from the blood by filtration through glass wool prevented the appearance of macrophages. More recently, Volkman and Gowans(19) used Rebuck's skin-window technique(20) in the rat, together with isotopic labeling of cells, to investigate the origin of macrophages at inflammatory sites. Their experiments showed that the macrophages were derived from blood and originated in bone marrow; macrophages did not arise from small lymphocytes that traversed the thoracic duct.

Our report further supports this conclusion. Macrophages did develop in cultures of whole blood but did not develop from cultures of either mesenteric lymph or monocyte-free blood. It is possible, however, that our culture conditions may only have been sufficient to support the minor morphological change of monocytes to macrophages and may not have been optimal for the stimulation and differentiation of all potential macrophage precursors.

Summary. 1. Rabbit blood or mesenteric lymph leucocytes cultured *in vitro* for 72 hours with PHA show blast forms and mitoses. 2. Spontaneous blastogenesis and mitosis of such cells takes place at the end of the fifth day. 3. Macrophages develop in 5-day blood cultures whether or not phytohemagglutinin is present. Macrophages do not arise from leucocyte cultures of monocyte-free blood or from lymph. This supports the view that macrophages arise from monocytes in the peripheral blood, and not from lymphocytes.

1. Hungerford, A. D., Donelly, J. A., Nowell, P. C., Beck, S., Am. J. Human Genet., 1959, v11, 215.

2. Berman, L., Stulberg, S. C., Lab. Invest., 1962, v11, 1322.

3. MacKinney, A. A., Jr., Stohlman, F., Jr., Boecher, G., Blood, 1962, v19, 349.

4. Carstairs, K., Lancet, 1962, vI, 829.

5. Marshall, H. W., Roberts, B. K., Quart. J. Exp. Physiol., 1963, v48, 146.

6. Bain, B., Vas, M., Lowenstein, L., Blood, 1964, v23, 108.

7. Bach, F., Hirschhorn, K., Science, 1964, v143, 813.

8. Cooper, H. E., Barkhan, P., Hale, J. A., Brit. J. Haemat., 1963, v9, 101.

9. Nichols, W. W., Levan, A., Blood, 1962, v20, 106.

10. Lycette, R. R., Permain, E. G., Lancet, 1963, vII, 385.

11. Ricke, O. W., Schwarz, R. M., Anat. Rec., 1964, v150, 383.

12. Hirschhorn, K., in Histocompatibility Testing, Nat. Acad. Sci., 1965.

13. Wintrobe, M. M., in Clinical Hematology, Lea & Febiger, 1956, pp212, 215.

14. Elves, M. W., Wilkinson, J. F., Nature, 1962, v194, 1257.

15. Tanaka, Y., Epstein, B. L., Brecher, G., Stohlman, F., Jr., Blood, 1963, v22, 614.

16. Sabesin, M. S., Science, 1965, v149, 1385.

17. Rabinowitz, Y., Schrek, R., Proc. Soc. Exp. Biol. and Med., 1962, v110, 429.

18. Schrek, R., Rabinowitz, Y., ibid., 1963, v113, 191.

19. Volkman, A., Gowans, L. J., Brit. J. Exp. Path., 1965, v46, 50, 62.

20. Rebuck, J. W., Crowley, J. H., Ann. N. Y. Acad. Sci., 1955, v59, 757.

Received January 13, 1966. P.S.E.B.M., 1966, v122.

Effect of Iodide-Deficiency on Thyrocalcitonin Secretion.* (31140)

H. MORII† AND ROY V. TALMAGE
Rice University, Houston, Texas

It has been demonstrated that thyrocalcitonin, the recently identified hypocalcemic principle(1) is released from the thyroid under experimental conditions of induced high calcium levels and is effective in increasing the rate of fall of serum calcium levels following experimental elevation(2). Perfusion of the thyroparathyroid complex, or of the isolated thyroid gland was reported to induce a

*Aided in part by grants from A.E.C. and N.I.D.R.

† Visiting Lecturer from Dept. of Medicine, Univ. of Tokyo.

TABLE I. Serum Calcium Values (mg/100 ml) of Rats Before and After Infusion of $CaCl_2$.

	Preinfusion value	30 min postinfusion	60 min postinfusion
1. Intact	9.9 ± .1 (18)	12.3 ± .2 (14)	10.9 ± .2 (14)
2. Iodide deficient a) 1 wk	9.8 ± .1 (4)	13.3 ± .2 (3)	11.6 ± .4 (3)
b) 2-4 wk	10.3 ± .1 (11)	15.1 ± .1 (8)	13.3 ± .3 (7)
3. I^{131} irradiated	10.1 ± .3 (5)	15.2 ± .1 (3)	14.9 ± .2 (3)
4. Thiouracil administered (2 wk)	9.9 ± .2 (6)	12.6 ± .1 (5)	11.2 ± .2 (5)

Standard error $= \pm \sqrt{\frac{\Sigma d^2}{n(n-1)}}$. Numbers in parentheses = No. of animals.

fall in systemic blood calcium in dogs and goats(3). The purpose of the study reported here was to investigate whether or not the secretion of thyrocalcitonin could be influenced by experimental conditions which are known to affect the established function of the thyroid. The experimental conditions were produced by maintenance of rats on an iodide-deficient diet, by administration of thiouracil, and by destruction of the gland by I^{131} radiation.

Materials and methods. Male Holtzman rats weighing 200-250 g were used in all experiments. To produce hypercalcemia, 2.5 ml/200 g body weight of 0.0739 M calcium chloride was infused at a rate of 0.8 ml/min through a polyethylene tube leading from a constant drive syringe containing the infusion solution and inserted into a lateral wall tail vein of rats anesthetized with ether. One ml of blood was taken by cardiac puncture before infusion and at two 30-minute intervals subsequent to the termination of the infusion. Calcium was determined fluorometrically by autoanalyzer (Technicon, Inc.). Unless otherwise indicated, animals were maintained on stock diet but were fasted overnight before the experiment.

The 3 experimental conditions were produced as follows: (a) Low iodide diet (Remington Diet, Nutritional Biochemicals Corp.) was administered for periods up to 4 weeks as indicated. By 2 weeks the thyroid glands had increased in weight by 50% and were hypertrophic in appearance, with follicles which contained reduced quantities of colloid. (b) Thiouracil was dissolved in slightly alkaline water and administered as a 0.1% solution in drinking water over a 2-week period (4). A slight retardation in body growth and a marked hypertrophy of the thyroid glands with scanty colloid and hypertrophic follicular epithelial cells were observed. (c) I^{131} was injected intraperitoneally as a single dose of 300 μc per rat, 3 days before experimental use in animals maintained on an iodide-deficient diet for 3 weeks(5). Grossly, the thyroid glands were small and firm. Histologically, the glands showed massive fibrosis with only small amounts of colloid remaining.

Results. Following intravenous infusion of calcium, serum calcium values in rats with iodide deficiency returned to normal at a significantly slower rate than their controls, as demonstrated by both the 30-minute and one-hour postinfusion values (Table I) ($p = < .001$). This retardation in the return of the calcium value to normal was only slightly less than that reported earlier(2) for the thyroidectomized animal. Maintenance of the rats on iodide-deficient diet for at least 2 weeks was required in order to produce this effect, though the trend was already observable, but at a borderline statistical significance at the end of one week.

Since thyroid destruction by irradiation was performed only in animals maintained on iodide-deficient diet, the data from the irradiated group suggests only that nearly complete cessation of the hypocalcemic principle was achieved by the iodide-deficient diet alone. The only difference in the serum calcium levels of the 2 groups was for the 60-minute postinfusion value. Since there were only 3 animals in the irradiated group, the statistical significance is questionable.

As has been reported previously by Aliapoulis and Munson(6), there were no significant differences in serum calcium values for either the 30- or 60-minute postinfusion peri-

ods between intact animals and those maintained on thiouracil.

Discussion. If one accepts the premise that an increase in circulating calcium level produces an increased formation and/or release of thyrocalcitonin from the thyroid as has been suggested(2,3), then any experimental condition affecting the thyroid which produces a less rapid return to the preinfusion level following calcium infusion can be presumed to have caused a decrease in the production and/or secretion of thyrocalcitonin. It seems probable from the results of the experiments reported here that prolonged iodide deficiency in some way disrupts the sequence of synthesis and/or release of this new hormone of the thyroid. This is based on the assumption that physiological effects of iodide deficiency are all thyroid mediated. In contrast, it must be pointed out that thiouracil which inhibits the process of oxidation of iodide and the iodination of the thyroglobulin could not be shown to have an effect on thyrocalcitonin release, at least under the experimental conditions used. Furthermore, while iodide has not been shown to be excluded from the thyrocalcitonin molecule, purified preparations(7,8) have not been reported to contain iodide at least on a per mole basis.

Prolonged iodide deficiency produces simple goiter(9,10). This is accompanied for the first few days, at least, by a marked increase in thyroid uptake of administered I^{131}(11) and a progressive increase in thyroid weight significant at least by the 11th day of diet (12). The effects of prolonged maintenance on iodide-deficient diet are less clearly elucidated. Unpublished work from this laboratory indicate that radioisotope uptake values become extremely variable after two weeks on the diet, suggesting thyroid changes other than simple hypertrophy. To explain the differences between thiouracil treatment and maintenance of the animals on iodide deficiency, one must postulate, therefore, that iodide deficiency of long duration produces a disruption of protein synthetic processes in the thyroid gland that includes the cessation of the formation of thyrocalcitonin. Since iodide is not considered to be an integral part of the thyrocalcitonin molecule, one can only conjecture as to the role of this element in the synthetic processes of this newly discovered hormone. From the present investigations, however, it is suggested that a normal content of iodide in the diet may be a prerequisite for the activation of the thyroid hypocalcemic principle.

Summary. Calcium chloride was infused intravenously in iodide-deficient, in I^{131}-irradiated, and in thiouracil-treated rats. Serum calcium values were determined prior to and 30 minutes and 60 minutes after the infusion. Greater responses to calcium loading and retardations of return to the preinfusion levels were observed in iodide-deficient and iodide-deficient I^{131}-irradiated animals. Thiouracil-treated animals showed normal response. It was concluded that normal content of iodide in diet may be necessary for activation of thyrocalcitonin, the thyroid hypocalcemic principle.

1. Hirsch, P. F., Gauthier, G. F., Munson, P. L., Endocrinology, 1963, v73, 244.

2. Talmage, R. V., Neuenschwander, J., Kraintz, L., ibid., 1965, v76, 103.

3. MacIntyre, I., Foster, G. V., Kumar, M. A., in The Parathyroid Glands: Ultrastructure, Function and Secretion, Gaillard, P. J., Talmage, R. V., Budy, A. M., eds., Univ. of Chicago Press, Chicago, 1965, 89.

4. Zarrow, M. X., Money, W. L., Endocrinology, 1949, v44, 345.

5. Goldber, R. C., Chaikoff, I. L., Lindsay, S., Feller, D. D., ibid., 1950, v46, 72.

6. Aliapoulis, M. A., Munson, P. L., personal communication, 1965.

7. Baghdiantz, A., Foster, G. V., Edwards, A., Kumar, M. A., Slack, E., Soliman, H. A., MacIntyre, I., Nature, 1964, v203, 1027.

8. Tenenhouse, A., Arnaud, C., Rasmussen, H., Proc. Nat. Acad. Sci., 1965, v53, 818.

9. Taylor, S., Poulson, E., J. Endocrinol., 1956, v13, 439.

10. Axelrod, A. A., Leblond, C. P., Isler, H., Endocrinology, 1955, v56, 387.

11. Money, W. L., Rall, J. E., Rawson, R. W., J. Clin. Endocrinol., 1952, v12, 1495.

12. Maloof, F., Dobyns, B. M., Vickery, A. L., Endocrinology, 1952, v50, 612.

Received January 14, 1966. P.S.E.B.M., 1966, v122.