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RELATIONSHIP OF GONADAL RECRUDESCENCE AND TESTICULAR MELANOGENESIS IN CALIFORNIA QUAIL

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The testes, but usually not the ovary, of many unrelated species of birds undergo considerable seasonal variation in color. Testes of these birds are generally pearl-white during the breeding season, but during testicular regression and postnuptial molt, they change in color, becoming dark to black. In other species of birds the testes may be yellow-orange in coloration (Serventy and Marshall 1956). The California Quail (*Lophortyx californicus*) in southeastern Washington exhibits a seasonal change in testis coloration. During fall and winter the testes of these quail are dark blue-black, but with the onset of recrudescence in the spring, they become lighter in color. At the height of breeding condition, the testes are pearl-white, and with testicular regression during late summer, the testes return to the blue-black color. This color change, which occurs with testicular recrudescence and regression, appears to be associated with the amount of melanin pigment in the interstitial tissue of the testis. Serventy and Marshall (1956) stated that this color change is the result of pigment dispersal with increase in testicular volume during recrudescence. According to J. King, Washington State University (pers. comm.), black testes seem to be unusual in birds, occurring in only a small proportion of specimens from a given species and locality. Studies on the gonadal cycle of *Lophortyx* spp. (Jones 1970; Williams 1967; Raitt and Ohmart 1966) do not mention a seasonal change in testes color; however, Lewin (1963) qualitatively described the abundance of pigment in microscopic sections of recrudescing and regressing testes of California Quail. The first author of this paper observed black testes in Gambel's Quail (*Lophortyx gambelii*) in southern Arizona and in Bobwhite (*Colinus virginianus*) in north-central Kansas during the fall months.

The reproductive biology of quail of the genus *Lophortyx* has previously been described (Jones 1970; Anthony 1970; Williams 1967; Raitt and Ohmart 1966; Lewin 1963). These studies outline the seasonal spermatogenic cycle or changes in interstitial cell activity of California Quail and Gambel's Quail. Fletcher (1971) studied the effect of vitamin A deficiency on the pituitary-gonadal axis and reproductive performance in California Quail. The purpose of this paper is to show that testis coloration is controlled, in part, by the interstitial melanophore, whose function is related to breeding condition in California Quail. The above papers have not described this relationship, even though they mention the abundance of melanin pigment in the interstitium of the testis. We will also suggest the effect of the hormones of reproduction on melanogenesis and the functioning of the "interstitial melanin unit."

MATERIALS AND METHODS

California Quail were collected in the field by shooting from February until October 1967. Testes were removed immediately after collection and placed in AFA solution. They were allowed to remain in this fixative for approximately 24 hr, at which time they were transferred to a 10% formalin solution for storage.

Testes were weighed (from formalin) to the nearest 0.01 g, and the length and width were measured to the nearest 0.1 mm. The volume of each testis was calculated using the formula for the volume of an ellipsoid: $V = \frac{4}{3} \pi ab^2$, where a is ½ the length and b is ½ the width. Microslides were prepared routinely of the left testis from each quail collected. Tissues were dehydrated through the standard alcohol-benzene series and embedded in Waterman's paraffin. Sections were taken from approximately the middle of each testis, cut at 8 μ, and stained with Heidenhain's hematoxylin.

Aggregated lipids were washed out by the alcohol-benzene series, so they would not appear in sections of testicular tissue. This treatment facilitated the analysis of melanin pigment in the interstitium.

The technique employed by Krumrey and Buss (1969) was used to analyze each testis slide. A reticule with a 200-square grid was inserted into the drawtube of a microscope. The reference points used in the cell counts were the 200 intersection points of the squares. The number of intersections which fell on melanin granules was counted and expressed as a percentage of the total number of intersections. These data were used as a relative indication of the amount of melanin in the interstitium. To eliminate bias, fields within slides were selected by the use of the table of random numbers obtained from Fisher and Yates (1949:104–109). Sample size was determined by Stein's formula (Steel and Torrie 1960:86).

RESULTS

Spermatogenesis. The histological sequence of recrudescence and regression in California Quail has been described by Lewin (1963) and quantified by Anthony

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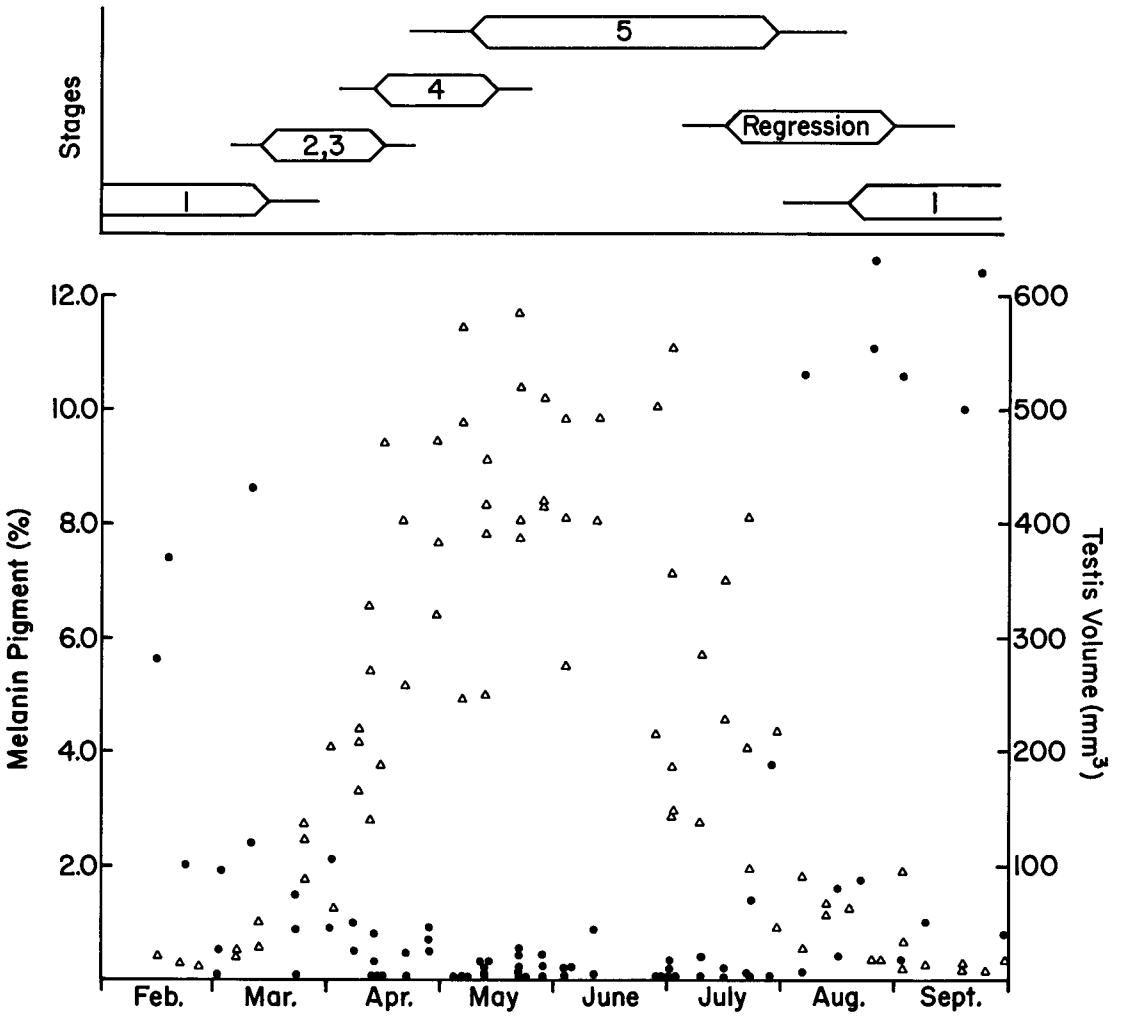

FIGURE 1. The relationship of testis volume to stage of spermatogenic activity and abundance of melanin pigment in the testicular interstitium of California quail. Closed circles indicate amount of melanin pigment and open triangles denote testis volume.

(1970) and Jones (1970). A brief summary of this sequence will facilitate a comparison to melanogenesis and the functioning of the "interstitial melanin unit." The stages of spermatogenic activity and their characteristics are as follows:

Stage 1: The seminiferous tubules and lumina are smallest at this stage. Spermatogonia are the most abundant cell type in the tubules, and a few primary spermatocytes can be seen. Melanin pigment is very abundant between tubules, and the interstitial cells appear to be inactive on the basis of the diameter of the nucleus of interstitial cells. The tunica albuginea is thick. This stage is typical of birds in nonbreeding condition.

Stage 2: The seminiferous tubules begin to enlarge, but their lumina remain small or absent. Spermatogonia are still abundant and line the periphery of the tubules. Spermatocytes can readily be seen and most are in synapsis; a few spermatids are also present. Melanin pigment is less abundant and more evenly distributed than in stage 1. The tunica albuginea is also thinner. Nuclei of interstitial cells are starting to enlarge.

Stage 3: The tubules are continuing to enlarge and the lumen is present but small. Spermatogonia are still the most abundant cell type, but speramtocytes and spermatids are becoming more common. Melanin pigment has become sparse. The nuclei of the interstitial cells have enlarged considerably and appear to be active.

Stage 4: The tubules are large, and their lumina have enlarged. Spermatogonia are no longer the most abundant cell type in the tubules; spermatocytes and spermatids are now equally most abundant. Sperm are present in the lumina of some individuals. Very little melanin pigment can be seen in the interstitial spaces, and the tunica albuginea is thinner than in previous stages. The interstitial cells are active.

Stage 5: The tubules and lumen are now at their greatest diameter. Spermatids and sperm are now the most abundant cell types, followed by spermatocytes and spermatogonia. Many mature sperm can be seen in the lumen. Melanin pigment is no longer present in most sections, and the tunica albuginea is very thin. The interstitial cells are now very active. Quail testes show these characteristics when they are in the height of breeding condition.

Regression: The tubules have collapsed, and no lumen is present. During this stage spermatids and sperm become less abundant, and spermatogonia increase relative to other cell types. Melanin pigment becomes more abundant as regression progresses to nonbreeding condition. Interstitial cells become inactive, and nuclei decrease in diameter. The tunica albuginea increases in thickness.

Melanogenesis. The relationship of testis volume to stage of spermatogenic activity and abundance of melanin pigment in the interstitium is depicted in figure 1. Quail in nonbreeding condition have a high percentage of melanin pigment in the interstitium. As recrudescence of the testis began, the percentage of melanin pigment in testicular sections decreased and at the height of breeding condition was near zero. The amount of melanin granules in the interstitium decreased to near zero during the initial stages of recrudescence and spermatogenic activity. The decrease in melanin pigment occurred during stage 2 and 3 of spermatogenic activity (discussed above) where spermatogenesis had begun and the

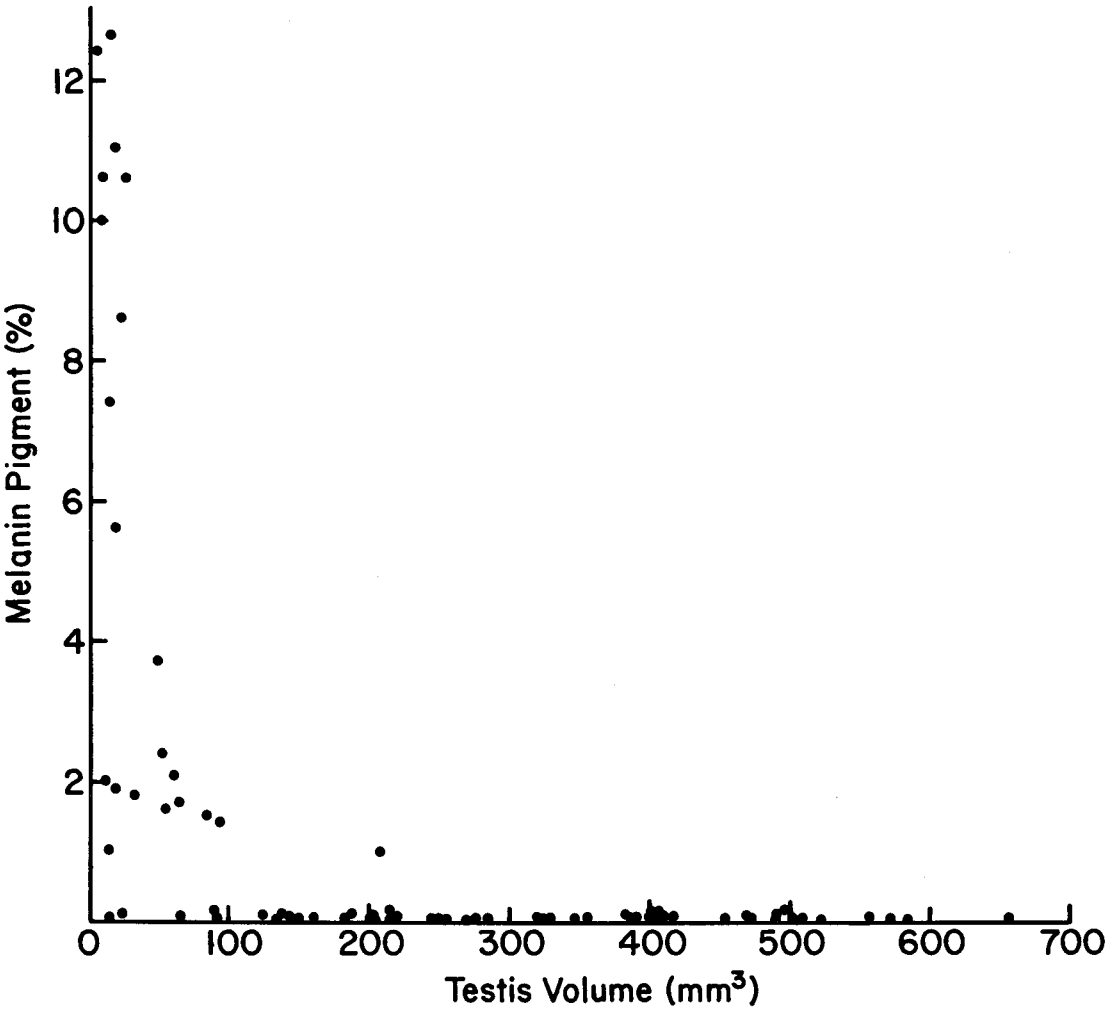

FIGURE 2. The relationship between percent of melanin pigment (dependent variable) and testes volume (independent variable) in California quail.

interstitial cells started to enlarge. Melanin pigment increased in late summer as testes regressed from breeding condition (i.e., when spermatogenesis ceased and the interstitial cells became inactive).

The relationship between the percentage of melanin pigment (dependent variable, M) and testis volume (independent variable, V) is presented in figure 2. This relationship is highly curvilinear and shows that increases in testis volume are associated with a marked decrease in percentage of melanin pigment in cross sections of quail testes. At high testicular volumes, the percentage of melanin pigment is near zero.

The above data were transformed into $\log_{10}$ (M + 1) and $\log_{10}$ V, so that the relationship between melanin pigment (M) and testicular volume (V) could be analyzed statistically by simple linear regression (Steel and Torrie 1960:161–182). By analysis of variance the regression of log (M + 1) on log V is highly significant ($F_{1,73} = 111.027$, $P \geqslant 0.005$) and is described by the equation $\log_{10}(M + 1) = 1.2328 - 0.4542 \log_{10} V$. The correlation coefficient, r, is 0.777; therefore the model accounts for 60% (r^2, coefficient of determination) of the variation.

The log-log transformation gave the best fit, since only 37% (r^2) of the variation could be accounted for by the model: $\log_{10}(M + 1) = B_0 + B_1 V$ and 51% (r^2) by the model $M = B_0 + B_1 \log_{10} V$. Much of the variation (40%), however, cannot be accounted for by the log-log model, suggesting that other factors besides volumetric changes and random variability are influential in the system. The amount of melanin pigment decreases to near zero during the early stages of recrudescence when volumetric changes have only begun (fig. 1). Testis coloration and abundance of melanin pigment in the interstitium, therefore, are not solely influenced by volumetric changes associated with recrudescence.

Melanin unit. During testicular recrudescence and

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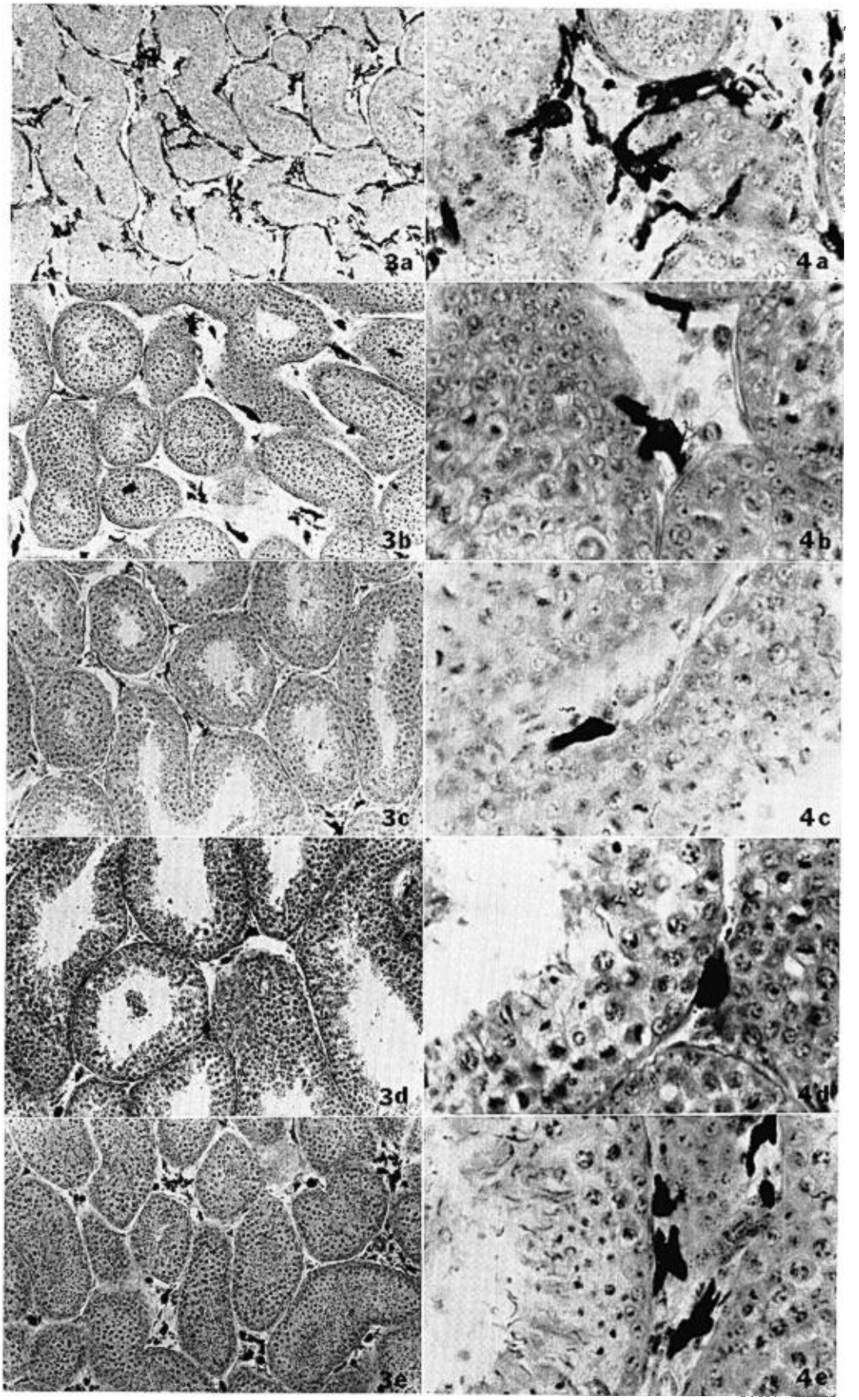
FIGURES 3a–4e. A series of photomicrographs of testes from California quail. Figures 3 and 4 were photographed at powers of 10× and 45×, respectively. Figures designated as a, b, c, d, and e correspond to stages 1, 2 and 3, 4, 5 and regression of spermatogenic activity, respectively.


3a
4a
3b
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3c
4c
3d
4d
3e
4e

regression there is also a change in the function of the "melanin unit" similar to that described by Bagnara et al. (1968) and Hadley and Quevedo (1966) for the "chromatophore unit." The photomicrographs in figures 3 and 4 display these changes. Figures 3 and 4 were photographed at 10× and 45×, respectively. Photomicrographs in figures 3a–4e display changes in abundance of melanin pigment, diameter of seminiferous tubules and tubule lumen, and spermatogenic activity during testicular recrudescence and regression. Melanocytes in testes of quail in nonbreeding condition (figs. 3a and 4a) are dendritic, with melanin granules dispersed into finger-like processes. Most of the pigment is located in the dendritic process, so that the melanocyte proper and its nucleus can be seen. Melanogenesis appears to be rapid in interstitial melanocytes in quail in nonbreeding condition.

As recrudescence of testes begins, melanin pigment is less abundant in the interstitium, and melanocytes are much less dendritic (figs. 3b and 4b corresponding to stages 2 and 3 of spermatogenic activity). Rates of melanogenesis appear to have slowed, and melanin granules are located closer to the nucleus of the melanocytes, so that the nucleus cannot be seen as readily as in stage 1 of spermatogenic activity. The number of dendritic processes containing melanin pigment has decreased.

At stage 4 of spermatogenic activity (figs. 3c and 4c) the pigment is sparse in the interstitium and aggregated in the melanocyte proper to the point that the nucleus of the melanocyte cannot be seen. The rate of melanogenesis has slowed considerably, and only a few dendritic processes contain melanin granules.

When quail reach maximum breeding condition, all melanin granules are tightly clumped within the melanocyte proper so that no dendritic processes are observable (figs. 3d and 4d). Melanogenesis appears to be proceeding at a very slow rate, and only a few melanocytes are present in each histological section.

Once testicular regression begins melanin pigment again becomes abundant in the interstitium (figs. 3e and 4e). Melanin granules can be seen in the dendritic processes of melanocytes, and melanogenesis appears to be proceeding at a higher rate. When testes have regressed fully, they resemble those depicted in photographs in figures 3a and 4a.

DISCUSSION

Results indicate that testes coloration and abundance of melanin pigment in the interstitium of quail testes are not solely influenced by volumetric changes associated with testicular recrudescence as suggested by Serventy and Marshall (1956). Instead, the functioning of the interstitial melanin unit is also responsible for the amount of melanin seen in histological sections of testes and, therefore, testes coloration. Our findings suggest that the hormones of reproduction, either follicle-stimulating hormone (FSH), interstitial-cell stimulating hormone (ICSH), or testosterone, have an inhibitory effect on melanogenesis in the quail testis.

The effect of FSH is to cause tubular growth and spermatogenesis in the testis (Sturkie 1965), so its influence is necessary for the secretion of testosterone. Since ICSH is necessary for the secretion of testosterone, one might consider its effect to be similar or additive. Hall (1966) and Ralph et al. (1967) have shown that ICSH increases tyrosinase activity in feather tracts of weaver birds and is responsible for the black plumage seen in the male during the breeding season. Evidence suggests that tyrosinase catalyzes the slow or rate-limiting step of melanin biosynthesis (Lerner 1953), and hence is likely to be a site of regulation for synthesis of this pigment. ICSH would have to decrease tyrosinase activity in the present system. To the best of our knowledge, no one has shown that ICSH can decrease tyrosinase activity, and thus have an opposite effect in other species and/or organ systems.

According to Pfeiffer et al. (1944) and Engels (1959), testosterone produced a local pigmentation of the beak in the English Sparrow (*Passer domesticus*) and the Bobolink (*Dolichonyx oryzivorus*), respectively. In Brown Leghorn fowl, however, testosterone has no effect on the pigmentary pattern in males (Juhn and Gustavson 1930), and Hamilton (1941) stated that testosterone inhibits the differentiation of black melanocytes in the chick. As a result of the proximity of interstitial cells to melanocytes in the quail testis, testosterone may affect melanogenesis in the interstitium. These speculations are avenues for further research.

The control of color in birds (Ralph 1969) and the functional significance of vertebrate integumental pigmentation (Hadley 1972) have been reviewed, but the adaptive significance of melanocytes in vertebrate viscera is controversial. Potter and Norris (1969) found that the shorter wavelengths of ultraviolet light penetrate the unpigmented body wall of desert iguanas (*Dipsosaurus dorsalis*), and the intensity of this ultraviolet radiation was potentially mutagenic when judged by bacterial standards. Pigmentation in testes of California Quail may act as protection against mutations from ultraviolet radiation, but the timing of this pigmentation in relation to spermatogenic activity makes this speculation most questionable. If testes of birds were scrotal like those for various mammals, light testis coloration would allow less heat gain and possibly greater fertility than darker colors.

Since little is known about the hormonal control of pigmentation in birds, it would be advantageous to determine what hormone(s) control melanogenesis in the quail testes, and therefore their coloration. Once the hormonal control for testicular melanogenesis has been determined, melanogenesis in the quail testis may serve as a valuable bioassay.

SUMMARY

The testes of California Quail undergo a seasonal variation in color which is related to recrudescence and spermatogenic activity. Amounts of melanin pigment in the interstitium decrease abruptly at the onset of recrudescence, and melanin granules migrate from the dendritic process of the interstitial melanophore to its center. The results suggest that one or more of the reproductive hormones are inhibitory to testicular melanogenesis in the interstitium. Hormonal relationships and the adaptive significance of this phenomena are discussed.

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SYSTEMATICS OF THE WHITE-THROATED TOWHEE (*PIPILO ALBICOLLIS*)

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The White-throated Towhee, *Pipilo albicollis*, is a Mexican endemic species of limited distribution. Most of the published information about this species can be found in two papers, those of Davis (1951) and Marshall (1964). Davis used the specific name *Pipilo rutilus*, but see Stresemann (1954).

Marshall's careful studies of vocalizations indicate that *Pipilo albicollis* is less distinct from the Brown Towhee, *P. fuscus*, than I had believed when I wrote of this genus and its relatives in 1957 (Parkes 1957). I called attention to certain striking similarities between *P. albicollis* and *Melozone kieneri* and proposed that, judging from skins, *P. albicollis* formed a link between *P. fuscus* and *M. kieneri*. Marshall found that some of the Mexican races of *P. fuscus* exhibited some of the characters that I had thought were confined, in this group, to *P. albicollis* and *M. kieneri*, but the latter two species still share characters not found in the other forms. One of the characters I invoked was the pattern of the juvenal plumage. I found that in true *Pipilo* (i.e., the *erythrophthalmus* group plus *chlorurus*) the juvenal plumage is distinctly streaked above and below (the ventral streaking corresponding in intensity, in general, to the pigmentation of these areas in adults), whereas all juveniles I saw of *P. fuscus* and Abert's Towhee, *P. aberti*, (which, with *P. albicollis*, form the "brown towhee" group) completely lacked dorsal streaking. In *M. kieneri* the dorsal markings appeared as bars (actually transverse expansions at the tips of somewhat suppressed streaks). The one juvenile of *P. albicollis* then available (Moore Collection 32696) showed a "faint barring" on the dorsum. Two additional juveniles now before me (Carnegie Museum 141364; A. R. Phillips 5195), as well as several in first prebasic molt that retain some dorsal feathers of the juvenal plumage, confirm this. The terminal barbs of the juvenal dorsal feathers have a few blackish barbules at the very tip, giving the effect of a faintly darker, narrow transverse barring or scalloping. This is presumably what Marshall (1964:354) meant in stating of *P. albicollis* that its "marks are broader than long." As stated in my 1957 paper and confirmed by additional specimens, the *ventral* markings of juvenile *albicollis* resemble those of the juvenile *Melozone kieneri* I examined in being irregularly distributed and vaguely shaped dense *spots*, quite unlike those of any other *Pipilo*. Marshall deprecated my statement that *P. fuscus* and *P. aberti* differed from *P. erythrophthalmus* and *P. chlorurus* in completely lacking dorsal streaks. He found that "many juvenal *fuscus* [he did not mention *aberti*] are liberally streaked above and below; and that juvenal *kieneri* are usually streaked, not spotted below." My 1957 findings were based