HORMONAL INDUCTION OF FEATHER PIGMENTATION IN PTARMIGAN

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ABSTRACT.—Willow Ptarmigan (*Lagopus lagopus*) in the winter plumage, on a regime under which control birds grew white feathers in plucked areas, grew pigmented feathers in the plucked areas, when injected with posterior pituitary extract or α MSH, LH, or FSH (only effective in females). White-tailed Ptarmigan (*Lagopus leucurus*), patch-plucked and released into the wild after implantation with a hormone-cholesterol mixture, grew pigmented feathers after implantation with α MSH, TSH, thyroxine (in one out of two birds tested), and an FSH/LH mixture. Controls implanted with cholesterol grew only white feathers. Both sets of experiments were carried out in winter.

A scheme of the control of ptarmigan plumage colors, which assumes that MSH and gonadotrophins and, in males, testosterone are involved in the natural control mechanism, is proposed.

ALL three species of ptarmigan have a white winter plumage that is followed by a pigmented summer (or nuptial) and a pigmented fall plumage. In Willow Ptarmigan (*Lagopus lagopus*), only the male further develops a pigmented breeding plumage preceding the summer plumage (Johnsen 1929). This plumage is not recognized as a distinct entity by some.

The experiments of Høst (1942) and of Novikov and Blagodatskaia (1948) demonstrated that daylength is the main external factor that determines timing of the molts as well as the feather pigmentation involved in the seasonal color changes. They reported that artificial long days could induce a premature molt into the pigmented breeding or summer plumage in captive Willow Ptarmigan, in spite of low normal winter temperatures.

Phenological observations on Rock Ptarmigan (*Lagopus mutus*) (Salomonsen 1939, Hewson 1973, Watson and Moss 1973) suggest that ambient temperature can also affect molts and feather color. Because long days can induce a premature spring molt in Willow Ptarmigan kept at a low temperature (Høst 1942, Novikov and Blagodatskaia 1948), the effect of temperature must be secondary to that of daylength. It is unlikely that external factors act directly on feather follicles and more probable that their effect is mediated by hormones.

Novikov (1939) was the first to investigate the role of hormones in ptarmigan molts and plumage color. He removed the gonads of Willow Ptarmigan of both sexes in winter and reported that these birds later molted at the usual time into the normal succeeding pigmented plumage. Stokkan (1979), however, found that the male breeding plumage of these birds depends upon testosterone. His castrates, like those of Novikov, were operated on in winter, but after the next molt they assumed the summer, not the breeding, plumage. Possibly Novikov did not distinguish these two plumages.

Novikov and Blagodatskaia (1948) investigated the role of the thyroid in the control of Willow Ptarmigan plumages. They reported that in three birds thyroidectomized during the spring molt the new feathers were white, but in controls they were pigmented. Feeding 200 mg thyroid powder daily to intact winter birds induced an unseasonal molt followed by pigmented new feathers, while controls under these

conditions grew white feathers. A daily dose of 50 mg of desiccated thyroid induced similar though less intense effects. They concluded that thyroid hormones control molts as well as feather pigmentation; with this view, however, it is difficult to explain why feathers grown after the fall molt are unpigmented, for it is known that avian molts in general are associated with increased thyroid secretion. In particular, Höhn and Braun (1977) found histological evidence of increased thyroid secretion in relation to all the molts in White-tailed Ptarmigan (*Lagopus leucurus*). If thyroid hormone alone controlled feather color, there would appear to be enough of it in the circulation about the time of the fall molt to cause pigmentation of the new winter feathers growing at this time.

These facts suggest that at least one nonthyroid hormone is also involved in the induction of feather color in both sexes of ptarmigan.

Materials and Methods

To study the effect of hormones on ptarmigan feather pigmentation independently of their possible effect on molting, we kept Willow Ptarmigan in the winter plumage on a regime of 8 h of light, 16 h of dark. Feathers were plucked in a small patch of about 3 × 3 cm on the head, neck, or breast. Within 2 weeks new feathers grew in these patches, and in untreated birds or controls injected only with hormone solvents the new feathers were always white. Experimental birds were injected daily with hormone solutions (amounts used are shown in Table 1) into the breast muscles for 8–10 days, i.e. until the new feathers had pushed through the skin. The birds were kept indoors in small wire cages measuring about 60 × 60 × 60 cm, one or two birds per cage, at a temperature not exceeding 20°C. They were fed *ad libitum* on a commercial pheasant breeder mixture supplemented daily with frozen peas, water, and grit.

The main difficulty with these simple experiments was the supply of birds. Willow Ptarmigan were captured in a wintering area about Uranium City, Saskatchewan by means of a noose on an extensible pole, as described by Zwickel and Bendell (1967). Not only would the birds often not allow sufficiently close approach, but about half of those caught died during the first 3 days of captivity. Those that survived this initial period thrived. When only one ptarmigan was being kept, it invariably died within 2–3 weeks. When a Japanese Quail (*Coturnix coturnix*) was caged together with a lone ptarmigan, the latter lived for over 2 yr until it died as a result of an accident.

To increase the experimental samples, White-tailed Ptarmigan were also used. These birds will not survive captivity but are much easier to catch than Willow Ptarmigan. Captured White-tails were marked individually, patch-plucked, implanted with solid pellets of a hormone-cholesterol mixture, released, and reobserved later through binoculars to ascertain the color of the new feathers. Pellets were prepared by Höhn and sent to Braun, who carried out these experiments in Colorado. As shown in Table 1, control implants of cholesterol resulted only in growth of white new feathers, among which there were (in one bird only) two faintly colored feathers. Finally, Höhn, with the cooperation of K.-A. Stokkan, was able to conduct a series of injection experiments on Willow Ptarmigan reared in captivity at the Field Station of the University of Tromsö (Norway) in 1978.

The hormone preparations used were: "Shizume preparation," made from mammalian posterior pituitary powder (Armour Pharmaceutical Co.) as described by Shizume et al. (1954); α MSH (synthetic) supplied by Ciba Co., Basel; sodium laevo thyroxine; thyroid stimulating hormone, TSH (Nordic Pharmaceuticals, Laval, Quebec); bovine follicle stimulating hormone, FSH (NIH BI), and bovine luteinizing hormone, LH (NIH BI), both supplied by the U.S. National Institutes of Health.

As Rust (1965, 1969) had shown that only MSH is required to induce the brown summer hair color in the short-tailed weasel (*Mustela erminea*), we speculated that hormones that affected ptarmigan feather color such as thyroid (reported to have this effect by Novikov and Blagodatskaia 1948) and gonadotrophins (which we found effective) might owe their effect merely to an increased secretion of MSH.

In the absence of sufficient numbers of ptarmigan, the experiment to test this possibility was carried out on leghorn hens (*Gallus gallus*) (birds of the same family as ptarmigan), which were injected daily with 0.6 mg of an FSH/LH mixture or with 0.3 mg thyroxine for 16 days. A blood sample was then withdrawn from the experimental as well as the solvent-injected controls; all were then killed, their pituitaries removed, and these glands as well as plasma samples from each bird freeze-dried and sent to Dr. A. J. Thody for assay of their α MSH content by the method described by Thody et al. (1975). The pituitary MSH content and the plasma MSH levels were found to be somewhat higher in the control as

TABLE 1. Effect of hormone administrations on Willow and White-tailed Ptarmigan. Willow Ptarmigan injected daily, doses shown. White-tailed Ptarmigan implanted with compressed Shizume preparation (first column) or hormone mixed with cholesterol. Color of new feathers: − = white; +− = colored less intensely than natural feather; ++ = color as intense as in natural feathers. Abbreviations: Rep. = repeated; prep. = preparation, freed of MSH (see text); chol. = cholesterol; F = female; M = male (birds not so marked could not be sexed); IU = international unit; Fe = a particular female, used for a number of experiments.

INJECTIONS: WILLOW PTARMIGAN

Posterior pituitary		α MSH		Thyroxine		FSH		LH	
Shizume prep. 3.3 mg	++	F 0.1 mg	++	50 μ	++	F freed of MSH	++	F freed of MSH	+++
Shizume prep. 3.3 mg	++	F 0.1 mg	++	M 50 μg	−	F 1 mg	+	M 1 mg	+++
Shizume prep. 3.3 mg	++	F 0.1 mg	++	Rep. on same bird	−	Rep. on same bird	+	M 1 mg	+++
		Rep. on same bird	++	F 50 μg	−	M 1 mg	−	F 1 mg	+++
		M 0.1 mg	++	Rep. on same bird	−	Rep. on same bird	−	F 1 mg	+++
		F 0.1 mg	++	Fe 50 μg	−				
		M 0.1 mg	−	Fe 100 μg	−				
		Rep. on same bird	++	Fe thyroid powder 0.33	−				
		Fe 0.1 mg	++	mg per os					
		Rep. on same bird	++						

IMPLANTATIONS: WHITE-TAILED PTARMIGAN

Posterior pituitary		α MSH		Thyroxine		FSH/LH mixture		Controls	
Shizume prep. 20 mg	−	2 mg in 24 mg	−	0.5 mg in 60 mg	−	2 mg FSH, 4 mg LH		60 mg chol.	−
Shizume prep. 20 mg	+−	2 mg in 24 mg	+−	0.5 mg in 60 mg	+−	in 60 mg	++	60 mg chol.	−
		2 mg in 24 mg	+−			2 mg FSH, 4 mg LH		60 mg chol.	−
		2 mg in 24 mg	+−	TSH		in 60 mg	+−	60 mg chol.	+−[a]
		2 mg in 24 mg	−	2 IU in 65 mg	+−			60 mg chol.	−
				2 IU in 65 mg	+				
				2 IU in 65 mg	++				

[a] Only two colored feathers.

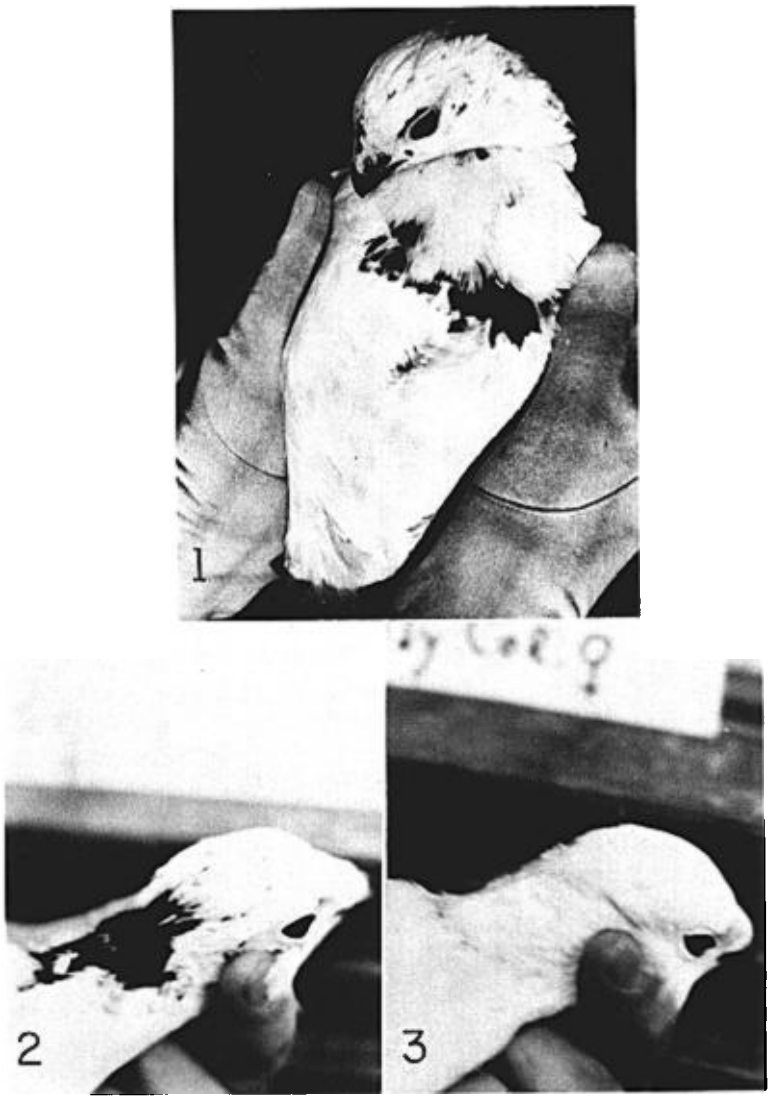

Fig. 1. 1. Willow Ptarmigan treated with α MSH while new feathers were growing in a plucked area of the breast; feathers that appear black in the photo were actually brown with darker cross bars. 2. Willow Ptarmigan treated with LH; the new feathers were uniformly dark brown. 3. Willow Ptarmigan control bird plucked in the same area as number 2; the new shorter feathers are unpigmented.

compared to the thyroxine or gonadotrophin treated hens. The reader must judge the relevance of these findings to ptarmigan.

When positive results were obtained with gonadotrophins and TSH in ptarmigan, we wondered whether the effects might actually be due to MSH or ACTH present as contaminants in preparations of the anterior pituitary hormones. To test this possibility, we applied solutions of TSH, LH, and FSH, respectively, to a Sephadex G50 column and collected the outflow in 10 fractions. The first of these contained substances of high molecular weight (e.g. one of the gonadotrophins), while later fractions, which hold smaller molecular weight substances, would be expected to contain MSH and ACTH. For each fraction, 0.5 ml was injected subcutaneously into a frog (*Rana pipiens*) kept before and during the experiment in the light and on a white background. Only those frogs injected with the later effluent

TABLE 2. The effect of daily light duration on the plasma α MSH level in a Willow Ptarmigan.

Date of sampling	Hours of light/24 h	Plasma α MSH/pg ml
10 April	8	217
17 April	16	737
20 April	16	1,834

fractions showed darkening of the skin, presumably due to MSH or ACTH or to both. The minimal dose of MSH that caused darkening in these frogs was determined by this rough-and-ready assay (used because a linear log dose response relationship was not obtained in trials of the bioassay of Shizume et al. 1954) to be about 0.15 μg. On this basis, we estimated that the anterior pituitary hormones used contained frog skin darkening substance equivalent to this amount of MSH in 0.05 IU of TSH 0.02 mg of FSH, and 0.03 mg of LH. Using the early fractions from the respective columns that had thus been freed of MSH and ACTH, one Willow Ptarmigan was injected with FSH and another with LH. As both birds grew pigmented new feathers (Table 1), it is clear that the effect of the two gonadotrophic preparations was not due to contamination with other hormones. An opportunity to test purified TSH in this manner did not arise, but, as injections of the "MSH ACTH" fractions obtained from LH and FSH, respectively, did not induce feather pigmentation when injected into a ptarmigan, it is unlikely that the TSH implanted in White-tailed Ptarmigan owed its effect to contamination with MSH and ACTH.

RESULTS

The effects of various hormones on the color of new feathers grown while the bird was under the influence of the hormone injected or implanted are shown in Table 1 and for some hormones in Fig. 1. Feathers recorded as colored in the case of Willow Ptarmigan were brown with darker, almost black, crossbars; they were colored like natural feathers, but we were unable to decide whether they were nearer to the summer or to the fall plumage in appearance. Birds treated with LH, however, grew feathers that were uniformly colored dark brown and thus did not resemble feathers of any of the normal plumages. In White-tailed Ptarmigan, Braun recorded new colored feathers as resembling the fall plumage, a plumage that in these birds shows virtually no sex differences.

Our findings indicate that posterior pituitary extract, α MSH, FSH (at least in females), and LH can all induce unseasonal feather pigmentation in Willow Ptarmigan. In White-tailed Ptarmigan feather pigmentation was induced by α MSH, a mixture of FSH and LH, or by TSH.

DISCUSSION

Before discussing the effect of thyroxine, a personal communication from Dr. Stokkan of relevant unpublished results must be reported. He found that, in six Willow Ptarmigan injected daily with 100 μg of thyroxine and in others injected daily with 50 μg of thyroxine, there was no pigmentation of the new feathers. The same result was obtained in six birds injected daily with 100 μg or 50 μg or triiodothyronine T3. In consideration of these findings, as well as those shown in Table 1, Thyroxine T4 and T3 must be considered to have no effect on feather color in this species. No definite conclusion about the effect of T4 in White-tailed Ptarmigan can be drawn from our two experiments.

Our results with thyroid agents contradict those reported by Novikov and Blagodatskaia (1948), who reported that the administration of thyroid powder induced feather pigmentation. We can see only two explanations for this discrepancy: either

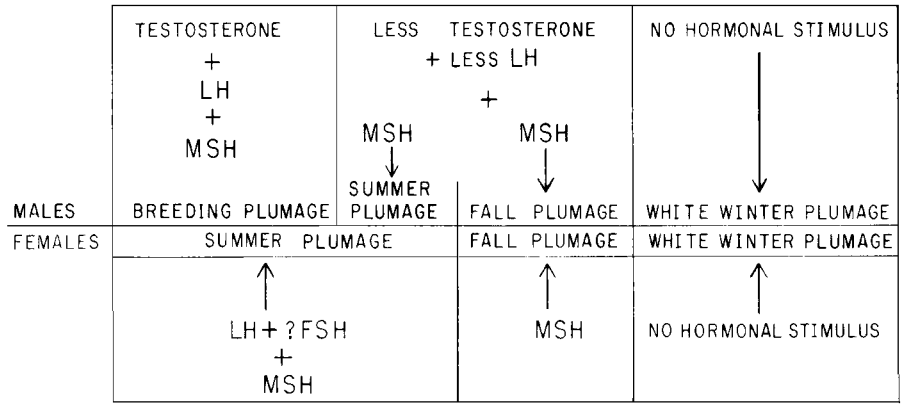

Fig. 2. A suggested scheme of the hormonal control of Willow Ptarmigan plumage color.

thyroid powder contains a hormone other than T4 and T3, which is unlikely, or the high dosage of thyroid used by Novikov and Blagodatskaia (50 mg and even 200 mg per day, compared to a human dose of 120–180 mg) may have had pharmacological rather than physiological effects.

TSH has been tested only in White-tailed Ptarmigan, but it is probably also effective in the other species considering the close relationship between them. Because T3 and T4 are ineffective, the effect of TSH must be attributed either to another thyroid hormone or to a direct action not involving the thyroid.

Our data on FSH in Willow Ptarmigan are too few to establish whether only females respond to this hormone or whether the trace amount of LH (0.01 units/mg) present in the preparation used was just enough to cause feather pigmentation in females but not in the larger males.

Novikov and Blagodatskaia (1948) reported that three thyroidectomized Willow Ptarmigan grew white feathers under daylength conditions in which control birds grew pigmented feathers. Apart from the small number of their experimental birds, they did not state whether or not their controls were subjected to sham operations. If not, the effect observed in their experimental birds might have been due to the nonspecific trauma of the operation; moreover, their observations have not to our knowledge been confirmed. If their observations were confirmed, it would follow that nonthyroid hormones effective in causing feather pigmentation need a facilitating or permissive effect of some thyroid hormone to manifest their action on pigmentation.

The experiment to test whether thyroxine or gonadotrophin injections would raise pituitary or plasma MSH concentration in hens failed to demonstrate any such effect. We feel that this makes it unlikely that it occurs in ptarmigan, but the point can only be established in experiments on ptarmigan.

An observation made only on a single Willow Ptarmigan so far indicates that long days induce a marked elevation of plasma MSH. Radioimmunoassays (Thody et al. 1975) carried out by Dr. A. J. Thody on freeze-dried plasma samples indicate α MSH values in relation to the number of hours of light per day to which the bird had been exposed before each sample was taken (Table 2). These strongly suggest that MSH secretion in ptarmigan, like their feather color, is affected by daylength

and is a point in favour of the view that this hormone plays a role in the natural control of feather pigmentation.

Leaving aside the possible involvement of a thyroid factor and of TSH, we may describe the role of the other hormones found to be effective in feather pigmentation in Willow Ptarmigan along the lines shown in Fig. 2.

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LITERATURE CITED

HEWSON, R. 1973. The molts of captive Scottish ptarmigan (*Lagopus mutus*). J. Zool. 171: 177–187.

HÖHN, E. O., & C. E. BRAUN. 1977. Seasonal thyroid gland histophysiology and weight in White-tailed Ptarmigan. Auk 94: 544–551.

HØST, P. 1942. Effect of light on the molts and sequences of plumage in the Willow Ptarmigan. Auk 59: 388–403.

JOHNSEN, S. 1929. Draktskiftet hos Lirypen (*Lagopus lagopus* L.) i Norge. Bergen Mus. Aarbok: 66–81.

NOVIKOV, B. G. 1939. Analyse der Saisonveränderungen am Federkleid bei (*Lagopus lagopus* L.). C.r. Acad. Sci. USSR 25: 554–556.

———, & B. I. BLAGODATSKAIA. 1948. [The mechanism of the development of protective seasonal coloration.] Dokl. Aka. Nauk USSR 41: 577–580.

RUST, C. R. 1965. Hormonal control of pelage cycles in the short-tailed weasels treated with melatonin. Science 165: 921–922.

RUST, C. R. 1969. Hair color, molt and testis size in male short-tailed weasels treated with melatonin. Science 165: 921–922.

SALOMONSEN, F. 1939. Molts and sequences of plumages in the Rock Ptarmigan (*Lagopus mutus*, Montin). Vidensk. Medd. Dansk. Naturh. Foren. 183: 95–142.

SHIZUME, K., A. B. LERNER, & T. B. FITZPATRICK. 1954. *In vitro* assay for the melanocyte stimulating hormone. Endocrinology 54: 533–560.

STOKKAN, K. A. 1979. Testosterone and daylength dependent development of comb size and breeding plumage of male Willow Ptarmigan (*Lagopus lagopus lagopus*). Auk 96: 106–115.

THODY, A. J., R. J. PENNY, D. E. CLARK, & C. TAYLOR. 1975. Development of a radioassay for melanocyte stimulating hormone in the rat. J. Endocrin. 67: 385–395.

WATSON, A., & R. MOSS. 1973. Molts of wild Scottish Ptarmigan in relation to sex climate and status. J. Zool. 171: 207–223.

ZWICKEL, F. C., & J. F. BENDELL. 1967. A snare for capturing Blue Grouse. J. Wildl. Mgmt. 21: 202–244.