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Is Calcitonin an Important Physiological Substance?

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That calcitonin (CT) at supraphysiological doses is hypocalcemic led to the mistaken conclusion that it was important for calcium homeostasis and this idea has persisted to this day. Despite these findings, there is no readily apparent pathology due to CT excess or deficiency and there is no evidence that circulating CT is of substantial benefit to any mammal. Mammalian CT at physiological doses is not essential and very likely the CT gene has survived because of the gene's alternate mRNA pathway to produce calcitonin-gene-related peptide found in neural tissues. CT is not involved in calcium homeostasis or any other important physiological function, except, possibly, as an adjunct to protection of the skeleton under conditions of calcium stress, and appears to be in the process of becoming vestigial. CT produced in other tissues has paracrine actions that modulate functions such as proton transport, acid–base balance, prolactin secretion, and gastrointestinal motility. C-cells in mammals evolved from the ultimobranchial body that secretes CT in all lower vertebrates. It is highly probable that changes in amino acid sequence during evolution are responsible for the loss of activity, as fish CT is about 40 times as potent as human CT. CT may have been very important to survival in seawater fish, but the presence of the parathyroid gland and other evolutionary changes occurring in tetrapods suggest that the function of CT is no longer important.

Key Words: Calcitonin; calcitonin gene-related peptide knockout mouse; calcitonin evolution; calcitonin physiology.

Historical Origins of a Misconception

Several experiments led investigators to conclude that a hypocalcemic hormone, named calcitonin (CT), exists and to consider that this hormone is an important calcium regulating hormone. Current evidence strongly indicates that this latter conclusion is not justified. The misconception that CT is an important calcium-regulating hormone has resulted in 40 yr of experiments to elucidate an important physiological role for it. How were we misled? Let us examine these experiments and their results and see how they led to the misconception that CT is important in calcium homeostasis in mammals. The history and description of the experiments relevant to the discovery of calcitonin were detailed by Munson *(1)*.

In 1962 Copp and associates published the first credible evidence for the existence of a hypocalcemic hormone *(2)*. Using a classical endocrine approach, they obtained evidence that a hypocalcemic agent was secreted by the thyroid–parathyroid apparatus. When blood high in calcium content was passed through the isolated thyroid–parathyroid glands and returned to the dog, the systemic concentration of calcium fell (see Fig. 1). The results could not be ascribed to inhibition of parathyroid hormone (PTH) secretion, because the fall in blood calcium was faster than that after thyroparathyroidectomy (TPTX). They named the putative factor "calcitonin" because they thought, "it was concerned with the maintenance of the normal level or 'tone' [of calcium] in body fluids" *(2)*. Copp et al. assumed that the hormone originated in the parathyroid gland, as several perfusion experiments through thyroid glands from which the parathyroid glands had been removed did not lower blood calcium in the recipient dogs. These experiments drew a fair amount of attention by workers in the field, especially after they were confirmed a year later by Kumar et al. *(3)*.

The thyroid origin of CT was discovered in 1963 *(4)*. Parathyroidectomy (PTX) in rats was performed by hot-wire cautery, a procedure routinely used to bioassay PTH *(5)*. Blood calcium in rats fed a low calcium diet fell within 6 h from a normal level of 10 to 6 mg/dL or lower. The surprising finding was that PTX by surgical excision only resulted in a fall in blood calcium to about 7.5 mg/dL. Experiments to resolve the difference in the fall in blood calcium led to the conclusion that cautery released a hypocalcemic agent from the thyroid gland. The active agent was easily extracted from thyroid glands of many mammals and was originally named thyrocalcitonin to distinguish it from calcitonin, then thought to be secreted by the parathyroid glands. The two-hormone hypothesis was eventually resolved by perfusion experiments on goats *(6)* and pigs *(7)* demonstrating that the thyroid secreted the hypocalcemic agent. Later, the thyroid parafollicular cells (C-cells) were identified as the source of the hypocalcemic hormone *(8)*, now referred to as calcitonin.

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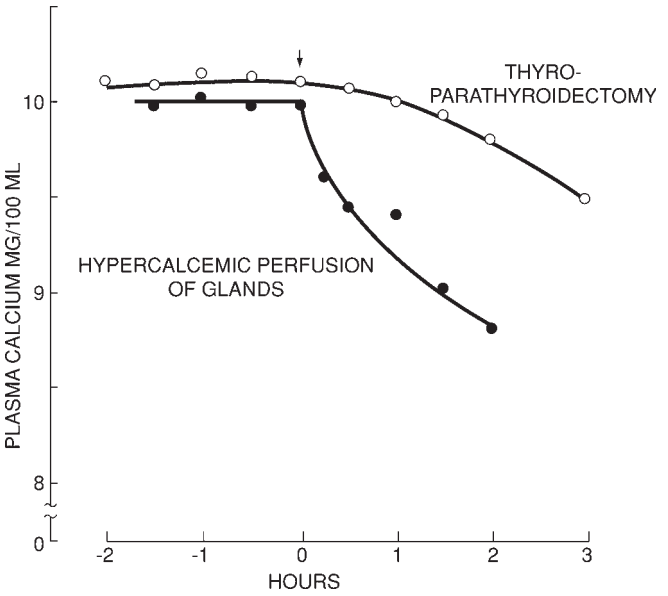

Fig. 1. Hypocalcemic response to hypercalcemic perfusion of the thyroparathyroid glands in dogs (solid circles).

Thus, these two experimental approaches, one by Copp et al. *(2)* and the other by Hirsch et al. *(4)*, have led investigators to perform experiments, most of which postulated or implied a major role for CT in calcium metabolism.

Unfortunately, the two experimental approaches described above were flawed because they were unrelated to physiological conditions. Copp's experiments involved the use of blood with calcium levels 30% above normal, a change not naturally experienced by normal animals. Even this high calcium content caused only a minor reduction of blood calcium, usually less than 10%. The cautery experiments by Hirsch et al. also did not at all resemble normal physiological conditions. Cautery released massive amounts of calcitonin, sufficient to reduce blood calcium of intact rats by 25%; rats, unlike humans, have a high rate of bone metabolism that was inhibited by CT *(9)*. Neither experiment provided evidence that calcitonin played a physiological role in the control of calcium metabolism. Investigators accepted Copp's concept that there was a need for a calcium-lowering hormone to maintain a constant level of blood calcium by preventing wide fluctuations in blood calcium when either PTH or CT was stimulated. Failure to evaluate these results critically in terms of the nature of the experiments and the putative need for a calcium-lowering hormone has hindered progress in this field.

We are concerned that chapters in textbooks on CT offer different roles for CT, some of them highly speculative with unsupported functions *(10,11)* and that this fact has quite undesirable pedagogical consequences. In some textbooks the authors of chapters on calcitonin concede that CT has an uncertain or no function in humans *(12–14)*. A plausible conclusion was offered by Shobak et al. *(15)* in the 6th edition of "*Basic and Clinical Endocrinology*" edited by Greenspan and Gardner and published in 2001. They wrote that "it is actually unlikely that calcitonin plays an essential physiologic role in humans and other terrestrial animals." Also in the chapter "Fetus and Neonate" Kovacs writes: "Fetal calcitonin levels [in mice] are higher than maternal levels and are thought to reflect increased synthesis of the hormone. Apart from responding appropriately to changes in the serum calcium concentration, there is little evidence of an essential role for calcitonin in fetal mineral homeostasis" *(16)*. Some authors concede that the function of CT has not yet been elucidated *(17)*.

Calcitonin and Calcium Metabolism of Mammals

After the thyroid gland had been established as the source of calcitonin in rats and other mammals, a considerable number of investigations were initiated to elucidate the physiological importance of the hormone and to determine the chemistry of the hormone. These latter studies were very productive and fruitful as calcitonin was isolated from many mammalian species and from the ultimobranchial bodies, identified as the source of CT in lower vertebrates. The calcitonins were purified, and the amino acid sequences were determined. In contrast to the chemical studies, attempts to identify the physiological function(s) of CT failed to produce definitive results. These studies fall into five different but related categories: (1) precise control of the level of blood calcium; (2) protection against hypercalcemia; (3) protection against hypercalciuria, formation of renal stones, and ectopic calcification; (4) protection of the skeleton; and (5) gastrointestinal interactions.

Maintenance of Blood Calcium Level

Copp viewed CT as a hormone that acted in concert with PTH to maintain the constancy of blood calcium *(18,19)*. In fact, this was called "Copp's calcitonin concept" *(20)*. Thus, without CT, the hypercalcemic response to PTH would overshoot normal calcium level and thereby cause large fluctuations in the blood calcium level. This concept has never been supported by experimental evidence and it seems important to emphasize that calcium in blood is supersaturated, and there is a physiochemical gradient that tends to lower blood calcium *(21)* by moving calcium into bone. PTH must overcome this gradient to maintain normal blood levels of calcium *(21)*. Thus, there really is no need for a hypocalcemic hormone.

Investigators have attempted to demonstrate that CT actually keeps blood calcium low in opposition to the calcium-raising action of PTH. Thyroidectomy (TX) in rats with functional parathyroid transplants did not result in a significant change in blood calcium *(22–24)*, strongly suggesting that CT is not required to maintain normal blood calcium. For example, 1 h after the operation, blood calcium had increased +0.9 in TX vs. +0.5 mg/dL for sham-

operated rats *(22)*. However, a better way to perform this type of experiment is to measure the change of blood calcium in the same animal before and after the removal of the thyroid gland. This type of experiment was done by Swaminathan et al. *(25)* in young pigs, an animal in which the thyroid is separate from the parathyroid glands. Removal of the thyroid led to a rise in blood calcium of at best 10%, an effect that disappeared within 24 h; IV infusion of CT at its approximate secretion rate effectively overcame the rise in blood calcium. While this is evidence that CT actually lowers blood calcium in opposition to the calcium-mobilizing action of PTH, albeit without exercising significant physiological control, another explanation for the findings is possible. One of the anesthetizing agents, barbital, used by Swaminathan et al. may have increased blood levels of PTH as occurred with this agent when used for rat surgery *(26)*. Thus, removal of the thyroid gland, which has been shown to restrict the calcium-mobilizing action of PTH (see below), could lead to a rise in blood calcium. Regardless of the mechanism, the change in calcium is not large and after a period of time, blood calcium returns to normal. The small change in blood calcium demonstrates that the maintenance of blood calcium is adequately handled by PTH alone *(27)*. Of course, calcium metabolism is influenced not only by PTH but also by metabolites of vitamin D as well as other hormones that affect bone, kidney, and the gastrointestinal tract *(12)*.

In summary, we agree with Kronenberg et al. *(27)* that it is PTH that controls calcium homeostasis: "Parathyroid hormone (PTH) controls calcium homeostasis through its actions on kidney and bone to raise blood calcium. Calcium, in turn, suppresses PTH secretion from the parathyroid gland. This negative feedback loop…serves to maintain the constancy of blood calcium from minute to minute."

Prevention of Hypercalcemia

Many investigators have found that the intact rat thyroid gland, by secreting CT, restricted induced hypercalcemia. Talmage et al. *(23)* were the first to demonstrate that the presence of the intact thyroid gland limited the hypercalcemia following intravenous or intraperitoneal injections of $CaCl_2$. Over the following few years other reports appeared showing that the thyroid gland by secreting CT, limited or prevented hypercalcemia induced by calcium, vitamin D, PTH, or during experimental acidosis in rats *(28)*. Experiments recently performed on CT/CGRP gene-deleted mice ($CT/CGRP^{-/-}$) by Hoff et al. *(29)* demonstrated results similar to those in TX rats. PTH given to such knockout mice (KO) increased serum calcium markedly, whereas serum calcium in the wild-type ($CT/CGRP^{+/+}$) control mice remained unchanged. Furthermore, injection of CT given to $CT/CGRP^{-/-}$ mice reversed the effect of PTH. Investigators interpreted these experiments, requiring supraphysiological doses of the hypercalcemic agent or high doses of calcium, as being important in defining the physiological function of CT as an antihypercalcemic hormone. However, it is unlikely that conditions exist in normal physiology under which humans or other mammals would be subjected to hypercalcemia. Thus, our current belief is that, while endogenous CT can indeed tend to prevent or reduce hypercalcemia, it is unlikely that this action has any physiological significance.

Prevention of Hypercalciuria and Soft Tissue Calcification

With the finding that endogenously secreted CT could attenuate or restrict hypercalcemia, investigators began to wonder whether CT might also restrict hypercalciuria, the formation of kidney stones, and soft tissue calcification. Such a mechanism for CT could also be beneficial for the skeleton. Experiments to support such actions were undertaken. For example, TPTX rats given PTH developed calcification of the kidney and heart, which could be somewhat ameliorated by the administration of calcitonin (reviewed in ref. *28*, p. 577). These experiments were not performed under physiological conditions and the results can easily be explained by the reduced blood level of calcium caused by exogenous CT. No evidence exists that endogenous CT alters soft tissue calcification in TX humans given adequate classical thyroid replacement therapy *(30)*.

Calcium nephrolithiasis is a multifactorial disorder that involves a number of contributing factors. Evidence to support a role of CT in the development of nephrolithiasis is lacking.

Protection of the Skeleton

That CT might be useful to conserve calcium for the skeleton has become the most favored hypothesis to explain its physiological role. Undoubtedly, this concept is strengthened by the well-known action of CT to inhibit osteoclastic activity. In fact, the calcium-sensing receptors on osteoclasts respond in vitro to physiological concentrations of CT *(31)*.

Using a special experimental protocol, Gray and Munson *(32)* were able to show that the removal of the thyroid gland increased blood calcium over that of the thyroid intact (TI) control. Rats with functional parathyroid transplants were given, after TX or a sham TX, either intragastric $CaCl_2$ or fed a 1% Ca diet that they had been trained to consume within 1 h, but were given access to the food for 90 min; TX rats had higher blood calcium level than TI rats within 1 h after administration of intragastric $CaCl_2$ or 90 min after consuming an average of 55 mg of dietary Ca. That special conditions were necessary was indicated by the finding (unpublished) that when the rats were fed a diet containing $CaCO_3$ in place of $CaCl_2$, TX did not result in a rise in blood calcium over that of TI rats, a result most likely due to the slower gastrointestinal absorption of $CaCO_3$ than that of $CaCl_2$.

Talmage, Matthews, and their collaborators *(33–35)* obtained considerable evidence that CT conserves calcium

by depositing a calcium-phosphate complex in intercellular fluid spaces of bone lining cells. VanderWiel and Talmage have proposed that the effect of CT is due to its action to conserve phosphate that complexes with calcium in the intercellular fluid spaces. This hypothesis and supporting evidence have been presented in detail *(34)*. Most important is that endogenous CT is implicated in the different appearance with electron microscopic observations in bone between thyroxin-treated TX and TI rats, both with functional parathyroid transplants. Also shown is the lower urinary calcium excretion in TI than TX rats, effects that were reversed by treating TX rats with exogenous CT *(35)*. Supporting arguments that phosphate plays a significant role in the conservation of calcium in bone by CT have been reviewed recently *(36)*.

As we see it, there are substantial problems associated with interpretation of these experiments. A very special experimental protocol for the rats must be established in order to observe the effects of CT ablation. The rats are trained to eat on a fixed feeding schedule, once a day, and are expected to consume all the food presented within 1 h. Thus, the calcium is absorbed over a short period of time and the largest differences are seen 4 h after eating. As with the antihypercalcemic effect of endogenous CT in PTH-treated rats, special, unusual conditions are necessary to demonstrate these effects. There is no evidence that the short-term effects observed have any benefits for the animals. Furthermore, TX rats, with functional parathyroid transplants and appropriately treated with thyroxin-replacement therapy, do not exhibit any discernable defects in bone *(36)*.

In this connection there have been reports indicating that TX resulted in lower bone mass, results that supported the concept that CT protected the skeleton. For example, evidence was presented indicating that CT deficiency in humans *(37)*, rats *(38)*, and goats *(39)* resulted in lower bone mass than in TI controls. On the other hand, Hurley et al. *(40)* found no bone changes in CT-deficient patients whether or not thyroxin replacement therapy was given; on the basis of their findings, they concluded that skeletal mass was not affected by endogenous plasma calcitonin in adults. If the administered dose of thyroxin is too high, then blood levels of TSH are suppressed and low bone mass can occur *(41–43)*. There is now a consensus that CT deficiency in TX humans does not result in detrimental effects on bone, provided they are given proper doses of thyroxin replacement therapy *(44)*.

The concept that the physiologic role of calcitonin is important during periods of calcium stress, such as pregnancy, lactation, and growth, is very popular. Concerning the putative effect in pregnancy, Kovacs states: "Serum calcitonin levels are also increased during pregnancy. It has been speculated that this increased level of calcitonin reflects its postulated role in protecting the maternal skeleton from excessive resorption of calcium, but this hypothesis remains unproved" *(16)*. The effects of lactation were the basis of several papers *(45–48)* and are mentioned in reviews *(49,50)* and textbooks *(10,11,51,52)*, but the evidence that CT is important was not considered convincing *(50)*. Convincing evidence to support this concept has been lacking until very recently when preliminary investigations in Kovacs' laboratory with *CT/CGRP*$^{-/-}$ mice demonstrated a much larger loss of bone during lactation than that occurring in the *CT/CGRP*$^{+/+}$ or the heterozygous (*CT/CGRP*$^{+/-}$) controls *(53)*. The loss of bone, about 45%, was quite similar to that occurring in lactating rats fed a low calcium diet (LCD) *(54)*. Despite the large loss of bone in *CT/CGRP*$^{-/-}$ mice, the recovery of bone after lactation ceased was rapid and reaches the same level as the recovering *CT/CGRP*$^{+/+}$ and *CT/CGRP*$^{+/-}$ controls *(53)*. How much of the loss of bone was because of an exacerbated response due to the loss of CT, CGRP, or both is not clear. Despite the increased loss of bone in *CT/CGRP*$^{-/-}$ mice during lactation, the consequence of the effect was not permanent, an indication that this effect is not physiologically important, although the short-term debilitating consequences upon the more fragile bones have yet to be determined.

In summary, the experimental evidence purporting to support the hypothesis that endogenous CT has a physiologically important role to conserve calcium for the skeleton has not been proven by definitive experimental data. Even though some experiments have shown differences between TX and TI rats, as well as between *CT/CGRP*$^{-/-}$ and *CT/CGRP*$^{+/+}$ mice, experiments on TX humans given adequate thyroxin therapy have not yet demonstrated any abnormalities due to the loss of CT. Clearly, no evidence has been obtained that shows an essential and important physiological role of CT on bone.

Gastrointestinal Interactions

There has been much interest in the possible involvement of the gastrointestinal tract in the function of CT. The gastrointestinal hormones, pancreozymin/cholecystokinin (PZ/CCK), caerulein, and gastrin, as well as the C-terminal octapeptide of PZ/CCK and pentagastrin, were found to be potent calcitonin secretagogues in pigs *(55,56)*; essential for activity was the C-terminal tetrapeptide Tryp-Met-Asp-PheNH_2.

These interesting observations have led to the development of a valuable clinical tool, a provocative test in which pentagastrin either alone or with calcium is administered to stimulate CT secretion. A high level of CT is pathognomic, and the test is used for the early detectection of medullary thyroid carcinoma (MTC) in patients and in families with a history of MTC or of multiple endocrine neoplasia Type 2, and to detect recurrence in patients who have undergone surgery for MTC *(57,58)*. There are, however, false positives with these tests *(59,60)*. The stimulation of CT secretion requires large doses of gastrin or its synthetic analog that imply quite strongly that the phenomenon is not a physiological action *(61,62)*.

There are a number of studies showing that calcitonin or Elcatonin, a synthetic analog of eel CT, can inhibit gastric or duodenal ulcers *(63,64)*. Again, it is highly likely that these effects are due to the high pharmacological doses administered and have no relationship to physiological function.

Evolutionary Considerations

That CT does not play an indispensable role in normal mammalian physiology should be apparent from the previous discussion that attempted to cover all aspects of the putative physiological actions of this hormone.

There is little doubt, however, that endogenous CT does, in fact, produce a number of well-established effects, as, for example, those upon the osteoclasts, as elucidated above. These effects appear to be neither sufficient nor necessary to make them important enough to be considered as regulating or controlling any aspect of mammalian life. Thus, a number of questions arise regarding the conservation of the *CT/CGRP* gene, in any form, and the nature of the calcitonin receptor (CTR), apparently in all mammalian species, for hundreds of millions of years. Deftos, in a very astute editorial *(65)*, raised the question of "the evolutionary enigma: why are fish CTs orders of magnitude more potent in mammals than mammalian CTs?" It has been well known for some time that the homology of the amino acid sequence between human CT and fish CTs is only approx 50%. On the other hand, CGRP is conserved to an extraordinary degree *(66)*, with a homology of approx 85–90%. "[H]uman CGRP is 86–89% homologous to chicken CGRP. This high degree of homology between mammalian and avian CGRPs supports the hypothesis that the physiological role of CGRP has not changed greatly during evolution and that this peptide plays a more primordial role than CT." It is this near-perfect homology that makes us propose that the survival of the gene should be ascribed to the activity of CGRP as a neuromodulator, and that it is CGRP that in fact has important physiological activity, such as its role in magnesium metabolism and the distinctly neurotic behavior of *CT/CGRP* knockout mice described by Kovacs and cited below.

Unfortunately, as of this writing, the nucleotide sequence of the salmon *CT/CGRP* (s*CT/CGRP*) gene is not available on NCBI; however, the sequence for *Fugu rubripes* (Japanese puffer fish) is available (NCBI Accession AJ309015) and the homologies of sCT and *Fugu* CT are over 90%, and, therefore, we shall use the nucleotide sequence of the *Fugu* as representative of teleost fishes. One of the more striking features of a comparison of *Homo* and *Fugu CT/CGRP* nucleotide sequences is the fact the human gene consists of 7637 base pairs, while the fish gene consists of slightly more than one third that number, i.e., 2581 base pairs. In addition to the great difference in the number of base pairs is the fact that the fish gene is comprised of four exons, while the human one consists of six exons. Thus, the evolutionary changes of the gene were much more severe than those occurring to the peptides, while the ability to synthesize both CT and CGRP was maintained.

CT/CGRP Knockout Mouse

There have been several reports on experiments with the *CT/CGRP*$^{-/-}$ mouse originally created by Gagel's group. Unfortunately, this knockout mouse necessarily produces a gene deletion that knocks out both CT and CGRP. Thus, it is not possible to determine from the results obtained with this mouse whether the changes are due to the loss of CT, CGRP, or both.

In their first abstract Hoff et al. *(67)* observed that mice homozygous null for the *CALCI* gene "were born normally, are fertile, and live a normal life span," although they "showed a significant reduction in cortical thickness" of tibia and lumbar spine "compared to normal animals at 14 months." In a subsequent abstract Catala-Lehnen et al. *(68)* described the surprising findings that bone formation in *CT/CGRP*$^{-/-}$ mice at 1 mo and 3 mo of age was significantly increased (twofold), that bone mass was 80% greater in both vertebral and long bone, and that ovariectomized (OVX) *CT/CGRP*$^{-/-}$ mice maintained a high bone mass "with no loss of bone following ovariectomy." They compared their results to "normal" OVX mice that lost 30–50% of bone volume *(68)*. The controls are not otherwise described and we do not know whether they were related to the experimental animals or were of the same strain. In a recent full paper, apparently based to a substantial extent on the same data presented in the Catala-Lehnen abstract *(68)*, Hoff et al. *(29)* confirmed the results of the earlier presentation.

It should be noted that the first report by Hoff et al. *(67)* is wholly consistent with the regnant paradigm that CT is protective of the skeleton, and that the subsequent publications are in stark contradiction to this and all other putative concepts of the physiological role of CT. Clearly, this conundrum requires a most careful evaluation. In the commentary accompanying the Hoff et al. paper *(29)*, Zaidi et al. *(69)* heap high praise upon the work ("…the elegant publication…"), while at the same time acknowledging that the results are difficult to explain and that a direct role for calcitonin in the dramatic increase in the rate of bone formation and the counteracting of the loss of bone due to OVX seems unlikely.

Generally accepted experimental protocols with genetically engineered mice require backcrossing for a minimum of four generations into the parental wild-type inbred strain, or, preferably, littermates, as control animals. The abstract announcing the creation of the *CT/CGRP*$^{-/-}$ mouse refers only to "age-matched controls" *(67)*. The second abstract *(68)*, announcing the high bone mass, as well as no loss of bone following OVX in *CT/CGRP*$^{-/-}$ mice, only mentions "normal ovariectomized animals" and makes no mention of the use of any controls for the high bone mass *CT/CGRP*$^{-/-}$

animals *(68)*. In the most recent full paper *(29)* it is made clear, although somewhat difficult to apprehend, that all the work reported in both the second abstract [Catala-Lehnen et al. *(68)*] and the detailed paper [Hoff et al. *(29)*] was performed on animals that came from a colony that was separately bred by mating together CT/CGRP$^{-/-}$ mice with other *CT/CGRP*$^{-/-}$ mice.

The last-mentioned practice is fraught with potentially major problems that appear not to have been considered by Hoff et al. *(29)* The first of these potential problems is stated unequivocally by Hertzog and Kola in their chapter, "Gene Knockouts," in *Gene Knockout Protocols (70)* as follows: "However, in general, it is important to remember that when a gene is knocked out in a mouse, the resultant phenotype is due to two major factors—first, the loss of function of the targeted gene and second, the reaction that the organism may initiate to compensate for that loss" *(70)*. It has been observed many times that (over)compensation may result in phenotypic traits that are the precise opposite of what might be predicted.

A second methodological flaw involved the creation of one population of *CT/CGRP*$^{+/+}$ mice and a separate population of *CT/CGRP*$^{-/-}$ mice. Repeated matings of *CT/CGRP*$^{-/-}$ animals will create a new, highly inbred strain that is not comparable to any controls. Clearly, it would be acceptable—albeit somewhat more time-consuming and expensive—to continue with a modified methodology of the first abstract *(67)* (by backcrossing, for example, into *C57BL/6* mice for at least the recommended number of generations) and then breed from *CT/CGRP*$^{+/-}$ mice, selecting from the offspring *CT/CGRP*$^{-/-}$ and *CT/CGRP*$^{+/+}$ siblings as controls. In this connection it should be mentioned that different mouse strains have substantially different bone mineral densities (BMD) in their skeletons *(71)* and an insufficiently backcrossed mutant may exhibit a BMD traceable to mouse strains other than the desired mutant.

The foregoing descriptions of the methodology utilized in both the Catala-Lehnen et al. abstract *(68)* and the Hoff et al. paper *(29)* suggest that alternative explanations, and not merely the loss of function of the targeted *CT/CGRP* gene, may account for the results reported in these publications and thus it appears that the experiments must be repeated under conditions free from these methodological flaws.

The recent publication by Woodrow et al. from Kovacs' laboratory *(53)* represents an acceptable protocol for experiments performed with *CT/CGRP*$^{-/-}$ mice and, not surprisingly, reports results consistent with the regnant paradigm concerning a physiological role for CT, i.e., that it tends to protect the maternal skeleton from excessive bone resorption during lactation (see above "Protection of the Skeleton").

The finding that elimination of the *CT/CGRP* gene decreased embryonic implantation *(72)* was supported by the results of McDonald et al. *(73)* showing reduced litter size in *CT/CGRP*$^{-/-}$ female mice. McDonald et al. *(74)* also reported that *CT/CGRP*$^{-/-}$ mice exhibited a selective defect in fetal magnesium but not in calcium metabolism: "…both serum Mg level and skeletal Mg accretion were reduced in the CT null" *(74)*. While the authors attributed the defect to loss of calcitonin, we believe it is as possible that the defect was due to the loss of CGRP because magnesium deficiency is associated with neural dysfunction *(75)*, such as hyperexcitability, hyperarousal with sensitivity to noise, bodily contact, and excitement *(76,77)*. These features of magnesium deficiency are highly similar to features that the adult *CT/CGRP*$^{-/-}$ mice exhibit, as described to us by Dr. C. S. Kovacs (personal communication). According to his description the *CT/CGRP*$^{-/-}$ mice were aggressive to the handlers, including biting, and also appeared neurotic, hypersensitive to light, overly sensitive and startled by clapping of hands, whereas the *CT/CGRP*$^{+/+}$ mice are comparatively calm.

Summary

The important physiological functions proposed over the years for calcitonin in mammals—to maintain a constant level of calcium, restrict hypercalciuria, nephrolithiasis, and ectopic soft tissue calcification—lack substantive supporting evidence. The recent work done in Kovacs' laboratory shows that CT (and/or CGRP) plays a role in tending to protect the skeleton during lactation *(53)*. The importance of this in terms of evolutionary fitness is not understood at this time, although the rapid post-lactational recovery of BMC casts doubt upon the assignment to CT of such an esential role.

It seems appropriate to refer to a review on CT by Austin and Heath *(78)* written 21 yr ago; they stated that they must echo a statement written 12 yr earlier by Foster *(79)* that "the physiological role of calcitonin is unknown." Now, 19 yr later, 40 yr after its discovery, it is time to recognize that these proposed functions, if they exist at all, do not appear to be physiologically important and that mammalian CT, although not yet vestigial, is likely to be in a gradual process of vestigialization.

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