SEASONAL THYROID GLAND HISTOPHYSIOLOGY AND WEIGHT IN WHITE-TAILED PTARMIGAN

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ABSTRACT.—Histological examination of thyroid glands from White-tailed Ptarmigan collected at various times of the year showed that thyroid cell height and percentage of epithelium and stroma are significantly higher in adult than in subadult males, as well as females of both age classes. Changes in these two measurements as well as gland weight indicated a moderate increase of gland activity during the coldest months of the year and a more marked increase at the time of the summer molt. The level of thyroid function was also high at the time of the prenuptial and fall molts but this might in the first case reflect an increase of general activity in connection with reproduction and in the second a response to the onset of cold weather. Relative thyroid gland weight in White-tailed and Willow Ptarmigan was not high compared to other gallinaceous birds but comparison of histophysiological measurements for the first-named species with such data for other birds from the literature suggests a high overall level of thyroid function in the ptarmigan.—*Department of Physiology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7. State of Colorado Wildlife Research Center, P.O. Box 2287, Fort Collins, Colorado 80522.*
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SEVERAL reports of increased thyroid function in birds during cold periods have appeared (Assenmacher 1973). Although failures to find such changes have also been reported, this may be due to differences of species and climate. A striking example of a thyroid response to winter cold was described in the White-crowned Sparrow, *Zonotrichia leucophrys gambelii*, by Wilson and Farner (1960); the observed natural winter activation of the gland could be imitated by experimental exposure to cold and, as shown by Oakeson and Lilley (1960), White-crowned Sparrows of another subspecies that live all year round in a more equable climate than those of the race *gambelii* do not show such a seasonal cycle of thyroid function.

Assenmacher (1973) also reviewed the considerable evidence for the existence of periods of raised thyroid function preceding or during the molt. This too has not been observed in all species studied. But the concept of a thyroid-molt relationship is further supported by the induction of unseasonal molts by thyroid administration and the prevention of subsequent molts by previous thyroidectomy in several species (Assenmacher 1973). Findings in the Mallard, *Anas platyrhynchos* (Höhn 1949) may be cited as a good example of seasonal thyroid activity related to the molt. The major annual molt of these birds occurs about a month earlier in males than in females, and thyroid gland histology indicated a corresponding sex difference in the onset of the late spring period of raised thyroid function.

Ptarmigan winter in very cold habitats, and during the warmer part of the year they undergo three molts, thus molting more often than most other birds. One might therefore expect evidence of thyroid activation in winter and in relation to the molts. One might also expect their thyroid glands to be larger, in response to a high overall demand for thyroid hormone, than in related birds.

MATERIALS AND METHODS

Braun collected White-tailed Ptarmigan, *Lagopus leucurus*, by shooting or noosing followed by killing by chest compression, at various times of the year from 1966 through 1969 in several alpine areas of Colorado, described by Braun and Rogers (1971). The birds were sexed by internal examination and separated into adults (at least 13–14 months old) and subadults (less than 13–14 months old) on the basis of pigmentation on the 9th and 10th primaries, as described by Braun and Rogers (1971). Within a few hours after death the birds were weighed and their thyroid glands were dissected and fixed in buffered 10% formol saline and preserved in this until 1974 when Höhn further processed them. Thorax compression caused blood suffusion in the neck in some cases, impairing identification of the thyroids. Further, some thyroids removed from birds so killed showed internal hemorrhage that made histological evaluation very difficult.

The tally of suitable glands ultimately available for study represented 17 adult males, 14 subadult males, 17 adult females, 22 subadult females and 3 male and 3 female chicks, a few up to 5 weeks old, collected between 12 August and 9 September. Glands of the chicks were examined microscopically but not weighed. The thyroid glands of 11 Willow Ptarmigan, *Lagopus lagopus* (2 adult and 1 subadult male, 5 adult and 3 subadult females, aged by size of the bursa) shot 28 and 29 October 1974, near Fort Resolution, N.W.T., Canada, were also studied; formol saline-preserved glands of 10 male and 10 female Blue Grouse, *Dendragopus obscurus*, collected by F. C. Zwickel on Vancouver Island, Canada, and of individuals of three other species of gallinaceous birds shot in the N.W.T. or Alberta were used solely to determine body and thyroid weights.

The fixed glands were weighed, after blotting on filter paper, and embedded in paraffin. Sections 8 μ in thickness were cut at right angles to the long axis of the gland at three levels: halfway between the upper pole of the gland and its middle, at the middle, and halfway between the lower pole of the gland and its middle. Sections were stained with Harris' hematoxylin and eosin.

An examination of sections from three levels of the glands of all birds showed a very similar histological appearance in the three zones from any one gland as well as in both glands of any one bird. Only sections from the midzone were therefore used for further study.

Assessment of the level of thyroid function was based on two histological parameters: mean cell height based on micrometer measurements of 5 cells in each of the two glands per bird as they were encountered at random when the slide was moved across the stage of the microscope in a search for cells with particularly clearly defined limits, and percentage of epithelium and stroma—% E + S, measured on a projection of one central zone slide per bird by the method described by Uotila and Kannas (1952) as applied to avian material by Wilson and Farner (1961). In our case, magnification of the image was restricted by the limitation of the apparatus available to ×170; correspondingly, the two lines along which measurements were taken traversed a larger number of follicles, namely 80–90. At the magnification available, follicular spaces could be demarcated with certainty, but the other two tissue elements of the gland, epithelium and stroma, were not so readily distinguishable.

An attempt to measure only obvious epithelium yielded percentage figures that, compared to the data of others (for mammalian and avian material) were evidently gross underestimates. Therefore all "solid" tissue, i.e. epithelium and stroma combined, was measured to establish its percentage area in the sections. The figures so obtained differ only slightly from the values for epithelium alone, for as Uotila and Kannas (1952) point out, under experimental but nonpathological conditions the quantity of stroma is not of great importance. In their data for guinea pigs (normal and treated with thyroxin and thyroid stimulating hormone) it ranged only from 1.2 to 3.9% of the thyroid tissue, compared to a range of 57 to 80.8% for epithelium. Further, as the stroma consists mainly of blood and lymph vessels, its amount in different functional states, in the absence of thyroid disease, varies directly with the amount of epithelium.

Results

Sex and age differences in mean cell height and thyroid weight.—The means and their standard errors for the three measurements described above for the four sex and age classes of all White-tailed Ptarmigan available are shown in Table 1. The values for all three parameters are manifestly higher for adult males than for the other three groups, which are rather similar to one another.

Mean cell height in adult males is significantly higher (T-test P value less than 0.05) than in any of the other groups. Mean % E + S for adult males also differs at the highly significant level (P values less than 0.001) from that for adult and subadult females (subadult males were not compared statistically with adult males as their % E + S and gland weight values were obviously similar to those of females).

Thyroid weight is a much more ambivalent indication of gland function than cell height or % E + S as a weight increase may be due to increased storage of colloid in

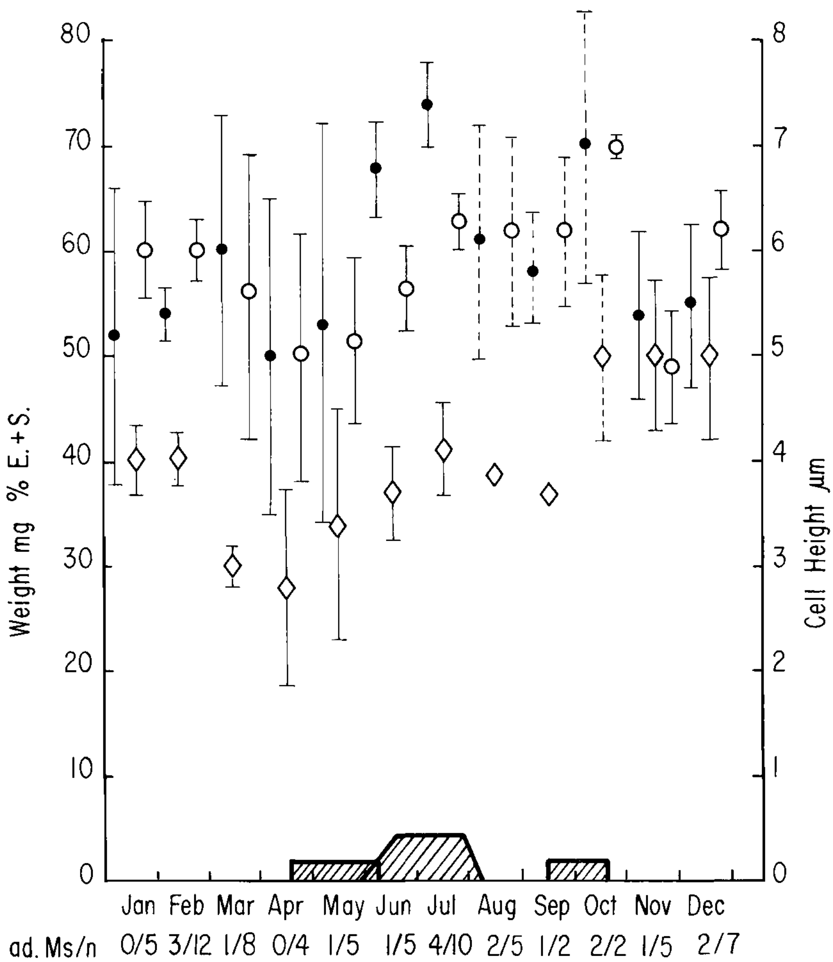

Fig. 1. Mean monthly cell height (black circles), % E + S (open circles), and thyroid weight (diamonds) of White-tailed Ptarmigan. Standard errors of means shown by bars except where data were insufficient for its calculation. The shaded areas above the abscissa indicate the three annual molts: from left to right, the prenuptial or spring molt, the major postnuptial or summer molt, and the fall molt. Figures below the month names indicate the number of adult males and the total number of birds examined in each month.

a relatively inactive gland or to cell hyperplasia in an active one. The higher mean gland weight of males than of adult and subadult females is of doubtful significance (T-test *P* values 0.200 and 0.100 respectively).

Hence adult males have a significantly higher cell height and % E + S than do birds of the other three groups, while their higher mean gland weight may or may not be significant. This indicates an overall higher level of gland function in mature as opposed to immature males and females of both age groups.

Seasonal differences in histological parameters of gland function and gland weight.—Initially data for cell height, % E + M, and gland weight were graphed separately according to date of collection for each of the four age and sex groups. Because of the rather small numbers in any one group, no clear picture of seasonal trends was obtained in this way. We therefore decided to establish means for each of

TABLE 1
MEAN EPITHELIAL CELL HEIGHT, PERCENT AREA OF EPITHELIUM AND STROMA, AND WEIGHT OF THE THYROID GLANDS IN WHITE-TAILED PTARMIGAN[1]

	Cell height μ	% E + S	Thyroid weight mg
Adult males	6.8 (15) ± 0.34	67.9 (17) ± 2.0	45.3 (16) ± 3.2
Subadult males	5.8 (14) ± 0.34	54.1 (14) ± 3.1	35.0 (14) ± 3.0
Adult females	5.8 (14) ± 0.33	53.1 (14) ± 3.1	39.2 (13) ± 3.0
Subadult females	5.7 (10) ± 0.38	58.7 (20) ± 2.1	37.9 (22) ± 2.7

[1] The figures in parentheses following the means indicate the number of measurements on which they are based.

these three measurements for each month of collection for all birds regardless of sex and age. This seemed justified because the seasonal cold affects all, and the seasonal incidence of the molts is essentially similar in all. The results obtained are shown in graphic form in Fig. 1. Since, as demonstrated above, values for adult male birds are somewhat higher than those for the other three groups, the number of adult males in relation to the total number of birds examined each month is indicated below the abscissa. As adult males show only moderately higher values than the other groups (Table 1) the moderate differences in the proportion of adult males to others in the monthly samples were disregarded in the study of Fig. 1 for evidence of seasonal trends.

It is evident that the values for all three parameters tend to change from month to month in a roughly parallel manner. Percent of epithelium in thyroid tissue (% E), is widely considered the most objective of the histophysiological measurements used in this study and reasons for considering % E + S as virtually identical to % E in nonpathological material were given in the preceding section. The degree of correlation between monthly mean % E + S and the corresponding data for cell height and gland weight respectively were therefore determined by calculation of the correlation coefficient between pairs of these values. The coefficient for % E + S and cell height was 0.5739 and for % E + S and gland weight, 0.6115; both values indicate correlation significant at the 0.05 probability level.

Since, as explained earlier, gland weight may not reliably indicate gland function, its good correlation with % E + S in this material was surprising. It suggests that the observed phases of increased gland weight were expressions of increased function and that glandular enlargement by colloid storage during periods of reduced function were absent or minimal.

Interpretation of seasonal trends was based on % E + S and cell height though, except in November, it is supported by similar trends in gland weight.

The low values for April and November may be taken as representing periods when the level of gland function is basal in the sense of being unaffected by particular seasonal stimuli such as marked cold, sexual activity, and molts. Gland weight, unlike % E + S and cell height, was high in November. It seems reasonable to suggest that gland weight may be less labile than the other features measured and that there may not have been time for it to decline from the October peak discussed below.

Compared to the basal level a very moderate increase of gland activity occurs during the coldest months of the year, i.e. December through February. Higher levels are reached in July and again in October. The July peak is preceded by progressively higher levels in May and June and it seems logical to relate it to the major of the three annual molts, which extends from late June to early August.

TABLE 2

RELATIVE THYROID WEIGHT IN TWO PTARMIGAN SPECIES AND SOME OTHER GALLIFORM BIRDS

Species	Number examined	Mean body weight g	Relative thyroid weight: Mean	Relative thyroid weight: Range	Source and remarks[1]
Japanese Quail *Coturnix coturnix*	3 6	113 123	0.0057 0.0116	0.0050–0.0063 0.0080–0.0165	[2] Bred in captivity
Bobwhite *Colinus virginianus*	9	195	0.0093		[3]
Spotted Wood Quail *Odontophorus guttatus*	9	323	0.0062		[2]
Gray Partridge *Perdix perdix*	4	385	0.0080	0.0042–0.0146	
White-tailed Ptarmigan	52	348	0.0118	0.0053–0.0163	
Willow Ptarmigan	27	540	0.0049	0.0020–0.0073	
Spruce Grouse *Canachites canadensis*	2	655	0.0037	0.0035 & 0.0039	
Ruffed Grouse *Bonasa umbellus*	10	668	0.0049	0.0025–0.0095	
Sharp-tailed Grouse *Pediocetes phasianellus*	3	770	0.0085	0.0078–0.0096	
Blue Grouse	20	965	0.0090	0.0055–0.0152	
Domestic Hen *Gallus gallus*	Not Stated	2,330		0.0040–0.0074	According to various authors

[1] Where no source is shown in the last column, present authors.
[2] Hartman and Brownell 1961.
[3] Hartman 1946.

Similarly the October peak, preceded by indications of somewhat decreased function in August and September, appears to be related to the fall molt, though it might, in part or in whole, represent a response to the onset of colder weather.

A reflection of the spring or prenuptial molt (mid-April to early June) in the level of gland function is less certain as the rising level of gland activity observed in May and June might well relate to the general increase of activity associated with display, mating, and breeding rather than to this molt, or it might relate to both phenomena.

On the basis of the histophysiological measurements used, the evidence for increased thyroid activity in association with the midsummer molt is clear, but it is more ambiguous in connection with the other two molts, while that for an association between seasonal cold and level of thyroid function is less convincing.

The glands of the six chicks were, by histological criteria, somewhat more active than those of fully grown birds collected during the same period; mean cell height for both sexes was 7.5 μ and % E + S was 64 for males and 75 for females. The metabolic demands for growth and the probability that the partially developed plumage of these birds was a less efficient insulator than that of older birds make this finding an expected one.

The mean values for the October sample of Willow Ptarmigan for both age groups of the two sexes were: males, cell height 5.6 μ, % E + S 50.7, weight 31 mg; for females, cell height 4.6 μ, % E + S 55.1, weight 38.3 mg. These values are somewhat lower than those in the same month in White-tailed Ptarmigan.

Thyroid gland weight in ptarmigan and other galliform birds.—In order to compare gland weights in species of widely different size, relative thyroid weights, i.e.

gland weights expressed as a percentage of body weight, in two species of ptarmigan are compared with data for such other galliforms as we were able to collect and gather from the literature in Table 2. Further details on dates and localities of collection and gland weights in particular age and sex groups are available from the senior author.

The value for White-tailed Ptarmigan is somewhat but not very strikingly high compared to that observed in the other species, but it is rather low in Willow Ptarmigan. The evidence in Table 2 as a whole indicates that ptarmigan do not have unusually high relative thyroid weights.

Discussion

The higher cell heights and percentages of E + S found in adult males compared to younger males and to females of both sexes may well be an indirect effect of greater androgen secretion in the former, i.e. related to their energy-consuming territorial display and mating behavior. According to Choate (cited *in* Höhn 1969: 78) males of this species are on territories from mid-June to mid-July but sexually related activities probably occur during good weather at some time of the day over a much longer period of the year. Watson and Jenkins (1964) reported this in Willow Ptarmigan (Red Grouse) in Scotland. In Scottish Rock Ptarmigan (*Lagopus mutus*) Watson (1972) recorded sexual behavior in every month, frequently in October to May and especially from January to April.

A comparison of the range of mean thyroid cell heights and % E + S observed in White-tailed Ptarmigan with reported values for other species from the literature is of interest. For cell height the values in μ are: White-tailed Ptarmigan 4.2–8.3; Mallard 3–4.3 (Höhn 1949); Red Fody (*Foudia madagascariensis*) 0.5–4.5 (Legendre and Rakotondrainy 1963); English Sparrow (*Passer domesticus*) 0.09–6.9 (Kendeigh and Wallin 1966); Tricolored Blackbird (*Agelaius tricolor*) 1.7–7.5 (Payne and Landolt 1970); three European passerines, *Fringilla coelebs*, *Parus major*, and *Lanius collurio* 3–5.2 (Munteanu and Baratz 1967). Comparison of % E + S of White-tailed Ptarmigan with published values for % E in other species shows the following: White-tailed Ptarmigan 31–79, 55.4 mean; Gambel's Quail (*Lophortyx gambelii*) 34 mean (Raitt 1963); Black-billed Magpie (*Pica pica*) 59.2–69 (Erpino 1968); White-crowned Sparrow 46–88 (Wilson and Farner 1960).

The comparison shows histophysiological evidence of a high general level of function in White-tailed Ptarmigan compared to the majority of the other species studied. This is what might be expected in view of the higher metabolic rates for two other species of ptarmigan, than would be predicted by body weight metabolic equations (based on data from a variety of other birds), found by West (1971).

Our finding of only slight activation of the thyroid during cold weather is also explicable on the basis of West's (1971) finding of a lower critical temperature (the temperature at which heat production is raised to balance heat loss) in Willow Ptarmigan than in any bird so far tested. This implies that their winter plumage provides such effective insulation that stimulation of metabolism would only occur when the external temperature falls below this extraordinarily low critical point.

Furthermore, Lee and Lee (1937) showed in experiments on geese that elevation of the metabolic rate in response to external cold is not dependent on participation in this response of the thyroid gland.

A more marked phase of raised thyroid function associated in time with a molt was evident in connection with the major of the three annual molts of White-tailed Ptarmigan. The existence of a similar association with the other two molts is not denied by the data in Fig. 1, but the evidence in favor is less ambiguous than that in connection with the summer molt.

To what extent can the thyroid activation be considered causally related to the more or less synchronous molt? In Willow Ptarmigan Novikov and Blagodatskaia (1948) found that addition of thyroid powder to the feed of males after the fall molt resulted in an unseasonal molt and that thyroidectomy before the normal time of the prenuptial molt could postpone this molt.

More experimental observations have been made on the thyroid molt relationship in domestic fowl than in ptarmigan; as ptarmigan are related to domestic fowl, findings in the latter are relevant to the present issue. Administration of dried thyroid or thyroxine leads to an unseasonal molt in about a week; it is easier to induce an unseasonal molt with thyroxine during the normal molting period than at other times of the year, the metabolic rate increases during the molting period (the authorities for these statements are given in Sturkie 1965). Further, there is histophysiological evidence of thyroid activation before the onset of the molt (Zawadowsky 1928) and surgical thyroidectomy prevents subsequent molts (Crew 1927), although administration of the antithyroid drug, thiouracil, merely postpones it (Glazener and Jull 1964). The failure of Tanabe et al. (1957) to observe an increase in the iodine uptake and release (indications of thyroid function) of molting hens induced to molt with progesterone, seems to be effectively countered by the demonstration of a seasonal peak in iodine uptake and conversion into protein-bound (hormonal) iodine in domestic duck within the month prior to the onset of a molt; Astier et al. (1970).

The evidence for a causal thyroid molt relationship in the chicken is clearly adequate; such information as is presently available supports its existence in ptarmigan as well.

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