Thyrocalcitonin: The Second Hormonal System of the Thyroid Gland

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This review of our state of knowledge of an aspect of the thyroid gland is published in order to keep biology teachers alert to something which will probably show up in general textbooks several years from now. The senior author is an Associate Editor.

Research into gross human anatomy was essentially completed by the end of the 19th century. Insulin was first crystallized from the pancreas in 1926 and its complete amino acid sequence established in 1954. In addition, it was the first naturally occurring protein ever to be synthesized, a major scientific event in 1965. Today, biologists are working out evolutionary sequences by comparing amino acid sequences in proteins from various organisms. Geneticists are working with products of single genes, and embryologists can obtain differentiation in tissue culture from a single cell. It is perhaps correct to estimate that most of today's endocrinologists are primarily concerned with either of two major problems: The mechanisms which control hormone release and the mode of hormone action at the cellular level.

However, within the last five years, it has been determined that yet another hormone is produced by the human body—one that has gone unrecognized until now in the biology research explosion. This hormone, which has the ability to lower blood calcium levels, is now called thyrocalcitonin and is the second hormone known to be produced by the thyroid gland. The well-known thyroid hormone from this gland, which regulates oxidative metabolism in mammals, has been recognized for centuries. Kendall isolated thyroxine, the major thyroid hormone component, from the thyroid gland on Christmas Day in 1914 and Hems and associates synthesized the amino acid thyroxine, starting with the tyrosine molecule, in the late 1940's. Our purpose is to describe the events that led to the recent discovery of the second hormone of the thyroid gland and to summarize what is currently known of its structure and function.

Until recently, it was assumed that calcium homeostasis was maintained solely by the presence or absence in the blood stream of parathormone, which mobilizes calcium from tissues. It appeared that a rise in the blood calcium level (hypercalcemia) would suppress the production and release of parathormone from the parathyroid glands, whereas a drop in blood calcium level (hypocalcemia) would stimulate the production and release of parathormone. In 1961, Rasmussen suggested that this hypothesis was untenable in that such a mechanism would result in wide fluctuations in calcium levels because parathormone acts relatively slowly. That same year, Copp and co-workers found that crude parathyroid gland extracts, upon being injected into dogs, caused an initial, rapid lowering in the plasma calcium level which was then followed by a rise to higher than normal calcium level. The second effect of an increased calcium level in the blood was ex-

pected because parathormone release, in response to the lowered calcium level would occur, and cause an increase in calcium mobilization. However, the first observation of a drop in blood calcium suggested that a second hormone was present in the parathyroid gland extract which had an antagonistic action to that of parathormone. This second hormone was then named *calcitonin.*

The apparent question at this point was: Does calcitonin act directly on blood calcium levels or does it simply inhibit parathormone release? To this end, Copp and his associates (1962) perfused the thyroid-parathyroid apparatus in the dog with solutions that were high in calcium. The resulting perfusate, when injected into the blood stream, lowered plasma calcium levels within 15 minutes. However, removal of the parathyroid glands (parathyroidectomy), which would be the same as loss of parathormone, did not result in a lowering of plasma calcium levels for one or two hours. Thus, apparently the thyroid-parathyroid perfusate included a humoral agent which acted by swiftly and directly lowering blood calcium levels rather than by means of parathormone suppression.

There is a good precedent for two hormones with opposite effects being produced by the same gland. Crude insulin extracts, which contain some glucagon, initially cause transient high blood sugar levels (hyperglycemia) in response to the glucagon, followed by a lowering in blood sugar levels (hypoglycemia) in response to insulin. The diabetic whose insulin production is impaired, must walk a tightrope between these two extremes.

However in retrospect, it now appears that the physical proximity of the thyroid and parathyroid glands (Fig. 1) was once again interfering with understanding the functions of the two glands. Until the last century, it was thought that in the human, the thyroid gland was essential for life because its extirpation resulted in a fatal tetany within a few weeks. Eventually, it was recognized that the parathyroid glands, which lie closely apposed to the thyroid gland, were also being removed unnoticed along with the thyroid gland. The resultant loss of parathormone caused a severe drop in blood calcium levels which soon caused tetany and death. The

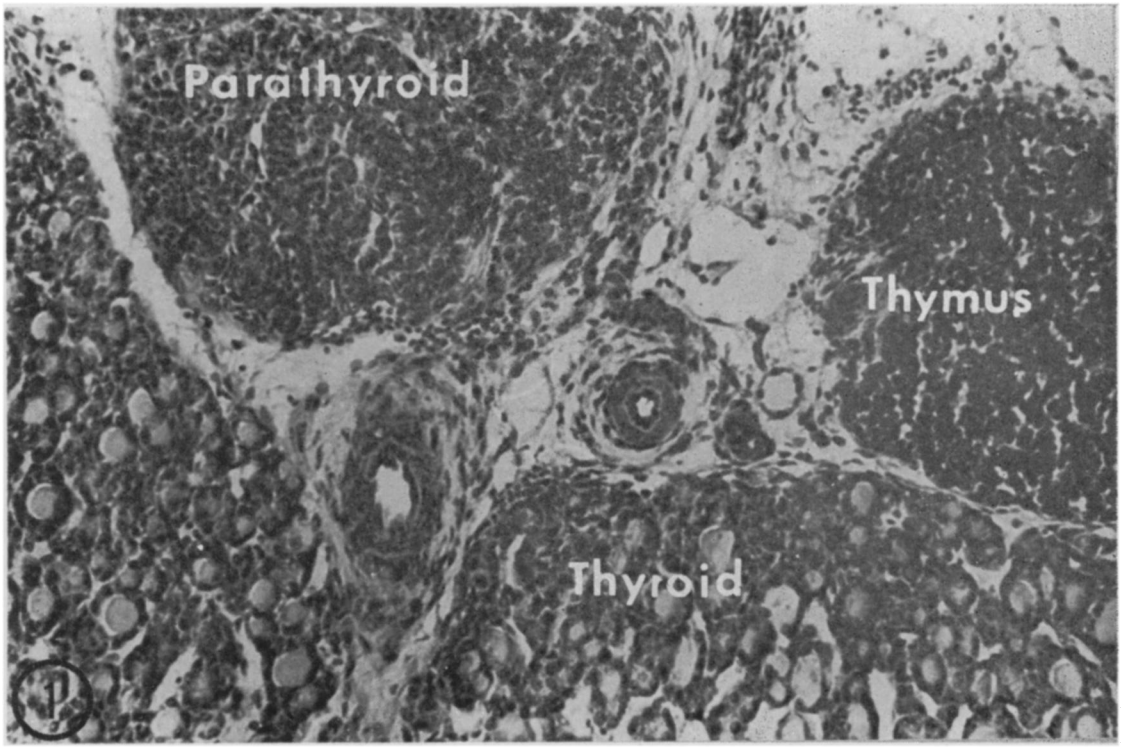

Fig. 1. Histological preparation of thyroid gland region from a 19-day chick embryo. Note close proximity of thyroid, thymus, and parathyroid glands. Numerous follicles, with colloid centers, may be observed in the thyroid. X 67. PAS Stain.

thyroid gland contributes to physiologic well-being, and its absence results in a slow onset of cretinism.

Thus, on the trail of the source of calcitonin, subsequent investigations were designed to differentiate between the thyroid and parathyroid glands as the possible sources of the hormone. Hirsch (1963) and co-workers found that in the rat, cautery of the thyroid gland resulted in an immediate lowering of plasma calcium levels, again more quickly and to a greater extent than the lowering resulting after parathyroidectomy. They suggested that cautery of the thyroid gland was provoking the release of a hypocalcemic substance from the thyroid gland and hence, it should be called *thyrocalcitonin* (also abbreviated as TCT). Further, these investigators found that an hydrochloric acid extract of thyroid tissue caused a significant decrease in the plasma calcium levels of parathyroidectomized animals.

A third type of experiment added confirmation to the thyroid gland as the source of calcitonin (Foster *et al.*, 1964). After perfusion of the goat thyroid gland (which is anatomically separate from the parathyroid gland in this species) with high calcium loads and returning the perfusate to the circulation, a drop in plasma calcium level occurred. Parathyroid gland perfusion failed to yield a perfusate with hypocalcemic ability.

Independently, other investigators confirmed the existence of thyrocalcitonin, a hormone with the ability to lower plasma calcium levels, whose origin is the thyroid gland. At this point, many workers were attracted into the field and imaginatively are now attacking the obvious questions that arise about thyrocalcitonin.

A. *What is the source of the hormone within the thyroid gland?*

In the dog and human thyroid gland, two cell populations may be recognized, the acinar or follicular cells, composing each thyroid follicle around a core of colloid, and the parafollicular cells which are external to the follicles. Included in the parafollicular cells are the so-called "mitochondrion-rich cells" or "C-cells" which display histochemical changes corresponding to fluctuations in plasma calcium levels indicating that they might be the source of thyrocalcitonin (Foster *et al.*, 1964; Pearse, 1966). We have observed distinct parafollicular cells in the 19-day old chick embryo (Fig. 2). On the other hand, Hargis *et al.* (1966) using fluorescent antibody techniques, found thyrocalcitonin to be present in essentially all of the follicle cells in the thyroid gland although it was absent in the colloid. If the purified thyrocalcitonin from which their antibody was derived had no thyroidal contaminants, this would suggest that thyrocalcitonin is elaborated by the same follicular cells that produce thyroid hormone. Homogenized thyroid glands yield a cytoplasmic fraction, which may contain secretory granules that have a hypocalcemic effect when injected into rats (Bauer and Teitelbaum, 1966).

B. *How is the output of thyrocalcitonin by the thyroid gland controlled?*

If the thyroid gland is removed from an animal, calcium levels in the blood gradually rise above normal (Care *et al.*, 1966). The simplest mechanism to control thyrocalcitonin output would be one based on blood calcium levels. An increase in blood calcium would elicit the release of thyrocalcitonin whereas a decrease would inhibit thyrocalcitonin release.

Thyroid hormone release is under the direct control of the pituitary gland which responds to yet another center, the hypothalamus. Thus far, these centers have not yet been implicated in the control of thyrocalcitonin release. This area should indeed receive much more exploration.

C. *What is the nature of the thyrocalcitonin molecules?*

Several laboratories, including those of pharmaceutical houses, are now attempting to isolate and to analyze the hormone. In working with any hormone, the most important parameter is that of hormone activity. Thus, the bioassay, or a quantitative measure of hormone effect in a living system, is essential so that various investigators can have a common ground for using a hormone and communicating with each other about it. Also, the elaborate biochemical procedures used to isolate a hormone or to cause its *in vitro* or total synthesis may result in a substance that has the proper identification signs but that has impaired activity. Since thyrocalcitonin

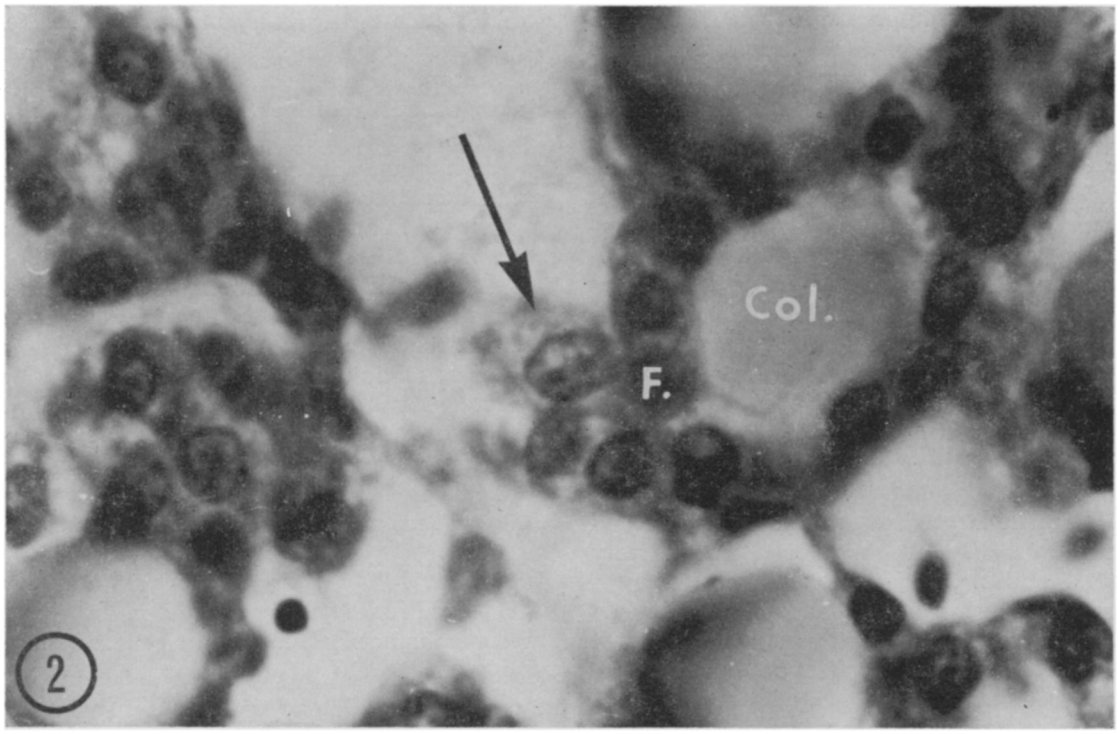

Fig. 2. Enlarged view of thyroid gland cross-section from a 19-day chick embryo. A probable parafollicular cell is indicated by the arrow. Note its large, pale nucleus and more abundant, light-staining cytoplasm in comparison to the follicle cells. (Col. = Colloid; F = Follicle cell) X 1066. PAS Stain.

is a hormone only by virtue of its activity, a bioassay for it was quickly established. The Medical Research Council Department of Biological Standards has prepared a standard thyrocalcitonin extract possessing one unit of activity per approximately 40 mg of material. Ten milliunits injected into a 150 g rat cause a 10% reduction in plasma calcium level after 50 minutes. A bioassay procedure based on the MRC unit and involving intravenous injection was proposed by Kumar *et al.* (1965). Recently, a simpler and faster bioassay method was developed which utilizes subcutaneous injection into the rat followed by a rapid fluorometric determination of plasma calcium level (Schlueter and Caldwell, 1966).

Thyrocalcitonin is soluble in acid solutions and loses its activity after incubation with trypsin and pepsin, which split protein molecules (Baghdiantz *et al.*, 1964). In 1965, Tenenhouse found that thyrocalcitonin is a single polypeptide chain with a molecular weight approximating 8700. As is usually true when a new protein is being isolated, the estimated molecular weight goes down as research proceeds. Very recently, density-gradient centrifugation techniques have indicated a molecular weight for thyrocalcitonin of between 5000 and 6000 (O'Riordan *et al.*, 1966).

D. *What is the site of action of thyrocalcitonin?*

Logically, there are two major systems which could respond to thyrocalcitonin and remove calcium from the blood: kidney and bone. Excretion of calcium by the kidneys and deposition of calcium in bones would serve to take calcium out of circulation. Although scientists disagree about aspects of thyrocalcitonin research, the work on the site of action all points to bone. Thyrocalcitonin is effective in rats whose kidneys have been removed. Also, when blood calcium levels lower in response to thyrocalcitonin, so do inorganic phosphate levels which is consonant with the notion that bone salts are being deposited (Hirsch *et al.*, 1964). In addition, after thyrocalcitonin injection, the kidneys, salivary glands and gut fail to incorporate additional calcium (Kenny and Heiskell,

1965). Parathormone is able to cause calcium resorption from bone into the blood stream. There is some indication that TCT may act by preventing calcium resorption from occurring, thus counteracting the effects of parathormone.

The stimulation of calcium release from bones that are in tissue culture by parathormone is inhibited by the addition of thyrocalcitonin (Friedman and Raisz, 1965; Aliapoulios *et al.*, 1966). Several other recent investigations not cited here add further support to the notion that TCT acts primarily on bone calcium.

E. *Will thyrocalcitonin have important therapeutic and clinical uses?*

Because large quantities of highly purified thyrocalcitonin are not yet available, very little clinical testing has occurred. However, as methods are developed which will yield the hormone in pure form and in sufficient quantity, clinical applications should increase. Thyrocalcitonin activity has been found in human thyroid glands (Aliapoulios *et al.*, 1966). Recently, the hormone was found to be helpful in treating idiopathic hypercalcemia in infant twins (Milhaud and Job, 1966).

In elderly persons, extensive calcium resorption from the bones often occurs. This condition, known as osteoporosis, causes bones to fracture easily and to require long periods in which to mend. Conceivably, thyrocalcitonin might prevent some of the deterioration that occurs in bones with aging. Thyrocalcitonin will undoubtedly be of use in managing the relatively few and rare diseases that are accompanied by high blood calcium levels.

F. *Other possible aspects of the biological role of thyrocalcitonin.*

The majority of research thus far on thyrocalcitonin has emerged from laboratories affiliated with medical centers and only mammals have been used in the studies. Thus, the question of thyrocalcitonin's evolutionary significance has yet to be answered. We have preliminary evidence that an hydrochloric acid extract of embryonic chick thyroid gland lowers plasma calcium levels in the newly hatched chick within 5 minutes. Ultimately, we hope to investigate lower vertebrates in addition to birds. Every mammalian species examined at this point, including the dog, rat, rabbit, pig, sheep, goat, ox, monkey, deer and man produces active thyrocalcitonin. However, the concentration of the hormone in the thyroid gland varies from species to species and there is some indication of species-specificity with respect to the degree of hormone activity.

It is hoped that possible roles of thyrocalcitonin in developing organisms will receive attention. Thyrocalcitonin could be active in bone formation during embryogenesis and in young animals. Foreleg bones from mouse embryos in tissue culture respond to thyrocalcitonin by increased bone deposition (Gaillard, 1965). Bone growth in regenerating systems may also be affected by thyrocalcitonin.

In the 1960's, a hormone was found that had been functioning perfectly well for most people, unnoticed, all of their lives. Hopefully, by the 1970's, the structure of thyrocalcitonin, its mode of action, and its control mechanisms in various organisms throughout their life cycles will have been determined.

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Summer Institutes for College Biology Teachers

The National Science Foundation continues its support of institutes already well established for secondary school teachers. The following are the opportunities available for college teachers.

Arizona State University, Tempe, Arizona 85281. Desert Biology; June 19-July 29. Dr. Gordon L. Bender, Department of Zoology.

Auburn University, Auburn, Alabama 36830. Biology; June 12-August 22. For junior college teachers. Professor Eldon J. Cairns, Department of Botany and Plant Pathology.

University of Illinois, Urbana, Illinois 61801. General Parasitology; June 21-August 1. Dr. Norman D. Levine, College of Veterinary Medicine.

Oregon State University, Corvallis, Oregon 97331. General Biology; June 26-August 4. Dr. David L. Willis, General Science Department.

University of Puerto Rico, Rio Piedras, Puerto Rico 00931. Marine Biology and Tropical Ecology; June 19-28. Dr. Herminio Lugo Lugo, Office of the Dean of Studies.

Stanford University, Pacific Grove, California 93950. Marine Biology; June 12-August 19. Professor John H. Phillips, Hopkins Marine Station.

Tulane University, New Orleans, Louisiana 70118. Parasitology; June 1-August 31. Dr. Franklin Sogandares-Bernal, Department of Biology.

Williams College, Williamstown, Massachusetts 01267. The Organism; June 26-August 4. Professor Allyn Waterman, Department of Biology.

Bacteriology Exercises

The Bacteriology Committee of the American Phytopathological Society has prepared a booklet of laboratory exercises on the use of plant bacterial pathogens and is prepared to send cultures of three pathogens. Inquiries should be addressed to Professor Robert N. Goodman. Department of Horticulture, University of Missouri, Columbia.

Upon receipt of such requests, two forms will be sent the teacher. One form is to be completed and returned with $2.00 to Professor Goodman. The booklet and cultures will then be sent.

Requests from Hawaii cannot be honored, and those from Michigan must be accompanied by a state permit.

Ovary

The weight of the human ovary increases to a maximum of 10 gm by age 20. It remains at this level until age 30 at which there is a gradual decline to about 4 gm by age 50.