CALCITONIN AND PARATHYROID HORMONE[1,2]

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The discovery of calcitonin in 1961 (1, 2) has stimulated great interest in the endocrine control of calcium metabolism. Two international symposia on calcitonin were held in 1967—the first on June 5 and 6 in Cambridge, Massachusetts (3) and the second from July 17 to 20 in London, England (4). The Third Parathyroid Conference, held at Mont Gabriel, Quebec from October 16 to 20 (5), dealt with research on both calcitonin and parathyroid hormone.

The present chapter will deal primarily with publications in 1967 and 1968, although some references to earlier work will be included to place the recent developments in perspective. For more detailed coverage, reference should be made to the published proceedings of the above conferences (3–5) and to a number of excellent recent reviews on parathyroid hormone (6–9) and calcitonin (10–14) including the chapter on thyrocalcitonin by Tenenhouse et al. (15) which appeared in the previous volume of this series.

Nomenclature and units.—The original term calcitonin[4] (CT) will be used for the hypocalcemic factor rather than the other widely used term thyrocalcitonin, since it is now apparent that the hormone is produced by cells of ultimobranchial origin and is not found in the thyroids of many animals (9). Biological activity will be expressed in terms of the MRC unit based on Thyroid Calcitonin Research Standard A or B as provided by the Division of Biological Standards, National Institute for Medical Research, Mill Hill, London, England. One MRC unit is equivalent to approximately 4 μg of pure porcine calcitonin.

Parathyroid hormone (PTH) will be used to designate the hypercalcemic factor rather than the equivalent term parathormone, and biological

[1] The survey of literature pertaining to this review was concluded in July 1968.

[2] Abbreviations used in this review are: CT (calcitonin) and PTH (parathyroid hormone).

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[4] Calcitonin has been designated as the generic name for the hormone by the World Health Organization and the U. S. Adopted Names Council (sponsored by the Am. Med. Assoc., Am. Pharm. Assoc., and the U. S. Pharmacopeial Convention).

activity will be expressed in USP units as specified in the U.S. Pharmacopoeia. One USP unit is equivalent to approximately 0.4 μg of pure bovine parathyroid hormone.

Calcitonin

History.—The brief history of this new field of endocrinology was reviewed recently (16). Although Sanderson, Marshall & Wilson (17) reported in 1960 that control of hypercalcemia was impaired following thyroparathyroidectomy in young dogs, the significance of these observations was not fully appreciated. In 1961, Copp et al. (1, 2), using a technique for perfusing the thyroparathyroid glands of the dog with high- or low-calcium blood (18),

from the glands. They named this newly discovered hormone calcitonin, since it was apparently involved in regulating the level or "tone" of calcium in body fluids. These observations were confirmed by Kumar, Foster & MacIntyre (19) two years later. It soon became apparent that the cells responsible for production of the hormone were present primarily in the mammalian thyroid rather than in the parathyroid as had been originally proposed. Hirsch, Gauthier & Munson (20) succeeded in extracting the hormone with dilute HCl from rat and hog (21) thyroid, and tentatively proposed the name thyrocalcitonin to indicate its thyroid origin and possible identity with calcitonin. Their work was of great importance, for it opened the way to purification of the hormone and to a study of its physiological action. The thyroid origin of calcitonin in mammals was confirmed by gland-perfusion experiments in the goat (22) and pig (23), and by peritoneal lavage studies in the rat (24).

C cells.—Foster, MacIntyre & Pearse (25) in 1964 found that the cells in dog thyroid which responded to hypercalcemia were the argyrophilic parafollicular cells of Nonidez (26) which were first described by Baber (27) in 1876. Pearse (28) proposed the name C cell (C for calcitonin) for these cells to indicate their role in production of the hormone. In the rat, Matsuzawa & Kurosumi (29) demonstrated by electron microscopy that a 4 hr period of hypercalcemia discharged many of the secretory granules present in the cells. Further evidence was provided by immunofluorescent studies of Bussolati & Pearse (30) which were subsequently confirmed using antibodies to pure porcine calcitonin (14). They showed that calcitonin was present in the C cells of dog and hog thyroid but was absent from the colloid-containing follicular cells.

The C cells have certain cytochemical characteristics in common with the beta cells of the pancreatic islets and the ACTH-producing cells of the anterior pituitary (31). These include a high content of cholinesterase and alpha glycerophosphate dehydrogenase, and an affinity for 5-hydroxytryptamine. The C cells are not restricted to the thyroid, but are also found in the internal parathyroid (parathyroid IV) and in the ultimobranchial (32) when present as a distinct gland.

Ultimobranchial origin of calcitonin.—The ultimobranchial origin of the parafollicular cells of dog thyroid was first suggested by Godwin in 1937 (33) and was confirmed in the mouse by Sato et al. (34) and Pearse & Carvalheira (35). Further evidence was provided in June, 1967 when Copp et al. (36, 37) observed high concentrations of calcitonin in extracts of the ultimobranchial glands of chickens and turkeys where the thyroid glands were devoid of hypocalcemic activity (38). These observations were confirmed independently by Tauber (39). The ultimobranchial glands of sharks (8, 40, 41), bony fishes (41), amphibians (41), and reptiles (41, 42) have now also been shown to contain calcitonin.

The extractable hormone concentrations in thyroid, parathyroid, and ultimobranchial glands of a number of representative vertebrates are given in Table I (43). The hormone was present in all mammalian thyroids tested (except for the upper pole of the rabbit thyroid which contains no C cells) and in the internal parathyroid glands of the dog and rabbit (41) and man (44). However, it is apparent that calcitonin is essentially an ultimobranchial hormone which can be extracted from these glands only because of the presence of C cells of ultimobranchial origin. It is interesting that the hormone concentration on a body-weight basis is very similar in the mammals, birds, and fishes studied.

In 1905, Greil (45) proposed the name ultimobranchial bodies for these glands, since they arise from the terminal branchial pouch. They develop in all jawed vertebrates and can be found in sharks and bony fishes, preceding the parathyroids in the phylogenetic scale. The parathyroids appear first in amphibians at the time of metamorphosis and migration from an aquatic to a terrestrial environment. As may be seen in Figure 1, both glands arise from a very similar *anlage*—the parathyroids from the third and fourth pouch and the ultimobranchials from the terminal pouch. In contrast, the thyroid develops as a midline structure from the endostyle (46).

There is growing evidence that the ultimobranchial glands are involved in calcium metabolism in nonmammals. Rasquin & Rosenbloom in 1954 (47) reported hypertrophy of the glands associated with bony deformities in Mexican cave fish (*Astyanax mexicanus*) raised in total darkness. Chan (48) observed ultimobranchial hypertrophy following a prolonged period of hypercalcemia produced by removal of the Corpuscles of Stannius in the eel (*Anguilla anguilla*). Robertson produced hypercalcemia in frogs (*Rana pipiens*) by maintaining them in 0.8 per cent $CaCl_2$ combined with vitamin D_2 injections. After two weeks, the ultimobranchials were hypertrophied (49) and showed signs of increased secretory activity (50). Similar changes were observed in chickens after 1 week of hypercalcemia produced by feeding a high-calcium diet (51).

All ultimobranchial extracts have a potent hypocalcemic effect when injected into young rats. It was found that the slopes of response plotted against the logarithm of the dose of extracts of rat, hog, beef, shark, and chicken calcitonin were essentially the same (52) suggesting that the bio-

TABLE I

CALCITONIN CONCENTRATION IN GLANDS FROM VARIOUS VERTEBRATES[a]

	(*MRC*) *Units/g fresh gland weight*			Units/kg body weight
	Thyroid	Ultimobranchial	Internal Parathyroid	
Mammalia				
Man (*Homo sapiens*)				
Normal thyroid	0.4		1.5[b]	0.16
Medullary cell carcinoma of thyroid	17			
Rat (*Rattus rattus*)	5–15			0.2–0.6
Hog (*Sus scrofa*)	2–5			0.4–0.8
Dog (*Canis familiaris*)	1–4		1.5–3.3	0.25–0.50
Rabbit (*Oryctolagus cunniculus*)				
Lower pole	9.5–2		2.1–2.5	
Upper pole	—*			
Aves				
Domestic fowl (*Gallus domestica*)	—*	30–120		0.5–0.8
Turkey (*Meleagra gallopava*)	—*	60–100		0.5–0.9
Reptilia				
Turtle (*Pseudemys concinna suwanienis*)	—*	3–9		0.002–0.006
Amphibia				
Bullfrog (*Rana catesbeiana*)		0.5–0.8		0.001–0.002
Teleosti				
Chum salmon (*Oncorhynchus seta*)		25–40		0.4–0.6
Grey cod (*Gadus macrocephalus*)		10–20		0.2–0.4
Elasmobranchii				
Dogfish shark (*Squalus suckleyi*)	—*	25–35		0.25–0.40

* No detectable hypocalcemic activity.

[a] Data from this laboratory (43).

[b] Based on an estimated content of 0.5 units/g dry weight (MacIntyre, personal communication).

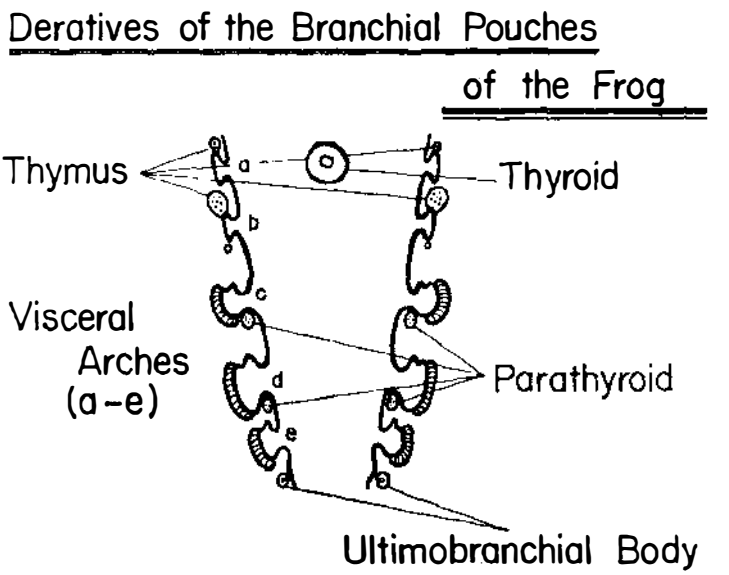

FIG. 1. The embryological development of the thyroid, parathyroid, and ultimobranchial glands in the frog (37). Reproduced through the courtesy of Heinemann Medical Books, London.

logical action in the rat was similar. However, the hypocalcemic effect of rather high doses of porcine calcitonin in lower vertebrates has been variable and disappointing. No hypocalcemic effect was observed in chickens (8) or in the teleost fish (*Fundulus heteroclitus*) (53), but significant lowering of plasma calcium was observed following injection of the hormone into catfish (*Ictalurus melas*) (54) and eels (*Anguilla anguilla*) (55). It is evident that much more research is necessary before the role of calcitonin in lower vertebrates is fully understood.

Assay.—Most bioassays for calcitonin are based on the hypocalcemic effect in young rats (21, 55–59) and mice (60). Schlueter & Caldwell (56) discussed some of the parameters involved in the assay, and Cooper et al. (57) described significant improvements and modifications of the original assay of Hirsch, Voekel & Munson (21). Sturtridge & Kumar (58) have modified the original procedure of Kumar et al. (55) by using 3-week-old rats, and were able to detect as little as 0.2 milliunit. The assay of Copp & Kuczerpa (59) is based on the integrated hypocalcemic response to intraperitoneal injection of the hormone, as measured by the area between the plasma-calcium curve, plotted against time following injection of the test preparation, and the control calcium level. The response is extremely age-dependent, in 9-month-old rats being less than 5 per cent of that found in 5-week-old animals. Diet was also an important factor. Young rats fed a

TABLE II

AMINO ACID COMPOSITION OF CALCITONIN AND PARATHYROID HORMONE

Amino Acid Residues	Calcitonin (66)	Parathyroid Hormone (129)
Alanine	1	6
Glycine	3	4
Leucine	3	7
Isoleucine	0	3
Serine	4	7
Threonine	2	0
Valine	1	8
Aspartic Acid	4(NH_2)	10
Glutamic Acid	1	12
Arginine	2	5
Lysine	0	9
Histidine	1	3
Tryptophan	1	1
Proline	2	3
Tyrosine	1	1
Phenylalanine	3	2
Cysteine	2	0
Methionine	1	2
Amino Acid Residues	32	83
Molecular Weight	3,585	9,476
Biological Activity per mg	200–250 MRC Units	2,500 U.S.P. Units

phosphate-deficient diet were less sensitive than those receiving a normal one.

An intriguing and very sensitive bioassay was developed by Raisz et al. (61) which is based on the inhibition of parathormone-stimulated release of ^{45}Ca from bone rudiments when calcitonin is added to the culture medium. The assay readily detected 1 milliunit/ml. An even more sensitive radioimmunoassay was developed by Deftos, Lee & Potts (62) using porcine calcitonin. This will detect $10^{-12}M$ concentrations of porcine, bovine, and human calcitonin (approximately 1 milliunit/l). In normal rabbits, the mean plasma concentration was 0.14 ± 0.07 mμg/ml, and this was increased 3 to 15 fold following calcium infusion. When large doses of calcitonin were injected, the half-life of the hormone in blood was very short (5 to 15 min), indicating a very rapid turnover.

Chemistry.—Great progress has been made in chemical studies of porcine calcitonin during the past year. Pure hormone was isolated in several laboratories (14, 63–68) and the amino-acid structure was determined (65–67). It is a straight-chain peptide with 32 amino acids and a molecular

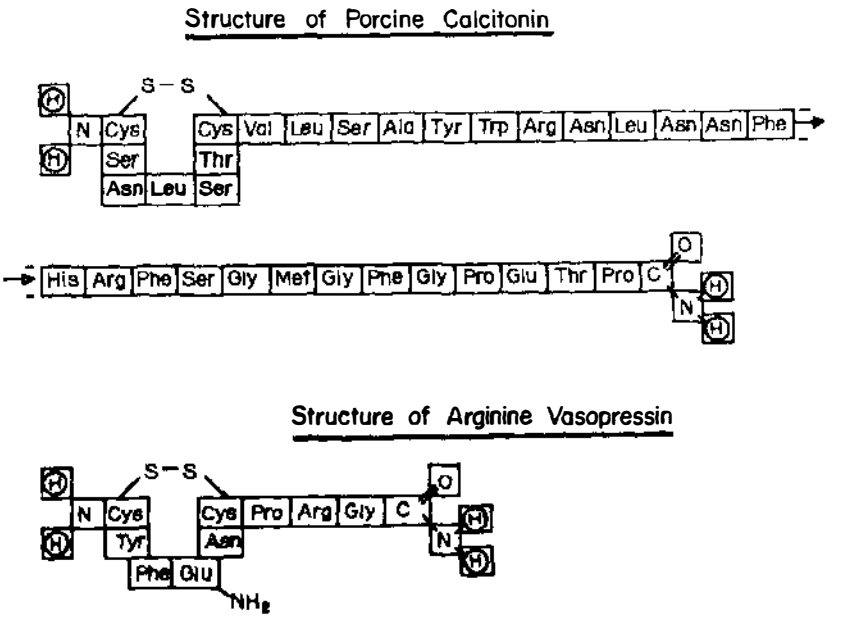

FIG. 2. Chemical structure of porcine calcitonin (66–68). The structure of arginine vasopressin is shown for comparison.

weight of 3585. The amino-acid composition is given in Table II and the structure is shown in Figure 2. It has certain unique features. It contains no lysine or isoleucine and the four aspartic acids are present as the amide, asparagine. There is a 1 to 7 intrachain disulfide bridge at the N terminus and the C terminus is prolinamide. The N-terminal disulfide loop and the alpha carboxyl amide are analogous to the structural features of vasopressin and oxytocin. The peptide chain of at least 25 amino acids, including methionine at the N terminus, is necessary for biological activity (66). Successful synthesis of porcine calcitonin, apparently identical to the natural hormone in all respects including biological potency, was announced by Lederle Laboratories on May 8 of this year (*New York Times*, May 9, 1968) quickly followed by a similar report from the scientists at Ciba Laboratories in Basel, Switzerland (69).

Using a 1.5 × 80 cm column of Sephadex G-50, Copp et al. (52) determined the elution pattern of hypocalcemic activity in partially purified extracts of hog, ox, rat, and human thyroids and chicken, turkey, salmon, and shark ultimobranchials as shown in Figure 3. The column was previously calibrated with standard protein markers. Porcine calcitonin and the major bovine activity came off in the region of glucagon (MW 3465), which is consistent with the molecular weight of pure porcine hormone. The calcito-

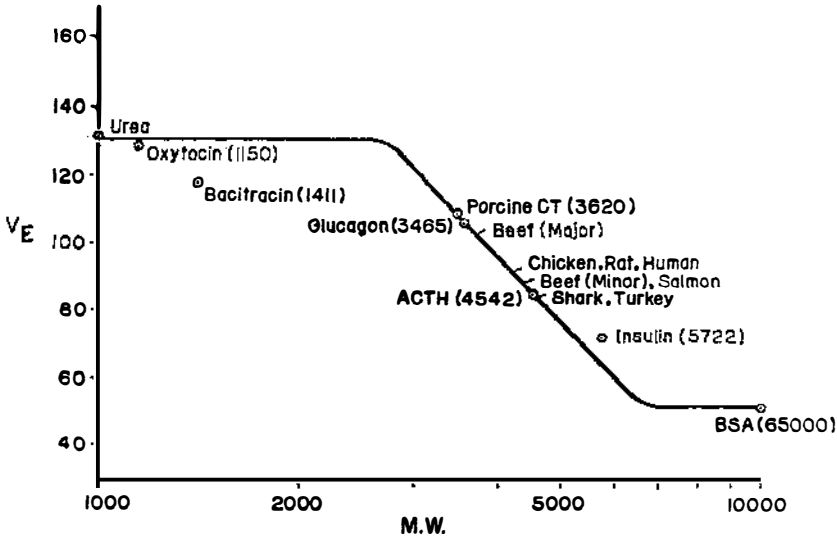

FIG. 3. The elution pattern of hypocalcemic activity in partially purified extracts of thyroid and ultimobranchial glands from a number of species (52). The determination was made with a calibrated 1.5 × 80-cm column of Sephadex G-50.

nin activity in the other preparations came off in the same region as ACTH (MW 4542), suggesting a molecular weight of approximately 4500.

Action.—In mammals, the primary action of calcitonin is on bone where it suppresses bone resorption, and in this sense antagonizes the action of parathyroid hormone. This is clearly evident in organ cultures of bone from embryos or newborn animals where addition of calcitonin to the medium suppresses bone resorption (70, 71) and ^{45}Ca release from previously labelled bone (72–75), particularly when these processes have been stimulated by the presence of parathyroid hormone. Gaillard (71) also observed an increase in osteoblastic activity. In cultures of calvaria from 6-day-old mice, Reynolds & Dingle (74) observed significant inhibition of calcium release within 30 min of the addition of CT to the medium. A concentration of 25 milliunits/ml of chicken calcitonin was as effective as 500 milliunits/ml of porcine hormone, suggesting possible differences in biological activity of hormone from these two sources.

In the intact rat, calcitonin also inhibits bone resorption, as indicated by reduced release of ^{85}Sr (75), ^{45}Ca, and ^{32}P (76, 77) which had been previously deposited in bone. Foster et al. (78) observed reduced osteoclast counts and bone resorption in young parathyroidectomized rats after 4 weeks of treatment with calcitonin. In addition, total bone mass was increased. They also observed (79) that chronic administration of calcitonin (100 milliunits/day) prevented the osteoporosis and weight loss which

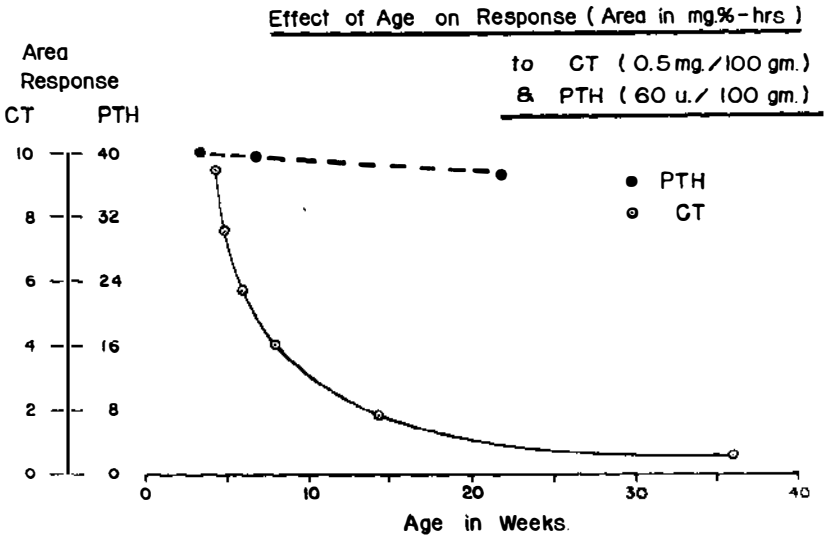

FIG. 4. Effect of age on the response to bovine calcitonin (CT) and parathyroid hormone (PTH) in rats (59).

otherwise occurred in young rats receiving toxic doses of vitamin A. Wase et al. (80) suggested that chronic administration of calcitonin not only suppressed bone resorption but also stimulated the formation of new bone. This would certainly be an important action if it proved effective in senile osteoporosis. In the isolated cat tibia, MacIntyre, Parsons & Robinson (81) found that perfusion with blood containing calcitonin resulted in a positive arteriovenous difference in calcium of up to 5 per cent, indicating a net retention of calcium in the bone. Reduced excretion of hydroxyproline following calcitonin administration (82, 83) provides further evidence for suppression of bone resorption.

Since the hypocalcemic effect of the hormone depends on suppression of bone resorption, it is not surprising that young actively-growing animals with rapid bone turnover are far more sensitive to the hormone than adults (59, 84–86). This is illustrated in Figure 4, where the integrated hypocalcemic response (59) in rats to a dose of 55 milliunits/100 g body weight is plotted against age. The effect of age on the CT response is striking; the effect on the corresponding PTH response is small.

Although the effects of CT and PTH on bone are antagonistic, the hypocalcemic response to calcitonin occurs in parathyroidectomized animals (87), so that its action is not dependent on the presence of PTH. Actinomycin D, while blocking the action of PTH, did not affect the response to calcitonin (87) which presumably does not require synthesis of new protein.

Little is known of the biochemical effect of calcitonin. Rasmussen & Ten-

enhouse (88) observed that PTH inhibited the pyrophosphatase activity released by Ehrlich ascites tumor cells. This inhibition was blocked when CT was added to the medium. They also observed that a suitable dose of calcitonin would prevent the nephrocalcinosis which was produced in rats by infusion of 5 μg PTH per hr for 16 hr. This suggests that CT may be of value in preventing metastatic calcification in hyperparathyroidism. It also prevented the development of skin calcification in the rat induced by intravenous injection of lead acetate followed by polymyxin subcutaneously (89).

Wells & Lloyd (90) found that pretreatment with theophylline or isoproterenol inhibited the hypocalcemic effect of porcine calcitonin. Both drugs increased the plasma-calcium level in parathyroidectomized rats. On the basis of these and other experiments, they suggest (91) that calcitonin may produce its effect by activating phosphodiesterase in bone cells, thus destroying the cyclic 3′5′-adenosine monophosphate which is thought to stimulate synthesis and release of osteolytic enzyme from bone cells, and to mediate the effect of PTH.

With respect to other tissues, calcitonin has a hypocalcemic effect in eviscerated and nephrectomized rats (92) so neither gut nor kidney is essential for its action. It has no effect on calcium absorption from the intestine in the dog (9).

Clinical Implications.—Calcitonin has been extracted from human thyroid glands (93–97), although the values obtained are variable. This may be due to the uneven distribution of C cells in the human thyroid where they are concentrated in the posteromedial area in and around the superior parathyroid (98). High concentrations of calcitonin have been observed in thyroids in patients with pseudohypoparathyroidism (94, 95) and medullary carcinomas of the thyroid (96, 97) which are presumably tumors of ultimobranchial C cells.

In normal adult human subjects, the hypocalcemic effect of injected porcine calcitonin is small (79, 99, 100), which is not surprising in view of the age effect on sensitivity to hormone (98–100). However, significant reductions in serum calcium were observed in patients with hypercalcemia secondary to osteolytic bone disease (79, 99, 101), idiopathic hypercalcemia (100), and that due to vitamin-D intoxication (102). The largest reductions (20 to 25 per cent) were obtained in patients with normal plasma-calcium levels but with generalized Paget's Disease (103). Calcitonin has been used to control hypercalcemia in the above conditions. However, the main therapeutic interest lies in the possibility that it may be of value in controlling nephrocalcinosis and the bone wasting which occurs with immobilization, chronic adrenal-steroid therapy, and advancing age.

Control of Secretion.—In the original experiments which led to the discovery of calcitonin (1, 2), the hormone was released when the isolated glands were perfused with high-calcium blood. The response did not require nervous connections or the mediation of other endocrine glands. In quantita-

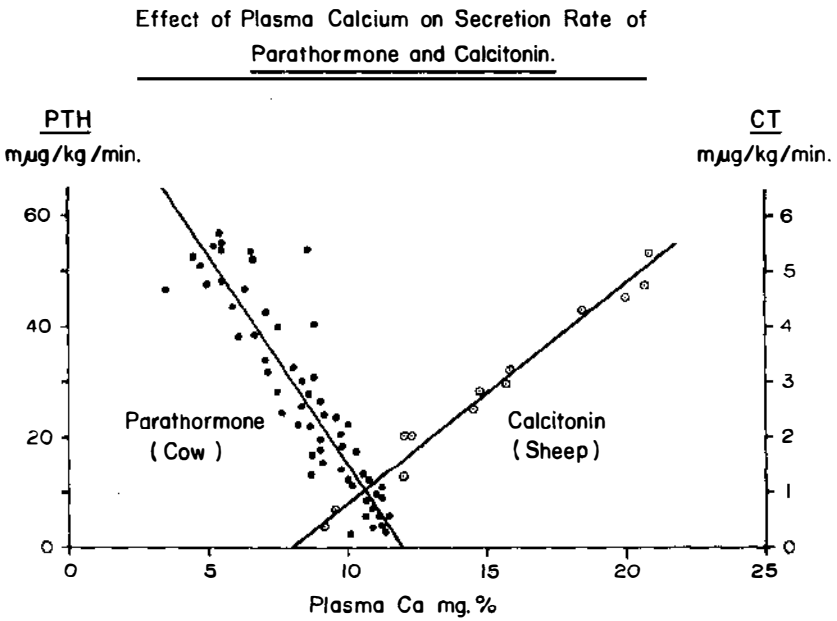

FIG. 5. Effect of the plasma calcium concentration on the rate of secretion of parathormone in the cow (144, 145) and of calcitonin in an adult sheep (105).

tive studies of CT secretion, Care (104, 105) perfused the isolated thyroid glands of hogs and sheep with blood of varying calcium concentrations. The calcitonin in the venous effluent from the glands was measured by bioassay (57) and the secretion rate was determined from the hormone concentration and volume flow. Variations in volume flow did not affect secretion rate which was found to increase in direct proportion to the increase in calcium concentration in the perfusing blood, as is shown in Figure 5. For this adult sheep, secretion rate can be expressed by the equation $d\text{CT}/\text{dt} = 0.40\ (\text{Pc}_a - 8.1)$ mμg/kg per min (in terms of equivalent porcine hormone), where Pc_a is the level of calcium in the perfusing plasma in mg per cent. For a 4-month-old pig, the equation was $dCT/dt = 0.36\ (\text{Pc}_a - 12.0)$. The resting reserve in a 25-kg pig was estimated to be approximately 30 units (104), which would be sufficient to maintain a secretion rate of 10 milliunits (or 40 mμg) per min for 48 hr, even if no new synthesis of hormone occurred. The response to hypercalcemia was very rapid, a 5-min "pulse" of hypercalcemia being sufficient to produce a significant release of hormone (105). In the sheep (105), rat (106), rabbit (62), and man (44, 107), calcitonin activity has been detected in plasma at normal calcium levels, suggesting that secretion is continuous. Indeed, MacIntyre (44) has estimated that the output in the adult human may be as high as

50 to 100 units per day. Calcitonin secretion has been demonstrated at normal plasma-calcium levels in the sheep (105) and rat (106), and the hormone has been detected in normal rabbit blood (62) at a mean concentration of 0.35 milliunit/ml, and in normal human plasma in a concentration of 0.10 milliunit/ml (107) to 0.5 milliunit/ml (44).

The precise proportional control of calcitonin secretion and its presence in normal plasma suggest that it could have a significant role in normal calcium homeostasis (108). However, Bronner (109) found no evidence for CT involvement in regulation of the steady-state value of blood calcium, and concluded that the hormone functioned primarily to refine the action of PTH on bone.

There is ample evidence that thyroparathyroidectomy with removal of the source of calcitonin grossly impairs the control of hypercalcemia with administration of large doses of PTH (110, 111, 120) or injection of calcium salts (17, 112–114). Impaired handling of a calcium load has also been observed in thyroid and hypothyroid patients (115, 116) for whom a calcium tolerance test has been devised to test calcitonin activity (117).

There is little threat of hypercalcemia in the normal adult, but calcitonin may have an important function in times of active bone turnover as a regulator of bone resorption. It will also help to control the effects of overactivity of the parathyroid.

Parathyroid Hormone

Although attention has been focussed on calcitonin in recent years, there has been substantial progress in research on parathyroid hormone, which must still be considered the principal hormone involved in calcium regulation in the mammal. It has a much longer history, since the endocrine role of the parathyroids in calcium metabolism was first recognized by McCallum & Voegtlin in 1909 (118) and the first biologically potent extract was prepared by Collip in 1925 (119).

Assay.—A number of new assay procedures have been developed in the rat (120–122) and mouse (123) to supplement the classical dog assay of Collip (124) and that of the rat by Munson (125). The hormone is antigenic, and sensitive immunoassays have been developed based on complement fixation (126) or radioimmunoassay (127). The intravenous infusion of antibodies to bovine PTH caused hypocalcemia in rats and rabbits but not in guinea pigs (128). The response in the first two species paralleled that following parathyroidectomy and was presumably due to neutralization of endogenous hormone. The effect lasted up to 24 hr.

Chemistry.—The most recent studies on the structure of parathyroid hormone indicate that it is a straight-chain peptide containing 83 amino-acid residues with a molecular weight of 9500 (129). The amino-acid composition is given in Table II. Significant biological activity and full immunological activity were observed in the peptide sequence of 35 amino acids

extending from the N terminus. This is in contrast to an earlier report (130) which indicated that the active portion was at the carboxyl terminus. Preliminary studies with partially purified porcine (131) and human (132) PTH indicate that they are similar in size to bovine hormone.

Action.—Although parathyroid hormone causes phosphaturia and promotes the absorption of calcium from the gut and renal tubules, its principal action is on bone. In organ cultures of calvaria or limb-bone rudiments from fetal or newborn rats and mice, addition of PTH to the medium increases bone resorption and calcium release (70–74). There is also an increase in the release and synthesis of acid protease and acid phosphatase (133). This may involve new protein synthesis since PTH administration increase RNA synthesis in bone cells (134, 135). In rats treated with actinomycin D, the initial hypercalcemic response to PTH is normal, but the effect beyond 6 hr is blocked (136). Presumably the initial response involves release of preformed enzymes, while the delayed response involves new enzyme synthesis (137).

There is now considerable evidence that the action of PTH is mediated by the "secondary messenger," cyclic 3′,5′-adenosine monophosphate (cAMP). Chase & Aurbach found that administration of PTH increased the excretion of cyclic AMP by the kidney (138). In bone cells, the hormone activated adenyl cyclase with a resulting increase in cyclic AMP (139). This is consistent with the observations of Rasmussen & Tenenhouse (140) that dibutyryl 3′,5′-AMP mimics the effect of PTH on kidney and bone. When they infused 5 μg PTH into the renal vein of parathyroidectomized rats, they observed a 3-fold increase in cyclic AMP in the kidney within 1 min, and an inhibition of isocitric dehydrogenase which they attributed to an increase in intracellular Ca^{++}. Borle (141) also observed that PTH caused a rapid and transient uptake of calcium by naked HeLa tumor cells *in vitro*. This effect of the hormone on cell uptake of Ca may account for the transient hypocalcemia, erroneously attributed to calcitonin, which occurred when parathyroid extract was injected intravenously in dogs (142). They concluded that cyclic AMP may function by regulating the permeability of membranes to Ca^{++} thus affecting the concentration of intracellular Ca^{++}. The above experiments provide support for the hypothesis of Wells & Lloyd (91) who suggest that the actions of both PTH and CT involve cyclic AMP.

Control of Secretion.—Direct humoral control of PTH production was demonstrated in gland perfusion studies in 1961 (18), but much more quantitative data are now available (143–145) based on EDTA-infusion studies in cattle. A sensitive radioimmunoassay was used to measure PTH levels (127) in plasma. In the cow, PTH increased in direct proportion to the reduction in plasma Ca and had a half-life in plasma of approximately 20 min (146). From this and the volume of distribution of the hormone, it is possible to calculate the secretion rate (145). This has been plotted

against plasma Ca in Figure 5. It will be noted that PTH is secreted continuously at normal plasma-Ca levels. The equation for secretion rate in the normal cow is $d\text{PTH}/dt = 7.6(12.0\text{-}P_{Ca})$ mμg/kg per min, where P_{Ca} is the plasma-Ca level in mg per cent. In the condition of post-parturient paresis in cows, which is associated with prolonged hypocalcemia, the parathyroid glands become hyperactive (147) and the slope of the curve for PTH secretion is increased 8 to 10 fold. This would reflect the increase in activity of the parathyroid glands.

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