2.519	2.483	2.670*	2.769*	2.795*
Serum Mg (meq/l)	1.72	1.79	1.57	1.86	1.79
Exchangeable Mg (meq)	73.6	54.5	51.4	42.3 *	77.5
Mg intake (meq/day)	23.1	19.6 *	20.7	20.2	20.3
Urinary Mg excretion (meq/day)	6.9	4.8	4.6 *	6.5	7.9
Urine volume (ml/day)	155	72 *	80 *	87 *	98 *

* Statistically significant difference when compared with mean baseline value.

† Propylthiouracil administered as 0.05% solution in distilled water, 250 ml daily.

‡ Mean values in 8 rabbits, 5 days on propylthiouracil.

§ " " " 7 " , 13 " " " .

‖ " " " 6 " , 20 " " " .

¶ " " " 6 " , 27 " " " .

activity of muscle, accompanied by increase in Mg_e. In contrast, the change in Mg^{28} uptake following administration of desoxypyridoxine, pyridoxine, or alloxan occurred in tissues other than muscle or bone, and was not associated with any significant alteration in Mg_e. In the present studies, although relative radioactivity in some soft tissues was increased by thyroxine, muscle and bone were not affected and no change occurred in Mg_e. Conversely, administration of propylthiouracil suppressed uptake of Mg^{28} in bone, and was associated with a significant decrease in Mg_e.

Previous studies showed that the largest body pool of magnesium is in muscle and bone. Magnesium in other tissues appears to exchange fairly rapidly with tracer dose of magnesium labeled with Mg^{28} and injected intravenously(6). In normal human subjects Mg^{28} does not enter muscle, bone, or erythrocytes in appreciable amounts, and Mg_e values are extremely low(7).

The results of our study suggest that, although thyroxine or propylthiouracil produces alterations in magnesium metabolism, which can be demonstrated by Mg^{28}, the changes are insufficient to be detected by usual external balance technic. In *potassium,* another cation which is predominantly intracellular in location, a decrease in *extra*cellular concentration reflects a depletion of the *intra*cellular store. Such a simple relationship does not seem to hold true with magnesium. Even though administration of propylthiouracil suppressed bone uptake of Mg^{28} sufficiently to decrease the exchangeable body content of magnesium, serum magnesium concentration did not change.

Summary. 1) In rabbits given thyroxine or propylthiouracil, external balance studies for magnesium were performed, and Mg^{28} used to measure exchangeable body content of magnesium. In 8 rabbits given 2 intravenous injections of thyroxine sodium (0.3 mg/kg) no significant changes were noted in body weight, magnesium balance, or exchangeable magnesium content, although a decrease in serum magnesium concentration occurred. In another group of 8 rabbits the tissue relative activity of Mg^{28} was determined following 3 intravenous injections of thyroxine (1.0 mg/kg) given within 4 days. The relative activity was increased in liver, skin, appendix, and heart. 2) Eight rabbits given propylthiouracil orally showed a decrease in exchangeable body content of magnesium at end of third week. A significant decrease was found also in relative radioactivity of liver and bone. 3) The value for exchangeable body content of magnesium appears related to rate of turnover of Mg^{28} in bone and muscle, the 2 largest body stores of magnesium.

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