4. Exposure of spleen microsomes to RNAase did not affect their antigenic activity. 5. Brief exposure of spleen microsomes to ultrasound waves yielded a semisoluble antigenic preparation.

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Evidence for Thyrocalcitonin in Man. (31380)

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Studies with several species of experimental animals have demonstrated that there is a calcium-lowering principle whose secretion is stimulated by hypercalcemia(1,2,3). It was initially thought that this hormone was secreted by the parathyroids(1), but subsequent investigations have shown that it is secreted by the thyroid gland(4,5,6). It has therefore been designated thyrocalcitonin(4). Apparently this hypercalcemia-induced calcium-lowering thyrocalcitonin and the long known hypocalcemia-induced calcium-elevating parathyroid hormone are both important in calcium homeostasis: by their combined, but opposite, effects, the two hormones maintain the plasma calcium concentration within a very narrow physiologic range. Most studies of the homeostatic effects of thyrocalcitonin have been carried out on experimental animals(1-3,5,6). However, a substance has been extracted from the human thyroid gland which is hypocalcemic in rats(7), and injection of porcine thyrocalcitonin into human subjects causes lowering of plasma calcium (8). Therefore it seems probable that thyrocalcitonin is present and has important physiologic effects in the human species. Thyrocalcitonin is a polypeptide(9) and is therefore biochemically and physiologically distinct from thyroxine, but apparently both hormones are secreted by the same thyroid cells(10). Therefore patients with hypothyroidism (of excessive surgical removal, excessive I^{131} therapy, or idiopathic etiology) may well have a deficiency of thyrocalcitonin. If these patients do have thyrocalcitonin deficiency, they should have impaired calcium homeostasis, especially in their ability to prevent or promptly reduce hypercalcemia when subjected to an excessive calcium intake.

This study was undertaken to determine whether a thyroid hypocalcemic principle, thyrocalcitonin, can be demonstrated in man, and whether a deficiency of thyrocalcitonin

can be demonstrated in patients with diminished or absent functional thyroid tissue.

Materials and methods. Seven patients with well-documented deficient or absent thyroid functional tissue, (but who were maintained in an euthyroid state with exogenous desiccated thyroid therapy) were studied. The etiology of the hypothyroidism was idiopathic in 3, post-I^{131} therapy for hyperthyroidism in 2, and post-surgical plus I^{131} therapy for thyroid carcinoma in 2 patients. There was no clinical evidence for parathyroid deficiency, and serum calcium and phosphorus were normal. Eight convalescing ambulatory patients with normal thyroid function and no evidence to suggest an abnormality of calcium homeostasis were studied as controls. After an overnight fast (and abstinence from desiccated thyroid for 24 hours in the treated hypothyroid group) each patient received an intravenous infusion of 4 mg per kilogram body weight of calcium ion as calcium gluconate over a 15-minute period. Two blood samples were drawn before the infusion to establish a plasma calcium base line. A blood specimen was taken 5 minutes after the infusion was completed and every 10 to 15 minutes for the following 2½ hours, and analyzed for plasma calcium(11) and phosphorus(12). The post-infusion plasma calcium concentrations for each patient were expressed as the per cent increment from his baseline or pre-infusion value. From these serial post-infusion values, the slope of disappearance of the plasma calcium increment was determined by least squares.

Results. The base line plasma calcium concentrations were within normal limits in all patients of both groups. Five minutes after completion of the calcium infusion, the treated hypothyroid group of patients had a mean plasma calcium increment of 24.9 ± 1.9%* above the baseline value as compared with 20.6 ± 1.9%* for the control group. This difference is not statistically significant. The arrays of individual disappearance slopes of the plasma calcium increment for the 2 groups (Fig. 1) were compared by the Wilcoxon 2-sample rank sum test(13) and were

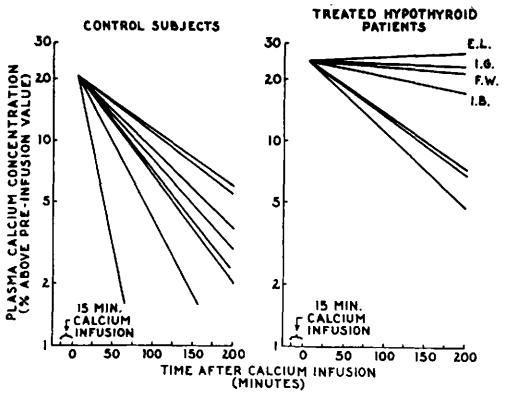

FIG. 1. Semi-log plot of individual disappearance slopes of plasma calcium increment after calcium infusion.

found to be significantly less ($P<0.01$) in the treated hypothyroid group. The half-time of disappearance of the plasma calcium increment, calculated from the mean slope, was 200 minutes for the treated hypothyroid group as compared with 48 minutes for the control group. Because of the small number of patients in each group and the variability within the treated hypothyroid group, the mean slopes were not statistically compared. The changes in plasma phosphorus were small and variable, with no significant difference between the groups.

Discussion. The observations indicate that patients with diminished or absent thyroid functional tissue have normal fasting plasma calcium concentrations, but are unable rapidly to reestablish a normal calcium concentration after a high calcium stimulus. Similar results were observed by Talmage *et al*(6) in thyroidectomized euparathyroid rats, by Sanderson(14) in some of his thyroparathyroidectomized dogs, and by Lowe *et al*(15) in untreated hypothyroid patients. These observations suggest that parathyroid hormone regulation, physico-chemical exchange relationships, and probably other factors can maintain the plasma calcium at a normal level in thyroid-deficient subjects in a non-stressed state, but that some calcium-lowering substance secreted by the thyroid gland is necessary to cope with the challenge of hypercalcemia. This explanation would account for the apparent infrequency of spontaneous hypercalcemia in hypothyroid patients. How-

* Standard error of mean.

ever, there are several reports of hypercalcemia occurring in hypothyroidism. Wilkins (16) reported 3 hypothyroid children who had hypercalcemia without hypophosphatemia. Lowe *et al*(15) reported a hypothyroid patient with spontaneous hypercalcemia while on only a moderate calcium intake. However, this patient became normocalcemic after 3 weeks of desiccated thyroid therapy. Several of their other hypothyroid patients on thyroid therapy had a normal response to high doses of oral calcium, whereas several patients in the hypothyroid state had greater than normal elevation of serum calcium and slow return to the baseline level in response to high doses of oral calcium. The apparent normalization of response of their patients to a calcium load when treated with desiccated thyroid is different from the observations of the present study and is not readily explained. All of the hypothyroid patients in the present study were on desiccated thyroid therapy and were functionally euthyroid. Therefore the observed defect in calcium homeostasis in this group could not be dependent upon hypometabolism or a lack of thyroxine *per se*. It is unlikely that thyrocalcitonin would remain potent in preparations of desiccated thyroid and also would not likely be absorbed intact *via* the gastrointestinal tract. Hahnemann and Friis(17) observed suggestive but not statistically significant differences in restoration of normocalcemia when control and partially thyroidectomized patients were studied at 4 and 20 hours after calcium infusion. However, their patients were apparently euthyroid without replacement therapy and therefore probably retained sufficient functional thyroid tissue to maintain its role in calcium homeostasis. In the present study, there was great variation in the disappearance slopes of the plasma calcium increment among patients in the treated hypothyroid group, suggesting variability in the ability of the thyroid in these patients to secrete a hypocalcemic substance. Two patients (Fig. 1, I.G. and I.B.) who had surgical and I^{131} thyroid-ablative therapy for thyroid carcinoma, and one idiopathic hypothyroid patient (F.W.) who had portrayed clinical and laboratory evidence of severe hypothyroidism, had very prolonged half-disappearance times (2000, 400, and 900 minutes respectively). One patient (E.L.) who had received I^{131} therapy for hyperthyroidism and who had had evidence for profound hypothyroidism before replacement therapy, showed no disappearance of calcium during the 2½ hour period of study. These observations strongly suggest a correlation between the amount of residual functional thyroid tissue and the degree of ability to secrete a hypocalcemic substance.

Hirsch *et al*(18) found that administration of large doses of porcine thyrocalcitonin to the rat caused hypophosphatemia as well as hypocalcemia. However, Foster *et al*(8) did not observe any significant change in serum phosphorus when hypocalcemic doses of thyrocalcitonin were administered to human subjects. Therefore the lack of serum phosphorus change in the present study is not considered evidence against thyrocalcitonin effect.

Summary and conclusions. The response to a hypercalcemic stimulus was studied in normal subjects and in treated hypothyroid patients. There was no difference between the groups in the fasting plasma calcium or the plasma calcium increment immediately following the calcium infusion. However, the disappearance of the plasma calcium increment was markedly prolonged in the treated hypothyroid patients. These observations are interpreted as indirect evidence for the presence and homeostatic function of thyrocalcitonin in man, and a deficiency of thyrocalcitonin in patients with reduced or absent functional thyroid tissue. The full physiological significance of thyrocalcitonin in human calcium homeostasis is not clear.

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Correlation Between Release of Individual Free Fatty Acids and Fatty Acid Composition of Adipose Tissue.* (31381)

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The control of free fatty acid (FFA) release from adipose tissue has been the subject of many studies. Much less attention has been given to the factors influencing the individual FA composition of the mobilized lipid from adipose tissue. The present study attempted to analyze the quantitative relationship between the individual FA released from adipose tissue when incubated *in vitro,* and the total FA composition of the same tissue.

Materials and methods. Young (50-100 g) and adult (more than 400 g) male rats of the Wistar strain were used after an overnight fast under ether anesthesia. Seven experiments were performed, 3 employing adult, and 4 using young rats. Epididymal fat pads of the adult rats were removed, divided into approximately equal parts, and used for the various incubation vessels. Fat pads of several young rats were pooled and aliquots from this pool were incubated.

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Tissues were incubated according to the method of Gordon and Cherkes(1) in Krebs-Ringer phosphate buffer at pH = 7.3-7.4 in the presence of 5% bovine albumin. No glucose or hormones were added to the incubation medium. In 2 experiments, half of the tissues were incubated in the presence of albumin previously treated according to Goodman(2) to remove most of the FFA. In the other half untreated bovine albumin was used. The results obtained with young or adult rats were very similar, as were the ones using untreated or pre-extracted albumin. Therefore data of all experiments were pooled.

To supply a wide range of concentration of the released FFA without employing hormones, in most of the experiments tissue aliquots from the same pool were incubated separately for 15, 45, and 90 minutes. At the beginning and end of incubation, FFA content of the medium was determined by the method of Dole and Meinertz(3) and the