Table 1. RNA synthesis in liver nuclei from rats injected with cyclic AMP or saline (controls). Nuclei were incubated for 5 minutes at 37°C in a medium containing 100 μM each of cytidine triphosphate; guanosine triphosphate, and uridine triphosphate; 12 μM [8-^{14}C]ATP (30 mc/mmole); 0.2M sucrose; 3.2 mM $MgCl_2$; and 8 mM tris-HCl (pH 8.3). Portions were placed on Whatman 3MM filter paper disks, washed three times in 5 percent trichloroacetic acid–1 percent sodium pyrophosphate, two times in ether, and dried; the radioactivity was determined by liquid scintillation counting.

Condition	Radioactivity in RNA (count/min per mg DNA)	Stimulation (%)
Control	2097	
Cyclic AMP	3077	47
Control	1768	
Cyclic AMP	2444	38
Control	1857	
Cyclic AMP	2288	23
Control*	698	
Cyclic AMP*	1128	62
Control*	807	
Cyclic AMP*	1362	69
Control*	1467	
Cyclic AMP*	2183	49
Control†	2912	
Cyclic AMP†	3950	36
Control†	1937	
Cyclic AMP†	2606	35
Control*†	2270	
Cyclic AMP*†	2870	26

* Adrenalectomized rats. † Incubation was performed in the presence of a saturating amount of *E. coli* RNA polymerase.

creased capacity for RNA synthesis as compared with controls. The results from nine experiments are summarized in Table 1, where it can be seen that the degree of stimulation ranged from 23 to 69 percent in different experiments. Similar results are obtained when the experiments are performed in adrenalectomized rats, an indication that we are not dealing with a secondary effect caused by the release of adrenal steroids. We have also considered the possibility that the observed stimulation is due to a release of insulin induced by cyclic AMP (*1*), which in turn causes the increase in RNA synthesis (*8*). However, unlike the results in our system, the effect of insulin on RNA synthesis cannot be observed in the absence of added *Escherichia coli* RNA polymerase (*8*); thus it appears unlikely that we are dealing with a secondary effect of insulin.

Comparison of the kinetics of RNA synthesis in nuclei from control animals and animals treated with cyclic AMP indicates that the stimulation is apparent within the first minute of incubation (Fig. 1); thus the differences we observe are probably not artifacts generated during prolonged incubation of the nuclei. The effect on RNA synthesis is highly specific for cyclic AMP, because treatment of animals with the closely related nucleotide, adenosine 5′-monophosphate (5′-AMP), causes no detectable change in RNA synthesis (Fig. 1).

In order to determine whether the observed stimulation was due primarily to an increase in the activity of RNA polymerase or to an increase in the template activity of DNA, experiments were performed in which the nuclei were incubated in the presence of saturating amounts of exogenous *E. coli* RNA polymerase (Table 1). Nuclei from cyclic AMP–treated animals retained their increased capacity for RNA synthesis, so that we are dealing with a possible increase in template acitvity. However, in view of the obvious differences in mammalian and bacterial polymerases, one must be cautious in interpreting such findings.

Our results indicate that under conditions where cyclic AMP is known to induce formation of specific hormone-inducible enzymes in rat liver a dramatic increase in the capacity for nuclear RNA synthesis occurs. Although this finding is consistent with the proposed role of histone phosphorylation induced by cyclic AMP in altering gene transcription, one must be cautious in interpreting such findings. First of all, the observed increase of roughly 25 to 50 percent in RNA synthesis is much greater than would be needed or expected during the induction of a small number of enzymes. Furthermore, there is reason to believe that many of the effects of cyclic AMP on the induction of enzyme synthesis occur at levels other than transcription (*1, 4*).

Nonetheless, our experiments do demonstrate that cyclic AMP administration has dramatic effects on the capacity for RNA synthesis in rat liver nuclei, and our results are consistent with the findings that cyclic AMP can alter gene transcription in bacterial systems (*1*). Thus the effect of cyclic AMP on transcriptional activity in higher organisms needs to be considered and its significance must be determined before we have a complete understanding of the role of this nucleotide in the induction of enzyme synthesis.

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Thyrocalcitonin: Stimulation of Secretion by Pentagastrin

Abstract. *Administration of a small dose of pentagastrin, a synthetic pentapeptide containing the biologically active portion of the native hormone gastrin, results in a marked, rapid, transitory increase in thyrocalcitonin secretion in the pig. Gastrin or a related gastrointestinal peptide may be important in the physiological secretion of thyrocalcitonin, such as that which occurs when calcium salts are introduced into the gastrointestinal tract.*

It is well established that secretion of the hypocalcemic, hypophosphatemic hormone, thyrocalcitonin, can be regulated directly by the blood calcium concentration and that secretion of this hormone by the mammalian thyroid gland in response to a calcium load may afford protection against hypercalcemia (*1*). The original evidence for these conclusions involved nonphysiological procedures unrepresentative of situations encountered in normal life, for example, injections of high doses of calcium salts, parathyroid hormone, or vitamin D. However, Gray and Munson (*2*) have demonstrated a protective action of the rat thyroid gland against hypercalcemia after oral administration of small doses of calcium salts; thyroid-intact rats remained normocalcemic because of increased secretion of thyrocalcitonin, while thyroidectomized rats rapidly developed hypercalcemia. Subsequently, direct measure-

ment of thyrocalcitonin secretion in pig thyroid vein blood by radioimmunoassay (*3*) showed that thyrocalcitonin secretion increased after oral administration of calcium even in the absence of a detectable hypercalcemia. Since the proposed stimulus for secretion, hypercalcemia, usually could not be demonstrated, the problem was to identify the nature of the physiological stimulus which caused the secretion of thyrocalcitonin after oral administration of calcium.

We had suggested (*3*) that thyrocalcitonin-secreting cells in the thyroid gland might be so exquisitely sensitive to the blood calcium concentration that they responded to minute, analytically undetectable increases in blood calcium resulting from intestinal absorption of calcium. We (*3*) and, independently, Care (*4*) also considered an attractive alternative hypothesis, that the presence of calcium in the gastrointestinal tract (or its absorption therefrom) might have caused secretion of a gastrointestinal hormone which, in turn, signaled the thyroid gland to secrete thyrocalcitonin. In his preliminary report, Care (*4*) indicated that addition of porcine pancreozymin to blood perfusing the isolated pig thyroid gland produced a modest (twofold) increase in thyrocalcitonin in the venous effluent.

We now report that a large increase in thyrocalcitonin secretion, as much as 40-fold, occurs in the pig after a small intravenous dose of pentagastrin (*5*), a synthetic pentapeptide containing the active carboxyterminal tetrapeptide amide of gastrin, the hormone secreted by the mucosa of the gastric antrum (*6*). A stimulation of this magnitude by calcium alone requires production of severe hypercalcemia (12 to 15 mg of calcium per 100 ml) by intravenous infusion of large doses of calcium chloride.

Table 1 summarizes results obtained in four separate consecutive experiments, each involving a single pig. With the animal under halothane-oxygen anesthesia, thyroid venous effluent blood was completely and continuously collected from the surgically isolated, in situ thyroid gland (*7*). Concentrations of thyrocalcitonin in the thyroid effluent plasma samples (collected at 10- to 15-minute intervals) were determined by radioimmunoassay (*8*). Plasma samples also were analyzed for total calcium and inorganic phosphate by automated methods (*9*). Injections or infusion of pentagastrin via the femoral vein at doses of 0.26 to 2.6 μg per kilogram of body weight (0.34 to 3.4 nmole/kg) caused rapid, striking increases in the concentration of thyrocalcitonin in blood leaving the thyroid gland. Injections of secretin, betazole, or pancreatic glucagon had little or no effect at the doses tested (Table 1). Increased secretion of thyrocalcitonin occurred within the first collection period (6 to 12 minutes) after administration of pentagastrin. Pretreatment baseline levels were regained within about 30 minutes after injection or cessation of infusion of pentagastrin. Single injections of as little as 0.26 μg of pentagastrin per kilogram produced a five- to tenfold increase in secretion of thyrocalcitonin while doses of 2.6 μg/kg produced a 20- to 40-fold increase. A single test of 0.026 μg/kg showed little effect. Blood calcium concentrations remained unaltered except at the end of

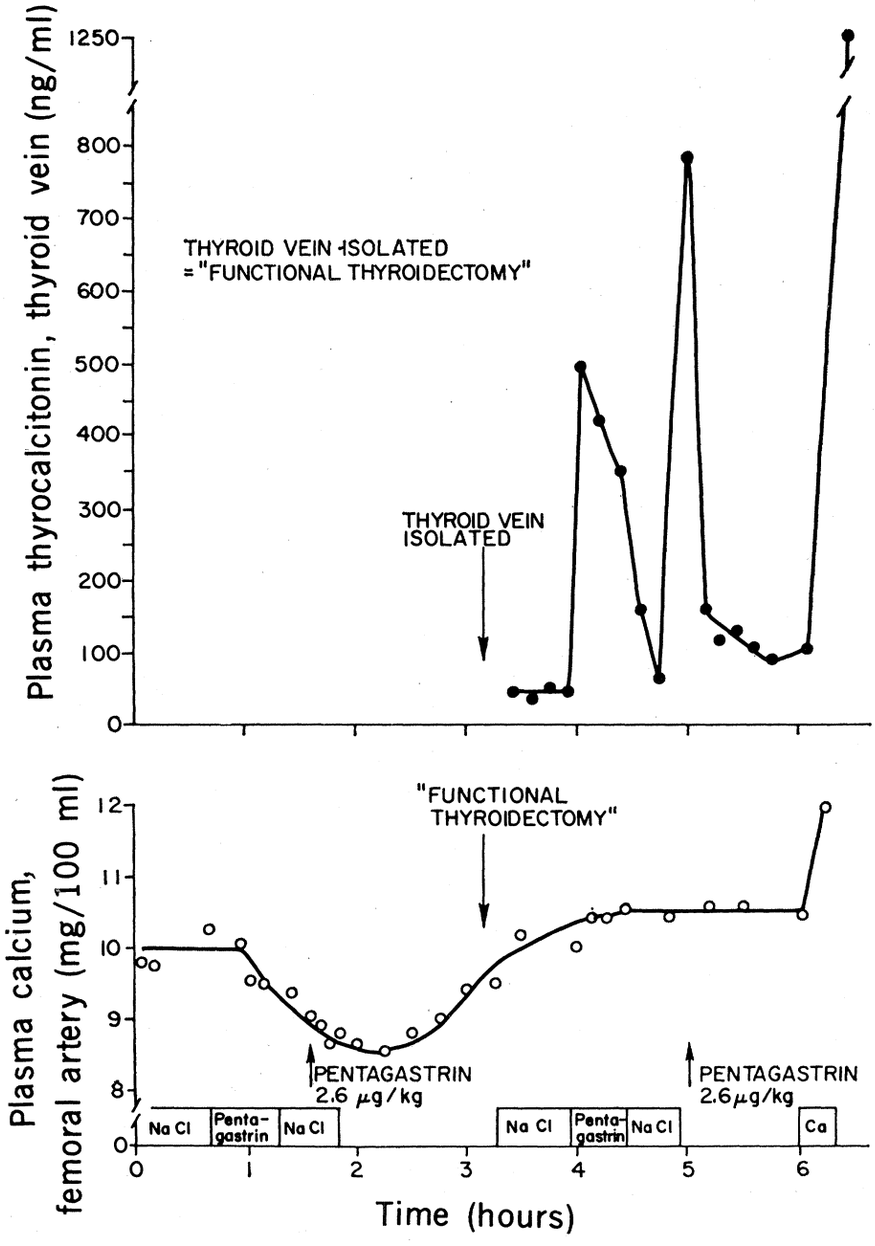

Fig. 1. Production of hypocalcemia in a pig by stimulation of secretion of thyrocalcitonin by pentagastrin before functional thyroidectomy. After beginning complete collection of thyroid venous blood, pentagastrin did not lower blood calcium, but markedly stimulated secretion of thyrocalcitonin. Blocks along the abscissa show durations of infusions (into a femoral vein) of 0.15*M* NaCl or pentagastrin (0.05 μg/kg per minute), and upward-pointing arrows indicate the time at which a single intravenous injection of pentagastrin was given. Downward-pointing arrows show the time of functional thyroidectomy when complete collection of thyroid venous blood was begun. The pig was male, weighed 28.5 kg, and had been fasted for 24 hours. See Table 1 legend and (*5*) for additional details.

Table 1. Concentration of thyrocalcitonin in the thyroid venous effluent plasma of pigs during control periods (control infusion) and immediately after administration of test substances. All substances were administered by a single intravenous injection (3 ml) or by intravenous infusion (where indicated). Sufficient time was allowed between treatments for control period baseline values to be approximately regained. The vehicle for all test substances was 0.15*M* NaCl. The pigs were young males and females, ranging in weight from 15.5 to 26.5 kg and had been fasted for 24 to 48 hours. Mean plasma thyrocalcitonin concentrations were obtained by radioimmunoassay (*8*) of three to five dilutions of each plasma sample, with pure porcine thyrocalcitonin used as both standard and labeled antigen. Radioimmunoassays were conducted under equilibrium conditions for 24 to 48 hours at 4°C, and antiserum was used at a dilution of 1 : 30,000.

Treatment	Plasma thyrocalcitonin (ng/ml)*
Pig No. 1	
Control infusion, 0.15*M* NaCl	27 (15 to 37)†
Pentagastrin, 2.6 μg/kg	898
Secretin, 1.6 unit/kg	46
Pig No. 2	
Control infusion, 0.15*M* NaCl	29 (24 to 40)†
Pentagastrin, 2.6 μg/kg	1208
Betazole, 0.5 mg/kg	37
Calcium infusion (14.8 mg of Ca per 100 ml)‡	848
Pig No. 3	
Control infusion, 0.15*M* NaCl	20 (18 to 23)†
Pentagastrin, 0.26 μg/kg	266
Pentagastrin, 2.6 μg/kg	622
Glucagon, 62.5 μg/kg	60
Calcium infusion (13.7 mg of Ca per 100 ml)‡	336
Pig No. 4	
Control infusion, 0.15*M* NaCl	18 (16 to 20)†
Pentagastrin, 0.026 μg/kg	41
Pentagastrin infusion, 0.05 μg/kg per minute for 11 minutes = 0.575 μg/kg total	198
Calcium infusion (13.4 mg of Ca per 100 ml)‡	688

* Values for thyrocalcitonin during calcium infusion represent peak values observed, that is, the first to third collection period after treatment. All other values in this column represent the first collection period after treatment. † Values in parentheses in this column give the range of baseline values during four to eight collection periods (30 minutes to 1 hour and 45 minutes) at the beginning of the experiment before treatments were started. ‡ Values in parentheses in this column are the calcium concentrations of plasma samples analyzed for thyrocalcitonin.

three of the experiments shown in Table 1, when isotonic calcium chloride was infused at a dose rate (14.75 mg of Ca^{2+} per minute) designed to produce severe hypercalcemia (13 to 15 mg of calcium per 100 ml). During stimulation of secretion of thyrocalcitonin by pentagastrin, blood calcium concentrations did not fall because the thyroid effluent blood containing the secreted hormone was completely collected and was not permitted to reenter the systemic circulation.

Figure 1 shows results in detail from an experiment involving a single pig. Peripheral (femoral arterial) blood calcium and inorganic phosphate concentrations were determined periodically both before and after diversion of thyroid venous effluent blood from the systemic circulation (functional thyroidectomy). Infusion and injection of pentagastrin produced hypocalcemia before, but not after, functional thyroidectomy. Blood phosphate levels (not shown) also fell after the first injection of pentagastrin from a mean baseline level of 10.5 mg/100 ml to 7.7 to 8.4 mg/100 ml. Baseline phosphate levels were regained at the same time that normocalcemia was reestablished (at about 3.5 hours). These results suggested that the hypocalcemia and hypophosphatemia, observed when the thyroid circulation was intact, occurred in response to an elevated secretion of thyrocalcitonin caused by pentagastrin, an interpretation supported by the marked elevations of thyrocalcitonin observed in thyroid venous effluent blood analyzed after isolation of the thyroid vein.

Throughout all experiments shown (Table 1 and Fig. 1), during collection of thyroid venous blood the plasma flow rate through the thyroid gland remained approximately constant at 1 to 2 ml/min. In control studies we have shown that direct addition of pentagastrin to porcine plasma or to pure porcine thyrocalcitonin does not interfere with the determination of thyrocalcitonin by radioimmunoassay. In one preliminary experiment, injection of pentagastrin directly into the thyroid artery at a dose level ineffective systemically (0.012 μg/kg) produced a rapid three- to fourfold increase in thyrocalcitonin secretion, which suggests that pentagastrin acts directly on the thyroid gland.

It is known that circulating levels of human gastrin are elevated during hypercalcemia produced by systemic infusion of calcium (*10*). Our results show that pentagastrin is an extremely potent stimulus for secretion of pig thyrocalcitonin. If native gastrin is of equal, or even greater, potency (*6*), an effective dose may well be in the physiological range. Therefore, we hypothesize that gastrin or a related gastrointestinal hormone, possibly in concert with the blood calcium concentration, may be important in the regulation of secretion of thyrocalcitonin by the mammalian thyroid gland.

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