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Effects of Calcium Chloride and Sodium Fluoride on Serum Levels of Calcium and Inorganic Phosphorous and Histology of Parathyroid Gland in *Acrochordus granulatus* (Schneider)

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Anita Warbhuwan and Arun Padgaonkar (1993) Effects of calcium chloride and sodium fluoride on serum levels of calcium and inorganic phosphorous and histology of parathyroid gland in *Acrochordus granulatus* (Schneider). *Bull. Inst. Zool., Academia Sinica* **32**(4): 236-241. We studied the effects of calcium chloride (hypercalcemic agent) and sodium fluoride (hypocalcemic agent) on the histology of the parathyroid gland and on serum levels of calcium and inorganic phosphorous in the snake, *Acrochordus granulatus*. Calcium chloride treatment (10 mg/100 gm body wt) resulted in hypercalcemia after 4 hrs as well as on the 4th and 7th day and reached a maximum on the 14th day of treatment. Serum inorganic phosphorous levels did not show any significant change after 4 hrs and on the 4th day, but decreased significantly on the 7th and 14th day of treatment.

Animals sacrificed 4 hrs following a single injection (20 mg/100 gm body wt) of sodium fluoride showed no effects on serum calcium levels. However, snakes which were given the same dose daily for 4 days (and sacrificed 4 hrs after the last injection) showed significant hypocalcemia. A serum inorganic phosphorous levels also showed a significant increase ($p < 0.05$) 4 hrs after and on the 4th day of treatment.

The administration of a hypercalcemic agent (calcium chloride) resulted in degenerative changes in the parathyroid gland; treatment with a hypocalcemic agent (sodium fluoride) resulted in hypertrophy of the parenchymal cells of the parathyroid. We suggest that parathyroid secretion is regulated by blood calcium levels.

Key words: Hyper and Hypocalcemic substances, Parathyroid gland, *Acrochordus granulatus*.

There have been few studies made on the control of parathyroid secretion in poikilothermous vertebrates. Since parathyroid hormone is a predominant hypercalcemic agent in reptiles (Clark 1972, Clark and Laverty 1985), any alteration in serum calcium levels should affect parathyroid secretion. Up to now, parathyroid gland activity in snakes in response to the exogenous administration of hypo and hypercalcemic substances has not been studied. We therefore attempted to record histological changes in the parathyroid gland following alterations in serum calcium and inorganic phosphorous levels induced by the administration of hyper and hypocalcemic substances in the estuarine snake, *Acrochordus granulatus*.

Tomiyama I. 1936. Gobiidae of Japan. Jap. J. Zool. **7(1):** 37-112.
Winterbottom R. 1984. A review of the gobiid fish genus *Trimma* from the Chagos Archipelago, central Indian Ocean, with descriptions of seven new species. Can. J. Zool. **62:** 675-715.

台灣產鰕虎科七種新記錄魚類

邵廣昭　陳義雄

本文記述採集於本省南部及小琉球一帶珊瑚礁區的七種鰕虎科之新記錄種。它們分別爲沖繩硬皮鰕虎*Callogobius okinawae* (Snyder, 1908)；長棘紡錘鰕虎*Fusigobius longispinus* Goren, 1978；肩斑頜鱗鰕虎*Gnatholepis scapulostigma* Herre, 1953；典範副短鰕虎*Paragobiodon modestus* (Regan, 1908)；黃身副短鰕虎*Paragobiodon xanthosomus* (Bleeker, 1852)；莫三比克腹瓢鰕虎*Pleurosicya mossambica* Smith, 1959；圓斑磨鰕虎*Trimma macrophthalma* (Tomiyama, 1936)。其中副短鰕虎屬*Paragobiodon*係本省之新記錄屬。文中除描述各種之形態特徵、地理分佈或附記外，並附以各魚種之彩色照片。

MATERIALS AND METHODS

Sixty snakes were kept in seawater tanks for 48 hrs prior to experiments; snakes were not fed during this period. Animals were separated into control and experimental groups (five animals in each group) for different experiments.

For short-term experiments, single injections of calcium chloride were given intraperitoneally to single groups of five animals at a dose of 10 mg/100 gm body weight. The five animals in the control group received equivalent doses of saline. Both control and experimental animals were sacrificed 4 hrs after injection. For long-term experiments, calcium chloride was injected intraperitoneally every day to three groups of five animals each for 4, 7, and 14 days, respectively. These animals were sacrificed 4 hrs following the final injection.

In a third set of experiments, sodium fluoride was administered intraperitoneally at a dose of 20 mg/100 gm body weight to a single group of five animals; five animals in the control group received equivalent doses of saline. Both control and experimental animals were sacrificed 4 hrs following injection. A second group of five animals received sodium fluoride intraperitoneally at a dose of 20 mg/100 gm body weight every day for 4 days; five control animals received equivalent doses of saline. Both control and experimental animals were sacrificed 4 hrs following the final injection. Experiments were not extended beyond four days, as animals died following prolonged treatment with sodium fluoride.

In all experiments, injections were given and blood samples collected at the same time of each day to avoid the circadian rhythm effects. In all experiments, blood samples were taken from the right systemic artery. After clotting, the blood serum was separated by centrifugation and analysed for serum calcium and serum inorganic phosphorous content according to methods described by Trinder (1960) and Gomorri (1942), respectively.

For histological observations, parathyroid glands from both control and experimental animals were fixed in Bouin's fluid. Paraffin sections of parathyroid tissue cut at 5 μ were stained using a hematoxylin-phloxin method. Results were evaluated for statistical significance using student's *t*-test.

RESULTS

Biochemical changes

It is clear that a single injection of calcium chloride caused significant hypercalcemia (Table 1). Similar results were also obtained on the 4th, 7th, and 14th day of calcium chloride treatment. The maximum increase in serum calcium was observed on the 14th day.

Levels of serum inorganic phosphorous in calcium chloride-treated snakes did not show any significant change after 4 hrs or on the 4th day; however, they decreased significantly on the 7th and 14th day following treatment (Table 1).

A single injection of sodium fluoride had no effect on the level of serum calcium. However, daily injections of sodium fluoride for four days resulted in significant hypocalcemia. Serum inorganic phosphorous levels in sodium fluoride-treated snakes showed significant increases ($p < 0.05$) after 4 hrs and on the 4th day of treatment (Table 1).

Histological changes

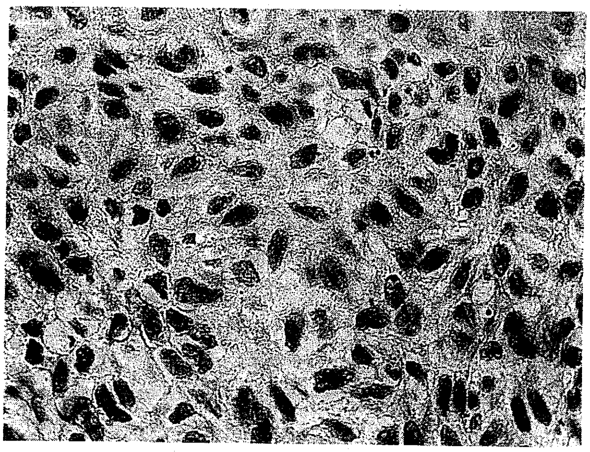
Snakes which were treated with calcium chloride for fourteen days showed degenerative changes in the parathyroid gland. The parenchymal cells and their nuclei became shrunken (Fig. 1b), and the cord-like arrange-

Table 1. Effects of calcium chloride and sodium fluoride on serum calcium and inorganic phosphorous levels in the snake, *Acrochordus granulatus*

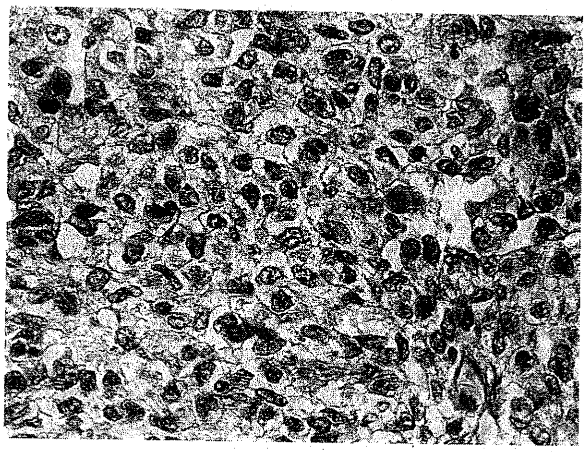
Treatment	Duration	Serum Calcium		Serum Inorganic Phosphorous	
		Control	Experimental	Control	Experimental
	4 hrs	11.78 ± 0.66	18.14 ± 0.36***	3.83 ± 0.6	4.5 ± 0.08
	4 days	11.06 ± 0.29	18.37 ± 0.43***	3.28 ± 0.16	3.83 ± 0.19
Calcium chloride	7 days	12.5 ± 0.62	18.63 ± 0.13***	5.15 ± 0.13	3.25 ± 0.19***
(10 mg/100 body weight)	14 days	12.65 ± 0.33	23.34 ± 0.59***	4.39 ± 0.23	3.36 ± 0.39*
Sodium fluoride	4 hrs	11.36 ± 0.56	10.88 ± 0.56	3.87 ± 0.43	6.11 ± 0.71*
(20 mg/ 100 body weight)	4 days	10.69 ± 0.60	5.95 ± 0.17***	4.70 ± 0.40	6.41 ± 0.62*

mean ± S.E.M.: five snakes were used in each experiment.
*** : $p < 0.001$
* : $p < 0.05$

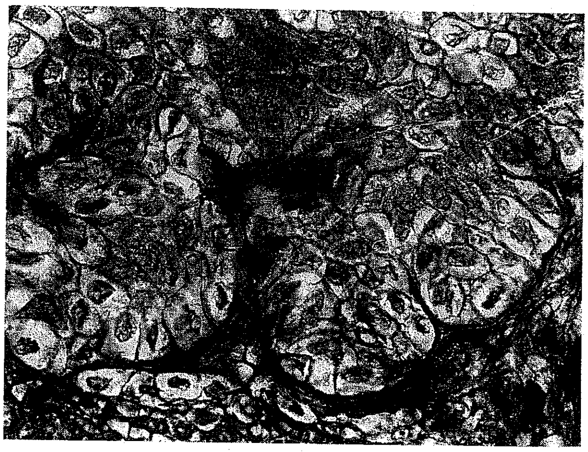
ment of cells was disturbed. Degenerative changes were less marked in the parathyroid glands of animals sacrificed on the 4th and 7th day of treatment. Prolonged treatment with sodium fluoride resulted in hypertrophy



(1a)



(1b)



(1c)

Fig. 1. Histological changes in the parathyroid glands of the snake, *Acrochordus granulatus*, following calcium chloride or sodium fluoride treatment. (a) Control; (b) fourteen days of calcium chloride treatment; (c) four days of sodium fluoride treatment. Note the degenerative change in (b) and hypertrophy in (c).

of the parenchymal cells of the parathyroid gland (Fig. 1c). These histological changes suggested parathyroid gland hyperactivity.

DISCUSSION

The administration of both single and multiple doses of calcium chloride in *A. granulatus* resulted in significant hypercalcemia which peaked on the fourteenth day. The observed degenerative changes in the parathyroid gland were progressive and due to its inactivity under conditions of chronic hypercalcemia. These observations are in agreement with previous observations made in various groups of vertebrates in which regressive changes were observed in the parathyroid gland following hypercalcemic stress (Stoeckel and Porte 1966 1973, Nakagami 1967, Capen and Rowland 1968, Kameda 1970, Rowland et al. 1971, Swarup and Das 1974 1978, Swarup and Srivastav 1979, Dubewar 1979, Swarup et al. 1987).

The administration of sodium fluoride in *A. granulatus* resulted in parathyroid gland hyperactivity. Similar results were previously obtained following injections of a different hypocalcemic substance (EDTA) in this snake (Warbhuwan and Padgaonkar 1991). Other researchers have demonstrated hyperplasia of the parathyroid gland in animals which were fed low calcium diets (Marine 1913, Ham et al. 1940). Our observations for snakes are also in agreement with those of Isona et al. (1976) for frogs and Dubewar (1979) for lizards. Although no direct measurements of blood parathyroid hormone levels in snakes are presently available, the hyperactivity of the parathyroid gland following the administration of a hypocalcemic substance and the hypoactivity of this gland following the administration of a hypercalcemic substance both suggest that parathyroid secretion is dependent on blood calcium levels.

A few researchers have emphasized the importance of the level of serum inorganic phosphorous in determining parathyroid hormone secretion (Tomblom 1949, Crawford et al. 1950). In our experiment a significant depletion in serum inorganic phosphorous levels was observed only on the 7th and 14th day of calcium chloride treatment. Necrotic changes in the parenchymal cells of the parathyroid gland were evident well in advance of the observed serum inorganic phosphorous depletion.

These results lead us to suggest that histological changes in the parathyroid gland following the administration of calcium chloride are due to high calcium levels rather than low inorganic phosphorous levels.

Furthermore, serum inorganic phosphorous levels showed significant increases following sodium fluoride treatment. Hypertrophy was observed in the parathyroid glands of these animals, suggesting over activity of these glands. In another experiment, the administration of EDTA (a hypocalcemic agent) did not have any effect on serum inorganic phosphorous levels, although the parathyroid glands of EDTA-treated snakes showed signs of hyperplasia (Warbhuwan and Padgaonkar 1991). Therefore, we believe that parathyroid gland activity is not related to serum inorganic phosphorous levels. Following the same line of argument, we conclude that the parathyroid gland hypertrophy observed following sodium fluoride treatment is due to low levels of serum calcium.

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REFERENCES

Capen CC, GN Rowland. 1968. The Ultrastructure of the parathyroid gland of young cats. Anat. Res.

162: 327-339.

Clark NB. 1972. Calcium regulation in reptiles. Gen. Comp. Endocrinol. **3:** 430-440.

Clark NB, G Laverty. 1985. Role of parathyroid hormone in regulation of calcium in reptiles. *In* Current Trends in Comparative Endocrinology, eds. B Lofts, WH Holmes. Hong Kong: University Press, pp. 843-845.

Crawford JD, MM Osborne, NB Talbot, ML Terry, MF Morrill. 1950. The parathyroid glands and phosphorous homeostasis. J. Clin. Invest. **29:** 1448-1461.

Dubewar DM. 1979. Effect of hyper and hypocalcemic substances on the parathyroid histology of the lizard, *Uromastix hardwickii*. Z. Mikros Anat. Forsch. **93:** 315-320.

Gomorri G. 1942. A modification of the colorimetric phosphorous determination for use with the photoelectric colorimeter. J. Lab. Clin. Med. **27:** 955-959.

Ham AW, M Littner, TGH Drake, EC Robertson, FF Tisdall. 1940. Physiological hypertrophy of the parathyroids: Its cause and its relation to rickets. Amer. J. Pathol. **16:** 277-286.

Isona H, S Shoumura, S Takai, T Yamahira, S Yamade. 1976. Electron microscopic observation on the parathyroid gland of the EDTA treated frog, *Rana rugosa*. Okaimas Fol. Anat. Jap. **53:** 127-149.

Kameda Y. 1970. Increased mitotic activity of the parafollicular cells of the dog thyroid in experimentally induced hypercalcemia. Arch. Histol. Jap. **32:** 179-192.

Marine D. 1913. Parathyroid hypertrophy and hyperplasia in fowls. Proc. Soc. Exptl. Biol. Med. **11:** 117-118.

Nakagami K. 1967. Comparative electron microscopic studies of the parathyroid gland II. Fine structure of the parathyroid gland of the normal and clcium chloride treated mouse. Arch. Histol. Jap. **28:** 185-205.

Rowland GN, CC Capen, HE Black, DM Young. 1971. Microradiographic evaluation of bone and ultrastructure of C-cells and parathyroid extract. Beitr. Pathol. **144:** 360-376.

Stoeckel ME, A Porte. 1966. Observations ultrastructurales sur la parathyroide de souris. II. Etude experimentale. Z. Zellforsch. **73:** 503-520.

Stoeckel ME, A Porte. 1973. Observations ultrastructurales sur la parathyroide de mammifere et d'oiseau dans les conditions normales et experimentales. Arch. D'Anat Microsc. **62:** 55-88.

Swarup K, VK Das. 1974. Effect of experimental hypercalcemia on the thyroid calcitonin cells of the Indian grey mangoose, *Herpestes edwardsi* (Geoffroy). Arch. Histol. Jap. **37:** 121-131.

Swarup K, VK Das. 1978. Calcitonin cells and unusual follicles in four-month-old wister rats. Z. Mikrosk Anat. Forsch. **92:** 212-221.

Swarup K, AK Srivastav. 1979. Studies of Calcitonin cells and parathyroid gland of house shrew, *Suncus murinus*, in response to experimental hypercalcemia. Z. Mirosk Anat. Forsch. **93:** 343-352.

Swarup K, AK Pandey, AK Srivastav. 1987. Calcemic responses in the yellow monitor, *Varanus flavescens*, to vitamin D_3 administration. Acta. Physical. Hung. **70:** 375-377.

Tomblom. 1949. On the functional relationship between the pituitary gland and the parathyroid. Acta. Endocrinol. suppl 4.

Trinder P. 1960. Colorimetric microdetermination of calcium in serum. Analyst **85:** 889-894.

Warbhuwan AP, AS Padgaonkar. 1991. Effects of hypocalcemic substance (EDTA) in the snake, *Acrochordus granulatus* (Schneider). Geobios **18:** 260-261.

氯化鈣與氟化鈉對蛇*Acrochordus granulatus* (Schneider) 血清中鈣離子與無機磷濃度及副甲狀腺組織學之影響

Anita Warbhuwan and Arun Padgaonkar

本研究在探討氯化鈣（升血鈣劑）與氟化鈉（降血鈣劑）對蛇*(Acrochordus granulatus)*之副甲狀腺組織學及血清中鈣離子與無機磷濃度之影響。氯化鈣處理(10 mg/100 gm體重)後，4小時、第 4 天、第 7 天血中鈣離子濃度升高，並在第14天達到最高峰；而血中無機磷濃度在 4 小時後及第 4 天並無顯著變化，但在第 7 天及第14天後則顯著下降。

單一劑量（20 mg/gm體重）注射氟化鈉，4小時後血中鈣離子濃度不受影響；但是同樣劑量連續注射 4 天，最後一次注射後 4 小時，則血中鈣離子濃度下降，而血中無機磷濃度 4 小時及 4 天後則顯著上升($p < 0.05$)。

注射升血鈣劑（氯化鈣）引起副甲狀腺之退化性變化，而注射降血鈣劑（氟化鈉）則引起副甲狀腺實質細胞肥大。本研究結果顯示蛇類副甲狀腺之分泌受血鈣調控。

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Seasonal Variation of the Activity and Range Use Patterns of a Wild Troop of Formosan Macaque in Kenting, Taiwan

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Hai-Yin Wu and Yao-Sung Lin (1993) Seasonal variation of the activity and range use patterns of a wild troop of Formosan macaque in Kenting, Taiwan. *Bull. Inst. Zool., Academia Sinica* **32**(4): 242-252. The activity and range use patterns of a wild troop of Formosan macaque in Kenting were studied from October, 1987 to August, 1990. Whenever possible, troop activity and location were recorded every 15 minutes. Troop activity was divided into five categories (active, inactive, traveling, feeding, and feeding while traveling) and the location of the troop was recorded on a grid map consisting of 0.25 ha quadrates. Inactive and feeding were the two most important troop activities, occupying 40% and 23% of the observation time, respectively. Significant differences in seasonal time budgets were found, and the diurnal patterns of troop activity are described in this paper. The home range of the study troop was 14 ha by quadrate method, or 19.5 ha by the minimum convex polygon method; range areas varied seasonally. The range use pattern of the troop was clumped; they spent 95% of their time in 12 quadrates. We found seasonal differences in the allocation of time by the troop in the core area quadrates; these differences may be related to both the characteristics of each quadrate and weather conditions.

Key words: Activity pattern, Range use pattern, *Macaca cyclopis*

Activity and range use patterns—the ways animals use time and space—reflect the adaptation of animals to their environments. Interspecific comparisons reveal that primate activity and range use patterns are related to body size, food habits, and troop size. Home range size and the amount of time spent feeding and traveling decrease with the amount of foliage in the diet (Clutton-Brock and Harvey 1977, Milton and May 1976). Environmental variables such as food distribution and abundance, weather patterns, habitat structure, and intraspecific interactions may also influence the activity and range use patterns of primates (Bercovitch 1983, Clutton-Brock 1974, Gittins 1982, Harding 1976, Harrison 1983, Isbell 1983, Iwamoto and Dunbar 1983, Post 1981, Rasmussen 1985). Studies have shown that primates have various strategies for adjusting their ranging and foraging patterns to accommodate changing food supplies or weather conditions. During a lean season

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