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SEASONAL SEX CHARACTERS IN BIRDS AND THEIR HORMONAL CONTROL*

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Considering the widespread interest in ornithological research, it is surprising, how little information we have concerning the causative factors controlling the conspicuous seasonal phenomena in bird life.

In the light of recent work on hormones, the seasonal changes obtain a renewed interest and I wish to present here, some of the results of experiments that I have carried out with the assistance of my students during the last three years.[1]

The first problem which we face is that of the *seasonal development of the gonads and the gonoducts.* During the sexually inactive period, the sex glands of most wild birds regress to tiny rudiments, resembling in every way, the corresponding organs in juvenile specimens. In the testis, at this time, one finds only inactive spermatagonia,

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[1]Hormones are substances produced in the glands of internal secretion. They are released into the blood stream and thus circulate in the whole body. However, they produce effects only in specially responsive, "tuned in" organs. These hormones can be prepared by extraction of the producing gland or they may be recovered from the blood serum. Some are eventually eliminated from the body through the kidneys and are therefore found in the urine. Three groups of hormones are considered in this paper. A. *Hormones of the hypophysis gland* which stimulate development of the gonads (so-called gonadotropic hormones, maturity hormones, or hebin). They are at least of two kinds, as becomes evident from the reaction that they produce in the rat ovary. A first type stimulates follicular growth while the second type produces corpora lutea (luteinizing hormone). B. *Hormones of the gonads* (or sex glands). The active ovary releases the "female sex hormone" or oestrin; the active testis releases the "male sex hormone". These sex hormones control the full development of many of the secondary sex characters. C. *The hormone of the thyroid* is very important for maintenance of body temperature and general life functions. In some way it has an influence also on feather growth and coloration. The function of the thyroid is controlled by the hypophysis (through the thyreotropic hormone). Hormones are highly potent substances. In most instances fractions of milligrams bring about the full reactions.

in the ovary only small ovocytes. At the approach of the breeding season, these glands enlarge very rapidly. The testis increases in weight 500 fold or more, while spermatogenesis proceeds quickly to the production of millions of ripe spermatozoa. Epididymis, vas deferens, and seminal glomus, the latter taking the rôle of true seminal vesicles, enlarge correspondingly and become filled with seminal fluid. In a similar way, though starting slightly later, develop the female genital organs. The ovary in the English Sparrow increases from less than 10 to 500 and more milligrams. The thin and straight oviduct becomes convoluted and very copious, due to the enormous development of its glandular endothelia.

The same developments can be evoked at any time of the year by injection of gonadotropic hormones (Figs. 15-18). The following Table I shows that any of the gonadotropic substances known to us can activate the quiescent bird gonad, though the reactions do not show a complete quantitative parallel to those in the rat. Equal

TABLE I

Effect of Gonadotropic Hormones on the Left Testis and the Ovary of the English Sparrow.

Source of Hormone	DRU	l. Testis	Ovary
Beef Hyp. F.	1	x 8 C	x 1.5
Beef Hyp. F.	3	x 72 S	x 80
Beef Hyp. F.+L.	3+(3)	x 80 S	x 40 d
Beef Hyp. L.	(3)	x 30 S	x 30 D
Sheep Hyp. L.	(2)	x 20 S	....
Human P.U.	10	x 2 c	x 1
Human P.U.	20	x 15 C(s)	x 1
Human P.U.	50	....	x 1.5 D
F.+P.U.	.5+5	x 12 c	....
F.+P.U.	.5+10	x 18 C	....
Preg. Horse Serum	1	x 15 C(s)	x 2
Preg. Horse Serum	4	x120 S	x 4
Preg. Horse Serum	20	x150 S	x 40 Ov.
Preg. Horse Serum	40	x 80 S	x 60 D

C, spermatocytes (c, only in small numbers); d, slight degeneration; D, high degeneration; F, follicle stimulating fraction of hypophyseal extract; L, luteinizing fraction of hypophyseal extract; Ov., ovulation; P.U., pregnancy urine; (s), spermatozoa in small numbers; S, spermatozoa in large numbers; DRU, daily rat units.

amounts in rat units of follicle stimulating hormone from beef hypophysis and of pregnant horse serum act similarly on the sparrow testis, but very differently on the ovary which responds more strongly to the former. Hebin from human pregnancy urine brings about complete spermatogenesis, if excessive amounts are injected. The ovaries

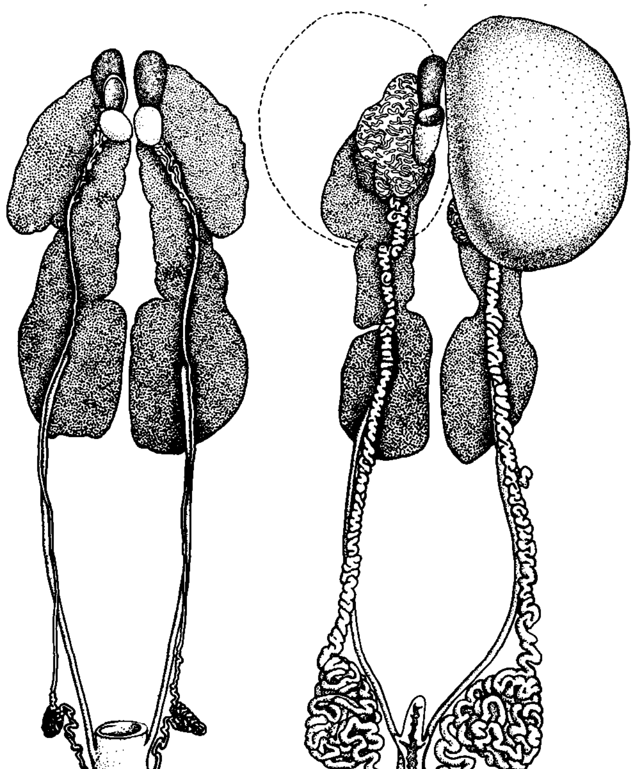

FIG. 15. 1, Urogenital organs of a male sparrow in the quiescent phase, x3. Weight of left testis, 0.5 mg., of left glomus, 0.4 mg. 2, Urogenital organs of a male sparrow in the period of sexual inactivity, after seventeen daily injections of 0.1 cc. (20 DRU) of pregnant mare serum; right testis removed to show the enlarged epididymis; x3. Weight of left testis, 345 mg., of left seminal glomus, 24 g. Note also the enlarged and convoluted vasa deferentia.

react but slightly and mainly by degenerative processes to the highest doses of urinary hebin. Purified luteinizer from beef and sheep hypophysis stimulates testicular as well as ovarial growth, though the latter soon ends in degeneration of the large ovocytes. *Summarizing*, we can say that in the bird, the quiescent gonads react most easily to hormones which in the rat produce follicle stimulation. Luteinizing hormones are less potent. However, they induce complete spermatogenesis if administered in sufficiently high doses. In the ovaries, they induce some developments which soon end in degeneration. In our finches, the most perfect results were obtained, by the injection of 0.1 cc. of pregnant mare serum. In the males, the testes and seminal ducts become filled with spermatozoa before the end of the second week (Figs. 16-17), while the females begin to ovulate and to lay normal eggs with colored shells (Figs. 18, 7). This result was obtained not only with the English Sparrow but also with African weaver finches (*Quelea quelea*) which otherwise never layed in captivity, not even during the breeding season.

These experiments indicate that the reproductive cycles of the birds are under hypophyseal control. It is known that this is true also for such mammals as the rat and man. The relationship is, however, a quite different one. In the rat, if the level of *sex hormones* (produced by testes and ovaries) falls, the hypophysis answers with the release of increased amounts of *gonadotropic hormones;* if the level rises, the hypophyseal output is lowered. In the finches, however, the hypophysis makes no attempt to keep the gonads and the sex hormones at a constant level. On the contrary, ebb and flood of sex and gonadotropic hormones are coincident and the hypophysis leads the gonadal development without reacting back on variations in sex hormones. We shall soon give further evidence in support of this statement. However, first let us consider the *factors that determine the seasonal cycles of hypophyseal activity.*

Popular opinion credits the rising temperatures in spring with the rôle of the first cause in bringing about bird migration, nesting, and breeding. Of course, the common citizen is highly impressed by the fact that in spring the coal bills become smaller and eventually fall off entirely. However, we must not forget that the migratory birds have spent the winter in tropical or subtropical countries and in fact move into cooler environments, at least during the time of actual migration. Rowan in his charming little book on "The Riddle of Migration" has conclusively ruled out the temperature factor. On the other hand, his experiments on the junco show that an artificial in-

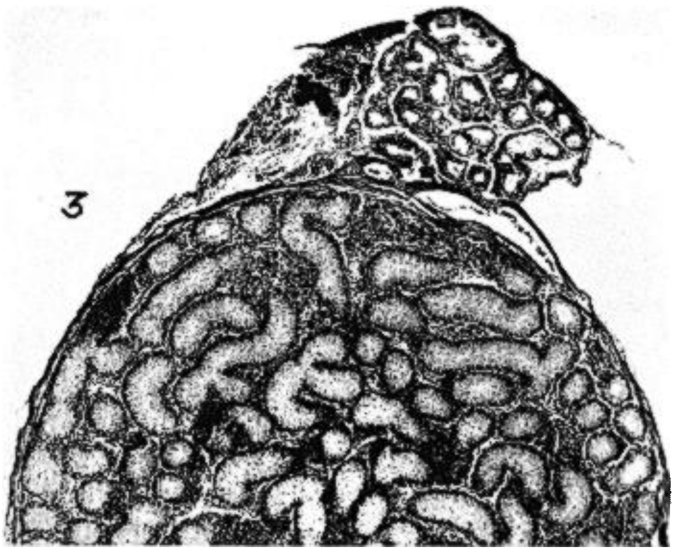

FIG. 16. Part of a cross section through testis and epididymis of a sparrow in the quiescent phase; x40.

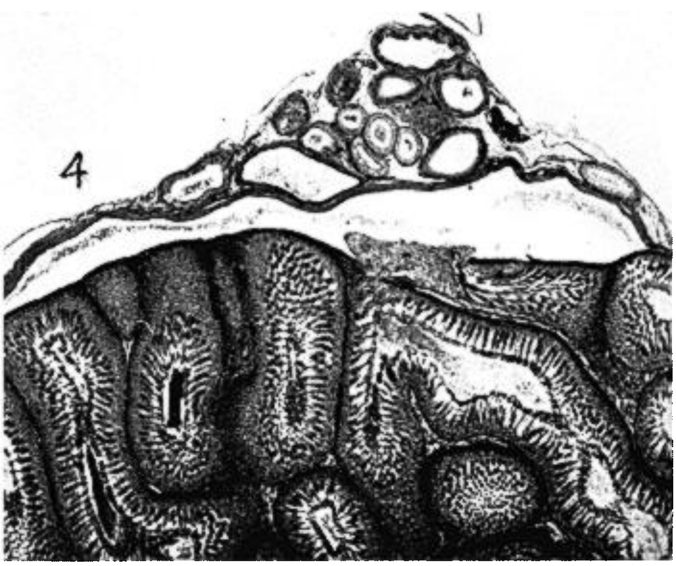

FIG. 17. Part of a cross section through testis and epididymis of the activated sparrow of Fig. 16; x40. Note the increased diameter of the seminal tubules, active spermatogenesis, and discharge of spermatozoa into the antrum of the epididymis.

crease in daylight beginning in November causes a development of testes and ovaries reaching the breeding size by January 9 instead of March, as under normal conditions. Miyazaki (1934) reports that in Japan, it is an old practice of owners of pet birds to induce singing early in winter by exposing the birds to candle light for three or four hours daily after sunset. This "Yogai" method is found especially effective in the White Eye (*Zosterops palpebrosa*). Bissonnette, working with the Starling, and Kirschbaum in experiments on the English Sparrow, find also that artificial lengthening of the day brings about a precocious development of the sex glands, especially in the male, during the winter months. We have ourselves repeated these experiments with similar effects. However, our observations seem to indicate, that the gonads respond only toward the approach of the normal breeding season and not in late summer nor in fall. It appears that the hypophyseal year cycle of the bird is as deeply rooted as the oestrus cycle of the human and other mammalian females. Changes in illumination may help to maintain the synchronism of the inborn cycle with the seasonal periods. We have observed that prolonged "Indian summers" with sunny days extending until late November bring about precocious developments of the testes in free living English Sparrows of Iowa. Yet the changing day in itself is not sufficient to explain entirely the breeding cycles, as becomes evident in the case of trans-equatorial migrants, like the Bobolink or the Golden Plover. Spending the winter south of the equator, they are exposed also to lengthening days; though their gonads remain quiescent. One should consider also that spring migration before the 21st of March takes the bird from longer to shorter days, as in the case of the Mourning Dove (Cole, 1933). Most interesting in this respect are possibly the tropical birds of which many have also very definite periodical breeding seasons. Different explanations have been proposed, most authors (Moreau, Bissonnette) agreeing, that changes in type or quantity of food most probably determine these cycles. This assumption, however, is definitely wrong. I have kept tropical African weaver finches for three years in the animal room at Iowa City, under constant food conditions and they have maintained their African cycles to this day. Oddly enough their breeding season is in the fall. If light has any influence, then this group of birds reacts to shortening and not to lengthening of the day. Even juvenile paradise whydahs which came into their first breeding period only after they had lived one full year in Iowa (and after having traveled through the shops of bird dealers in different parts of the northern hemisphere) unhesitatingly fell in line with their adult companions, coming in breeding condition in August-September of their second year. According to Delacour and Edmond-Blanc, the breeding season of these birds in Africa is coincident with the rainy season. If light has any regulating effect, these birds obviously react to decrease rather than to increase of illumination. Experiments to test this question are in preparation.

It is an interesting fact that the hypophysis of the bird reacts at least in a limited extent to light, that is, to sensory stimulation. This case, however, stands not alone. Cole has shown in very convincing experimental series that the mere sight of the incubating female induces in the male pigeon the changes necessary for crop milk secretion. We must conclude, therefore, that a specific optic perception stimulates, in this case, the release by the hypophysis of the lactation hormone, prolactin. Furthermore, it is a known fact, again borne out by our own observations, that the sparrow female in the breeding season lays four or five eggs, and then becomes broody. Her ovaries during the incubating period regress rapidly through degeneration of the larger eggs. According to Riddle, this regression seems to be due also to the release of prolactin. If, however, one removes daily the egg that the sparrow deposits, she goes on laying up to fifty eggs in succession, often twelve to nineteen on directly consecutive days. Whether the female "counts" by eye or by tactile perceptions of the ventral body surface, is not clear in this case; though in a similar observation by Phillips on continuous egg laying in the flicker, the latter alternative has the greater probability. Somewhere, obviously there is a bridge between the nervous system and the hypophysis transmitting stimuli that direct the release, by the latter, of gonadotropic hormones.

From the consideration of gonadal cycles, let us turn to the *secondary sex characters*. We mentioned already that concomitant with the development of the sex glands goes the enlargement of the gonoducts. The castrate female has an oviduct as thin as that characteristic for the quiescent phase. Injections of daily doses of one to twenty rat units of oestrin bring about a rapid growth. With the maximum dose, the full breeding size is attained within one week. Similarly in the male, the epididymis and the vas deferens of castrates react on injections of male sex hormone. In the normal course of events, quite obviously, the hypophysis stimulates the gonads; and the growing gonads, by release of sex hormones, stimulate secondarily the gonoducts.

In many birds, the *color of the bill* changes during the breeding season. In the English Sparrow and some other finches, both sexes have a horn-brown bill during the quiescent phase. During the reproductive phase, this changes in males to a jet black. Castrates have permanently lightly colored bills, but injections of male sex hormone (extracted from male human urine) in minute quantities bring about the blackening of the bill in castrates as well as in males and females of the quiescent phase. On the contrary in the Red-bill Weaver, males and females have brilliantly red bills during the inactive phase, which turn to yellow during the breeding season in the female only. These bills change to yellow in either sex, or in castrates, at any season, upon the prolonged injection of female sex hormone (from female human urine). In the love birds, or parokeets, the sexes differ mainly in the waxy skin over the root of the bills. It is blue in the male and brown in the female, at least during the breeding season. Castrated males maintain the blue color which, however, changes to brown after injection of female sex hormone. Obviously, one of the alternate bill colors always is neutral, not hormone controlled, and persists in castrates and in both sexes during the quiescent phase. The other color, appearing during the breeding season only, is conditioned by sex hormones. Surprising is the fact that in some species it is the male sex hormone, in others, the female sex hormone that brings about the dimorphic effect.

The greatest puzzle was offered by the *plumage*. Instead of describing the zigzag course of our experiments, I shall try to describe in the most direct way the results that we have in hand now, at the end of three years of observation and investigation. Matters are relatively simple in the case of the English Sparrow. The plumage in this species is the simple expression of hereditary constitution. Castrated males and females maintain and repeatedly regenerate their inherited male or female plumage. Even if injected with hormones of the opposite sex or if implanted with contrary sex glands, they always regenerate according to the hereditary type (Keck '34). Sex hormones play no part in the determination of either sex type in plumage.

Things become more interesting in the case of the birds which put on a special breeding plumage in one sex. I mention the well known case of the Indigo Bunting. The female carries a modest brown habit throughout the year. The male, however, is iridescent blue nearly all over during the breeding season and brown with just a few traces of blue, if out of season. The bill of the female is always light brown, that of the male is brown out of season and daintily black and

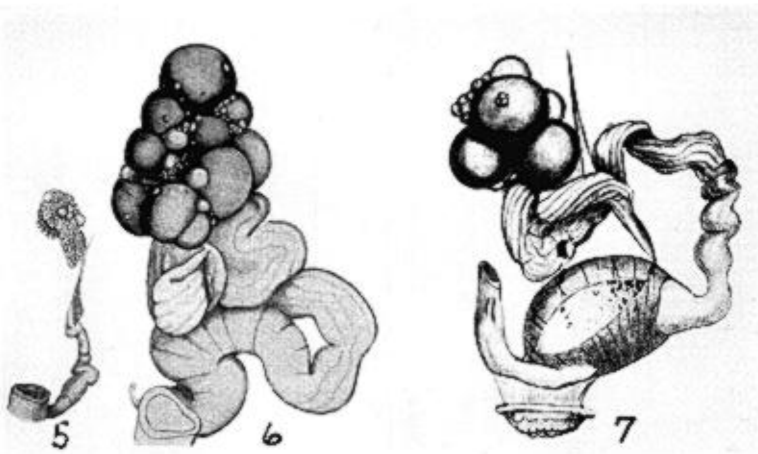

FIG. 18. 5, Ovary and oviduct of a female sparrow in the quiescent phase; x2. Weight of ovary, 10 mg. 6, Ovary and oviduct of a female sparrow in the period of sexual inactivity, after sixteen daily injections of two rat units (2DRU) of hypophyseal extract; x2. Weight of ovary about 500 mg. 7, Ovary and oviduct of a female sparrow after seventeen daily injections of 0.1 cc. (20DRU) of pregnant mare serum; x1. Weight of ovary, 940 mg. A window is cut into the uterine part of the oviduct to show the enclosed egg with its normal shell.

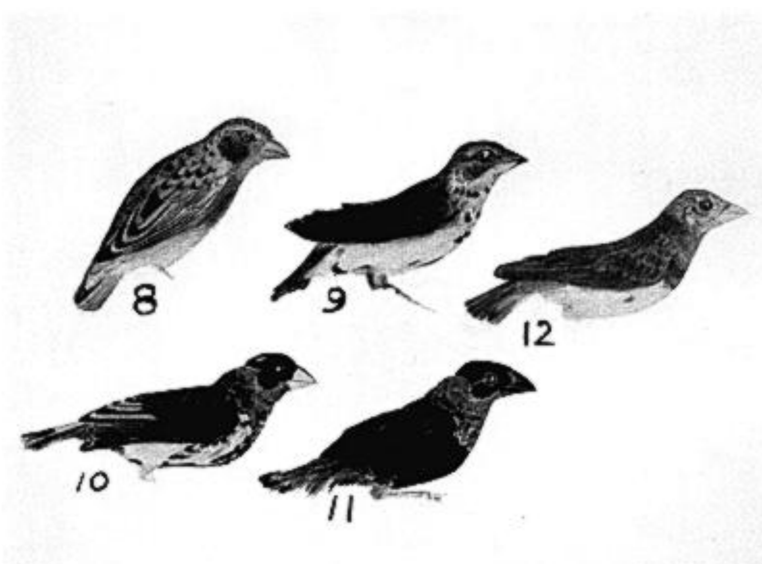

FIG. 19. Color phases of the African weaver finch, Pyromelana (Euplectes) orix franciscana. 8, Henny plumage, carried by females all year round and by males outside of the breeding season (January-May); the bill is ivory colored. 9, 10, Molt of the male and assumption of the cock's plumage (June-August) at the approach of the breeding season; the bill purple, changing to black. 11, Fully developed cock's plumage carried during the breeding season (August-October); the bill is black. 12, Castrated male outside of breeding season which, exceptionally, carries an intermediate type of plumage; the bill is ivory.

white during the breeding season. Castrated females so far have not exhibited any changes. Castrated males show a light brown bill throughout the year, indicating that no sex hormones are produced any longer. But to our great surprise, castrated males go on changing from brown to blue plumage and vice versa in the same rhythm as their normal male cage companions.

African weaver finches show similar phenomena. For experimental purposes they prove more valuable, because they are more hardy in captivity. At least in the case of the orange weaver, we know that the male passes through two molts, the female through one only. Probably the same condition prevails in the whole group of weaver finches. Both sexes molt at the end of the breeding season and acquire the modest henny plumage. At the beginning of the breeding season the male sheds this sober garment and dons a flashy nuptial plumage of black, and orange-red. The new pattern does not show any regard for the design of the cast off henny plumage but runs across feather tracks and natural boundary lines. At the same time, the bill of the male changes from ivory to black (Fig. 19). Castrated males permanently assume or maintain the light colored bill indicating absence of male sex hormone; though they go on changing, rhythmically, their plumages. It is interesting, however, that a few castrated males do not acquire a completely henny plumage during the inter-season, but a plumage with a mixture of nuptial and henny characters (Fig. 19, 12). The cock's plumage of the breeding season is always perfect. Females as mentioned above, do not change their modest garb at the beginning of the breeding season. Female castrates observe the same economy and even plucked feathers regenerate only rarely. However, the few that do so are of the cock's type. They are always shed at the end of the season, when a new henny plumage is acquired.

These observations suggested on the one side that the plumage type is controlled by hormones; on the other hand it was obvious that sex hormones are not the ones concerned. Consequently we suspected that the plumage type might be directly controlled by the hypophysis. We were, however, only recently able to prove our point, when we injected some of our birds with pregnant mare serum, containing that powerful gonadotropic principle. Injected at the daily dosage of 1/10 cc. of serum into normal and castrated male and female weaver finches out of the breeding season, it induced regeneration of cock's (in place of henny) plumage in all but the normal females. In the latter the change of the bill color from red to yellow (in red bill weavers) and the rapid development of the genital organs with subsequent egg laying, clearly indicated the presence of female sex hormone (produced by the enlarging ovaries). Injection of female sex hormone into males in breeding season has always a feminizing effect on the plumage. We can conclude, therefore, from our experiments that: (1) the henny plumage of weaver finches is the neutral (not hormone conditioned) type; it is found, consequently, in both sexes and in castrates, out of season. (2) The cock's plumage is due to a high level in gonadotropic hormones. (3) Female sex hormone counteracts the "masculinizing" effect of gonadotropic hormone on the plumage. These investigations show further, (4) that castration in birds stimulates only slightly, if at all, the hypophyseal activity, and (5) that the hypophyseal seasonal cycles are wholly independent of progressive or regressive changes in the sex glands.

There is just one more point which I wish to bring out. I remarked above, that success in our work on the plumage came with the application of pregnant mare serum. Before that, we had used other gonadotropic substances, especially extracts from beef hypophysis. These gave nearly as good gonadal development in normal males and females, but the plumage reaction was of the henny type in both sexes and also in castrates. The solution of this riddle came through the examination of the whole endocrine system, which showed that the extracts brought about an enormous enlargement and an apparent rapid discharge of the *thyroids*. The thyroid hormones, however, have a very decided "feminizing" effect on the bird plumage. This has been suggested already by earlier work of Cole and others on the chick, and is brought out again by extensive studies by one of my students on the plumage of the sparrow (Miller '35). It is especially interesting that in the sparrow, where male and female sex hormones show not the least effect on the plumage, injections of thyroxin change the developing feather from the male to the female type.

The most remarkable fact brought out by these investigations on seasonal sex dimorphic plumages is their dependence on other than sex hormones. Whether the sex difference in molting periods, which is also maintained after castration, is under hypophyseal or possibly, under genetical control, we do not know at the present time. Miyazaki's experiments seem to indicate that shortening of day length causes molting in the White Eye.

If time would permit, a discussion of the different mechanisms of determination of plumage patterns should follow this presentation of mere experimental data. In concluding, may I again emphasize the here established fact that even within the taxonomic family of the finches, the sex type of the plumage is determined in one species (sparrow) directly by the genetical constitution of the feather forming cells, and in another species (orange weaver) through free circulating hormones in the blood stream. It might well be that, through the closer study of such cases of substitution of hormonic by direct genetical control, we shall be able to learn more about the physiological nature and the mode of action of the hereditary element, the mysterious gene.

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NESTING OF THE RAVEN IN VIRGINIA

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Dark shadows were forming in the deep hollows leading down from the Shenandoah Mountains. The sun had already passed out of sight on the other side, and now twilight prevailed. Silence reigned everywhere over a snow-clad landscape which appeared to be devoid of life.

High up on the side of the mountain perched on an old dead snag leaning over a steep rock cliff sat a Raven in owl-like posture, seemingly in deep thought. Perhaps he was dreaming of bygone days when his kind inhabited all of the eastern mountain ranges in a land of plenty where game of all kinds was abundant and food easily procured. What now? Gone forever were the millions of Passenger Pigeons whose flight at times darkened the skies. The bugling of the elk was no longer heard. The land of abundance and plenty had van-