

Importance of Progesterone to Induction of Sexual Receptivity in Spayed Female Rats.*

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Young and his co-workers¹⁻³ have reported that although estrogen may induce heat behavior in spayed female rats, the administration of progesterone to estrogen-treated animals markedly increases sexual receptivity. Ball^{4,5} was unable to confirm these results. She was of the opinion that her failure may have been due to peculiarities in the strain of rats used. In all of the studies listed the behavior of females has been recorded as nonreceptive, slightly receptive or receptive.

In the course of a longer experiment we have collected some data which tend to resolve some of the differences between the findings of Ball and those of Young and his co-workers. Further, our results include complete and sensitive records of different degrees of receptivity in treated females.

Procedure. Virgin female rats were raised in segregation and ovariectomized at 3 or 4 months of age. Hormone injections and mating tests were initiated approximately 3 weeks after castration.

The effects of 3 types of injections upon sexual receptivity were tested. Seven spayed females were injected with 500 R.U. of estrogen,[†] and tested for sexual receptivity 66-68 hr later. Eight days after the first estrogen treatment the same 7 animals were injected with 500 R.U. of estrogen followed

48 hr later by 0.5 mg of progesterone.[‡] Sex tests were conducted 16-18 hr after the progesterone treatment. The second injection schedule was repeated 3 times at 2-week intervals, and mating tests were carried out in each instance.

Six spayed females were injected with 100 R.U. of estrogen followed 48 hr later by 0.5 mg of progesterone. Sex tests were conducted 16-18 hr after the progesterone injection. Two weeks later these same 6 females were injected with 500 R.U. of estrogen followed 48 hr later by 1 mg of progesterone, and mating tests occurred 16-18 hr after the injection of progesterone. After a 2-week interval the second injection schedule was repeated, and sex tests were given again.

Fourteen spayed females received 500 R.U. of estrogen followed 48 hr later by 0.5 mg of progesterone and were tested for receptivity 16-18 hr after the progesterone treatment. Members of this group were given 3 sex tests at 2-week intervals, each test being preceded by the hormone treatment described above.

In the conduction of sex tests the female was placed with a sexually-receptive male in a circular metal cage 30" in diameter. During the 5-min observation period the following items were recorded: times the male mounted the female, times the female displayed lordosis, frequency of the hopping reaction, of the ear-wiggling reaction and of the back-kicking response of the female. Rapid head movements which produce vibration of the ears, abrupt hopping movements,

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¹ Boling, J. L., Young, W. C., and Dempsey, E. W., *Endocrinology*, 1938, **23**, 182.

² Boling, J. L., and Blandau, R. J., *Endocrinology*, 1939, **25**, 359.

³ Boling, J. L., Blandau, R. J., Rundlett, B., and Young, W. C., *Anat. Rec.*, 1941, **80**, 155.

⁴ Ball, J., *Proc. Soc. Exp. Biol. and Med.*, 1936, **35**, 416.

⁵ Ball, J., *J. Comp. Psychol.*, 1939, **28**, 273.

[†] Estradiol benzoate (Progynon-B) was generously supplied by the Schering Corp., Bloomfield, N.J.

[‡] Ciba Pharmaceutical Products, Inc., Summit, N.J., kindly provided the Lutoeylin employed.

TABLE I.
Comparison of Sexual Behavior Produced in Spayed Female Rats by the Various Amounts of Estrogen and Progesterone.

Hormone dosage	No. rats	Tests per rat	Times mounted	Lordosis responses	Average per rat per test		
					Ear wiggling responses	Hopping	Back-kick
500 R.U. estrogen	7	1	17.4	2.4	0.3	0	4.0
500 R.U. estrogen + 0.5 mg of progesterone	7	3	13.4	10.8	5.5	8.4	0.8
100 R.U. estrogen + 0.5 mg of progesterone	6	1	10.3	3.5	0.2	0.7	3.3
500 R.U. estrogen + 1 mg of progesterone	6	2	7.4	6.2	1.9	2.3	2.3

and the exhibition of lordosis in response to mounting by a second animal are characteristic items in the estrus behavior of the normal female rat. Back-kicking is a form of resistance against the male's copulatory attempts.

Results. Data presented in Table I show plainly that rats of our experimental strain are only slightly responsive to estrogen. Two of 7 animals exhibited some indication of receptivity following estrogen injection. For the group as a whole only 14% of the male's mounts elicited lordosis. The tendency to resist the male by back-kicking was strong. The administration of estrogen followed by progesterone resulted in marked increase in sexually receptive behavior in all cases. For the entire group 74% of the male's mounts elicited lordosis. Ear wiggling and hopping behavior were greatly increased, and back-kicking was reduced. Two females which had shown some receptivity under the influence of estrogen alone displayed a much higher degree of behavioral estrus when progesterone was employed.

The administration of 100 R.U. of estrogen followed by 0.5 mg of progesterone induced heat behavior in 5 of 6 rats. Data shown in Table I reveal that when these same animals were given 500 R.U. of estrogen followed by 1 mg of progesterone sexual receptivity was increased and resistance to the male decreased. Under the influence of the first dosage employed the females showed lordosis in response to 34% of the male's mounts. When the larger amounts were administered 84% of the male's mounts elicited lordosis.

The 14 females receiving 500 R.U. of

estrogen and 0.5 mg of progesterone were mounted a total of 465 times in 3 tests. Lordosis occurred in 389 instances. In this case 83% of the male's mounts elicited lordosis. The scores on hopping and ear wiggling and back-kicking were closely comparable to those exhibited by the 7 females whose responses to the administration of 500 R.U. of estrogen followed by 0.5 mg of progesterone are shown in Table I.

Discussion. Results reported above indicate that in spayed females from our rat colony sexual receptivity depends upon the synergistic action of estrogen and progesterone. At this point it may be stated that normal estrus females from this colony tested with the same technics herein employed usually exhibit lordosis in response to 80% to 90% of the male's mounts. Individual differences are marked and there is some variation for the same individual in different cycles. However, it is safe to state that degrees of receptivity induced in spayed animals by 500 R.U. of estrogen and 0.5 mg of progesterone are closely comparable to those observed in normal estrus females from the same stock. Our findings are therefore comparable to those reported by Young and his co-workers, and dissimilar to the observations described by Ball.

Our colony was originally derived from Wistar stock, but in the past 12 years wild males have been used as breeders at approximately 4-year intervals. In the intervening periods the colony has been inbred. We are inclined to agree with Ball in her conclusion that animals with which she worked are somewhat unique, and we regard it as prob-

able that in the majority of laboratory strains a high degree of receptivity depends upon the administration of estrogen and progesterone.

The method of counting the male's mounts, the females' lordosis responses, and the frequency of other estrus reactions is to be strongly recommended in all studies of heat behavior. Scores thus obtained provide the basis for a direct quantitative comparison of the effects of various types of hormone treatment. By employing the ratio of lordosis responses to mounts the degree of heat behavior induced by 100 R.U. of estrogen plus 0.5 mg of progesterone may be described as approximately 40% as intense as that which follows the administration of 500 R.U. of estrogen and 1 mg of progesterone. Receptivity induced by 500 R.U. of estrogen alone is only 19% as intense as that resulting from the administration of the same amount of estrogen plus 0.5 mg of progesterone. If these technics are applied to a sufficiently large number of

animals results obtained will have high reliability. In the present instance the groups were small and comparisons must be regarded as approximate.

Summary. Three groups of spayed female rats were injected with various amounts of estrogen and of progesterone, and the sexual receptivity thus induced was measured. 500 R.U. of estrogen produced some receptivity in 2 of 7 cases. The same amount of estrogen followed by 0.5 mg of progesterone resulted in a much higher degree of receptivity in all 7 animals. 100 R.U. of estrogen and 0.5 mg of progesterone induced a low degree of receptivity in 6 of 7 cases. 500 R.U. of estrogen and 1 mg of progesterone caused the same animals to exhibit much more intense heat behavior. It is concluded that in the strain of animals studied the production of estrus behavior of normal intensity involves the synergistic action of estrogen and progesterone.

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Relation of Anesthetics to Experimental Shock Syndrome Produced by Venous Occlusion.

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A major difficulty encountered in studies of the factors affecting the onset and course of experimental traumatic or circulatory shock has been that of failure to bring about a fairly constant and readily reproducible syndrome. A partial solution, at least, seems to be offered by the technic reported by Perlow *et al.*¹ who modified the method of regional venous occlusion (Mann)² for this purpose. This approach to the problem has been utilized in our laboratory in a considerable number of animals with gratifying results, and certain experimental variables have been studied. The present report deals with

the influence on the experimental picture of certain anesthetic agents.

Methods. The technic of venous occlusion as outlined by Perlow *et al.* consisted of ligation, under anesthesia, of the common and the internal iliac veins, followed by injection of a suspension of animal charcoal either into the external iliac or the femoral vein; the latter, in our hands, has seemed preferable in view of easier access. Anesthetics were given at dose levels biologically standardized for each animal (Wiggers);³ these usually approximated the calculated doses, which were: (1) morphine sulfate, 2%, 1 cc subcutaneously followed by 1.5 g/kg urethane, 50% intravenously; (2) barbital sodium,

¹ Perlow, S., Killian, S. T., Katz, L. N., and Asher, R., *Am. J. Physiol.*, 1941, **134**, 755.

² Mann, F. C., *Am. J. Physiol.*, 1918, **47**, 231.

³ Wiggers, C. J., *Physiol. Rev.*, 1942, **22**, 74.