EFFECT OF SEASON AND GONADOTROPIN ON TESTICULAR INTERSTITIAL CELLS OF CALIFORNIA QUAIL

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THAT the interstitial (Leydig) cells of the testis are the major source of androgens in birds is generally concluded (Marshall, 1961; van Tienhoven, 1961), although a few authors (e.g. Pfeiffer and Kirschbaum, 1943) once favored the germinal epithelium as the secretory site of these hormones. Testicular interstitial cells have been described in some avian species, but in others these cells appear to be absent (Pfeiffer and Kirschbaum, 1943), and often seasonal changes in their number do not correlate with other evidence of androgen secretion (e.g. Sarkar and Ghosh, 1964). The scarcity of interstitial cells in breeding testes has led some authors to conclude that androgen secretion is relatively low at this time (Oslund, 1928). Others (Marshall, 1961) have ascribed this scarcity to dispersion of the interstitial elements by the expanding seminiferous tubules, and have concluded that interstitial cell secretory activity increases along with spermatogenesis.

Although Lewin (1964) describes the seasonal spermatogenic cycle in the California Quail (*Lophortyx californicus*), he refers only briefly to changes in interstitial cell activity. The purpose of the present study was to confirm, in this species, the hypothesis that the secretory activity of the interstitial cells increases with the vernal advance in spermatogenesis by comparing the quantity and appearance of these cells with histological and behavioral evidence of androgen production. I also attempted to increase interstitial cell activity with pregnant mares' serum gonadotropin (PMSG). Inasmuch as male California Quail inhabiting arid regions often fail to exhibit testis growth and pairing behavior (Grinnell et al., 1918; McMillan, 1964; Francis, 1967), data from this study hopefully will serve as a standard against which the condition of the interstitial cells of these "inhibited" males can be judged.

MATERIALS AND METHODS

I collected wild California Quail from the Sierra Nevada and coastal California foothills and performed the gonadotropin experiment on quail obtained from Poisal's Rare Bird Farm, Pleasanton, California. Only data from adult males were used in this study.

After killing quail in the field or laboratory, I removed the left testis and gonaduct complex and fixed them in 10 per cent neutral-buffered formalin. I then measured the testes to the nearest 0.1 mm using a dial micrometer, and calculated their volume using the formula for the volume of an ellipsoid: $V = 4/3\pi a^2 b$, where "a" is ½ the shorter diameter and "b" is ½ the longer diameter. One-half of each testis and

TABLE 1

SEASONAL VARIATIONS IN TESTIS VOLUME, INTERSTITIAL CELL NUMBER AND NUCLEAR SIZE, AND HEIGHT OF THE EPIDIDYMIDAL EPITHELIUM IN QUAIL

			Mean ± SE mean[1]					
Testis stage	No. of speci mens	Dates collected	Testis volume (mm^3)[2]	No. inter-stitial cells/ field[2]	Index no. interstitial cells[3]	Interstitial cell nuclear diameter (μ)[2]	Ht. epidid ymidal epi-thelium (μ)[3]	Inter-stitial lipid[4]
1-spermatogonia	4	17 Aug.–20 Feb.	16.6 ± 3.6	114.2 ± 11.4	466.5 ± 138.7	3.57 ± 0.08	7.9 ± 1.0	A-M
2-primary spermatocytes	3	20 Feb.–12 March	37.6 ± 19.8	46.6 ± 5.9	360.3 ± 135.9	3.86 ± 0.26	11.3 ± 0.8	A-M
3-secondary spermatocytes	4	12 March	95.1 ± 6.6	21.6 ± 1.2	488.5 ± 25.9	5.09 ± 0.40	15.4 ± 3.0	M-S
4-spermatids	4	25 March	314.9 ± 7.9	20.8 ± 1.7	1559.5 ± 37.4	5.26 ± 0.24	15.9 ± 2.3	S-0
5-spermatozoa	4	25 March–10 June	347.4 ± 30.5	17.4 ± 1.9	1418.5 ± 149.8	5.31 ± 0.29	14.5 ± 1.8	S-0
6-early regression	5	24 June	73.2 ± 20.7	31.5 ± 1.6	534.0 ± 147.0	3.34 ± 0.55	6.8 ± 0.4	S-M
7-late regression	4	24 June–3 Dec.	12.2 ± 2.8	54.1 ± 5.9	148.7 ± 17.0	3.76 ± 0.45	7.3 ± 1.4	S-M

[1] SE computed for average of mean values for each specimen in a representative stage.
[2] Correlates with predicted cycle peaking at stage 5, $P < 0.05$.
[3] Correlates with predicted cycle peaking at stage 4, $P < 0.05$; modified Kendal Rank Correlation (Siegal, 1956).
[4] A, abundant; M, moderate; S, slight; 0, absent.

gonaduct complex was then embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin. The other halves were embedded in gelatin, frozen, sectioned at 12 μ, and stained with sudan-black B for lipid determination. I also subjected some of these sections to the Schultz test for cholesterol and its esters (Davenport, 1960).

After examining the testes for the presence of spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa, I counted the number of interstitial cells visible in five microscopic fields. The mean number was then multiplied by a factor (a^2b) proportional to testis volume to provide a relative index of the total number of these cells in each testis (Threadgold, 1956; Selander and Hauser, 1965). In addition I measured, with an ocular micrometer and under oil immersion, the longest diameters of 30 to 50 interstitial cell nuclei selected at random; I also noted the amount of lipid in the cytoplasm of these cells and measured the height of the epididymidal epithelium in 15 to 20 areas selected at random.

To determine the effects of PMSG (Ayerst Laboratories) on the interstitium of nonbreeding quail, I injected eight males intramuscularly with 0.2 ml saline containing 150 IU PMSG every 3rd day beginning 9 January and ending 28 January. Three control males received 0.2 ml saline alone.

Results

Spermatogenesis.—This study verifies Lewin's (1964) conclusions concerning the seasonal spermatogenic cycle in this species. Table 1 presents the volumes and stages of spermatogenesis of the testes in males collected at various times of the year.

Interstitial cells.—In stage 1 (inactive) testes, the interstitial cells were relatively abundant in one microscopic field, but their total number was low because of the small testis volume at this time. The nuclei of these inactive cells were small and dark and their nucleoli hard to see (Figure 1A). Their cytoplasm was packed with cholesterol-positive lipid. As the testes passed through stages 2 to 5, the number of interstitial cells in one microscopic field decreased, the total number of these cells increased, their nuclear diameters increased, and the amount of lipid contained in their cytoplasm decreased. The nuclei of interstitial cells in stages 3 to 5 testes were very large and lightly stained with prominent nucleoli (Figure 1C, 1D, 1E). The cytoplasm of these large epithelioid cells contained little if any cholesterol-positive lipid. During testis regression the above mentioned changes reversed, and the interstitial cells returned to the inactive condition (Table 1, Figure 1F, 1G).

Epididymis.—In the winter (testis stages 7 and 1) the epididymis was lined with wedge-shaped epithelial cells having nuclei at their base. The lumen was often occluded. As the breeding season approached (stages 2 and 3), some epithelial cells were sloughed into the lumen. In the breeding condition (stages 4 and 5), the duct was lined with ciliated pseudostratified columnar epithelium that secreted acidophilic droplets into the now enlarged lumen. During testis regression (stages 6 and 7),

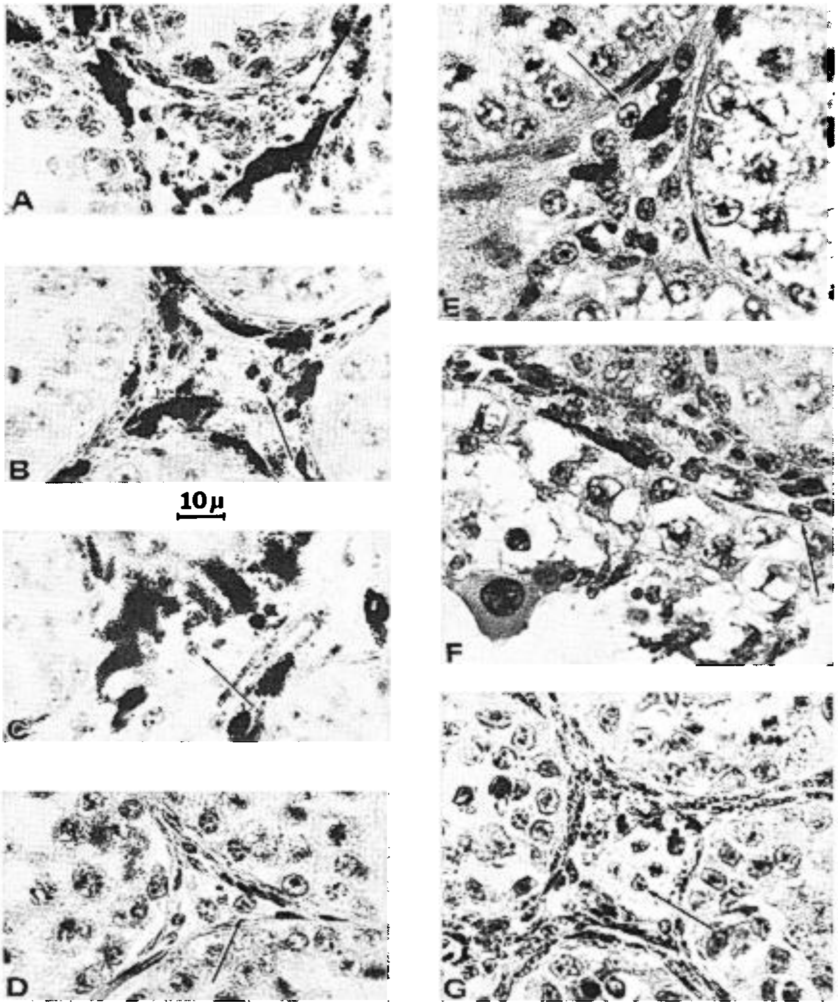

Figure 1. Interstitium of quail testes in various stages of spermatogenesis showing seasonal changes in appearance of interstitial cell nuclei (arrows). Hematoxylin and eosin. ×1200. A, stage 1: small dark nuclei and melanophores; B, stage 2: slightly enlarged nuclei; C, stage 3: moderately enlarged nuclei; D, stage 4: large nuclei with prominent nucleoli; E, stage 5: very large nuclei with prominent nucleoli; F, stage 6: nuclei which appear slightly pycnotic (note capillary with erythrocytes); G, stage 7: pycnotic nuclei and abundant fibroblasts.

necrotic cells were sloughed into the lumen, and the duct returned to the inactive condition. These qualitative changes in epididymal epithelium were reflected by variations in epithelial height (Table 1).

Effects of gonadotropin.—PMSG caused a significant increase in testis

TABLE 2
EFFECT OF PMSG ON TESTIS VOLUME AND INTERSTITIUM OF NONBREEDING MALE QUAIL

			Mean ± SE mean				
Treatment	No. of specimens	Testis stages[1]	Testis volume (mm^3)	No. interstitial cells/field	Index no. interstitial cells	Interstitial cell nuclear diameter (μ)	Interstitial lipid[2]
Saline	3	7 and 1	10.0 ± 1.8	76.5 ± 4.7	225.2 ± 53.0	3.25 ± 0.14	S-M
PMSG	7	2 and 3	111.9 ± 14.9[3]	70.6 ± 5.4	1917.0 ± 239.9[2]	4.88 ± 0.21[2]	A-M

[1] Stages described in Table 1.
[2] Abbreviations explained in Table 1.
[3] Differ significantly from controls, $P < 0.001$ (Student's t-test).

volume, advanced spermatogenesis to stage 2 or 3, increased the number of interstitial cells to an abnormally high level, and caused an increase in interstitial cell nuclear size (Table 2, Figure 2A, 2C). The interstitium in the testes of the PMSG-treated birds appeared to contain more lipid (Figure 2B, 2D) than did that in the control testes (stage 1). Although most of the gonaducts from the PMSG-treated birds were lost during the histological procedure, the two I examined appeared similar in activity to the ducts of breeding birds.

DISCUSSION

California Quail show a definite increase in interstitial cell size and number in early spring (stages 3 and 4 of the spermatogenic cycle). Because in many endocrine cells an increase in nuclear size reflects an increase in secretory activity (Muschke, 1953; Alfert et al., 1955), the hyperplasia and hypertrophy of these interstitial cells probably results in an increase in androgen secretion, a conclusion supported by the following associated phenomena: 1) initial depletion of interstitial cell lipid content, this cholesterol-positive lipid probably being converted into androgens (Arvy, 1962) and released from the cells; 2) first signs of pair formation in the covey (Raitt, 1960), a process that, in male California Quail, is androgen-dependent (Emlen and Lorenz, 1942); and 3) start of gonaduct activity, which is also dependent on androgen in this species (Jones, 1968) and others (e.g. Bailey, 1953). Along these same lines, a correlation exists among the decrease in size and number of interstitial cells in regressing testes and 1) absence followed by a gradual increase of lipid in the cytoplasm of these cells, 2) atrophy of the gonaducts, and 3) brooding behavior (Jones, 1968). The termination of interstitial cell activity with the resultant decrease in circulating levels of androgen may facilitate brooding, the aggressiveness associated with high androgen levels (Emlen and Lorenz, 1942) possibly being detrimental to the raising of young. The indirect indices of circulating androgen levels used in this study

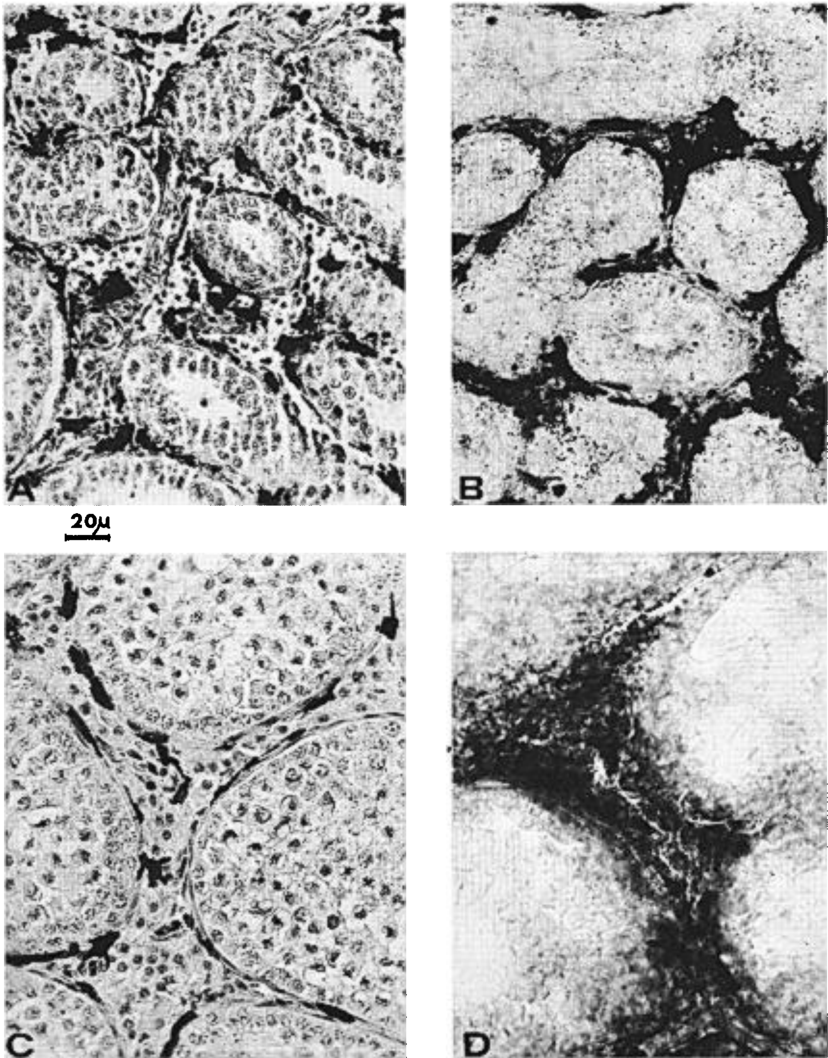

Figure 2. Effect of PMSG on the seminiferous tubules and interstitium of the nonbreeding quail testis. A, stage 1 (control) testis. Interstitial cell nuclei are abundant but small and dark. Hematoxylin and eosin. ×480. B, same testis as in A but stained with sudan-black B. Interstitial cells contain a moderate amount of lipid. ×480. C, stage 3 testis of PMSG-treated male. Note abundance of interstitial cells with large nuclei. Hematoxylin and eosin. ×480. D, same testis as in C but stained with sudan-black B to show abundance of lipid in the interstitial cell cytoplasm. ×480.

render the above conclusions tentative. Both enzyme-histochemistry of the interstitium and direct measurement of blood levels of androgen would certainly add to their validity.

I cannot say whether the increase in number of interstitial cells during stages 4 and 5 is due to differentiation of these cells from fibroblasts, as has been reported for other species (e.g. Pfeiffer and Kirschbaum, 1943), or to mitotic division of the preexisting interstitial cells. It is also possible that the regressing interstitial cells divide to produce the new crop of lipid-filled but nonsecretory cells in stages 7 and 1, or that this new crop is derived from fibroblasts which are abundant during these stages (Figure 1G). According to Marshall (1961) the regeneration of this rehabilitated interstitium is independent of hypophysial influence.

Sluiter and van Oordt (1949) provide evidence that two types of epitheloid cells exist in the interstitium of birds, a lipid-containing, nonsecretory "Leydig cell" and a fuchsinophilic "secretory cell," though it is generally concluded that the former differentiates into the latter (Marshall, 1961). Nalbandov et al. (1951) demonstrate that hypophysectomized roosters have two types of interstitial cells that respond differently to gonadotropin treatment. Inasmuch as I used no stains that demonstrate the fuchsinophilic cell in this study, I obtained no evidence of more than one epitheloid cell type.

PMSG has both FSH and LH activity in a 1 to 5 ratio (Schmidt-Elmendorff et al., 1962). Hence, it is not surprising that PMSG increased both the number of interstitial cells and the size of their nuclei, but advanced spermatogenesis only to stage 2 or 3. If the two gonaducts were representative of the group, PMSG stimulated the interstitial cells to secrete androgen. Similar results were obtained by Sluiter and van Oordt (1949) in the Chaffinch (*Fringilla coelebs*), but PMSG causes sperm production as well as androgen secretion in the House Sparrow, *Passer domesticus* (Witschi, 1935; Pfeiffer and Kirschbaum, 1943). Further research is needed to determine if variability in response of the avian testis to PMSG is due to differences in methods (dose levels, length of treatment) or in species sensitivity. Nevertheless the seasonal increase in interstitial cell size, number, and secretory activity in the California Quail is probably controlled by increasing levels of endogenous gonadotropin, as is also true in other vertebrates (van Tienhoven, 1968).

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SUMMARY

The effects of season and PMSG on testicular interstitial cell activity were studied in the California Quail (*Lophortyx californicus*) using various histochemical and histometric techniques. The testicular interstitial cells of nonbreeding males were relatively scarce, their nuclei were small and darkly stained, and their cytoplasm was packed with cholesterol-positive lipid. As spermatogenesis began in spring, the interstitial cells became more abundant and their nuclei became larger and lighter staining with prominent nucleoli. Lipid was slowly depleted from these cells as spermatogenesis progressed. These trends reversed during testis regression.

The start and end of pairing behavior and sex accessory duct activity was correlated with an increase and decrease in the size and number of interstitial cells, respectively, indicating that the cellular changes reflect changes in androgen production.

PSMG caused an increase in size and number of interstitial cells with the resultant increase in gonaduct activity.

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