

Seasonal changes in plasma calcium and inorganic phosphate levels in relation to parathyroid structure of the grey quail, *Coturnix coturnix* Linnaeus

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Abstract: Plasma Ca concentration (annual mean) in males *Coturnix coturnix coturnix* was 10.27 ± 0.14 mg/100 ml while it was slightly higher (11.85 ± 0.15 mg/100 ml) among females. Plasma Pi levels (annual mean) in males and females were 5.62 ± 0.12 mg/100 ml and 6.52 ± 0.20 mg/100 ml, respectively. While the males did not exhibit marked fluctuation in plasma Ca and Pi levels either in winter or summer, the females did record significant elevation in the levels of both these electrolytes during breeding season. The peak values of plasma Ca (17.66 ± 0.38 mg/100 ml) and Pi (8.64 ± 0.22 mg/100 ml) in females were observed during June. Parathyroid gland of the grey quail exhibited hyperactivity (hypertrophy and hyperplasia) during breeding season, however, the activity was more conspicuous among females than in males. The maximum increase in cell and nuclear diameters were observed in females during May - July. The follicles were also filled with AF- and PAS-positive materials during these months. The glands depicted signs of hypoactivity and degeneration during peak winter season (November-December) as evident by decrease in cell and nuclear diameters as well as vacuolation in the chief cells.

Key words: Seasonal changes, Parathyroid gland, Plasma calcium, Plasma inorganic phosphate, Grey quail.

Introduction

Endocrine regulation of plasma calcium (Ca) in non-laying birds is achieved by the interactions of parathyroid hormone (PTH), calcitonin (CT) and active metabolite of vitamin D₃ (1, 25-dihydroxycholecalciferol) (Feinblatt, 1982; Pang and Schreiber, 1989; Dacke *et al.*, 1996; Suryawanshi *et al.*, 1997; Dhande *et al.*, 1997 a, b; Dacke, 2000). However, birds do have very high rate of Ca turnover during egg-laying (Simkiss, 1967; Dacke, 1979, 2000; Scanes *et al.*, 1982). A domestic hen utilizes Ca equivalent to 10% of its body weight per day during this period. The large storage of Ca in yolk or egg shell is subsequently utilized for the ossification of developing embryo (Simkiss, 1967; Feinblatt 1982). Among birds egg production is associated with the concomitant increase in plasma Ca and inorganic phosphate (Pi) levels (Mori and George, 1978; Dacke, 1979). Estrogen has specific effects upon Ca metabolism of birds related to the ovulatory cycle and accumulation of yolk in the ovarian follicles (Urist, 1967; Wallace, 1985). Administration of estradiol in pigeon, cocks, capons and Japanese as well as grey quails induced marked increase in non-ultrafiltrable (protein-bound) fraction of plasma Ca (Simkiss, 1967; Dacke, 1979; Dhande *et al.*, 1999). Though parathyroid hormone (PTH) appears to play major role in synchronizing egg formation by altering the concomitant changes in skeletal metabolism (Mueller *et al.*, 1973), no major seasonal changes in the avian parathyroid structure have been observed except lipid inclusions which are more common in winter birds (Stoekel and Porte, 1973; Clark *et al.*, 1986). Though there exist reports on enhanced cytological activity in

parathyroid gland of the laying birds but annual cyclic variations in the plasma electrolytes has not yet been documented (Roth and Schiller, 1976; Clark *et al.*, 1986; Dacke, 2000). An attempt has, therefore, been made to record the seasonal changes in the plasma Ca and Pi levels in relation to parathyroid structure of the grey quail.

Materials and Methods

Adult quails, *Coturnix coturnix coturnix* Linnaeus weighing 60-75 gm (both sexes) were procured from the local markets of Nagpur. They were kept under the laboratory conditions for a week and fed on millet (*Sorghum vulgare*) grains. Five birds (both sexes) were killed in the fourth week of every month throughout the year for recording the seasonal changes. Blood samples from both the groups were collected in centrifuge tubes from the cut made on the jugular vein. Plasma was separated by centrifugation at 3,500 rpm. Plasma Ca and Pi values were estimated by the methods described by Wootton (1974).

Parathyroid, ultimobranchial, thyroid and thymus glands of the birds from both the sexes were surgically removed and fixed immediately in freshly prepared Bouin's as well as Carnoy's solutions. After 24 hours, the tissues were washed thoroughly in running tap water, dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax at 60°C. Serial sections were cut at 6 µm and stained in hematoxylin-eosin (H&E), aldehyde fuchsin (AF), lead-hematoxylin (PbH) (Solcia *et al.*, 1968) and periodic acid-Schiff's reagent (PAS). Cell and nuclear diameters were measured

Table – 1: Annual variations in the plasma calcium (Ca) and inorganic phosphate (Pi) levels (mg/100 ml) of *Coturnix coturnix coturnix*.

Months	Female		Male	
	Plasma calcium	Plasma inorganic phosphate	Plasma calcium	Plasma inorganic phosphate
January	10.78 ± 0.09	5.82 ± 0.07	9.86 ± 0.06	5.18 ± 0.08
February	11.54 ± 0.07	6.84 ± 0.08	10.60 ± 0.17	6.60 ± 0.06
March	11.78 ± 0.13	6.80 ± 0.06	11.22 ± 0.08	6.06 ± 0.09
April	12.52 ± 0.10	7.56 ± 0.41	10.70 ± 0.17	6.50 ± 0.09
May	13.88 ± 0.12	8.24 ± 0.16	11.26 ± 0.13	6.80 ± 0.08
June	17.66 ± 0.38	8.64 ± 0.22	11.20 ± 0.18	5.78 ± 0.22
July	13.64 ± 0.22	6.54 ± 0.15	11.50 ± 0.29	5.10 ± 0.21
August	11.46 ± 0.20	5.94 ± 0.12	10.62 ± 0.16	4.92 ± 0.09
September	9.62 ± 0.13	5.66 ± 0.15	8.50 ± 0.11	4.90 ± 0.22
October	10.14 ± 0.12	5.84 ± 0.13	9.42 ± 0.12	5.20 ± 0.15
November	9.80 ± 0.12	5.14 ± 0.45	9.64 ± 0.15	5.54 ± 0.13
December	9.46 ± 0.15	5.24 ± 0.10	8.82 ± 0.17	4.88 ± 0.06
Annual mean	11.85 ± 0.15	6.52 ± 0.20	10.27 ± 0.14	5.62 ± 0.12

Values shown are mean ± S.E of 5 determinations.

Table – 2: Annual changes in the cell and nuclear diameters (µm) of the parathyroid glands of *Coturnix coturnix coturnix*.

Month	Female		Male	
	Cell diameter	Nuclear diameter	Cell diameter	Nuclear diameter
January	9.32 ± 0.34	4.69 ± 0.30	8.70 ± 0.22	4.28 ± 0.26
February	9.28 ± 0.28	4.99 ± 0.42	9.12 ± 0.34	4.88 ± 0.30
March	9.72 ± 0.40	5.94 ± 0.26	8.16 ± 0.14	3.77 ± 0.16
April	9.94 ± 0.24	6.24 ± 0.28	8.20 ± 0.18	4.80 ± 0.18
May	10.55 ± 0.36	6.64 ± 0.36	8.90 ± 0.37	5.10 ± 0.22
June	10.50 ± 0.26	6.64 ± 0.22	9.47 ± 0.24	5.85 ± 0.22
July	10.85 ± 0.18	6.26 ± 0.16	9.70 ± 0.20	5.68 ± 0.32
August	9.84 ± 0.40	5.35 ± 0.20	8.67 ± 0.43	5.40 ± 0.38
September	8.75 ± 0.32	5.12 ± 0.24	8.62 ± 0.42	5.10 ± 0.11
October	8.67 ± 0.25	4.62 ± 0.40	8.34 ± 0.26	4.42 ± 0.20
November	8.72 ± 0.11	4.46 ± 0.26	8.56 ± 0.21	4.35 ± 0.14
December	8.56 ± 0.11	4.30 ± 0.18	8.42 ± 0.20	4.30 ± 0.24
Annual mean	9.52 ± 0.27	5.35 ± 0.27	8.73 ± 0.26	4.82 ± 0.24

Values shown are mean ± S.E. of 5 determinations.

with the help of ocular micrometer (PZO, Poland) along its long and short axes and the mean values were calculated. 250 cells and nuclear measurements (25 from each bird) were randomly recorded from both the sexes at monthly intervals.

Results and Discussion

Normal plasma Ca concentration (annual mean) in males *Coturnix coturnix coturnix* was 10.27 ± 0.14 mg/100 ml whereas it was slightly higher (11.85 ± 0.15 mg/100 ml) in females (Table 1). These values are comparable to those of non-reproducing female or male pigeon, *Columbia livia* (9.0 mg/100 ml), ring dove, *Columbia palumbus* (9.00 mg/100 ml), house sparrow, *Passer domesticus* (10.00 mg/100 ml), Canada goose, *Branta canadensis* (10.00-11.00 mg/100 ml), male or immature domestic fowl, *Gallus domesticus* (12.00 mg/100 ml), Japanese quail, *Coturnix japonicus* (10.4 mg/100 ml) and rain quail, *Coturnix coromondalica* (9.5 mg/100 ml)

(Simkiss, 1967; Boelkins and Kenny, 1973; Clark *et al.*, 1976; Mori and George, 1978). However, there are records of higher plasma Ca in bomb white quail, *Colinus virginianus* (13.0 mg/100 ml) and pullet (17.4 mg/100 ml). The average mean of plasma Pi concentration in male and female grey quails during non-breeding season were 5.62 ± 0.12 mg/100 ml and 6.52 ± 0.20 mg/100 ml, respectively (Table 1) which is rather similar to that of newly-hatched chick (5.2 mg/100 ml) and rooster (5.8 mg/100 ml) (Simkiss, 1967). Though there were no marked change in plasma Ca and Pi values in males either in winter or summer, the female grey quails exhibited marked increase in both of these metabolites during summer (April-July) which coincides with breeding season of the bird. Concentration of plasma Ca in female grey quails reached to maximum (17.66 ± 0.38 mg/100 ml) in June (Table 1) which was less as compared to the laying hen (25-29 mg/100 ml), Bobwhite quail (29 mg/100 ml), Canada goose (22 mg/100 ml) and Japanese quail (26

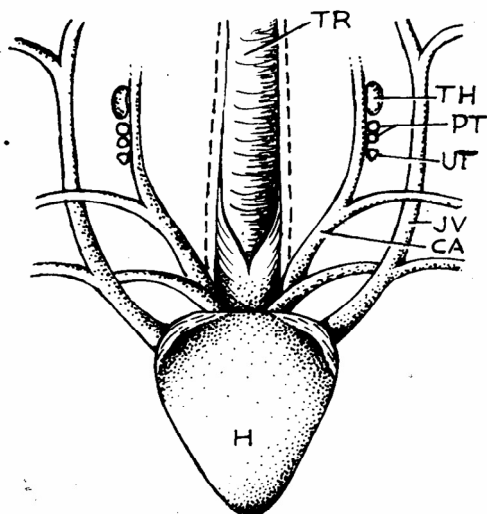


Fig. 1: Diagrammatic representation of the neck region of *Coturnix coturnix coturnix* showing the location of thyroid (TH), parathyroid (PT) and ultimobranchial (UT) glands. (TR trachea, CA carotid artery, JV jugular vein, H heart).

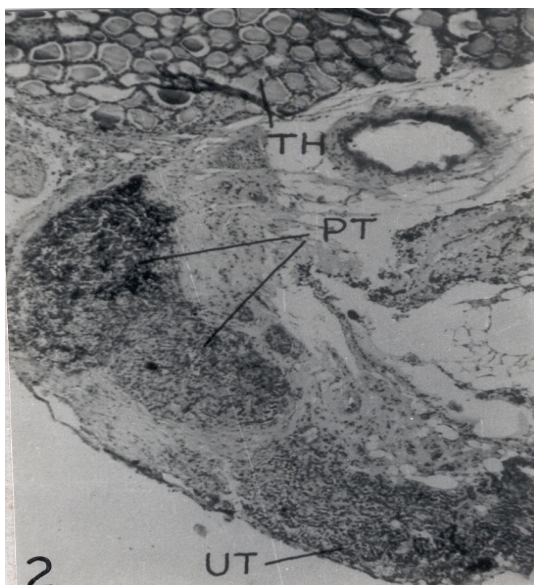


Fig. 2: Section of the neck region of *Coturnix coturnix coturnix* showing relationship of parathyroid glands with thyroid and ultimobranchial glands. H&E x 75.

mg/100 ml). The highest plasma Pi level (8.64 ± 0.22 mg/100 ml) was also recorded during June in the female grey quails (Table 1) which is comparable to plasma Pi concentration of the laying hen (Simkiss, 1967). In the present study, only the female birds showed marked increase in plasma Ca and Pi levels during breeding season. Increase in the levels of Ca and phosphorus have also been reported during breeding season or egg-laying in several avian species (Simkiss, 1967; Urist, 1967; Mori and George, 1978).

Most of the birds possess two pairs of parathyroid glands but in some species the glands on each side fused to form a single mass. Moreover, considerable variations occur in precise location of the glands (Roth and Schiller, 1976; Clark *et al.* 1986). *Coturnix coturnix coturnix* possesses two pairs of small, oval or spherical yellowish parathyroid glands - anterior (parathyroid III) and posterior (parathyroid IV) which are situated near the caudal region of the thyroid glands just above the junction of the common carotid with subclavian artery (Fig. 1,2). The arterial blood is supplied by branches of common carotid artery and venous blood collected by the jugular vein (Fig. 1). Histologically, the parathyroid gland of bird appears to be similar to those of mammals but without oxyphil cells (Roth and Schiller, 1976; Clark *et al.*, 1986). The parathyroid gland of grey quail was encapsulated by connective tissue layer. The parenchymal chief cells were arranged in elongated and branching cords separated by thin connective tissue stroma, capillaries and sinusoids (Fig. 3,4). Sometimes cords were arranged in whorls forming follicles with a central cavity filled with AF- and PAS-positive material (Fig. 3,5). The parenchymal chief cells of parathyroid were ovoid, elongated with little cytoplasm and poor cellular boundaries. Each cell contained a distinct centrally-situated large nucleus occupying major portion of the cell (Fig. 5,6). Generally, these cells lacked secretory granules but during enhanced activity of gland, the granules were present which stained pink with eosin. The histological observation in *Coturnix coturnix coturnix* revealed uneven staining of the chief cells as some of them stained dark and light giving false appearance of two cell types (Fig. 7). However, electron microscopic studies on other avian parathyroids have conclusively shown the presence of single cell type in two different secretory - dark and light phases (Nevalainen, 1969; Fuji and Isono, 1972; Fuji, 1975; Chan, 1977; Isono *et al.*, 1979). The average cellular and nuclear diameters of the chief cells ranged between 8.16 to 10.85 μm and 3.77 μm to 6.64 μm , respectively (Table 2). The oxyphil cells were totally absent in the parathyroid gland of the grey quail.

Though seasonal variations in parathyroids have been studied extensively in the poikilotherms, such cyclic changes (though of less magnitude) are also reported in homeothermic mammals such as ground squirrel and hedgehog (Roth and Schiller, 1976; Clark *et al.*, 1986). A careful histological study of the parathyroids of *Coturnix coturnix coturnix* throughout the year revealed cyclic changes as the gland exhibited hyperactivity (hypertrophy and hyperplasia) during breeding season (April-July). This activity was more conspicuous in females than in males. During this period, there was a gradual increase in the cell and nuclear diameters of the chief cells. The maximum increase in cell and nuclear diameter was observed during May-July in females (Fig. 9; Table 2). Occasionally, the chief cells of *Coturnix coturnix coturnix* were arranged in whorls forming follicles filled with AF- and PAS-positive material (Fig. 8). Such follicles were usually observed in females during breeding season (April - July). Hypertrophies as well as hyperplasia have been recorded in egg-laying hen (Urist, 1967).

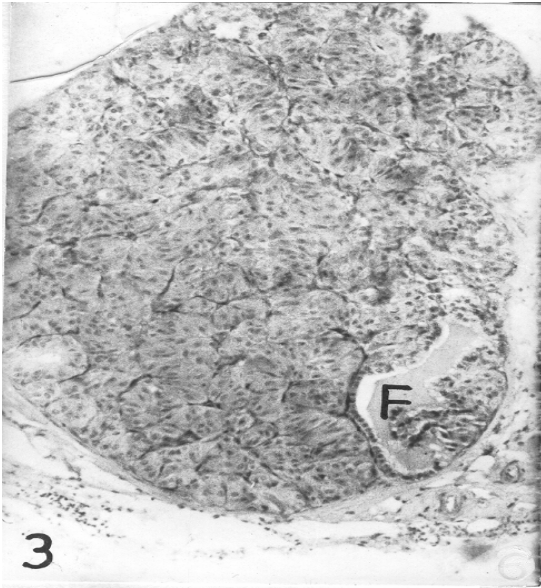


Fig. 3: Parathyroid gland of male grey quail in April showing follicular arrangement of the chief cells. Note the follicle (F) with homogenous colloid-like material in the lumen. H&E x 200.



Fig. 5: Parathyroid gland of female grey quail in June showing follicular arrangement of chief cells and AF-positive material in the lumina (arrow). AF x 200.

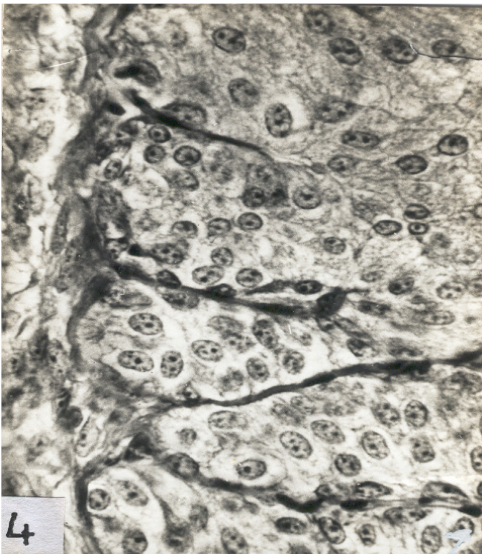


Fig. 4: Parathyroid gland of female grey quail in May exhibiting the connective tissue sheath and arrangement of chief cells in cords. Mark the hyperplasia in the gland. H&E x 800.

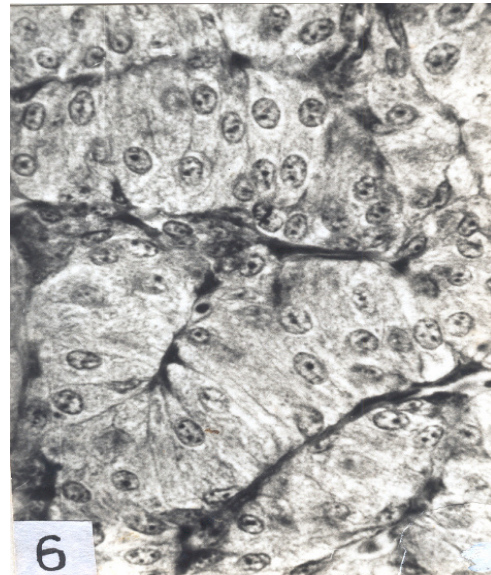


Fig. 6: Parathyroid gland of female grey quail in July exhibiting cord-like arrangement of the hypertrophied chief cells. H&E x 1,000.

van der Velde *et al.* (1984) have also recorded enhanced parathyroid hormone (PTH) levels in hen during egg-shell formation. On the other hand, the parathyroid glands of grey quail showed signs of hypoactivity and degeneration during winter peak (November-December) as evident by the decrease in cell as well as nuclear diameters (Table 2), cytoplasmic vacuolation of the chief cells and disruption of the cord-like arrangement. Though the degree of degeneration varied from

individual to individual, the chief cells possessed pycnotic nuclei and lacked cytoplasmic granules during this period (Fig. 10).

Accessory parathyroid tissue was observed in the ultimobranchial glands of *Coturnix coturnix coturnix* (Fig. 8). Presence of such parathyroid tissue has also been reported in the ultimobranchial gland of other birds (Roth and Schiller, 1976; Clark *et al.*, 1986). Though there exist reports on presence of the accessory parathyroid tissue in the caudal lobe of thymus of birds (Roth and Schiller, 1976; Clark *et al.*, 1986)

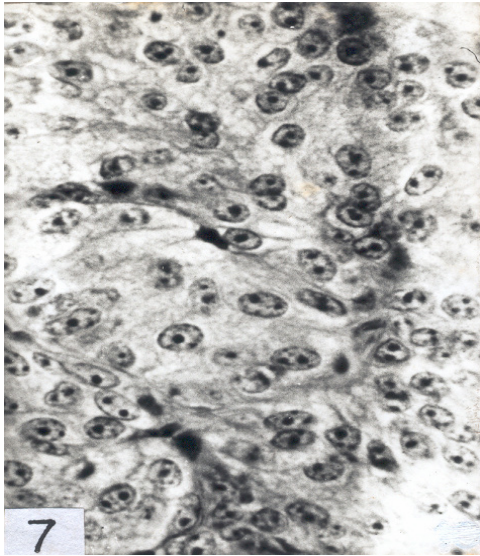


Fig. 7: Parathyroid gland of the male grey quail showing single type of active chief cells. H&E x 1,000.

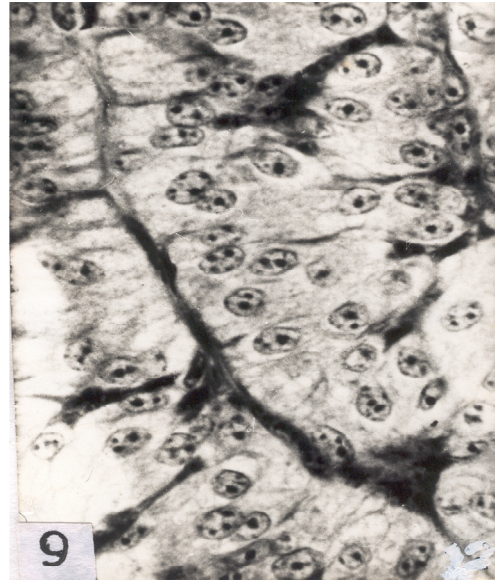


Fig. 9: Parathyroid gland of the female bird during breeding season showing marked increase in nuclear and cell diameter of chief cells. H&E x 1,000.

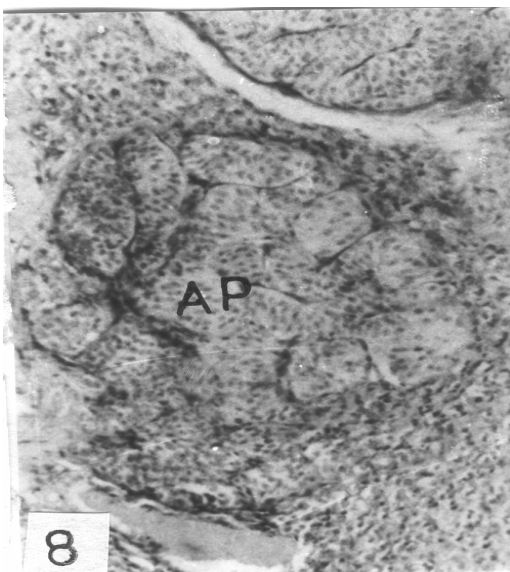


Fig. 8: Ultimobranchial gland of the female grey quail depicting the presence of accessory parathyroid tissue (AP). H&E x 200.

but such tissue was not encountered in the thymic lobe of the grey quail. Presence of groups of ultimobranchial tissue within the parathyroids of birds has also been recorded (Le Douarin and Le Lievre, 1970) but no such cells could be observed in the glands of *Coturnix coturnix coturnix*.

The source of Ca for egg-shell formation in birds is either from the dietary sources or the labile medullary bone (Simkiss, 1967; Urist, 1976; Dacke, 1979). In the present study, only females showed marked hypercalcemia during breeding season and this may probably be due to formation of medullary bone (Urist, 1976; Dacke, 1979). It may be likely that the female

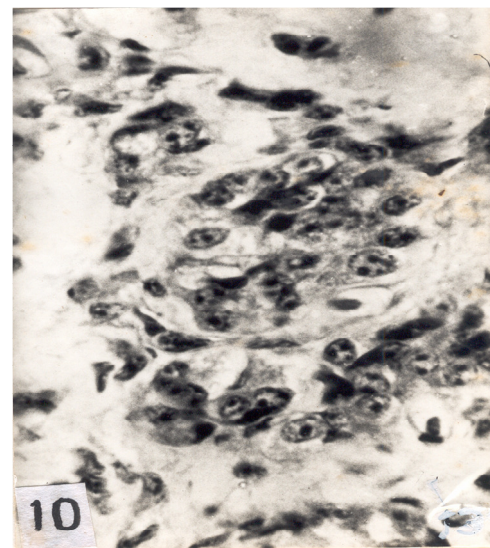


Fig. 10: Parathyroid gland of the female bird showing degenerating chief cells with pycnotic nuclei during December. Some cells also exhibit vacuolation in cytoplasm. H&E x 1,000.

sex steroid hormone acts synergistically with parathyroid hormone to enhance resorption of Ca from the bone. The grey quail shows hyperactivity of the ovaries during breeding season (Saxena and Saxena, 1980) suggesting elevation in the endogenous estrogen levels due to increased number of rapidly developing ova. Similarly, exogenous administration of estrogen during egg-laying period increased the plasma Ca and Pi levels in birds (Simkiss, 1967; Urist, 1967; Dacke, 1979). The parathyroids of the female grey quail also showed signs of

hyperactivity during breeding season. It may be concluded that both estrogen and parathyroid increased mobilization of Ca from long bone resulting in elevation of plasma Ca level which may be transported through blood to the uterus for egg-shell formation (Dacke et al., 1979, 1996).

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