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Effect of Calcium Rich Environment on the Ultimobranchial Bodies of *Rana cyanophlyctis* Treated with Calciferol

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The ultimobranchial bodies of the frog, *Rana cyanophlyctis* injected with calciferol, and placed in 0.8% aqueous solution of calcium chloride, or kept in tap water showed an increase in the height of the follicular epithelium, formation of secondary follicles and obliteration of the follicular lumen when compared with that of controls. Further, the frogs treated with calciferol and kept in 0.8% calcium chloride solution showed heavy deposition of calcium carbonate in the paravertebral lime sacs. The observations indicate that hypercalcemic condition might stimulate the ultimobranchial gland to secrete calcitonin which may prevent excessive calcium mobilization.

Key Words: Calcium, Environment, Ultimobranchial bodies, Calciferol, Frog

Introduction

Calcium homeostasis in mammals is maintained by parathormone, calcitonin and vitamin D. It is accepted that the homologues of calcitonin producing cells are found in the ultimobranchial body (Robertson 1968a,b, 1971b) which is the main source of calcitonin in non-mammalian vertebrates (Copp et al. 1967, Copp & Parkes 1968, Moseley et al. 1968, Van Noorden & Pearse 1971).

The present investigation is an attempt to study the normal structure of the ultimobranchial body in a tropical frog *Rana cyanophlyctis* and to evaluate the effect of induced hypercalcemia on the gland.

Material and Methods

Adult female *R. cyanophlyctis* weighing between 25–30 gms were locally collected during the months of December-January. The frogs were acclimatized to laboratory conditions for about a week and were force-fed on frog thigh muscles. Ten frogs were used for routine histological studies, while forty frogs were divided into four equal groups for experimentation for a period of two weeks.

Group I: Ten frogs formed the control group and were placed in tap water containing 65 ppm calcium at room temperature (24–25°C).

Group II: Ten frogs were intramuscularly administered with calciferol, 100,000 IU in 0·25 ml olive oil/week, for two weeks (calciferol-trade name: Sterogyl, manufactured by Roussel, Franco-India Ltd., Bombay). They were kept in tap water containing 0·8% calcium chloride at room temperature.

Group III: Ten frogs were injected with 100,000 I U of calciferol/week for two weeks and were placed in tap water at room temperature.

Group IV: Ten frogs were placed in tap water containing 0·8% calcium chloride at room temperature.

After two weeks, the ultimobranchial bodies of the frogs were fixed in Bouin's fluid, embedded in wax and 5–6 μm thick serial sections were stained with Harris haematoxylin and eosin and also by PAS method. The population of different cell types per unit area in the ultimobranchial bodies was determined by using standard ocular grid under 500 magnification. The values of the different groups were compared to find out the significance of differences, if any. At the end of the experiment, the frogs of all the groups were subjected to X-rays and radiographs were taken to evaluate the degree of $CaCO_3$ deposition in the paravertebral lime sacs. Further, they were dissected for physical observation of the paravertebral lime sacs.

Results and Discussion

The ultimobranchial body in *R. cyanophlyctis* consists of a large primary follicle associated with a small secondary follicle lying lateral to the glottis, enclosed in fibrous connective tissue capsule, the follicles being lined by a pseudostratified epithelium (figure 1) which is similar to those found in some other amphibians (Robertson & Swartz 1964, Robertson 1971a). The follicular lumen contains heterogeneous PAS-positive colloid. The follicular epithelium in control frogs shows three types of cells, viz. the basal, the secretory and the degenerating cells. The basal cells are oval, have lightly staining cytoplasm and do not extend up to the lumen of the gland. The columnar secretory cells form the major part of the epithelium, are provided with deeply stained nuclei, highly eosinophilic granular cytoplasm and extend from the basement membrane to the free luminal border (figure 2). The secretory cells may also form a fan like configuration. The degenerating cells contain pycnotic nuclei and scanty vacuolated cytoplasm and are located near the luminal border of the follicles (figure 2). Earlier Robertson (1965, 1968a, b) has recognised three types of cells in the ultimobranchial body of *R. pipiens*.

Structurally ultimobranchial body of the control frogs was similar to that of frogs collected from the natural water bodies. The ultimobranchial bodies of the group II, treated with calc ferol + $CaCl_2$ showed hyperplasia of the cells in the follicles, suggesting hyper activity of the gland (figure 3). There was a significant increase in the cell population per unit area ($P < 0{\cdot}001$) of the basal cells, the secretory cells and the degenerating cells (table 1). In a majority of the glands the lumen was very much reduced or obliterated. A large number of secondary follicles containing degenerating cells were found (figure 4). The lumen of the follicles contained heterogeneous colloidal material, cell debris and degenerating nuclei. In Group III the glands showed wavy infoldings and there was a significant increase in the cell population/unit area (table 1). The glands of Group IV appeared similar to that of control group (I) in structure. However, there was a slight but statistically insignificant increase in the cell population/unit area (table 1). The present study revealed that the ultimobranchial bodies in *R. cyanophlyctis* showed hyperactivity when the animals are subjected to continuous high calcium environment.

Table 1 *Different cell types per unit area of the ultimobranchial body in control and treated* Rana cyanophlyctis

Group	Treatment	Basal cells Mean ±SE	Secretory cells Mean ±SE	Degenerating cells Mean±SE
I	Control	1.70±0.40	8.58±0.21	1.47±0.08
II	Calciferol+$CaCl_2$	3.23±0.29	13.80±0.52	5.18±0.66
III	Calciferol	3.39±0.23	10.41±0.31	3.53±0.20
IV	$CaCl_2$	2.34±0.07	7.18±1.00	1.72±0.09

	P. values		
Groups	Basal cells	Secretory cells	Degenerating cells
I : II	<0.02	<0.001	<0.001
I : III	<0.01	<0.01	<0.001
I : IV	>0.05	>0.05	>0.05
II : III	>0.05	<0.001	<0.05
II : IV	<0.02	<0.001	<0.001
III : IV	<0.01	<0.02	<0.001

The hyperactivity seen in the ultimobranchial body of frogs subjected to continuous high calcium environment may be due to calciferol which stimulates rapid calcium absorption through intestine. Our results are similar to those reported by Robertson (1968) except that we have noticed secondary follicle formation in frogs subjected to high calcium environment whereas Robertson (1971a) has reported the presence of secondary follicles in the larvae of *R. catesbiana*. Further, with regard to calcium absorption our work provides support to the report by Nicolaysen (1943) and Gorbman et al. (1983) that vitamin D stimulates intestinal calcium absorption.

Deville and Lopez (1970) reported 'S' shaped infolding of the epithelium in hyperactive ultimobranchial bodies of *Salmo salar*. The 'S' shaped infoldings can be compared with the wavy infoldings seen in the glands belonging to Group II and Group III in *R. cyanophlyctis*, suggesting the hyperactive state of the glands. Robertson (1968) has reported the presence of increased calcium deposits in the paravertebral lime sacs in *R. pipiens* during induced hypercalcemia. Similarly in the present study, the frogs of Groups I and IV contained thin deposits of calcium in the paravertebral lime sacs, whereas in Groups II and III the paravertebral lime sacs were large and contained heavy calcium deposit which appeared as dense segmental opacities in radiographs. The lime sacs were ve ry prominent in frogs belonging to Group II.

It is clear that the activity of the ultimobranchial bodies as well as the deposit of calcium in the paravertebral lime sacs is maximal in Group II, which may be due to the combined effect of calciferol and a calcium rich environment. On the other hand, in Group III calciferol is available but the amount of calcium in tap water seems to be insufficient for maximal stimulation of the ultimobranchial bodies, whereas in Group IV the animals may lack the capacity to absorb the available calcium due to absence of calci-

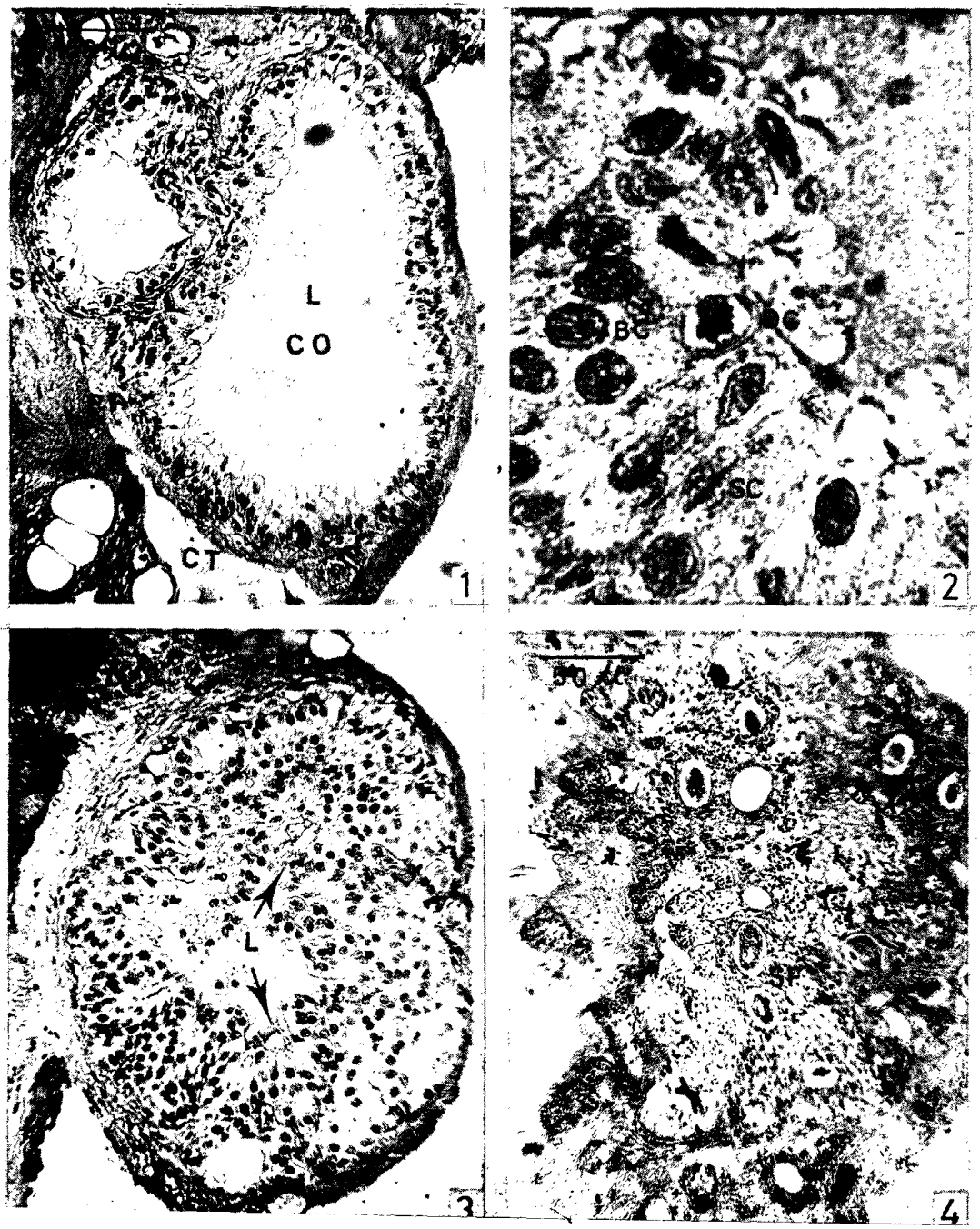

Figure 1 C S of the ultimobranchial body of normal/control of *R. cyanophlyctis* showing the connective tissue capsule (CT), the primary follicle (PF), the secondary follicle (SF), Lumen of the follicle (L) and colloid (Co)

Figure 2 C S of the normal/control ultimobranchial body magnified showing different types of cells, the basal cell (BC), the secretory cell (SC) and the degenerating cell (DC). Magnification: 1,000 ×

Figure 3 C S of the ultimobranchial body of group II, showing hyperplasia and wavy in foldings (arrows). Note the formation of secondary follicles (SF) and reduced lumen (L)

Figure 4 C S of the ultimobranchial body of group II, showing hyperplastic condition. Note the complete obliteration of the lumen and formation of many secondary follicles (SF)

ferol. The induced hypercalcemia might activate the ultimobranchial bodies to secrete calcitonin which prevents excessive calcium mobilization from the paravertebral lime sacs in frogs.

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