THE CONDOR

VOLUME 66 NOVEMBER-DECEMBER, 1964 NUMBER 6

TESTOSTERONE-INDUCED NUPTIAL FEATHERS IN PHALAROPES

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Although hormonal control of plumage dimorphism has been investigated in several species of birds, no study has been published on species in which the female wears the bright nuptial plumage and the male the dull plumage (Mathews, 1960). Typically the nuptial plumage of the male is more brilliantly colored than that of the female, although in many species, their plumages are quite similar. In the Phalaropodidae, Jacanidae, and Rostratulidae, however, the female is more colorful than the male and there is a reversal in the role of the sexes in courtship and incubation behavior (Gilliard, 1958).

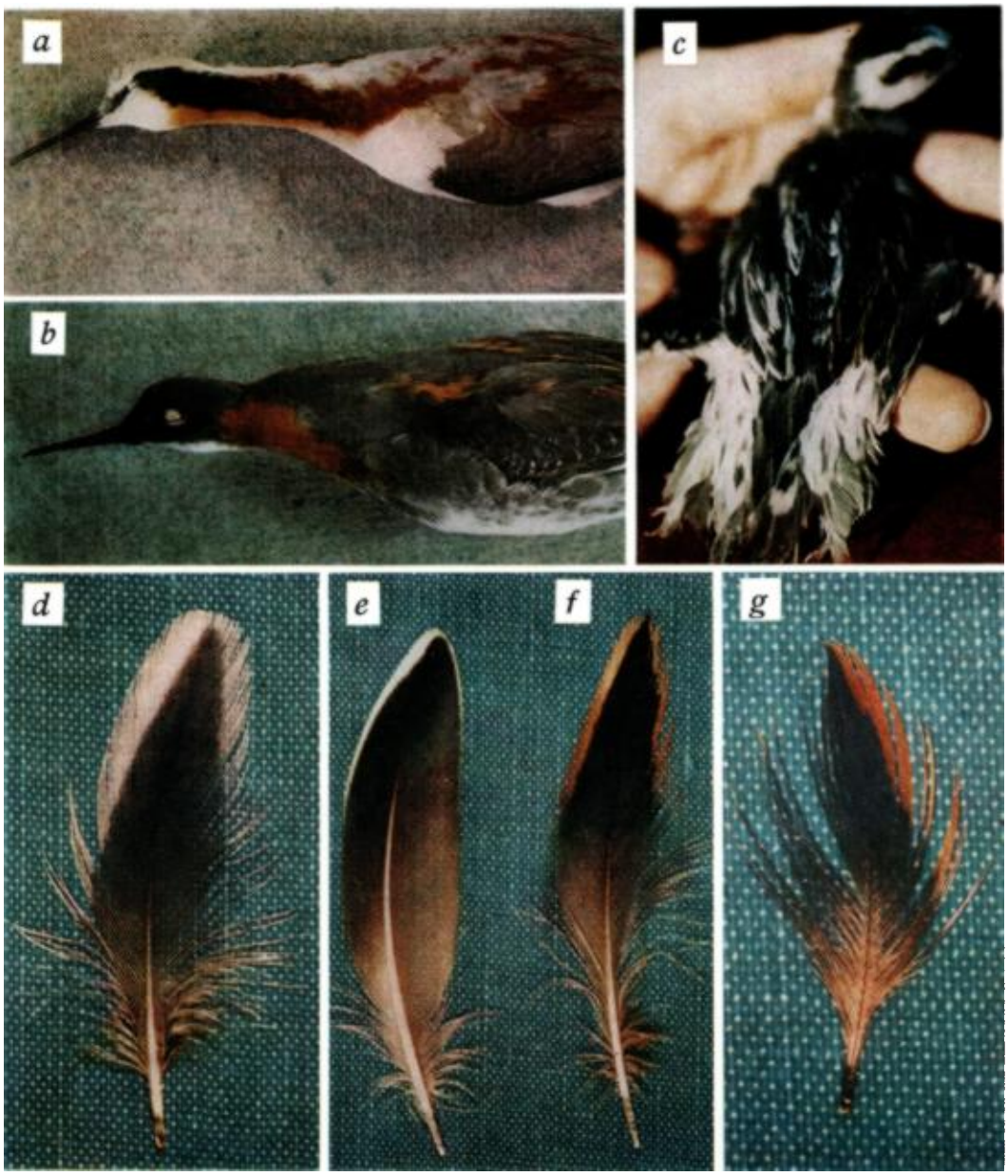
In order to determine what hormones, if any, are involved in the control of plumage dimorphism in phalaropes, experiments were conducted from June, 1961, through August, 1963, on captive Wilson Phalaropes (*Steganopus tricolor*) and Northern Phalaropes (*Lobipes lobatus*).

Previous studies have shown that a wide variety of hormones may be responsible for the difference between male and female plumage in different species of birds. For example, the action of estrogen in domesticated Brown Leghorn chickens, pituitary and estrogenic hormones in certain African weaver finches, and androgens in the Ruff (*Philomachus pugnax*) have been shown to bring about sex differences (Witschi, 1961). In the phalarope, androgenic hormones, probably produced by the ovary, were suspected to be an essential factor in the development of the colorful female nuptial plumage, because the growth of this plumage is correlated with the onset of aggressive courtship behavior at the start of the breeding season.

METHODS

Cages and maintenance.—To test the hypothesis that androgen produces the brilliant nuptial plumage in female phalaropes, Northern and Wilson phalaropes of both sexes were captured in northwestern Montana. Trapping was accomplished by a horizontal mist net method (Johns, 1963) or by the method of Serventy, Farner, Nicholls, and Stewart (1962). Sixty-one adult Wilson Phalaropes in nuptial plumage were captured in May and June of 1961 and 1962, and thirteen, eight of which were juvenal birds, were captured in May, June, and July of 1963. In addition, six Northern Phalaropes in winter plumage were captured in October, 1962. Many of these served as experimentals, a number served as controls, some were killed for anatomical studies, and a few were utilized exclusively in other experiments (Johns and Pfeiffer, 1963).

All birds were maintained in individual cages for periods of time ranging from one to five months. Cages measured 24 x 16 inches in floor space and were constructed of wood covered by ¼-inch mesh hardware cloth. This gauge proved to be sufficiently small to keep the head of the bird inside while allowing the long, thin bill to pass freely through. Toenail loss and crippling, common to wild birds maintained in wire floored cages, was minimized by the use of several layers of newspapers over the cage floors. Each cage was provided with a small, flat water pan (8 x 12 x 2 inches) which was cleaned and refilled with fresh water twice daily at feeding times.



PLUMAGES OF PHALAROPES

a, Wilson Phalarope, female in nuptial plumage.
b, Northern Phalarope, female in nuptial plumage.
c, Female Northern Phalarope with left scapular region regrown without hormone treatment and right region regrown during treatment with testosterone propionate.
d-g, Contour feathers of Northern Phalarope: *d*, winter plumage, wild grown; *e*, regrown in captivity without treatment; *f*, grown under testosterone propionate; *g*, nuptial plumage, wild grown.

The normal phalarope diet consists largely of assorted arthropods (Jewett, Taylor, Shaw, and Aldrich, 1953), and after some experimentation, it was found that a daily ration of one ounce of frozen brine shrimp (*Artemia salinas*) and two ounces of fat-free ground beef maintained body weight and plumage condition satisfactorily. The most acceptable feeding procedure was to place fresh food and water together in a flat pan at least twice daily. Food placed in a dish, or on the floor of the cage, usually remained untouched or was swallowed with considerable difficulty. Apparently, in phalaropes, as in many species of aquatic and shore birds, the process of swallowing solid foods is facilitated by mixture with water.

TABLE 1

WILSON PHALAROPES IN NUPTIAL AND JUVENAL PLUMAGE

No. of experimentals	Sex		No. receiving prolactin pretreatment		Hormonal treatment	Plumage before treatment		Plumage regrown during treatment	
	Female	Male	Female	Male		Nuptial	Juvenile	Nuptial	Winter
4	1	3	0	1	None	4	....	....	4
2	1	1	0	0	None	....	2	....	2
3	1	2	0	1	Estradiol benzoate	....	3	....	3
8	3	5	2	2	Testosterone propionate	8	....	8	....
4	2	2	0	0	Testosterone propionate	....	4	4	....
10	5	5	5	5	Prolactin	10	....	....	*
2	1	1	0	0	Testosterone propionate and estradiol benzoate	....	2	2	....

* No plumage replacement during the period of treatment.

Experimental procedure.—For the experiments, adult and juvenal Wilson Phalaropes were divided into seven groups (table 1), with representatives of both sexes in each. Three of these groups were comprised entirely of adult birds in nuptial plumage, and four were of juvenal birds not yet in their first winter plumage. Two groups consisted of nontreated controls, a third received injections of estradiol benzoate, the fourth and fifth received testosterone propionate, the sixth prolactin, and the seventh estradiol benzoate and testosterone propionate.

In addition, six adult Northern Phalaropes in winter plumage (3 males and 3 females) were maintained each in a separate cage. These received successively various hormonal combinations as shown in table 2. Between each hormonal combination, this group was photographed and given a rest period of several days with no treatment.

Because experimental birds were exposed to continuous artificial light, the gonadotrophic hormones of most of those in nuptial plumage were suppressed by preliminary daily injections of prolactin for a minimum period of ten consecutive days, beginning immediately after their capture (treated birds indicated in table 1). It is well established among birds that physiological levels of prolactin for this period of time are sufficient to inhibit the production of gonadotrophic hormones by the anterior lobe of the pituitary (Bates, Riddle, and Lahr, 1937; Bailey, 1950; Lofts and Marshall, 1956, 1958).

In phalaropes, as in other species, this inhibition results in profound gonadal regression, similar to that which occurs naturally in the fall (Johns and Pfeiffer, 1963). For this reason, the gonads of prolactin-treated Wilson Phalaropes, which were in the nuptial plumage at the time of capture, were considered to be essentially in the same regressed condition as were those of Northern Phalaropes in winter plumage. It is probable that gonadal regression occurred at least slowly in all captive Wilson Phalaropes, regardless of treatment, because all uninjected birds regrew winter-type feathers, and the gonads of all birds, both prolactinized and nonprolactinized, were greatly regressed at the termination of the experiments.

All hormones were administered intramuscularly until feather regrowth had proceeded to the condition were pigmentation could be easily determined. Plumage replacement to this stage required from 5 to 30 days, with a mean of 9 days. Hormones were administered in the following daily doses: 20 international units (I.U.) of prolactin (ovine) in 0.1 ml. of pyrogen-free water; 100 I.U. of estradiol benzoate in 0.04 ml. of sesame oil; and 1.0 mg. of testosterone propionate in 0.04 ml. of sesame oil. These amounts were not varied, whether given alone or in combination.

TABLE 2

NORTHERN PHALAROPES IN WINTER PLUMAGE

No. of experimentals	Sex		Hormonal treatment	Plumage regrown during treatment	
	Female	Male		Nuptial	Winter
3	1	2	None	....	3
2	1	1	Estradiol benzoate	....	2
2	1	1	Estradiol benzoate and prolactin	....	2
3	1	2	Estradiol benzoate and testosterone propionate	3	....
6	3	3	Testosterone propionate	6	....
2	1	1	Testosterone propionate and prolactin	2	....
4	3	1	Prolactin	....	4
2	1	1	Prolactin, estradiol benzoate, and testosterone propionate	2	....

At no more than four days prior to injection of each hormonal combination, all Wilson and Northern phalaropes had patches of contour and down feathers unilaterally plucked from two separate areas. The selection of the two sites was based on the desire to utilize the areas which in the nuptial plumage are normally most brilliantly colored. One of these included the right half of the cervical region of the spinal pteryla, as well as the down in right cervical apterium. The other encompassed the right interscapular region of the spinal pteryla, the down of the adjoining lateral apterium and the right humeral pteryla. The nuptial plumages of the female Wilson and Northern phalaropes are shown in figures *a* and *b*, respectively, of the color plate.

All plucked feathers were carefully labeled and retained for later comparison to feathers regrown in the same region. In addition, the same areas on the left side of some birds were plucked and the feathers were allowed to regrow without influence of injection. The right side was then plucked and these feathers were regrown during a period of hormone injection. The left side of these birds, therefore, served as a control for the feathers grown on the right side under exogenous hormonal influence (fig. *c*).

RESULTS

The results of the experiments are summarized in tables 1 and 2. These show that testosterone propionate, whether used alone or in any combination with prolactin or estradiol benzoate, produced regrowth of nuptial-type feathers in both species of phalaropes. This hormone was equally effective in male and female birds, and it produced in the plucked body area feathers which were indistinguishable from normal female nuptial feathers of that area (figs. *f*, *g*). With the exception of the regrowth of white, winter-type feathers on the neck area of uninjected Wilson Phalaropes in nuptial plumage, no plumage replacement was noted in the plucked neck area of any bird (tables 1 and 2 refer to feather replacement in the plucked body area only). Without exception, feathers regrown on the bodies of uninjected birds were winter-type (fig. *e*), as were those receiving prolactin (results achieved in Northern Phalaropes only), estradiol, or prolactin and estradiol.

Although prolactin produced no observable effect on the plumage of phalaropes, there was some evidence that phalaropes which are plucked after the nuptial plumage has entirely developed and then treated with physiological levels of prolactin, may fail to regenerate replacement plumage until the approximate time of normal postnuptial molt. While this was the case for all of the Wilson Phalaropes in nuptial plumage, it was not true for one Wilson Phalarope in winter plumage (not included in table 1), nor was it true for four Northern Phalaropes in winter plumage (see table 2). Five male and five female Wilson Phalaropes in nuptial plumage which were unilaterally plucked and then injected daily with prolactin for from four to twelve weeks failed to regrow feathers in the plucked areas during the period of prolactin injections (see table 1).

DISCUSSION

Since female Wilson Phalaropes possess nuptial plumage strikingly more colorful than the male, and since these females are already in nuptial plumage at the time of arrival on the breeding grounds in northwestern Montana, it is probable that endogenous androgen levels are higher in female than in male phalaropes during the normal time of development of this plumage prior to departure for, or enroute to, the breeding grounds. This view is directly supported by hormonal injection which shows that testosterone induces colorful nuptial feathers, as well as by the indirect evidence of preliminary data compiled by Dyrenfurth and Höhn (1963) which show that in phalaropes already on the breeding grounds there is a higher androgenic level in phalarope ovarian tissue than in testicular tissue. Because it has been shown that androgens induce aggressive sex behavior in some birds (Noble and Wurm, 1940), these findings may also explain the aggressive courtship behavior of phalarope females.

It is particularly significant that male Wilson Phalaropes react to exogenous testosterone in the same manner as do the females by donning the bright plumage more typical of the females. Because at the time of their arrival in early May, the gonads of all males are already greatly enlarged and spermatogenesis is in process (Johns and Pfeiffer, 1963), there can be little doubt that endogenous testosterone levels are relatively high. Male Wilson Phalaropes in nuptial plumage show a wide range of coloration, with the most brilliantly colored males closely resembling the females. It could be hypothesized that, because of age factors, inherent physiological differences, or differences in external environmental factors of the winter ranges of individual male birds, testosterone levels during prenuptial feather growth vary considerably from one male to another, producing the nuptial plumage variation among males. It should also be noted that the docile nature of male phalaropes, who never take part in the frequent

courtship battles (Tinbergen, 1958), suggests low androgen production, which has been tentatively confirmed by Dyrenfurth and Höhn (1963).

Earlier studies have shown that a synergistic action of testosterone and prolactin is capable of activating the production of the incubation patch in both sexes of phalaropes (Johns and Pfeiffer, 1963). Since the patch is normally produced only by the male in these species (Bailey, 1952), and since it has been shown that testosterone induces colorful female nuptial feathers, it is probable that the female lacks sufficient amounts of prolactin. This hypothesis is supported by study of the breeding behavior of female phalaropes. The association of a high prolactin level with broody behavior is well known (Riddle, Bates, and Lahr, 1935; Breitenbach and Meyer, 1959; Höhn, 1961; Lehrman and Brody, 1961; Medway, 1961), and, therefore, the complete lack of broody behavior in these females during the breeding season is indicative of a low, or nonexistent, prolactin level.

As was stated above, there was some evidence that birds deplumed after the nuptial plumage has entirely developed and subsequently treated with prolactin may fail to regenerate replacement plumage until the normal time of postnuptial molt. It may be that premature feather growth is prohibited by the ability of high levels of prolactin to bring about a temporary refractory condition of the adenohypophysial function, similar to that which occurs normally during the breeding season. This physiological condition in wild birds, at least as it affects the production of gonadotrophic hormones, seems to develop naturally during the period of incubation in single brooded species (Lofts and Marshall, 1956, 1958; Bailey, 1950) and terminates when day length becomes sufficiently short in late fall to provide an effective period of darkness (Wolfson, 1959). The inhibitory effect on the production of gonadotrophic hormones by the anterior lobe of the pituitary, caused by prolactin, is well known, and it seems possible that the effects of prolactin may also include an inhibition of the production of thyrotrophic hormone by this same gland. The resulting reduction of thyroxine secretion by the thyroid could result in suppression of molt and decreased feather growth rate, for, according to a hypothesis by Höhn (1961:101), thyroxine "is required for the stimulation of growth of new feathers, an essential factor in the molting mechanism" (for a review of the effects of hypothyroidism on feather structure and growth, see Blivaiss, 1947*a* and 1947*b*). Significantly, Northern Phalaropes already in winter plumage (table 2) showed no inhibition of plumage regeneration resulting from prolactin treatment. This may indicate, as Lofts and Marshall (1958:91) suggest, that prolactin may only temporarily suppress the output of the anterior pituitary and that the "seasonal inhibition of adenohypophysial function" and the resulting multiple effects "is probably essentially under neural control with external stimuli ultimately involved."

ACKNOWLEDGMENTS

This research was supported by a grant from the National Science Foundation (GB–659). Prolactin (ovine) was generously provided by the Endocrinology Study Section, Division of Research Grants, National Institutes of Health, Bethesda 14, Maryland.

In addition, I would like to thank Dr. E. W. Pfeiffer of Montana State University for his counsel and confirmatory data which were invaluable to the completion of the work.

SUMMARY

Testosterone propionate injected daily in Wilson and Northern phalaropes produced growth of brilliant nuptial feathers. The plumage replacement in plucked areas did not

vary in color or form among birds in nuptial, winter, or juvenal plumages, nor did it vary significantly between male and female birds. Testosterone propionate alone, or in combination with estradiol, prolactin, or both, was equally effective. Prolactin or estradiol singly, or in combination, appeared to have no effect in inducing nuptial feathers. It is therefore concluded that androgen is a major factor in production of the brilliant nuptial plumage in female phalaropes as well as in the varying but lesser degree of color in the breeding plumage of male phalaropes.

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