SEASONAL VARIATION IN TESTIS-STIMULATING ACTIVITY OF MALE PHEASANT PITUITARY GLANDS

BY FREDERICK GREELEY AND ROLAND K. MEYER

THIS paper is a study of seasonal pituitary-gonad relationships in game farm Ring-necked Pheasants (*Phasianus colchicus*). Investigations of the seasonal breeding physiology of birds have customarily been approached through study of gonadial activity. The data obtained in this way are abundant, but there is little information on the seasonal variation in gonadotrophic activity of the pituitary gland, measured by direct means. In this paper we shall present data from the bio-assay of gonadotrophin in dried pheasant pituitary gland collected monthly throughout the year.

Burger (1949) emphasized the need for further direct work on the pituitary gland as the master control of breeding activity. How closely the quantity of gonad-stimulating hormone produced by the pituitary gland follows the seasonal changes in gonadial activity has not been shown. Seasonal changes in the gonads may be due to parallel changes in pituitary production of gonadotrophic hormone or may reflect changes in the sensitivity of the gonads themselves to this hormone. No data on the level of gonadotrophic hormone in the pituitary gland or the blood stream at various seasons of the year are available. However, the presence of gonad-stimulating substances in the pituitary gland has been demonstrated in most domestic birds, and Benoit (1935) demonstrated that the amount of these substances increased in the pituitaries of ducks which were subjected to increased day-length. Phillips (1942) found no gonad-stimulating activity in the pituitary gland of the Ring-necked Pheasant but did not state at what season the material was obtained. A higher level of pituitary gonadotrophin has been demonstrated in older and reproductively more active male chickens and pigeons than in younger, inactive individuals (Breneman, 1944; Kato, 1938; Schooley, 1937; Smith and Engle, 1927). In domestic hens, on the other hand, more pituitary gonadotrophin was shown in non-laying than in laying birds (Phillips, *op. cit.*; Riley and Fraps, 1942). The answers to some of these problems can be surmised from what is known, but direct evidence is fragmentary.

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MATERIALS AND METHODS

The pheasants used in this study were pen-reared. For each of 12 months a dozen birds of each sex were transported on the day of autopsy from the State Game Farm at Poynette, Wisconsin, to the laboratory, a distance of 25 miles. All birds were sexually mature (9 to 24 months). They were decapitated, and the endocrine organs were weighed on a torsion balance to the nearest 0.5 mgm., or, in the case of heavier organs, on a gram balance. After weighing, each pituitary gland was crushed between two glass slides, air dried, and stored at room temperature. Only anterior lobes were used. Subsequently, all of the pituitary material from a given sex and month was scraped from the slides into a mortar and ground to a fine powder. The powder was stored in a corked vial placed in a desiccator in darkness at room temperature. When material for the entire year had been collected, it was assayed for testis-stimulating potency in one-day-old male chicks from a redbar strain (Lloyd and Hill, 1946). The pituitary powder was suspended in an aqueous solution then injected subcutaneously twice daily for 4.5 days. Each chick received a total of 2.0 mgm. of pituitary powder. Therefore, all comparisons were made on the basis of hormone concentration in 2.0 mgm. of dried pituitary. The changes which occur are the same when the data are calculated as the amount of hormone per pituitary gland as they are when expressed as concentration in the dried tissue. The chicks were sacrificed 24 hours after the last injection, and the testicular and body weights recorded. This technique measures primarily the follicle-stimulating hormone (FSH).

The dried weight of the pituitaries was low in winter, so the powdered samples were pooled by pairs of months as follows: September-October, November-December, and January-February.

The basic statistical methods used in the study are those of Snedecor (1946). A significant positive correlation was found between the body weights and testis weights of the assay chicks, and the regression line of one on the other was calculated. The variation of the testicular weights from the regression line was used as a measure of the response to the pituitary preparations. The original variation in the raw data is not limited by this method as it is when organ weights are expressed as a ratio of body weight.

A detailed examination of the histology of the testes of the donor pheasants was not made except during periods of rapid change in weight. Testes were sectioned at six micra and stained with Harris' haematoxylin. All testes from the donors of pituitaries in January and five from donors in February, including the heaviest (1.125 gms.) and the lightest (0.263 gms.), were examined. It was assumed, because of their large size (0.784 to 11.7 gms.), that most of those from donors in March, April, May, and June were producing mature sperm,

TABLE I

PITUITARY GONADOTROPHINS AND TESTIS WEIGHT OF PHEASANTS AT DIFFERENT SEASONS AND DAY-LENGTHS

	Gonadotrophic Activity		*Testis Weight (in gm.)*				
Date	*Number of Chicks*	*Testis Response (in mgm.) Mean* $\pm\sigma_m$	*Number of Pheasants*	*Mean* $\pm\ \sigma_m$		*Day-length Including Civil Twilight (Hrs.:mins.)*	
Jan. 20	9	9.0* ±1.3	12	0.2233 ±0.0738	0.32 ±0.05	10:36	11:10
Feb. 20			12	0.4138 ±0.0649		11:47	
Mar. 20	6	14.5* ±1.5	12	4.0866 ±0.7150		13:04	
Apr. 16	6	18.1* ±3.7	12	8.2167 ±0.5490		14:28	
May 18	7	18.0* ±2.9	11	8.1364 ±0.5346		15:54	
June 19	5	15.7* ±3.2	12	5.7583 ±0.6075		16:36	
July 20	4	2.3 ±1.0	12	1.7178 ±0.2434		16:06	
Aug. 19	6	6.6 ±1.7	12	0.2944 ±0.0136		14:49	
Sept. 20	9	11.7* ±2.3	12	0.3364 ±0.0194	0.236 ±0.0122	12:13	12:05
Oct. 17			10	0.2488 ±0.0141		11:58	
Nov. 19	5	8.7* ±1.5	12	0.1797 ±0.0121	0.180 ±0.0098	10:40	10:24
Dec. 20			12	0.1825 ±0.0126		10:09	

* Significantly different from uninjected chicks.
σ_m Standard error of the mean.

but no histological study was made of these testes. Five testes from July, including the heaviest (3.5 gms.) and lightest (0.287 gms.), and all of those from August were sectioned. All preparations were examined at 900× under oil immersion and classified by stages according to Blanchard (1941) and Blanchard and Erickson (1949). Testes from a group of 12 juveniles and 12 adult male pheasants from the game farm sacrificed on October 1 the year following the study were examined for age differences.

RESULTS

The data for cock pheasant pituitary gonadotrophin are shown in table 1 and in figure 1, with the mean gonadial weight of the donor pheasants for each month. The spermatogenic status of the pheasant testes was determined by histological examination in some cases and assumed in others. The period during which spermatozoa were observed or assumed to be present in the seminiferous tubules is indicated in figure 1. The values plotted for gonadotrophic potency are the mean differences between uninjected assay chicks and those receiving pheasant pituitary powder.

Cock pheasants from the game farm were sacrificed for the first time on July 20, 1947, when their testes were rapidly involuting. Spermatozoa were still present in three of the five testes examined but not in the two lightest. The pituitary glands at that time were at their minimum gonad-stimulating potency for the 12 months studied.

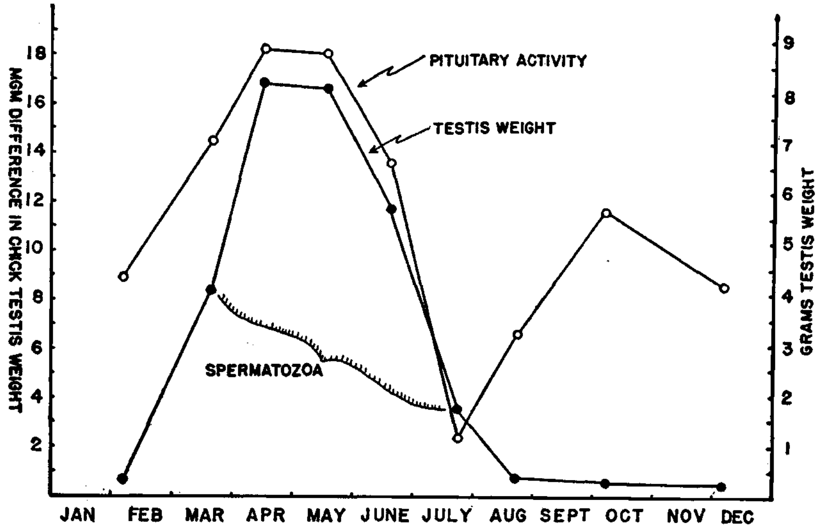

FIGURE 1. Activity of Testis and Pituitary in Pheasants.

A slight increase in potency was found in August when all the testes were in late regression. Pituitaries taken from birds in September and October produced a statistically significant increase in the testes of the assay chicks. The material from both winter groups (November-December and January-February) was significantly active though slightly less so than material from birds in September and October. In November and December, the weights of the donors' testes were minimal. There may still have been some activity in the interstitial cells, but no histological data are available. The presence of primary spermatocytes in adult pheasants has been observed throughout the year (Hiatt and Fisher, 1947). In this study, testes from pheasants obtained in January and February showed some activity, those from January having reached Blanchard's stages two and three (a few primary spermatocytes and occasional Leydig cells) and those from February, stages three and four (some tubules with synaptic primary spermatocytes and increase in Leydig cell development).

In the spring and early summer months the potency of pituitary material varied directly with gonadial weight: both reached a maximum in April and May and decreased during June and July. The testes examined in July were still in Blanchard's stage 7 (full spermatogenesis) or early regression, although average weights had declined markedly. Widespread involution of tubular tissue and pyknosis of interstitial cell nuclei were evident in the smallest testes.

A study of hen pheasants was carried out simultaneously and in a similar manner except that no histological work was done. During the spring egg-laying period, the pituitaries were collected on the basis of the number of eggs laid: those birds which had laid one egg, those which had laid more than 30 eggs, and those which had ceased laying. In addition on July 20, a mixed group of game farm hens was collected, some of which were still laying. Hen pituitaries generally were not as potent as cock pituitaries: significant stimulation of domestic chick testes was obtained only from the January-February, March, and June pituitary preparations. Pituitary glands collected in April from hens which had laid but one egg (and in some cases ovulated a second) gave no significant stimulation of chick testes with the 2 mgm. dose used.

Discussion

Most investigations of pheasant reproduction have divided the behavioristic and physiological processes into phases. Hiatt and Fisher (*op. cit.*) described three phases based primarily on testis weight. The processes involved are continuous so that partitioning of them into phases is a superficial but necessary technique for describing the observations. We shall discuss in order (1) the breeding season, (2) the post-reproductive season, and (3) the fall recovery and winter seasons.

The breeding season.—Two problems suggested by the data described in this paper and other data on the reproductive season of birds are: (1) the relationship of measured endogenous gonadotrophin to the function of the testes of the donor birds; and (2) the relationship of environmental stimuli to the production and secretion of pituitary gonadotrophin. In this study, the close parallel between gonadotrophic content of the pituitary gland and the function of the testes of the donor pheasants (based primarily on weight) indicates that gonadotrophin was entering the blood in direct proportion to the amount in the pituitary gland. Hence, when the gonadotrophic content increased in March, there was a concurrent advance in testicular weight. Both remained high in April and May. This relationship held for other phases of the reproductive cycle, although at lower levels of gonadotrophin the rate of recrudescence of the testes was slower.

Pheasants are capable of breeding any time from October to June (Bissonnette and Csech, 1937) and perhaps at other times of the year under proper manipulation of day-length. Bissonnette and Csech (*op. cit.*) found 14 hours of artificial and natural light per day for eight days in early October sufficient to cause an immature cock pheasant to tread immature hens which produced fertile eggs in November. They were able to obtain fertile eggs from November through March by increasing day-lengths. In our data, the first major increase in pituitary gonadotrophin and in testicular weights over winter levels was from January-February to March when the days, including Civil Twilight, were from 11.2 to 13.1 hours long (Suppl. to the American Ephemeris 1946, U. S. Naval Observatory). A 62 per cent increase in the level of gonadotrophin and a 1200 per cent gain in mean testis weight were associated with these day-lengths in March. Although day-length continues to increase until late June, the amount of gonadotrophin in the pituitary apparently reaches its maximum by April and decreases in June. The nature of pituitary function during the long days of summer is not well understood but probably is related to what has been called the "refractory state" of the testes, and/or the pituitary gland, by many writers (Bissonnette, 1937).

The post-reproductive period.—This period begins in June. The quantity of gonadotrophin in the pituitary was the same in April and May; June was the first month in which a decline was evident. The pituitary gonadotrophin decrease 12.5 per cent from May to June and 87 per cent from May to July (table 1). A simultaneous decline of 79 per cent in mean testis weight occurred in the latter period. We can reasonably conclude that the decline in the production of gonadotrophic hormone brings about gonadial involution in game farm pheasants whether or not the testes still are sensitive to gonadotrophin during this period.

Other investigators have clearly shown that the species of birds which can be brought into reproductive condition by increased day-lengths cannot be maintained in this condition. Involution of the testes ultimately occurs and molting follows in spite of continued long days. A drop in mean testicular weight and molting occurred in June in our experimental cock pheasants, although day-lengths were far in excess of those which produced an increase in March.

Various writers have suggested explanations for the cyclic regression of activity in the gonads. Several investigators have proposed an inherent cycle: most recently Wolfson (1952) writes of the inherent rhythm as an alternation of activity and inactivity, the rate, amplitude, and duration of which is controlled by day-length.

Several agents have been observed to alter the reproductive cycle of birds. Thyroxin accelerates the reproductive cycle of English Sparrows, *Passer domesticus* (Miller, 1935), producing premature regression; adrenalin inhibits the effect of light or causes regression in English Sparrows (Perry, 1941; Lyman, 1942); and prolactin causes regression of testes in the chicken and pigeon (Bates, Lahr, and Riddle, 1935; Bates, Riddle, and Lahr, 1937) and inhibits gonadial development in White-crowned Sparrows, *Zonotrichia leucophrys* (Bailey, 1950). Selye (1946) suggests that environmental stress stimulates the secretion of adrenocorticotrophic hormone (ACTH) by the pituitary gland while at the same time suppressing the output of gonadotrophin. Whatever the phenomenon associated with regression of pituitary gonadotrophin and the testes, it is clear that we need more direct evidence of the levels of the other pituitary hormones (thyrotrophin, ACTH, prolactin) during the course of the reproductive cycle. Not until then can we understand the nature of the rhythm whether inherent or otherwise.

The duration of the refractory period of pheasants under normal seasonal day-lengths is not well established. In this study gonadotrophic content of the pituitary was greater, but not significantly so, on August 19 than at the low point on July 20. The combined September-October sample contained significant amounts of gonadotrophin. Thus, the pituitary gland of adult male pheasants may be sensitive to day-length by the latter period if not before. The work of Bissonnette and Csech (*op. cit.*) demonstrated that immature pheasants were sensitive by October, but data on the testes of pheasants taken by Hiatt and Fisher (*op. cit.*) indicate that the testes of adults do not undergo spermatogenesis in fall while the testes of juvenile birds sometimes do. We have similar data for pheasants, but not from those from which pituitaries were taken.

The fall resurgence and winter period.—The resurgence of reproductive activity in fall among pheasants and other birds is well known. Significant amounts of pituitary gonadotrophin similar to those in the September-October sample were also found during the winter months, but this was not reflected in a mean increase in testicular weight until January. It should be noted that the slow growth of the testes from November through February is associated with gonadotrophic levels only half those of April. In early October, the testes of pheasants which we have examined histologically show considerable advancement over those taken in August. We suggest that these moderate levels of pituitary secretion produce a slow development of the testes.

Wolfson (*op. cit.*) pointed out that nine hours of day-light were sufficient to produce spermatogenesis in Slate-colored Juncos (*Junco hyemalis*) in 11 months, and it is to be emphasized that the pheasants used in our study were subject to not less than 10 hours of light per day (including Civil Twilight) throughout the winter. Moderate levels of pituitary activity are associated with these day-lengths in pheasants.

SUMMARY

Dried pituitary glands of sexually mature game farm pheasants sacrificed during 12 months of the year were assayed for their gonadotrophic hormone content as determined by bio-assay in one-day-old male domestic chicks. The testicular weights of the male pheasant donors followed closely the fluctuations of pituitary gonadotrophic content from late winter to mid-summer.

A decline in amount was underway by June 20, so it was reasoned that the pituitary gland was refractory to day-length by that time. The decrease in the production of gonadotrophic hormone was accompanied by a decline in testis weight with complete involution in July and August. Production of gonadotrophin appeared to increase in August, and reached statistically significant levels in combined dried pituitaries taken in September and October.

No weight increase of the pheasant testes was found in fall and winter although appreciable amounts of gonadotrophin were present in the three samples taken in September-October, November-December, and January-February. No histological study of donors' testes taken in the fall and early winter was made; but testes of non-donor birds killed October 1 had reorganized tubular and interstitial tissue, and testes from the donors of January 20 contained active Leydig cells. The data indicate that day-lengths of this period are sufficiently long to maintain moderate pituitary gonadotrophic production which may act slowly on the testes.

The cyclic nature of reproduction in pheasants and other birds was discussed in relation to the effect of light on the production of gonadotrophins by the pituitary gland. It was emphasized, however, that more information concerning the seasonal fluctuation of other pituitary hormones is needed before adequate evaluation can be made of the rôle of the environment in the control of reproduction, especially the refractory phase of the cycle.

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