sorption was markedly inhibited. It would not take much time for EDTA to be conjugated by polyvalent ions in the loop, and some of the B_{12} should have a chance of absorption after excess Ca ions start entering the gut, provided IF kept its activity. Further studies are required to see if EDTA would not directly affect IF or damage the mucosal receptor site. As far as the binding capacity of rat IF preparations was concerned, EDTA did not appreciably reduce it in the test tube.

The time study demonstrated a long interval required for absorption to become unaffected by chelation, suggesting that the reaction involving Ca and possibly Mg is a slow and reversible process. As Herbert(1) first suggested, such divalent cations might act as a bridge to aid mucosal adsorption of IF-B_{12} complex(11), but the evidence has been meager. The failure to demonstrate any effect of gastric juice administration in the duodenum is in line with the assumption that IF activity is present in adequate quantities in the human intestinal canal(12).

Summary. The effect of metal chelation on intestinal adsorption of B_{12} was studied in man and in rats. When a physiological dose of B_{12}-Co^{60} was instilled through an indwelling tube into the duodenum in man, immediately followed by 2 g of EDTA in a dilute solution, absorption was reduced markedly as studied by the Schilling test. Administered shortly after EDTA, Ca^{++}, Mg^{++}, Sr^{++}, but not Fe^{++}, Fe^{+++}, or Zn^{++} counteracted the inhibition by EDTA. Similar results were obtained with rat intestinal loops. When there was interval between the dosage of B_{12}-Co^{60} and EDTA, about 3 hours in man and 45 minutes in the case of rat loop were necessary for the absorption to become unaffected by EDTA.

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Effect of Hyperthyroidism on Intestinal Absorption of Calcium in the Rat.* (30432)

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Several manifestations of abnormal calcium metabolism have been observed in hyperthyroidism. Hypercalcuria(1-3), and, less frequently, hypercalcemia(4-8) have been found. Rarefied bone has been observed, most often in hyperthyroidism of long duration(1,4,9-12). In the past this laboratory studied 2 patients with long-standing recurrent Graves' disease associated with demineralized bone. In both patients, serum calcium was normal, serum alkaline phosphatase was elevated, and urinary calcium excretion was low, even on a high calcium diet. These observations led to consideration of the possibility that the

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gastrointestinal tract may play a significant role in the abnormal calcium metabolism of hyperthyroidism.

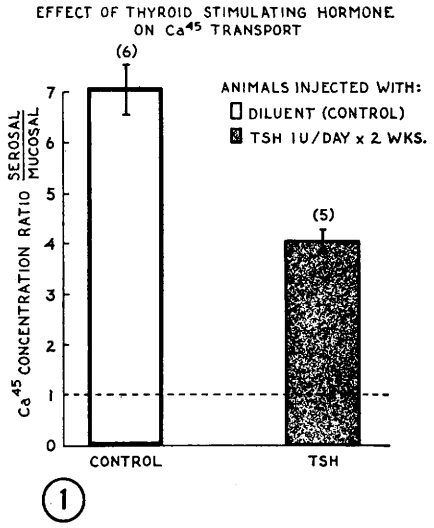
Calcium metabolic balance studies of hyperthyroid patients reported by several investigators have shown increased fecal calcium loss to contribute to the observed negative calcium balance(1-3,5). This would indicate either a greatly increased endogenous excretion, or decreased intestinal absorption, of calcium. Endogenous excretion was considered normal in one study in which it was indirectly measured(2). Impaired intestinal absorption of calcium could not explain the instances of hypercalcuria or hypercalcemia, but could at least partially explain the observed increased fecal calcium loss and negative calcium balance; and, when present for a prolonged period, could cause the rarefied bone seen in some hyperthyroid patients.

The present study was designed to determine whether an excess of thyroid hormone results in decreased intestinal absorption of calcium. To eliminate effects of bone, kidney, or endogenous excretion on the observations, intestinal active transport of calcium was determined by an *in vitro* gut sac technic (13).

Materials and methods. Groups of male weanling (45-50 g) Sprague-Dawley rats were given daily subcutaneous injections of either one unit of thyroid stimulating hormone (TSH) for 14 days or 10 μg L-triiodothyronine (T^3) for 7, 14, or 28 days. (These doses rendered the animals moderately hyperactive and decreased the slope of their weight curves. However, the animals remained otherwise normal in vigor and appearance. The resin sponge T^3-I^{131} uptake was significantly elevated(14). Serum calcium values were normal in all groups.) Also studied were a group of animals treated for 1 day, which received a single injection of 100 μg of T^3; and a group treated for 98 days, which received 10 μg of T^3 daily during the first 54 days and 20 μg daily during the last 44 days. A control group of animals, which received daily injections of diluent, was studied simultaneously with each treatment group. During the injection periods, all animals were maintained on an *ad lib* intake of tap water and a nutritionally complete diet containing 1.7% calcium, 0.8% phosphorus, and 148 units vit D/100 g diet. After the varying injection periods (and after a 12-hour period without food but with water *ad lib*) the rats were killed by decapitation. The proximal 3 cm segment of small intestine was rapidly removed and everted. A sac was prepared from the everted segment by tying both ends, after placing in the lumen 0.35 ml of a solution of the following composition: 0.148 M NaCl, 0.008 M sodium phosphate of pH 7.4, 0.02 M glucose, 4×10^{-5} M $CaCl_2$, and 0.02 μc/ml $Ca^{45}Cl_2$ (Oak Ridge Nat. Labs., specific activity $>$ 1000 mc/g). The filled sac was placed in an Erlenmeyer flask containing 2.5 ml of solution identical to that placed in the lumen. The flask was aerated for one minute with oxygen, stoppered, and placed in a shaking incubator for 2 hours at 37°C. Following incubation, the sac was removed from the flask, and the luminal contents withdrawn. Ca^{45} concentration was determined on equal aliquots of the solution on the serosal side (or inside) and the mucosal side (or outside) of the sac by a liquid scintillation counting technique. The ratio of the Ca^{45} concentration of the serosal solution to that of the mucosal solution (S/M ratio) was calculated. Since by design, the Ca^{45} S/M ratio was 1 before incubation, any post-incubation ratio greater than 1 indicated active calcium transport against a concentration gradient(13).

Tibiae were removed from representative groups of rats for histologic study.

Results. As shown in Fig. 1, the mean S/M ratio for 14-day TSH-treated rats was 4.1, and for the control rats was 7.1, indicating a significant decrease ($P<0.001$) in active calcium transport in the treated group. The S/M ratio for rats treated with T^3 for 14 days was 3.3 compared with the control S/M ratio of 6.0 (Fig. 2), also indicating a significant decrease in active transport in the T^3-treated animals. The S/M ratios of rats treated with T^3 for periods varying from 1 to 98 days, along with the simultaneously-studied control animals, are shown in Fig. 3. There is a variation in calcium transport in the control animals in different age groups.



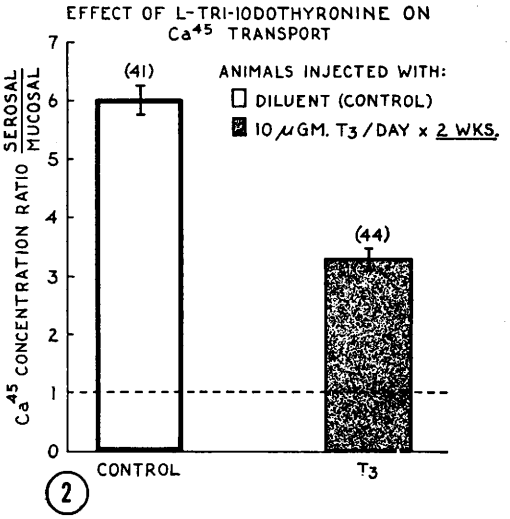


FIG. 1. Effect of TSH on intestinal active transport of calcium. Dotted line indicates S/M ratio existing in all sacs before incubation. Each bar represents mean post-incubation value, with standard error, for number of animals studied (in parenthesis).

FIG. 2. Effect of T^3 on intestinal active transport of calcium.

Especially apparent is the minimal degree of active calcium transport in the group injected for 98 days, a phenomenon previously reported in rats of advanced age(15). However, at each time period of study the S/M ratio in the T^3-treated group was significantly less than that in its respective control group ($P<0.001$ in 1-, 7-, 14- and 98-day studies, and $P<0.01$ in the 28-day study).

Histologic sections of the proximal tibial area of rats treated with T^3 for 98 days showed fewer and smaller bone trabeculae than comparable bone sections from control animals of the same age.

Discussion. An excess of endogenous or exogenous thyroid hormone resulted in significantly decreased intestinal active transport of calcium. This effect of exogenous hormone occurred promptly (within 24 hours), and still persisted after 98 days of T^3 injections, which was the longest treatment period studied. The importance of the active transport process in total calcium absorption by the intestine *in vivo* is still uncertain. It is possible that in the intact animal under physiologic conditions, diffusion may play a major role. However, it has been demonstrated that intestinal active calcium transport occurs in the intact rat, as well as *in vitro*(16).

Several investigators(1,3,5) have proposed that a direct catabolic effect of excess thyroid hormone on bone is the primary cause of the abnormalities of calcium metabolism found in hyperthyroidism. In the present study the observed decrease in trabecular bone of the T^3-treated rats could have re-

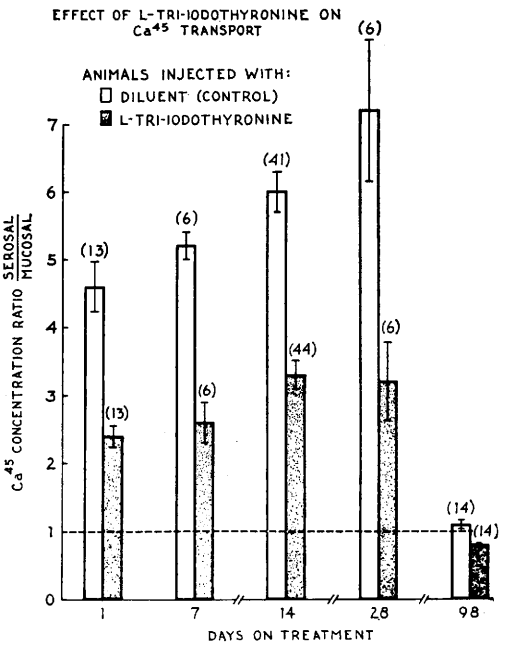


FIG. 3. Intestinal active transport of calcium after varying periods of T^3 injections.

sulted from a direct effect of T^3 on bone. However, other investigators(17,18) have observed this type of bone lesion in rats fed calcium-deficient diets. It is therefore possible that excess thyroid hormone has both a) a direct effect on bone, promoting resorption, and b) an indirect effect whereby decreased intestinal calcium absorption results in insufficient calcium to maintain proper mineralization of bone.

Summary and conclusions. Intestinal active transport of calcium was studied by an *in vitro* gut sac technic in rats with induced hyperthyroidism. An excess of thyroid hormone resulted in a prompt and persistent decrease in calcium transport, and also in a decrease of trabecular bone. The mechanism of this action of thyroid hormone and its physiologic significance is yet to be determined. It is suggested that decreased calcium absorption may be a significant factor in the negative calcium balance and bone changes found in some hyperthyroid patients.

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Thymidine Kinase in IDU Resistance.* (30433)

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It has been established that serial passages of herpes simplex viruses in increasing concentrations of 5-iodo-2′-deoxyuridine (IDU) can result in the development of mutant viruses which are resistant to IDU as well as bromodeoxyuridine (BDU)(1-3). The action of both drugs is dependent on their phosphorylation and incorporation into DNA: Cells which are deficient in thymidine kinase (TK—) are resistant to BDU(4) and can also withstand high concentrations of IDU which are lethal to normal LM cells(5).

Herpes simplex virus induces infected cells to produce large amounts of thymidine kinase and it has been postulated that virus resistance to IDU may be caused by the loss by the virus of the ability to induce thymidine kinase synthesis in its host. The extent to which the virus strains are inhibited by IDU

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