the heart; both leads I and III from this region are the inverse of normal. (It is to be noted that "extrinsic" waves are lacking in each lead arising from the region of stimulation, *e. g.*, leads A I and II and B I and III. Here is evidence that only adjacent activity is recorded by this method.)

Premature beats have been recorded 41 times in 5 dogs, where direct electrodes were arranged as shown by the inset, Fig. 1. Twenty times the premature beats were excited at or near the posterior horn of the left ventricular apex and in each instance the main deflection was in the same direction as that of the spontaneous beats. Twenty-one times the premature beats were entered at the base of the heart and in each instance the main deflection was opposite in direction to that of the spontaneous beats.

Interpretation. The cephalic and caudal ends of the external fibers of the superficial bulbo-spiral muscle were stimulated alternately. Records from direct contacts on these fibers show that apical premature excitation resembles the normal sequence, whereas the product of basal stimulation is the inverse of normal.

This behavior conforms with the anatomical observation[1] that the superficial bulbo-spiral muscle is innervated by the posterior division of the left ramus of the bundle of His, entering that muscle in the posterior papillary muscle of the left ventricle and emerging to the surface in the posterior horn of the apex, to supply that muscle.

9505

Action of Male Sex Hormone With and Without Estrin in the Female Rat.*

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It has recently been reported that injection of male sex hormone simultaneously with estrin into normal female rats partially suppressed the characteristic histologic reaction of the anterior pituitary to estrin.[1, 2] The degree of weight increase in the pituitary and the

[1] Robb, Greene, and Robb, *J. Tech. Methods and Bul. Assn. Int. Ass. Med. Mus.*, 1937, **17**, 91-92.

* These studies were aided by a grant from the International Cancer Research Foundation made to J. B. Hamilton.

[1] Wolfe, J. M., and Hamilton, J. B., *Anat. Rec.*, 1937, **67**, 55 (suppl.).

[2] Wolfe, J. M., and Hamilton, J. B., *Endocrinology*, 1937, **21**, 603.

degree of degranulation of the chromophilic cells, together with a reduction in their relative percentages and an increase in that of the chromophobes, were more marked in the rats which received estrin alone than in those which received both estrin and male hormone. Previously, it had been demonstrated that injection of sufficient amounts of estrin into normal female rats resulted in the formation of large, active corpora lutea in the ovaries.[3-6] Estrin apparently induced such luteinization indirectly by stimulating the production or the release of a luteinizing factor by the anterior hypophysis.[5] Therefore, it was considered possible that in rats which received male hormone simultaneously with estrin there would be not only a suppressed histologic reaction of the anterior lobe to estrin but a reduction in the degree of luteinization in the ovary. In the experiments described below this assumption has been tested. The action of male hormone alone on the ovaries was also studied.

Series 1. Seventeen mature female rats received daily injections of both 500 gamma of male sex hormone† and 200 r.u. of estradiol-benzoate† for a period of 10 days while a second group of 42 females received daily injections of 200 r.u. of estrin alone for a like period. The mean pituitary weight of the rats receiving both male hormone and estrin was 11.7 mg.; that of rats receiving estrin alone was 20.4 mg. The mean ovary weight of rats receiving the combined injections was 54 mg.; the mean of those receiving estrin alone was 66 mg. The vaginal epithelium of the rats which received both male hormone and estrin was with 2 exceptions markedly mucified; these 2 exceptions showed general stratification with evidence of early mucification. The epithelium in the majority of the vaginæ of rats which were given estrin alone was stratified, with evidence of early mucification. However, a few showed stratification and in some instances cornification, while a few were markedly mucified. As a whole, vaginal mucification was much more marked in those rats which received combined injections than in those which received estrin alone.

Serial sections were cut of all ovaries studied. The 5 largest corpora lutea in each ovary were measured with a micrometer eyepiece and their average diameter calculated in millimeters. The

[3] Hohlweg, W., *Klin. Woch.*, 1934, **13**, 93.

[4] Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 757.

[5] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1377.

[6] Nelson, W. O., *Anat. Rec.*, 1935, **64**, 61 (suppl.).

† Testosterone-propionate and estradiol-benzoate (Progynon-B) were furnished by the Schering Corp. through the courtesy of Dr. Erwin Schwenk.

mean diameter of the corpora lutea in the ovaries of rats which received both male hormone and estrin was 1.6 mm.; the range was from 1.4 to 1.9 mm. The mean diameter of the corpora in the rats which received only estrin was 1.7 mm.; the range was from 1.4 to 2.0 mm. In 33 uninjected controls it was found that the mean diameter of the corpora lutea was 0.93 mm. These data indicate that male hormone administered with estrin at the most only slightly reduces the capacity of estrin to stimulate luteinization.

Series 2. The above experiments indicate that injection of male hormone does not possess the capacity to decrease materially luteinization induced by injection of estrin. Therefore, the capacity of testosterone-propionate to stimulate luteinization was tested. Our studies confirm and extend those of others[7, 8] who have reported that injection of male hormone increases the size of the corpora lutea.

Ten adult female rats received 500 gamma of male hormone daily for 10 days; they were killed one day after the last injection. Examination of serial sections of the ovaries revealed that in 4 animals the corpora lutea were moderately increased in size; the mean diameter was 1.4 mm. On the other hand, the corpora lutea in the ovaries of a second group of 4 of these rats were as small as those found in the ovaries of normal untreated cyclic females. The ovaries of the remaining 2 rats contained corpora lutea which were slightly increased in size. These results indicate that male sex hormone possesses the capacity to induce luteinization in some instances and suggests the possibility that the capacity of this substance, given in the amounts used in these experiments, to stimulate the formation of large active corpora lutea might be correlated with the phase of the estral cycle during which injections were initiated. This hypothesis was tested in the experiments recorded below.

Forty adult rats received daily injections of 2000 gamma of male hormone for 10 days; all were killed one day after the final injection. Previous to the beginning of injections vaginal smears were made for a period of 3 weeks and were continued throughout the injection period. In 15 rats injections were begun in estrus; in 6 the rats were in metestrus and in 19 they were in diestrus (2 days postestrus) when the injections were started.

Our data indicate that the response of the ovaries to the injections of male hormone differed, depending on the phase of the estral cycle during which injections were started. The ovaries of rats

[7] Korenchevsky, V., Dennison, M., and Hall, K., *Biochem. J.*, 1937, **31**, 780.

[8] Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 823.

which received their first injection either in estrus or metestrus contained corpora lutea which were definitely and consistently larger than those found in the ovaries of untreated rats killed during the normal estral cycle. Individual data on the mean diameters of the corpora lutea are presented in Table I. Confirming the results of previous investigators[7, 8, 9] it was found that injection of male hormone resulted in a suppression of the estral cycle. In these 2 groups the suppression was immediate; the rats failed to exhibit the next expected estrus and remained in diestrus throughout the injection period. Histologic examination revealed that the vaginal epithelium was markedly mucified.

TABLE I.
Detailed Data on Degree of Luteinization of Ovary When Injections Were Started During Various Phases of Estral Cycle.

Aver. diam. of corpora lutea in mm.	Control	Time of Initiation of Injection		
		Estrus	Metestrus	Diestrus
.8 and below	9			9
.9	13			
1.0	4			2
1.1	7			3
1.2				2
1.3				1
1.4		6	1	1
1.5		5	2	1
1.6		1	2	
1.7		2	1	
1.8		1		
Mean diameter	.93	1.5	1.5	1.0

In the rats which received their first injection during diestrus the results were more variable. The ovaries of 9 rats contained corpora lutea which were very small (Table I). Ten rats presented corpora lutea which showed various degrees of enlargement, although only 2 were as large as those found in rats which received their first injection either in estrus or metestrus (Table I).

Histologic study showed that in general in those rats in which the corpora lutea were very small the vaginal epithelium was low with the innermost cells mucified; however, one rat with very small corpora presented a moderately thick vaginal epithelium. In those rats in which there was a definite stimulation of the corpora lutea the vaginal epithelium was thicker and more definitely mucified although the degree of mucification was seldom as great as in those rats which received their first injection either in estrus or metestrus. Vaginal

[9] Robson, J. M., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 49.

smears of the animals receiving their first injection during diestrus revealed that with 2 exceptions, they failed to present a fully cornified smear after injections were started; in several, however, there was a mixed smear of nucleated epithelium and cornified cells on the second or third day of the injection period, the normal time of the next expected estrus. After this initial attempt at estrus in some of the rats, diestrus was prevalent throughout the remainder of the injection period. These studies indicate that testosterone-propionate possesses the capacity to stimulate luteinization when injected in sufficient amounts and that the degree of luteinization obtained is greatest when injections are initiated in estrus or metestrus. Furthermore, the degree of mucification seems in our experiments to be correlated with the degree of luteinization induced.

Studies on the pituitaries of these rats are incomplete. In the glands studied the basophiles have been completely or almost completely degranulated. In most instances the eosinophiles also presented evidence of degranulation and in some of the glands the relative level of these cells was reduced. In these latter glands there was an increase in the percentages of the chromophobes; some were enlarged and possessed enlarged negative images of the Golgi apparatus. The changes in the eosinophiles and chromophobes noted in some of these rats are in contrast to previous findings in which in normal or castrated immature rats 10 daily injections of 500 gamma of male hormone induced degranulation of the basophiles but no noteworthy changes in the eosinophiles or chromophobes.[1, 2] It should be noted that in the experiments here reported we have used a dosage of 10 daily injections of 2000 gamma of male hormone and a different type of test animal.

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Some Properties of the Antigonadotropic Factor.

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While continuing our studies on the antigonadotropic factor of the blood[1] we examined the following properties:

1. *Thermolability.* The antigonadotropic factor was heated in a

[1] PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708, 712.