TABLE I.
Average Data Obtained in a Series of 27 Hyperthyroid and 8 Normal Control Rats.

	Normals	Hyperthyroid
Initial body wt., gm.		224
Final body wt., gm.	197	178
Wt. of ventricles, mg.	510.4	768.8
Ventricular wt./body wt. ratio	2.59	(3.43)* 4.32
Creatine concentration in ventricles, mg. %	198.3	104.4
Total creatine in ventricles, mg.	.996	.825

* The upper figure is calculated on the basis of original body weight; the lower on the basis of the final weight.

hyperthyroid rats showed hypertrophy, signs of acute degeneration, mononuclear cellular infiltration, and occasionally fatty metamorphosis.

Not infrequently death overtook the thyrotoxic animals in a sitting posture, sometimes following relatively slight exertion, and without being preceded by the usual signs of serious illness.

Our acknowledgment is due to Eli Lilly and Company for their generous cooperation in supplying a part of the desiccated thyroid used in these experiments.

7780 C

Differential Effect of Some Gonadotropic Substances on Development of Cyclical Sex Characters in the English Sparrow.*

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From the State University of Iowa.

The English Sparrow, like all other wild birds of the temperate zone, has a cyclical sexuality. During the breeding season in spring and early summer the sex glands are large and active; in July or August they undergo a rapid involution. In the males the testes shrink to approximately one-thousandth of their maximal size and the ovary of the females regresses to about the condition characteristic of immature birds 2 or 3 months old. Some of the secondary sex characters, especially bill color and gonadal ducts, follow this seasonal cycle. During the period of gonadal involution the bill of the male is as light as that of a castrate and the oviduct of the female is as thin and straight as that of an immature or an ovariotomized

* This investigation was supported by grants from the Committee for Research in Problems of Sex of the National Research Council.

bird. On the other hand during the breeding season the male bill is jet black and the female oviduct is voluminous and convoluted. Keck[1] has shown that these secondary sex characters are directly controlled by the corresponding male and female sex hormones. It is evident, therefore, that the involuted gonads of fall sparrows release sex hormones either not at all or in subthreshold quantities.

In an attempt to determine the possible rôle played by the hypophysis in this seasonal sex cycle, gonadotropic substances were injected into sparrows in sexual inactivity. In the winter of 1932 a group of quiescent males was injected with extracts from pregnancy urine, prepared and standardized in rat units in our own laboratory. No response was obtained, even though the exorbitant dosage of 50 units was administered daily during the whole month to some of the birds (Table I). Success came, however, when extracts of the anterior

TABLE I.
Male sparrows in quiescent phase injected with gonadotropic extracts.

Source of hormone	Daily amt. in rat units	No. injections	No. animals	Response
Horse Hyp. (Hill 4)	2	33	1	+
" " "	2	13	1	+
" " "	2	18	1	+
" " "	2	3	1	+
" " "	2	5	1	+
" " "	2	7	2	+2
" " "	2	17	1	+
Beef Hyp. (Kamm A.)	1	24	2	+2
" " "	1	20	1	+
" " "	2.5	7	1	+
" " "	2.5	17	1	+
Pregnancy Urine	1	30	4	—4
" "	10	30	4	—4
" "	25	30	4	—4
" "	50	30	3	—3
Pregnancy Urine (Kamm A.S.)	10	18	3	—3
" " " "	20	23	1	(+)

lobe of horse pituitary (preparation R. T. Hill) were given. As early as 4 hours after the third injection (52 hours after the first) the testes are considerably enlarged. The seminal tubules are inflated; their diameter is 1.5 times that maintained in the quiescent phase. Spermatogonial mitoses, never observed in the controls, are abundant. At the end of one week, primary spermatocytes make their appearance. During the third week, secondary spermatocytes and spermatids are added and in a male injected for 33 days even mature spermatozoa are present. The growth of the testes is shown in Fig. 1. At 17 to 18 days testes of 2 males have increased their

[1] Keck, W. N., *J. Exp. Zool.*, 1934, **67**, 315.

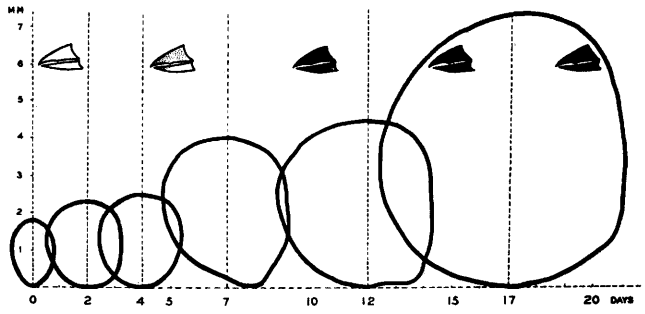

FIG. 1.
Effects of daily injections of 2 rat units of "follicle stimulating" hormone extracted from anterior lobe of hypophysis on testicular size and bill pigment. (The bills are not drawn to scale.)

volume by 100 to 150 times. The testes of the male with 33 injections (not represented in Fig. 1) are about 210 times as large as those of the controls. As one would expect, the growing testes liberate increasing amounts of male sex hormone which presently are recorded by the changing bill color (Fig. 1). On the fifth day a purplish tint is first noticeable which soon turns to blue, due to the accumulation of black pigment in the deeper strata of the horny bill. As time passes, this pigment is moved toward the surface and the bill appears darker and darker. The deepest jet black is not acquired before the end of the third week. These striking effects were obtained with daily injections of about 2 rat units only (Table I). Recently we received through the courtesy of Dr. Oliver Kamm 2 products from the Research Laboratories of Parke, Davis and Company, which made it possible to repeat our experiments (Table I). The first ("Antuitrin") is an extract of beef anterior lobe, containing mainly the follicle stimulating fraction of the gonadotropic hormones. Three males were injected with 1 rat unit daily. At the end of 20-24 days the testes show an increase to about 60 times the volume of controls. Two males injected with 2.5 rat units daily for shorter periods show about the same rate of development as those of our first series. The second product ("Antuitrin S") was extracted from pregnancy urine. Three males injected during 18 days with 10 rat units daily do not show even a trace of growth. However, the male that received 20 units during a period of 23 days had moderately enlarged testes (20 times the volume of controls). This raises the question whether we deal with an irregular spontaneous activation or with a "synergetic" effect (Evans, *et al.*[2, 3]). It can

[2] Evans, H. M., Meyer, K., and Simpson, M. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 845.

[3] Evans, H. M., Pencharz, R. I., and Simpson, M. E., *Endocrinology*, 1934, **18**, 601 and 607.

safely be assumed that this male, which had been newly caught, was slightly stimulated by recent sunny weather. However, since the bird was kept in captivity this alone should have remained without consequences—as it did in a large number of controls caught on the same day. Further experiments will have to prove whether the combination of a subthreshold dose of hypophysis hormone with the otherwise inactive principle of pregnancy urine can stimulate testicular development in the sparrow. It is of interest that in the present case the thyroid is not enlarged (see below).

Experiments with females give equally striking results (Table II). Fig. 2 represents the effect of 16 daily injections of 2 rat units

TABLE II.
Female sparrows in quiescent phase injected with gonadotropic extracts.

Source of hormone	Daily amt. in rat units	No. injections	No. animals	Response	Weight mg.
Horse hyp. (Hill 4)	2	33	1	+	140*
" " "	2	16	1	+	**
Beef hyp. (Kamm A)	1	24	3	+1 (?)2	58.6; 10; 9.4
" " "	2.5	24	1	+	700
Preg. urine (Kamm A.S.)	10	18	2	—2	6.8; 1.6
" " " "	10	20	1	—	10
" " " "	20	23	1	—	6.1

* Estimated 1000 mg. at biopsy on 22nd day. ** Estimated 500 mg.; fig. 2 (right).

FIG. 2.
Effects of 16 daily injections of 2 rat units of "follicle stimulating" hormone extracted from anterior lobe of hypophysis on the development of the ovary and the oviduct of the English Sparrow. Left: largest of controls. Right: injected bird. × 2.

of Hill's extract. The ovary has attained about 50 times the weight of controls. A larger number of eggs than ever seen in normal lay-

ing females have entered the final growth phase. The oviduct shows an equally high development, indicating the production of female sex hormone by the ovary. A second female which received the same treatment was biopsied on the 22nd day. Its ovary was found about twice as large. Some of its largest follicles were punctured at that occasion. Injections were discontinued on the 33rd day. When on the 35th day the bird was killed its ovary contained at least a hundred middle-sized eggs (diameter 1-2 mm.), while the largest ones had all disappeared. The oviduct was still of maximal size. Administration of 2.5 units of Dr. Kamm's beef-antuitrin gave a similar result. On the 24th day the ovary had 70 times the weight of the heaviest of controls. Ten eggs had diameters of from 4 to 6 mm. Injections of 1 rat unit daily give only inconstant threshold reactions. Of three females treated with this dosage one shows a good response. Its ovary has 6 times the weight of the best controls and the largest egg measures 4 mm. The oviduct shows a very marked though not the maximal development. The 2 other females have ovaries not heavier than those of the heaviest controls. Their thyroids, however, are very much enlarged, like those of all males and females injected with hypophysis extracts (more than 3 times the volume of those from birds injected with urine preparations). Four females injected with relatively high doses of Antuitrin S do not show growth of ovaries or thyroids.

The failure of pregnancy urine (prolan or Antuitrin S) to stimulate the quiescent and involuted gonads of the sparrow is in harmony with the statement by Riddle and Polhemus[4] and by Schockaert[5] of its ineffectiveness to bring about precocious development of sex organs in immature doves, pigeons, ducks and chicks. The question arises, therefore, whether the bird differs from the mammal by the responsiveness of its gonads or by the failure of "synergetic" action of the hypophysis. No final answer can be offered yet, though a comparison with conditions in hypophysectomized rats suggests fundamental differences in the mechanism of gonadal reactions. Starting with a preliminary note for Evans' laboratory[3] a number of excellent studies, mainly by Collip, Smith, Evans and their respective coworkers, have recently come forth which disclose that the ovaries and testes of hypophysectomized rats are slow to react on pregnancy urine injections. With relatively high doses

[4] Riddle, O., and Polhemus, I., *Am. J. Physiol.*, 1931, **98**, 121.

[5] Schockaert, J. A., *Am. J. Physiol.*, 1933, **105**, 497.

[6] Reichert, F. L., Pencharz, R. I., Simpson, M. E., Meyer, K., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 843.

the endocrine system and consequently the development of secondary sex characters can be stimulated. Follicular growth seems never to follow and spermatogenesis is only incompletely restored. It is evident that the gonads of birds are even less responsive to pregnancy urine extracts than those of hypophysectomized rats. At the present it seems that this principle is entirely foreign to and not utilizable by the bird.

A most characteristic feature of the endocrinology of the seasonal cycles of the sparrow is the near coincidence of minima and maxima in hypophyseal and gonadal activity. Our experiments indicate that gonadal development is stimulated by increased hypophyseal activity; but on the other hand, gonadal involution and cessation of the production of sex hormones do not incite renewed production of gonadotropic hormones. The sexual cycle seems to be entirely and one-sidedly directed by seasonal changes in hypophyseal functions.

7781 C

Origin of Functional Differences Between Male and Female Hypophyses.*

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Recent investigations have shown that there is a functional, and even an histological difference between male and female hypophyses. This presupposes either (1) a primary difference which is genetical and determined by the sex genes, or, (2) a secondary difference which is determined by the gonad function and is, therefore, dependent upon whether an ovary or a testis is present.

In order to investigate these two possibilities, testes of new-born male rats were transplanted into littermate females and the resulting disturbance in the endocrine system followed by the vaginal smear method. Since in the newborn rat the heat regulatory mechanism has not been established, animals can be operated after being rendered insensible and immotile by cooling on ice. Out of 150 females raised to puberty, 56 had the grafts resorbed; 59 established normal oestrus cycles even though grafts persisted; however, 35 females, all with well-growing grafts, gave evidence of an altered

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