THYROID HISTOLOGY OF TRICOLORED BLACKBIRDS (*AGELAIUS TRICOLOR*) IN THE ANNUAL CYCLE, BREEDING, AND MOLT

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Seasonal changes in thyroid activity have been observed in several species of wild birds in temperate regions (Wilson and Farner 1960; Voitkevich 1966). Some species undergo increased histological thyroid activity in winter during periods of cold temperature, and the association of thyroid activity and metabolic rate in warm-blooded vertebrates (Turner 1966) suggests an adaptive response by the thyroid involved in physiological adjustment of the metabolic rate during cold weather. Other phases of the annual cycle of birds also are thought to involve an increase in the level of energy utilization. For example, the processes of breeding, raising the young, and molting in particular are energy-demanding periods for wild birds, and it is of interest to know whether thyroid activity is increased in these conditions. Few studies of thyroid activity in wild birds have been based on specimens of known breeding status or of known molt condition. The use of specimens collected on such an individual basis rather than on a chronological basis is desirable since not all birds are in the same state of breeding or molt at the same time. Few such studies have been made. Erpino (1968) has examined thyroids of Black-billed Magpies (*Pica pica*) collected from nests, and Raitt (1968) has worked with wild Gambel Quail (*Lophortyx gambelii*) of known status, whereas Voitkevich (1966) has noted progressive changes in the thyroids of birds in experimentally induced molt. This paper presents a report of the thyroid activity of Tricolored Blackbirds (*Agelaius tricolor*) through the year and especially includes specimens of known breeding or molt condition. Tricolored Blackbirds are of particular physiological and ecological interest because a portion of the population in California regularly breeds in autumn as well as in spring (Payne 1969). These blackbirds afford an opportunity to separate the effects of breeding condition from other seasonal changes in the environment which are independent of the reproductive condition of the birds. The central question in this study was to determine whether the proposed increased energy demands of wintering, breeding, and molt are reflected in histologically evident changes in thyroid activity.

The annual cycle of behavior, gonadal changes, molt, fat deposition and utilization, weight changes, and reproductive photoresponsiveness of Tricolored Blackbirds in California has been described elsewhere in detail (Payne 1969), and a summary of this information will be helpful in interpreting the thyroid cycle of the birds. Gonadal enlargement in both sexes begins in late January, and breeding commences in April. Nesting continues until June or July, and after completion of breeding the birds molt from late July through early September.

A second period of sexual activity involving a recrudescence of the regressed gonads occurs in some individuals in late September, and autumn nests are built from late September through early November by a minority of the blackbirds. No molt follows this autumnal nesting; that is, the summer molt is the only molt of the year. Gonads of birds from November through December are small, and, as in summer, show no histological or histochemical evidence of steroid secretion in the interstitium. A small degree of fat deposition occurs before breeding, and both nesting and brooding birds then undergo a continuous loss of fat through the nesting period. A second period of increased fat and weight occurs near the end of the annual molt before the autumnal breeding period.

METHODS

Blackbirds were collected in central California from 1960 through 1965. The thyroids and gonads were

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fixed in the field, usually within 5 min of the time birds were shot or were removed from nests. The fixative used was neutral buffered formalin. Several dozen birds were shot from nests in various stages of the breeding cycle in order to obtain samples of thyroid activity in nestbuilding, laying, and incubating birds and in birds feeding their young. Molting birds were scored for the stage of molt. The molt scores range from 0 to 100 and indicate the percentage of molt which had been completed; i.e., a bird with a molt score of 20 had completed about 20 per cent of its molt. The details of this system of scoring molt are given in Payne (1969).

Samples of thyroids from six adult males and six adult females for nearly each month, nesting category, and molt class were prepared for histological examination. Thin sections were cut at 6 μ and stained with hematoxylin and eosin. Thyroid activity was then assessed in terms of three readily observed histological features. Firstly, cell heights were measured with an ocular micrometer in 10 cells selected from the central portion of the gland of each bird. Secondly, the nuclear size (radial height) of each of these 10 cells was recorded. Thirdly, follicular diameter (colloid only, excluding the surrounding cells) was measured in 10 follicles selected from the center of the thyroid.

An attempt at unbiased sampling was made by selecting each point for measurement by means of moving the mechanical stage without looking through the eyepiece until 10 appropriate measurements were made of each parameter for each thyroid. When the point under the micrometer mark fell on a blank area or on a thyroid follicle located within two follicles' distance from the periphery of the gland, the stage was moved to the nearest more central follicle along either the x- or y-axis of the stage according to the nearest central follicle.

The variations in the 10 measurements for each bird were rather large. The mean coefficient of variation for eight individuals (January-February males) was 5.95 for nuclear height, 8.94 for cell height, and 9.08 for follicle diameter. The somewhat smaller CV for nuclear diameter may indicate that this parameter is the best single histological measurement of thyroid activity in these birds. Certain variation was noted in the occurrence and extent of vacuolated peripheral follicular colloid; however, the more heavily vacuolated, frothy substance appeared only sporadically through the samples and did not appear to vary consistently with the seasons or breeding status. The use of direct measurements of the thyroid cells and follicles rather than an index of thyroid activity based on ratios was considered to be sufficient to show any striking differences in histological activity among samples.

The use of histological data in the interpretation of thyroid activity is here considered appropriate even in the absence of correlative data on rates of radioiodine uptake and conversion. Saatman and van Tienhoven (1964) compared the response of histological features of the thyroid and of uptake and release of radioiodine to treatment with exogenous thyrotrophic hormone and found that the response of the thyroid was first evident on the histological level. Von Faber (1967) compared the thyroxine secretion rate (TSR) determined in bioassay systems to the histological correlates and concluded that the histological appearance (nuclear size) of the gland gives a clear indication of TSR; e.g., in the Mallard (*Anas platyrhynchos*) a large nuclear volume was evident in birds with higher TSR. Following Voitkevich (1966) and von Faber (1967), we interpret large cell heights and nuclear diameters and decreased follicular diameters to indicate increased rates of secretion of the thyroid hormone which is stored in the colloidal material.

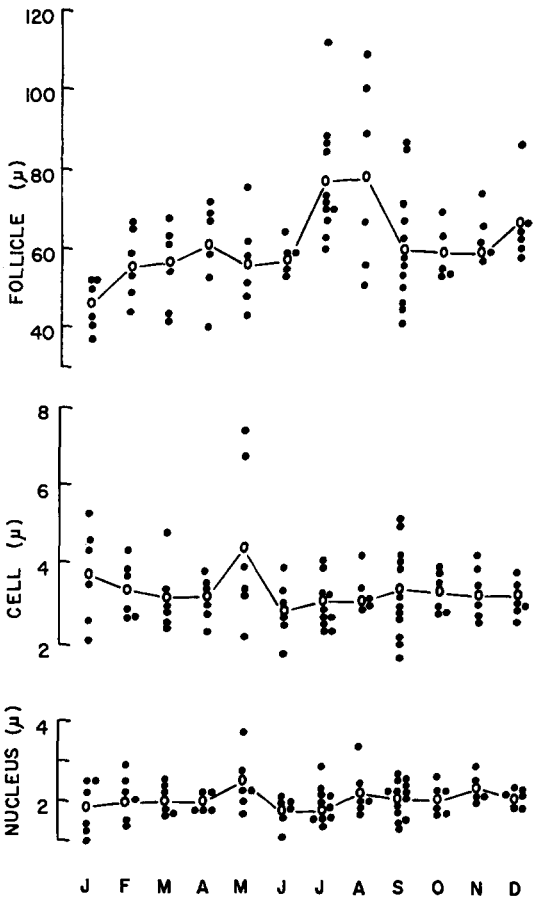

FIGURE 1. Seasonal variation in thyroid histology of male Tricolored Blackbirds. Dots give the mean activity values for each bird; open circles give the monthly mean values.

RESULTS

SEASONAL VARIATIONS OF THYROID HISTOLOGY

Little seasonal change in the histological appearance of the thyroid is evident in male Tricolored Blackbirds (fig. 1). No significant monthly changes in mean cell height occurred, although mean nuclear diameter increased in May, which is the time when most males establish territories and are active in sexual and territorial displays. Prior to the molting period of late summer the mean nuclear diameter decreased. The nuclei increased again in August and remained about the same in size through the periods of autumnal breeding and winter. Mean follicular diameter showed more pronounced

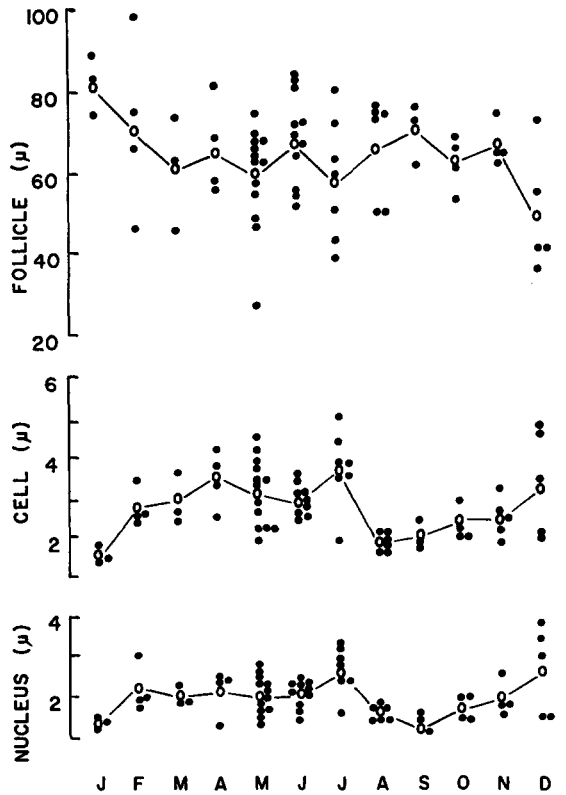

FIGURE 2. Seasonal variation in thyroid histology of female Tricolored Blackbirds.

changes, with an increase in size in the summer agreeing with the decrease in nuclear size in suggesting a decreased rate of thyroid secretion.

Females showed an insignificant change in thyroid histology in late summer, when cell size and nuclear diameter both decreased slightly (fig. 2). Activity slowly increased through the autumn and was similar in December to that in the spring. No peak of activity was associated with the autumnal breeding in individual females shot from nests in October. Follicular diameter showed no clear trends or seasonal differences, although December thyroids had somewhat smaller follicles than did January thyroids, and these differences were paralleled by larger cells and nuclei in December females as compared with January birds.

Thyroid histological activity remained unaltered in periods of colder weather. Using the daily minimum temperatures for the weather station nearest the site of collection, based on published U. S. Climatological Data, the weekly mean minimum temperatures in November, December, and January periods sampled ranged from −1 to +8°C. When the mean daily minimum temperatures

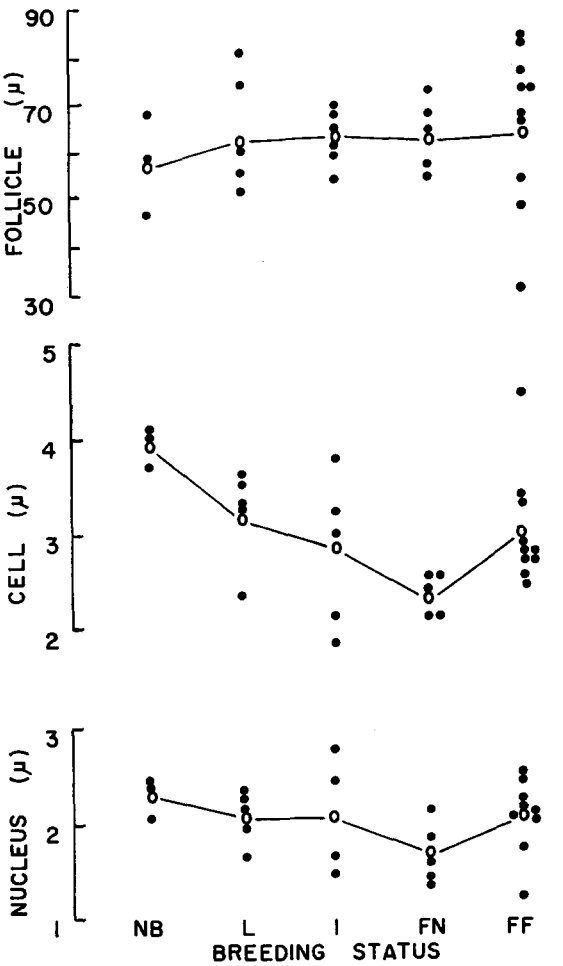

FIGURE 3. Variation in thyroid histology in female Tricolored Blackbirds through the nesting cycle. NB = nestbuilding, L = laying, I = incubating, FN = feeding nestlings, and FF = feeding fledglings.

of the preceding week were graphed with the nuclear diameter, cell height, and follicular diameter, no correlation of thyroid activity with temperature was evident.

THYROID ACTIVITY IN RELATION TO THE NESTING CYCLE

Female Tricolored Blackbirds alone build the nest and incubate. Males sometimes assist them in feeding the young, but at nests under observation where both sexes fed, the females were more active in bringing food to the nestlings. For these reasons the variation in thyroid activity through the breeding cycle was examined only in females.

The thyroid activity of females shot from marked nests or from begging young is recorded in fig. 3, and the status of each female was confirmed by examination of the ovary and brood patch. Little change in

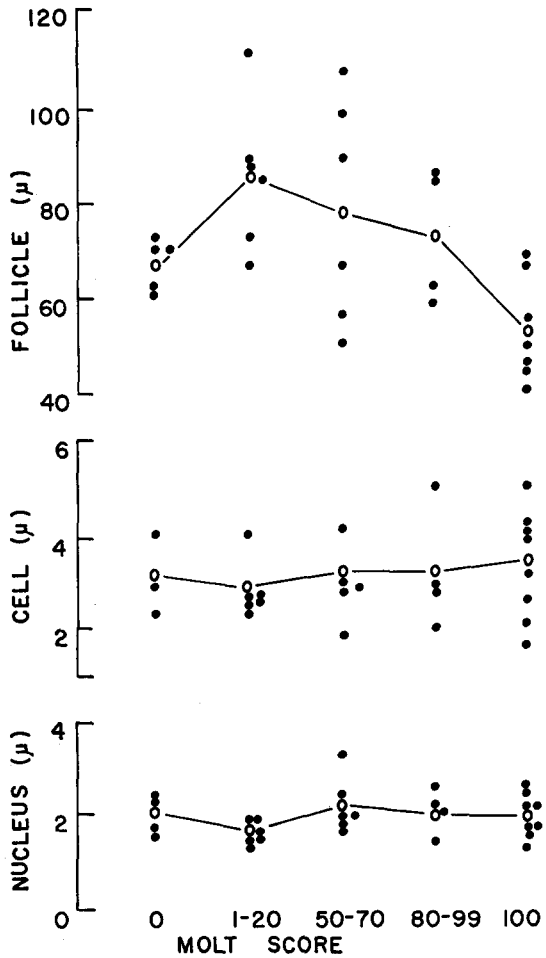

FIGURE 4. Variation in thyroid histology in molting male Tricolored Blackbirds. Birds in col. 1 are July males that had not begun molt; birds in col. 5 are September males that had completed molt.

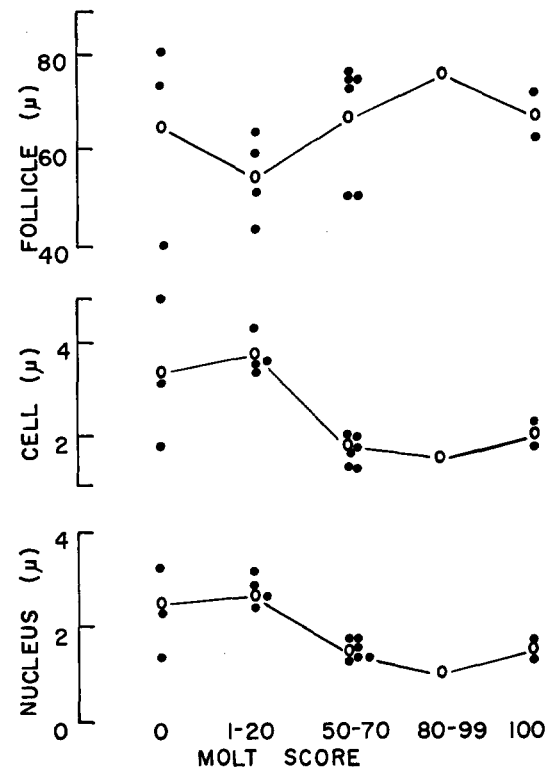

FIGURE 5. Variation in thyroid histology in molting female Tricolored Blackbirds. Birds in col. 1 are July females that had not begun molt; birds in col. 5 are September females that had completed molt.

thyroid activity is evident in breeding females, the only suggestion of increased activity during nesting being the large size of cells and nuclei and the small follicular diameter of females building nests in spring. Nestbuilding is completed in Tricolored Blackbirds in four days, and in this period, each female carries to the nest from a distance of up to several hundred meters, a weight of nesting material nearly equal to her own weight. Nestbuilding also coincides with yolk deposition. No increase in thyroid activity is evident in birds caring for their young.

THYROID ACTIVITY IN RELATION TO MOLT

Little evidence of changing levels of thyroid activity in molt is seen in male blackbirds (fig. 4). No significant differences in nuclear diameter or cell height are apparent in birds in early, middle, or late stages of molt, and no conspicuous peak of increased thyroid activity prior to the molt is apparent. Nonmolting males taken in July had a somewhat smaller but statistically insignificant mean follicle diameter than did males with a molt score of 1–20 in July. A trend of decreasing mean size of the thyroid follicles through the period of molt itself does suggest a continued increase in thyroid activity through the molting period.

Females had somewhat more active thyroids immediately prior to and during early molt than in the later stages (fig. 5). The mean nuclear diameter and the mean cell height of non-molting July females plus other females with molt scores of 1–20 were, on the average, larger than those of females with molt scores of 50–99; however, no significant change in follicle diameter is apparent in these same birds through the period of molt. The differences betwen sexes in thyroid activity during molt were not significant.

DISCUSSION

The great variation in all of the examined histological measures of thyroid activity through the year suggests no conspicuous response by the thyroid to cold temperatures in

winter, even though central California has temperature extremes in summer and winter which differ by more than 40°C and mean monthly temperatures which may vary more than 20°C through the year. No clear correlation of thyroid activity and the temperature during the preceding seven days was evident in individual blackbirds taken in the period of November to March. In the House Sparrow (*Passer domesticus*) and in the White-crowned Sparrow (*Zonotrichia leucophrys nuttalli*), two other resident wild birds studied in central California, thyroid activity in winter was similarly variable, and no clear trends for increased activity were apparent (Davis and Davis 1954; Oakeson and Lilley 1960). Comparably, Wilson and Farner (1960) have summarized thyroid studies for many species of temperate songbirds and found an increase in thyroid histological activity in winter in only a third of species living in a highly seasonal environment. Whether the apparent lack of response of thyroid activity in such birds as the Tricolored Blackbirds is due to changes in metabolic rate not mediated through the thyroid, to adaptation by means other than change in metabolic rate, or to an insensitivity of the histological approach, has not been determined. Metabolic rates in the closely related Redwinged Blackbird (*Agelaius phoeniceus*) are approximately 60 per cent higher at cold (0°C) temperatures than at hot summer temperatures (Lewies and Dyer 1969). This significant change in metabolic activity with temperature suggests that considerable metabolic change may occur in the absence of equally conspicuous changes in thyroid activity. Nor are thyroid changes likely to be important in long-term acclimation to cold, inasmuch as metabolic rates of *A. phoeniceus* in individuals on constant cold temperatures were similar to those on changing temperatures (Lewies and Dyer 1969).

The peak of thyroid activity in female blackbirds at the beginning of nesting suggests a possible increased metabolic rate related to the work involved in nestbuilding and to the formation of yolk in the ovary in preparation for the laying of eggs. Increased thyroid activity prior to nesting has been noted also in female Pintails (*Anas acuta*) (Phillips and van Tienhoven 1962) and in Black-billed Magpies (*Pica pica*) (Erpino 1968) and in samples taken on a chronological basis in non-migratory White-crowned Sparrows (*Zonotrichia leucophrys nuttalli*) (Oakeson and Lilley 1960) and in European Blackbirds (*Turdus merula*) (Fromme-Bouman 1962).

It was of interest to find no increase in thyroid activity during the period of parental care. Avian ecologists have generally accepted the viewpoint of Lack (1954) that reproductive rates in temperate-region birds are limited by the amount of food which the parents can provide for their young in the nest; thus, one might expect birds to be most active, to bring as much food as possible to their young, and consequently to have a higher metabolic rate at this time. Since metabolic rate is mediated in part through thyroid activity in birds (Ringer 1965, Turner 1966), this increased activity might be evident upon histological examination of the thyroids. Orians (1961) has estimated an increase of energy utilization of 250 per cent of that of nonbreeding adults during the period of parental care in Tricolored Blackbirds when the adults collect the food for the young in feeding areas remote from the nesting colonies. However, as in the case of thyroid activity in reference to cold temperatures, no direct relationship between behavioral activity and thyroid histology is evident, suggesting that changes in thyroid activity may not mediate the changes in behavior of the adult blackbirds. More recently, Lack (1968) has reviewed evidence that provision of energy for the formation of the eggs prior to laying may be important in control of the timing of breeding and in clutch size, and the histology of the thyroids does indicate a more active secretory condition during the nestbuilding and egg-forming periods than during the period of parental care.

The process of molt in birds has often been thought to be controlled by thyroid activity (Assenmacher 1958; Voitkevich 1966). Voitkevich (1966) has reviewed the considerable experimental work of his laboratory and concludes that a peak of thyroid activity commonly precedes molt by a few weeks, whereas the thyroidectomy studies of other workers often involved removal of the gland well after this peak in activity had occurred (Assenmacher 1958). Through long-term plucking experiments Voitkevich also found the regrowth of feathers to involve a hyperactive thyroid (large cells, small amount of follicular colloid). This observation is of interest since avian biologists have long noted in many kinds of birds a separation of the times of breeding and molting (Dwight 1900; Stresemann and Stresemann 1966), and pre-

sumably the non-overlapping schedules are an adaptation to the high energy demands of each of these processes. More direct evidence of the increased rate of energy expenditure in molting birds is available in the increase in body temperature in molting Bullfinches (*Pyrrhula pyrrhula*) (Newton 1968) and in the increased metabolic rate in several species of songbirds (King and Farner 1961; Blackmore 1969). The activity of the thyroid in relation to molt is of interest on two accounts: the correlation of thyroid activity changes with the onset of molt and the relationship between thyroid activity and the energy demands of molting.

Female Tricolored Blackbirds showed a small increase in thyroid activity prior to molt and in the first stages of feather replacement, and these results are in agreement with similar data from most other passerines (Wilson and Farner 1960; Voitkevich 1966) though not for some non-passerines (Raitt 1968; Ljunggren 1968). The evidence is less clear for increased activity in males, although the follicle size data are suggestive. A sexual difference in the thyroid-molt relationship is suggested in the changing activity of the thyroid through the molting period. Males show no change in nuclear or cell sizes but may have an increase in follicle size in early molt and then a decrease. Females, on the other hand, have somewhat more active thyroids both before and during early molt than in later stages of molt, and if the energy demands of the birds are adequately reflected in the thyroid measurements, this means that molt may not be an energetically demanding period of the annual cycle of Tricolored Blackbirds. The peak in thyroid activity at the onset of molt may then perhaps be a direct stimulus for molting rather than an indication of the energy-demanding requirements of molt.

SUMMARY

The histological activity of the thyroids of Tricolored Blackbirds was studied in birds collected in the field in central California. Little seasonal change in nuclear or cell size or in follicle diameter was evident, and thyroid activity did not increase with the cold temperatures of winter. Thyroid activity of adults increased in the early stages of nesting but not during the period of parental care. Neither sex showed a clear correlation of thyroid activity with molt. The histological pattern of thyroid activity generally showed no increase during stages of the annual cycle such as cold winters, parental care, or the middle of molt when ecological considerations suggest an increase in metabolic rate in Tricolored Blackbirds. Changes in metabolic rate in the blackbirds are probably in large part mediated through routes other than changing levels of thyroid activity.

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