the placenta of the elephant and those of the carnivores probably represents convergent rather than divergent evolution (*1*).

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Thyrocalcitonin: Hypocalcemic Hypophosphatemic Principle of the Thyroid Gland

Abstract. *A factor that lowers serum calcium and inorganic phosphate in rats has been purified 500-fold from 0.1*N *HCl extracts of hog thyroid glands. It is distinct from thyroxine and triiodothyronine and appears to be a polypeptide.*

Thyrocalcitonin (*1*) is the name given to a hypocalcemic principle readily extracted from thyroid tissue of numerous mammalian species. It was discovered (*2*) as an outgrowth of an investigation of the difference in the acute effect on serum calcium in the rat between parathyroidectomy by cautery and parathyroidectomy by surgical excision (*3*). Apparently, cautery of the thyroid gland during the operation of parathyroidectomy stimulates release of thyrocalcitonin, provoking a greater fall in serum calcium than that which occurs after simple removal of the parathyroid glands.

Since extracts of numerous other tissues, liver, kidney, and salivary gland of the rat, and thymus, pituitary gland, and parathyroid gland of the ox were found not to be hypocalcemic in the rat, thyrocalcitonin-like activity appears to be present in high concentration only in the thyroid gland. Furthermore, the effects of thyrocalcitonin are not duplicated by thyroxine or triiodothyronine.

We have demonstrated thyrocalcitonin activity in extracts of thyroid tissue of the rat, rabbit, dog, hog, ox, and monkey. In addition, Foster *et al.* have prepared an extract of goat thyroid gland that is hypocalcemic in the goat (*4*), and Foster and Hirsch have reported hypocalcemic effects of thyroid extracts in the dog (*5*). Marked hypocalcemic activity in the rat was found in the extract of one sample of human thyroid tissue obtained at surgery, but six other human samples were inactive.

Subcutaneous injection of the extract of as little as one-third of a single rat thyroid gland into an intact rat results in a fall of 20 to 30 percent in serum calcium below the normal level within 1 hour. Hog thyroid extract, selected for purification because of easy availability in quantity, is also very active; the extract of one hog thyroid gland is sufficient to produce a significant fall in serum calcium in more than 1000 rats. Contamination with parathyroid tissue was not a problem since the parathyroid glands of the hog are embedded in the thymus gland at sites remote from the thyroid gland (*6*).

Chemical and physical properties, including behavior during purification, indicate that thyrocalcitonin is a polypeptide. Ashing destroys the activity, an ether extract is inactive, and the activity is lost during treatment with pepsin or trypsin.

A three-step procedure consisting of ultracentrifugation, fractionation on Sephadex G-50, and fractionation on carboxymethyl-Sephadex G-25 has resulted in a 500-fold purification of thyrocalcitonin from the starting extract, which is prepared by homogenizing fresh hog thyroid tissue with 10 ml of 0.1*N* HCl per gram of tissue for 30 seconds in a Waring blender in the cold. The supernatant obtained by centrifuging the homogenate at 600*g* for 30 minutes in an International refrigerated centrifuge can be stored frozen for at least 6 months without loss of activity. A nine- to tenfold purification of this starting extract is achieved by centrifuging it at 100,000*g* for 24 hours in the No. 40 rotor of the Spinco preparative ultracentrifuge. Presumably, the inactive sedimented material is largely denatured thyroglobulin.

A biological assay method was developed to guide purification. The test animals are intact male rats (150 to 180 g) (Holtzman Co.) maintained on a purified diet low in calcium for 4 days before the assay. (Rats on a stock diet react in a similar manner, but their responses are somewhat more variable.) Standard and unknown solutions are injected subcutaneously into parallel groups of test rats. One hour later, blood samples are drawn by cardiac puncture under ether anesthesia, and the serum calcium is analyzed (*7*). A dose-response curve for thyrocalcitonin is shown in Fig. 1. The relative potencies of the unknowns in arbitrary units per milligram of nitrogen are calculated from the results of the bioassay by standard statistical procedures (*8*) and from analysis of nitrogen content by

Table 1. Effect of thyrocalcitonin on serum calcium and inorganic phosphate in rats. There were five test rats in each group, treated as described for thyrocalcitonin assay. Thyrocalcitonin or vehicle was injected subcutaneously 3 hours after parathyroidectomy by surgical excision. Blood was drawn for analysis 1 hour after the injection. The standard errors were: for calcium, 0.2 mg/100 ml; for phosphate, 0.4 mg/100 ml.

Thyrocalcitonin (units/rat)	Serum values (mg/100 ml)	
	Ca	Inorganic P
	Intact	
0	9.3	11.1
10	7.2	8.5
	Parathyroidectomized	
0	8.1	12.4
10	6.0	10.8

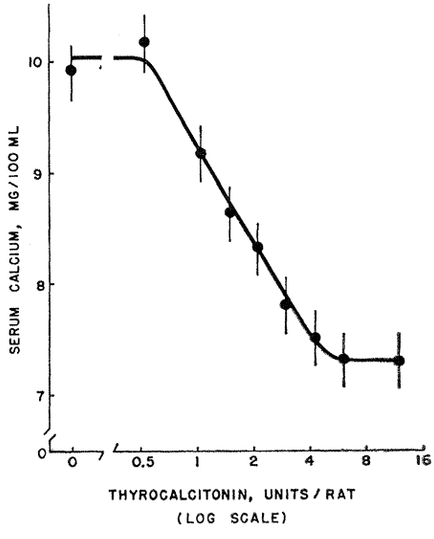

Fig. 1. Log dose-response curve for hog thyrocalcitonin in young male rats. Blood was drawn for calcium analysis 1 hour after subcutaneous injection of the extract. Each point represents the mean value for seven rats, and the vertical lines represent the standard errors.

the method of Lowry *et al.* (*9*). The mean index of precision (λ) of ten consecutive recent assays is 0.23 ± 0.025.

The arbitrary unit is 10 μg of nitrogen of a standard preparation (THV-44-D) of supernatant from ultracentrifuged hog thyroid extract. One unit produces a statistically significant fall in serum calcium (about 1 mg/100 ml) in test rats.

Additional purification of thyrocalcitonin was achieved by gel filtration. In a typical preparation, 5 g of lyophilized ultracentrifuged supernatant fluid containing 300 mg of nitrogen (obtained from 170 g of thyroid tissue) was applied to a column of Sephadex G-50 (3.8 by 90 cm), equilibrated with 0.05*M* sodium acetate buffer (*p*H 3.8). On elution with the same buffer, a large inactive protein peak first emerged, then a smaller protein peak, and then a nucleotide fraction (*10*). The hypocalcemic activity was associated with the descending limb of the smaller protein peak and the ascending limb of the nucleotide fraction. When the most active fractions (containing 15 mg of nitrogen) were pooled, they represented a tenfold increase in specific activity with a yield of 40 to 60 percent. This product was applied to a column of carboxymethyl-Sephadex G-25 (1.9 by 14 cm), equilibrated with the same buffer as before. On elution with the same buffer, increased stepwise from 0.05*M* to 0.2*M*, only nucleotides were eluted. Protein was eluted on changing to a gradient of sodium chloride increasing to 2.0*M*, in 0.2*M* sodium acetate (*p*H 3.8). The hypocalcemic activity was associated with the descending portion of the protein peak. The pooled active fractions showed an additional fivefold improvement in specific activity with a yield of 70 percent in this step.

Although the site of action of thyrocalcitonin is not yet known, that there may be a direct effect on bone, important physiologically, is an intriguing possibility. The kidney is not essential for the hypocalcemic effect, since thyrocalcitonin is active in nephrectomized rats. The activity of thyrocalcitonin in rats fed a low calcium diet suggests that decreased absorption of calcium from the gut is not responsible for the hypocalcemic effect. Since thyrocalcitonin is active in parathyroidectomized rats (*2*), it does not produce its effect by an action on the parathyroid gland or on parathyroid hormone. The parallel decreases in serum inorganic phosphate (*11*) and serum calcium (Table 1), also noted by Kenny (*12*), are consistent with an effect on the deposition of bone salt.

Elegant experiments on the perfusion of the thyroid and parathyroid glands of dogs (*13*), later extended to sheep (*14*), led Copp *et al.* to introduce the concept of a hypocalcemic hormone, named calcitonin, working antagonistically and in concert with the parathyroid hormone in the normal regulation of the calcium concentration in plasma. This work was confirmed independently by Kumar *et al.* (*15*). Although many of the experiments by Copp *et al.* did not discriminate between the thyroid gland and the parathyroid gland as the site of origin of calcitonin, some experiments pointed to the parathyroid gland as the source of calcitonin. On the other hand, studies in the goat (*4*) and in the rat (*16*) support the conclusion that the newly recognized hypocalcemic principle originates in the thyroid rather than the parathyroid gland. Whether or not thyrocalcitonin is the same as calcitonin, its marked activity and easy availability encourage further investigation of its chemistry, pharmacology, and therapeutic potentialities.

Note added in proof: Since this report was submitted, Baghdiantz *et al.* (*17*) have published an alternative method of partial purification of thyrocalcitonin.

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25 August 1964

Chromatography of Ribonuclease-Treated Myosin Extracts from Early Embryonic Chick Muscle

Abstract. *Chromatography, on diethylaminoethyl cellulose, of leg muscle extracts from older chick embryos yields three myosin-containing fractions, whereas material from muscle extracts of 11-day embryos cannot be eluted under the same conditions. A major fraction having the adenosine triphosphotase activity characteristic of myosin can be eluted when 11-day-old embryo muscle extracts are given prior treatment with ribonuclease.*

The preparation of myosin from chick embryos in the form of a single homogeneous fraction has not yet been achieved. The preparation of such material is essential for immunological studies and measurements of tracer incorporation into myosin during embryonic development. Column chromatography, on diethylaminoethyl (DEAE)-cellulose, of muscle extracts from chick embryos 14 days old and older yielded a chromatographically heterogeneous myosin fraction. Under the same conditions of fractionation, extracts from muscle of embryos 11 days old or less gave no protein in the eluate with properties of myosin. Because of the high RNA content of embryonic muscle tissue and the presence of RNA-like material (extractable with hot trichloroacetic acid, and showing a positive orcinol reaction) in myosin fractions from older embryos, ribonuclease treatment of the 11-day embryo muscle extracts was carried out prior to chromatography. Extracts from 11-day chick embryos, from which no myosin fraction could be recovered