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Effect of Thyrocalcitonin on Duodenal Calcium Transport.* (32282)

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The gastrointestinal tract is unnecessary for mediation of the hypocalcemic effect of thyrocalcitonin(1). It is possible, however, that this hormone does exert an effect upon intestinal calcium absorption, but reports in the literature regarding this possibility are conflicting(2-4). This investigation was undertaken to determine the effect of thyrocalcitonin on calcium fluxes in the rat duodenum in acute studies using an *in vivo* perfusion technique.

Methods. Male Sprague-Dawley rats weighing 230-300 g which were fed normal laboratory chow (calcium content 1.2%) served as experimental and control animals. Food was withheld for 24 hours before study but the rats were permitted tap water *ad libitum*.

Anesthesia was produced with intraperitoneal *Dial*® with urethane solution (Ciba) (0.7-1.0 ml/kg). Through a midline abdominal incision the distal end of the bile duct was ligated and transected. The proximal 8-13 cm of small intestine were cannulated with metal adapters attached to polyvinyl tubing. The inflow cannula was inserted through an incision in the pylorus and the tubing tied in place with suture. Similarly, the outflow cannula was positioned and tied 1-2 cm distal to the ligament of Treitz. Before perfusion the isolated segment of gut was washed with 0.9% NaCl and then emptied with air. The abdomen was closed over the cannulas and body temperature was maintained with a heating pad.

Fluid was perfused by recirculation at a rate of 2.0 ml/min for one hour from a reservoir containing 12 ml of solution by use of a proportioning pump (Technicon). The perfusing solution of pH 6.5 contained 0.85 mM $CaCl_2$, 137 mM NaCl, 20 mM THAM, 0.2% polyethylene glycol and 0.02 μc/ml $^{45}CaCl_2$ (Nuclear Chicago, specific activity $>$ 10 curies/g).

Just before the start of perfusion 1.0 ml of blood was withdrawn from the inferior vena cava. At the beginning of the perfusion, thyrocalcitonin† (approximately 250 MRC milliunits/kg body weight in a total volume of 2.5 ml/kg of body weight) was injected rapidly through a femoral vein. The control animals received the vehicle (0.9% NaCl of pH of 3.0, 2.5 ml/kg body weight) only. At the end of the perfusion period, blood was withdrawn from the inferior vena cava. The gut was removed and the contents and any excess fluid expressed by gently pressing the tissue flat on absorbent paper. The perfused segment of gut was weighed on a torsion balance (Bethlehem Instrument Co.).

Polyethylene glycol was analyzed turbidometrically with a spectrophotometer (Research Specialties Model 4000 Spectromatic). Calcium was determined by atomic absorption spectrophotometry (Perkin-Elmer Model 303) and radioactivity was estimated in a

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liquid scintillation spectrometer (Packard Tri-Carb).

In this paper, the term "absorption" refers to the net movement of calcium from the lumen of the gut into the experimental animal, while lumen to plasma flux (LP) and plasma to lumen flux (PL) are unidirectional movements. The following equations adapted from Wasserman *et al*(5) were used to calculate absorption, LP, and PL fluxes:

$$\text{Absorption} = \frac{V[{}^{40}Ca_i - ({}^{40}Ca_f)(PR)]}{(W)(t)}$$

$$\text{LP Flux} = \frac{V[{}^{45}Ca_i - ({}^{45}Ca_f)(PR)]}{[(SA_i + SA_f)/2](W)(t)}$$

$$\text{PL Flux} = \text{LP Flux} - \text{Absorption}$$

where ^{40}Ca = the chemical calcium content of the fluids in μmol/ml; ^{45}Ca = the radioactive calcium content of the fluid in cpm/ml; V = the volume of perfusate in ml; W = the wet weight of the gut in grams; SA = the specific activity of calcium in cpm/μmol; t = time in hours and PR = the polyethylene glycol ratio, which corrects for water movement and is calculated by dividing the polyethylene glycol concentration of the initial perfusate by its concentration in the final perfusate. The subscripts "i" and "f" refer to the initial and final values.

Results. The data for all experiments are summarized in Table I. The mean change in serum calcium concentration in per cent ± standard deviation for the thyrocalcitonin treated animals (n = 19) was — 15.3% ± 6.2. The mean change in the control animals (n = 12) was + 0.02% ± 5.2. The mean absorption rate ± standard deviation was 3.9 ± 1.2 μmol/g wet weight of intestine/hr for the thyrocalcitonin treated animals compared with a mean of 4.9 ± 1.3 for the control animals. Mean LP flux in the thyrocalcitonin treated rats was 5.4 ± 1.4 while that of the control animals was 6.5 ± 2.0. The PL flux mean for the thyrocalcitonin treated animals was 1.5 ± 0.8 as compared with a control flux of 1.5 ± 0.9. Analysis of these means by a two sample t-test(6) revealed a statistically significant difference for the per cent change in serum calcium concentration ($p < 0.001$), but no statistically significant difference for absorption ($p = 0.05$), LP flux ($p > 0.05$), or PL flux.

Discussion. In these acute experiments, thyrocalcitonin had no effect on calcium transport in the rat duodenum, a small intestinal segment where calcium is avidly absorbed(5,7). The slight decreases in absorption and lumen-to-plasma flux in the thyrocalcitonin treated animals were not statistically significant.

Care and Keynes(2) found decreased absorption of calcium from an ileal loop in one sheep after intravenous injection of a calcitonin preparation, but did not specify how much of a decrease had occurred. Kenny and Heiskell(3) found no change in tissue calcium in the rat small intestine one hour after subcutaneous injection of a crude bovine thyrocalcitonin preparation in animals which had been fasted 48 hours. Milhaud and Moukhtar (4) studied the effect of a long acting thyrocalcitonin preparation over a 3-day period from combining balance data and ^{40}Ca and ^{45}Ca measurements. Based on coefficient of absorption (calcium absorbed/calcium ingested), they found increased absorption in the thyrocalcitonin treated animals. Endogenous fecal calcium output was diminished, but this could have been explained by either a decrease in calcium movement into the gut, an increase in absorption, or a combination of the two. They could not rule out the possibility of increased parathyroid activity in response to prolonged hypocalcemia as an alternative explanation for their findings. These changes may have resulted from alterations of total body calcium metabolism occurring in chronically treated animals and

TABLE I. Changes in Serum Calcium Concentration, Absorption Rates, Lumen-to-Plasma and Plasma to Lumen Fluxes (Mean ± Standard Deviation) in the Rat Duodenum.

	Serum calcium, % Δ	Absorption	LP flux	PL flux
		μmol g^{-1} hr^{-1}		
Thyrocalcitonin treated (n = 19)	−15.3 ± 6.2	3.9 ± 1.2	5.4 ± 1.4	1.5 ± .8
Control (n = 12)	+ 0.2 ± 5.2	4.9 ± 1.3	6.5 ± 2.0	1.5 ± .9
p	<.001	=.05	>.05	—

may account for the differences in findings from our acute studies measuring duodenal transport.

Summary. The effect of thyrocalcitonin on calcium transport in the rat duodenum was investigated in acute studies by an *in vivo* perfusion method. Absorption and lumen-to-plasma flux were slightly decreased in the thyrocalcitonin treated animals, but the differences were not statistically significant.

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Growth of Rubella Virus in BHK21 Cells. I. Production, Assay, and Adaptation of Virus.* (32283)

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The demonstration(1) that rubella virus (RV) grows in baby hamster kidney (BHK21) cells has been followed by the successful application of this system to virus assay(1), purification(2,3), the production of complement-fixing antigen(4), and the development of RV hemagglutinin(5). The principal advantages of using BHK21 cells in rubella virus work have been the high titers developed in this cell-type, and the feasibility of preparing large volumes of virus by infection of suspended cells. An additional advantage has been the development of a sensitive plaque assay made possible by the cytopathogenicity of RV in the BHK21 cells.

This paper describes the basic methodology of RV studies using BHK21 cells.

Materials and methods. Media. Stock cultures of the 2 cell lines were propagated at 36.5°C in monolayer in BHK21 medium which is based on Eagle's basal medium but contains double-strength amino acids and vitamins, and 0.1 mg/l of $Fe_2NO_3 \cdot 9H_2O$, 4.5 g/l of d-glucose, supplemented with 10% tryptose phosphate broth (Difco) and 10% heat-inactivated (56°C, 30 min) fetal calf serum(6). All media contained 100 IU/ml of penicillin-G, 50 μg/ml of streptomycin sulfate and 25 IU/ml of nystatin. The stock cultures were split twice a week in a ratio of 1:6 to 1:8 using as the cell-dispersing agent a mixture of 0.25% trypsin and 0.01% versene in Dulbecco's phosphate buffered saline (PBS) without Ca++ and Mg++.

Cell lines. Two lines of BHK21(6) were used in these studies. The first, 13S, is a rapidly growing cell derived from Stoker and MacPherson's Clone 13. Morphologically these cells are short fibroblasts. Although 13S grows on glass, its adherence to glass is poor and it does not form a confluent monolayer. Rather, growth proceeds by clumping of cells and subsequent release of clumped cells into the medium. Monolayer-grown 13S cells readily grew in suspension culture in the growth medium described above without adaptation. An increase in cell density from 5 $\times$ 10^5 to 1.5-2.0 $\times$ 10^6 cells/ml took place in 1.5-2 days.

The second cell line, WI-2, was derived as a clone from a population of BHK21 cells by the method of MacPherson and Montagnier (7). The WI-2 line morphologically consists

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