CALCITONIN AND ELECTROLYTE HOMEOSTASIS

by

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Abstract

The effects of calcitonin on plasma and urinary electrolytes were studied in rats and sheep.

1. Twice-daily administration of salmon calcitonin (250 mU(50 ng)/100g rat, i.p.) to young rats over a period of 10 days resulted in increases in urine volume and the excretion of Na, Ca, P, and Mg. Single injections of graded doses of salmon calcitonin (100-2000 mU(20-400 ng)/100g rat, i.p.) resulted in dose-related increases in urine volume and Na excretion over a six-hour period. Phosphate excretion was also found to increase. However, in contrast to the chronic studies, net decreases in the excretion of Ca and Mg were found. Significant decreases in plasma Ca, inorganic P, and Mg were found one hour after injection of 100 mU(20 ng) salmon calcitonin/100g rat, i.v. No significant decreases in plasma Na and K were observed. The urinary electrolyte changes that occurred following calcitonin administration also occurred in thyroparathyroidectomized rats and rats pretreated with large doses of DOCA (1 mg/100g rat, i.m.), and were not associated with increases in the excretion of endogenous creatinine.

2. A one-hour infusion of Ca (10 mg Ca/kg) in intact conscious sheep was accompanied by a rapid rise in plasma Ca levels which rapidly returned to normal when the infusion was stopped. Plasma P levels immediately decreased following the start of the Ca infusion. Following thyroidectomy (TX), Ca infusion resulted in a significantly greater increase in plasma Ca ($\Delta 2.31 \pm 0.10$ mg%) as compared to intact sheep ($\Delta 1.60 \pm 0.07$ mg%), and the return towards normal levels was delayed.

Plasma P levels in the TX sheep rose following the start of the infusion, and at 1/2 hour post infusion were significantly higher (Δ+0.42±0.07 mg%) than plasma P in the intact sheep (Δ-0.66±0.22 mg%). In intact sheep, increases in urine volume and the excretion of Na, Ca, P, and Mg were observed during and following the Ca infusion. The TX sheep showed no increase in urine volume, and despite a larger increase in Ca excretion, the excretion of Na in the TX sheep was significantly less than that found in the intact animals.

3. Volume expansion in intact rats (5 ml 0.9% NaCl/100g rat, i.p.) was followed by significant increases in urine volume and the excretion of Na, K, Ca, P, and Mg, but these changes did not occur in TX rats in the three hours following the saline load.

Dextran infusion (6% Gentran 75 in 0.9% NaCl) in intact sheep resulted in a sustained fall in plasma Ca levels. When dextran was infused into TX sheep, plasma Ca fell initially but returned to normal within three hours. In both groups of sheep, the volume expansion resulted in a similar increase in Na excretion. However, an increase in Ca excretion did not accompany the natriuresis in the TX sheep, which was in contrast to the findings in the intact animals.

4. Administration of parathyroid extract to TPTX rats (100 U/100g rat, s.c.) decreased the urinary excretion of Na, Ca, and Mg, and increased the excretion of P. In similar studies using highly-purified PTH (3000 U/mg), the excretion of Ca and Mg also decreased and the excretion of K as well as P was found to increase. However, no significant decrease in Na excretion was found.

These studies indicated that exogenous administration of

salmon calcitonin alters the excretion of Na, Ca, P, and Mg in the urine, in addition to decreasing the levels of Ca, P, and Mg in the plasma. Infusion of Ca salts in intact sheep resulted in certain changes in both plasma and urinary electrolytes which were not found following thyroidectomy in these animals. Since elevation of plasma Ca levels would increase circulating levels of calcitonin in intact but not TX sheep, endogenous release of calcitonin was implicated in the electrolyte changes that were observed. Calcitonin may also be implicated in the plasma and urinary changes that occur following volume expansion since removal of the calcitonin-containing "C" cells altered the pattern of certain electrolyte changes that occurred in the intact animals following volume expansion. Studies with various PTH preparations suggested that calcitonin and PTH may be acting in antagonistic ways in the renal handling of Na, Ca, and Mg.

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INTRODUCTION

The electrolytes in the extracellular fluid (ECF) are maintained at constant levels despite wide alterations in mineral intake or excretion. This constancy depends on the existence of efficient homeostatic mechanisms, many of which are humoral. This thesis is primarily concerned with the role of calcitonin as one of the hormones which can modify the levels of certain body electrolytes in the ECF. However, consideration of the action of parathyroid hormone (PTH) is also included in this investigation since it is becoming increasingly apparent that calcitonin and PTH can act in antagonistic ways.

Bone is a target site for both of these hormones and serves as a storehouse or reserve supply of mineral which, in contrast to the fluctuating source of mineral obtained from dietary sources, is constantly available to the body. Most of the body's Ca (99%) and inorganic P (85%), 50% of the total body Na and Mg, and about 8% of the K in the body is in bone. Some of this mineral is readily exchangeable, and release of the more firmly bound fraction can also occur coincident with factors which may initiate bone breakdown or resorption. Hormones which can influence either the resorption or accretion of bone influence the movement of those minerals found in bone.

The parathyroid glands have a critical role in maintaining a normal plasma Ca level. Parathyroid hormone from the chief cells of these glands is secreted in response to a hypocalcemic stress and acts by enhancing

osteolysis (1), resulting in the release of Ca from bone to the ECF. Hypomagnesemia was also shown in in vitro studies to cause release of this hormone (2), and PTH administration results in movement of Mg as well as Ca from bone to ECF (3, 4). Phosphate is also released from bone by PTH. However, the observed effect of a lowered plasma P level following PTH administration is secondary to the phosphaturic action of this hormone (5, 6). Parathyroid hormone has also been implicated in the movement of Na from bone in response to Na depletion (7). Thus, it would appear that PTH is one of the major hormones which can initiate the movement of electrolytes from the bone pool.

The second major hormone involved in the dynamics of bone mineral is calcitonin. This was discovered more recently than PTH although it is an older hormone phylogenetically. Calcitonin is described as a hormone that depresses bone resorption (8). It has been reported to enhance osteoblastic activity and thus may also be involved in mineral accretion and formation of new bone (9). It is released in response to an elevated plasma Ca level, depresses bone resorption, and thus reduces the input of bone Ca to the ECF so that plasma Ca levels are returned to normal (10). During recent years, the involvement of calcitonin in phosphate homeostasis has also been recognized. The hypophosphatemia which is observed following calcitonin treatment is apparently a direct action of the hormone and is not secondary to PTH stimulation (11 - 14). The role of calcitonin in Mg homeostasis is still not clear. There is some evidence that it acts in a similar way in the control of this ion as it does with Ca, that is by lowering plasma Mg levels as a result of decreasing bone resorption (4, 15). Others have found no plasma Mg lowering effect of calcitonin (16 - 18). Nevertheless, the fact that a Mg load results in a hypocalcemic response, provided the

calcitonin-containing "C" cells are intact, suggests that calcitonin may be involved in Mg metabolism as well (19).

Thus there are two major hormonal systems, PTH and calcitonin, which appear to be working in opposite directions with respect to bone resorption and bone accretion. The dual-hormonal concept (20) of the control of plasma Ca by PTH and calcitonin is now well accepted. However, the interrelationship of these two hormones in the control of the other major electrolytes found in bone is far from clear.

Besides the input of mineral from the bone pool, the ECF also receives an input of mineral from the gut via intestinal absorption. Once the electrolytes are in the ECF, they can either be taken up by cells or bone, or can be lost from the body via various excretory mechanisms. An efficient control mechanism must therefore have not only some influence on the input to the ECF, but also some influence on the output from it.

Parathyroid hormone raises plasma Ca and Mg to normal levels by increasing the rate of bone resorption and by decreasing the loss of Ca (21, 5, 6, 22-26) and Mg (24-26) in the urine. An increased reabsorption of these ions from the kidney tubule, under the influence of PTH, would protect the bone from undue loss of mineral and would help restore normal levels of Ca and Mg in the ECF. The phosphaturic effect of PTH has been known for many years. Albright and Ellsworth (27) first observed that a phosphaturia occurred immediately following PTH treatment. This in turn resulted in a hypophosphatemia which they erroneously considered as the indirect cause of the Ca release from bone. Numerous studies since then, stimulated by the investigations of Barnicot (28) and Gaillard (29), have shown that the action of PTH on bone is a direct one. However, the major effect on phosphate exerted by PTH is not via bone but by kidney.

The role of the kidney as a possible target organ of calcitonin is only just becoming recognized. Calcitonin decreases the amount of Ca in the ECF by inhibiting bone resorption and, as has been suggested, by decreasing the reabsorption of this ion from the kidney tubule (14, 30). Calcitonin may also decrease the reabsorption of Mg by the kidney (30) which would enhance the plasma Mg lowering effect which is sometimes observed following calcitonin treatment. Calcitonin has also been shown to cause a phosphaturic response. Since this effect is also found in parathyroidectomized animals, it is not secondary to hypocalcemic stimulation of the parathyroid glands (11-14). The observation that calcitonin administration is followed by a natriuretic response (31, 14, 32, 16) has implicated this hormone in Na homeostasis as well.

The changes in the renal excretion of certain electrolytes following either PTH or calcitonin administration indicate that the kidney is a second major target organ of these hormones. The apparent changes in reabsorptive capacity of the kidney tubule cells to certain ions following intervention of either hormone can enhance or modify the plasma changes that occur as a result of the action of PTH or calcitonin on bone. However, the experimental evidence is far from complete for clarifying the action of PTH or calcitonin on the kidney.

The participation of PTH and calcitonin in Na homeostasis has not been intensively explored. Aldosterone and the renin-angiotensin system are considered to be the primary hormones concerned with the maintenance of a normal amount of Na in the ECF. Alterations in the circulating levels of aldosterone, in addition to changes in glomerular filtration rate (GFR), hemodynamics, or "Starling forces" within the kidney are thought to be the

major controlling mechanisms in Na regulation (33-35). However, this explanation for the control of body Na is not entirely satisfactory. When all these variables are controlled, an increased loss of Na can still occur in response to ECF volume expansion (36, 37), and evidence from cross-circulation (38, 39) and other studies suggests that the factor(s) involved is humoral in nature. Also, a Na-losing factor has been found in the plasma and urine of salt-loaded man and sheep which caused a natriuresis when injected into assay rats (40). Therefore, a search for further control systems in the regulation of this important ion is indicated.

The observation that a natriuresis accompanies the administration of calcitonin suggests the value of an intensive study of this hormone and its relation to Na control. A parallelism seems to exist in the renal handling of Ca and Na (41-44), and at least in some part of the nephron Ca and Na may share a common reabsorptive pathway (45). However, it is not known if hormonally-induced changes in the renal handling of Ca are paralleled by similar changes in Na.

A great deal of experimental work has been performed in an attempt to understand the action of PTH on the kidney. Many of the findings resulting from this work have been reviewed by Rasmussen (46). These studies have concentrated on the Ca, P, and Mg changes that occur following PTH administration, or the clinical abnormalities that result from malfunction of the parathyroid gland. However, possible involvement of PTH in regard to other electrolytes has not been widely investigated.

The studies reported in this thesis explore the electrolyte changes that occur in plasma and urine following exogenous calcitonin administration, or during maneuvers designed to promote endogenous calcitonin release.

It was hoped that a concurrent study of plasma and urine following calcitonin involvement would lead to a greater understanding of the mechanism of action of this hormone.

These investigations were prompted by the author's observation that a profound diuretic response to salmon calcitonin occurred during a 10-day period of treatment in rats. Repeat studies confirmed these initial observations and showed that the diuretic response was accompanied by a natriuresis and also changes in other urinary electrolytes. Earlier studies by Ardaillou *et al.* (14) and Rasmussen *et al.* (31) with porcine calcitonin revealed a natriuretic response following hormone administration, but at that time no investigation of the renal effects of salmon calcitonin had been reported.

These initial findings with salmon calcitonin led to further investigations in order to establish:

(a) Was the effect of the hormone on the kidney a direct one or was it, for example, related to release of PTH in response to hypocalcemia?
(b) Was the plasma Ca level *per se* the initiating factor?
(c) Were the observed renal responses a result of changes in GFR?
(d) Were changes in mineralocorticoid activity implicated in any way?
(e) Would electrolyte changes similar to those found following exogenous calcitonin treatment occur with endogenously-released hormone in response to elevated plasma Ca levels which are known to increase release of calcitonin (47, 48)?
(f) If the calcitonin-containing "C" cells are removed, are the electrolyte changes that occur following Ca stress altered?

(g) Is the natriuresis which accompanies the increased Ca excretion following Ca stress (49-54) related to calcitonin secretion?

(h) Is calcitonin involved in the natriuresis and calciuresis that occur following volume expansion (41, 43, 55, 44, 56)?

The experiments reported in this thesis were designed in an attempt to answer these queries.

GENERAL METHODS AND MATERIALS

Experimental animals and diets

Rats: Male rats of the Long-Evans strain were used in all rat studies and were supplied by Blue Spruce Farms, Charles River, Ont. They were maintained on Purina Rat Chow and water and were without food during collection periods unless otherwise stated.

Sheep: Female lambs, five-six months of age, were obtained locally and were studied during the ensuing six-month period. These animals were given free access to crushed oats, Omolene (Purina), alfalfa hay, and water at all times except for 24 hours prior to and during experimental periods when food was removed. An iodized salt lick was also provided.

Both rat and sheep diets contained Vitamins A and D in amounts considered adequate for normal nutrition and metabolism as recommended by the Food and Nutrition Board, N.R.C., Canada.

Procedures for plasma and urine collections

Rats: Blood samples were obtained either from the tail vein or by cardiac puncture, depending on the number of analyses to be performed. Urine was collected while animals were housed in metabolism cages (Acme Metal Products, Cincinnati, Ohio) or in some studies by fractional collection via a bladder cannula.

Sheep: Blood samples were obtained from a catheter (Abbocath 18-guage iv. catheter, Abbott Laboratories, Montreal, Que.) inserted into the lateral

saphenous vein. The catheter allowed frequent blood sampling and was removed at the end of each experiment. A bladder catheter (Bardex Foley Catheter, 14 FR, C.R. Bard International Ltd., England) was also inserted prior to each experiment and urine was collected during 15-minute intervals with uninterrupted flow following an equilibration period of one hour. These catheters were held in position by inflation of an internal 5-cc balloon.

Surgical Procedures

Rats: All surgery was performed with the aid of a dissecting microscope while the animals were anaesthetized with ether. Thyroparathyroidectomy involved the removal of both lobes of the thyroid with the attached parathyroids and the connecting isthmus. Thyroidectomy included the separation of the superior parathyroids from underlying thyroid tissue on each lobe and their immediate transplantation deep into the muscle mass of the posterior aspect of the thigh. The thyroid tissue was then completely removed. Control animals were subjected to sham surgery which simply consisted of exposure of the thyroid complex. The effectiveness of the surgery was determined two weeks after surgery by plasma Ca measurements following overnight starvation. Those animals with plasma Ca levels below 7.5 mg% were considered successfully thyroparathyroidectomized (TPTX), and the transplant animals with plasma Ca levels greater than 9.0 mg% were considered effectively thyroidectomized (TX). Plasma Ca levels in intact control animals were also determined at this time.

Those rats with thyroids removed were immediately started on thyroxine (Synthroid-Sodium Levothyroxine, U.S.P., Flint Laboratories, Alliston, Ont.)

which they obtained from their drinking water at a concentration of 100 µg/l. This dose was based on a study by Kennedy and Talmage (57).

Sheep: Thyroidectomy in sheep was performed under halothane-nitrous oxide anaesthesia following initial induction with Pentothal Sodium (Abbott Laboratories, Montreal, Que.) which enabled insertion of an endotracheal tube. Since the parathyroids in sheep are completely separate* from the thyroid gland, thyroidectomy was performed without the necessity of parathyroid transplants and consisted of removal of the two thyroid lobes and the connecting more caudally-placed isthmus. Following surgery, the animals were given replacement thyroxine (Synthroid-Sodium Levothyroxine, U.S.P., Flint Laboratories, Alliston, Ont.) in a daily oral dose of 1 mg (58).

Hormones and Infusates

Calcitonin: Pure salmon calcitonin (Armour Pharmaceutical Co., Kankakee, Ill.) having an activity of 5000 MRC U/mg was used for all calcitonin administration studies. The activity of the hormone was determined by bioassay in young rats according to a modification of the method of Kumar et al.(59) in which the hypocalcemic response was compared to that of Medical Research Standard (MRC) B of porcine calcitonin. The vehicle used for calcitonin was 1% NaAc in 0.1% glycine, pH 4.6.

Parathyroid Hormone: Bovine PTH was used for all PTH administration studies and was obtained in various degrees of purity. Lilly Parathyroid Extract (PTE) (Para-thor-mone, U.S.P., Eli Lilly Co. Canada, Ltd., Toronto, Ont.),

*Occasionally, the small inferior parathyroids may be incorporated in the thyroid mass. However, the predominant superior parathyroids are quite distinct and are found high up in the thymus tissue.

TCA-precipitated PTH (240 U/mg) and a further highly purified preparation (3000 U/mg), kindly provided by Dr. Nadine Wilson of this laboratory, were used for the different investigations. Bioassays for PTH were based on the method of Causton et al.(60).

Desoxycorticosterone acetate (DOCA): Percorten (Ciba Co., Ltd., Dorval, Que.), a synthetic mineralocorticoid, was used to simulate the activity of aldosterone.

Dextran: 6% Gentran 75 in 0.9% NaCl solution (Travenol Laboratories, Inc., Morton Grove, Ill.) was used for the volume expansion studies in sheep.

Dextrose-Saline: 5% dextrose and 0.9% NaCl, U.S.P. (Baxter Laboratories, Malton, Ont.) was used as the infusion vehicle in the $CaCl_2$ studies in sheep.

$CaCl_2$ solution: $CaCl_2$ was dissolved in dextrose-saline solution to give a dose of 10 mg Ca/kg/hour.

Plasma and urine analyses

Blood samples were collected in heparinized tubes, immediately centrifuged, and the plasma separated. Calcium (Ca), inorganic phosphorus (P), magnesium (Mg), sodium (Na), and potassium (K) concentrations were determined the same day and osmolality was also measured in some studies.

Individual timed urine samples were collected and the volume measured. The analyses were carried out immediately following collection. In addition to the analytical procedures which were carried out on plasma samples, urine analyses also included a measurement of endogenous creatinine excretion ($U_{Creat.}V$). This measurement gave an indication of changes in

GFR and also of completeness of bladder emptying. Urine osmolal concentrations were also determined in some studies.

Analytical Methods

Calcium: Plasma Ca was measured on the Technicon Auto Analyzer, Method N-31 P, modified by Newsome (61). Urine Ca concentrations were measured by Atomic Absorption Spectrophotometry using the Jarrell-Ash Model 280 Atomsorb.

Phosphorus: Both plasma and urine samples were measured on the Technicon Auto Analyzer, Method N-4 b which is based on the method of Fiske and Subbarow (62).

Magnesium: Plasma and urine samples were measured by Atomic Absorption Spectrophotometry using the Jarrell-Ash Model 280 Atomsorb.

Sodium and Potassium: Plasma and urine samples were measured by emission on Instrumentation Laboratories Model 143 flame photometer.

Creatinine: Creatinine was estimated as the alkaline picrate, the method being modified for use on automated colorimetric analyzers (Carlo Erba Automatic Analyzer, Model CLA 1510).

Osmolality: Osmolality of plasma and urine samples was measured by freezing point depression using the Osmette Precision Osmometer, Model 2007 Precision Systems, Inc.

Statistical Methods

Student's t test for small, unpaired groups was used for statistical evaluation of the data. Where p values of ≤ 0.050 were found, the results were considered significantly different.

Abbreviations

$C_{\underline{x}}$ Clearance of $\underline{x}$; the number of ml of plasma completely cleared of substance $\underline{x}$ per minute:

$$C_{\underline{x}} = \frac{U\ V}{P} = \frac{\text{Urine concentration}_{\underline{x}} \times \text{Volume of urine excreted (ml)} \times \text{minute}^{-1}}{\text{Plasma concentration}_{\underline{x}}}$$

CT Calcitonin

ECF Extracellular fluid

GFR Glomerular filtration rate

mg% mg per 100 ml solution

Na/Creat. The ratio of sodium to endogenous creatinine in the urine. This ratio gives an approximation of the fractional excretion of sodium, assuming plasma sodium and creatinine concentrations are unchanged during the experimental period.

Na/K The ratio of sodium to potassium in the urine.

P The chemical abbreviation for phosphorus is used when referring to plasma and urinary phosphate since the chemical analysis measures inorganic phosphorus.

PTE Lilly parathyroid extract.

PTH Parathyroid hormone. This term is used when referring generally to parathyroid hormone.

TCA-precipitated PTH Trichloroacetic-acid precipitated parathyroid hormone. The abbreviations PTE and TCA-precipitated PTH (240 U/mg), in addition to the term highly-purified PTH (3000 U/mg) are used then comparing the responses to hormone preparations of varying degrees of purity.

$U_{\underline{x}}V$ The rate of excretion of a given substance $\underline{x}$ in the urine: $U_{\underline{x}}V$ = urine $\text{concentration}_{\underline{x}}$ X Volume of urine excreted X time^{-1}.

SPECIFIC METHODS AND RESULTS

I. EXOGENOUS CALCITONIN ADMINISTRATION

A. Studies on Rats During Chronic Administration of Calcitonin

Methods

Thirty-day-old rats were individually housed in metabolism cages for 10 days and urine was collected over 24-hour periods. They had free access to food and water during this time period. Ten rats were each given 250 mU salmon calcitonin i.p. in vehicle (1% NaAc in 0.1% glycine, pH 4.6) every 12 hours and a control group of 10 animals was given vehicle only at the same time. A preliminary study on rats of a similar age was performed in order to determine the hypocalcemic response to this dose of hormone and to determine the constancy of the hypocalcemia during a 10-day period.

Results

In Fig. 1 it can be seen that salmon calcitonin exerts a long-lasting hypocalcemic response following a single injection of the hormone. Twice-daily administration of calcitonin maintained a hypocalcemic state throughout the experimental period as revealed by random spot checks.

The polyuria which accompanied chronic administration of the hormone was the initial observation that indicated a possible involvement of calcitonin in renal function. In some instances, 75-g rats excreted as much as 45-50 ml of urine per day. Figure 2 shows the persistent and progressive polyuria during the 10-day period of treatment with salmon calcitonin, the difference in urine volume between the calcitonin-treated

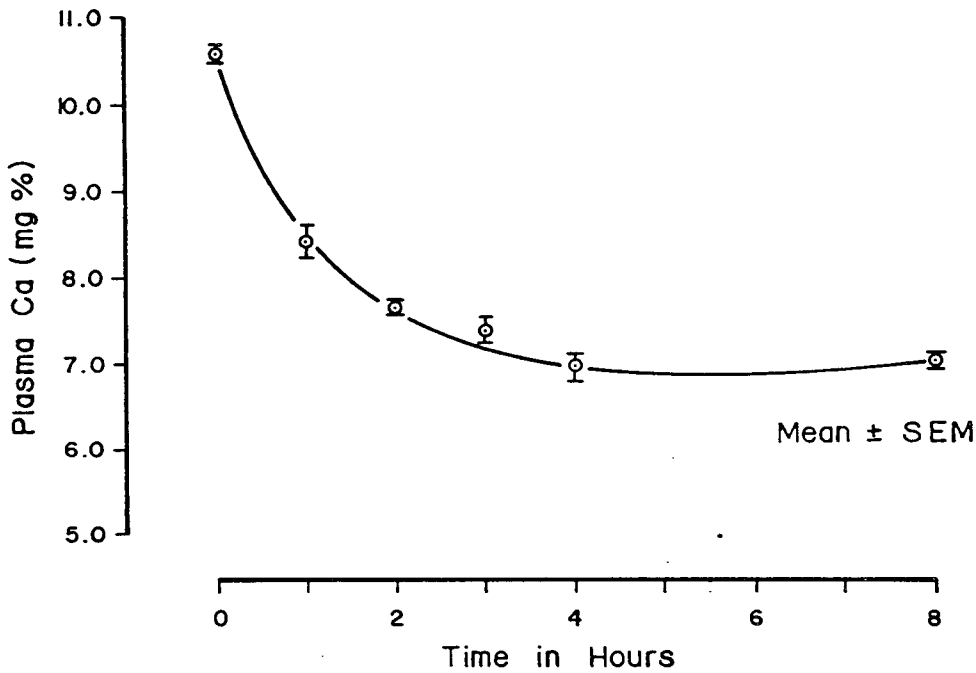

Fig 1. Time-course of action of a single injection of salmon calcitonin (250 mU/100 g body weight, i.p.) on plasma Ca levels in six-week-old rats. Each point represents the Mean ± SEM of five animals.

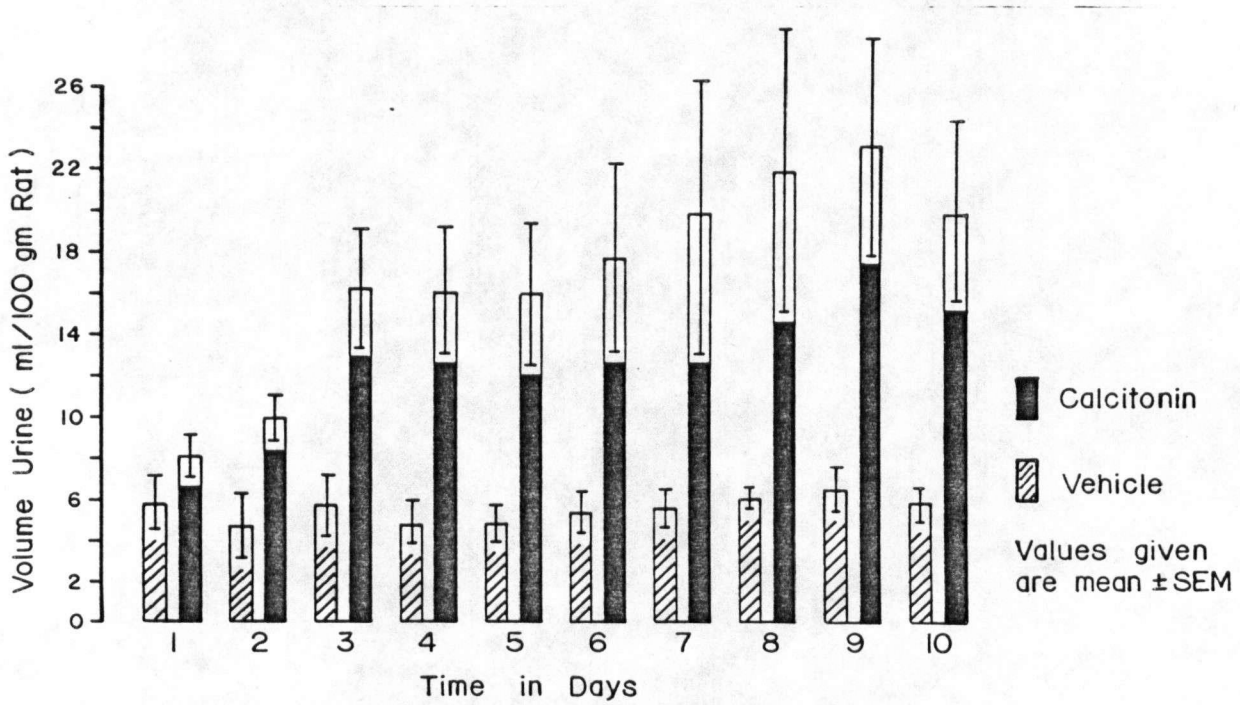

Fig 2. Urine volume changes in rats treated chronically with salmon calcitonin (250 mU/100 g body weight, i.p. twice daily). (n = 10 rats per group.)

and control animals being significantly different from Day 2.

Urinary excretion of Na ($U_{Na}V$), Ca ($U_{Ca}V$), Mg ($U_{Mg}V$), and P (U_PV) were also found to increase in the calcitonin-treated rats as is seen in Fig. 3. An initial transient increase in $U_{Na}V$ was followed by an elevated mean output from Day 4 - Day 10. Significant increases in $U_{Ca}V$ were found to occur from Day 4 of treatment, whereas the greatest increase in $U_{Mg}V$ was found to occur from Day 2 - Day 7. An increased U_pV in the calcitonin-treated rats occurred only during the first five days of treatment.

This study indicated that chronic treatment with salmon calcitonin in young rats could increase the normal excretion of certain urinary electrolytes as well as urine volume.

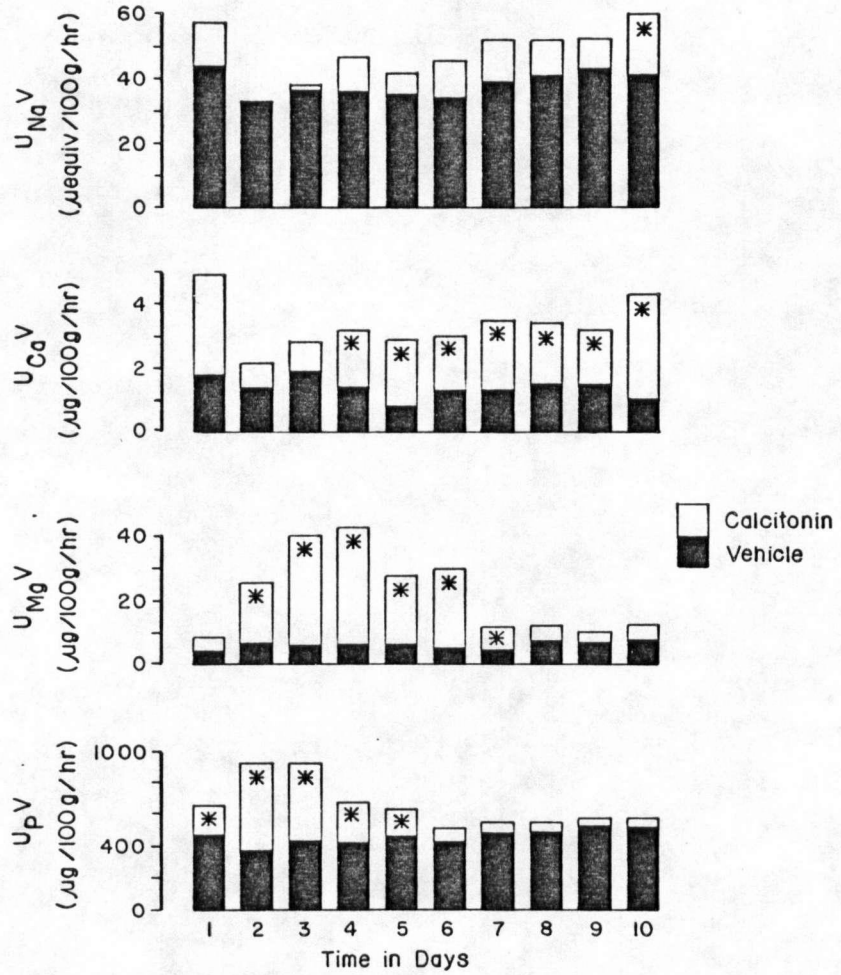

Fig 3. The effect of chronic treatment with salmon calcitonin (250 mU/100 g body weight, i.p., twice daily) on urinary electrolyte excretion in young rats. Mean outputs are shown from groups of ten rats. Significant increases in electrolyte excretion are indicated by an asterisk (*).

B. Studies on Rats Following Single Injections of Calcitonin

1. Intact Rats

Methods

In order to determine the time-course of the urinary electrolyte changes that occur following a single injection of calcitonin, urine was collected during timed intervals from individual rats in which the bladder was cannulated and a constant infusion of 2.5% dextrose or dextrose (2.5%)-saline(75 meq/l) at a rate of 3 ml/hour was given via the external jugular vein. These rats were restrained in a holding device during the collection period and were allowed to equilibrate for several hours following the start of the infusion before calcitonin was given. In some of these studies, the animals were pre-loaded with large doses of DOCA (1-1.5 mg/rat).

Since the major electrolyte changes appeared to occur within a six-hour period following hormone administration, this period was chosen for urine collection in subsequent studies.

Graded doses of calcitonin were then given to separate groups of rats and one group was given vehicle only. Electrolyte changes were measured at these various dose levels in addition to changes in endogenous creatinine excretion.

Plasma electrolyte levels were also determined in a further series of experiments with animals starved 12 hours prior to hormone administration. Blood samples were obtained by cardiac puncture on separate groups of 10-11 rats at one hour and three hours after hormone injection. Control groups were given vehicle and were bled at similar times.

In order to determine if changes in mineralocorticoid levels could block or potentiate the natriuretic response to calcitonin, DOCA was injected into groups of intact rats with and without calcitonin administration. Appropriate control groups were followed simultaneously.

The effect of age on the urinary response to calcitonin was also studied since it is known that the hypocalcemic effect of calcitonin is far greater in young rapidly-growing animals than in adults (63). In this study, groups of 5-week-old and 28-week-old rats were given calcitonin, and urine was collected over a period of 24 hours in six-hour intervals. Control groups of the same age difference were given vehicle only.

Results

As can be seen in Table I, and in another experiment shown graphically in Fig. 4, a natriuretic response occurred in the first half hour following hormone administration. At this time, urine volume, U_pV, and the ratios of Na/K and Na/Creat. were also increased. However, a general decrease in $U_{Ca}V$ and $U_{Mg}V$ was found. These responses were most marked at about 1 1/2-2 hours following calcitonin administration. In comparing the changes in $U_{Ca}V$ and $U_{Mg}V$ in Table I and Fig. 4 following calcitonin administration, it is seen that the decrease in the excretion of these two ions was immediate in the three-month-old rat studied in Table I, whereas a transient increase in the excretion of Ca and Mg was observed in the seven-month-old rat studied in Fig. 4.

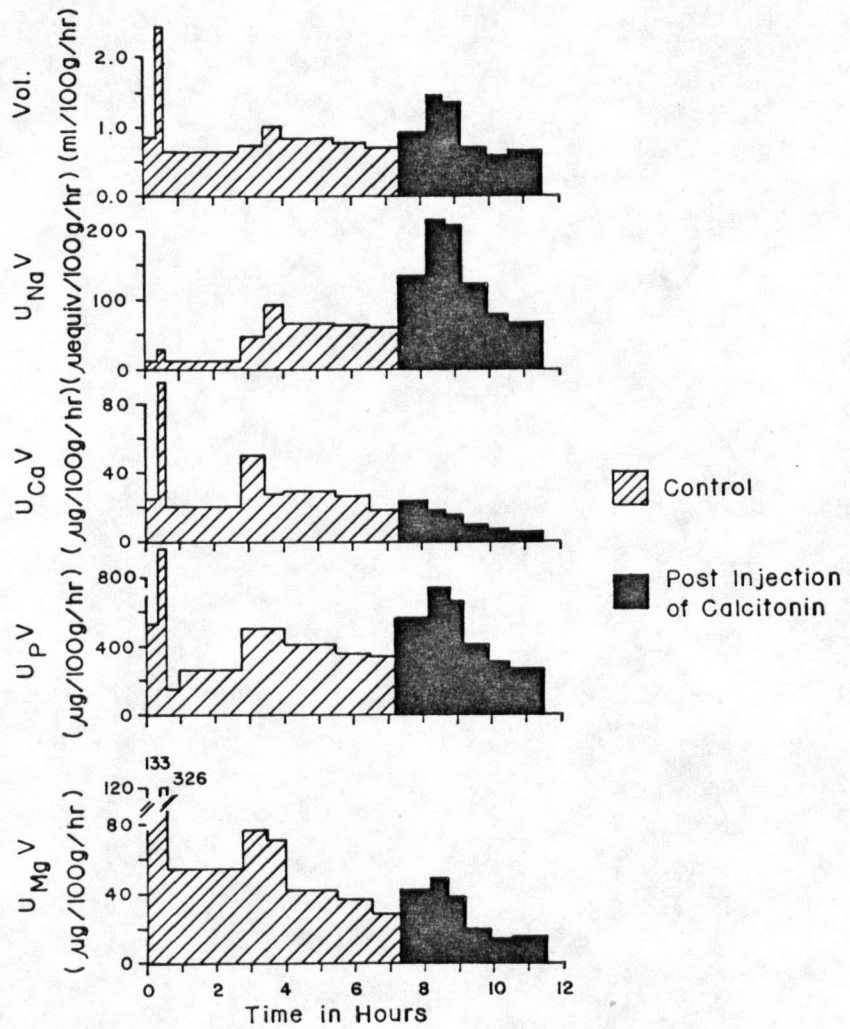

Fig 4. Electrolyte changes in 460-g rat (age seven months) given 500/mU calcitonin, i.v. during dextrose(2.5%)-saline (75 meq/l) infusion given at a rate of 3ml/hour.

TABLE I

The effect of salmon calcitonin on urinary electrolytes in dextrose-infused rat*

Time (hr)	Vol/100g/hr (ml)	Na	K	Ca	P	Mg	Creat.	Na/K	Na/Creat.
		µequiv/100g/hr		µg/100g/hr					
Control									
-2½ - 2	0.594	38.0	69.5	14.4	250	121.3	309	0.547	0.123
-2 - 1½	1.862	7.4	46.6	9.2	279	100.5	205	0.160	0.036
-1½ - 1	2.683	13.4	37.6	14.5	215	102.0	188	0.357	0.071
-1 - ½	2.972	17.8	47.6	16.7	208	136.7	208	0.375	0.085
-½ - 0	1.538	23.1	89.2	11.8	461	152.3	200	0.258	0.115
Calcitonin									
0 - ½	1.241	54.6	120.4	8.8	918	114.2	211	0.453	0.258
½ - 1	1.958	68.5	101.8	7.5	1410	76.4	215	0.673	0.318
1 - 1½	2.220	62.2	66.6	6.2	1043	42.2	178	0.933	0.350
1½ - 2	1.556	82.5	70.0	4.9	902	56.0	187	1.177	0.441
2 - 2½	1.792	52.0	52.0	6.9	860	35.8	179	1.000	0.290
2½ - 3	1.136	36.4	37.5	5.1	608	40.9	148	0.969	0.246
3 - 3½	2.194	35.1	39.5	3.9	899	43.9	198	0.888	0.177
3½ - 4	1.503	37.6	33.1	8.1	736	76.7	188	1.136	0.200
4 - 4½	1.294	38.8	33.6	8.4	725	88.0	181	1.153	0.214
4½ - 5	2.281	25.1	25.1	10.3	707	93.5	205	1.000	0.122
5 - 5½	1.040	46.8	36.4	10.5	385	171.6	198	1.285	0.236
5½ - 6	0.760	52.4	41.0	10.1	502	126.9	190	1.277	0.275
6 - 6½	1.084	41.2	41.2	10.0	650	124.7	173	1.000	0.238

*Age of rat, 3 months; weight, 229 g. Pretreated with 1.5 mg DOCA, i.m. and infused with 2.5% dextrose at 3ml/hour. Calcitonin (250 mU, i.v.) given at time 0 hour.

Changes in urinary excretion over a six-hour period are shown in Fig. 5 where responses to 0, 10, 100, 500 and 2000 mU calcitonin per 100 g body weight are indicated. It will be noted that increases in urine volume and the excretion of Na were related to the does of hormone given. Increases in P excretion and decreases in the excretion of Ca and Mg were also found. However, at the highest dose level (2000 mU) the reduction in the excretion of Ca and in part that of Mg was overcome.

Analysis of another study at the 100 mU dose level in Table II shows an increase in the Na/K ratio which invariably occurred following calcitonin treatment since the increase in Na excretion was accompanied by little or no increase in K excretion. The ratio of Na/Creat., which gives an indication of the fractional excretion of Na, also increased following hormone administration.

Significant decreases in the plasma concentrations of Ca, P, and Mg were found following calcitonin administration (100 mU/100 g body weight, i.v.) at one hour as is seen in Table III. Plasma Na and K concentrations were not changed. The decrease in plasma Ca and P was also significant at three hours after injection of the hormone although plasma Mg levels at this time were not significantly different from the vehicle-injected controls.

Table IV compares the electrolyte outputs in rats with and without DOCA administration. It also compares the relative responses when calcitonin was given in addition to DOCA treatment. It can be seen that DOCA caused a significant decrease in $U_{Na}V$, accompanied by a slight decrease in $U_{K}V$. However, the excretion of Ca, P, and Mg was unaffected by the DOCA. When calcitonin was administered at the same time as DOCA, the absolute increase in $U_{Na}V$ was virtually the same as that found in the non-DOCA, calcitonin-treated rats (non-DOCA, calcitonin-treated: 11.4 μequiv/100g/hr; DOCA,

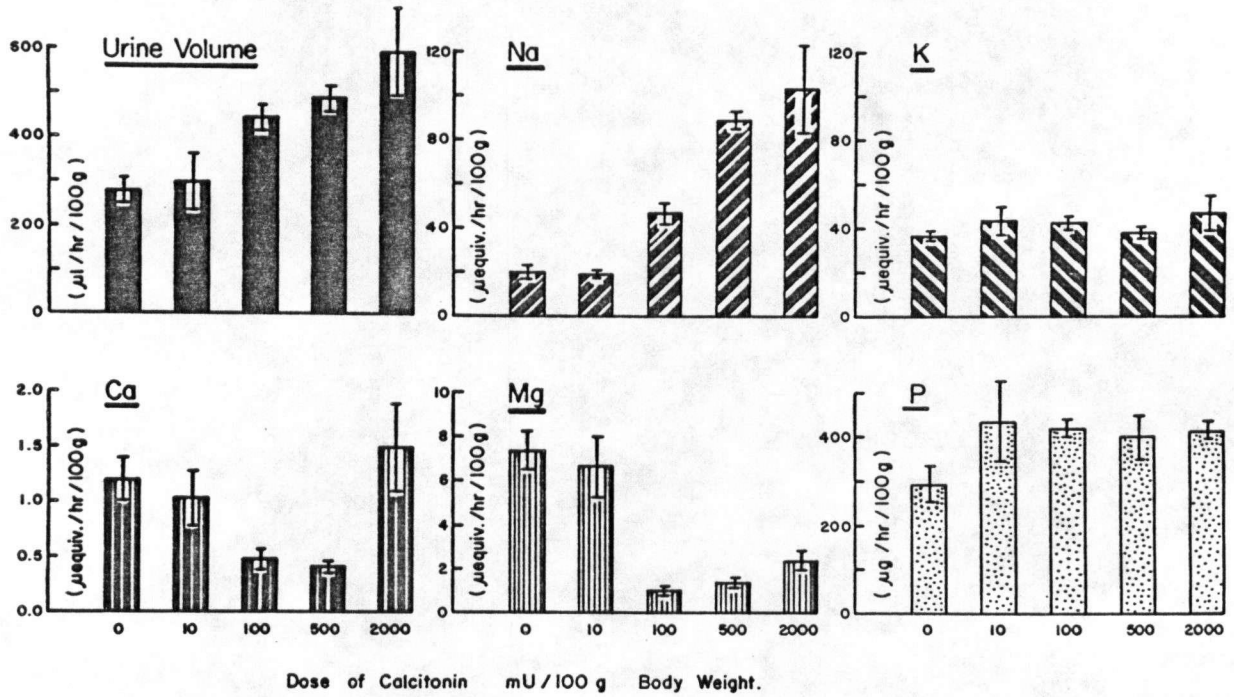

Fig 5. Urinary changes in young rats in response to graded doses of salmon calcitonin administered i.p. Urine was collected over a six-hour period. Each bar indicates the Mean ± SEM in six rats.

TABLE II

Effects of salmon calcitonin on urinary electrolyte excretion in intact rats*

Treatment	Volume	Na	K	Ca	P	Mg	Creat.	Na/K	Na/Creat.
	ml/100g/hr	μequiv/100g/hr		μg/100g/hr					
Vehicle (9)	0.264 ±0.054	27.3 ±3.1	41.3 ±2.2	12.1 ±1.1	414 ±29	86.4 ±8.3	120 ±4	0.658	0.225
Calcitonin (12)	0.542 ±0.059	57.9 ±5.7	36.9 ±1.5	6.6 ±1.0	455 ±29	19.2 ±3.7	130 ±4	1.569	0.440
p	<0.005	<0.001	NS	<0.005	NS	<0.001	NS		

*Age of rats, 3 months. Urine collected over six hours following injection of salmon calcitonin, 100 mU/100 g body weight, i.p. Mean ± SEM; n = number in parenthesis.

TABLE III

Plasma electrolyte changes following salmon calcitonin in intact rats*

Treatment	Na	K	Ca	P	Mg
	meq/l			mg%	
One-hour sample					
Vehicle (10)	141±1	4.1±0.2	8.98±0.14	7.41±0.26	2.339±0.077
Calcitonin (10)	138±1	3.8±0.1	7.19±0.15	5.31±0.19	2.027±0.083
p	NS	NS	<0.001	<0.001	<0.025
Three-hour sample					
Vehicle (11)	128±1	4.1±0.1	8.55±0.14	8.55±0.14	2.096±0.077
Calcitonin (11)	129±1	4.0±0.1	6.14±0.06	6.22±0.08	1.983±0.054
p	NS	NS	<0.001	<0.001	NS

*Age of rats, 26 days. Dose of calcitonin, 100 mU/100 g body weight, i.v. Animals bled by cardiac puncture and starved 12 hours prior to study. Mean ± SEM; n = number in parenthesis.

TABLE IV

The effects of simultaneous administration of DOCA and calcitonin on urinary electrolyte excretion in intact rats*

Treatment	Volume	Na	K	Ca	P	Mg	Creat.	Na/K	Na/Creat.
	ml/100g/hr	μequiv/100g/hr		μg/100g/hr					
Vehicle (6)	0.239 ±0.025	22.6 ±3.2	49.1 ±5.5	18.0 ±1.8	226 ±58	94.7 ±3.5	146 ±8	0.484	0.155
Calcitonin (6)	0.348 ±0.041	34.0 ±4.6	51.6 ±3.3	6.9 ±1.5	468 +56	63.5 ±12.9	162 ±11	0.689	0.216
DOCA + Vehicle(6)	0.205 ±0.035	8.6 ±1.4	42.3 ±2.4	20.8 ±6.9	308 ±19	91.4 ±9.6	134 ±8	0.201	0.067
DOCA + Calcitonin (6)	0.201 ±0.035	18.7 ±3.7	50.0 ±3.4	7.8 ±2.9	435 ±39	47.4 ±8.8	147 ±4	0.367	0.126

*Age of rats, two months. DOCA, 1 mg/100 g body weight, i.m.; Calcitonin, 100 mU/100g body weight, i.p. Urine collection over six hours. Mean ± SEM; n = number in parenthesis.

calcitonin-treated; 10.1 μequiv/100g/hr). Changes in $U_{Ca}V$, $U_{Mg}V$, and U_pV in DOCA-treated rats given calcitonin were not appreciably different from those found in non-DOCA, calcitonin-treated animals. The absolute changes in urinary electrolyte excretion in the non-DOCA and DOCA-treated rats given calcitonin are shown in Fig. 6.

In Table V, it is seen that the natriuretic response to a single injection of calcitonin was much larger during the six-hour period following administration of the hormone in the 5-week-old rats than it was in the 28-week-old rats. Also during this time period the % drop in $U_{Ca}V$ and $U_{Mg}V$ was greater in the younger rats. However, in these studies on rats of different ages the urinary changes in response to calcitonin administration persisted for a longer period of time in the older rats so that the more acute and immediate response to the hormone in the younger rats was balanced by a more prolonged response in the older animals.

In summary, the experiments in intact rats given a single injection of calcitonin indicated that:

(a) Increases in urine volume, $U_{Na}V$, and U_pV occurred following a single injection of calcitonin, but in contrast to the findings in the chronic studies, there were net decreases in $U_{Ca}V$ and $U_{Mg}V$.

(b) The changes in urinary volume and $U_{Na}V$ were related to the dose of calcitonin given.

(c) The plasma changes which occurred within the time interval of the urinary collections indicated significant decreases in plasma Ca, P, and Mg. However, a significant decrease in plasma Mg was not found at three hours after injection of hormone, and no significant changes in plasma

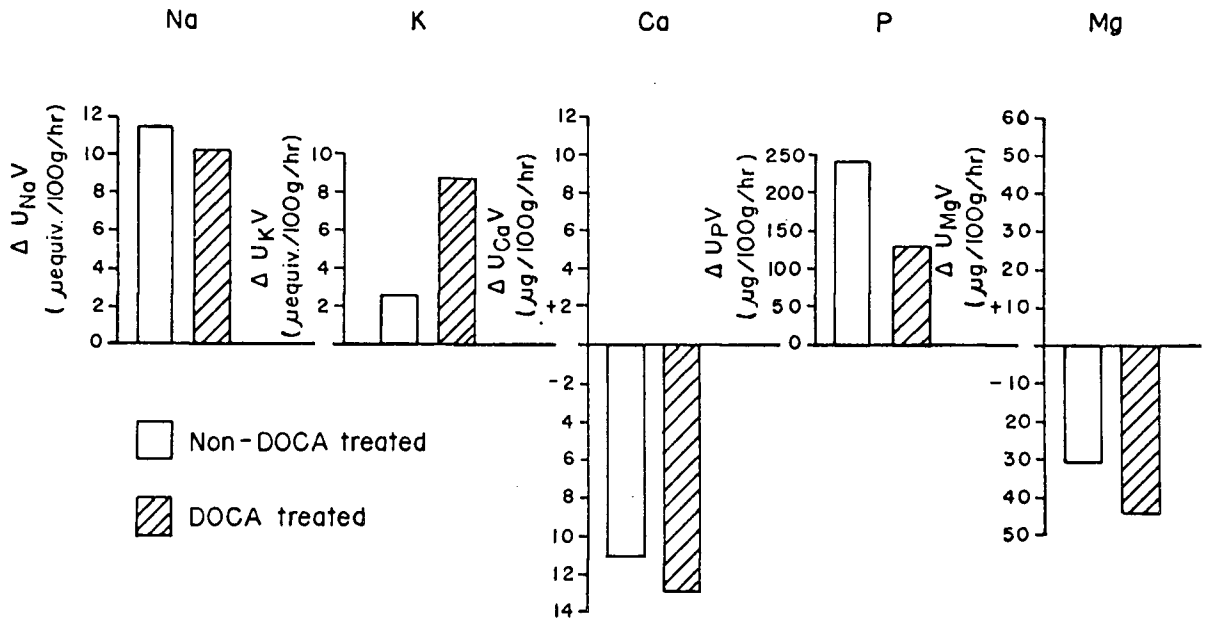

Fig 6. Comparison of absolute changes in urinary electrolyte excretion in response to a single injection of calcitonin (100 mU/100 g body weight, i.p.) into non-DOCA treated and DOCA treated rats. (Compiled from Table IV)

Table V

Effect of calcitonin on urinary electrolyte excretion in rats of different ages*

Age (wks)	Weight (g)	Treatment	Time (hr)	Volume ml/100g/hr	Na µequiv/100g/hr	K µequiv/100g/hr	Ca ug/100g/hr	P ug/100g/hr	Mg ug/100g/hr	Creat. ug/100g/hr	Na/K
28	474 ±18	Vehicle (5)	0-6	0.195 ±0.024	13.8 ±1.7	24.5 ±2.0	11.1 ±1.9	223 ±18	16.1 ±4.3	115 ±2	0.567
			6-12	0.119 ±0.410	4.5 ±0.68	11.1 ±1.6	5.3 ±1.1	127 ±20	17.4 ±4.8	101 ±11	0.437
			12-24	0.151 ±0.350	4.2 ±0.54	6.3 ±1.4	3.4 ±0.6	181 ±25	8.1 ±1.4	92 ±7	0.800
	460 ±23	Calcitonin (5)	0-6	0.299 ±0.019	24.3 ±3.5	26.5 ±1.5	5.4 ±0.8	322 ±18	2.6 ±0.3	128 ±11	0.915
			6-12	0.162 ±0.029	12.1 ±1.5	8.0 ±1.1	3.4 ±0.7	189 ±22	1.7 ±0.3	90 ±8	1.633
			12-24	0.371 ±0.124	18.4 ±5.2	4.9 ±1.4	3.7 ±0.5	190 ±42	16.6 ±2.5	121 ±11	5.208
5	135 ±6	Vehicle (5)	0-6	0.314 ±0.055	20.0 ±1.4	41.0 ±3.2	68.6 ±18.4	362 ±66	79.6 ±14.3	122 ±5	0.502
			6-12	0.256 ±0.061	9.5 ±0.4	24.3 ±4.0	18.8 ±4.4	352 ±58	58.9 ±8.3	95 ±7	0.447
			12-24	0.244 ±0.041	11.2 ±1.7	15.6 ±1.7	4.6 ±1.9	508 ±32	22.2 ±9.4	87 ±6	0.790
	130 ±6	Calcitonin (5)	0-6	0.704 ±0.086	63.8 ±5.3	50.9 ±3.7	21.7 ±4.3	525 ±25	9.1 ±2.5	123 ±11	1.272
			6-12	0.314 ±0.105	15.3 ±1.2	19.5 ±2.5	5.4 ±1.1	509 ±66	16.3 ±5.9	102 ±11	0.826
			12-24	0.377 ±0.071	7.7 ±2.1	14.8 ±2.7	10.8 ±3.7	769 ±119	59.0 ±18.5	98 ±8	0.498

*Calcitonin, 100 mU/100g, i.p. given at time 0 hour. Mean ± SEM; n = number in parenthesis.

Na and K were found at either one or three hours after injection of calcitonin.

(d) The absolute increase in $U_{Na}V$ in response to calcitonin was unaffected by large doses of the synthetic mineralocorticoid, DOCA.

(e) Calcitonin caused a greater and more immediate response in the changes in the excretion of Na, Ca, P and Mg in young rats than it did in older rats.

2. Thyroparathyroidectomized rats

Since hypocalcemia is known to stimulate PTH release, and since calcitonin in the intact rats induced a long-acting hypocalcemic response, experiments in TPTX rats were carried out in order to determine whether the electrolyte changes in the urine following calcitonin treatment were a primary response to the hormone or were perhaps secondary to parathyroid stimulation. Were they related to the altered plasma Ca level per se?

Methods

Groups of rats were thyroparathyroidectomized and thyroidectomized according to Surgical Procedures in the GENERAL METHODS section. Since both groups of rats were without thyroid tissue, and thus lacked "C" cells, the only difference between them was the presence in the TX group of functioning parathyroid transplants, as indicated by the ability of these rats to maintain a normal plasma Ca level. Thus, the TX animals were an appropriate control group for the TPTX rats. Both groups were then given calcitonin and their urinary electrolyte outputs were

measured over a period of six hours. A further comparison of the response to calcitonin was made between TPTX and intact rats.

Control urine outputs during various time periods from 3-24 hours were also examined in the TPTX and TX rats in order to determine if an altered pattern of electrolyte excretion was evident in these two groups with widely different resting plasma Ca levels.

Results

Table VI shows the effects of calcitonin on urinary electrolyte excretion in TPTX and TX rats. It is evident from this study that no reduction in the natriuretic response occurred in the TPTX rats despite the absence of the parathyroid glands. In fact, an even greater response was found. The potentiation of the natriuretic response in TPTX rats, as compared to those rats having parathyroid tissue (TX and intact), was a consistent finding in these studies.

The pattern of changes in other urinary parameters in the TPTX rats in response to calcitonin administration was similar to that found in the TX and intact rats as is seen in Tables VI and VII. Thus in all three groups of rats (TPTX, TX, and intact), the direction of the electrolyte changes following exogenous calcitonin was unaltered by the presence or absence of the parathyroid glands.

As shown in Table VIII, despite the altered resting plasma Ca levels in the TPTX rats (plasma Ca: 5.78±0.32 mg%) as compared to the normal plasma Ca levels in the TX and intact rats (plasma Ca, TX: 10.13±0.12 mg%; intact: 10.20±0.13 mg%), the excretion of Na and K over a 24-hour period was remarkably similar in the three groups of rats.

TABLE VI

Urinary electrolyte excretion in TPTX and TX rats following salmon calcitonin*

Group	Plasma Ca	Volume	Na	K	Ca	P	Mg	Creat.	Na/K	Na/Creat.
	mg%	ml/100g/hr	µequiv/100g/hr		µg/100g/hr					
TPTX	6.16									
1st 3 hr	±0.14									
Vehicle (8)		0.507 ±0.073	43.3 ±8.3	57.6 ±8.2	71.5 ±16.4	136 ±23	101.2 ±16.8	139 ±14	0.773	0.312
Calcitonin (10)		0.536 ±0.067	81.6 ±10.5	77.9 ±8.0	36.3 ±8.6	356 ±38	53.2 ±9.5	133 ±11	1.064	0.611
2nd 3 hr										
Vehicle		0.236 ±0.036	24.9 ±5.5	27.1 ±5.5	39.7 ±6.8	51 ±10	50.6 ±10.8	87 ±17	0.963	0.286
Calcitonin		0.633 ±0.079	93.1 ±15.8	46.5 ±5.3	13.6 ±1.7	317 ±38	41.5 ±7.9	106 ±8	1.970	0.835
TX	10.12									
1st 3 hr	±0.33									
Vehicle (7)		0.413 ±0.070	33.9 ±10.4	57.9 ±13.7	88.1 ±30.7	196 ±70	133.2 ±16.5	148 ±20	0.479	0.229
Calcitonin (7)		0.252 ±0.031	48.3 ±7.9	49.6 ±6.6	37.0 ±10.6	257 ±41	43.0 ±4.9	116 ±11	0.964	0.399
2nd 3 hr										
Vehicle		0.219 ±0.061	12.5 ±5.5	23.8 ±6.3	29.3 ±9.2	83 ±23	63.6 ±14.4	125 ±23	0.539	0.100
Calcitonin		0.333 ±0.044	59.3 ±11.4	31.7 ±4.3	7.6 ±0.8	213 ±31	43.5 ±7.4	116 ±7	1.800	0.492

*Age of rats, 2 1/2 months. Calcitonin, 100 mU/100 g body weight, i.p. Mean ± SEM; n = number in parenthesis.

TABLE VII

The effect of calcitonin on urinary electrolyte excretion in TPTX and intact rats*

Group	Volume ml/100g/hr	Na	K	Ca	P	Mg	Creat.	Na/K	Na/Creat.
		µequiv/100g/hr		µg/100g/hr					
TPTX									
Vehicle (5)	0.408 ±0.066	44.3 ±6.7	61.2 ±2.6	51.4 ±10.5	124 ±27	68.0 +12.4	161 ±7	0.716	0.271
Calcitonin (6)	0.570 ±0.074	62.5 ±6.7	55.0 ±4.3	11.9 ±3.3	205 ±55	39.6 ±6.4	128 ±12	1.169	0.495
Intact									
Vehicle (5)	0.469 ±0.049	40.9 ±10.3	67.4 ±5.1	26.2 ±7.7	433 ±63	85.9 ±16.9	165 ±3	0.575	0.247
Calcitonin (5)	0.428 ±0.064	52.9 ±7.2	69.1 ±4.8	4.3 ±0.45	599 ±35	62.3 ±15.6	163 ±15	0.761	0.329

*Age of rats, 1 1/2 months. Calcitonin, 100 mU/100g body weight, i.p. Urine collected over six hours. Mean ± SEM; n = number in parenthesis.

TABLE VIII

Control urinary electrolyte excretion in TPTX, TX, and intact rats during a 24-hour collection*

Group	Plasma Ca mg%	Volume ml/100g/hr	Na	K	Ca	P	Mg	Creat.	Na/K	Na/Creat.
			µequiv/100g/hr		µg/100g/hr					
TPTX (12)	5.78 ±0.32	0.257 ±0.031	16.7 ±1.7	26.0 ±1.9	47.7 ±4.4	120 ±10	61.1 ±5.4	118 ±3	0.647	0.140
TX (6)	10.13 ±0.12	0.459 ±0.109	15.4 ±3.2	22.7 ±3.1	36.7 ±10.9	206 ±27	51.6 ±8.2	129 ±10	0.650	0.114
Intact (12)	10.20 ±0.13	0.153 ±0.017	17.4 ±1.5	33.4 ±1.5	14.5 ±1.9	306 ±27	102.3 ±18.7	110 ±3	0.523	0.153

*Age of rats, two months. Animals were without food during collection period. Mean ± SEM; n = number in parenthesis.

The excretion of Ca over a 24-hour period in the TPTX rats was similar to that found in the TX rats as is seen in Table VIII in spite of the lower plasma Ca level in the TPTX group. However, the excretion of P was less in the TPTX group as compared to either the TX or the intact groups where parathyroid tissue was present.

In summary:

(a) The presence of functioning parathyroid tissue was not essential for the increase in the excretion of Na and P and the decrease in the excretion of Ca and Mg observed in young rats following a single injection of calcitonin.

(b) An altered resting plasma Ca level did not affect the net excretion of Na and K over a 24-hour period.

II. CALCIUM INFUSION STUDIES (ENDOGENOUS CALCITONIN RELEASE)

In the rat experiments, plasma studies were necessarily performed separately from the urine studies because of the trauma involved in blood sampling and the effect this might have on kidney function. Also, the size of the rat imposed a limit on the number of blood samples that could be taken from any one animal in a single study.

It seemed of great advantage to be able to follow simultaneous changes in plasma and urine since this would enable a clearer concept of the relationship between the two and show how changes in one parameter might be reflected by alterations in the other. Thus, a larger animal was thought to offer distinct advantages. The sheep was chosen not only for its suitable size but also because its thyroid gland is separate from the large superior parathyroids. Therefore, surgery in these animals would not require transplantation of the parathyroids in those experiments where thyroidectomy was required. In addition, in the larger animal, any manipulations regarding catheterization for sampling or infusion could be performed without the use of anaesthesia so that the animals could be studied for long periods of time in a conscious state.

The rat studies all involved an investigation of the electrolyte changes following intervention of <u>exogenous</u> calcitonin from another class of animal. The sheep studies were designed in order to assess the effects of <u>endogenous</u> calcitonin secretion.

Since the infusion of Ca salts is known to cause a proportional increase in circulating calcitonin levels (47, 48), a comparison of the plasma and urinary electrolyte changes in intact and thyroidectomized animals following a Ca stress should indicate the degree of involvement of endogenous calcitonin in the responses found.

Plasma and Urinary Electrolyte Changes in Conscious Sheep Following Calcium Infusion

Methods

All sheep studies were performed while the animals were loosely restrained on a stand by a shoulder and hip harness. This allowed considerable freedom of movement and the animals were quiet and relaxed during the 6-8 hour period of the experiment.

Following preparations according to procedures in the GENERAL METHODS section, a maintenance infusion of dextrose-saline was given at a rate of 23 ml/hour which balanced the mean hourly urine outputs determined in preliminary control collections. This infusion rate was maintained throughout the experiment.

Blood and urine collections began at the completion of a one-hour equilibration period, blood samples being taken at the midpoint of each 15-minute urine collection.

After a control collection period of 1 1/4 hours, Ca was added to the infusate ($CaCl_2$ in dextrose-saline) in a concentration to give 10 mg Ca/kg body weight for one hour. Blood and urine collections then continued for a further three hours following completion of the one-hour Ca infusion so that

the total collection time was 5 1/4 hours. Catheters were then removed and individual sheep were given a 10-day to 2-week recovery before being used again.

Upon completion of studies in intact sheep, the same animals were thyroidectomized and subjected to a further series of Ca infusions.

The final series of experiments included the addition of salmon calcitonin, given at a rate of 2 U/kg body weight/hour for a two-hour period beginning at the start of the Ca infusion in the TX sheep.

Results

Plasma changes

Following the start of the Ca infusion in the intact sheep, plasma Ca levels immediately rose with an increase of 1.60±0.07 mg% after one hour of the infusion. As is seen in Fig. 7, the plasma Ca levels rapidly returned to normal values when the infusion was stopped. Calcium infusion in the TX sheep was followed by a significantly greater absolute rise in plasma Ca levels of 2.31±0.10 mg%. The return of plasma Ca to base-line levels was delayed in this group and was not achieved during the experimental period. When salmon calcitonin was infused for two hours beginning at the start of the Ca infusion in the TX sheep, the absolute rise in plasma Ca levels was less, as compared to the TX sheep without calcitonin, and the return to base-line control levels was accelerated. Plasma Ca levels at 1,2, and 4 hours after the start of the Ca infusion in the three groups of sheep are shown in Table IX.

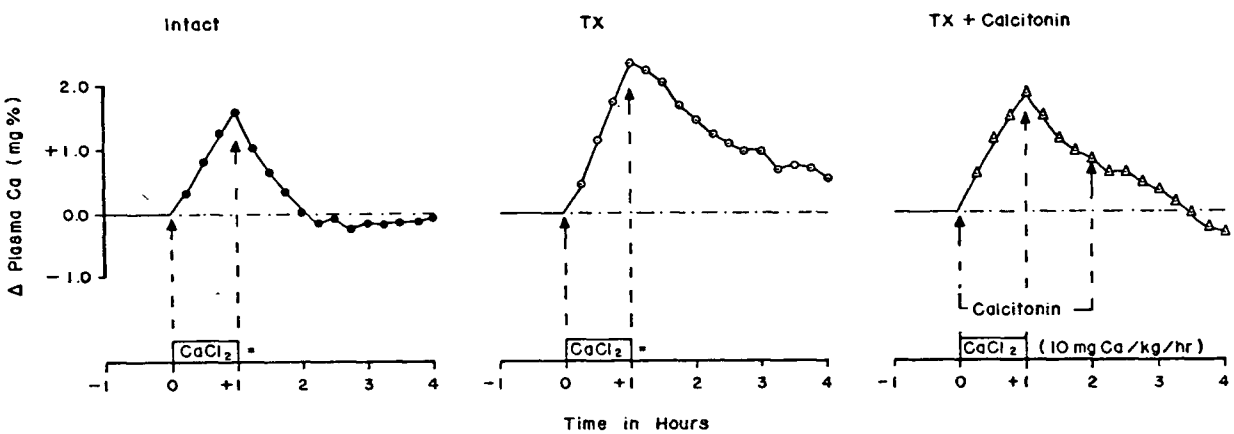

Fig 7. Changes in plasma Ca levels following Ca infusion in intact, TX, and TX sheep infused with salmon calcitonin (2 U/kg/hr). Changes in plasma Ca expressed as mg% changes from base-line control values. (Mean values are indicated; for n see Table IX.)

TABLE IX

Plasma changes following Ca infusion in intact, TX, and TX + CT sheep*

Time after start of Ca infusion (hr):	1	1 1/2	2	4
Δ Plasma Ca				
Intact (5)	1.60 ± 0.07		0.03 ± 0.18	-0.08 ± 0.03
TX (6)	2.31 ± 0.10		1.42 ± 0.18	0.52 ± 0.30
TX + CT (4)	1.82 ± 0.26		0.83 ± 0.30	-0.32 ± 0.31
p Intact vs TX	<0.001		<0.001	
Δ Plasma P				
Intact (4)	-0.24 ± 0.15	-0.66 ± 0.22	-0.71 ± 0.27	-1.29 ± 0.21
TX (5)	0.18 ± 0.07	0.42 ± 0.07	0.36 ± 0.07	-0.80 ± 0.19
TX + CT (4)	-0.38 ± 0.12	-0.73 ± 0.19	-0.86 ± 0.30	-1.42 ± 0.45
p Intact vs TX	<0.050	<0.005	<0.005	
TX vs TX+CT	<0.005	<0.001	<0.005	
Δ Plasma Mg				
Intact (3)	-0.108 ± 0.027		-0.224 ± 0.040	-0.201 ± 0.050
TX (6)	-0.096 ± 0.055		-0.251 ± 0.073	-0.261 ± 0.097
TX + CT (4)	-0.218 ± 0.072		-0.498 ± 0.092	-0.563 ± 0.063
p				
TX vs TX+CT				<0.050
Intact vs TX+CT				<0.010

* mg% changes from baseline control values are shown. Mean ± SEM; n = number in parenthesis.

As the plasma Ca levels were rising in the intact sheep, plasma P levels were found to fall as is shown in Fig. 8. However, in the TX animals, this decrease in plasma P levels was delayed and was preceded by a slow rise reaching a peak about 1/2 to 3/4 hour after the end of the Ca infusion. Plasma P levels in the TX sheep showed an increase of +0.42±0.07 mg% at this time as compared to the intact group where levels showed a decrease of -0.66±0.22 mg%. When calcitonin was infused in the TX group during and following the Ca infusion, an immediate fall in plasma P was observed which was similar to that seen in the intact sheep. Table IX shows the absolute changes in plasma P levels in the three groups of sheep at 1, 1 1/2, 2,and 4 hours following the start of the Ca infusion.

In Fig. 9, changes in plasma Mg levels are shown following Ca infusion. It is seen that the fall in plasma Mg which occurred following the start of the Ca infusion in the intact sheep was similar to that found in the TX sheep. Only when calcitonin was infused in this latter group were significantly greater decreases in plasma Mg levels found (Table IX).

Urine changes

In response to the Ca infusion, the intact sheep showed a diuretic response which was not observed in the TX animals. In fact, the TX sheep put out less urine than they did during the pre-infusion period. These changes are shown in Fig. 10 and Table X where the data were treated according to changes in urine volume from the mean of base-line control levels. The infusion of calcitonin in the TX sheep was accompanied by an increase in urine volume.

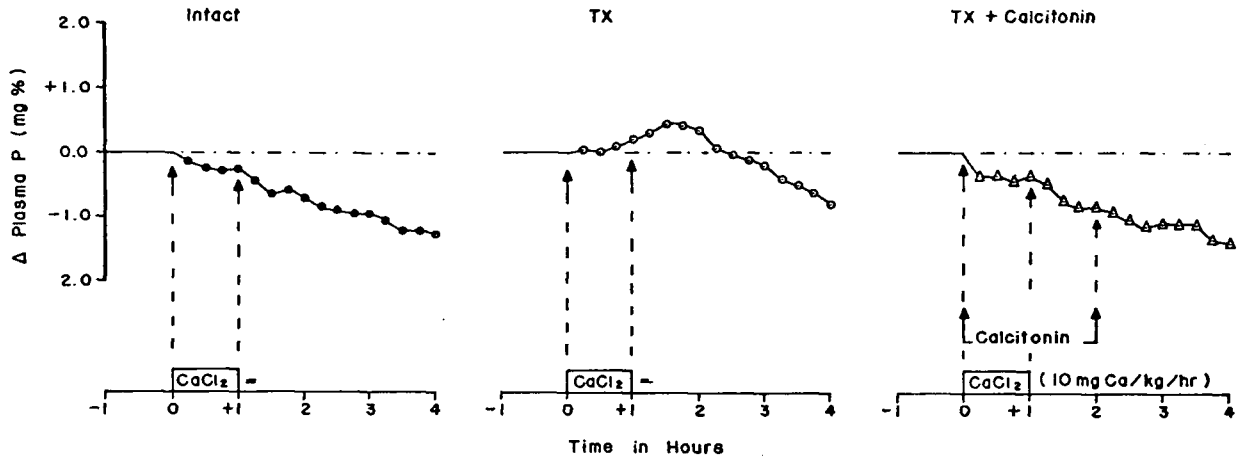

Fig 8. Changes in plasma P levels following Ca infusion in intact, TX, and TX sheep infused with salmon calcitonin (2 U/kg/hr). Changes in plasma P expressed as mg% changes from base-line control values. (Mean values are indicated; for n see Table IX.)

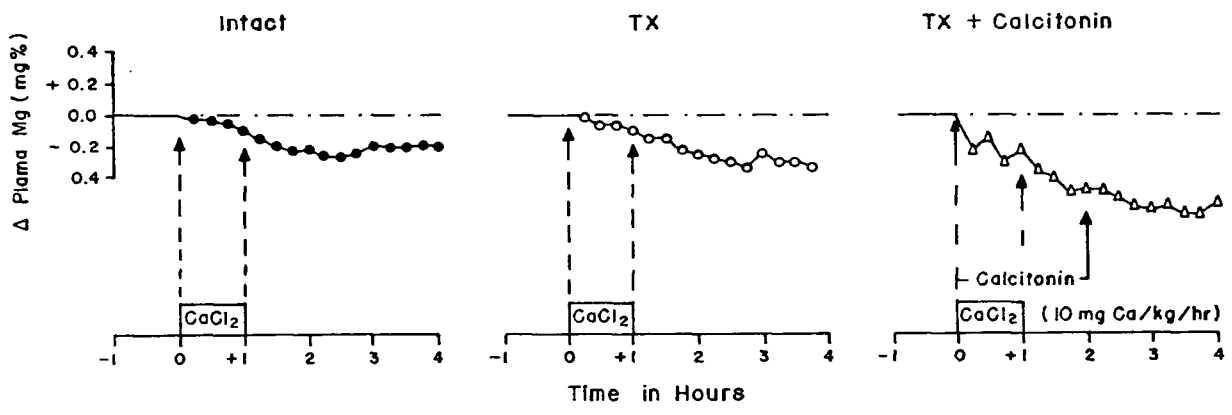

Fig 9. Changes in plasma Mg levels following Ca infusion in intact, TX, and TX sheep infused with salmon calcitonin (2 U/kg/hr). Changes in plasma Mg expressed as mg% changes from base-line control values. (Mean values are indicated; for n see Table IX.)

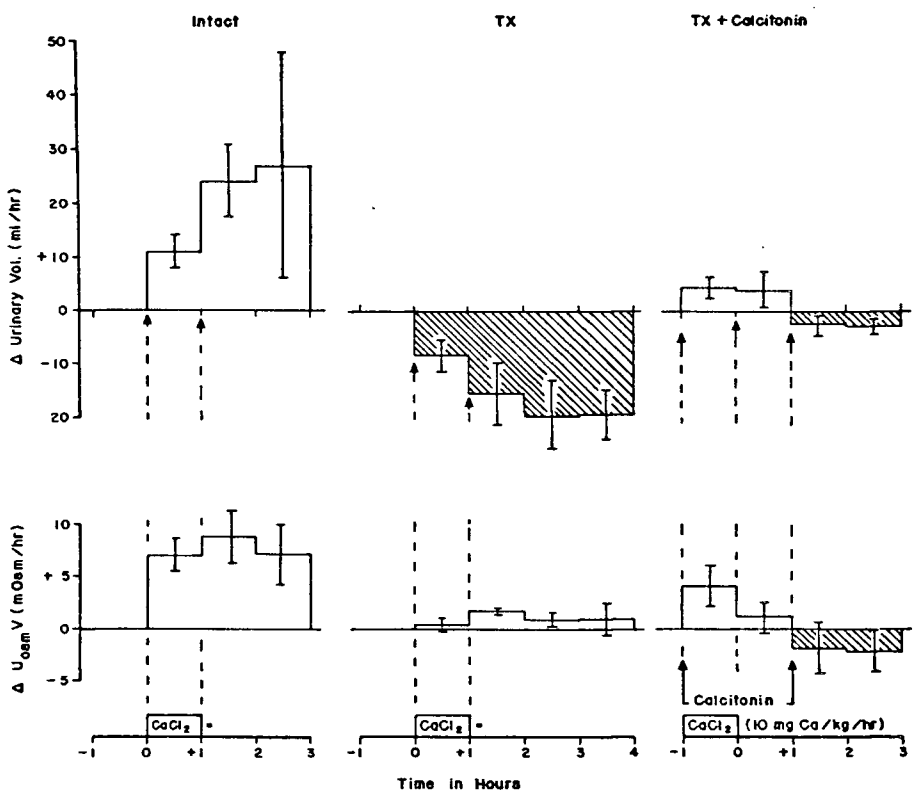

Fig 10. Changes in urine volume (upper trace) and osmolal output (lower trace) following Ca infusion in intact, TX, and TX sheep infused with salmon calcitonin (2 U/kg/hr). Changes from base-line control outputs are indicated. (Mean ± SEM; for n see Tables X and XI.)

TABLE X

Changes in urine volume following Ca infusion in intact, TX, and TX + CT sheep*

Time after start of Ca infusion (hr):	0 - 1	1 - 2	2 - 3	3 - 4
Changes in volume output from base-line control values (ml/hr)				
Intact (3)	11.0 ± 3.2	24.1 ± 6.8	27.0 ± 21.1	
TX (6)	-8.3 ± 3.1	-15.4 ± 5.9	-19.5 ± 6.6	-19.3 ± 4.7
TX + CT (4)	4.3 ± 1.9	3.8 ± 3.3	- 2.5 ± 2.3	- 2.7 ± 1.5
p Intact vs TX	<0.010	<0.005	<0.050	
TX vs TX+CT	<0.025	<0.050		<0.050
Intact vs TX+CT		<0.050		

*Mean ± SEM; n = number in parenthesis.

The diuresis observed in the intact sheep was accompanied by a large increase in osmolal output as is seen in Fig 10 and Table XI. The increase in osmolal output in the TX sheep was considerably less by comparison, but was enhanced (during the period of the Ca infusion) by calcitonin. The changes in urinary electrolyte excretion are shown in Figs 11, 12, 14, and 15 where 15-minute changes are recorded. Changes in U_PV are shown in Fig 13 and Table XII.

Changes in $U_{Ca}V$ appeared to reflect the plasma Ca levels. The amount of Ca excreted by the TX sheep was greater than that excreted by the intact or TX+CT groups as is seen in Table XIII. Not only was the hourly output in the TX group greater, but also the hypercalciuria was of longer duration.

Increases in $U_{Na}V$ in the TX sheep did not parallel the enhanced $U_{Ca}V$ found in this group. In fact, $U_{Na}V$ in the TX animals was less than that found in the intact sheep and significantly so during the period of the Ca infusion ($p < 0.005$). In two studies with the TX sheep, a large increase in $U_{Ca}V$ was accompanied by no change in $U_{Na}V$.

Resting U_PV levels varied considerably, not only between the different sheep but also in the individual sheep from day to day. Absolute changes in response to the Ca infusion also showed a wide variation. Therefore, it was impossible to group the data from any one series of experiments. Outputs from individual studies are shown, therefore, in order to obtain some concept of the direction of changes that occurred following Ca infusion rather than an absolute quantitative evaluation. Fig 13 shows the changes in U_PV that occurred during the period of the Ca

TABLE XI

Changes in osmolal output following Ca infusion in intact, TX, and TX + CT sheep*

Time after start of Ca infusion (hr):	0 - 1	1 - 2	2 - 3	3 - 4
Changes in $U_{osm}V$ from base-line control values (mOsm/hr)				
Intact (3)	6.88 ± 1.69	8.76 ± 2.57	7.05 ± 2.93	
TX (6)	0.43 ± 0.72	1.73 ± 0.27	0.92 ± 0.70	0.98 ± 1.62
TX + CT (4)	4.20 ± 2.08	1.23 ± 1.40	-1.71 ± 2.65	-2.09 ± 2.14
p Intact vs TX	<0.005	<0.005	<0.050	
Intact vs TX+CT		<0.050		

*Mean ± SEM; n = number in parenthesis.

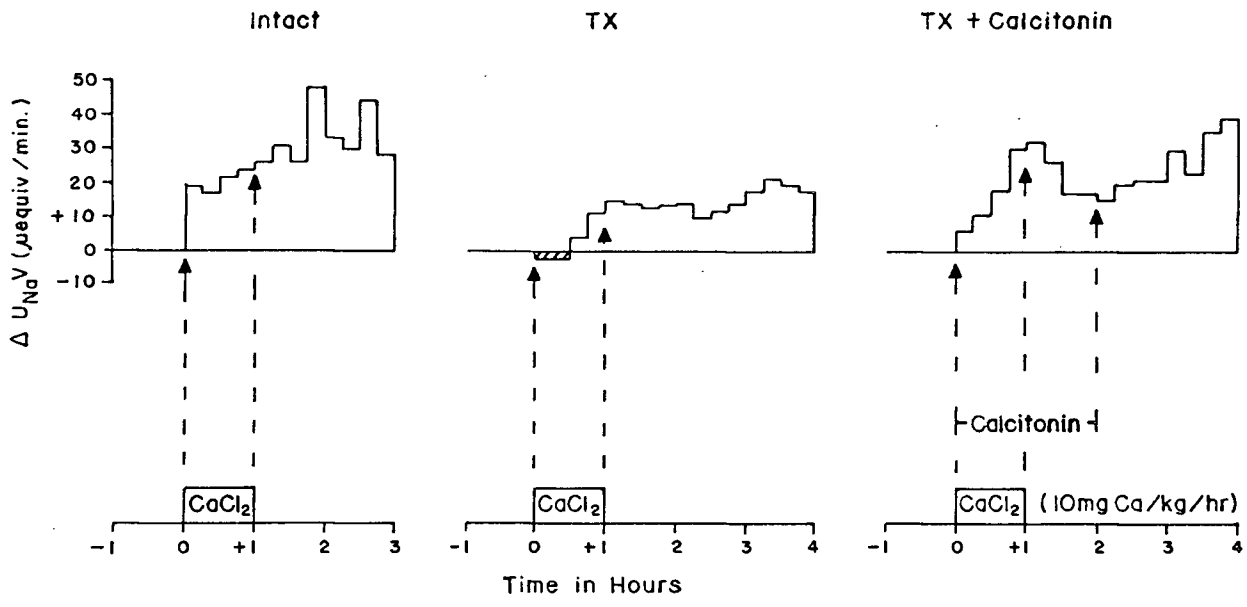

Fig 11. Changes in $U_{Na}V$ following Ca infusion in intact, TX, and TX sheep infused with salmon calcitonin (2 U/kg/hr). Changes from base-line control outputs are indicated. (Mean values are shown; for n see Table XIII.)

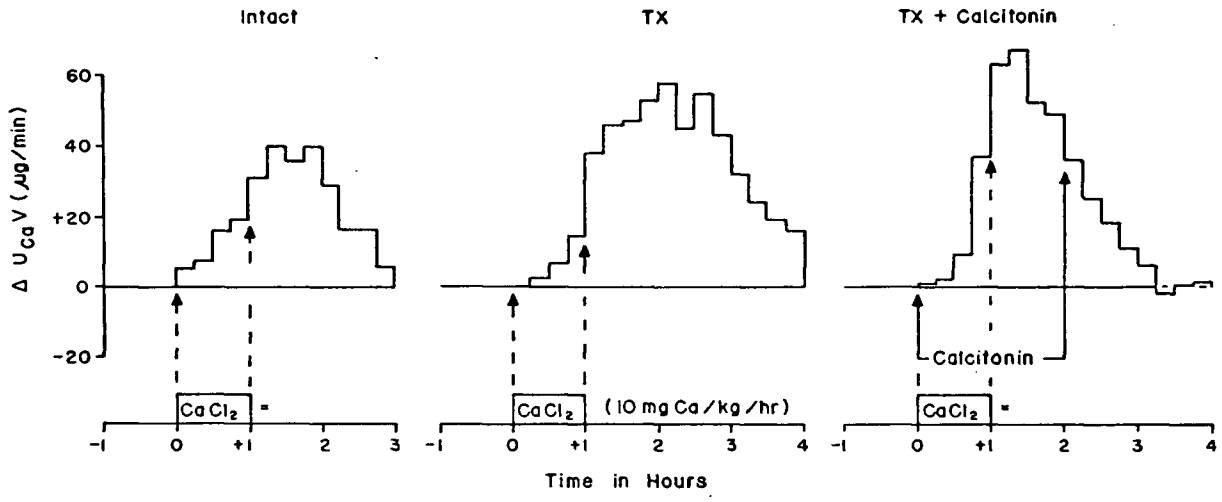

Fig 12. Changes in $U_{Ca}V$ following Ca infusion in intact, TX, and TX sheep infused with salmon calcitonin (2U/kg/hr). Changes from base-line control outputs are indicated. (Mean values are shown; for n see Table XIII.)

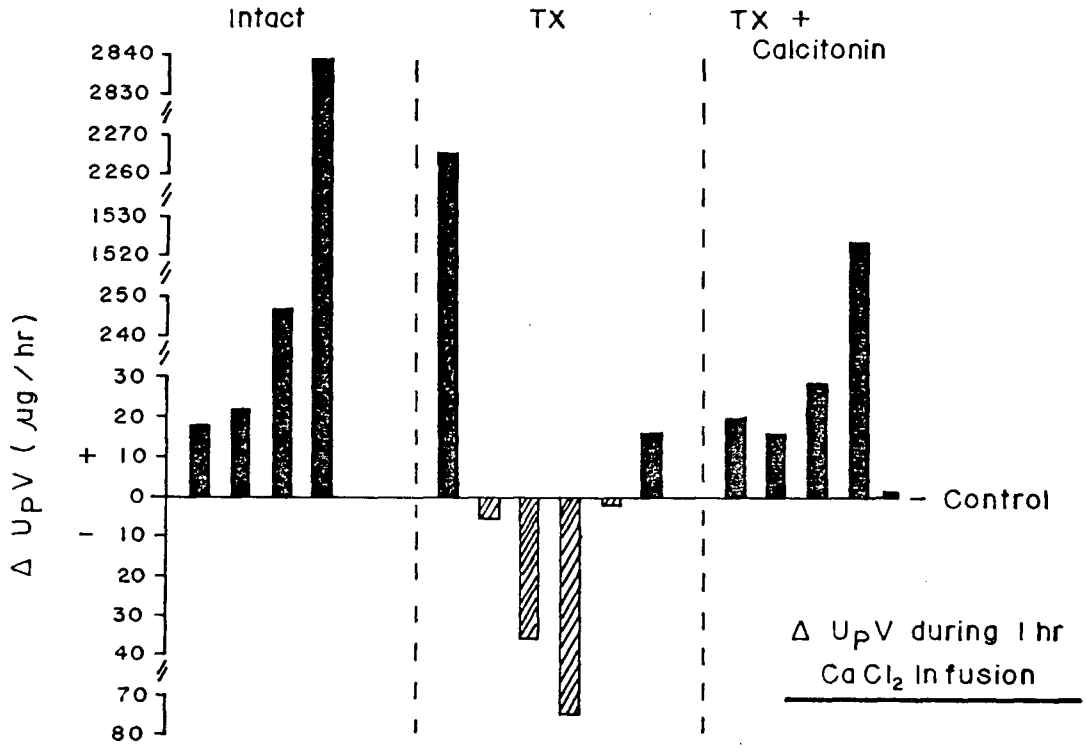

Fig 13. Changes in U_pV during the period of Ca infusion in individual intact, TX, and TX sheep infused with salmon calcitonin (2 U/kg/hr).

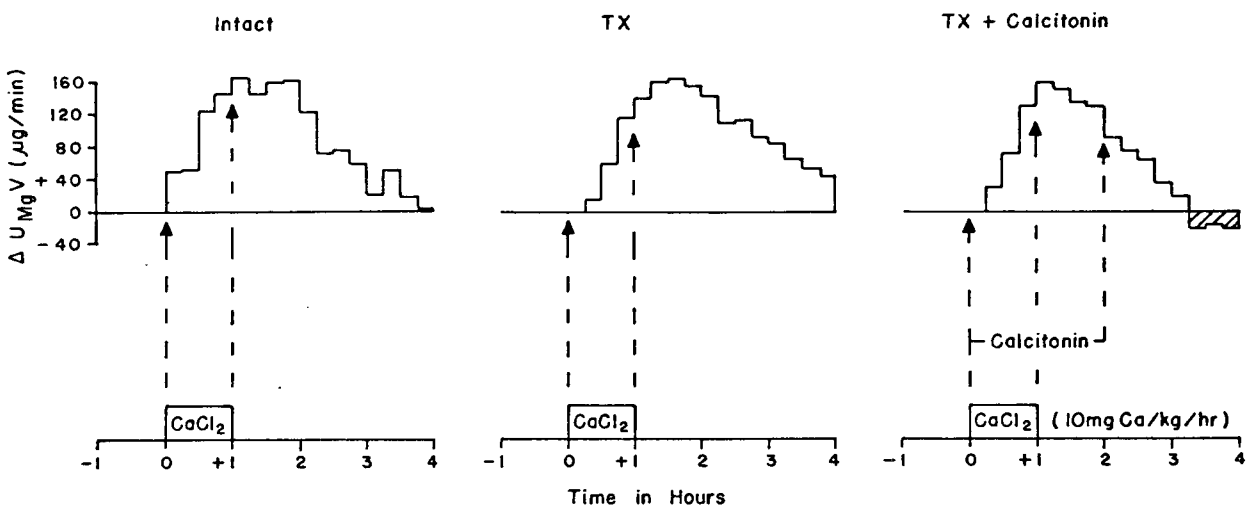

Fig 14. Changes in $U_{Mg}V$ following Ca infusion in intact, TX, and TX sheep infused with salmon calcitonin (2 U/kg/hr). Changes from base-line control outputs are indicated. (Mean values are shown; for n see Table XIII.)

TABLE XII

Urinary P excretion following calcium infusion in intact, TX, and TX + CT sheep

	Control	Calcium infusion	Post infusion		
Time (hr)	-1 - 0	0 - 1	1 - 2	2 - 3	3 - 4
U_pV (mg/hr)					
Intact	0.182	0.429 ↑	0.951 ↑	0.269 ↑	
	0.202	3.041 ↑	6.216 ↑	6.330 ↑	
	0.055	0.073 ↑	0.057 ↑	0.060 ↑	0.072 ↑
	0.075	0.097 ↑	0.084 ↑	0.077 ↑	0.066 ↓
TX	2.601	4.867 ↑	12.491 ↑	8.299 ↑	1.836 ↓
	0.182	0.176 ↓	0.203 ↑	0.177 ↓	0.111 ↓
	0.383	0.347 ↓	1.411 ↑	0.596 ↑	0.295 ↓
	1.371	1.296 ↓	1.517 ↑	0.929 ↓	1.214 ↓
	0.114	0.112 ↓	0.103 ↓	0.101 ↓	0.099 ↓
	0.097	0.113 ↑	0.102 ↑	0.108 ↑	0.113 ↑
TX + CT	0.084	0.104 ↑	0.074 ↓	0.065 ↓	0.064 ↓
	34.119	35.643 ↑	29.826 ↓	21.123 ↓	18.210 ↓
	0.116	0.132 ↑	0.109 ↓	0.110 ↓	0.102 ↓
	0.065	0.094 ↑	0.047 ↓	0.051 ↓	0.079 ↑
	0.089	0.090 ↑	0.085 ↓	0.071 ↓	0.091 ↑

Arrows indicate either increase (↑) or decrease (↓) from control period values.

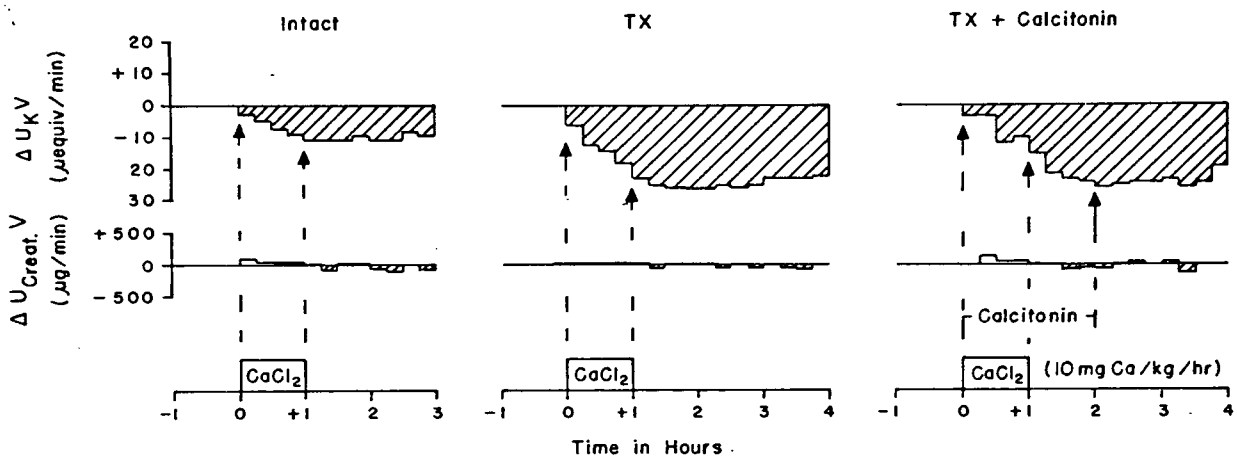

Fig 15. Changes in $U_K V$ (upper trace) and $U_{Creat.} V$ (lower trace) following Ca infusion in intact, TX, and TX sheep infused with salmon calcitonin (2 U/kg/hr). Changes from baseline control outputs are indicated. (Mean values are shown; for n see Table XIII.)

TABLE XIII

Changes in urinary electrolyte excretion following Ca infusion in intact, TX, and TX + CT sheep*

Time after start of Ca infusion (hr):	0 - 1	1 - 2	2 - 3	3 - 4
$\Delta U_{Na}V$ (meq/hr)				
Intact (3)	1.64±0.40	2.34±0.87	2.42±1.27	
TX (6)	0.45±0.09	1.08±0.25	1.04±0.31	1.45±0.65
TX + CT (4)	0.98±0.28	1.39±0.50	1.13±0.35	1.88±0.81
p Intact vs TX	<0.005			
$\Delta U_{Ca}V$ (mg/hr)				
Intact (3)	0.64±0.44	2.12±0.49	0.91±0.38	
TX (6)	2.87±2.54	2.74±0.47	3.19±0.97	1.37±0.56
TX + CT (4)	0.74±0.27	3.47±1.17	1.34±0.76	0.08±0.33
$\Delta U_{Mg}V$ (mg/hr)				
Intact (5)	5.48±0.80	9.31±1.98	4.89±0.94	1.24±0.52
TX (6)	2.66±0.50	8.96±1.19	6.83±1.82	3.65±1.72
TX + CT (4)	3.82±0.76	8.97±2.31	4.35±1.98	-0.25±0.67
p Intact vs TX	<0.025			
$\Delta U_{K}V$ (meq/hr)				
Intact (5)	-0.13±0.13	-0.41±0.28	-0.37±0.35	-0.68±0.43
TX (6)	-0.69±0.21	-1.45±0.36	-1.52±0.38	-1.31±0.35
TX + CT (4)	-0.04±0.43	-1.22±0.29	-1.86±0.55	-1.89±0.63

*Changes in output from base-line control values are shown. Mean ± SEM; n = number in parenthesis.

infusion. Table XII shows the hourly outputs before, during and following the period of the Ca infusion.

Whereas $U_{Ca}V$ in the three groups of sheep appeared to reflect the plasma Ca changes, this was not observed when relating U_PV to plasma P changes. In the first, second, and third hours following the start of the Ca infusion, the intact sheep showed an increase in P excretion as is seen in Table XIII. This occurred <u>despite</u> the decreased plasma P levels that were found in this group at this time. Increases in U_PV were also found in the TX+CT sheep when plasma P levels were falling. However, this occurred only during the period of the Ca infusion.

In the TX sheep, no consistent change in U_pV was found during the period of the Ca infusion, and in four of the six studies, a slight drop was found as is seen in Fig 13. Plasma P levels in the TX sheep during this time were just beginning to rise, and in the second hour following the start of the Ca infusion, the hyperphosphatemia was reflected by a transient increase in P excretion (Table XII).

The increase in $U_{Mg}V$ which occurred in the intact animals in the four hours following the start of the Ca infusion was similar to that found in the TX sheep (Fig. 14). However, during the actual period of the Ca infusion, $U_{Mg}V$ in the TX groups was significantly less than that found in the intact sheep. When calcitonin was given to the TX sheep, $U_{Mg}V$ was increased during this same time period as is seen in Table XIII.

During preliminary control urine collections in intact and TX sheep, U_KV was found to decrease over a period of 4-5 hours. The decrease in the excretion of this ion in the two groups during control studies was similar. The infusion of Ca in the intact sheep appeared to have no effect on the normal pattern of K excretion in this group, and the administration of exogenous calcitonin did not affect the magnitude of the decrease in U_KV in the TX group. Figure 15 shows the mean changes in U_KV in the three groups of sheep. These changes were not significant. This figure also shows changes in $U_{Creat.}V$ and, as can be seen, no consistent changes were observed in response to Ca infusion in the three groups of sheep.

III. EXTRACELLULAR VOLUME EXPANSION

A natriuretic and diuretic response is commonly observed following a saline load and can occur apparently without any concomitant increase in GFR (64). It has been suggested by some (65, 38, 66, 36, 39, 67) that a hormone may be implicated in some way in this response. Calcitonin administration also results in a natriuretic and diuretic response. Therefore, it seemed possible that there might be an association between this hormone and the response to saline loading.

Saline loading a TPTX rat resulted in a typical natriuresis (32) which suggested at first that calcitonin was not involved. However, the more definitive experiment of saline loading an animal in the absence of the major source of calcitonin had not been performed. Therefore, a series of experiments was carried out in order to compare the responses following saline loading in intact, TX, and TPTX rats to see if any alteration in Na excretion occurred in those animals without calcitonin. A further series of experiments was carried out on conscious intact and TX sheep to compare the electrolyte changes in both plasma and urine in response to volume expansion by dextran loading.

A. Urinary Electrolyte Changes in Rats Following a Saline Load

1. The effects of saline loading in intact, TX, and TPTX rats.

Methods

Groups of intact, TX, and TPTX rats were given an i.p. load of saline (5 ml 0.9% NaCl/100 g body weight) and immediately placed

in metabolism cages for urine collection during two consecutive three-hour periods.

Results

Table XIV shows the urinary electrolyte changes following a saline load in intact, TX, and TPTX rats. A graphical representation of this data is shown in part in Fig 16 where the absolute changes in electrolyte excretion are shown. (The absolute changes were determined by comparison with electrolyte excretion in non-loaded control animals.)

Both intact and TPTX rats responded to the saline load by increasing urine volume and $U_{Na}V$ in the first three hours following loading. However, these changes in output in intact and TPTX rats were very different from those found in the TX animals where urine volume and $U_{Na}V$ were not significantly altered during this time period.

During the second three-hour collection period, both intact and TPTX rats showed a continued increase in urine volume and $U_{Na}V$. During this same time period, TX animals also showed a large increase in urine volume and $U_{Na}V$.

The transitory reduction (during the first three hours following a saline load) in urine volume and $U_{Na}V$ in the TX rats as compared to intact and TPTX rats was a consistent finding in all loading studies in these animals. Groups of rats thyroidectomized in June, November, and April all showed this type of response, therefore it could not be attributed to either one particular "batch" of rats, nor could it be attributed to seasonal variation.

TABLE XIV

Effects of saline loading on urinary electrolyte excretion in intact, TX, and TPTX rats*

Group	Volume ml/100g/hr	Na µequiv/100g/hr	K µequiv/100g/hr	Ca µg/100g/hr	P µg/100g/hr	Mg µg/100g/hr	Creat. µg/100g/hr	Na/K	Na/Creat.
1st 3 hr									
Intact									
Non-loaded (10)	0.255 ±0.026	31.9 ±4.3	62.6 ±4.8	38.7 ±5.6	191 ±30	102.3 ±10.5	162 ±8	0.502	0.193
Saline load (10)	0.567 ±0.084	84.4 ±9.6	85.4 ±6.7	98.4 ±13.4	359 ±57	152.9 ±13.8	175 ±5	1.011	0.485
p	<0.005	<0.001	<0.025	<0.001	<0.025	<0.010	NS		
TX									
Non-loaded (10)	0.307 ±0.047	37.6 ±5.1	60.2 ±5.2	48.7 ±8.4	158 ±31	86.1 ±16.9	150 ±7	0.615	0.243
Saline load (10)	0.271 ±0.037	41.1 ±3.7	47.8 ±3.7	59.9 ±9.6	99 ±13	61.9 ±11.6	125 ±5	0.917	0.333
p	NS	NS	NS	NS	NS	NS	<0.010		
TPTX									
Non-loaded (5)	0.199 ±0.025	34.5 ±6.3	32.0 ±4.0	38.4 ±5.8	46 ±14	39.8 ±10.3	107 ±7	1.117	0.324
Saline load (6)	0.481 ±0.103	82.2 ±17.9	47.3 ±4.7	63.8 ±9.1	76 ±16	60.1 ±10.7	132 ±13	1.702	0.621
p	<0.050	< 0.050	< 0.050	NS	NS	NS	NS		

TABLE XIV (continued)

Group	Volume ml/100g/hr	Na	K	Ca	P	Mg	Creat.	Na/K	Na/Creat.
		µequiv/100g/hr			µg/100g/hr				
2nd 3 hr									
Intact									
Non-loaded	0.167 ±0.031	14.1 ±2.7	29.3 ±3.4	12.9 ±2.8	172 ±14	59.4 ±9.6	118 ±9	0.464	0.116
Saline load	0.686 ±0.114	89.5 ±11.0	48.4 ±6.0	53.9 ±9.2	338 ±57	103.4 ±13.4	158 ±7	1.892	0.557
p	<0.001	<0.001	<0.025	<0.001	<0.025	<0.025	<0.005		
TX									
Non-loaded	0.229 ±0.031	26.6 ±4.5	36.3 ±3.8	23.1 ±4.5	169 ±28	61.6 ±9.0	138 ±8	0.765	0.196
Saline load	0.527 ±0.062	56.4 ±8.3	42.8 ±5.1	64.7 ±9.3	193 ±25	64.3 ±12.4	143 ±7	1.303	0.385
p	<0.001	<0.010	NS	<0.001	NS	NS	NS		
TPTX									
Non-loaded	0.189 ±0.036	20.0 ±2.3	24.8 ±6.1	52.8 ±9.1	19 ±9	63.5 ±2.1	132 ±14	0.940	0.160
Saline load	0.752 ±0.186	82.7 ±14.1	35.6 ±5.0	91.6 ±10.6	33 ±12	87.1 ±5.6	150 ±12	2.281	0.553
p	<0.025	<0.005	NS	<0.025	NS	<0.010	NS		

*Age of rats, two months. Five ml 0.9% saline/100g body weight, i.p. was administered prior to start of 1st 3-hr collection. Mean ± SEM; n = number in parenthesis.

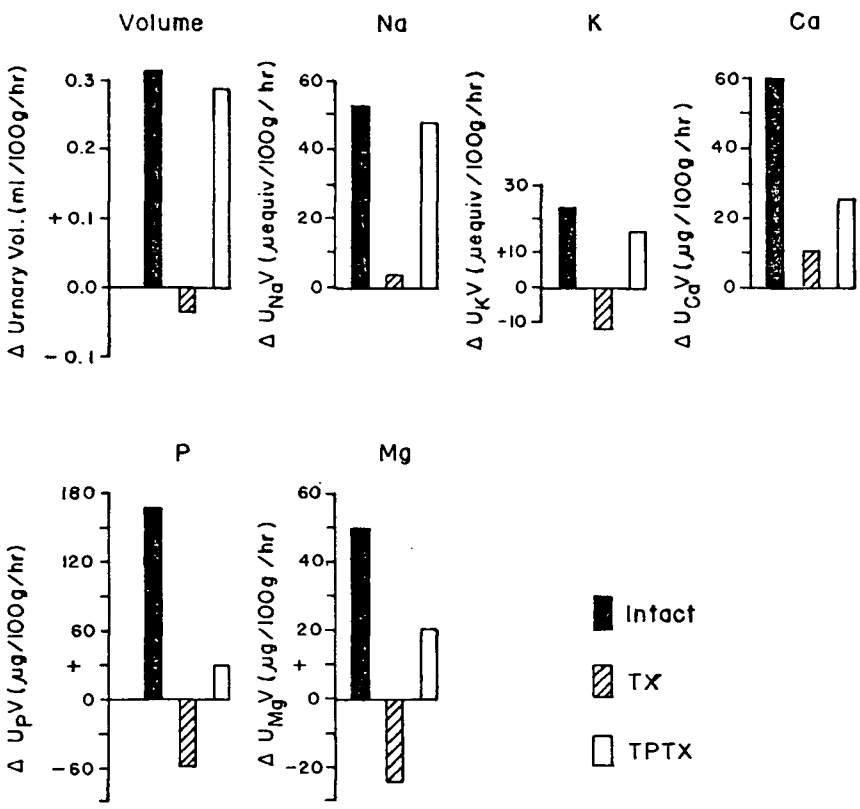

Fig 16. Changes in urinary electrolyte excretion in intact, TX, and TPTX rats during the first three hours following a saline load (0.9% NaCl, 5ml/100g body weight, i.p.). (Compiled from Table XIV.)

Significant increases in U_KV, $U_{Ca}V$, U_PV, and $U_{Mg}V$ accompanied the natriuretic and diuretic responses in the intact rats in the first three hours following the saline load. However, in the TPTX rats no significant increases in $U_{Ca}V$, U_PV, and $U_{Mg}V$ were found when urine volume, $U_{Na}V$, and U_KV were significantly increased. In TX rats, no significant changes in any of the urinary electrolytes measured were observed in the first three hours following the saline load. In the second three-hour period in the TX rats, however, a significant increase in $U_{Ca}V$ accompanied the increases in urine volume and $U_{Na}V$ that were found at this time.

2. The effects of PTH (Lilly PTE) on the response of TPTX rats to saline loading

Methods

Thyroparathyroidectomized (TPTX) rats were divided into three groups where the responses to saline loading were compared with and without the addition of PTE given at the same time as the saline load. The third group of TPTX rats was given PTE only.

A second series of experiments compared the relative responses following a saline load in those animals having functional parathyroid tissue (TX rats) with TPTX rats given PTE. Lilly PTE was used in all PTH administration studies in this section, given as a single injection of 100 U/100 g body weight, s.c.

Results

In Table XV it is seen that the administration of PTE to saline-loaded TPTX rats resulted in a reduction in urine volume and the excretion of Na, similar to that exhibited by the TX animals (functioning parathyroid transplants) during the first three hours following a saline load. Lilly PTE in non-loaded rats reduced $U_{Na}V$ to about 1/6th the normal Na output in the first three hours following injection. As was observed in experiments in part I of this Section, the reduction in $U_{Na}V$ which occurred in the first three hours of urine collection was followed by an increased $U_{Na}V$.

Figure 17 compares the response to a saline load in TX rats with that in TPTX rats given PTE. Both of these groups were without calcitonin, one having functional parathyroid transplants and the other replacement therapy with extract of the parathyroid glands (PTE). The responses of these two groups are compared to those found in intact (both thyroid and parathyroid tissue) and TPTX rats (no source of calcitonin or parathyroid tissue). As is seen in Fig 17, those rats with PTH (either endogenous or exogenous) and <u>no</u> calcitonin showed a transient reduction of $U_{Na}V$ following ECF volume stress.

TABLE XV

The effect of PTE on urinary electrolyte excretion following a saline load in TPTX rats*

Treatment	Volume	Na	K	Na/K
	ml/100g/hr	μequiv/100g/hr		
Saline load (5)				
1st 3 hr	0.402±0.109	49.0±14.7	38.3±4.4	1.284
2nd 3 hr	0.421±0.044	60.3± 8.0	40.3±5.5	1.628
Saline load + PTE (6)				
1st 3 hr	0.227±0.044	17.0± 4.4	28.1±5.7	0.614
2nd 3 hr	0.602±0.094	87.1±18.6	49.2±7.7	1.924
Non-loaded + PTE (6)				
1st 3 hr	0.123±0.020	5.8± 1.4	22.6±3.6	0.261
2nd 3 hr	0.372±0.031	60.3±10.8	61.9±9.2	0.600

*Age of rats, 2 1/2 months. Lilly PTE, 100 U/100 g body weight, s.c. given at same time as saline load, 5 ml 0.9% NaCl/100 g body weight, i.p. Mean ± SEM; n = number in parenthesis.

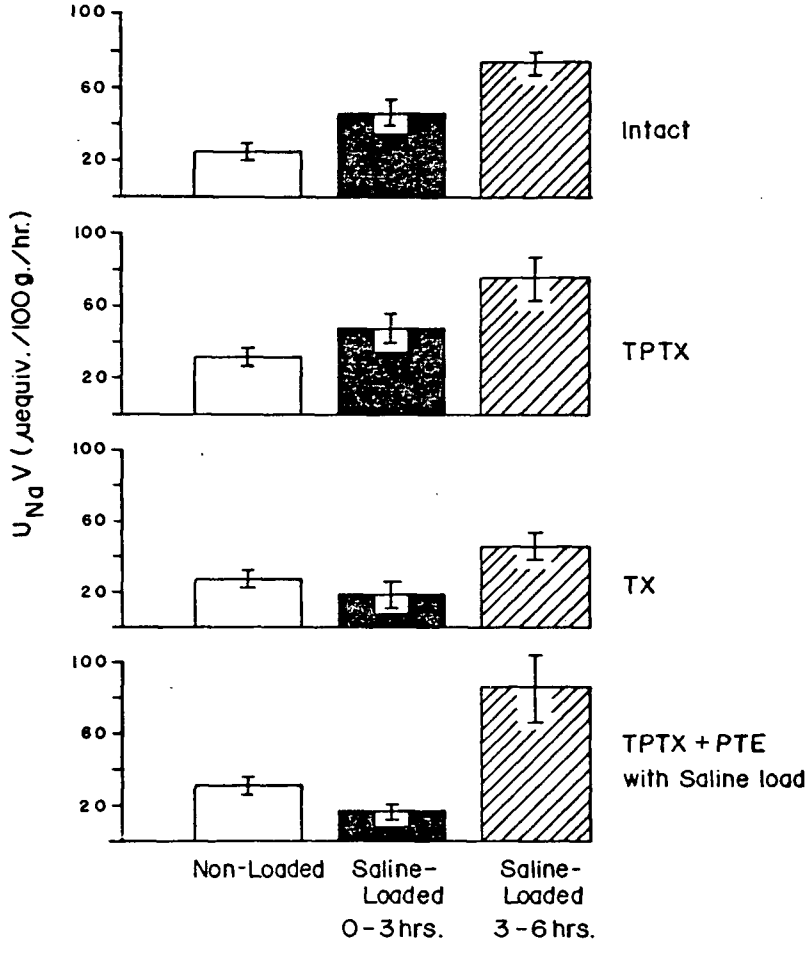

Fig 17. The effect of Lilly PTE (100 U/100 g body weight, s.c.) on the response of TPTX rats to a saline load as compared to the response in TX rats with functioning parathyroid transplants. (Values shown are Mean ± SEM; n = eight rats per group.)

B. The Effect of PTH on Plasma Ca and Urinary Electrolytes

The findings from the experiments in part A of this section suggested that the transplanted parathyroid tissue or the parathyroid extract (Lilly PTE) was involved in the reduction in Na excretion, or antinatriuretic activity. It seemed appropriate to study the relative effects of purified PTH preparations with that of Lilly PTE in order to clarify whether it was PTH itself or some other substance produced by the transplanted parathyroid tissue or present in the PTE which was responsible for the antinatriuresis.

Methods

In order to study the relative effects of purified PTH (TCA-precipitated PTH) with that of Lilly PTE, three groups of intact rats were pre-loaded with saline and at one hour after loading were injected with either TCA-precipitated PTH (activity, 240 U/mg), Lilly PTE, or vehicle. Urine outputs were determined at hourly intervals for four hours following the saline load.

Serial dilutions of Lilly PTE and highly-purified PTH (activity, 3000 U/mg) were then made and given to TPTX rats so that the urinary responses to graded doses of the hormone could be determined.

In a further series of experiments, the time-course of action of Lilly PTE on plasma Ca levels was studied in order to compare this with the three-hour time period during which an inhibition of $U_{Na}V$ occurred. Groups of TPTX rats were given a single s.c. injection of Lilly PTE (100 U/100 g body weight) and were bled at various time intervals for determination of plasma Ca

levels. Graded doses of Lilly PTE were also given to TPTX rats in order to compare the magnitude of the hypercalcemic response with that of the $U_{Na}V$ following different doses of the hormone.

Results

Both purified PTH (TCA-precipitated PTH; activity, 240 U/mg) and Lilly PTE caused a reduction in $U_{Na}V$ following a saline load as is seen in Table XVI. In addition, decreases in $U_{Ca}V$ and $U_{Mg}V$ were found to occur with both hormone preparations. In the third hour following hormone injection, however, the decrease in $U_{Ca}V$ and $U_{Mg}V$ was not apparent in the 'TCA' group, although it was still evident in the 'Lilly' group.

Table XVII shows the increasing reduction in $U_{Na}V$ with increasing doses of Lilly PTE. This Table also shows the decreased $U_{Ca}V$ and $U_{Mg}V$ and the phosphaturic effect that normally occurs following PTH administration. Table XVIII shows the response of TPTX rats to graded doses of highly-purified PTH (activity, 3000 U/mg). It is seen that no significant decrease in $U_{Na}V$ occurred following administration of highly-purified PTH, in contrast to the findings with similar doses of Lilly PTE. Significant increases in K excretion were found, however, with highly-purified PTH and increases in P, and decreases in Ca and Mg excretion were also found in this study.

The changes in urinary electrolyte excretion that were found following administration of highly-purified PTH occurred without apparent changes in GFR since $U_{Creat.}V$ was not altered in the three dose ranges. A control group of three rats was given Lilly PTE (100 U/100 g body weight) in the same experiment shown in Table XVIII in order to compare

TABLE XVI

Effect of TCA-precipitated PTH and Lilly PTE on the response of intact rats to saline loading*

Group	Time (hr)	Na (µequiv/100g/hr)	K (µequiv/100g/hr)	Creat. µg/100g/hr	Na/K	Na/Creat.
Vehicle	-1 - 0	50.0± 7.2	63.5±12.3	157± 6	0.823	0.314
TCA**	"	49.8± 3.3	55.5± 4.3	166±10	0.908	0.304
Lilly†	"	53.5± 4.5	63.0± 4.1	159±12	0.865	0.349
Vehicle	0 - 1	79.5±13.5	57.0±10.8	191± 8	1.418	0.416
TCA	"	26.3± 3.9	37.0± 3.5	142± 3	0.710	0.186
Lilly	"	11.5± 1.2	16.5± 0.5	70± 2	0.713	0.170
Vehicle	1 - 2	43.3± 3.9	37.5± 5.5	162±10	1.208	0.269
TCA	"	21.0± 4.9	23.0± 4.7	130±21	0.908	0.168
Lilly	"	5.9± 1.0	32.3± 2.5	164±13	0.178	0.036
Vehicle	2 - 3	48.3± 6.8	39.3± 9.1	155±18	1.388	0.325
TCA	"	37.5± 3.8	34.3± 3.8	146±18	1.100	0.270
Lilly	"	8.0± 0.9	35.8± 6.5	152±12	0.260	0.054

TABLE XVI (continued)

Group	Time (hr)	Ca	Mg
		µg/100g/hr	
Vehicle	- 1 - 0	8.4±2.6	67.8±16.3
TCA	"	16.4±2.6	95.6±14.0
Lilly	"	7.0±1.6	52.2± 9.2
Vehicle	0 - 1	10.2±3.0	66.0± 6.9
TCA	"	5.0±0.3	47.0± 7.5
Lilly	"	2.6±0.5	29.4± 5.1
Vehicle	1 - 2	4.8±1.4	46.8± 6.0
TCA	"	2.7±0.9	40.2± 6.7
Lilly	"	1.0±0.02	30.6± 2.8
Vehicle	2 - 3	6.2±2.4	51.0±11.0
TCA	"	8.6±2.6	68.4±13.4
Lilly	"	1.0±0.02	41.0± 9.1

*Mean weight of rats, 345 g. Time -1 hr: 10 ml 0.9% NaCl/rat, i.p. Time 0 hr: TCA-precipitated PTE, PTE, or vehicle (0.2% phenol in 20 meq/l saline).

**TCA-precipitated bovine PTH, 200 U/rat, s.c. in vehicle.

†Lilly PTE, 200 U/rat, s.c. Mean ± SEM; n = four rats per group.

TABLE XVII

Log-dose response to Lilly PTE in TPTX rats*

Dose PTE U/100 g	Volume ml/100g/hr	Na µequiv/100g/hr	K µequiv/100g/hr	Ca µg/100g/hr	P µg/100g/hr	Mg µg/100g/hr	Creat. µg/100g/hr	Na/K	Na/Creat.
1 (5)	0.573 ±0.122	28.6 ±6.0	48.6 ±6.4	101.2 ±13.7	221 ±23	113.9 ±11.2	114 ±9	0.587	0.251
10 (5)	0.379 ±0.134	19.0 ±6.2	49.8 ±11.5	43.4 ±14.8	498 ±77	79.3 ±37.3	113 ±19	0.295	0.168
100 (5)	0.223 ±0.044	10.3 ±3.1	34.7 ±6.5	23.4 ±4.8	332 ±37	43.7 ±7.3	98 ±8	0.263	0.105

*Age of rats, two months. Urine collected over a three-hour period following s.c. injection of Lilly PTE, serially diluted with water. Mean ± SEM; n = number in parenthesis.

TABLE XVIII

Log-dose response to highly purified PTH in TPTX rats*

Dose PTH U/100g	Volume ml/100g/hr	Na	K	Ca	P	Mg	Creat.	Na/K	Na/Creat.
		μequiv/100g/hr			μg/100g/hr				
1 (5)	0.325 ±0.088	31.7 ±7.8	28.4 ±1.8	13.2 ±0.9	174 ±17	69.7 ±8.2	163 ±13	1.085	0.198
10 (6)	0.252 ±0.025	29.5 ±2.5	40.9 ±2.6	13.4 ±4.5	574 ±63	65.7 ±9.0	160 ±5	0.721	0.185
100 (6)	0.260 ±0.059	26.8 ±5.9	42.4 ±4.7	5.3 ±1.2	759 ±42	37.6 ±7.0	169 ±11	0.600	0.156
Lilly PTE									
100 (3)	0.105 ±0.024	8.1 ±3.2	23.4 ±4.3	4.1 ±0.5	435 ±64	11.7 ±3.1	125 ±16	0.350	0.065

*Age of rats, four months. Urine collected over a three-hour period following s.c. injection of highly purified PTH (activity, 3000 U/mg) in 0.5 ml vehicle (20 meq/l saline), or Lilly PTE (100 U/100g, s.c.) Mean ± SEM; n = number in parenthesis.

responses under identical conditions. Here it is seen that $U_{Creat.}V$ was reduced as compared to the $U_{Creat.}V$ in the rats given highly-purified PTH. The excretion of Na, K, Ca, P, and Mg was also less with the Lilly PTE by comparison with the 100 U dose range of highly-purified PTH.

The hypercalcemic response to a given dose of Lilly PTE (100 U/100 g body weight) is a long-lasting one as is shown in Fig 18 where plasma Ca levels did not decrease until 10-12 hours after injection of the hormone. A linear relationship was found between the plasma Ca levels and the urinary excretion of Na, and the log-dose of Lilly PTE, plasma Ca levels rising and $U_{Na}V$ falling with increasing doses of the hormone as is seen in Fig 19.

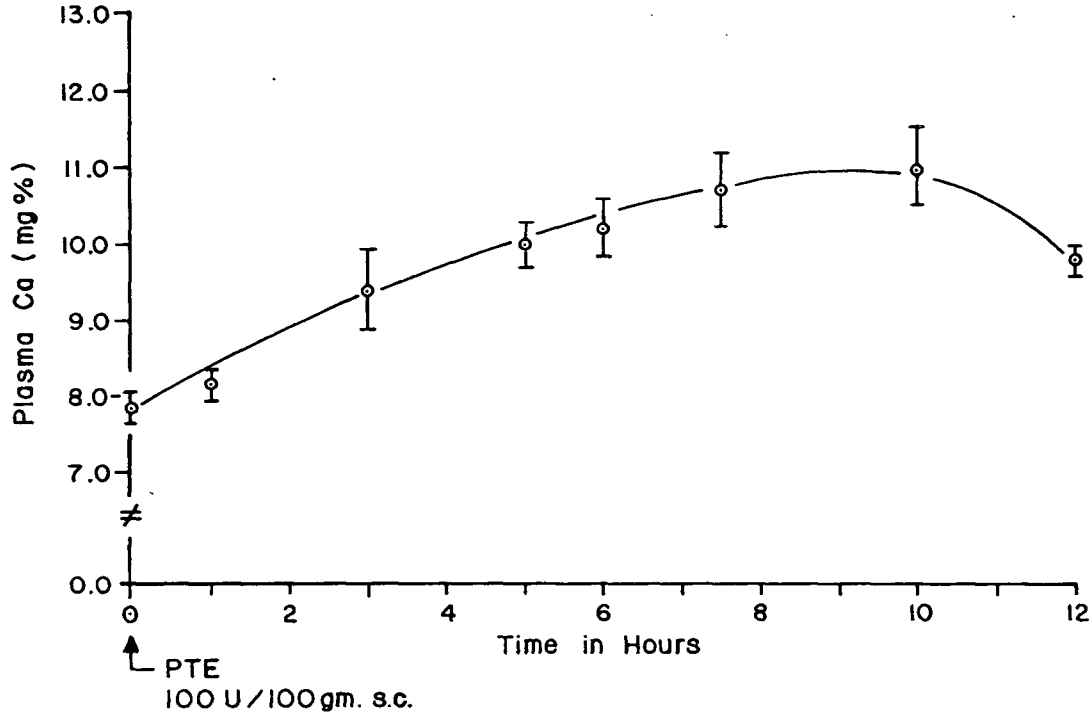

Fig 18. Time-course of action of Lilly PTE (100 U/100 g body weight, s.c.) on plasma Ca levels in TPTX rats. (Each point represents Mean ± SEM from separate groups of five rats.)

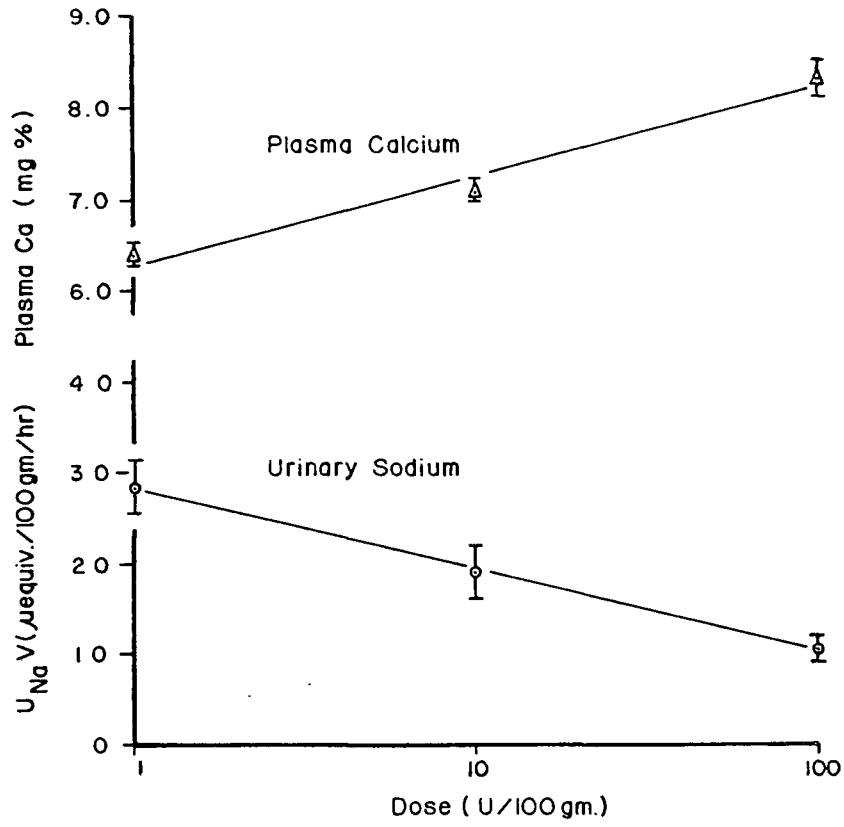

Fig 19. Log-dose response to Lilly PTE in TPTX rats. (Each point represent Mean ± SEM from five rats.)

C. Plasma and Urinary Electrolyte Changes in Conscious Sheep Following Dextran Infusion

Methods

The sheep were prepared for the volume-expansion studies with dextran using a similar protocol to that used in the Ca-infusion studies (see Section II, Methods), with the exception that they were not given a dextrose-saline infusion.

Following a control plasma and urine collection over a period of 1 1/4 hours, dextran in a volume equivalent to 1% body weight was infused over a period of 15 minutes. Plasma and urine samples were collected for a further 2 3/4 hours so that the entire collection period covered 4 1/4 hours. Catheters were then removed and the individual sheep were given a 10-day to 2-week recovery before being used again.

This experiment was performed with the intact sheep and then was repeated in the TX animals. The final series of experiments was carried out in the same TX sheep with the addition of salmon calcitonin which was administered in two doses of 50 U each. The first dose was given at the completion of the 15-minute dextran infusion, and the second one hour later.

Results

Plasma changes

Following the completion of the 15-minute dextran infusion, plasma Ca levels were decreased as is seen in Fig 20. In the intact sheep, this decrease was maintained throughout the experimental period. At three hours post infusion, the change in plasma Ca level was -0.72 ± 0.22 mg%. In

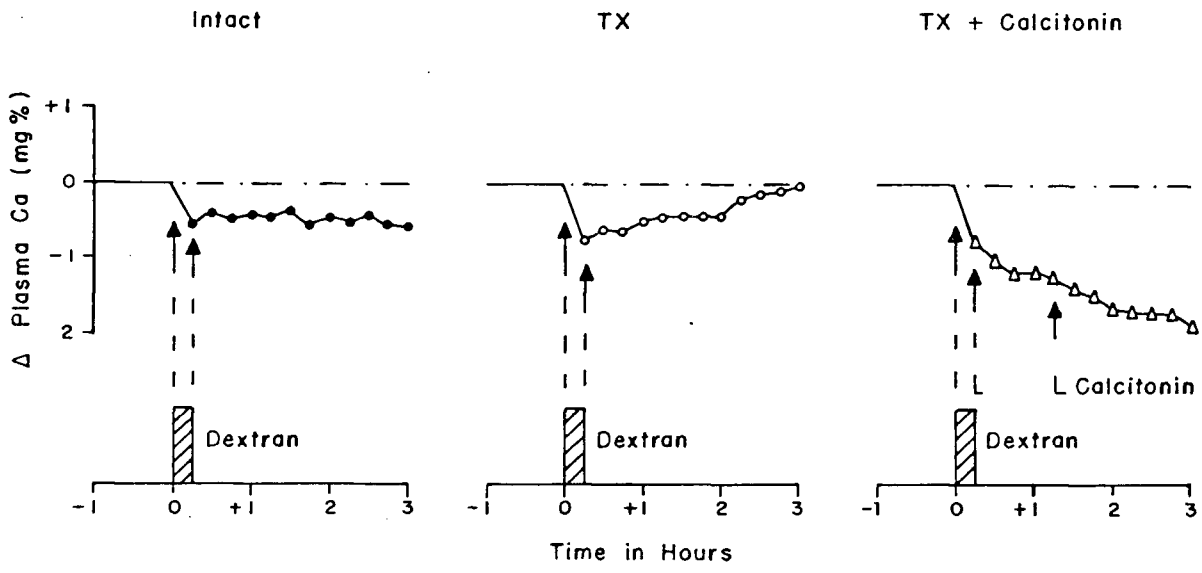

Fig 20. Changes in plasma Ca levels following a 15-minute dextran infusion in intact, TX, and TX sheep treated with salmon calcitonin (2 X 50 U). Changes in plasma Ca levels expressed as mg% changes from base-line control values. (Mean values are indicated; for n see Table XIX.)

the TX sheep, however, the plasma Ca levels gradually returned to normal and by three hours following the dextran load showed a change of only -0.18±0.11 mg% from normal base-line levels as is seen in Table XIX. The plasma Ca levels in the intact and TX sheep at this time were significantly different ($p<0.050$). When calcitonin was given to the TX sheep, plasma Ca levels showed a progressive fall as is seen in Fig 20.

Plasma P and Mg changes are shown in Fig 21. Following the dextran infusion, both plasma P and Mg levels decreased in the intact sheep and were maintained at this lower level during the three-hour period following the load. Plasma P in the TX animals showed a progressive fall in the hour following completion of the dextran infusion. A similar fall in plasma P was observed when these animals were given calcitonin but was continued for a longer period. Plasma Mg changes in the TX and TX + CT sheep were very similar but showed a slightly greater fall in levels than was found in the intact animals. This change was not significant (Table XIX).

Urine changes

Similar increases in urine volume and osmolal output were observed in the intact and TX sheep following dextran infusion, as is seen in Fig 22 and Tables XX and XXI. However, when calcitonin was given to the TX animals, a larger increase in urine volume and osmolal output was observed.

The changes in $U_{Na}V$ in the three groups of sheep reflected the osmolal changes that were observed, as is shown in Fig 23. The administration of calcitonin was followed by a greater increase in Na excretion as compared to that observed in the intact and TX sheep (Table XXIV).

TABLE XIX

Plasma changes following dextran infusion in intact, TX, and TX + CT sheep*

Time after start of dextran load (hr):	1/4	1	2	3
ΔPlasma Ca (mg%)				
Intact (3)	-0.72±0.06	-0.58±0.07	-0.60±0.15	-0.72±0.22
TX (6)	-0.95±0.04	-0.66±0.15	-0.56±0.13	-0.18±0.11
TX + CT (3)	-0.88±0.21	-1.25±0.22	-1.72±0.36	-1.97±0.34
p Intact vs TX	<0.025			<0.050
TX vs TX+CT			<0.010	<0.001
Intact vs TX+CT		<0.050	<0.050	<0.050
ΔPlasma P (mg%)				
Intact (3)	-0.20±0.15	-0.40±0.06	-0.35±0.10	-0.70±0.15
TX (6)	-0.38±0.12	-0.97±0.16	-1.23±0.22	-1.26±0.19
TX + CT (3)	-0.20±0.15	-0.37±0.16	-0.68±0.23	-1.03±0.27
p Intact vs TX		<0.050	<0.050	
ΔPlasma Mg (mg%)				
Intact (3)	-0.16±0.02	-0.17±0.03	-0.21±0.05	-0.22±0.08
TX (6)	-0.20±0.03	-0.29±0.09	-0.34±0.09	-0.32±0.09
TX + CT (3)	-0.22±0.03	-0.24±0.03	-0.35±0.04	-0.29±0.01

*Dextran (6% Gentran 75 in 0.9% NaCl), 1% body weight, infused over 15 min. Changes in plasma levels from base-line control levels. Mean ± SEM; n = number in parenthesis.

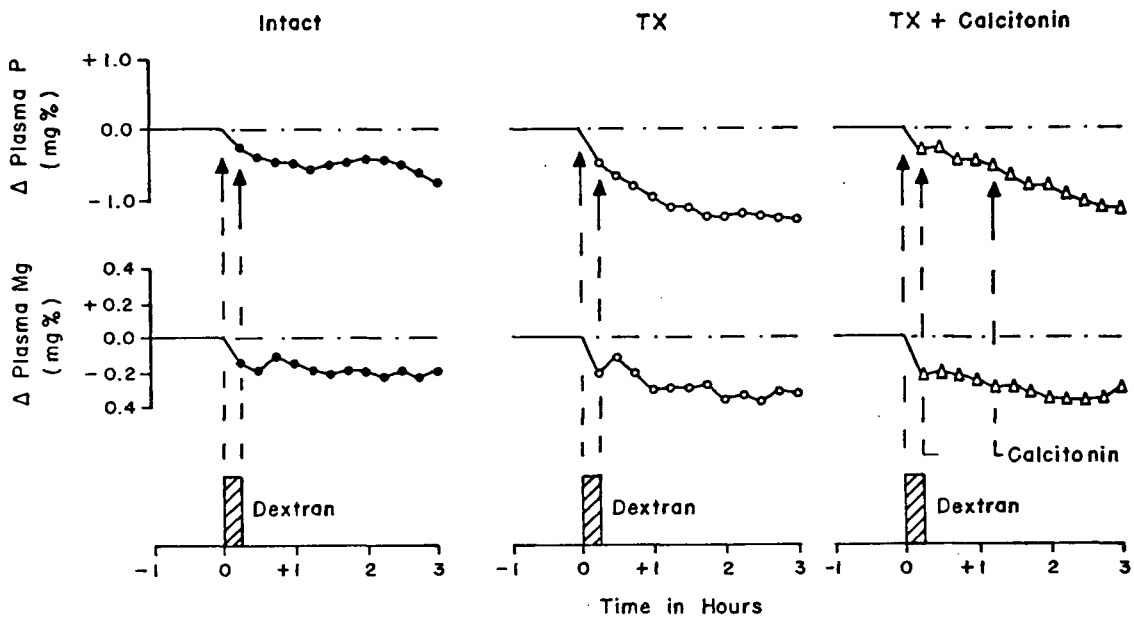

Fig 21. Changes in plasma P and plasma Mg following a 15-minute dextran infusion in intact, TX, and TX sheep treated with salmon calcitonin (2 X 50 U). Changes in plasma levels expressed as mg% changes from base-line control values. (Mean values are indicated; for n see Table XIX.)

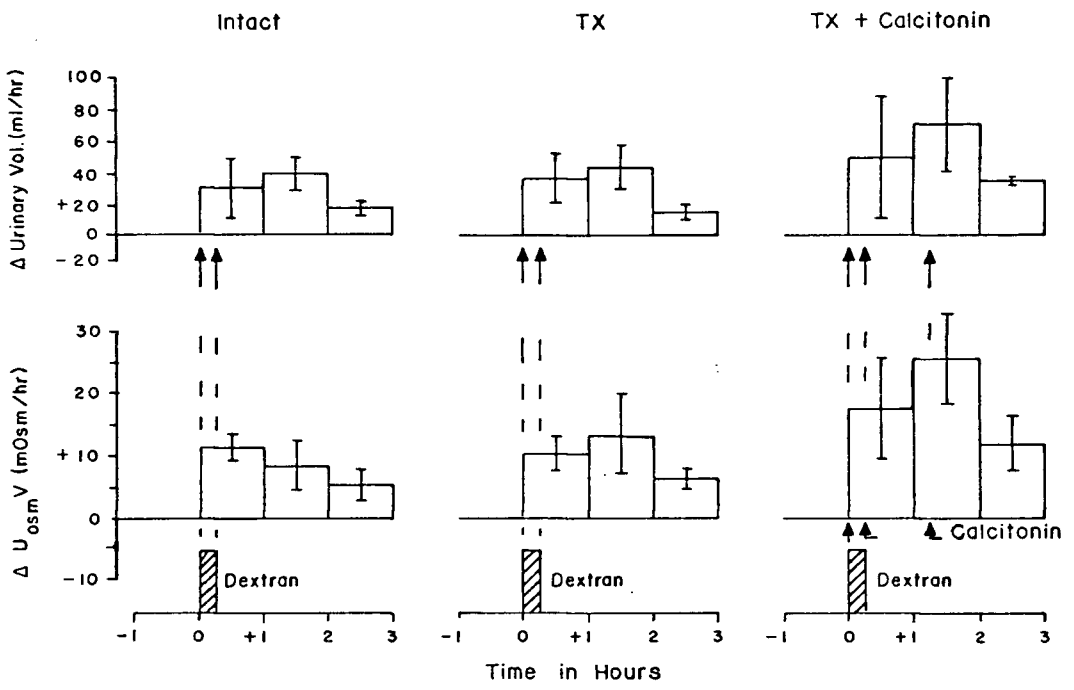

Fig 22. Changes in urinary volume (upper trace) and $U_{osm}V$ (lower trace) following a 15-minute dextran infusion in intact, TX, and TX sheep treated with salmon calcitonin (2 X 50 U). Changes from base-line control values are indicated. (Values expressed as Mean ± SEM; for n see Tables XX and XXI.)

TABLE XX

Changes in urine volume following dextran infusion in intact, TX, and TX + CT sheep*

Time after start of dextran load (hr):	0 - 1	1 - 2	2 - 3
Changes in urine volume from baseline control values (ml/hr)			
Intact (5)	30.6±18.9	38.7±10.5	18.3±5.3
TX (6)	37.1±15.9	44.3±14.3	15.3±4.8
TX + CT (3)	49.0±38.3	69.9±29.1	35.8±2.3
p TX vs TX+CT			<0.025

*Mean ± SEM; n = number in parenthesis.

TABLE XXI

Changes in osmolal output following dextran infusion in intact, TX, and TX + CT sheep*

Time after start of dextran load (hr):	0 - 1	1 - 2	2 - 3
Changes in osmolal output from base-line control values (mOsm/hr)			
Intact (5)	11.3±2.3	8.4±4.1	5.5±2.6
TX (6)	10.5±2.7	13.4±6.7	6.5±1.7
TX + CT (3)	17.3±8.0	25.6±7.5	11.9±4.4

*Mean ± SEM; n = number in parenthesis.

A calciuria was found to accompany the natriuretic response to the dextran infusion in the intact sheep as is shown in Fig 23. However, this was not observed in the TX sheep despite a natriuresis of similar magnitude to that found in the intact animals. When calcitonin was administered to the TX sheep, a calciuria was observed although it was of smaller magnitude than that found in the intact animals. The increase in Ca excretion in the TX + CT sheep occurred despite the significantly greater fall in plasma Ca which was found in this group. The clearance of Ca in the intact and TX + CT groups was found to increase in response to the dextran infusion, as is seen in Table XXII. In the TX sheep, clearance rates did not change.

Control U_PV and absolute changes in U_PV showed considerable variation. Outputs from individual sheep are shown, therefore, and are compared to control outputs before the dextran infusion in Table XXIII.

In four of the five studies in the intact sheep, an increase in U_PV was observed following the dextran infusion. When calcitonin was given to the TX sheep, a phosphaturic response was also seen. In the TX sheep without calcitonin, very little change was observed in U_PV in four of the studies, and an increase in U_PV was found in the remaining two although this was only transient in one of these animals.

Changes in $U_{Mg}V$ in the three groups of sheep were far better defined as is seen in Fig 24. A magnesuria was observed in the intact animals following the dextran infusion. This response was diminished in the TX sheep but was restored in part when these animals were given calcitonin. Table XXIV shows the hourly changes in Mg excretion in the three groups of sheep, in addition to changes in $U_{Na}V$, $U_{Ca}V$, and U_KV.

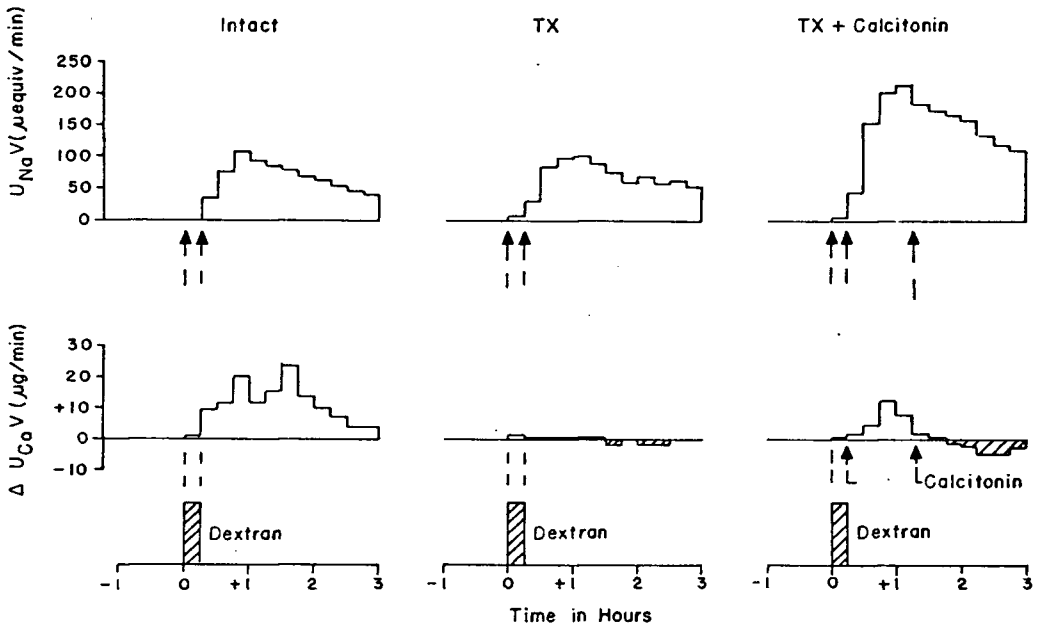

Fig 23. Changes in $U_{Na}V$ (upper trace) and $U_{Ca}V$ (lower trace) following a 15-minute dextran infusion in intact, TX, and TX sheep treated with salmon calcitonin (2 X 50 U). Changes from base-line control values are indicated. (Mean values are shown; for n see Table XXIV.)

TABLE XXII

Clearance of Ca following dextran infusion in intact, TX, and TX + CT sheep*

Time (hr):	Control -1 - 0	Post Infusion** 0 - 1	1 - 2	2 - 3
Clearance of Ca (ml/min)				
Intact (3)	0.124±0.066	0.272±0.091	0.379±0.125	0.237±0.052
TX (6)	0.086±0.034	0.091±0.023	0.081±0.023	0.079±0.025
TX + CT (3)	0.081±0.039	0.158±0.063	0.138±0.039	0.062±0.022

* Clearance, $\frac{[\text{Urine}] \times \text{Vol.(ml)/min}}{[\text{Plasma}]}$

** Dextran infusion given at time 0 hour over a period of 15 min.
Mean ± SEM; n = number in parenthesis.

TABLE XXIII

Urinary P excretion following dextran infusion in intact, TX, and TX + CT sheep

Time (hr):	Control -1 - 0	Post infusion* 0 - 1		1 - 2		2 - 3	
U_pV (mg/hr)							
Intact	0.107	0.165	↑	0.131	↑	0.073	↓
	0.112	0.222	↑	0.114	↑	0.096	↓
	0.134	0.272	↑	0.248	↑	0.147	↑
	0.142	0.993	↑	2.926	↑	3.657	↑
	20.772	20.027	↓	19.517	↓	8.561	↓
TX	0.092	0.089	↓	0.098	↑	0.082	↓
	0.097	0.113	↑	0.091	↓	0.081	↓
	0.237	0.232	↓	0.211	↓	0.153	↓
	0.278	0.265	↓	0.200	↓	0.210	↓
	1.289	2.337	↑	3.120	↑	6.012	↑
	17.865	25.223	↑	14.853	↓	17.025	↓
TX + CT	0.023	0.097	↑	0.100	↑	0.064	↑
	0.046	0.080	↑	0.050	↑	0.056	↑
	0.516	1.791	↑	6.645	↑	6.813	↑
	0.615	0.758	↑	0.579	↓	0.146	↓

*Dextran load given at time 0 hr over a period of 15 minutes. Arrows indicate either increase (↑) or decrease (↓) from control period value.

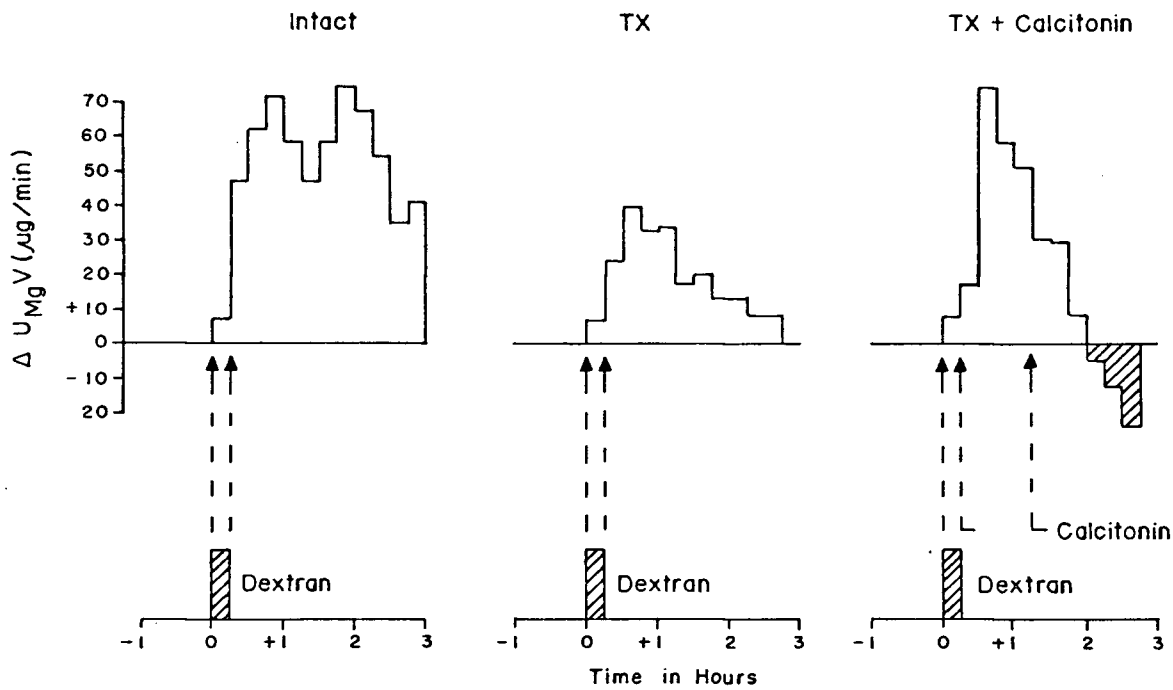

Fig 24. Changes in $U_{Mg}V$ following a 15-minute dextran infusion in intact, TX, and TX sheep treated with salmon calcitonin (2 X 50 U). Changes from base-line control values are indicated. (Mean values are shown; for n see Table XXIV).

TABLE XXIV

Changes in urinary electrolyte excretion following dextran infusion in intact, TX, and TX + CT sheep *

Time after start of dextran infusion (hr):	0 - 1	1 - 2	2 - 3
$\Delta U_{Na}V$ (meq/hr)			
Intact (4)	3.27±0.74	4.92±1.84	3.03±1.42
TX (6)	3.23±1.24	4.88±1.64	3.65±1.02
TX + CT (3)	6.10±3.64	10.74±3.94	6.44±1.82
$\Delta U_{Ca}V$ (mg/hr)			
Intact (5)	0.50±0.13	0.77±0.60	0.25±0.19
TX (6)	-0.02±0.10	-0.09±0.13	-0.09±0.11
TX + CT (3)	0.33±0.37	0.19±0.20	-0.19±0.16
p Intact vs TX	<0.025		
$\Delta U_{Mg}V$ (mg/hr)			
Intact (5)	2.02±0.73	2.32±1.22	0.45±0.61
TX (6)	1.21±0.54	0.94±0.68	0.20±0.42
TX + CT (3)	2.47±1.37	1.78±0.23	-0.98±0.13
$\Delta U_{K}V$ (meq/hr)			
Intact (5)	0.14±0.15	-0.34±0.14	-0.35±0.29
TX (6)	-0.15±0.13	-0.47±0.20	-0.85±0.28
TX + CT (3)	-0.06±0.18	-0.83±0.18	-1.64±0.08
p Intact vs TX+CT			<0.025

*Changes in output from baseline control values are shown.
Dextran infused over a period of 15 minutes from time 0 hour.
Mean ± SEM; n = number in parenthesis.

Decreases in $U_K V$ were observed in all three groups of sheep during the period of urine collection. The transient increase in $U_K V$ following the dextran infusion paralleled the transient increase in $U_{Creat.} V$ observed at this time as is seen in Fig 25.

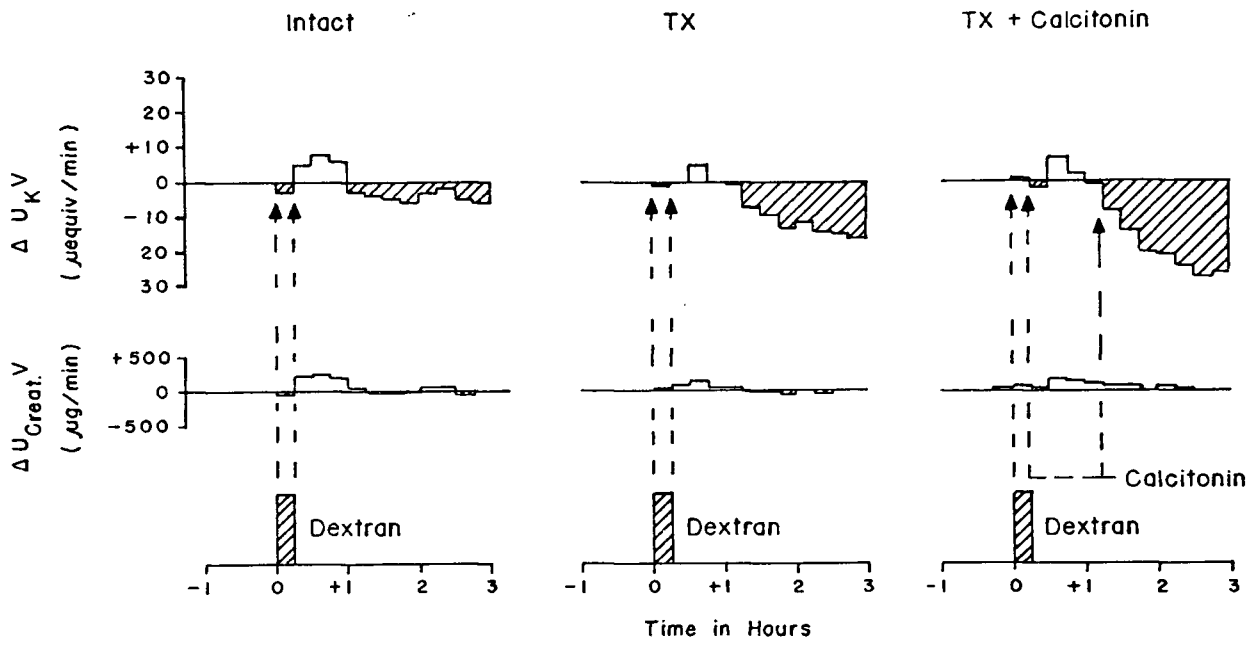

Fig 25. Changes in $U_K V$ (upper trace) and $U_{Creat.} V$ (lower trace) following a 15-minute dextran infusion in intact, TX, and TX sheep treated with salmon calcitonin (2 X 50 U). Changes from base-line control values are indicated. (Mean values are shown: for n see Table XXIV.)

DISCUSSION

I. EXOGENOUS CALCITONIN ADMINISTRATION

A. Chronic Studies

The finding of a potent natriuretic and diuretic effect of salmon calcitonin in young rats during chronic administration of the hormone was of considerable interest. At that time, no studies on the urinary changes that occur following chronic treatment with calcitonin had been reported, and no evidence was available to show that salmon calcitonin could exert such profound alterations in urinary excretory patterns.

The natriuretic and diuretic response, in addition to the increased output of other electrolytes which included Ca, P, and Mg, suggested that the hormone, besides its known effect on bone, may be exerting a renal effect which persists with continued administration of the hormone. Recent studies in rats by Sørensen and Hindberg (68) support these findings. They found that porcine calcitonin in a dose of 100 mU/100 g body weight every three hours for three days caused a progressive increase in urine volume, and the excretion of Na, Ca, P, and Mg. They attributed these effects to a direct action of calcitonin on the renal tubules.

Bijvoet et al.(69) showed that the continual infusion of porcine calcitonin in man (63 MRC U/day) during a period of 6 to 39 days resulted in a transient diuresis and natriuresis which was accompanied by weight loss in the first two days. It would appear that the hormone was exerting a continued effect on Na and volume, however, since it was observed that aldosterone secretion rates and plasma renin activity were above normal

during the experimental period. This increase in compensatory mechanisms could obscure a continued action of the hormone on the kidney. Bijvoet et al.also observed an increase in Ca and P excretion and ruled out the intervention of PTH since no increase in excretion of cAMP was observed.

It would appear from these chronic studies in rat and man that calcitonin is capable of causing a volume stress which persists as long as the hormone is being administered. In man (69) and possibly rats, mechanisms may be evoked in order to compensate for this condition. In addition to the effects of this hormone on urinary Na and volume during chronic administration of calcitonin, loss of other electrolytes which includes Ca, P, and Mg can also occur and these effects appear to be less readily compensated for.

B. Acute Studies

1. Plasma changes

The hypocalcemic effect of calcitonin is well documented and is the basis for the bioassay of this hormone (59). The response is dose-dependent and is related to the age of the animal and more directly to the rate of bone turnover (62). In Table III, a progressive drop in plasma Ca levels is shown from 0 to 3 hours which was highly significant ($p < 0.001$). Since this response was found at the dose of hormone used for many of the six-hour urinary studies (100 mU/100 g body weight), the rats were in a hypocalcemic state during the periods of urine collection.

The highly significant decrease in plasma P at one and three hours as shown in Table III ($p < 0.001$) is consistent with the hypophosphatemic effect of calcitonin observed by other workers (70, 11, 71). The

hypophosphatemic effect is apparently not abolished by nephrectomy or parathyroidectomy as shown by Kennedy et al.(71), and thus appears to be a direct effect of the hormone on bone.

Table III also shows a decrease in plasma Mg. Consistent decreases in plasma Mg have not always been found following calcitonin treatment by other workers. Studies in man by Sørensen et al.(72) revealed a hypomagnesemic effect with porcine calcitonin which was more pronounced in hypercalcemic patients than in normocalcemic patients. Haas et al.(30) also found a decrease in plasma Mg in hypoparathyroid patients. Negative results were reported by Bijvoet et al.(73). Nielsen et al.(15) using synthetic porcine calcitonin observed a significant hypomagnesemic effect in young rats (70-110 g) as did Palmieri et al.(4) using partially purified porcine calcitonin. These findings were in contrast to those of Aldred et al.(16) using salmon calcitonin in 220-250 g rats. Williams et al.(18) using synthetic human and salmon calcitonin also reported no hypomagnesemic response three hours following the start of a one-hour infusion of hormone. These differences in response to calcitonin treatment may be related to the age of the animal and thus the rate of bone remodelling, a hypomagnesemic effect being more obvious in those animals where bone turnover is occurring at a faster rate. In addition, the time at which the blood sample is taken following hormone administration may be an important factor. The data in Table III were derived from studies in three-week-old rats (ca. 40 g body weight) from which samples were taken at one and three hours after a single injection of hormone. Only at one hour was the plasma Mg decrease significant ($p < 0.025$).

Significant decreases in plasma Na or K in response to calcitonin treatment have not been consistently observed and this is in agreement with data shown in Table III.

The decrease in plasma levels of Ca, P, and Mg could readily be accounted for by the inhibition of bone resorption which occurs following calcitonin administration. The lowering of plasma P could, in addition, be a result of a general movement of this ion into soft tissues as has been suggested by Talmage and Anderson (74). An increased loss of these ions from the kidney in response to calcitonin could enhance the plasma changes found.

2. Urine changes

Young rats treated with single injections of salmon calcitonin exhibited a marked natriuretic and diuretic response. Increases in P excretion were also observed at this time. However, Ca and Mg outputs were found to decrease, although in some fractional-collection studies, this decrease was preceded by a transitory increase in $U_{Ca}V$ and $U_{Mg}V$. The changes in the excretion of urinary electrolytes following single doses of calcitonin were found to occur within the range of a six-hour period, and the magnitude of some of the changes that occurred was related to the dose of hormone given as is seen in Fig 5.

The decrease in $U_{Ca}V$ and $U_{Mg}V$ which was observed in the acute studies was at variance with findings from the chronic studies where $U_{Ca}V$ and $U_{Mg}V$ increased. It is possible that the net changes found in the acute studies were secondary to the effects of the hormone on bone.

The decreased plasma Ca level, as a result of decreased bone resorption, would result in a decreased filtered load* of Ca (assuming GFR unchanged). This would mask any decreases in tubular reabsorption of Ca that might occur. If decreases in the filtered load of Ca were smaller, the calciuric effect of the hormone would become evident as was suggested by the findings of Sørensen and Hindberg (67). They found that during chronic administration of porcine calcitonin, the hypocalcemic effect of the hormone was reduced after the first day of treatment, and as plasma Ca levels returned towards normal, the initial decrease in urinary Ca excretion was replaced by an increase in the excretion of this ion.

Decreases in the filtered load of Mg could also mask any decrease in reabsorption of this ion by the renal tubules. Since Mg is normally reabsorbed at a maximal rate (25), very small decreases in filtered load could be reflected by large decreases in $U_{Mg}V$. The increase in Mg excretion which was found during chronic calcitonin administration may be explained in a way similar to that described above for Ca.

Although the net excretion of Ca and Mg was reduced in the acute studies, it will be noted, as shown in Fig 5, that large doses of calcitonin tended to reverse the trend. In addition, as mentioned above, a biphasic response in the output of these ions was observed in some fractional-collection studies. In this instance, the transitory increase in excretion of Ca and Mg may have reflected a direct action of calcitonin

*filtered load = the amount of a given substance filtered through the glomerulus in a given time:

$$\text{ultrafiltrable concentration}_{\underline{x}} \times \text{GFR}.$$

on the renal tubules in decreasing reabsorption of these ions before decreases in filtered load were sufficient to obscure the effect.

The urinary electrolyte changes that occurred following a single injection of calcitonin can not be accounted for by an increase in GFR since they occurred when endogenous creatinine excretion was unchanged. This is in agreement with the findings of Rasmussen et al.(31) who found no increase in endogenous creatinine excretion or inulin excretion (in rats receiving a constant infusion of inulin) in response to calcitonin. Sørensen et al.(72) in man also found no change in $U_{Creat.}V$ which they could attribute to calcitonin treatment. In studies on rats, Aldred et al.(16) found $U_{Creat.}V$ elevated as did Williams et al.(18). However, when Williams et al.gave either synthetic human or salmon calcitonin in doses which caused a similar rise in $U_{Creat.}V$, Na excretion was increased by the salmon calcitonin but not by the human peptide. Ardaillou et al.(14) and Haas et al.(30) found GFR, as indicated by clearance of inulin or creatinine, unaltered by calcitonin administration when increases in electrolyte excretion were found (14, 30). It is likely that the changes in electrolyte excretion observed following calcitonin treatment were primarily a reflection of changes in the reabsorptive capacities of the renal tubules, although these studies cannot localize any particular area of the nephron where these changes might have occurred.

The DOCA studies did suggest that the Na effect exerted by calcitonin might be occurring at a site in the nephron proximal to the site of action of this synthetic mineralocorticoid. This was indicated by the findings that when calcitonin was given to the DOCA-treated rats,

an increase in U_KV was found (Table IV). An increased delivery of Na to this distal site could result in an increased exchange for K (75).

The DOCA studies also revealed that calcitonin can counteract the Na-retaining effect of large doses of mineralocorticoid. Thus, it can be ruled out that changes in mineralocorticoid activity are responsible for the changes in $U_{Na}V$ that were observed. The increase in $U_{Na}V$ following calcitonin treatment in DOCA-treated or non-treated rats was virtually the same, and the changes in the excretion of Ca, P, and Mg were unaltered by DOCA pretreatment.

Parathyroid hormone causes a decrease in the reabsorption of phosphate from the kidney tubule as demonstrated by micropuncture studies (76) and an increased reabsorption of Ca and Mg (23-25, 77). An increase in U_PV and a decrease in $U_{Ca}V$ and $U_{Mg}V$ is subsequently observed. It is unlikely, however, that changes observed in intact rats following calcitonin administration could be secondary to stimulation of the parathyroid glands as a result of the hypocalcemia since an increase in U_PV and a decrease in $U_{Ca}V$ and $U_{Mg}V$, in addition to a natriuretic and diuretic response, were also found in animals with their parathyroid glands removed (TPTX rats). In fact, an even greater natriuretic response was consistently observed following calcitonin administration to TPTX rats as compared to either TX or intact rats where parathyroid function was normal.

It is possible that the decreasing plasma Ca level which was found during the period of natriuresis following calcitonin administration might in itself be responsible for the increase in $U_{Na}V$. However, this is in direct conflict with the findings of others. Wolf and Ball (49)

showed that an increasing plasma Ca level resulted in a prompt increase in the rate of Na excretion in the dog. This was also observed by Levitt et al.(78) in man and monkey. Levitt et al. also demonstrated that a fall in plasma Ca induced by Na EDTA was associated with an immediate decrease in Na excretion. If a lowered plasma Ca level per se could affect Na excretion, this would have been apparent in the studies with the TPTX rats with plasma Ca levels ranging from 5-7 mg% as compared to TX or intact rats with normal plasma Ca levels of approximately 10 mg%. In Table VIII, it is seen that $U_{Na}V$ in the three groups of rats was not appreciably different over a 24-hour period. In addition, in studies in man where plasma Ca levels were not significantly altered following calcitonin administration, a natriuresis was still observed (79, 14, 17).

The electrolyte changes that were observed in the acute studies with rats were in general agreement with those observed by Rasmussen et al.(31) in 1967. However, these workers observed a minimal natriuretic effect using high doses (ca. 70 U/kg body weight/hour) of porcine calcitonin in rats. Ardaillou et al.(14) in studies in man using porcine calcitonin at much lower doses (ca. 6 mU/ kg body weight/hour) reported a much greater natriuretic response in addition to increases in U_PV, $U_{Ca}V$, and $U_{Cl}V$.

Independent observations by Aldred et al.(16) with salmon calcitonin substantiated our findings (32) and established the dramatic effect that salmon calcitonin has on Na and water excretion. Recent studies by Williams et al.(18), comparing the relative effects of synthetic human and salmon calcitonin in rats, demonstrated the far greateer renal

effect of the salmon preparation on a weight basis as compared to that of human calcitonin. This they attributed to not only differences in structure of the two peptides, but also to the greater stability of the salmon preparation. In limited comparative studies in man, Haas et al.(30) also demonstrated a more pronounced renal effect with synthetic salmon calcitonin than that obtained with either the porcine or human peptides.

It would appear from these studies in rats, and from the many recent investigations in both man and animals, that calcitonin can cause profound changes in the urinary excretion of Na, Ca, P, and Mg. These changes are not dependent on concomitant changes in GFR, aldosterone or parathyroid activity, and may reflect a direct action of the hormone on the renal tubules. Recent studies by Marx and Aurbach (80) localize specific binding sites for calcitonin in the kidney, concentrated in the inner cortex or outer medulla. These authors also found that salmon calcitonin had a higher affinity than the mammalian calcitonins for these tissue receptors.

The absolute changes exerted by calcitonin on the renal excretion of certain electrolytes appear to be related to the particular calcitonin used, the duration of administration, and to the rate of bone turnover that is found, the effects being more pronounced in younger animals where bone turnover is occurring at a greater rate. As shown in Table V, an immediate and greater change in urinary electrolytes was observed in 5-week-old rats as compared to the 28-week-old rats. This increased renal response in the young rapidly growing animal is consistent with the greater hypocalcemic response which is also found in the young rat (63). It will be observed, however, that the renal effect in the older rats was more

prolonged and this tended to compensate for the smaller immediate response (as compared to the younger rats).

II. CALCIUM INFUSION STUDIES (ENDOGENOUS CALCITONIN RELEASE)

Changes in plasma concentrations of Ca, P, and Mg have been observed following exogenous administration of calcitonin as well as by induced release of endogenous calcitonin following treatment with Ca (81, 82, 47, 83, 84, 57) or Mg salts (19). However, a possible correlation between induced changes in the circulating levels of endogenous calcitonin and the excretion of urinary electrolytes has not been investigated. There is some question whether physiological release of the animal's own hormone can result in electrolyte changes similar to those found following exogenous hormone administration. In other words, does the physiological release of calcitonin have any real effect on excretion of such ions as Na, Ca, P, and Mg via the kidney in the normal animal?

Numerous studies have been performed which demonstrate that the infusion of Ca salts does result in dramatic changes in the excretion of certain urinary electrolytes both in man (51, 85, 86, 53, 87) and in animals (49, 50, 88, 52, 54). The urinary changes that are found following Ca infusion include an increase in urine volume, and an increase in Na, Ca, P, and Mg excretion. These changes have not been considered in relation to changes that would occur in circulating levels of calcitonin following an induced hypercalcemia. Care *et al.* (47) and Deftos *et al.*(48) have shown that a proportional relationship exists between the degree of hypercalcemia and the rate of secretion of calcitonin. Since the urinary changes that occur following Ca infusion in intact animals are very similar to those found following exogenous calcitonin administration, it is possible that calcitonin is participating in such changes.

Calcium infusion in intact, conscious sheep produced alterations in plasma Ca and P levels that would be expected if a concurrent increase in endogenous calcitonin had occurred. That is, a hypercalcemia was found which was rapidly corrected following cessation of the Ca infusion, and a hypophosphatemia similar to that found following exogenous hormone administration was also observed. Plasma Mg levels were also found to decrease. The urine changes in the intact sheep were consistent with findings of others following Ca infusion in man or animals. These changes included an increased volume of urine and an increase in the excretion of Na, Ca, P, and Mg.

The removal of the calcitonin-containing "C" cells of the sheep by thyroidectomy resulted in changes in plasma and urinary electrolytes in response to Ca infusion which were different from those found in the intact sheep. The impairment which was observed in the control of plasma Ca levels following the hypercalcemic stress in the TX sheep was similar to that observed by Sanderson et al. in 1960 (89) in dogs. However, these authors failed to realize the significance of their findings. It remained for Copp et al. in 1961 (90) to demonstrate the presence of a hypocalcemic factor in the thyro-parathyroid gland complex which was released in the presence of hypercalcemia and could account for the improved control of hypercalcemia in animals with intact glands. These findings have been confirmed by many workers and Inskeep and Kenny (83) have also observed an impaired control of hypercalcemia in TX sheep following an i.p. Ca gluconate load.

The rise in plasma P which occurred following the start of the Ca infusion in the TX sheep, in contrast to the fall found in the intact group, suggested that endogenous release of calcitonin could also affect

plasma P levels in a similar way to that found following exogenous hormone treatment. Kennedy and Talmage (57) also found a rise in plasma P levels in TX rats following an i.p. Ca load. Talmage et al.(91) suggest that calcitonin causes a general movement of P into soft tissues as well as bone, in addition to stopping the movement of this ion from bone. They attribute the rise in plasma P in the TX animals following a Ca stress to an impaired phosphate transport into cells secondary to Ca accumulation on cell membranes. They suggest calcitonin can counteract the inhibition of phosphate transport into cells and thus reduce plasma P levels (74).

A fall in plasma Mg levels in the TX sheep was observed which was not significantly different from that found in the intact sheep. Since this fall was similar, the involvement of endogenous calcitonin could not be associated with these observations. The decrease in plasma Mg in both the intact and the TX groups could be associated with the decrease in PTH activity following the hypercalcemia, and thus the decreased movement of this ion from bone. However, when calcitonin was given to the TX sheep, a significantly greater fall in plasma Mg was seen which suggests a possible hypomagnesemic effect of this hormone.

In contrast to the increased volume of urine that was found following the start of the Ca infusion in the intact sheep, a significant and maintained decrease in urine volume was found in the TX animals. Accompanying this decrease in urine volume, significantly smaller increases in osmolal output were also observed in the TX group.

In the TX sheep, changes in $U_{Na}V$ were reduced as would be expected from the decreased osmolal output which was observed in this group. In addition, decreased changes in U_PV and $U_{Mg}V$ were also seen. The small increase in $U_{Na}V$ in the TX sheep as compared to that found in the intact animals occurred despite the fact that the mean output of Ca was greater in the TX group. This increased $U_{Ca}V$ would be expected since plasma Ca levels reached a higher absolute level in the TX group and were maintained for a longer period of time. Thus, a dissociation of urinary Ca and Na was apparent in the TX sheep. In fact, two of the TX sheep showed no increase in $U_{Na}V$ when a large increase in $U_{Ca}V$ occurred. This suggests that calcitonin may play a role in maintaining a parallelism in the renal control of these two ions.

Phosphate outputs in the sheep were very variable, not only in resting control values between the different animals, but also in the absolute changes observed following the Ca stress. Variations in U_PV were also observed in a given animal from day to day. Thus, it was impossible to pool results from any one series of experiments. Nevertheless, analysis of the data from individual animals indicated that whereas $U_{Ca}V$ in the intact and TX sheep appeared to reflect the plasma Ca changes, this did not apply to changes in U_PV in relation to the plasma P changes that were seen. In the intact sheep, despite a falling plasma P, the urinary P excretion was increased in each of the four animals observed. Since the sheep were hypercalcemic at this time, PTH-induced phosphaturia is not likely to be the cause of this finding since PTH secretion rates diminish with rising plasma Ca levels as has been shown by Sherwood *et al.*(92). It is more likely that a decreased

reabsorption of P had occurred in the kidney since a decreased filtered load of P was indicated. A phosphaturic response has been found by a number of workers following administration of calcitonin (11, 14, 30, 16), and these effects are also found where PTH intervention can be ruled out (12, 13, 31, 15). It is possible that induced increases in endogenous calcitonin levels could cause a phosphaturic effect similar to that seen following exogenous hormone treatment.

When salmon calcitonin was given to the TX sheep, the pattern of both plasma and urinary changes following the Ca infusion was restored in part to one similar to that found in the intact sheep. Plasma Ca levels were limited in their absolute rise and the return to base-line levels was accelerated. Plasma P levels immediately fell following the start of the Ca infusion and, in addition, there was a greater fall in plasma Mg than that found in either the intact or TX sheep.

Increases in urine volume and the excretion of Na, P, and Mg were also observed in the TX sheep when calcitonin was administered during and following the Ca infusion. A decrease in the excretion of Ca was also found which appeared to be secondary to the calcitonin-induced changes in plasma Ca levels. The changes in plasma Ca and urinary excretion of Ca when calcitonin was administered to the TX group were similar to those found in the intact sheep.

The alterations in both plasma and urinary responses in the TX sheep as compared to those found in the intact (endogenous calcitonin) or TX+CT (exogenous calcitonin) animals suggest that the release of calcitonin in response to a Ca stress affects plasma Ca, P, and possibly Mg, as well as urinary volume and the excretion of Na, Ca, P, and Mg.

III. EXTRACELLULAR VOLUME EXPANSION

The "strain on volume homeostasis" (93) which is brought about following calcitonin administration suggests that this hormone may play a significant role in the control of body Na. Studies in both man and animals following exogenous hormone administration have shown that a considerable loss of Na and volume occurs as a result of the treatment. However, an impairment in control of body volume in man or animals without the calcitonin-containing "C" cells has not been demonstrated. It is difficult to correlate the activities of this hormone in relation to Na control without such evidence.

A. Volume Expansion in Rats

The volume expansion studies in rats implicated calcitonin and the parathyroid glands in Na homeostasis. This was not immediately apparent, however, since a natriuretic and diuretic response following saline loading occurred in TPTX rats similar in magnitude to that found in the intact animals (Fig 16). Only when calcitonin was absent in these animals (TX rats) was an inhibition of the normal natriuretic response evident following volume expansion. The difference in response between the TPTX and TX rats suggested that the presence of the transplanted parathyroid tissue might be responsible for the delayed natriuresis. To support this suggestion it was hypothesized that any possible antinatriuretic activity exhibited by parathyroid tissue in the intact rats (where an immediate natriuresis was found) could be antagonized by the action of calcitonin released in response to volume expansion.

The possibility that the presence of the parathyroid tissue was responsible for the antinatriuretic response in the TX rats was strengthened by the finding that exogenous administration of PTE to TPTX rats resulted in a similar inhibition of Na output following a saline load (Fig 17).

In the TPTX rats, the increases in Ca, P, and Mg outputs during the first three hours following the saline load were not significant, in contrast to the significant increases in the excretion of these ions in the intact rats (Table XIV). However, the volume and Na increases were not appreciably different between the intact and TPTX groups. It is possible that the urinary changes in the TPTX rats in response to the saline load were secondary to an increase in GFR. It was found that a 23% increase in $U_{Creat.}V$ occurred in these animals in the first three hours following the saline load (Table XIV) as compared to an 8% increase in the intact rats. The absence of functioning parathyroid tissue may also have been a factor.

The finding that a natriuresis occurs in intact rats in response to a saline load and is inhibited in those animals which are "C"-cell deficient (TX rats) suggests that calcitonin may be released in response to volume expansion. In these studies where isotonic saline was used to cause the extracellular volume expansion, one might have expected exactly the opposite to occur. A dilution of the extracellular Ca concentration by the saline load would be expected to decrease the release of calcitonin and enhance parathyroid secretion. However, the urinary changes that occur following volume expansion with saline (44),

even when GFR is reduced (53), are not consistent with the known urinary changes that occur following PTH intervention. The only renal response found that is consistent with the PTH activity is a phosphaturia, but a phosphaturic response is also observed with calcitonin. The changes that are found following volume expansion in intact animals are consistent with the renal responses observed in animals following the administration of exogenous calcitonin. The fact that these changes did not occur in thyroidectomized rats lends support to this hypothesis.

B. PTH and Renal Electrolyte Changes

In 1929, Albright and Ellsworth (27) first noted that a decrease in urinary excretion of Ca occurred, in addition to an increase in phosphate excretion, following the administration of PTE in a hypoparathyroid patient. The decrease in Ca excretion continued until the serum Ca level rose to a "critical value" of 8.5 mg%, above which point urinary Ca increased. Talbot et al.(21) in re-evaluating this data along with their own findings suggested that PTH acts "to boost the lowered serum Ca concentration towards normal, in part by increasing the efficiency of Ca reabsorption by the renal tubules".

Talmage and Kraintz (5) observed an increase in Ca excretion in rats following acute parathyroidectomy which was reversed by the administration of PTE. Since serum Ca levels were falling at this time, they concluded that PTH has a direct effect on the renal tubules, enhancing Ca reabsorption. Similar findings were observed by

Kleeman et al.(22) in man and dogs. They stated that "the excretion of Ca is a function of the filtered load of ionic and complexed Ca and the homeostatic factors regulating active tubular reabsorption." One of the factors involved in homeostatic regulation of tubular reabsorption is PTH which they found enhanced Ca reabsorption. In hyperparathyroid patients, for example, they found that the C_{Ca} was decreased, and in hypoparathyroid patients or in PTX dogs, despite a decreased filtered load of Ca, C_{Ca} was increased. Massry et al.(25) also found an increased C_{Ca} in TPTX dogs.

Stop-flow studies by Widrow and Levinsky in 1962 (23) indicated that the site of action of PTH on Ca reabsorption was in the distal tubule. Microperfusion and micropuncture studies by Frick et al. in 1965((94) substantiated these findings by demonstrating that PTH does not affect Ca reabsorption proximally. Therefore, the changes in reabsorption of this ion modified by PTH must occur at a more distal site.

Changes in Mg reabsorption have also been found in conjunction with changes in Ca reabsorption following PTH administration. In 1963, MacIntyre et al.(24), using purified PTH or Lilly PTE, observed decreases in both $U_{Ca}V$ and $U_{Mg}V$. Studies in hypoparathyroid man by Gill et al.(77) and Haas et al.(30) also showed that decreases in urinary Ca and Mg occurred following PTE. Gill et al.(77) found that as plasma Ca and Mg rose following PTE, the initial decrease in urinary Ca and Mg was reversed. They suggested that the increased filtered load of these ions masked any change in reabsorptive capacity of the renal tubule cells. Peacock et al.(95) also stated that PTH promotes tubular reabsorption of Ca. When calciuria is evident in hyperparathyroidism, it

is secondary to the increased filtered load of Ca.

The phosphaturic action of PTH has been recognized for many years. In a recent micropuncture study in dogs by Goldberg et al.(76), the decrease in phosphate reabsorption that occurred following PTH administration was found to occur in the proximal tubule. They found that the rejected phosphate from this site was then excreted in the urine with little further alteration. These authors also found that the decrease in phosphate reabsorption at the proximal tubule was accompanied by a parallel decrease in Na reabsorption at this same site. However, a natriuresis was not found to accompany the phosphaturia, and this they attributed to a reabsorption of this ion in the distal nephron. They suggested that PTH via its mediator cAMP may be initially acting on Na reabsorption and that the inhibition of proximal phosphate reabsorption is a consequence of this event. However, this hypothesis does not indicate what factor(s) may be involved in the increased reabsorption of Na that occurred in a more distal site so that no net increase in Na excretion was observed.

These findings by Goldberg et al.(76) could be interpreted somewhat differently since they do not confirm that PTH was acting directly on Na reabsorption at the proximal tubule site. A decreased Na reabsorption was simply observed in conjunction with a decreased phosphate reabsorption. It is possible that PTH's action on Na reabsorption was at a more distal site (where PTH has been found to increase the reabsorption of Ca (23)), enhancing the reabsorption of Na, counteracting the increased delivery of Na to this region so that the expected natriuresis did not occur.

In many of the studies where an increase in Na excretion is observed following PTH administration, factors secondary to PTH action have not always been considered. For example, (a) is the natriuresis which is sometimes observed a result of renal hemodynamic changes (96); (b) is it secondary to an increase in plasma Na which may follow PTH activity (7); (c) is the natriuresis secondary to the release of calcitonin in response to PTH-induced hypercalcemia? Such factors may be pertinent in interpreting the Na changes in the urine following PTH intervention.

If one is to accept that the immediate effect of PTH is to decrease urinary Ca excretion before any changes in plasma Ca occur which may obscure the effect, a closer examination of some of the studies following PTH intervention relating the simultaneous changes in Na and Ca might clarify the problem.

In studies on TPTX dogs, Massry et al.(52) found that in response to PTE, $U_{Ca}V$ fell on Day 1 of treatment despite a rising serum Ca. Increases in $U_{Na}V$ were minimal at this time even when serum Na was elevated. On Day 2 of treatment, $U_{Ca}V$ was increased only when the dog was hypercalcemic. $U_{Na}V$ on Day 2 was reduced or moderately elevated despite a large increase in serum Na concentration.

In a similar study in man, Wills et al.(53) found that on Day 1 treatment with PTE, $U_{Ca}V$ increased with no measured change in serum Ca. $U_{Na}V$ was also increased at the same time but was accompanied by an increase in serum Na. On Days 2 and 3, $U_{Ca}V$ was still elevated but serum Ca levels

were also elevated at this time. $U_{Na}V$ decreased during Days 2 and 3 and was not significantly different from control values when normal serum Na levels were found.

In a more recent study by Paunier et al.(26) in man, Na and Ca showed similar changes in their excretory patterns following PTE infusion. The ratios of Na and Ca outputs to control Na and Ca outputs showed a similar increase in the first hour following PTE, and in the second hour showed similar decreases. That is, when Ca excretion was decreased, a similar decrease in Na excretion was found. Results from such studies in animals and man do not convincingly demonstrate that exogenous administration of PTH causes a primary natriuretic response.

In Table XVII, the changes in the excretion of Ca, P, and Mg in TPTX rats following administration of PTE are consistent with the findings of others. Parathyroid hormone by increasing the reabsorption of Ca and Mg and decreasing the reabsorption of P causes an initial decrease in the excretion of Ca and Mg and a phosphaturic response. Accompanying the urinary Ca, P, and Mg changes, a decrease in Na excretion and urine volume was observed with increasing doses of hormone. As is seen in Fig. 19, $U_{Na}V$ was linearly related to the log-dose of PTE.

Previous loading with saline did not alter the responses of rats to either TCA-precipitated PTH or PTE, as is seen in Table XVI. An antinatriuresis was observed with both hormone preparations, although a greater and more prolonged antinatriuretic activity was observed with the Lilly PTE. The inhibition of Ca and Mg excretion was also more marked with PTE.

In Table XV, the antinatriuretic response to Lilly PTE in non-loaded and saline-loaded TPTX rats exhibited during the first three hours following treatment was followed by an "escape" whereby a natriuresis and a diuresis were observed. Thus the antinatriuretic activity that was found following PTE was a transient inhibition which suggested that during this time period, PTE, in addition to increasing the reabsorption of Ca and Mg from the kidney tubules, also increased Na reabsorption. The possibility that decreases in GFR accompanied these changes can not be overlooked, although decreases in $U_{Na}V$ were not always associated with decreases in $U_{Creat.}V$.

In studies by Levitt et al.(78) in man and monkey, the infusion of a Ca-chelating agent which lowered plasma Ca levels resulted in similar electrolyte changes to those observed in the TPTX rats following PTE treatment (Table XVII). That is, immediate decreases in $U_{Na}V$ and $U_{Ca}V$ were found to occur in addition to a concurrent phosphaturia which persisted even after the chelating infusion was stopped. The authors suggested that the phosphaturia was secondary to stimulated release of PTH in response to hypocalcemia but did not comment on the cause of the other urinary changes that occurred at the same time. It is equally possible that release of endogenous PTH could have caused the decrease found in the urinary excretion of Na and Ca, as well as being responsible for the phosphaturia.

Highly purified PTH did not cause a significant decrease in $U_{Na}V$ when given in graded doses to TPTX rats (Table XVIII). However,

significant increases in U_KV were observed in the 10 and 100 U/100 g dose range which suggested the possibility that an increased reabsorption of Na in exchange for K was occurring. It is possible that PTH may alter the reabsorption site for Na from the proximal to the distal tubule thus reducing exchange for hydrogen ion (at a proximal site) in preference for exchange for K at a more distal site. Goldberg et al.(76) found that a decreased reabsorption of Na occurred in the proximal tubule following PTH administration. Since this is the major site for Na-hydrogen exchange, an inhibition of this mechanism as postulated by Hellman et al.(97) is not unreasonable.

The relative effects of the different PTH preparations on net Na excretion could be attributed to changes in GFR. In some studies with PTE, for example, a decreased excretion of endogenous creatinine was observed when urinary Na was decreased. The reason for this apparent decrease in GFR in some of these studies cannot be accounted for. The fact that $U_{Na}V$ was not increased in saline-loaded TX rats (with functioning parathyroid transplants) as compared to TPTX rats suggests that PTH can compensate for a decreased proximal reabsorption of Na which has been shown to occur following volume expansion (76) by increasing the reabsorption of this ion at a more distal site. The increased excretion of K which was observed in the studies with highly purified PTH (Table XVIII) substantiated the concept that PTH may enhance distal tubular reabsorption of Na.

C. Volume Expansion in Sheep

1. Plasma changes

The initial dilution of the plasma volume by a factor of 23%* as a result of the dextran infusion resulted in decreases in the concentrations of total Ca, P, and Mg in the plasma. Since a dilution of saline only causes small increases in the ultrafiltrable fraction of Ca and Mg according to Blythe et al.(44), it is likely that in these studies the ultrafiltrable concentrations of Ca, Mg, and P (which is totally ultrafiltrable) were also decreased.

In the intact sheep (Figs. 20 and 21), these plasma electrolyte changes were maintained throughout the experimental period. In the TX animals, a similar dilution effect was observed. However, in this group plasma Ca levels slowly returned to normal in the three-hour period following the dextran load. The observation that a calciuria was found in the intact group which did not occur in the TX group suggests that the renal handling of this ion contributed in part to the plasma changes found.

If calcitonin was released in response to volume expansion in the intact sheep, plasma Ca levels would be expected to be unaltered

*Based on the assumption that in sheep plasma volume = 3.4% total body weight (98).

or even decreased further following the initial dilution with the dextran load. Calcitonin by depressing bone resorption and by inducing a calciuria could cause such plasma changes as were observed. If PTH was released in response to the hypocalcemia in the TX animals, unapposed by calcitonin, the observed changes in the plasma and urine would also be expected. It is not possible to determine without knowledge of the circulating levels of these two hormones, calcitonin and PTH, what actual mechanism may have been operating, if indeed these hormones were involved at all. However, the observed changes in plasma and urinary Ca in the intact and TX sheep did suggest that calcitonin might be involved. This was substantiated by the finding that when the TX sheep were given calcitonin, a progressive and sustained fall in plasma Ca occurred. In addition, a calciuria was observed in spite of the low plasma Ca level.

2. Urine Changes

The infusion of a saline load with the addition of the macromolecule dextran increases the colloid osmotic pressure of the plasma so that the increased volume is maintained. Dextran (Gentran 75) is a glucose polymer with an average molecular weight of 75,000 and is only slowly degraded to glucose which can then be eliminated from the vascular space. As a result, infusion of a saline load with the addition of dextran increases the vascular volume for a longer period of time than that of saline alone. Nizet (99) found that the infusion of dextran following a saline load depressed the increase in Na and volume excretion that occurred with saline alone. This he attributed to a measured increase in plasma colloid osmotic pressure which may have been

responsible for a simultaneous decrease in GFR. However, Nizet could not rule out haemodynamic intra-renal changes as also being involved in the response. Howards et al.(100) in micropuncture studies in the dog found that dextran decreased Na reabsorption by the proximal tubule but net Na excretion was still depressed. Therefore, it is possible that the depression of Na excretion following dextran infusion as compared to saline expansion alone can be attributed to haemodynamic changes within the kidney enhancing reabsorption of Na in sites distal from the proximal tubule. It would appear that dextran is simply modifying the changes found in response to saline loading and offers some advantages in such studies by compensating for the dilution of plasma proteins and, thereby, reducing the degree of electrolyte loss which could be secondary to a shift of the "Starling equilibrium" in the kidney.

If dextran affects the absolute changes that are found in response to volume expansion, it does not alter the direction of electrolyte changes that normally occurs following saline loading in normal animals. Increases in urinary volume, Na, Ca, P, and Mg excretion were found in the intact sheep following the dextran load. The natriuretic and diuretic responses which occurred in the intact sheep were also found in the TX sheep. Only when salmon calcitonin was given to this latter group was an altered response found. At this time, exogenous calcitonin administration doubled the increase in Na and volume excretion following dextran infusion.

The most significant differences that occurred in the urine of these three groups of sheep were those in relation to Ca excretion. Whereas the changes in $U_{Na}V$ in the intact and TX sheep were of a similar

magnitude, a calciuria did not accompany the natriuresis in the TX sheep as was found in the intact animals. In fact, $U_{Ca}V$ actually decreased in these animals following the dextran load, and clearance values as shown in Table XXII indicated no significant change. Calcitonin administration to the TX sheep was followed by a calciuria and an increased C_{Ca}, although these changes were not as great as those found in the intact animals. This could be explained by the greater fall in plasma Ca levels which occurred in the TX sheep treated with salmon calcitonin.

Blythe et al.(44) in studies in dogs following volume expansion with saline observed increases in $U_{Na}V$ and $U_{Ca}V$ and inconsistent changes in $U_{K}V$, $U_{P}V$, and $U_{Mg}V$ when GFR was reduced by inflation of a balloon in the aorta. Despite the decrease in GFR, the fraction of the filtered load of all these ions excreted in the urine was increased. In relating Ca and Na, Blythe et al.suggested that decreased reabsorption of either Ca or Na might consequently affect the reabsorption of the other. But, whatever the mechanism, an increased excretion of Ca is an "integral part" of the kidneys response to volume expansion.

Antoniou et al.(45) found that a progressive increase in the clearances of Na, Ca, and Mg occurred in dogs in response to saline loading. Similar changes were previously observed by Walser (41) and Duarte and Watson (43). Since these ion changes occur irrespective of changes in GFR, mineralocorticoid or ADH activity (44), it would appear that they are caused by some other factor(s) affecting their reabsorption as they pass through the nephron.

The dissociation between Ca and Na excretion in the TX sheep was at variance with the many observations that demonstrate the parallel relationship of these two ions in their excretion by the kidney. This dissociation could be attributed to the lack of calcitonin since it was only found in thyroidectomized sheep. When calcitonin was present, either endogenously or exogenously, both excretion and clearance of Ca were found to increase following the dextran load when Na excretion was increased.

The excretion of Mg was also reduced in the TX sheep as compared to the intact and TX+CT animals. Plasma changes could not account for the excretory changes in this ion. Massry et al.(25) found that PTE decreased the percent of filtered Mg excreted which supports the concept that PTH can enhance tubular reabsorption of Mg. The changes that were observed in the excretion of Ca and Mg in the TX sheep couldalso be modified by the intervention of PTH unopposed by calcitonin in these animals.

If this argument is continued in relation to P excretion, the results should indicate similar patterns of P excretion in the three groups of animals since both calcitonin and PTH can cause a phosphaturia. Both increases and decreases or no change at all in P excretion were observed (Table XXIII).

Calcitonin appeared to have no direct influence on K excretion in these studies. In preliminary control studies in sheep where no dextran infusion was given, U_KV was found to decrease over a period of 4-5 hours of urine collection in a way similar to that observed in the dextran infusion studies. This is probably a reflection of dietary input

since the animals were starved. It could also be related to the diurnal pattern of aldosterone secretion. Only when transient increases in $U_{Creat.}V$ were observed following the dextran load were small increases in U_KV observed. These changes are probably directly related to transient increases in GFR. Evidence is not conclusive that calcitonin has any direct effect on K metabolism. Potassium is not a bone-seeking mineral and all of the K in bone is readily exchangeable. Changes in K excretion, apart from changes in dietary input, are related to the movement of Na and the concentration of this ion in the distal portion of the nephron where K is secreted. Increased concentrations of Na in the distal nephron can increase the passive movement of K in response to the establishment of a more favourable electro-chemical gradient (75).

The urinary electrolyte changes in the TX sheep in response to volume expansion with dextran infusion showed certain similarities to the changes found in the TX rats in response to volume expansion with an i.p. saline load. Both animals showed an impairment in the calciuretic and magnesuric response that normally follows volume expansion. In the TX rats, a phosphaturia was not observed and in the TX sheep this was the finding in 4/6 of the studies performed in these animals. Whereas the rats showed a highly significant impairment in the natriuretic and diuretic response, this was not observed in the sheep. This disparity in the Na and volume responses could be a result of the different methods used for volume expansion in the two animals, or then again it may be a species difference. Nevertheless, it was apparent in both the rat and the sheep studies that alterations in the normal excretion of certain body electrolytes following volume expansion occur when calcitonin is not present. This suggests that this hormone may play some role in the

electrolyte changes that occur following extracellular volume expansion.

SUMMARY AND CONCLUSIONS

1. Exogenous Calcitonin Administration

1. The administration of salmon calcitonin lowered the concentration of certain electrolytes in the plasma. These included, in addition to Ca, inorganic P and Mg. Decreases in the concentrations of these ions following calcitonin treatment were observed in the rat and the sheep. The plasma-lowering effect of calcitonin on Ca, P, and Mg is attributed to the decrease in bone resorption which occurs following administration of the hormone. However, concurrent changes in the renal excretion of these ions could also contribute to the net plasma-lowering effect. It has also been suggested that calcitonin can enhance the movement of P into cells (91).

2. Calcitonin appears to decrease the reabsorption of Ca, P, and Mg from the kidney tubule so that a calciuria, phosphaturia, and magnesuria are found following hormone treatment. The magnitude of the urinary changes may be dependent on the plasma changes that occur. Where calcitonin is causing decreases in the plasma concentrations of Ca, P, and Mg, secondary to its action on bone, the subsequent decrease in filtered load of these ions could modify or even obscure the effects of a decrease in tubular reabsorption at the renal site.

3. Accompanying the changes in the renal excretion of Ca, P, and Mg following calcitonin treatment, a diuretic and natriuretic effect was also observed. Calcitonin also caused an increase in Na excretion in rats pre-treated with large doses of the synthetic mineralocorticoid, DOCA, which did not differ from the increase in Na excretion found in non-DOCA treated rats. This suggests that the natriuretic effect of this hormone is independent of changes in mineralocorticoid activity.

4. Since increases in the excretion of endogenous creatinine were not consistently found to accompany the natriuretic and diuretic responses, it is unlikely that increases in GFR were primarily responsible for the urinary electrolyte changes found.

5. The renal responses observed following calcitonin treatment were not secondary to stimulation of the parathyroid glands. Following calcitonin administration, TPTX rats (without parathyroid tissue) showed similar electrolyte changes to those found in intact rats. Since the natriuretic response in the TPTX rats following calcitonin treatment was consistently greater in response to a given dose of hormone than that found in rats with intact parathyroid tissue (intact and TX rats), it would appear that the presence of parathyroid tissue can alter the magnitude of the natriuretic response.

6. The urinary electrolyte changes that occurred immediately following the administration of calcitonin were greater in the young rat as compared to the older animal.

II. Calcium Infusion Studies (Endogenous Calcitonin Release)

1. Plasma Ca levels in intact sheep were rapidly restored to normal values following the completion of a one-hour Ca infusion. Plasma P levels showed an immediate fall following the start of the Ca infusion. These affects on plasma Ca and P are attributed to an increased endogenous release of calcitonin which is known to occur following a hypercalcemic stress induced by the infusion of Ca salts. This is substantiated by the observation that in the TX sheep, an impairment in the control of plasma Ca was observed, and plasma P levels were found to rise during and following the Ca infusion. When salmon calcitonin was administered to the TX sheep during and following the Ca infusion, the changes in plasma Ca and P were similar to those found in intact sheep.

2. An increased endogenous release of calcitonin may be responsible for some of the changes in urinary electrolyte excretion that occurred following Ca infusion in intact sheep. These changes included an increase in Na, P, and Mg, in addition to an increase in urine volume. This was indicated by the observation that the changes in Na excretion were significantly reduced in the TX sheep when large increases in Ca excretion were found. Smaller changes in the excretion of P and Mg in the TX sheep were also observed, in addition to a decrease in urine volume. It would appear that a dissociation in the renal excretion of Ca and Na can occur in sheep when calcitonin is not present.

III. Calcitonin and Volume Homeostasis

1. An impairment in the normal excretion of certain body electrolytes was observed in response to volume expansion in rats and sheep when the calcitonin-containing "C" cells were removed. In intact animals, volume expansion is followed by increases in urine volume and the excretion of Na, K, Ca, P, and Mg. Thyroidectomized rats showed no significant increases in any of these parameters in the first three hours following volume expansion. In the TX sheep, both the C_{Ca} and the excretion of Ca were unaltered by volume expansion and only small increases in the excretion of Mg were observed as compared to the intact sheep.

2. The finding of a maintained reduction in plasma Ca levels following dextran infusion in the intact sheep, which was not observed in the TX sheep, suggested further that the release of calcitonin may be involved in the response to volume expansion.

3. The presence of functioning parathyroid tissue in addition to lack of calcitonin was implicated in the inhibition of normal electrolyte excretion in TX rats following volume expansion. Exogenous administration of PTE to TPTX rats prevented the natriuretic and diuretic responses which were found in these rats following volume expansion. It is postulated that PTH may enhance the reabsorption of Na in addition to that of Ca and Mg from the renal tubule. It is further postulated that following volume expansion, endogenous release of calcitonin (in addition to decreasing proximal reabsorption of Na, Ca, P, and Mg) may antagonize the action of PTH at a more distal

site. Thus, an increased urine volume and electrolyte excretion is observed.

The evidence from the volume expansion studies in rats and sheep implicated calcitonin and PTH in the electrolyte changes that were found. However, this evidence was indirect. The definitive experiment would be a concurrent evaluation by means of reliable assay methods of the circulating levels of both calcitonin and PTH in response to volume expansion or volume depletion. This approach would clarify to what extent the two hormones are involved in volume control.

Stop-flow and micropuncture studies should enable a determination of the site of action of calcitonin at the tubular level. Such techniques would also clarify to what extent PTH is affecting tubular reabsorption of Na at various sites in the nephron, and might also establish whether the action of either hormone can antagonize the action of the other at the renal site.

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