ASPECTS OF THYROID HISTOLOGY IN BLACK-BILLED MAGPIES

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INVESTIGATIONS of seasonal changes in thyroid histology in wild birds have shown that increases in activity occur in the period preceding and during the annual molt (Davis and Davis, 1954; Assenmacher, 1958; Voitkevich, 1966) and in response to low temperature (Wilson and Farner, 1960; Kendeigh and Wallin, 1966). Involvement of the avian thyroid in migration and reproduction has also been suggested (Höhn, 1961). Although Phillips and van Tienhoven (1962) collated changes in thyroid activity with events within the breeding season in female Pintails (*Anas acuta*), information of this type is not available for other species. Few studies have considered sex differences in thyroid metabolism.

This paper reports observations of seasonal variations in thyroid histology in a nonmigratory population of Black-billed Magpies (*Pica pica hudsonia*) near Laramie, Wyoming. Special attention was accorded (1) changes in thyroid activity in connection with major events in the breeding season, and (2) differences in thyroid dynamics as related to sex and age.

METHODS

Thyroid glands were removed from 82 magpies collected for investigation of reproductive physiology between November 1964 and February 1967. Divisions of the annual cycle represented included winter (November–February), nest-building or "March-prelay" interval (March–April, unless laying or incubating eggs), egg-laying (April–May when known to be laying), incubation (April–June), parental or nestling (May–July), and postbreeding molt (July–September). Mean January temperatures during the study averaged –3.9°C; mean July temperatures averaged 19.3°C. Mean monthly temperatures during most of the study were equivalent except that temperatures in March 1965 averaged about 9°C lower than in March 1966. For this reason data from birds collected in March 1965 are considered separately from those obtained in March 1966.

Organs were excised in the field, placed immedately in Bouin's fluid, and were later embedded in paraffin by usual procedures. Selected 6-micron sections from the central part of the gland were stained with hematoxylin and eosin.

A number of histologic methods have been employed in assessing thyroid activity. Most of these depend upon height and/or appearance of epithelial cells (Davis and Davis, 1954; Oakeson and Lilley, 1960; Phillips and van Tienhoven, 1962). Percentages of epithelial cells (Wilson and Farner, 1960) or colloid (Saatman and van Tienhoven, 1964) according to the technique of Uotila and Kannas (1952) within the thyroid have also been used as criteria of activity. Barrington (1963: 185–186) suggests that avoidance of subjectivity and detection of minor fluctuations in thyroid activity might be more readily accomplished by methods where relative amounts of epithelium and colloid are considered.

In the present study epithelial cells, colloid material, and other tissue (including connective and vascular tissue) falling under nine fixed intersections of a Whipple ocular disc at high power (450×) were scored in randomly selected fields until a total of 100 was reached on a section from the center of one thyroid from each bird. Both glands were examined in several individuals and found to be similar. Resultant counts were equivalent to per cent of the gland composed of epithelium, colloid, and other material. Intersections falling on space between colloid and epithelial cells were scored as colloid (cf. Uotila and Kannas, 1952). This technique does not differ in essentials from the Chalkley (1943) method of tissue analysis which has been used by Roosen-Runge (1955) and Johnson (1966) for analysis of testicular tissue.

As this procedure apparently has not been employed in previous investigations of thyroid histology, a random sample of thyroids from 15 male magpies was also analyzed for per cent colloid according to the accepted and widely used method of Uotila and Kannas (1952) for purposes of comparison. Their method involves projection of tissue section images on a white paper upon which either two intersecting lines or a series of parallel lines have been drawn. The relative length of each line occupied by epithelium, colloid, and other tissue is directly related to the volume of the gland comprised of these materials (Uotila and Kannas, 1952).

Comparison of the two techniques revealed (1) a coefficient of correlation of $r = 0.79$, (2) insignificant differences ($P > .05$) between mean per cent colloid (means and standard deviations of 32.2 ± 9.5 according to the method of Uotila and Kannas and 29.4 ± 10.5 according to the present procedure), and (3) no significant differences ($P > .05$) according to analysis of variance when methods were compared and seasons of collection blocked. Thus the present method yields results mathematically comparable to those of Uotila and Kannas (1952) with considerably less effort and equipment.

It should be noted that changes in thyroid histology do not necessarily correlate with variations in other parameters of thyroid activity. Tashima (1965) reports that although *in vivo* thyroid uptake of radioiodine and conversion ratios of plasma radioiodine to protein-bound radioiodine differed significantly between control, cold-exposed, and hibernating golden hamsters (*Mesocricetus auratus*), these differences were not evident in histologic preparations. Saatman and van Tienhoven (1964) found that a histologic method permitted detection of thyroid responses to exogenous thyroid-stimulating hormone in smaller amounts than did methods involving determination of uptake and release of radioiodine. It thus seems prudent to compare results of the present study only with other studies of variation in thyroid histology.

Statistical tests to which data were subjected include homogeneity of variance (Snedecor, 1946: 249–252) of mean epithelial cell values among and between males and females, comparison of mean epithelial cell values ("t" test) among and between males and females, and analysis of differences in proportions of glands composed of epithelial cells and colloid material (chi-square contingency test) among males and females. The latter tests were based upon procedures outlined in Lewis (1966). Values used in the chi-square analysis represent total amounts of epithelium and colloid in glands from all birds obtained in a particular collection period. Chi-square was also used to compare proportions of epithelial and colloid material in recently fledged birds with those in postbreeding molting birds of the same sex.

RESULTS

Table 1 presents data on seasonal variations in thyroid histology in 79 birds according to age and sex. Additional data from three individuals obtained in March 1965 are presented below. Total amounts of thyroid tissue other than epithelium and colloid were small (averaging 6.02 per

TABLE 1
VARIATIONS IN THYROID HISTOLOGY IN BLACK-BILLED MAGPIES BY SEASON, SEX, AND AGE

	Winter	*March-prelay*[1]	*Laying*	*Incubating*	*Nestling*	*Post-breeding molt*
Adult males						
N	7	5	4	4	2	5
Average per cent epithelium	64.3±15.1[3]	64.6±6.7	62.0±10.2	69.0±10.4	62.5±7.8	62.0±4.6
Average per cent colloid[2]	31.1	29.0	30.3	27.5	33.0	31.8
Epithelial to colloid ratio	2.07	2.23	2.05	2.51	1.89	1.95
First-year males						
N	5	6	–	–	–	–
Average per cent epithelium	59.2±13.0	62.0±9.4	–	–	–	–
Average per cent colloid	35.2	28.8	–	–	–	–
Epithelial to colloid ratio	1.68	2.15	–	–	–	–
Adult and First-year females						
N	5	5	10	5	8	2
Average per cent epithelium	65.2±10.0	69.6±2.8	62.5±9.1	71.0±8.2	58.5±4.8	58.5±0.7
Average per cent colloid	31.8	27.4	33.4	26.4	36.3	36.0
Epithelial to colloid ratio	2.05	2.54	1.87	2.69	1.61	1.63
Recently fledged males						
N	–	–	–	–	–	4
Average per cent epithelium	–	–	–	–	–	49.3±2.4
Average per cent colloid	–	–	–	–	–	48.0
Epithelial to colloid ratio	–	–	–	–	–	1.03
Recently fledged females						
N	–	–	–	–	–	2
Average per cent epithelium	–	–	–	–	–	49.0±1.4
Average per cent colloid	–	–	–	–	–	50.0
Epithelial to colloid ratio	–	–	–	–	–	0.98

[1] Does not include data from March 1965.

[2] Variability not indicated because analyses were based upon epithelial values.

[3] Standard deviation.

cent in males and 4.02 per cent in females) and were not included in the analysis.

Adult males—Comparison of mean epithelial cell counts in males showed no real differences between birds from various collection periods. This is attributable to (1) minor actual fluctuations in mean values and (2) relatively high variability in epithelial cells counts during most collection periods. Variability was significantly greater ($P<.05$) in winter than either the March-prelay or postbreeding molt intervals. As an example of winter variability, four adult males killed simultaneously at a single trap location on 31 December 1966 had glands with 44, 56, 71, and 73 per cent epithelium respectively. Chi-square analysis of observed and expected proportions of epithelium and colloid showed no significant deviations among the six collection periods.

First-year males.—Mean values of thyroid epithelium in first-year males did not differ for the two collection periods. As in adult males, this reflected high variability within each sample. Proportion of epithelium was not significantly greater in the March-prelay sample than in winter. Thyroids of one first-year male collected in March 1965 contained 47 per cent epithelium.

Females.—As females of both age classes participate actively in breeding, data from all but recently fledged females were pooled. As noted in males, variation in mean epithelial cell values in winter was great, being significantly higher ($P<.05$) than variability in the March-prelay and nestling periods. Variability among laying females was also significantly greater ($P<.01$) than that in birds collected in the March-prelay and nestling periods. Mean epithelial cell counts were greater ($P<.005$) in both the March-prelay and incubation periods than during the postbreeding molt. Mean epithelial cell values in two females obtained in March 1965 were significantly less ($P<.005$) than in females collected in March 1966. Chi-square analysis of relative proportions of epithelium and colloid indicated significant differences ($P<.01$) between various collection periods with high epithelial values occurring in March-prelay and incubation periods and high colloid values occurring in nestling and postbreeding molt periods.

Sex differences.—Variation in mean epithelial values among females, although averaging somewhat less than in adult males during each collection period, was in no period significantly less than that observed in males. In addition only during the March-prelay interval were mean epithelial cell values significantly higher ($P<.05$) in females than in males. Proportions of epithelium and colloid were similar in males and females in all collection periods. Thyroid histology in recently fledged males corresponded to that in recently fledged females.

Recently fledged birds.—Thyroid activity in birds obtained shortly after fledging was uniformly low. Proportion of epithelium was significantly greater ($P<.001$) in molting males than in recently fledged males and was also greater ($P<.02$) in molting females than in recently fledged females.

Discussion

Variability in thyroid histology in male magpies suggests no clearcut pattern of activity relative either to environmental or physiological events. In particular no typical thyroid response to low temperature is apparent. In this context it is interesting that Davis and Davis (1954) report highly variable winter thyroid activity in a resident population of *Passer domesticus*. Kendeigh and Wallin (1966) similarly note that although thyroid weights (where increased weight indicates decreased activity) averaged less in winter than in summer in five of six species investigated, the differences in mean values were statistically significant in only one case. Oakeson and Lilley (1960) report greater individual variation in thyroid activity in a resident race of *Zonotrichia* than in a migratory race. Wilson and Farner (1960) and Farner (1964) point out that a valid thyroid response to cold has been shown in only about one-third of the species investigated. Thus although Höhn (1950) proposes that exposure of birds to cold is generally accompanied by increased thyroid activity and further suggests (Höhn, 1961) that a lack of thyroid response to cold in Mourning Doves (*Zenaidura macroura*) might necessitate autumnal migration to a milder climate in that species, it is apparent that at least some magpies and some members of many other species are able to compensate for relative inactivity of thyroids in winter by other means.

The lack of a thyroid response to low temperature was evident in female magpies obtained in winter and also in individuals collected in March of 1965 and 1966. Significantly less epithelial tissue was observed in thyroids from females in March 1965 than in 1966 in spite of temperatures averaging 9°C lower in 1965 than in 1966. Obviously clarification of the thyroid response to cold in magpies and other birds requires experimental attention. The possibility exists that thyroid metabolism during cold weather is related more to behavioral or nutritional factors than to temperature. Flickinger (1961), for example, found significantly heavier thyroids in domestic fowl (*Gallus domesticus*) kept in groups of six than in those kept in pairs. Other physiological mechanisms such as fat deposition, which is common in wintering birds at high latitudes (King and Farner, 1966), may obviate the necessity for a thyroid-generated increase in metabolic rate.

In contrast to males, female magpies exhibited two well-defined periods of increased thyroid activity. One occurred in the March-prelay interval and suggests possible involvement of the thyroid in reproduction or preparation for reproduction. Höhn (1961) reviews instances in which thyroid hormone augmented gonadal development in birds and Phillips and van Tienhoven (1962) report that female Pintails show relatively high thyroid activity prior to incubation. The second peak in thyroid activity in female magpies, that during incubation, preceded onset of the molt by about 4 weeks and suggests a possible relationship between the thyroid and the molting process. Assenmacher (1958) and Höhn (1950, 1961), for example, note that increased thyroid activity commonly precedes the molt. Farner (1964) however, suggests that the role of the avian thyroid in molting may be overemphasized. Increased thyroid activity during incubation might also be a metabolic corollary of heat loss through the incubation patch. Male magpies, which do not incubate, showed no significant increase in thyroid activity during the incubation period.

Published information comparing thyroid metabolism of fledgling birds with that of adults in the same season is not available for other species. Dawson and Allen (1960) found a decrease in thyroid activity in fledgling as opposed to nestling Vesper Sparrows (*Poocetes gramineus*), while Voitkevich (1966: 109–110) reports a decline in thyroid activity in cormorants (*Phalacrocorax carbo*) upon attainment of adult size.

SUMMARY

Histology of thyroid glands was studied in resident Black-billed Magpies near Laramie, Wyoming from winter through the postbreeding molt. No real differences in proportions of epithelium and colloid material were detected in males from different collection periods. This is attributable to high variability in thyroid activity during all collection periods with particularly high histologic variation in winter. Female thyroids also showed high variability in winter and during egg-laying, but had significantly high proportions of epithelium in nest-building and incubation periods. Fledgling magpies showed low thyroid activity.

It is proposed that magpies of either sex do not show a specific thyroid response to low temperature and that factors other than temperature may influence thyroid activity. In females, high thyroid activity preceding laying may reflect involvement of thyroxine in reproductive development; high activity during incubation may relate to heat loss through the incubation patch.

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