Effect of Chronic Calcitonin Deficiency on the Skeleton of the Chicken* (33709)

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The role of calcitonin in the regulation of serum calcium has been extensively documented (1–3). Its serum calcium lowering effect is mediated by its inhibition of bone resorption. This mechanism of action has been demonstrated *in vivo* (4, 5) as well as *in vitro* (6, 7). However, there is little evidence that calcitonin is essential for the normal processes of bone growth and remodeling during early development. We have studied this relationship by removal of the chicken ultimobranchial body which is the source of calcitonin in birds (8).

Materials and Methods. White leghorn cockerels were obtained from the Ghostley Chicken Hatchery (Anoka, Minn.) shortly after hatching and were fasted until 4 hr after surgery. One-day-old male chicks were anesthetized by hypothermia and the ultimobranchial glands were removed by blunt dissection under a dissecting microscope. Sham operations were performed by exposure of the glands without further dissection. There were 12 birds in each group. The location of the ultimobranchial glands was invariably dorsal to the bifurcation of the brachiocephalic artery into the carotid and the branchial arteries and proximal to the carotid artery (9). Bleeding was minimal, and there was no evidence of interference with the blood supply to nearby vital structures. All chickens were allowed free access to tap water and to a standard poultry feed (Purina, Co., St. Louis, Mo.) containing 18% protein, 1.2% calcium, and 0.7% phosphorus.

Serum calcium was determined by atomic absorption (10), phosphorus by a modification of the method of Fiske and Subbarow (11), and alkaline phosphatase by the Kind and King modification (12) of the King, Armstrong method. Bovine parathyroid hormone (Eli Lilly Co., Indianapolis) was injected subcutaneously after an 18-hr fast, and serial blood samples from each bird were taken from the wing vein at 0, 3, 6, and 9 hr.

X-Rays of the tibia were taken at a fixed distance from the source and the ratios of the diameters of the medullary space to the cortical plus medullary space were estimated as a crude parameter of cortical bone thickness. The measurements were taken with a measuring magnifier (Bausch and Lomb, Hastings 7X) at two fixed distances from the distal end of each tibia of each bird thus providing four measurements for each bird. Microradiographs of the midshaft and of the metaphysis were prepared by the method of Jowsey (13).

Results. The body weights, serum calcium, phosphorus and alkaline phosphatase values for the ultimobranchialectomized and the sham-operated chickens at various postoperative intervals are presented in Table I. There were no significant differences between the groups in any of these parameters. The mortality of all birds was under 10% and was equal for both ultimobranchialectomized and sham-operated birds throughout the duration of the study.

The X-Rays of the tibia of both groups appeared to be similar grossly. Furthermore, removal of the ultimobranchial gland did not seem to affect the thickness of the cortical bone at 3 months of age as estimated by the ratio of medullary to the total bone diameters. The mean of the ultimobranchialecto-

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mized group was 0.66 ± 0.02 (1 SD) as compared with 0.66 ± 0.02 for the sham-operated birds. Microradiographs of sections of the metaphysis and of the midshafts of the tibias at 1 and 3 months after surgery revealed no striking alteration in the appearance of the bone or of mineral density in the ultimobranchialectomized vs the sham-operated birds (Fig. 1).

Despite the apparent lack of effect of chronic calcitonin deficiency upon the appearance of bone in the growing chicken, there was evidence that deficiency of calcitonin prevented a normal response to a hypercalcemic stimulus. Hence, the hypercalcemic response after the injection of 500 units of bovine parathyroid hormone 3 months postultimobranchialectomy was followed by a lowering of the serum calcium to a mean of 10.2 mg/100 ml at 9 hr as compared with 7.6 mg/100 ml in the sham-operated birds (Fig. 2). There were no differences between the two groups of the preinjection or the peak calcium levels.

Discussion. The mechanism of calcitonin induced decrease in resorption of bone is not understood. There are several observations of the effect of calcitonin on bone which may have bearing on the significance of calcitonin in the maintenance and growth of the skeleton. Calcitonin increases the formation of trabecular bone in the metaphysis of parathyroidectomized animals chronically treated with calcitonin although no changes are produced in intact animals (14). The growth of cortical bone is enhanced in intact rats treated with calcitonin (15). Furthermore, calcitonin decreases the number of osteoclasts *in vitro* and *in vivo* (16, 17), while some studies have demonstrated that calcitonin treatment increased the numbers of osteoblasts (17). Other evidence suggests that synthesis of glycosaminoglycan, a mucopolysaccharide constituent of bone matrix, is increased by calcitonin *in vitro* (18).

Few attempts have been made to examine the effects of chronic calcitonin deficiency upon the skeleton. In one study bone formation surfaces were reduced in animals made calcitonin deficient by thyroidectomy despite adequate thyroxine replacement (19). There is no evidence for an abnormality in skeletal structure of the thyroidectomized human treated with replacement thyroxine. The

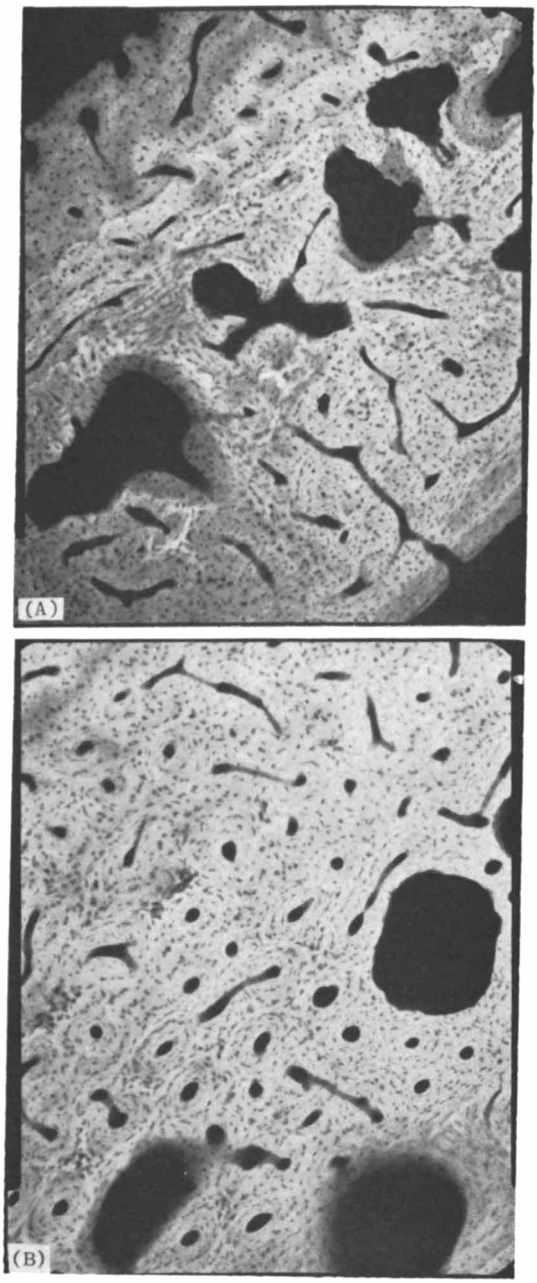

FIG. 1. Microradiograph of the midshaft of the tibia 3 months after removal of the ultimobranchial gland (A) as compared with a sham-operated bird (B).

TABLE I. The Effect of Ultimobranchialectomy on Body Weight, Serum Calcium, Phosphorus and Alkaline Phosphatase.

Age (weeks)	Item	Ubectomy[a]	Sham[b]
1	Body wt. (g)	58 (43 - 71)	60 (53 - 67)
2	Serum Ca (mg/100 ml)	10.2 (8.3- 12.0)[c]	9.8 (7.3- 12.5)
4	Serum Ca		10.7 (10.3- 11.3)
	Serum alk. phos. (KAU/100 ml)	251 (147 -427)	248 (108 -605)
	Serum P (mg/100 ml)	6.6 (5.9- 7.1)	6.0 (4.8- 7.6)
	Body wt.	162 (147 -200)	146 (124 -173)
6	Serum Ca	9.6 (9.2- 10.0)	9.6 (8.5- 10.7)
8	Body wt.	613 (568 -682)	582 (482 -682)
14	Serum Ca	10.7 (9.2- 12.4)	10.9 (10.3- 11.7)
	Serum P	5.6 (4.3- 6.7)	5.9 (5.5- 6.7)
	Serum alk. phos.	114 (51 -301)	86 (38 -248)

[a] Ultimobranchialectomy.
[b] Sham operated.
[c] Mean (range of values).

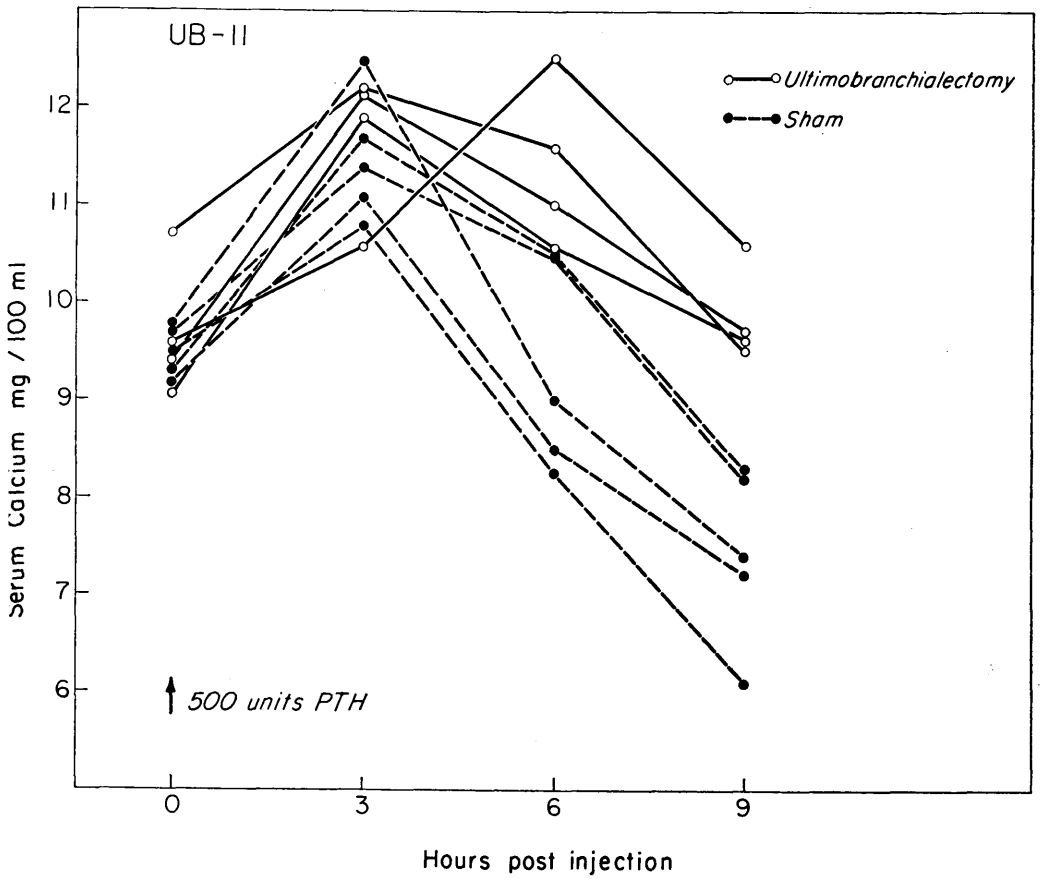

FIG. 2. Effect of bovine parathyroid hormone on the serum calcium of ultimobranchialectomized chickens as compared with sham-operated controls.

present study suggests that growing bones are not dependent upon the effects of calcitonin to maintain normal structure and remodeling. The presence of normal serum alkaline phosphatase levels and the absence of increased bone resorption in ultimobranchialectomized birds indicate that calcitonin effects are not essential in inhibiting the effects of parathyroid hormone on bone. In addition, there is no evidence that calcitonin is important in the maintenance of normal serum levels of calcium and phosphorus. However, the stressful stimulus of hypercalcemia produced by parathyroid hormone elicits a hypocalcemic response in the intact birds presumably due to calcitonin secretion. Calcitonin deficiency prevents this response to hypercalcemia. The studies have confirmed the role of calcitonin in the maintenace of serum calcium values in response to hypercalcemia but do not indicate that the hormone is essential to skeletal or calcium metabolism under basal conditions.

Summary. The effect of ultimobranchialectomy upon the maintenance of normal skeletal structure in the young chicken has been examined. Mortality, body growth, serum calcium, phosphorus, alkaline phosphatase, X-Ray appearance and microradiographs of the tibia of calcitonin-deficient animals was not different from sham-operated birds. It was demonstrated that the fall of serum calcium to hypocalcemic levels following parathyroid hormone induced hypercalcemia is calcitonin dependent. Similar observations were made after the infusion of calcium studies indicate that calcitonin is essential in in thyroidectomized animals. (20). These studies indicate that calcitonin is essential in the prevention of hypercalcemia but has no major role in the growth of the skeleton under normal conditions.

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