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# CALCIUM METABOLISM IN THE FEMALE TOAD, *BUFO MELANOSTICTUS*: EFFECT OF AGING

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**A. K. Pandey** (1988) Calcium metabolism in the female toda, *Bufo melanostictus*: effect of aging. *Bull. Inst. Zool., Academia Sinica* **27**(3): 145-150. With advancing age, there are changes in the activities of the two major calcium regulating glands—the ultimobranchial body and the parathyroid gland of the female toad, *Bufo melanostictus.* The activity of the ultimobranchial body declines while that of the para thyroid gland is enhanced in elderly individuals. Measurement of serum calcium and inorganic phosphorus levels shows significant hypocalcemia and hypophosphatemia in aged specimens when compared with that of adults.

**Key words:** Age, Ultimobranchial body, Parathyroid gland, Female toad.

Recent studies in mammals (Orimo and Hirsch, 1973; Kalu and Foster, 1976; Kalu and Hardin, 1984), Japanese quail (Baksi and Kenny, 1981) and lizard (Kline, 1982) have shown the occurrence of numerous disturbances in calcium metabolism associated with aging, many of them being related to changes in bone structure and metabolism (Stevenson, 1984). So far, there is no record concerning the effect of age on calcium and inorganic phosphorus metabolism of the amphibians. The present attempt is first to describe the effect of aging on serum calcium and inorganic phosphorus levels in relation to the structure of the ultimobranchial body and parathyroid gland of the female toad, *Bufo melanostictus.*

## MATERIAL AND METHODS

The common Indian toad, *Bufo melanostictus* Schneider, were collected from the University Campus during the end of July (the period marks its breeding peak). Females were segregated and divided into seven equal groups according to the size and weight. A rough estimate of the age was made by measuring the length, weight and gonosomatic index (GSI) (Table 1). Furthermore, the aged individuals were sluggish with a declined jumping episode.

Six individuals from each group were sacrificed. Blood samples were collected under ether anaesthesia directly by the cardiac puncture. The analyses of serum calcium and inorganic phosphorus were made by Trinder (1960), and Fiske and Subbarow (1925) methods, respectively. Areas adjoining to the glottis region were extirpated and fixed in Bouin's fluid. Sections were cut at 6-8 $\mu$ and stained in hematoxylin and eosin.

## RESULTS

The ultimobranchial body of juvenile toads (Groups 1, 2) comprises follicles without coagulum in their lumina and the cells

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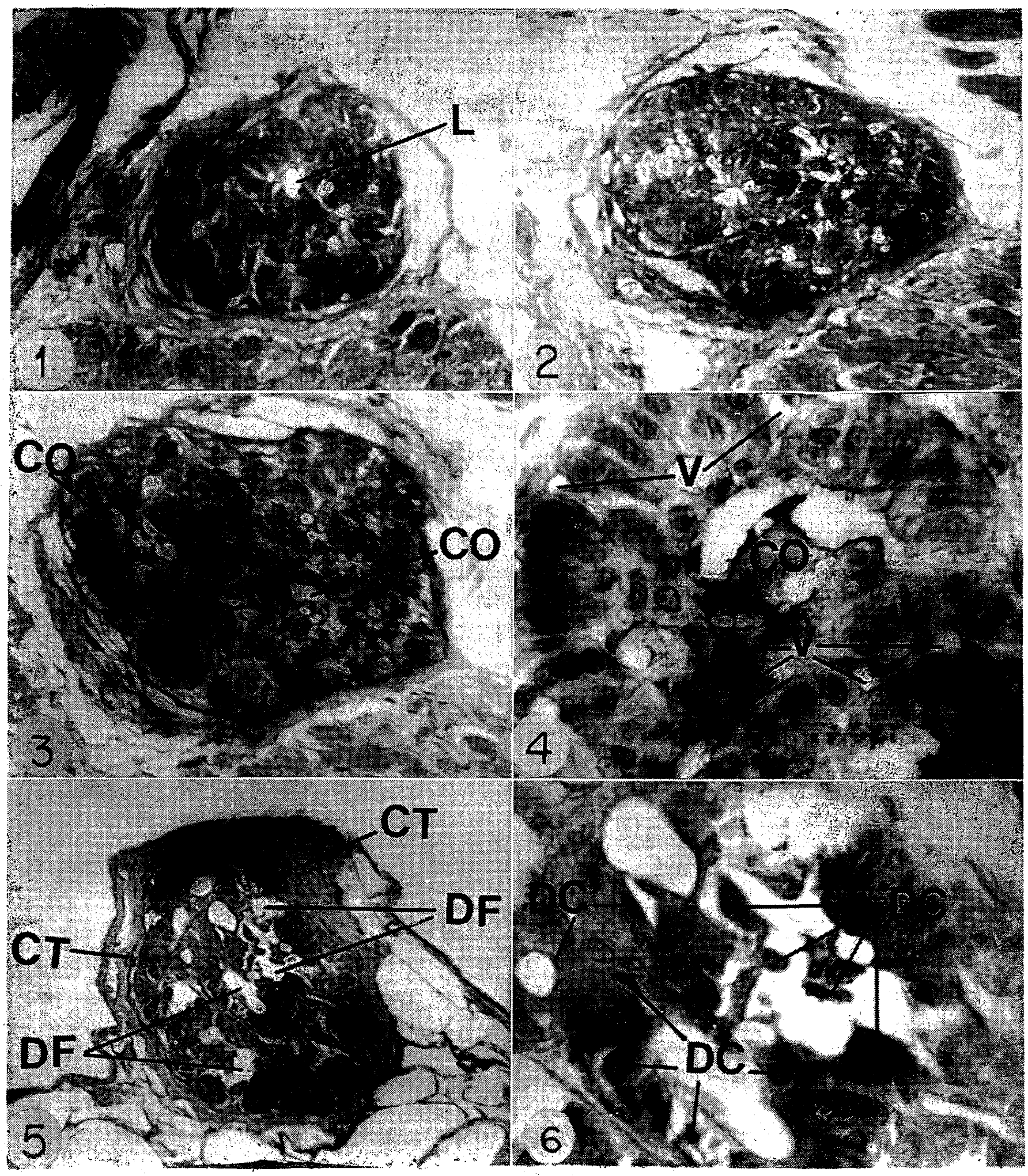
with hyperchromatic nuclei (Fig. 1). Size of the gland increases (Fig. 2) in the individuals of the Group 3. In mature individuals (Groups 4, 5), the ultimobranchial body exhibits the heightened activity with an increase in its size, cells with hyperchromatic nuclei (Fig. 3) and presence of coagulum in lumina of the follicles. Some cells also display vacuolation (Fig. 4). In senile toads (Groups 6, 7), the gland displays the signs of declined activity as it gets encapsulated within an thick connective tissue with its follicular structure distorted and the lumina without coagulum (Fig. 5). Also, numerous degenerating cells are seen in lumina of the follicles (Fig. 6).



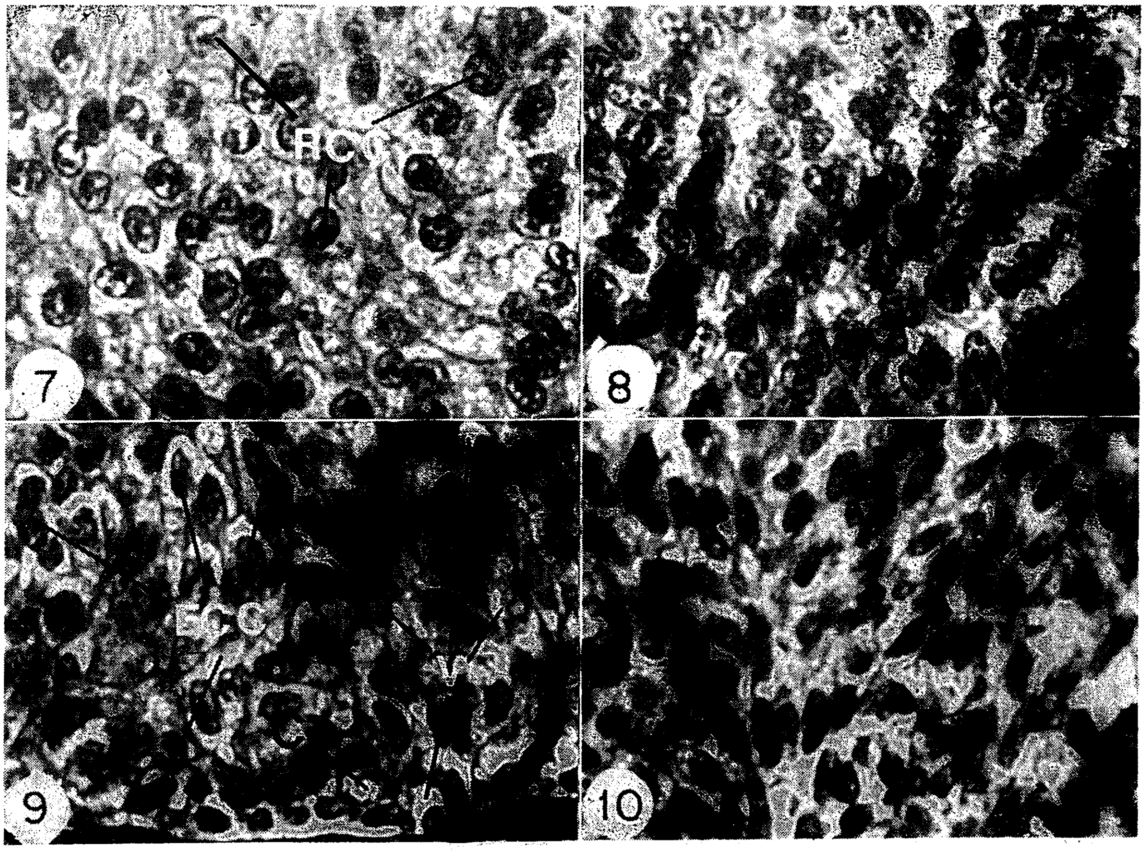



Fig. 1. The ultimobranchial body of juvenile female *Bufo melanostictus* (Group 1) displaying follicular structure and lumen (L) without coagulum. Hematoxylin-eosin ×360.

Fig. 2. The ultimobranchial body of female *Bufo melanostictus* (Group 3). Note an increase in its size and the lumina without coagulum. Hematoxylin-eosin ×360.

Fig. 3. The ultimobranchial body of mature female *Bufo melanostictus* (Group 4) exhibiting the presence of coagulum (CO) in lumina of the follicles. Hematoxylin-eosin ×360.

Fig. 4. A portion of the ultimobranchial body of mature female *Bufo melanostictus* (Group 5) magnified to show lumen with heterogeneous colloid material (CO) and cells with rounded nuclei. Some cells also display vacuolation (V). Hematoxylin-eosin ×680.

Fig. 5. The ultimobranchial body of the senile female *Bufo melanostictus* (Group 7) gets encapsulated within a thick connective tissue (CT). Mark the presence of distorted follicles (DF). Hematoxylin eosin ×180.

Fig. 6. A portion of the ultimobranchial body (Fig. 5) magnified to show the degenerating cells (DC) in lumina of the follicles. Hematoxylin-eosin ×680.

Fig. 7. The parathyroid gland of juvenile female *Bufo melanostictus* (Group 1) showing sparsely distributed rounded chief cells (ROC). Hematoxylin-eosin ×680

Fig. 8. The parathyroid gland of female *Bufo melanostictus* (Group 3) with increased number of rounded chief cells per microscopic field (hyperplasia). Hematoxylin-eosin ×680.

Fig. 9. The parathyroid gland of mature female *Bufo melanostictus* (Group 5) comprises predominently elongated clief cells (ECC). Note the occurrence of cytolysis (C) and vacuolation (V) towards periphery. Hematoxylin-eosin ×680

Fig. 10. The parathyroid gland of sensile female *Bufo melanostictus* (Group 7) exhibiting elongated chief cells with hyperchromatic nuclei. Also the number of cells increases per microscopic field (hyperplasia). Hematoxylin-eosin ×680.

TABLE 1
The female toad, *Bufo melanostictus*, studied

| Group | Length of animal (mm) | Weight of animal (gm) | Weight of ovary (gm) | Sexual status | Gonosomatic index (GSI) | Serum calcium (mg/100 ml) | Serum inorganic phosphorus (mg/100 ml) |
|---|---|---|---|---|---|---|---|
| 1 | 32.000±0.940 | 13.156±1.011 | 0.52±0.049 | Immature ovary | 3.956±0.155 | — | — |
| 2 | 51.333±0.543 | 23.620±0.774 | 1.606±0.112 | —do— | 7.602±0.153 | 9.445±0.147 | 3.278±0.121 |
| 3 | 72.333±0.834 | 33.175±1.058 | 7.141±0.314 | Maturing ovary | 26.140±1.201 | 10.908±0.069 | 3.736±0.097 |
| 4 | 79.833±1.249 | 44.425±0.976 | 11.216±0.195 | Fully grown ovary | 33.801±0.316 | 11.483±0.676 | 3.836±0.096 |
| 5 | 84.833±0.882 | 55.066±0.532 | 13.833±0.156 | —do— | 33.714±0.230 | 12.376±0.112 | 4.750±0.057 |
| 6 | 93.833±0.595 | 82.061±0.471 | 10.466±0.153 | Ovary regressed | 14.609±0.152 | 10.533±0.533 | 3.385±0.114 |
| 7 | 103.500±1.760 | 91.216±0.753 | 8.383±0.105 | —do— | 10.123±0.067 | 9.835±0.123 | 3.226±0.108 |

The values are mean±SE of 6 animals.

Parathyroid gland of the juvenile toads (Groups 1.2) consists of sparsely distributed rounded chief cells (Fig. 7). The number of these cells increases (hyperplasia) in individuals of the Group 3 (Fig. 8). In mature specimens (Groups 4, 5), the gland displays the signs of heightened activity which is expressed by the presence of elongated chief cells (hypertrophy) with hyperchromatic nuclei, and the occurrence of cytolysis and vacuolation towards periphery of the gland (Fig. 9). The gland of senile toads (Groups 6, 7), comprises elongated chief cells with an increase in their number per microscoscopic field (hyperplasia). The cells also possess hyperchromatic nuclei (Fig. 10).

Serum calcium and inorganic phosphorus levels display variations in different groups. Considering animals of Groups 2, 3 as immature, Groups 4, 5 as mature and Groups 6, 7 as senile; adding both the mean values (mean±SE) and taking average of their calcium: 10.177±0.108, 11.930±0.394 and 10.184±0.331, and inorganic phosphorus values: 3.507±0.109, 4.293±0.077 and 3.306 ±0.116, respectively, the senile toads record significant ($p<0.05$) hypocalcemia and hypophosphatemia when compared with that of adult values.

## DISCSSION

In mammals, calcitonin protects the skeleton from calcium loss (Stevenson, 1984). Shamonki *et al.* (1980), Lore *et al.* (1984) and Meller *et al.* (1984) reported a progressive decline in immunoreactive calcitonin concentrations with aging in human. Further, the response of calcitonin secretion to exogenous calcium infusion may be impaired in postmenopausal osteoporotics (Taggart *et al.*, 1982; Zse'li *et al.*, 1982). In older rats, exogenous calcitonin produces smaller hypocalcemic responses than in young ones (Orimo and Hirsch, 1973; Kalu and Foster, 1976). Also, the young chukwalla, *Sauromalus obesus*, records a marked hypocalcemia after synthetic salmon calcitonin treatment while 'a dose twice the original also had no effect in older intact lizards' (Kline, 1982).

The ultimobranchial body of toad is responsible for calcitonin secretion (Treilhou-Lahille *et al.*, 1984). The present observations regarding an increase in the activity of the ultimobranchial body of female *B. melanostictus* oconcurrently with gonosomatic index is likely to be due to an increase in circulating estrogen levels (Stevenson, 1984). The exhausted ultimobrancial body of the aged toad provides an explanation for the declined secretion of calcitonin after exogenous calcium infusion in the postmenopausal osteoporotic women (Taggart *et al.*, 1982; Zse'li *et al.*, 1982) as well as the decreased hypocalcemic response of exogenous calcitonin administration in older rats (Orimo and Hirsch, 1973; Kalu and Foster, 1976) and the lizard (Kline, 1982).

Parathyroid hormone induces hypercalcemia by stimulating bone resorption (Yoshida and Talmage, 1962). With advancing age, there is an increase in the immunoreactive parathyroid hormone in human (Insogna *et al.*, 1981; Chapuy *et al.*, 1983) and rat (Armbrecht *et al.*, 1984; Kalu and Hardin, 1984). Setoguti (1977), and Castleman and Roth (1978) recorded an increase in the number of oxyphil cells in the parathyroid gland of the senile dog and man, respectively. Tsuchiya and Tamate (1982) have also noted an enhanced cytochemical activity in the parathyroid gland of the old domestic fowl. The present observation on *B. melanostictus* about the increased parathyroid activity in the aged females derive support from the above findings. It also provides an explanation for the observed hypophosphatemia in aged individuals (Table 1) which might, perhaps be due to an increase in the parathyroid function (Sasayama and Clark, 1984).

Vitamin $D_3$ induces hypercalcemia by increasing the calcium absorption through the small intestine. Calcium absorption decreases with aging in human (Gallagher *et al.*, 1978), rat (Horst *et al.*, 1978; Armbrecht *et al.*, 1984) and Japanese quail (Baksi and Kenny, 1981) which may be due to decline in the renal 1 $\alpha$-hydroxylase activity (Armbrecht *et al.*, 1984). Since this enzyme is also found in the anuran kidney and have a physiological role in the regulation of calcium (Baksi *et al.*, 1978), it is very likely that a decrease in calcium absorption through intestine may also occur in amphibians with advancing age. In female *B. melanostictus* the lower levels of serum calcium in aged toads as compared to those of active mature individuals (Table 1) is perhaps indicative of this phenomenon.

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# 雌蟾蜍 (*Bufo melanostictus*) 鈣之代謝：年齡的影響

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雌蟾蜍 (*Bufo melanostictus*) 兩個主要調節鈣之腺體，後鰓體及副甲狀腺，其活性隨年齡之增加而改變。高齡個體之後鰓體活性下降， 而副甲狀腺活性却增高。 高齡個體血淸之鈣及無機磷，較成熟個體爲低。