Calcitonin

A review of experimental and clinical investigations

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Summary

In 1962 Copp proposed the existence of a hypocalcaemic hormone. Subsequent studies have shown him to be right.

Calcitonin is a potent inhibitor of bone resorption. By its action it lowers systemic blood calcium. It is secreted in response to hypercalcaemia by a distinct endocrine system within the thyroid in mammals and within the ultimobranchial bodies in fish, amphibia and reptiles. The active principle from pig has now been isolated and found to be a polypeptide composed of thirty-two amino acid residues. Together with parathyroid hormone, the hormone provides precise regulation of calcium concentration in the blood and controls bone remodelling and mineral turnover. Calcitonin in man may be secreted in excessive amounts by medullary carcinoma of the thyroid. In addition, aberrations in its secretion may play a role in pseudohyperparathyroidism and osteopetrosis.

Introduction

Calcitonin is a blood-calcium-lowering hormone. In mammals it is secreted by the thyroid in response to hypercalcaemic stimulation (Foster *et al.*, 1964a). The active principle is now recognized to be identical both to the hormone originally attributed to the parathyroids by Copp and his associates (Copp *et al.*, 1962; Copp & Henze, 1964) and to thyrocalcitonin, the hypocalcaemic constituent of rat thyroid described by Hirsch, Gauthier & Munson (1963). It acts on bone by inhibiting mineral resorption thereby reducing blood calcium. Clinically the hormone is of interest since it is of use in the treatment of hypercalcaemia; it may prove helpful in the management of osteoporosis (Foster *et al.*, 1968b).

A new endocrine system

The cells which secrete calcitonin are of ento-

dermal origin. Embryologically they derive from the ventral portions of the last pharyngeal pouches (Pearse & Carvalheira, 1967). In fish, amphibians, reptiles and birds these cells are found in the ultimobranchial bodies, organs of hitherto unknown function.

Hypocalcaemic extracts have been prepared from the ultimobranchial bodies of fish, lizard, turtle, pigeon and chicken (Copp, Cockcroft & Kueh, 1967; MacIntyre, 1967; Tauber, 1967; Moseley *et al.*, 1968; Parkes & Copp, 1968). These structures contain as much if not more hormone than the thyroid (Table 1). In mammals they fuse with the thyroid and become incorporated within the gland before birth. Here they constitute an endocrine system distinct from the follicular cells secreting thyroxine and triiodothyronine.

TABLE 1
Calcitonin content of the thyroid and the ultimobranchial body

	Ultimobranchial body (MRC milli-units/mg)	Thyroid (MRC milli-units/mg)
Pig	—	10.0
Chicken	37.8	0.9
Pigeon	3.5	3.4
Lizard	3.6	1.0

In view of the multi-organ distribution of the secreting cells, the name 'calcitonin' for the hormone is to be preferred. Adoption of this terminology does not detract from the contributions of other investigators who have used the alternative term, 'thyrocalcitonin'.

Secretion

Stimulus

In 1962 Copp and his associates demonstrated

that falls in blood calcium occur when the thyroid, together with the parathyroid glands, is perfused with hypercalcaemic blood (Copp *et al.*, 1962). This finding, first observed in dogs, has been confirmed (Kumar, Foster & McIntyre, 1963; Bernstein, Kleeman & Pine, 1965) and shown to be due to stimulation of the thyroid (Foster *et al.*, 1965; Care, Duncan & Webster, 1967). Isolated perfusion of the parathyroid in the goat (Foster *et al.*, 1964a) and the rat (Dale, Roth & Garcia, 1965) have excluded the latter gland as an additional source of the hormone.

Experimental evidence suggests the hormone is secreted under normocalcaemic conditions (Talmage, Neuenschwander & Kraititz, 1965; Minkin & Talmage, 1968). The secretion rate, however, is greatly increased by hypercalcaemia and may, in some animals, approach one-quarter the total hormonal content of the thyroid gland over a 4-hr period (Care *et al.*, 1968).

Interrelation with other endocrine organs

No conclusive evidence exists for extrathyroidal regulation of calcitonin secretion. Nonetheless, various reports have implicated the parathyroid and the pituitary.

Parathyroids

To reconcile early claims for a thyroid and parathyroid origin of the hormone, Gittes & Irvin (1965) proposed a parathyroid releasing factor. It was suggested this substance stimulated thyroid secretion of calcitonin. Cumulative evidence, however, has failed to support this thesis. (Baghdiantz *et al.*, 1965; Foster, 1966; Gittes, Wells & Irvin, 1967).

Pituitary

The number of calcitonin-secreting cells increases following hypophysectomy (Thompson, Isler & Sarkar, 1962; Kracht *et al.*, 1968). The findings are consistent with pituitary control of calcitonin secretion. The results, however, may also be interpreted as a decrease in the number of follicular cells. Failure to detect a difference in the amounts of extractable calcium-lowering activity from the thyroids of hypophysectomized and intact rats is presumptive evidence that calcitonin secretion is not under pituitary control (Baghdiantz *et al.*, 1965; Milhaud & Moukhtar, 1965).

The C cells

The calcitonin-secreting cells were first identified by Pearse and his colleagues (Foster, MacIntyre & Pearse, 1964b; Pearse, 1965), who observed that the levels of certain enzymes in the parafollicular cells of the dog thyroid were sen-

sitive to changes in calcium concentration. Immunofluorescent studies have confirmed their conclusions (Bussolati & Pearse, 1967; Kracht *et al.*, 1968). In earlier literature these cells have been referred to as light cells, mitochondrion-rich cells, ovoid cells, interfollicular cells, parenchymatous cells and protoplasm-rich cells. As none of these terms describes a general cell-type, they have been renamed 'C cells' to identify them with calcitonin (Pearse, 1966a). Their histological and cytochemical characteristics are as follows:

- (1) Preferential staining with Mallory's aniline blue and Cajal's silver nitrate (Pearse, 1966a).
- (2) Relatively high content of α -glycerophosphate dehydrogenase, esterase (Fig. 1) and cholinesterase (Foster, MacIntyre & Pearse, 1964b; Pearse, 1966a; Carvalheira & Pearse, 1967).
- (3) high concentration of ribonucleic acid (Carvalheira & Pearse, 1967).
- (4) Ability to concentrate 5-hydroxytryptan and DOPA *in vivo* (Pearse, 1966b; Owman & Sundler, 1968).

FIG. 1. C cells in the guinea-pig thyroid stained by the Cajal silver nitrate method, $\times 370$. (Courtesy of Professor A. G. E. Pearse, unpublished.)

(5) Toluidine blue metachromasia (Solcia & Sampietro, 1968).

(6) Inability to concentrate iodine (Azzali, 1968).

Despite variations in morphology these distinctive features are shared by widely different species.

The C cells are located near the basement membrane of the thyroid follicle but not in contact with the colloid. In contrast with follicular cells, they are larger and have clear cytoplasm. In the dog they are found in association with capillaries into which they presumably secrete. Their ultrastructure consists of small electron-dense mitochondria, a high level of free ribosomes and numerous spherical vesicles bounded by a single membrane (Pearse, 1966a). The latter contain electron-dense material sometimes in the form of discrete granules. Similar granules with calcitonin-like activity occur in thyroid-gland homogenates (Bauer & Teitelbaum, 1966; Cooper & Tashjian, 1966). These particles probably are the secretory products and have been shown to be released by hypercalcaemic stimulation (Matsuzawa & Kurosumi, 1967; Cameron, 1968).

Changes suggestive of increased cellular activity are also observed in the ultimobranchial body of frogs made hypercalcaemic (Robertson, 1968a, b). The induced hypertrophy and hyperplasia of the epithelial cells are consistent with an ultimobranchial source of the hormone in amphibia.

Assay

Purification of calcitonin has depended upon a reliable assay. Of the various methods employed, all have depended on the measurement of falls in blood calcium produced in the rat (Hirsch, Voelkel & Munson, 1964; Kumar *et al.*, 1965). Within limits this fall is related linearly to the logarithm of the dose given. Recent modifications of the basic assay to improve sensitivity have included the use of very young rats (Milhaud, Pérault-Staub & Moukhtar, 1965b; Sturtridge & Kumar, 1968), appropriate diets (Copp & Kuczerpa, 1968) and more sensitive animal strains (Cooper *et al.*, 1967).

Different laboratories are now able to express their results in terms of a single unit. The Medical Research Council have made internationally available a biological standard known as 'Research Standard B for Thyroid Calcitonin'. The unit is defined as the calcium-lowering activity contained in one ampoule. This unit is equivalent to approximately 1000 'Hammersmith Units' (Baghdiantz *et al.*, 1964) or 100 'Hirsch

Units' (Hirsch *et al.*, 1964). Ten MRC milli-units will lower the blood calcium roughly 10% in a starved 150-g rat bled 1 hr after intravenous injection. (Requests for Research Standard B should be sent to the Division of Biological Standards, National Institute for Medical Research, Mill Hill, London, N.W.7, attention of Dr J. A. Parsons.)

Biological estimation of potency can be made providing precautions are taken to prevent surface adsorption of the hormone (Parsons & Woodward, 1968). Preparations of high specific activity readily lose potency in this way, while crude preparations do not. The loss can be prevented by addition of protein to solutions used to dissolve and dilute the hormone (Parsons, 1968).

Purification

Porcine calcitonin has been isolated independently by several research teams (Putter *et al.*, 1967; Hawker, Rasmussen & Glass, 1967; MacIntyre, 1967; Gudmundsson *et al.*, 1968; Potts, 1967; Kahnt *et al.*, 1968; Franz *et al.*, 1968; Bell, 1968). Purification requires a combination of techniques including solvent extraction, precipitation, gel filtration, partition chromatography, counter-current distribution and preparative gel electrophoresis. Three or four separate active fractions are found (Gudmundsson *et al.*, 1968; Putter *et al.*, 1967; Neher & Kahnt, 1968; Bell, 1968). The amino acid composition of the major component has now been characterized (Table 2).

TABLE 2
Amino acid composition of porcine calcitonin

Amino acid	Residues
Aspartic acid	4
Threonine	2
Serine	4
Glutamic acid	1
Proline	2
Glycine	3
Alanine	1
Valine	1
γ-Cystine	2
Methionine	1
Isoleucine	0
Leucine	3
Thyrosine	1
Phenylalanine	3
Lysine	0
Histidine	1
Arginine	2
Tryptophan	1
Total	32

Putter *et al.* (1967), MacIntyre (1967) and Kahnt *et al.* (1968).

The hormone is a polypeptide (Baghdiantz *et al.*, 1964; Hirsch *et al.*, 1964). The major active fraction is composed of thirty-two amino acid residues suggesting a molecular weight of 3600. This figure is in essential agreement with previous estimates (MacIntyre, Foster & Kumar, 1965; O'Riordan *et al.*, 1966; Potts *et al.*, 1967). Its composition is distinguished by: (1) the absence of isoleucine and lysine, (2) no free terminal amino group, and (3) the presence of five of six potential carboxyl groups as amides (Putter *et al.*, 1967).

Action on bone

The hormone acts by inhibiting calcium resorption. Whether it affects bone formation remains uncertain.

A number of techniques have been used to study the effect on bone. Since the possible clinical usefulness of the hormone is dependent upon these basic observations, each deserves brief comment.

Culture studies

The hormone prevents the release of calcium from cultured embryonic bone *in vitro*. Resorption of calcium-45 in pre-labelled bone is inhibited by calcitonin (Friedman & Raisz, 1965). Likewise, gross loss of mineral is prevented by the hormone (Aliapoulos, Goldhaber & Munson, 1966a). Inhibition is most apparent when resorption is stimulated by either parathyroid hormone (Friedman & Raisz, 1965; Aliapoulos *et al.*, 1966a) or vitamin A (Aliapoulos, 1966a; Reynolds, 1968; Reynolds *et al.*, 1968).

Calcitonin does not appreciably affect resorption of bone-matrix hydroxyproline in acute studies (Raisz *et al.*, 1968). Neither does it increase the uptake of labelled proline, a precursor of collagen, into cultured bone (Goldhaber *et al.*, 1968). This latter finding suggests that the hormone does not increase bone formation rate. Changes in the relative distribution of bone cells, however, indicate that the reverse should be true. Calcitonin decreases the number of osteoclasts and increases the number of osteoblasts (Gaillard, 1967). The former supports an inhibitory effect on resorption; the latter is consistent with promotion of bone formation.

Kinetic studies

Calcitonin prevents the release of radioisotopes of calcium and strontium from pre-labelled rat bone *in vivo* (Johnston & Deiss, 1966; Robinson *et al.*, 1967; Wallach *et al.*, 1967; Hirsch, 1967). The results accord well with the earlier conclusions of Milhaud, *et al.* (1965) who related hormone-induced changes in cal-

cium-45 specific activity in the rat to a hypothetical four-compartment model.

Bone perfusion studies

By measuring arterio-venous differences in the isolated cat's tibia, MacIntyre, Parsons & Robinson (1967) demonstrated that calcitonin increases net calcium retention. While this method has not clarified the mode of action of the hormone, it confirms that the effect observed on the isolated bone can account for the systemic hypocalcaemia produced by the hormone.

Urinary hydroxyproline studies

Martin, Robinson & MacIntyre (1966) have observed that the urinary excretion of hydroxyproline is markedly reduced in the rat by prolonged calcitonin infusion. Likewise, hydroxyprolinuria induced by parathyroid hormone is inhibited by the hormone (Pechet *et al.*, 1967; Rasmussen & Tenenhouse, 1967). Since approximately half the hydroxyproline in the body is present in bone, its appearance in urine is a useful index of bone resorption. Diminished excretion is presumptive evidence of inhibition of bone breakdown.

Effects of chronic calcitonin treatment

In vivo changes produced by calcitonin are most marked in the metabolically active areas of bone. Foster *et al.* (1966a) have demonstrated that the hormone increases bone mineral in the metaphyseal areas of caudal vertebrae of parathyroidectomized rats (Fig. 2). In bones of treated animals the relative number of osteoclasts is decreased and the number of osteoblasts increased (Matrajt *et al.*, 1968). These findings are in agreement with the 'cellular shift' observed by Gaillard (1967) in bone-culture studies and are consistent with a possible increase in bone formation rate induced by the hormone. In addition, Foster and his associates (Foster *et al.*, 1967; Matrajt *et al.*, 1968) have found that not only is total metaphyseal bone increased in calcitonin-treated animals, but that the relative percentage of osteoid, the precursor of mineralized bone, is also increased. This observation likewise suggests the hormone may promote new bone formation.

Calcitonin-deficiency studies

Kumar and his colleagues (1968) have demonstrated changes in bone in chronically thyroidectomized rats adequately maintained on replacement thyroxine. They conclude that bone formation surfaces are reduced in the periosteal and endosteal regions of the femur in the absence of endogenous calcitonin secretion.

Unquestionably calcitonin inhibits bone mineral resorption. Further investigations are required to assess definitively the role of the hormone on bone formation rate.

FIG. 2. Longitudinal sections of the 9th to 11th caudal vertebra of a control rat (a) and a calcitonin-treated animal (b). Trabecular bone central to the growth cartilages in the treated animal is markedly increased relative to the control (Foster *et al.*, 1967).

Action on kidney

Calcitonin increases the urinary excretion of phosphate when given in doses in excess of those required to lower blood calcium. The definitive observation was made in rats by Robinson, Martin & MacIntyre (1966), who drew attention to the need that such studies be carried out in parathyroidectomized animals. In intact animals the calcitonin-induced hypocalcaemia would otherwise stimulate the secretion of parathyroid hormone, which itself produces phosphaturia. In a re-examination of the problem Russell & Fleisch (1968) perfused the renal artery of dogs and pigs. Their results confirm that phosphaturia

is only produced by relatively large doses of the hormone.

The phosphaturic role of calcitonin raises the question of the interpretation of phosphate excretion tests in man. The studies of Robinson *et al.* (1967) suggest that of the two sites of action, the action on the skeleton usually predominates. By inhibiting bone resorption, plasma calcium and phosphate are lowered even in the absence of the kidneys (Hirsch *et al.*, 1964; Gudmundsson, MacIntyre & Soliman, 1966). Only when the rate of bone resorption is low, as for example following parathyroidectomy, is the phosphaturic action dominant. This may well be true. In intact animals, however, calcitonin-induced phosphaturia is increased in conditions of rapid bone turnover (Milhaud, Tsien-Ming & Moukhtar, 1967). Presumably the phosphaturic action of increased parathyroid hormone secretion is responsible for this finding.

In view of the interrelation of the two hormones and their common effect on renal excretion, interpretation of phosphate excretion tests must be made with reservations.

Possible mechanisms of action

The calcium-lowering effect of calcitonin is secondary to a direct action of the hormone on the skeleton. Possible effects on the gastro-intestinal tract, kidney and soft tissues have been excluded. Calcitonin is active in degutted animals (Aliapoulos, Savery & Munson, 1965) and does not affect calcium transport in ligated intestinal loops *in vivo* (Robinson, Matthews & MacIntyre, 1968). Similarly the hormone is active in nephrectomized animals (Hirsch *et al.*, 1964; Gudmundsson *et al.*, 1966) and does not promote calcuria in intact animals (Kenny & Heiskell, 1965). Failure to demonstrate an increase in calcium in soft tissue following calcitonin administration likewise excludes an intra-cellular accumulation of the ion as a cause of hypocalcaemia (Gudmundsson *et al.*, 1966; Kenny & Heiskell, 1965).

Clearly calcitonin does not act by simple inhibition of parathyroid hormone since it effectively lowers blood calcium in parathyroidectomized animals (Tashjian, 1965; Gudmundsson *et al.*, 1966). Neither does it appear to affect synthesis of messenger RNA as many other hormones do. Actinomycin D, an inhibitor of RNA synthesis, does not impair its hypocalcaemic activity (Tashjian, 1965; Gudmundsson *et al.*, 1966). How then does calcitonin act to prevent bone resorption? Two possibilities have been proposed:

(1) Calcitonin may directly affect bone cells, regulating their activity. The studies of both

Gaillard (1967) and Foster and his associates (Foster *et al.*, 1966a; Matrajt *et al.*, 1968) indicate that the hormone decreases the number of osteoclasts and increases the number of osteoblasts.

(2) Wells & Lloyd (1968) suggest that calcitonin may produce hypocalcaemia by activating phosphodiesterase. Activation of phosphodiesterase enhances degradation of cyclic-3'5'-adenosine monophosphate which may act directly on bone to promote resorption. Theophylline, which inhibits this enzyme, raises blood calcium in parathyroidectomized rats. Similarly, imidazole, which inhibits it, lowers both calcium and phosphate. While no direct evidence has been found, this is an intriguing possibility.

These two alternatives for the action of the hormone need not be mutually exclusive.

Calcitonin in man

Calcitonin is present in man. That the hormone is involved in pathological conditions has been inferred by: (1) measuring extractable calcium-lowering activity from human thyroid tissue, (2) studying the response to alterations in systemic blood calcium, and (3) investigating disease conditions in animals. Definitive conclusions concerning secretion rates, however, will have to await further development and application of methods of measuring circulating hormone.

The active principle from human thyroid has been extracted (Hirsch *et al.*, 1964; Milhaud *et al.*, 1965a; Aliapoulos, Voelkel & Munson, 1966b; Chausmer, Mittleman & Wallach, 1966; Haymovits & Rosen, 1967). Extracts of normal glands are much less active in the rat than similarly prepared extracts from pig thyroid. This, however, does not necessarily imply that less hormone is present in man. Factors such as assay-animal sensitivity, enzymatic inactivation of the hormone and relative difficulty of extraction may explain the apparent differences in activity.

Hyperparathyroidism

Tashjian & Voelkel (1967) have reported that less calcium-lowering activity is extractable from thyroids from patients with hyperparathyroidism than from normal glands. This observation can be accounted for in one of two ways: either continuous hypercalcaemic stimulation of the thyroid may deplete the gland of the hormone or initially excessive secretion of calcitonin might stimulate hypersecretion of the parathyroid hormone and eventually result in autonomous parathyroid function. While extractable activity does

not necessarily reflect changes in secretion rate, the results are provocative.

Pseudohypoparathyroidism

This condition was previously considered to be due to failure of bone to respond to parathyroid hormone. The recent studies of Jowsey (1968) suggest this concept is no longer tenable.

An excess of calcitonin could explain the hypocalcaemia found in this condition (Fischer, Binswanger & Frey-Wettstein, 1967). Thyroids from two patients have contained approximately 100 times as much calcium-lowering activity as normal glands (Aliapoulos *et al.*, 1966b; Tashjian, Frantz & Lee, 1966). Thyroidectomy in one patient with the disease resulted in a return of calcium to normal levels (Mazzuoli *et al.*, 1968b). Thyroidectomy is not, however, recommended since alleviation of the disease was not permanent (Mazzuoli, Coen & Antonozzi, 1968a).

An increase in calcium-lowering activity may also be explained by accumulation of the hormone due to inadequate stimulation. In rats made hypocalcaemic by parathyroidectomy, the thyroid content of calcitonin is greatly increased (Gittes, Munson & Toverud, 1966).

Osteopetrosis

Osteopetrosis is a naturally occurring condition in the grey-lethal mouse, an inbred genetic mutant. Affected animals have metaphyseal sclerosis similar to that found in man. Their bones resemble those of rats treated with calcitonin (Foster *et al.*, 1966a), suggesting the disease is due to an excess of secretion of the hormone. Supporting this aetiology, Walker (1966) has found increased numbers of C cells in affected mice relative to their unaffected litter mates.

Thyroid disorders

There is histological and other evidence that medullary carcinoma of the thyroid is a hyper-secreting calcitonin tumour. Greatly increased levels of calcium-lowering activity in both blood and tumour tissue have been demonstrated in two patients (Milhaud *et al.*, 1968; Melvin & Tashjian, 1968).

Excessive secretion of calcitonin may also be associated with certain non-neoplastic conditions (Mazzuoli, Coen & Baschieri, 1966; Chimènes and Klotz, 1967). Surgical removal of the thyroid in several patients appears to have corrected pre-existing hypocalcaemia. Milhaud *et al.* (1965a), however, have not found appreciable differences in extractable calcitonin activity in goitrous and normal thyroid tissue. Clearly, no conclusions can be drawn at this time.

Thyroidectomy impairs calcium homeostasis (Woodhouse & Barnes, 1968; Smith & Laljee,

1967; Williams *et al.*, 1966). The findings support the thesis that calcitonin is physiologically important in man. Following intravenous calcium, athyroidal patients require longer for their blood calcium levels to return to normal than control patients. Reservations must be made, however, in the interpretation of the results as thyroidectomy can impair parathyroid function and the rate of bone mineral turnover if adequate thyroxine replacement is not given.

Calcitonin in animals

In 1963 Hirsch, Gauthier & Munson observed that acid extracts of the rat thyroid produce hypocalcaemia and hypophosphataemia when injected into laboratory animals. Their now classical investigations were stimulated by the finding that cauterization of the thyroid during parathyroidectomy lowered serum calcium in the rat. Calcium-lowering activity has since been found in the thyroid or ultimobranchial body of fish, lizard, turtle, pigeon, chicken, guinea-pig, rabbit, dog, pig, goat, sheep, deer, ox and monkey (Baghdiantz *et al.*, 1964; Foster *et al.*, 1964a; Hirsch *et al.*, 1964; Foster *et al.*, 1965; Chausmer *et al.*, 1966; Moseley *et al.*, 1968; Parkes & Copp, 1968).

The hormone may play a role in three unique conditions in animals: parturient paresis, hibernation and artificially induced soft-tissue calcification.

Parturient paresis

Parturient paresis is a spontaneous afebrile disease of dairy cows characterized by hypocalcaemic tetany and, in the later stages, paralysis and coma. Unless treated by intravenous calcium solutions the disease is invariably fatal. The investigations of Capen & Young (1967) suggest that increased calcitonin secretion may be responsible for this condition. Two factors support their conclusion. First, only a quarter as much extractable calcium-lowering activity is found in thyroids of diseased cows as in healthy animals. Second, calcitonin-secreting cells are less obvious in this condition and have the appearance of having discharged their secretory products.

Hibernation

A relation between calcitonin secretion and hibernation appears to exist. As many physiological functions undergo seasonal changes in hibernating animals, this would not be surprising were it not that hypocalcaemia is present in this condition. Nunez and his associates (1967) have identified cells in the thyroid of the bat similar to the C cells described by Pearse (1966a). Characteristically they contain secretory

granules in their cytoplasm. During the months shortly before the bat hibernates these granules vary greatly in size, some becoming many times larger. With the onset of hibernation the secretory product is released and only empty vesicles remain. Similar findings are observed in the thyroid of another hibernating animal, the bear (Azzali, 1968). In the latter species hypercalcaemia is present prior to hibernation which may account for the release of these secretory granules which are presumed to be calcitonin.

Soft-tissue calcification

Calcitonin greatly reduces or completely prevents soft tissue calcification induced artificially. Gabbiani and her co-workers (1968) have produced ectopic calcified lesions by four methods: exogenous administration of parathyroid hormone to thyroparathyroidectomized rats, inducing increased endogenous parathyroid hormone activity by nephrectomy in intact animals, cutaneous calcergy stimulated by intravenous lead acetate and subcutaneous polymyxin, and calciphylaxis provoked in dihydrotachysterol-treated animals by challenge with either depilation or subcutaneous injection of ferric dextran. In each study the lesions produced were minimized by pretreatment with calcitonin. As inhibition of mineral resorption cannot account for the findings, the more likely explanation is that calcitonin-induced hypocalcaemia protects against soft-tissue calcification.

Measurement of calcitonin in biological fluids

The measurement of calcitonin blood-levels is a prerequisite to the study of the role of the hormone in health and disease. Several approaches to the problem are under study.

Bone culture

Au, Niemann & Raisz (1967) have measured calcitonin in rat sera by assaying the effects of the hormone on parathyroid-hormone-induced bone resorption *in vitro*. As endogenously secreted parathyroid hormone interferes, the assay is limited to measuring calcitonin in sera from parathyroidectomized animals. At present the method is relatively insensitive and will not be applicable to the assay of the hormone in man unless interfering serum parathyroid hormone can be removed.

Bioassay

Sturtridge & Kumar (1968) are able to detect calcium-lowering activity from extracts of human plasma using an improved bioassay in young rats. The active substance is partially purified by precipitation, solvent extraction and gel filtration. Calcitonin-like activity has been detected in two

healthy volunteers before and after an intravenous calcium infusion. A two- to three-fold increase in activity was detected following stimulation. If the calcium-lowering activity can be shown to be due to calcitonin, this technique may prove applicable to resolving many of the unanswered questions in man.

Immunoassay

As yet no suitable radioimmunoassay for calcitonin is available. Arnaud & Littledyke (1966) have reported the measurement of a peptide in the blood of pigs, the levels of which rise when the animal is stimulated with exogenous calcium and which cannot be detected in the absence of the thyroid. Since their iodinated hormone was later recognized not to be calcitonin, their observation cannot be interpreted.

The physiological importance of the hormone

Calcitonin has two possible roles: control of calcium blood levels and regulation of bone mineralization. As these functions are closely inter-related, a distinction between the two is difficult. Both are probably operative, their relative importance depending upon the needs of the organism.

Calcium is essential for the normal maintenance of cardiac rhythmicity, nerve conduction, muscular contraction and blood coagulation, as well as many important enzymatic reactions. Endocrine regulation of calcium was previously

thought to be mediated through changing rates of secretion of parathyroid hormone. Secretion is known to be stimulated by hypocalcaemia. Hypocalcaemia, in turn, is corrected by parathyroid hormone mobilization of calcium from bone. This negative feed-back system is unquestioned. It now appears a second feed-back system exists (Fig. 3). Calcitonin, secreted by thyroid or ultimobranchial bodies, acts in opposition to parathyroid hormone. Its secretion is stimulated by hypercalcaemia. By inhibiting bone resorption it lowers blood calcium. Acting together, both hormones provide the precise control required to maintain blood calcium within physiological limits appropriate to its functions.

Calcitonin is probably also essential for the control of remodelling processes and the maintenance of adequately mineralized bone. During growth, increased secretion of both calcitonin and parathyroid hormone may account for the more rapid bone turnover. A high rate of secretion of parathyroid hormone could account for the increased gastro-intestinal absorption of calcium; calcitonin would prevent hypercalcaemia and facilitate bone mineralization. Possibly increased calcitonin secretion is associated with 'physiological goitre'. Enlarged thyroids are frequently found during infancy, puberty and pregnancy when the needs of the body for calcium are greatest. This thesis is in agreement with the suggestion of Hirsch & Cooper (1968) that calcitonin may also act to preserve the in-

FIG. 3. Diagram of hormonal control of calcium metabolism.

tegrity of the skeleton during periods of dietary deprivation of calcium when presumably bone would be more sensitive to the effects of the hormone.

Therapeutic uses

Hypercalcaemia

Porcine calcitonin is active in man. The hormone reduces the hypercalcaemia of malignancy, hyperparathyroidism, Paget's disease and hypervitaminosis D as well as idiopathic hypercalcaemia (Foster *et al.*, 1966b; Milhaud *et al.*, 1965a; Bijvoet & Jansen, 1967; Milhaud, 1968). Doses of less than 1 MRC unit produce falls in blood calcium. The hypocalcaemic effect appears related to the magnitude of the pre-existing hypercalcaemia (Hass & Dambacher, 1968; Foster *et al.*, 1968d). Two explanations are tenable. Either hypercalcaemia suppresses secretion of parathyroid hormone allowing calcitonin to act unopposed or hypercalcaemia is associated with more rapid bone turnover, and therefore, the effect on calcium blood levels of inhibition of bone resorption is more apparent. Both interpretations may be correct. The former concurs with present concepts of parathyroid hormone secretion (Sherwood *et al.*, 1966). The latter is consistent with the greater calcium-lowering effect of calcitonin in conditions favouring increased bone turnover (Milhaud, 1968).

FIG. 4. Changes in serum calcium in an osteoporotic patient treated with 33 MRC units of calcitonin three times a day (Foster *et al.*, 1968.)

Osteoporosis

Whether the hormone will prove effective in the treatment of osteoporosis in man has yet to be shown. Preliminary studies suggest the hormone acts on the skeleton in this condition (Foster *et al.*, 1968b) and that its effect is sustained (Foster *et al.*, 1968c, Fig. 4). Prolonged clinical trials, however, will be required to determine if a beneficial effect is produced on bone.

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