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Short Note

Effects of Salmon Calcitonin and Bovine Parathormone on the Levels of Serum Calcium and Inorganic Phosphorous, and the Histology of the Parathyroid Gland in the Snake, *Acrochordus granulatus* (Schneider)

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Anita P. Warbhuwan and Arun S. Padgaonkar (1997) Effects of salmon calcitonin and bovine parathormone on the levels of serum calcium and inorganic phosphorous, and the histology of the parathyroid gland in the snake, *Acrochordus granulatus* (Schneider). *Zoological Studies* **36**(1): 64-69. Administration of salmon calcitonin in the snake, *Acrochordus granulatus*, at a dose of 10 IU/100 g body weight, resulted in a slight rise in the levels of serum calcium and inorganic phosphorous on day 1 as compared to the controls. Thereafter, these levels remained within normal limits throughout the course of the experiment. Snakes treated with calcitonin showed hypertrophy of the parenchymal cells of the parathyroid gland. The nuclei of these cells increased in size. The lack of hypocalcemic response in *A. granulatus* after calcitonin administration has been attributed to an adequate secretion of parathormone from the parenchymal cells of the parathyroid gland which counteracts the action of calcitonin. The hypertrophy of the parenchymal cells in the treated snakes supports such a view.

Administration of parathormone in *A. granulatus* evoked a hypercalcemic response on day 1, which reached a peak on the 2nd day and thereafter, showed a decline (4th day). The serum inorganic phosphorous level in the parathormone-treated snake showed a slight decrease on the 2nd day of treatment. The chief cells of the parathyroid glands of the parathormone-treated snakes showed extensive vacuolation in the cytoplasm. The nuclei of the cells decreased in size. The above experiments lead us to conclude that parathormone plays a pivotal role in the control of calcium and phosphate metabolism in the snake under study, and that calcitonin counteracts the action of parathormone.

Key words: Calcitonin, Parathormone, Snake.

There is limited information about the physiological role of calcitonin in reptiles, a group possessing functional ultimobranchial glands which secrete calcitonin (Galan-Galan et al. 1981, Boudbid et al. 1987). Reptilian ultimobranchial gland extracts cause hypocalcemia in a rat bioassay (Clark 1968, Uchiyama et al. 1978), but are ineffective in individuals of their own species (Clark 1968, Moseley et al. 1968, Clark 1971, Laverty and Clark 1982). According to Boudbid et al. (1987), the calcitonin present in the ultimobranchial gland of the turtle, *Pseudemys scripta*, appears to be related to salmon calcitonin. Recently, Srivastav et al. (1986) observed a hypocalcemic response to the administration of synthetic salmon calcitonin in the freshwater snake, *Natrix piscator*. More studies are needed along similar lines. Hence, in the present study an attempt has been made to investigate the effects of salmon calcitonin on the levels of serum calcium and inorganic phosphorous as well as on the histology of the parathyroid gland of the estuarine snake, *Acrochordus granulatus*. The study further assesses the effects of parathormone on the calcium and phosphate metabolism in the snake. *A. granulatus*, since the ablation of the parathyroid glands in this species of snake results in hypocalcemia and hyperphosphatemia (Warbhwan 1993).

Materials and Methods—Seventy adult male *A. granulatus* weighing between 150 and 200 g used for this study, were procurred locally from suburbs of Bombay and were acclimatized in tanks containing sea water under laboratory conditions for 4 days prior to the experiments at a room temperature (27 °C – 28 °C). They were not fed during the entire course of the experiments. Animals were separated into control and experimental groups (5 snakes in each group) for different experiments. Both the experiments were carried out in the month of August-September.

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Calcitonin treatment

A group of 5 snakes each received a single intraperitoneal injection of calcitonin (Sandoz India Ltd., Batch R. No.007456) at a dose of (10 IU/0.1 ml)/100 g body weight. Five control snakes each received a single injection of the vehicle (0.1% gelatin) at a dose of 0.1 ml/100 g body weight by the same route. Both control and experimental snakes were sacrificed 4 h after the injection.

Other 3 groups of snakes received daily injections of calcitonin (Sandoz India Ltd., Batch R. No. 007956) intraperitoneally at a dose of (10 IU/0.1 ml)/100 g body weight for different durations of time. Snakes in the control groups received equivalent quantities (0.1 ml/100 g body weight) of the vehicle (1% gelatin) by the same route. These control and experimental snakes were sacrificed 4 h following the last injection on days 4, 7, or 14 for these respective experiments.

Parathormone treatment

A group of 5 snakes received a single intraperitoneal injection of parathormone (bPTH, Lot No. 74/557, National Hormone and Pituitary Agency, U.S.A.) at a dose of (10 USP/0.1 ml)/100 g body weight. The animals in the control group received equivalent quantities of the vehicle (L-cysteine hydrochloride solution at 5 mg/ml by the same route. Both control and experimental snakes were sacrificed 4 h after the injections. Other 2 groups of snakes were given daily parathormone injections intraperitoneally, at a dose of (10 USP/0.1 ml)/100 g body weight for 2 and 4 days, respectively. Control groups of snakes received daily intraperitoneal injections of the vehicle (L-cysteine hydrochloride solution at 5 mg/ml). Both control and experimental snakes were sacrificed 4 h after the last injection on the 2nd and 4th days.

To avoid the effects of circadian rhythms the animals were always injected at 9 a.m. Blood samples were also collected at approximately the same time. Blood samples from the control and experimental snakes were withdrawn from the right systemic artery, while the subjects were under mild ether anaesthesia, received in centrifuge tubes, allowed to clot at room temperature, and then the serum was separated by centrifugation (3 500 rpm). The serum samples were analyzed for calcium and inorganic phosphorous using the methods described by Trinder (1960) and Gomorri (1942), respectively.

For histological observations, parathyroid glands of the control and experimental animals were removed and fixed in Bouin's solution. After routine dehydration, they were embedded in paraffin wax. Paraffin sections of the parathyroid glands were cut at 5 μm processed in a routine way, and stained by the hematoxylin-phloxin method. The nuclear diameters of the chief cells were measured with an ocular micrometer. Each nucleus was measured along its long and short axes and a mean value was calculated. From each group 250 nuclei were randomly measured (50 nuclei from each specimen). The mean values presented in Tables 1 and 2 refer to the mean of the mean values per animal.

The differences between serum calcium and inorganic phosphorous and the mean nuclear diameter of the parathyroid cells of both the control and experimental groups were evaluated for statistical significance using Student's *t*-test.

Results—Changes in the serum calcium and inorganic phosphorous levels and nuclear diameters of the chief cells of

Table 1. Effects of calcitonin on levels of serum calcium and inorganic phosphorus, and on nuclear diameter of cells of the parathyroid gland in the snake, *Acrochordus granulatus*

Duration in days	Serum Calcium (mg%)		Serum inorganic phosphorous (mg%)		Nuclear diameter (μm)	
	Control	Experimental	Control	Experimental	Control	Experimental
1	10.94 ± 0.16[a]	13.95 ± 0.37***	4.62 ± 0.29	7.33 ± 1.25*	3.54 ± 0.10	3.45 ± 0.10
4	11.35 ± 1.12	11.99 ± 0.16	4.41 ± 0.25	5.25 ± 0.25	3.54 ± 0.10	3.30 ± 0.11
7	11.14 ± 0.09	11.45 ± 0.42	4.81 ± 0.27	5.43 ± 0.37	3.57 ± 0.10	3.60 ± 0.10
14	11.09 ± 0.62	11.23 ± 0.27	4.82 ± 0.20	5.10 ± 0.48	3.69 ± 0.11	4.08 ± 0.15

*, *** indicate significant response at the $p < 0.05$ and $p < 0.001$ levels, respectively.
[a]Values are mean ± S.E; 5 snakes were used in each group.

Table 2. Effects of bovine parathormone on levels of serum calcium and inorganic phosphorous, and on nuclear diameter of cells of the parathyroid gland in the snake, *Acrochordus granulatus*

Duration in days	Serum Calcium (mg%)			Serum inorganic phosphorous (mg%)			Nuclear diameter (μm)		
	Control	Experimental	*p*-value	Control	Experimental	*p*-value	Control	Experimental	*p*-value
1	10.14 ± 0.08[a]	13.85 ± 0.86*	< 0.02	4.61 ± 0.03	5.13 ± 0.09**	< 0.01	3.60 ± 0.10	3.33 ± 0.09	N.S.
2	11.14 ± 0.10	14.29 ± 0.63	< 0.01	4.54 ± 0.33	3.81 ± 0.52	NS	3.45 ± 0.10	2.07 ± 0.10***	< 0.001
4	11.23 ±0.06	12.45 ± 0.44	NS	4.57 ± 0.34	5.34 ± 0.21	NS	3.60 ± 0.10	3.39 ± 0.10	NS

*, **, *** indicate significant responses at the $p < 0.02$, $p < 0.01$, and $p < 0.001$ levels, respectively.
[a]Values are mean ± S.E; 5 snakes were used in each group.

the parathyroid gland in the experimental and control groups have been summarized in Tables 1 and 2.

Levels of serum calcium and inorganic phosphorous of the calcitonin-treated snakes showed a slight rise on day 1 as compared to the controls (Table 1). Thereafter, these levels remained within normal limits throughout the course of the experiment.

Administration of parathormone evoked a hypercalcemic response on day 1, which reached a peak on the 2nd day (Table 2). The serum inorganic phosphorus level in the parathormone-treated snakes showed a slight decrease on the 2nd day of the treatment.

The snakes treated with calcitonin for 7 and 14 days showed hypertrophy of the parenchymal cells of the parathyroid gland (Fig. 2). The nuclei of these cells increased in size (Table 1). These changes were less marked in the parathyroid glands of the animals treated for lesser durations of time, i.e., 1 and 4 days.

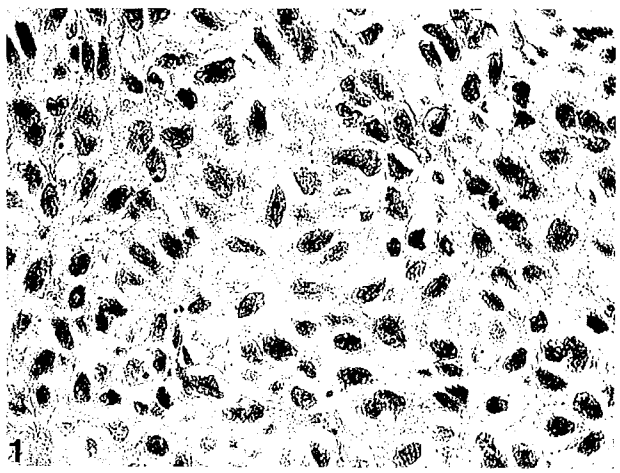

Fig. 1. Photomicrograph of the parathyroid gland of the control snake, *Acrochordus granulatus*. 1000 X.

The chief cells of the parathyroid glands of parathormone-treated snakes showed extensive vacuolation (Fig. 3). The mean nuclear diameter of these cells significantly decreased as compared to the controls (Table 2, Fig. 1).

Discussion—Administration of salmon calcitonin failed to induce a hypocalcemic response in the snake, *A. granulatus*. On the other hand, several prior reports indicate that calcitonin administration can induce marked hypocalcemia in species of lizards and snakes. Salmon calcitonin injections produced hypocalcemia in *Iguana iguana* (Kline, 1981) and in the snake, *Natrix piscator* (Srivatstav et al. 1986).

Similarly, porcine calcitonin produced hypocalcemia in the lizard, *Varanus flavescens* (Swarup and Pandey 1990), and the snake, *Sinonatrix piscator* (Srivastav and Rani 1989).

On the other hand, administration of mammalian calcitonin had no effect on the plasma calcium and inorganic phosphorous levels in the turtle, *Chrysemys picta* (Clark 1968), in the lizard, *Anolis carolinensis* (Dix et al. 1970), in the desert iguana, *Dipsosaurus dorsalis* (Kiebzak and Minnich 1982), in the snake, *Thamnophis sirtalis* (Clark 1971), and in 4 species of the genus *Natrix* (Clark and Dantzler 1975).

Available reports on the effects of mammalian calcitonin administration in lower vertebrates generally indicate conflicting results. In fish, the injection of calcitonin has been reported to result either in hypocalcemia (Chan et al. 1968, Lopez et al. 1971, Suryawanshi and Mahajan 1976, Wales 1984, Das et al. 1990), hypercalcemia (Glowacki et al. 1985, Fouchereau Person et al. 1987), or no effect on plasma calcium levels (Pang and Pickford 1967, Urist 1967, Pang 1971, Chan 1972, Urist et al. 1972, Yamauchi et al. 1978, Srivastav and Swarup 1980, Fenwick and Lam 1988).

These varied results have led many workers to conclude that calcitonin performs no major role in the maintenance of plasma calcium levels in this group of vertebrates (Hirano et al. 1981, Clark 1983, Pang and Pang 1986, Das et al. 1990).

In amphibians, the results of administration of calcitonin are also often contradictory. Many researchers (Bentley 1984, Guardabassi et al. 1968, Dore et al. 1969) have observed that porcine calcitonin-treated *Bufo bufo* become hypocalcemic in the month of April but hypercalcemic in June and July.

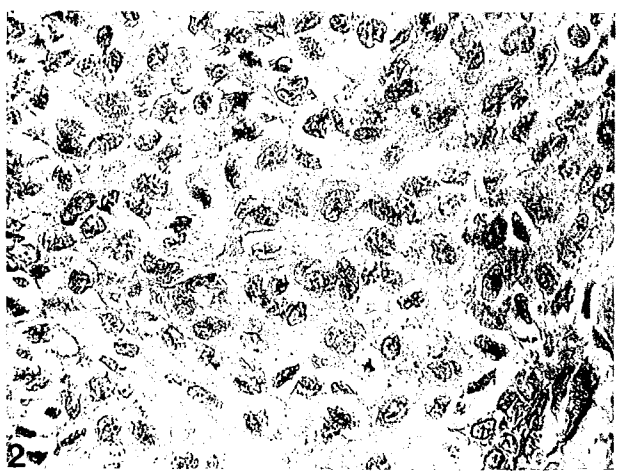

Fig. 2. Photomicrograph of the parathyroid gland of calcitonin-treated *Acrochordus granulatus* showing hypertrophy of the parenchymal cells after 14 days. 1000 X.

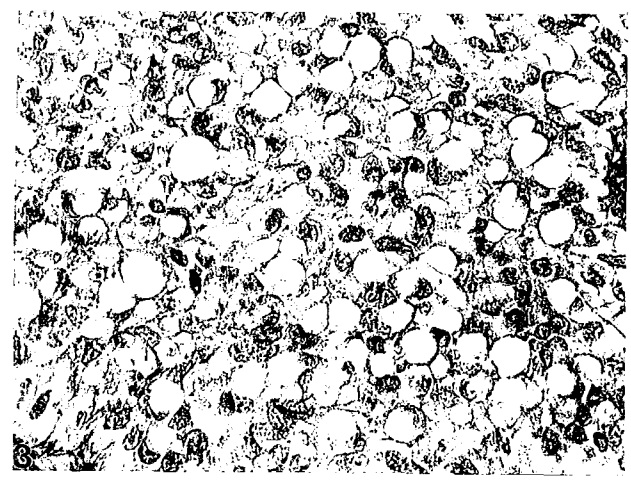

Fig. 3. Photomicrograph of the parathyroid gland of parathormone-treated *Acrochordus granulatus* showing extensive vacuolation in the cytoplasm after 2 days. 1000 X.

Boschwitz and Bern (1971) have reported that calcitonin induces hypocalcemia in *Bufo marinus*, but its administration had little effect in *Bufo boreas*. McWhinnie and Scopelliti (1978) have reported hypocalcemia and hypophosphatemia after the administration of low doses and hypercalcemia and hyperphosphatemia after the administration of high doses of calcitonin in *Rana pipiens*. No significant changes in the plasma calcium level of *Xenopus laevis* were observed after the administration of salmon calcitonin (McWhinnie and Scopelliti 1978). Bentley (1983) observed no change in the levels of plasma or urinary calcium in *B. marinus* after the administration of salmon calcitonin. On the other hand, Krishna and Swarup (1985) have reported hypocalcemia in *Rana cyanophlyetis* after the administration of salmon calcitonin.

A possible explanation for the lack of response after calcitonin administration in the present investigation may be that an adequate amount of parathormone is secreted from the chief cells of the parathyroid gland to counteract the action of injected calcitonin. The hypertrophied chief cell of the parathyroid gland of the calcitonin-treated snakes and an increase in their nuclear size support such a view. The initial transient hypercalcemia observed after calcitonin treatment may be due to the overproduction of parathormone as an immediate response to exogenously administered calcitonin.

It has been reported that the action of calcitonin is influenced by age, diet, and difference in species. Effectiveness of the hormone decreases with advancing age of the subject (Hirsch and Munson 1969). In the present investigation only adult specimens of the snake, *A. granulatus*, were used. The drug was not administered to younger specimens, and therefore it is not possible to comment on this aspect.

Laverty and Clark (1982) have convincingly suggested a physiological role for calcitonin in the calcium homeostasis of reptiles. In the present study the increased activity observed in the chief cells of the parathyroid gland in response to calcitonin administration appears to indicate an antagonistic role to parathormone in regulating the calcium metabolism of the snake, *A. granulatus*.

Administration of parathormone in the snake, *A. granulatus*, resulted in hypercalcemia, which reached its peak on the 2nd day. A similar hypercalcemic response was also noticed in lizards (McWhinnie and Cortelyou 1968) and frogs (Cortelyou and McWhinnie 1967, Swarup and Krishna 1979, Pandey and Swarup 1987) following parathormone administration. Parathormone administration also resulted in hypophosphatemia in frogs (Cortelyou and McWhinnie 1967) and toads (Pandey and Swarup 1987, Pandey 1988). However Clark et al. (1969) observed no alterations in plasma calcium and inorganic phosphate levels in the lizard, *Anolis carolinensis*, in response to parathyroid extract administration.

A rise in the serum calcium level following parathormone administration is due to the release of calcium ions from bones, which are a major site of its action. Parathyroid hormones also decrease the serum phosphorous levels. The decline in the serum calcium level observe on the 4th day following the administration of parathormone may be due to one of the following factors. 1. Feedback mechanisms in which the endogenous secretion of this hormone is decreased may result in less quantity of this hormone in the blood. This assumption gets support from the structural changes seen in the parenchymal cells of the parathyroid gland of parathormone-treated snakes. 2. It may be due to the secretion of calcitonin, a hypocalcemic factor from the ultimobranchial glands.

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鮭魚抑鈣素及牛副甲狀腺素對蛇(*Acrochordus granulatus*)血清鈣及無機磷濃度及副甲狀腺組織學之影響

Anita P. Warbhuwan[1] and Arun S. Padgaonkar[1]

注射抑鈣素(10 IU/100g體重)至蛇*Acrochordus granulatus*，導致其血清鈣及無機磷當天稍爲昇高。但在此後十四天試驗期間內與對照組相似，並無昇高。而鮭魚抑鈣素促使該蛇之副甲狀腺實質細胞肥大，細胞核亦增大。鮭魚抑鈣素處理後，未發現血鈣降低，應歸結於該動物副甲狀腺分泌足夠的副甲狀腺素因而對抗鮭魚抑鈣素之作用。

注射牛副甲狀腺素至*Acrochordus granulatus*引發高血鈣反應；注射後第一天開始昇高，第二天達高峰，第四天才降低。血清無機磷在注射後第二天的濃度，則稍降低。副甲狀腺主細胞在注射牛副甲狀腺素後，其細胞質廣泛出現空泡；而細胞核變小。本研究所得結論是副甲腺素在*Acrochordus granulatus*扮演調節鈣磷代謝，而抑鈣素可對抗副由狀腺素之作用。

關鍵詞：抑鈣素, 副甲狀腺素, 蛇。

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