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Short Note

Effects of Vitamin D_3 Administration on the Levels of Serum Calcium and Inorganic Phosphorus in the Smooth Water Snake, *Enhydris enhydris* (Schneider)

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Mrinalini V. Kagwade and Arun S. Padgaonkar (1994) Effects of Vitamin D_3 administration on the levels of serum calcium and inorganic phosphorus in the smooth water snake, *Enhydris enhydris* (Schneider). *Zoological Studies* **33**(4): 319-321. The effect of Vitamin D_3 administration (12,000 IU/100 g body wt.) on levels of serum calcium and inorganic phosphorus levels in the smooth water snake, *Enhydris enhydris* was investigated. Hypercalcemia and hyperphosphatemia was observed daily for 14 days. Maximum values were recorded on the fourth day, a steady decline followed thereafter.

Key words: Hypercalcemia, Snake.

Vitamin D metabolites play an important role in the control of calcium metabolism in vertebrates. In birds and mammals Vitamin D_3 is formed in the skin through a reaction involving ultraviolet light. It is then hydroxylated in the liver and kidneys to form the active metabolite, 1,25 dihydroxycholecalciferol (1,25 DHCC). Low plasma calcium or phosphorus stimulates the production of 1,25 DHCC. The rate of calcium absorption from the gut is increased by 1,25 DHCC; it also facilitates the osteolytic action of parathormone (PTH) on bone.

The role of Vitamin D in calcium regulation in the lower vertebrates appears to be the same as in mammals. Vitamin D_3 administration in *Clarias batrachus* and *Rita rita* displayed hypercalcemia and hyperphosphatemia (Swarup and Srivastav 1982, Swarup et al. 1984, Das et al. 1990). Hypercalcemia in response to Vitamin D_3 is also observed in *Rana pipiens* (Robertson 1975a, b) and *Bufo marinus* (Bentley 1983). The metabolism of Vitamin D has not been well documented in reptiles (Dacke 1979, Taylor 1985), especially snakes. Recently, however Srivastav and Rani (1988) and Warbhuwan and Padgaonkar (1993) recorded hypercalcemia and hyperphosphatemia in response to Vitamin D_3 in the fresh water snake *Natrix piscator* and the estuarine snake *Acrochordus granulatus* respectively. Therefore in the present study an attempt has been made to record the changes in serum calcium and inorganic phosphorus levels in *Enhydris enhydris* after administration of Vitamin D_3.

Materials and Methods—Adult specimens of both sexes of *E. enhydris* were collected from the seacoast near Bombay. They were acclimatized to laboratory conditions for a week prior to the experiment. The snakes each weighed about 65-80 g and were divided into control and experimental groups. The experimental group was injected intramuscularly with Vitamin D_3 ('Arachitol' Dupar-Interfran Ltd. Bombay. Batch No. B2313) with a dose of 12,000 IU/100g body weight. The control specimens were intramuscularly injected with the vehicle (arachis oil) with a dose of 0.01 ml/100g body weight. Animals from both groups were sacrificed 4 hours after the last injection on the 1st, 4th, 7th and 14th day. Blood samples were collected from the aortic arch and the sera were separated and analysed for serum calcium and inorganic phosphorus according to the methods described by Trinder (1960) and Gomori (1942) respectively. The animals were not fed during the entire course of the experiment. The injections were given at approximately the same time each day throughout the course of the experiment to avoid upsetting circadian rhythm. Differences in the values of serum calcium and inorganic phosphorus were tested for statistical significance using the Students '*t*' test for group comparison.

Results—The administration of Vitamin D_3 in *E. enhydris* resulted in hypercalcemia (Table 1). The serum calcium level increased steadily from the first day and reached its maximum on the 4th day. Thereafter, a gradual decline in the serum calcium level was noticed; but, the values still remained above the normal level on the 14th day.

Vitamin D_3 injections also resulted in a significant hyperphosphatemia (Table 1). The serum inorganic phosphorus level increased steadily from the first day and reached a maximum on the 4th day. The fall in the serum inorganic phosphorus values after the 4th day followed the same trend as that of the serum calcium values.

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Table 1. Effects of Vitamin D_3 on serum calcium and inorganic phosphorus levels (mg/100 ml) of *Enhydris enhydris*

Days	Serum Calcium mg/100 ml		Serum Inorganic Phosphorus mg/100 ml	
	Control	Exptal	Control	Exptal
1	11.19 ± 0.80	12.71 ± 0.30**	4.14 ± 0.50	4.48 ± 0.56
4	11.53 ± 0.39	14.43 ± 0.36***	4.54 ± 0.15	5.38 ± 0.13***
7	11.19 ± 0.08	13.92 ± 0.24***	4.22 ± 0.33	4.65 ± 0.10
14	11.21 ± 0.26	13.12 ± 0.44**	4.06 ± 0.33	4.14 ± 0.26

The values are mean ± SD of six determinations.
** and *** indicate significant responses: $p \leq 0.01$ and $p \leq 0.001$ respectively.

Discussion—These results clearly show that Vitamin D_3 is effective in inducing hypercalcemia and hyperphosphatemia in the smooth water snake, *E. enhydris* and agree with similar results in other vertebrate groups (Robertson 1975a,b, Swarup and Srivastav 1982, Bentley 1983, Swarup et al. 1984, Das et al. 1990). Hypercalcemia following Vitamin D_3 injections probably is not due to dietary intake since the animals were not fed during the entire course of the experiment. This hypercalcemic response however, be attributed to the mobilization of calcium from its stores (Bentley 1984).

The exogenous Vitamin D_3 injected into the animals acts in concert with parathormone resulting in bone osteolysis and hence results in hypercalcemia. The decrease in hypercalcemia after the 4th day may be due to a feedback mechanism whereby increasing levels of calcium inhibit the synthesis of PTH which in turn affect 1,25 DHCC synthesis. PTH is necessary for the stimulation of renal 1 – hydroxylase, an enzyme necessary for the formation of the active metabolite of Vitamin D, i.e., 1,25 DHCC (Henry and Norman 1975).

The decline in the serum calcium levels on the 14th day may be due to the secretion of a hypocalcemic factor. Calcitonin, a hypocalcemic hormone in the mammals, is present in the ultimobranchial glands of lower vertebrates (Clark 1971, Sasayama et al. 1984).

Hyperphosphatemia observed in response to administration of Vitamin D_3 in *E. enhydris* may be due to the mobilization of phosphorus from the bones or soft tissue.

These results are in agreement with those for the fresh water snake, *Natrix piscator* (Srivastav and Rani 1988). However, they differ from those for the estuarine snake, *Acrochordus granulatus* (Warbhuwan and Padgaonkar 1993). The hypercalcemic response of *A. granulatus* to Vitamin D_3 continued up to the 7th day, followed by a decline on the 14th day. Hyperphosphatemia, was noted only on the 14th day of the treatment.

These observations show that the smooth water snake, *E. enhydris* differs from *A. granulatus* in its response to Vitamin D_3.

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投與維他命 D_3 對水蛇，*Enhydris enhydris* (Schneider)，血漿鈣及無機磷之影響

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本研究探討投與維維他命 D_3 (12,000 IU/100 g體重)對水蛇 *Enhydris enhydris* 血漿鈣及無機磷含量之影響。從第一天至十四天實驗結束止，一直有高鈣及高磷之現象。血漿鈣、磷含量之最高值在第四天出現，隨後則穩定的降低。

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