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PLUMAGE DIFFERENTIATION OF NORMAL AND SEX-ANOMALOUS RING-NECKED PHEASANTS IN RESPONSE TO SYNTHETIC HORMONE IMPLANTS

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Several investigators observed in the late 1920's that in some species of birds and mammals a number of secondary sexual characteristics, including color, seemed to be dependent on hormonal factors and not at all on the genetic sex of the individual. This led to the enunciation of a theory of "equipotentiality" which assumed that male and female tissues were identical in their reactions, particularly to the sex hormones (Lillie, 1927).

Allen and Doisy (1923) were the first to suspect that the ovarian follicular hormone was an estrogenic agent in mammals. Allen *et al.* later (1924) found that a similar substance could be extracted from the follicular liquor in the ovary of the domestic hen (*Gallus domesticus*). Much research, mostly with domestic animals, followed these discoveries. The domestic fowl served as a popular subject. Since the color and shape of feathers seemed more sensitive to hormonal changes during their growth than other secondary sexual characters, they were studied at great length. As a consequence of this it was found that castrated male chickens showed little or no changes in feather color or shape; yet ovariectomized females acquired male plumage. The injection of ovarian extracts, however, feminized both intact and castrate males as well as ovariectomized females. Much emphasis was placed on the theory of equipotentiality; but as experimental work expanded into other avian species, it was found that other relations obtained. The European House Sparrow, *Passer domesticus* (Keck, 1934) and the Brewer Blackbird, *Euphagus cyanocephalus* (Danforth and Price, 1935) did not respond to the estrogen theelin. The Black-headed Gull, *Larus ridibundus* (van Oordt and Junge, 1933) is ambisexual, responding to both male and female hormones and the African Weaver Finch (*Pyromelana franciscana*) has its plumage regulated by an hypophyseal hormone (Witschi, 1935, 1936). Obviously then, genetic as well as hormonal factors must interact to produce these different effects.

Previous studies of specific effects of genes in relation to their expressivity under estrogenic influence in Red Junglefowl, *Gallus gallus* (Morejohn, 1953, 1955) prompted the authors to investigate the nature of the difference in plumage between the sexes of Ring-necked Pheasants (*Phasianus colchicus*). Three "off-colored" birds were noticed among normal-appearing cocks and hens in an incoming group of pheasants used in other experiments. The three birds were caught, examined, and identified as sex anomalies of one type or another. Two of the birds were colored like hens, and one was cock-colored. A review of the literature revealed that several types of so-called "sex anomalies" had been reported in Ring-necks and other pheasants. Some were called hermaphrodites, others intersexes, and still others sex-inverts. From published descriptions and photomicrographs of internal organs it was difficult to decide in which of these categories to place the birds we had examined. Hermaphrodites were cock-feathered birds with spurs,

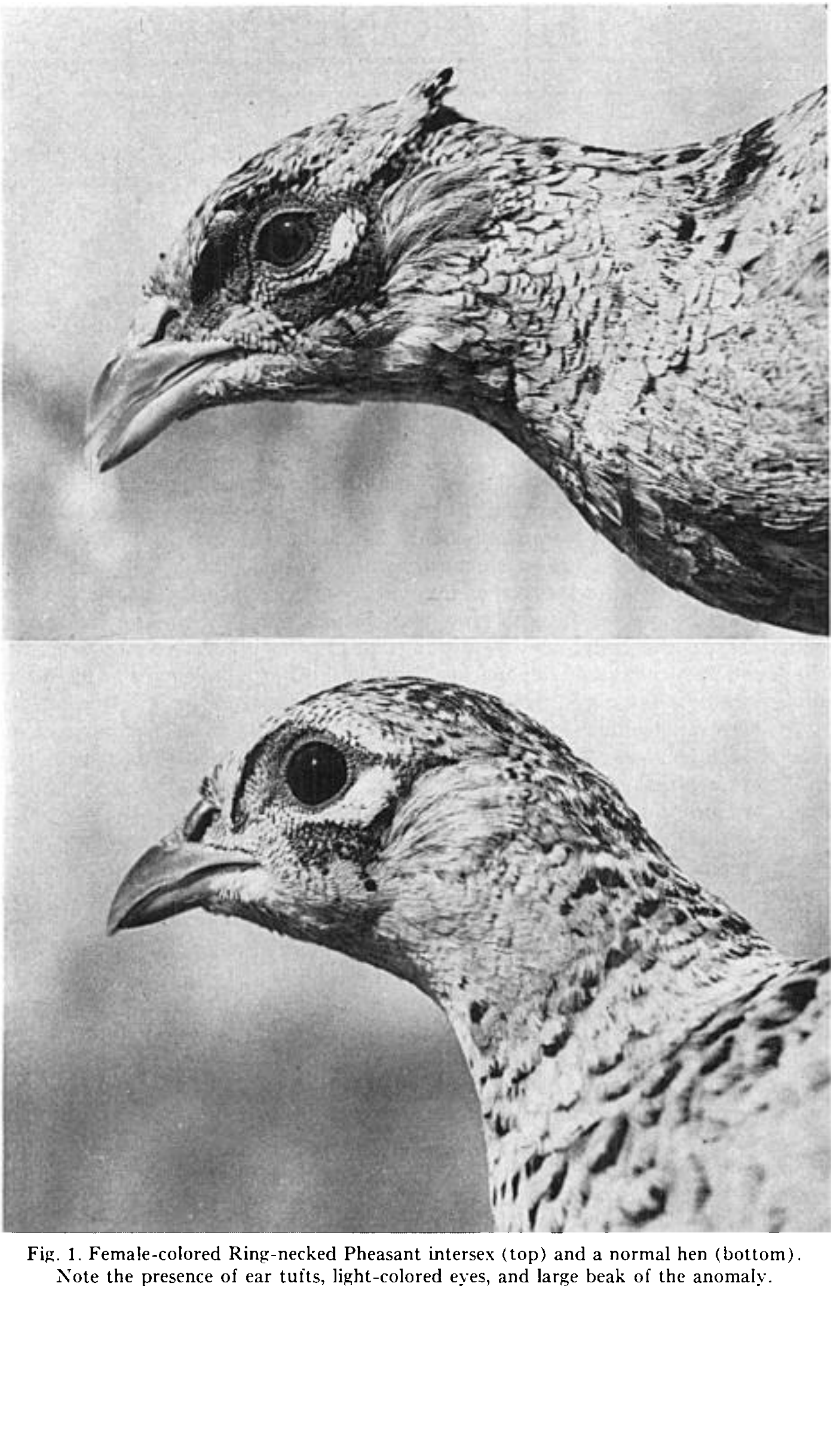

Fig. 1. Female-colored Ring-necked Pheasant intersex (top) and a normal hen (bottom). Note the presence of ear tufts, light-colored eyes, and large beak of the anomaly.

and these birds had both male and female organs. Intersexes were birds with supposed abnormal chromosomal complements which had mixed male and female feathers (sometimes called gynandromorphs), and sex-inverts were birds undergoing change from one sex to the other—usually female to male.

Previous investigators have given much thought and speculation to the origin and true nature of these sex anomalies but inadequate experimentation has been employed. Yarrell (1871–1885) was the first to report that a diseased state of the ovaries often induced production of male-type feathers in female Ring-necks in England. Gurney (1888) figured abnormal female organs from a cock-colored hen in his publication. Other investigators (Bond, 1914; Harrison, 1932; Rosen, 1948) have reported similar cases. Bissonnette (1940, 1941), however, reported birds that were essentially female-colored but possessed spurs, ear tufts, and gave the cock call. He also reported cock-colored hens with decidedly diseased ovaries. However, he believed both types were merely extremes of one phenomenon, that is, the ovaries became diseased, ovarian hormone secretion was curtailed, and the bird assumed cock plumage. This plumage is gradually lost as the bird's rudimentary right gonad begins to develop into an ovotestis or similar structure. During this time spur formation takes place and the bird gradually acquires a type of hen's plumage. In view of the work reported herein, this hypothesis seems untenable. It is believed that two separate conditions exist and that cock-feathered and hen-feathered individuals are not necessarily different stages of a deranged sexual progression.

Our three anomalous birds fit Bissonnette's description rather well. Two (fig. 1) were female-colored, possessed spurs, were large in size, had yellow irises and well developed ear tufts. The other (fig. 2) was cock-colored, had no spurs, was small with brown irises and no ear tufts. It can be seen, on the one hand, considering color and feather shape only, that two birds were female-like and one was cock-like; on the other hand, considering other so-called secondary sexual characteristics such as size, ear tufts, spurs, and iris color, two birds were male-like and one was female-like. This peculiar mixture of male and female characters will be dealt with later.

METHODS

The feather tracts of anomalous birds and normal males and females were compared. Examination of the cocks revealed that there were 22 distinct feathered areas on the normal bird. The normal hen was found to differ from the male in all of the recognized areas. Grossly, the differences (largely color and shape) between these feathered areas in cocks are clearly evident because of the difference in the color. In the hen, however, they may be distinguished only upon close examination. Feather samples were taken from each area as shown in figure 3 and mounted on sheets of cardboard. This was done for the normal cock and hen and for each of the anomalous birds. An evaluation of the individual feathers of the anomalous birds was made in relation to their degree of maleness or femaleness. Index values from zero to 4 were applied. Typical normal hen feathers were equated to zero; normal cock feathers were equated to 4. Intermediates were given the value of 2; those which tended to be more male-like the value of 3; and those which tended to be more female-like the value of 1 (table 1).

Instead of sacrificing the birds for studies of internal organs, it was decided to study their responses to subcutaneous implants of estrogen (diethylstilbestrol, 15 mgm. pellets) and androgen (testosterone propionate, 10 mgm. pellets). An experiment to determine responses to the hormones was set up as shown in table 2.

At the time of implantation of the hormone pellets approximately a dozen feathers were removed from each of the 22 different feather tracts. In this manner, the feathers that would subsequently replace those plucked would develop in a different hormonal

TABLE 1

A COMPARISON OF FEATHERS FROM VARIOUS PARTS OF THE BIRD

Feather samples were rated as follows: normal female, zero; mostly female, 1; intermediate (male-female), 2; mostly male, 3; and normal male, 4

Feather tracts	Normal hen	Anomalies 2775	2753	4025*	Normal cock
Dorsal head	0	0	0	1	4
Auricular	0	0	0	0	4
Dorsal lower neck	0	1	0	2	4
Lateral neck	0	0	0	4	4
Neck collar	0	0	0	4	4
Ventral lower neck	0	1	2	3	4
Upper back	0	1	1	2	4
Mid-back	0	1	1	1	4
Lower back	0	1	1	1	4
Upper rump	0	0	0	1	4
Lower rump	0	0	0	1	4
Anterior breast	0	2	1	3	4
Mid-breast	0	3	3	3	4
Posterior breast	0	0	1	3	4
Humeral	0	1	1	1	4
Lesser coverts	0	0	0	0	4
Median coverts	0	0	0	2	4
Greater coverts	0	0	0	2	4
Lower femoral	0	1	1	2	4
Upper femoral	0	1	1	1	4
Upper tail coverts	0	1	1	3	4
Caudal	0	1	1	3	4
Rating totals:	0	15	15	42	92

* Cock-colored anomaly.

background and the effect of the implanted hormone would be seen readily on the newly emerging feathers. The effects of the hormones lasted about six or seven weeks, and most feathers had grown about two-thirds of their full length in this time. Little chance of error occurred in plucking out the experimental feathers since much of the basal feather shaft was still growing and was bluish and soft. The feathers grown under the

TABLE 2

EXPERIMENTAL PROCEDURE USED TO DETERMINE RESPONSES TO HORMONES IN NORMAL AND SEX-ANOMALOUS RING-NECKED PHEASANTS

Sex type	Experiment I Diethylstilbestrol response	Experiment II Testosterone response
1. Normal intact ♂ (control)	......	......
2. Normal ♀ (control)	......	......
3. Normal ♀	No	No
4. Normal intact ♂	Yes	No
5. Normal castrate ♂ (control)	......	......
6. Normal castrate ♂	Yes	Yes
7. Anomaly 4025 (cock-colored)	Yes	No
8. Anomaly 2775 (hen-colored)	Yes	Yes
9. Anomaly 2758 (hen-colored) (control)	......	......

influence of the hormone were then compared with the feathers which had been plucked from the same site previous to implantation.

RESULTS

Results of estrogen implantation.—The effects of estrogen were evident in all birds except the normal hen (no. 3 in table 2). Contrary to expectation, however, males did not assume typical female feathers. The feathers were dull and brownish, and the pattern of pigment distribution was not at all similar to that found in the normal female. Moreover, to complicate the matter further, the castrated males developed some feathers

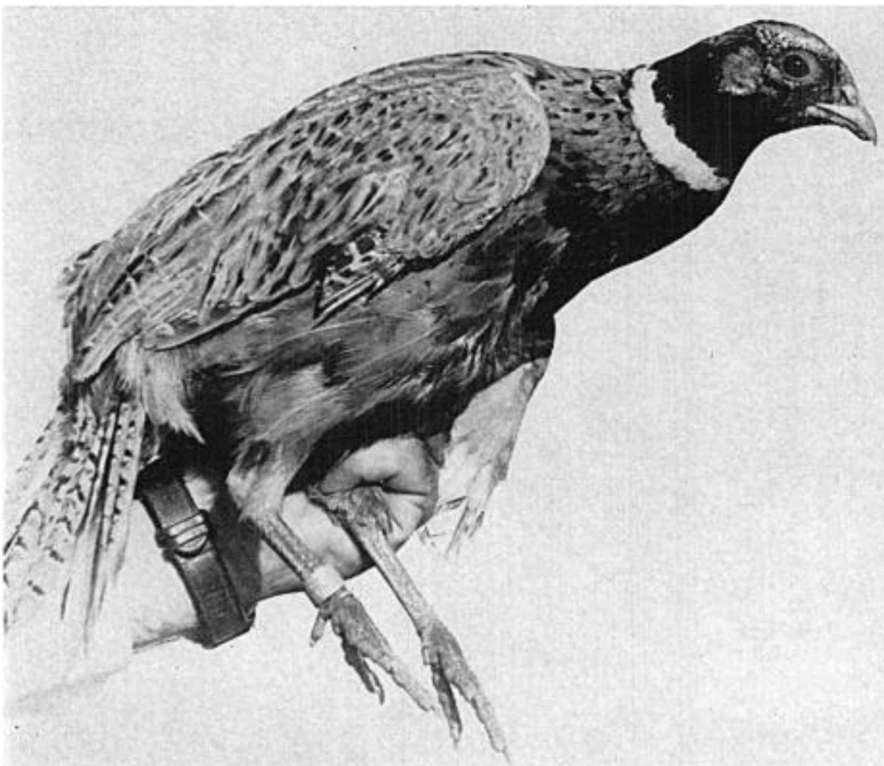

Fig. 2. Cock-colored female of the Ring-necked Pheasant. This bird had a diseased ovary and was believed to be a genetic female. Disregarding color compare the morphological features of the head with the normal female in figure 1 (bottom).

that differed from those developed by the intact males. These differences were most clearly evident in the dorsal lower neck, upper back and mid-back feather tracts. The lateral neck, neck collar, lower back, upper rump and lower rump were similar in both. The other tracts showed only minor differences between intact and castrated males.

All regenerated feathers of the hen-colored anomaly closely resembled the normal female type with the exception of the mid-back, lower back, upper rump and lower rump. These feathers approximated those produced in the same areas under estrogen influence by castrated and intact males.

The cock-colored anomaly produced feathers under the influence of estrogen similar to those of the hen-colored anomaly. The feathers of the back and rump, however, were

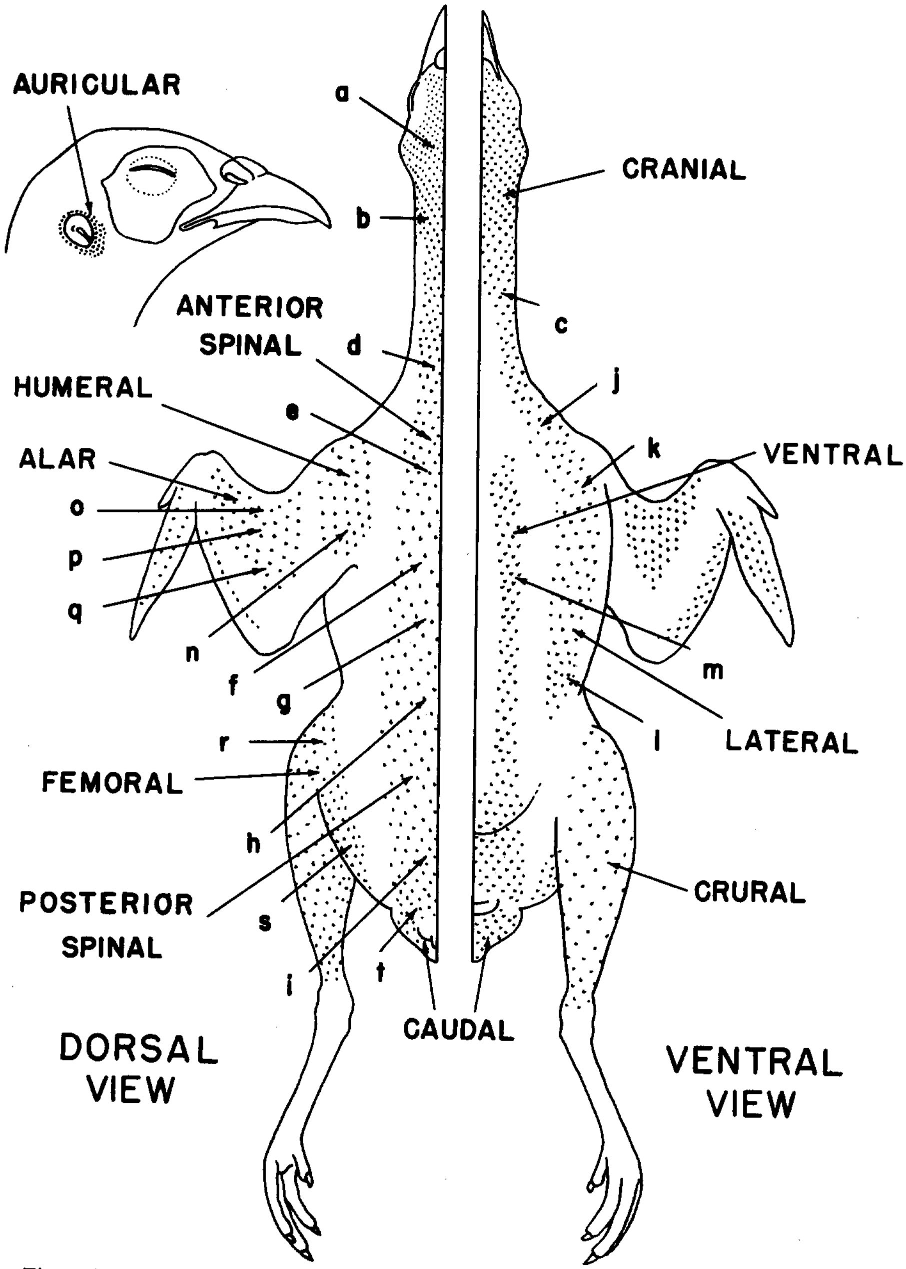

Fig. 3. Diagram of dorsal and ventral views of the major feather tracts of *Phasianus colchicus*. Feathers from specific areas in the feather tracts were employed. The areas, indicated by letters, are as follows: CRANIAL TRACT, (a) dorsal, (b) lateral neck, (c) neck collar; SPINAL TRACT, (d) dorsal lower neck, (e) upper back, (f) mid-back, (g) lower back, (h) upper rump, (i) lower rump; LATERAL TRACT, (j) ventral lower neck, (k) anterior breast, (l) posterior breast; VENTRAL TRACT, (m) mid-breast; HUMERAL TRACT, (n) posterior; ALAR TRACT, (o) lesser coverts, (p) median coverts, (q) greater coverts; FEMORAL TRACT, (r) lower femoral, (s) upper femoral; CAUDAL TRACT, (t) upper tail coverts. The drawing at the upper left demonstrates position of the auricular tract.

more nearly like those of the normal female than the feathers produced by the other birds. Other pre-treatment feather samples were more female-like than those produced post-treatment.

Results of testosterone implantation.—All remains of implanted pellets were removed from the skin of the neck on the experimental birds to rid them of any lingering effects of estrogen. The birds were then replucked in several sites. Males were examined two weeks later and the new emerging feathers were still hen-like. Four weeks after removal of the pellets the same feathers were re-examined and their bases were seen to be cock-colored. The effects of estrogen were obviously gone. At this time, the birds were re-implanted with testosterone and replucked.

Testosterone produced no response at all on the feathers of the normal female. In fact, the hen given testosterone had been laying and continued to do so. Noticeable redness and increased size of face wattles, however, were evident. No plumage change was seen in the intact male, but he became very active and had greatly expanded face wattles and erect ear tufts. This male actually dominated the normal control male. The castrated male behaved similarly to the normal male, but he was by no means as active and had smaller facial wattles.

The feathers on the hen-colored anomaly were affected by testosterone and somewhat resembled the feathers on the cock-colored anomaly. The facial wattles became very large, and the ear tufts were prominent and carried erect. In spite of its overall female-like color, it strutted about the pen and behaved like a normal male. The only striking feather changes seen in this bird were in the region of the neck collar, lateral neck and upper back. The feathers in these regions were masculinized: the neck became greenish and the collar feathers became white. The feathers of the back and rump, however, responded in an unexpected way. They became more female-like than those in the same area previous to any hormone treatment. The feathers in the other parts of the body remained somewhat feminine in character and showed only slight masculinization. Regenerating feathers of the cock-colored anomaly under testosterone treatment were no different than pre-treatment feathers.

Sexual status of the anomalies.—At the end of the experiments the anomalies were sacrificed and their gonads examined. The cock-colored anomaly proved to be a female with a diseased ovary. Histological sections revealed a non-functional ovary with no follicles present, but numerous small, cellular circular areas resembling degenerate follicles were present beneath the germinal epithelium. Black pigment granules were distributed throughout the organ. Intermingled throughout the tissue of the ovary were cords of adrenal-like tissue. The oviduct appeared normal.

Both hen-colored anomalies possessed an ovary-like structure, a testis, vasa deferentia, and an oviduct. In section, the ovary was much like the one described above, and the testis had many irregularly shaped seminiferous tubules without lumina, the cells of which were undergoing mitosis. Many tubules of the epididymis were also in evidence. The left adrenal was absent. Abnormal sexual conditions such as these have been observed by Crew (1923), Domm (1927), Fell (1923), and Lillie (1927).

DISCUSSION

Consideration must first be given to the responses to hormones in the group of normal birds studied. The normal intact male reacted somewhat differently to estrogen than the normal castrate, and both produced feathers mostly unlike those of the normal female (see frontispiece). This response to estrogen is unlike that which is usually found in the domestic chicken, such as the brown leghorn and other breeds which have the same plumage pattern as found in the Red Junglefowl. In these birds, as in most other phasianids which differ sexually in the color and shape of feathers, there is no evidence of sex-linked inheritance of plumage differences between males and females. The difference in sexually dichromatic breeds of fowl seems to be the direct effect of the hormone estrogen in females. In other domestic breeds of fowl and many semi-domestic birds, mutations affecting feather form and color are well known (Hutt, 1949). Genes affecting these characteristics are located in autosomes as well as in the sex chromosomes. The dominant sex-linked gene which determines barring of feathers in some domestic chickens, for example, has a different effect on males and females depending upon whether the individual has one or two genes for barring. Since the gene is located in the sex chromosome, females can have usually only one because they are hemizygous. Males have two sex chromosomes and when homozygous for this gene usually have a lighter appearance than heterozygous males since a double "dose" of the gene has a greater inhibitory effect on the deposition of melanin which forms the dark bars. Barring, however, is not known to be greatly affected by gonadal hormones. It seems likely that the major sexual differences in feathers in the Ring-necked Pheasant are produced in two ways: (1) by autosomal genes having different expressivity under hormonal influence, and (2) by sex-linked genes having different expressivity dependent upon whether they are single genes (as in the hemizygous female) or paired genes (as in the male). A single or double dose of the gene would give different responses to the hormone. Apparently, the feather tracts of the back and rump in this pheasant are governed by sex-linked genes since there is such a pronounced effect evidenced in males under the influence of estrogen (see frontispiece). The other regions are probably governed by autosomal genes for they approach more nearly the female feather pattern and shape under the effects of estrogen. The feathers of the dorsal lower neck, upper back, mid-back, and humerals seem to respond differently to estrogen in the absence of testosterone. The presence of testosterone seems to inhibit in these feather tracts the complete action of estrogen in intact males. In the absence of testosterone, estrogen is able to exert its feminizing effects more clearly, thus resulting in feathers resembling closely the typical female. It seems likely, therefore, that the feather tracts of the back and rump (upper back, mid-back, lower back, upper rump, and lower rump) are governed by genes in the sex-chromosomes, and that the dorsal lower neck, upper back, and mid-back tracts are much more sensitive to the action of estrogen in the absence of testosterone. Feather samples representing responses to hormones are shown in the frontispiece.

Our interpretation of sex-linked genes controlling plumage differentiation is also supported by the work of Smith and Thomas (1913), especially their observations in regard to the intergeneric hybrids which they produced. They found that sterile hybrid female pheasants, which were comparable to birds with non-functional ovaries (as in the cock-colored anomaly), often showed cock-colored feathers in certain tracts. These male-like feathers were similar to the types found in the male parent. In other words, the F_1 hemizygous female received its sex chromosome from the male of one genus and the genes governing certain feather tracts in that genus exerted their effects on the sterile hybrid female plumage. Obviously, sex-linked genes controlling plumage differentiation are found in other pheasants as well. Danforth's (1937) skin transplantation experiments on *Phasianus* provide the following support of the hypothesis of dosage effects on plumage: (1) Female skin of the rump transplanted to the rump of the male host produced feathers which were not completely masculinized. Iridescent green color was absent and feather form was not perfect. This is in accord with the hypothesis that hemizygous female skin would not show complete dosage effects of testosterone. (2) Male hosts carrying female skin and given estrogen produced typical female feathers in the graft, whereas adjacent feathers were incompletely feminized.

The responses given by the anomalies are not as easily explained. In fact, some results were exactly opposite of what was expected. The anomalies would not be expected to respond simply, so that at best a complex response seemed likely. The hen-colored anomaly or intersex may have possessed an abnormal chromosomal condition initially. There is no proof of this. An interpretation of responses to hormone implants was difficult since the bird had elements of both testis and ovary and perhaps low titers of both gonadal hormones. It responded, as might be expected, in a feminine way to estrogen in both the upper back and dorsal lower neck tracts. In fact, the resemblance to the castrated male given estrogen was marked (see frontispiece). The mid-back tract, however, which closely approximated the normal female before treatment, responded to estrogen much as did the castrated male given estrogen. The dorsal lower neck behaved likewise. The response of the upper back tract to testosterone, as one might predict, resembled the normal male. The mid-back tract reacted to testosterone as it should have reacted to estrogen and vice versa. This may be an example of the so-called exceptional cases of masculinization by estrogens and feminization by androgens. Generally all other tracts showed responses to testosterone similar to that which obtained in the dorsal lower neck tract—some masculinization together with intricate patterns not uniformly found in the tracts themselves. The cock-colored anomaly, however, gave responses similar to that which might be observed in an ovariectomized hen. This bird, as a matter of fact, may be considered an example of a gonadless female. That is, the plumage she carried may well represent the resultant plumage produced in an ovariectomized normal female. The incomplete feminization of plumage may be due to an unknown substance or to exogenous hormones. The differences in response found in the upper back tracts as compared to the males given estrogen may, perhaps, be accounted for as a dosage effect, since this bird was probably a genetic female. The responses to testosterone were virtually identical to pre-treatment plumage, or it may be said that there was no response to testosterone in regenerating feathers in this bird.

SUMMARY

The responses to implantation of the hormones, synthetic diethylstilbestrol and testosterone propionate, in Ring-necked Pheasants demonstrated that plumage differentiation of both sexes is dependent upon genetic as well as hormonal factors, and that the genetic nature of this differentiation is associated with autosomal genes as well as genes in the sex chromosomes. The latter genes exert their effects through their intermediaries, hormones, and the differences in plumage between the sexes is one of genetic dosage. The three anomalies that were studied indicated an aberrant intersexual condition in one type, the hen-colored form, probably genetic in nature, and in the other type, the cock-colored form, a genetic hen with a diseased ovary.

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