

Short Note

Effects of Partial and Total Parathyroidectomy on Serum Calcium and Inorganic Phosphorus Levels in the Snake, *Acrochordus granulatus* (Schneider)

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(Accepted March 14, 1996)

Anita P. Warbhuwan and Arun S. Padgaonkar (1996) Effects of partial and total parathyroidectomy on serum calcium and inorganic phosphorus levels in the snake, *Acrochordus granulatus* (Schneider). *Zoological Studies* 35(4): 286-291. The removal of either 1 or both rostral parathyroids or 1, 2, or 4 caudal parathyroids in the snake, *Acrochordus granulatus*, had no effect on the levels of serum calcium and inorganic phosphorus. Similarly, removal of rostral and caudal parathyroids together in various combinations (maximum of 3) did not alter the serum calcium or inorganic phosphorus levels for up to 14 days. Removal of 5 parathyroid glands, however, resulted in the depletion of serum calcium and elevation of serum inorganic phosphorus levels on the 14th day. Removal of all 6 parathyroid glands caused significant hypocalcemia and hyperphosphatemia from the 7th day till the end of the experiment on the 28th day. Parathyroidectomy was not fatal to the snakes. Tetany was not observed at any stage of the experiment in any of the snakes operated upon.

The present study shows that the parathyroid glands can regulate calcium and phosphate metabolism in the snake, *A. granulatus*. It further shows that as little as 1 of the 6 parathyroid glands is sufficient for maintaining the normal levels of serum calcium and inorganic phosphorus.

Administration of bovine parathormone (bPTH) in the parathyroidectomized *A. granulatus* restored serum calcium to levels before the decrease caused by the total parathyroidectomy. This strongly suggests that the parathyroid gland of the snake, *A. granulatus*, secretes a parathormone-like factor.

Key words: Parathyroidectomy, Serum calcium, Serum phosphorus.

Reptiles comprise the most diverse group of vertebrates and they occupy a key phylogenetic position. Nevertheless, very little has been known till recently about the endocrine regulation of calcium and phosphate metabolism in this group of animals.

Parathyroidectomy has been reported to cause tetany and hypocalcemia in lizards (Peters 1941, Sidky 1966, Clark 1968, Clark et al. 1969, Dubewar et al. 1975, Oguro 1975), in snakes (Oguro 1970 1972, Clark 1971, Clark and Dantzler 1972) and in crocodiles (Oguro and Sasayama 1976). Clark (1965) however observed neither tetany nor hypocalcemia in parathyroidectomized freshwater turtles, while significant hypocalcemia was noticed in parathyroidectomized turtles and tortoises by Oguro and his coworkers (Oguro and Tomisawa 1972, Oguro et al. 1974). The variations in the results of parathyroidectomy in these groups of reptiles generated interest for the present study, which is a brief account of the effects of partial and total parathyroidectomy on serum levels of calcium and inorganic phosphorus in the estuarine snake, *Acrochordus granulatus*. Partial parathyroidectomy was per-

formed in order to know how many parathyroid glands are required for maintaining normal levels of serum calcium and inorganic phosphorus.

Materials and Methods—Snakes were acclimatized to laboratory conditions for 4 days prior to parathyroidectomy. Only male snakes were used; female snakes were not used as they may have high blood calcium levels, perhaps due to estrogen secretion during estrus. A total of 250 snakes was used for this experiment which was conducted in both winter and summer months. Snakes were randomly divided into experimental and control groups. Snakes in the experimental group were further divided into batches of 5 snakes each, according to the nature of the operation to be performed on them (Table 1). Animals were anesthetized with ether before the operation. The snakes were not fed during the entire course of the experiments. Both surgical and electrocautery procedures were used in removal or destruction of the glands.

Acrochordus granulatus has 3 pairs of parathyroids, a rostral pair situated near the hinges of the jaws and 2 caudal

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Table 1. Effects of partial parathyroidectomy on the levels of serum calcium in the snake, *Acrochordus granulatus*

Parathyroid ablated	Duration (day)	Sham-operated control (mg%) ^a	Experimental (mg%) ^a	p-value
Rostral 1	1	10.90 ± 0.28	11.52 ± 0.51	NS ^b
	4	11.45 ± 0.12	12.10 ± 0.43	NS
	7	11.90 ± 0.23	11.48 ± 0.53	NS
	14	11.32 ± 0.11	11.57 ± 0.26	NS
Rostral 2	1	10.65 ± 0.32	12.74 ± 0.81	NS
	4	11.20 ± 0.19	11.75 ± 0.22	NS
	7	11.19 ± 0.23	11.37 ± 0.13	NS
	14	11.41 ± 0.13	10.88 ± 0.59	NS
Caudal 1	1	11.44 ± 0.13	11.70 ± 0.62	NS
	4	11.07 ± 0.28	11.51 ± 0.57	NS
	7	11.14 ± 0.21	11.45 ± 0.02	NS
	14	11.14 ± 0.20	11.22 ± 0.31	NS
Caudal 2	1	10.90 ± 0.28	11.72 ± 0.68	NS
	4	11.07 ± 0.28	11.59 ± 0.57	NS
	7	11.14 ± 0.23	11.45 ± 0.02	NS
	14	11.14 ± 0.21	11.22 ± 0.31	NS
Rostral 1 + Caudal 1	1	11.08 ± 0.27	10.63 ± 0.51	NS
	4	11.24 ± 0.20	11.35 ± 0.33	NS
	7	11.22 ± 0.22	10.34 ± 0.42	NS
	14	11.35 ± 0.27	10.54 ± 0.58	NS
Rostral 1 + Caudal 2	1	11.07 ± 0.28	11.34 ± 0.26	NS
	4	11.07 ± 0.28	10.92 ± 0.17	NS
	7	11.03 ± 0.27	10.87 ± 0.35	NS
	14	11.32 ± 0.11	10.84 ± 0.15	NS
Rostral 2 + Caudal 1	1	11.12 ± 0.27	11.41 ± 0.35	NS
	4	11.28 ± 0.22	10.24 ± 0.26	NS
	7	11.07 ± 0.28	11.82 ± 0.33	NS
	14	11.31 ± 0.21	11.48 ± 0.36	NS
Caudal 4	1	11.07 ± 0.28	12.63 ± 0.81	NS
	4	11.28 ± 0.23	12.97 ± 0.48	NS
	7	11.22 ± 0.22	11.14 ± 0.33	NS
	14	10.60 ± 0.19	10.74 ± 0.32	NS
Caudal 4 + Rostral 1	1	10.58 ± 0.39	11.58 ± 0.19	NS
	4	10.52 ± 0.21	10.64 ± 0.70	NS
	7	10.58 ± 0.17	10.62 ± 0.18	NS
	14	11.31 ± 0.21	09.67 ± 0.31	≤ 0.001

^aValues are mean ± S.E., 5 snakes were used in each group.

^bNS = not significant.

pairs embedded in, or located near, the lobes of the thymus glands (Warbhuwan 1993). Various types of operations were performed as shown in Tables 1 and 2. The rostral and caudal pairs of parathyroids were removed through separate incisions. Two small incisions were made near the angle of the jaws and the 2 rostrals were destroyed by cauterization. Another incision was made just anterior to the heart and the caudal parathyroid glands were removed along with the lobes of the thymus as these tissues look almost identical. After extirpation of the glands, incisions were sprayed with "Helex" antiseptic spray and sutured with surgical thread. Totally parathyroidectomized snakes were removed from their tanks at 2-day intervals and 'given exercise' in order to test for the appearance of tetanic convulsions. Sham-operated controls were also maintained. Both sham-operated controls and parathyroidectomized snakes were sacrificed after 1, 4, 7, 14, 21, and 28 days depending upon the nature of the operations.

Blood samples were collected from the right systemic artery and received in centrifuge tubes. Blood was allowed to clot at room temperature, then sera were separated by centrifugation (3 500 rpm) and analyzed for serum calcium and in-

organic phosphorus contents by using Trinder's (1960) and Gomorri's (1942) methods, respectively. Student's *t*-test was used for statistical evaluation.

In another experiment bovine parathormone (bPTH) (Lot No. 74/557, National Hormone and Pituitary Agency, U.S.A.) was injected intraperitoneally in the totally parathyroidectomized snakes at a dose of (10 USP/0.1 ml)/100 gm body weight. The parathyroidectomized snakes which were kept as controls received the vehicle, namely, L-cysteine hydrochloride (0.1 ml/100 gm). The experimental and control animals in this experiment were parathyroidectomized 28 days prior to their respective injection and sacrificed 4 h after it.

Results—Results of the parathyroidectomy experiments were compiled and are presented in Tables 1, 2, and 3. The removal of either 1 or both rostrals, or 1, 2, or 4 caudal parathyroids in the snake, *Acrochordus granulatus*, had no effect on the levels of serum calcium or inorganic phosphorus. Similarly, extirpation of rostral and caudal parathyroids together in various combinations.

Tables 1 and 2 did not alter serum calcium or inorganic

phosphorus levels for up to 14 days. Removal of 5 parathyroids however resulted in the depletion of serum calcium and elevation of serum phosphorus levels on the 14th day. Total parathyroidectomy (removal of all 6 parathyroid glands) caused

significant hypocalcemia and hyperphosphatemia from the 7th day till the end of the experiment on the 28th day (Table 3). Parathyroidectomy (either partial or total) was not fatal to the snakes. Tetany was not observed at any stage of the experi-

Table 2. Effects of partial parathyroidectomy on the levels of serum inorganic phosphorus in the snake, *Acrochordus granulatus*

Parathyroid ablated	Duration (day)	Sham-operated control (mg%) ^a	Experimental (mg%) ^a	p-value
Rostral 1	1	4.59 ± 0.33	3.80 ± 0.24	NS ^b
	4	4.67 ± 0.58	4.57 ± 0.67	NS
	7	4.31 ± 0.32	4.37 ± 0.22	NS
	14	4.65 ± 0.23	4.30 ± 0.57	NS
Rostral 2	1	4.53 ± 0.21	4.69 ± 0.56	NS
	4	4.47 ± 0.70	4.77 ± 0.60	NS
	7	4.50 ± 0.28	4.39 ± 0.64	NS
	14	4.40 ± 0.32	4.69 ± 0.20	NS
Caudal 1	1	4.51 ± 0.29	4.16 ± 0.17	NS
	4	4.67 ± 0.58	4.82 ± 0.17	NS
	7	4.31 ± 0.32	4.20 ± 0.08	NS
	14	4.42 ± 0.36	4.61 ± 0.55	NS
Caudal 2	1	4.53 ± 0.21	4.46 ± 0.48	NS
	4	4.64 ± 0.36	4.21 ± 0.34	NS
	7	4.51 ± 0.49	5.09 ± 0.67	NS
	14	4.42 ± 0.36	5.05 ± 0.66	NS
Rostral 1 + Caudal 1	1	4.67 ± 0.38	4.10 ± 0.59	NS
	4	4.27 ± 0.32	4.27 ± 0.42	NS
	7	4.50 ± 0.28	3.87 ± 0.38	NS
	14	4.48 ± 0.21	4.50 ± 0.36	NS
Rostral 1 + Caudal 2	1	4.41 ± 0.44	4.50 ± 0.45	NS
	4	4.64 ± 0.35	3.98 ± 0.31	NS
	7	4.64 ± 0.36	4.99 ± 0.76	NS
	14	4.32 ± 0.30	4.83 ± 0.16	NS
Rostral 2 + Caudal 1	1	4.53 ± 0.21	4.18 ± 0.23	NS
	4	4.47 ± 0.70	4.89 ± 0.49	NS
	7	4.46 ± 0.36	4.61 ± 0.21	NS
	14	4.32 ± 0.30	4.83 ± 0.16	NS
Caudal 4	1	4.55 ± 0.50	4.81 ± 0.69	NS
	4	4.42 ± 0.16	4.72 ± 0.42	NS
	7	4.51 ± 0.49	4.08 ± 0.29	NS
	14	4.43 ± 0.32	4.19 ± 0.32	NS
Rostral 1 + Caudal 4	1	4.47 ± 0.29	4.42 ± 0.06	NS
	4	4.36 ± 0.48	4.49 ± 0.65	NS
	7	4.51 ± 0.49	4.70 ± 0.19	NS
	14	4.34 ± 0.24	6.02 ± 0.36	≤ 0.001

^aValues are mean ± S.E., 5 snakes were used in each group.

^bNS = not significant.

Table 3. Effects of total parathyroidectomy on the levels of serum calcium and inorganic phosphorus in the snake, *Acrochordus granulatus*

Days after operation	Serum calcium (mg%) ^a			Serum inorganic phosphorus (mg%) ^a		
	Sham-operated control	Experimental	p-value	Sham-operated control	Experimental	p-value
1	11.12 ± 0.27	11.13 ± 1.02	NS ^b	4.44 ± 0.32	4.16 ± 0.33	NS ^b
4	10.74 ± 0.15	10.62 ± 0.09	NS	4.58 ± 0.38	4.75 ± 0.42	NS
7	11.54 ± 0.31	9.83 ± 0.29	≤ 0.01	4.24 ± 0.31	6.17 ± 0.22	≤ 0.001
14	11.16 ± 0.35	8.62 ± 0.14	≤ 0.001	4.31 ± 0.34	6.22 ± 0.45	≤ 0.01
21	11.43 ± 0.46	7.97 ± 0.27	≤ 0.001	4.27 ± 0.32	7.57 ± 0.84	≤ 0.001
28	11.82 ± 0.44	7.29 ± 0.27	≤ 0.001	4.52 ± 0.32	8.01 ± 1.10	≤ 0.01

^aValues are mean ± S.E., 5 snakes were used in each group.

^bNS = not significant.

ment in any of the snakes operated upon.

A single injection of (10 USP/0.1 ml)/100 gm body weight of parathormone in the parathyroidectomized snakes which were sacrificed 4 h later resulted in significant hypercalcemia (Table 4). The level of serum inorganic phosphorus, however, showed no change.

Discussion—Total parathyroidectomy in *A. granulatus* resulted in significant hypocalcemia and hyperphosphatemia. These results are in agreement with those observed in other ophidian species (Oguro 1970 1972, Clark 1971, Singh and Kar 1983b). Clark and Dantzler (1972) have also reported similar changes following parathyroidectomy in 4 species of the genus *Natrix* (*N. sipedon*, *N. rhombifera*, *N. cyclopion*, and *N. erythrogaster*).

Hypocalcemia following parathyroidectomy has been reported in several reptiles. Oguro and Tomisawa (1972) reported a significant decrease in the plasma calcium level in the turtle, *Geoclemys reevesii*, 28 days after parathyroidectomy. In *Testudo graeca* the plasma calcium concentration declined to 60% of the control value following parathyroidectomy, but tetany was not observed (Oguro et al. 1974). In the lizard, *Varanus griesus*, a 52% decrease in the plasma calcium concentration was noticed 12 to 28 days after parathyroidectomy (Sidky 1966). In the lizard, *Uromastix hardwickii*, the plasma calcium concentration fell to about 60% of the concentration in the sham-operated controls between 7 to 10 days following parathyroidectomy (Dubewar et al. 1975). In *Anolis carolinensis* and *Gecko japonicus* the decreases in serum calcium levels were about 44% and 37%, respectively, of those in sham-operated controls (Clark et al. 1969, Oguro 1975). The serum calcium level declined to about 38% of the control value in the lizard, *Calotes versicolor*, 10 days following parathyroidectomy (Pathan and Nadkarni 1987). Serum calcium levels of the snake in the present study, had declined to about 38.3% of those in the sham-operated controls, 28 days after parathyroidectomy. These observations are similar to those made in turtles and lizards mentioned above, but differ from those in the turtles *Pseudemys scripta* and *Chrysemys picta* in which parathyroidectomy did not cause any significant changes in serum calcium concentrations (Clark 1965).

In the present investigation total parathyroidectomy did not produce tetanic convulsions, though their appearance is one of the most characteristic symptoms of parathyroidectomy. In the turtles *P. scripta* and *C. picta* neither tetany nor hypocalcemia were evident following parathyroidectomy (Clark 1965). Oguro and co-workers (Oguro and Tomisawa 1972, Oguro et al. 1974) observed a 60% decline in serum calcium levels in 2 species of turtles, *Testudo graeca* and *Geoclemys reevesii*, but no tetanic convulsions were noticed. Tetanic convulsions following parathyroidectomy were noticed in many species of lizards (Sidky 1966, Clark 1968, Dubewar et al. 1975, Pathan and Nadkarni 1987) and a few species of snakes

(Oguro 1970 1972, Clark 1971, Clark and Dantzler 1972, Singh and Kar 1979a,b). In the snake, *Elaphe quadrivigata*, tetany occurred after 7 days in 5% of the snakes and in 10% after 20 days. In *Rhabdophis tigrinus tigrinus* tetany occurred in 8% of the animals operated upon after 28 days (Oguro 1972). In *Natrix piscator* tetany occurred in only 1 of 5 animals 7 days following parathyroidectomy (Singh and Kar 1979a). Clark (1971) has reported tetany in the majority (70%) of *Thamnophis sirtalis* 10 days after parathyroidectomy. It appears therefore, that the period prior to the onset of tetany varies in different species depending upon their susceptibility. The delay in manifestation of tetany in the snake under investigation here may also be attributed to its relatively sluggish metabolism, though this was not actually measured.

Total parathyroidectomy in the snake, *A. granulatus*, resulted in hyperphosphatemia which was progressive and reached its maximum on the 28th day (the final day of the experiment). These results coincide with those reported for the lizards, *Anolis carolinensis* (Clark 1968), and *Calotes versicolor* (Pathan and Nadkarni 1987); and the snake, *Thamnophis sirtalis* (Clark 1971), 4 species of *Natrix* (Clark and Dantzler 1972) and *Natrix piscator* (Singh and Kar 1979b); but differ from those reported for the turtles, *Chrysemys picta* and *Pseudemys scripta* (Clark 1965), and *Testudo graeca* (Oguro et al. 1974) in which no changes in the levels of serum phosphorus occurred following parathyroidectomy.

Our results indicate that the parathyroid glands of this species are involved in regulating calcium and phosphate metabolism. The role of the parathyroids and calcium levels in normal neuromuscular co-ordination could not be ascertained due to the absence of tetanic convulsions following parathyroidectomy.

In the present study it was also seen that the removal of 5 out of 6 parathyroid glands caused significant hypocalcemia after a week. Singh and Kar (1979a) also reported hypocalcemia after 1 week following the removal of 3 out of 4 parathyroid glands in the snake, *N. piscator*, while Clark (1971), on the other hand, noted no significant change after removal of 3 parathyroids even after 6 weeks in her animals. Our findings show that at least 1 of the 6 parathyroid glands is required for maintaining the normal levels of serum calcium and inorganic phosphorus in the snake *A. granulatus*.

In the present study, levels of serum calcium dropped following parathyroidectomy, but were restored by the administration of bovine parathormone (bPTH). Similar observations also have been made in parathyroidectomized birds after the administration of parathyroid extract (PTE) (Anderson and Consuegrs 1970, Mueller et al. 1973, Kenny and Dacke 1974). The reversal of symptoms of parathyroidectomy after the administration of bovine parathormone in the snake under study strongly suggests that the parathyroid glands of this snake may secrete a parathormone-like factor.

Table 4. Effects of bovine parathormone (bPTH) on the levels of serum calcium and inorganic phosphorus in the parathyroidectomized snake, *Acrochordus granulatus*

Group	Serum calcium (mg%) ^a	Serum inorganic phosphorus (mg%) ^a
Control (parathyroidectomized)	7.12 ± 0.23	7.82 ± 1.16
Experimental (parathyroidectomized + bPTH)	13.24 ± 0.61	8.12 ± 0.84
<i>p</i> -value	≤ 0.001	NS ^b

^aValues are mean ± S.E., 5 snakes were used in each group.

^bNS = not significant.

The hyperphosphatemia in the parathyroidectomized snakes, however, was not restored to normal levels following injection of bovine parathormone. This may be because full restoration requires a longer duration of time or a higher dose of bovine parathormone.

Acknowledgements—We are thankful to the National Hormone and Pituitary Agency, U.S.A. for the kind gift of bovine parathormone, and grateful to the University Grants Commission, India for financial assistance to one of the authors (A.S.P.).

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副甲狀腺完全及部份切除對印度蛇(*Acrochordus granulatus*) 血清中鈣及無機磷含量之影響

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切除 *Acrochordus granulatus* 印度蛇一個或二個嘴側副甲狀腺，或一、二、四個尾側副甲狀腺，對其血清中鈣及無機磷含量沒有影響。類此，一併切除其嘴側及尾側副甲狀腺（最多三個），亦不影響其血清鈣及無機磷含量，一直持續至第 14 天。然而，一次切除五個副甲狀腺，卻降低血鈣，但提升無機磷。一次切除六個副甲狀腺，導致於低血鈣症及高血磷症，在切除後第 7 天開始，持續至 28 天。印度蛇在切除副甲狀腺，並不致死；在實驗期間內，亦不引發痙攣症狀，本研究提示印度蛇副甲狀腺對鈣磷有調節功能；亦提示，在六個副甲狀腺之中的一個腺體，即足夠調節鈣磷之代謝。

注射牛副甲狀腺素至切除副甲狀腺的印度蛇之後，可使血鈣恢復至完全切除副甲狀腺之前的含量。提示，該印度蛇之副甲狀腺可分泌類似副甲狀腺素因子。

關鍵詞：副甲狀腺切除，血鈣，血磷。

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