

Maternal Behavior Induced in Virgin Rats by Prolactin.

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Many observations of the past twenty years suggested that ovarian hormones activate or develop the maternal instinct in mammals. Wiesner and Sheard,¹ however, have recently given almost conclusive proof that those hormones are not capable of effecting an exhibition of maternal behavior in the rat; their studies also made it quite probable that in rats conditioned by previous "concaveation" the effective agent in the excitation of maternal behavior is some substance, or combination of substances, found in the anterior pituitary. The pituitary extracts used by Wiesner and Sheard were mixtures of the several anterior lobe hormones and though they could not identify the active agent or agents they state: "It seems indeed exceedingly unlikely that the same factor is responsible both for mammary secretion and maternal behavior."

Elsewhere we^{2, 3} have shown that the broody instinct in laying fowl is produced by prolactin, and apparently by that anterior pituitary hormone only; neither estrin nor corpus luteum induces broodiness and care of young in fowl. Since the "incubation instinct" of lower vertebrates and the maternal instinct of mammals are probably phyletic equivalents the above-cited result strongly suggests that prolactin enters into the chain of reactions resulting in the exhibition of maternal behavior. The study of Wiesner and Sheard indicated that young virgin rats are particularly favorable animals on which to test the rôle of prolactin in maternal behavior.

We have used younger animals than were used by Wiesner and Sheard, have much reduced the period or amount of conditioning by concaveation, have sought (and found) maternal behavior while actively continuing injections, and have considered it advisable to arouse the rat ovaries to one full cycle of activity (estrin and progesterin production) before attempting to induce maternal behavior. In this "priming" process we used Prolan (Elberfeld) on one-half

¹ Wiesner, B. P., and Sheard, N. M., *Maternal behavior in the rat*. Oliver and Boyd, London, 1933.

² Riddle, O., Bates, R. W., and Lahr, E. L., *Anat. Rec. (Abstract)*, Supplement, 1934, **60**, 49.

³ Riddle, O., Bates, R. W., and Lahr, E. L., *Am. J. Physiol.*, 1935, **111**, 352.

and follicle-stimulating hormone (F.S.H.) from anterior pituitaries of cattle on the other half of the test animals. The F.S.H. and prolactin used were made essentially by methods previously described.⁴ The F.S.H. preparation (No. 267) contained some thyreotropic, luteinizing (and growth?) principles in addition, but was practically free from prolactin. The prolactin preparation used (No. 324) was free from F.S.H. (immature dove testis test) and thyreotropic (dove thyroid test) principles, and was heated to 60° for 5 hours (pH 8.0) to destroy growth hormone.

In our tests of behavior most of the technique used by Wiesner and Sheard was adopted. In all tests a young rat (1-7 days) was dropped into each cage daily and the behavior of the treated rat observed during 10 to 30 minutes. In a preliminary test 4 "unprimed" virgins 59 days old (and one multiparous rat isolated for 60 days) were injected for 5 days with 30 bird units daily of (unheated) prolactin; the multiparous rat received 60 units daily. At the end of the period of injection a 2-day-old young was left overnight (concaveation) with each of the treated rats of this series, and behavior was observed for 2 days after terminating the injections. No maternal behavior was observed in the case of any of these 5 rats. We thus obtained evidence that a quick response from "unprimed" virgins is probably not to be expected from single daily subcutaneous injections of prolactin; and also some evidence that a "spontaneous" occurrence of maternal behavior is not very frequent in our stock of virgin rats of this age. These preliminary tests are not further considered here. Our later tests involved priming with Prolan (or with F.S.H.), and in a short report Tietz⁵ has suggested that urine of pregnancy may indirectly excite the nesting instinct in rabbits. If that is correct we assume that such dosage ultimately leads to a release of prolactin from the rabbit's pituitary.

Table I gives data obtained on 18 virgin rats, from 4 litters, aged 67-81 days at the beginning of treatment. They had been isolated from males since they were 30 days old; in 8 rats the vagina was closed and in 10 it was open. Nine were primed with Prolan (15 R.U. daily) for 5 days; 9 others were primed with F.S.H. (3 mg. daily) for 5 days. Thereafter, 10 rats were injected with prolactin, 15 units daily; 4 were used as a control without further injection; and 4 were treated with F.S.H. (+thyreotropic, etc.), 3 mg. daily. Two of the rats used (Nos. 222, 224) in Series I had been included

⁴ Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

⁵ Tietz, E. B., *Science*, N. S., 1933, **78**, 316.

TABLE I.
Maternal Behavior in Virgin Rats after A.P. Hormones.

DAYS	INJ.	—	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
S E R I E S I																											
PROLAN	222								N	N	N	C															⊕ +
CONTROL	235								C	C		C															
	231																										
	265																										
PROLAN	201								C	N	C	N	C	N	N		N							N	N		N
	239								C	C		C	N	⊕	⊕	⊕	⊕	+	+	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
PROLACT.	236								N	N		C							○				N				
	243											C	+	⊕	⊕	+	⊕	⊕	⊕	⊕	⊕	+	⊕	⊕	⊕	⊕	⊕
	237								C	C		C															
F.S.H.	210								C	C		C	N														
	263											C	⊕	⊕	+	⊕	⊕	+	+	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
PROLACT.	232								C	C	N	C	N	○	○	○	N	N				○	○	N	○		
	268											C										○	N	○	⊕	⊕	⊕
	267								N	N	C	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
F.S.H.	224								C	C	N	C					⊕	+	+	⊕	⊕	+	+	+	⊕	+	⊕
	233											C					N								N?		
F.S.H.	230											N	C				N	+	+	+				+	N	N	
	252											N	C									N	N	N	N	N	N
S E R I E S I A																											
F.S.H.	230								⊕	⊕	⊕	⊕	⊕	⊕	⊕	+	⊕	⊕									
PROLACT.	233								+	+	⊕	⊕	+	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
	262								N	N		N	N	N	N	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
PROLAN	231								N	N		N	N		N	+											
PROLACT	235											+	○	⊕		⊕	⊕	⊕									
	265																N	○		○							
PROLACT	222								+			⊕	⊕	⊕	○	⊕	⊕	⊕									
F.S.H.	201								N																		
WHOLE A.P.	236																										
	237																										
PROLAN	210																										
WHOLE A.P.																											

⊕ RETRIEVES AND CUDDLES + RETRIEVES ONLY ○ CUDDLES ONLY
 N NEST-BUILDING OVER YOUNG N NEST-BUILDING
 C CONCAVEATED FOR 8-12 HOURS AT NIGHT

* Injected, by mistake, 14 hours earlier with prolactin.

in the preliminary series of prolactin injections; one was placed in the control group, the other in the F.S.H. group. It will be noted that these 2 rats—prolactin injected, and concaveated for 12 hours at 17-22 days previous to this later test—both gave exceptional results in their respective groups. We note that neither the nest-

building nor the care of young in nest (of course the rats can not suckle young) is the full equivalent of that usually observed in the multiparous rat.

Our experience indicates that the fullest expression of maternal behavior obtainable in these young virgin rats consists in the retrieving of young followed by cuddling or covering the young with the rat's own body. These two things may occur separately and are indicated by suitable symbols—separate or combined—in Table I. Nest-building, when the nesting material is placed directly over the young, seems definitely to indicate a true but lower level of development of the maternal instinct. In rat No. 201 this was the only expression of maternal behavior obtainable with prolactin; in rats 230, 237 and 210 even this grade of response was not obtained. These cases show that, with the small amount of concaveation employed and under other conditions of our test, some virgin rats do not show maternal behavior following prolactin administration. It is of course well known that some rats do not show such behavior after normal parturition, and there was a high percentage of failures with the mixed (or whole) pituitary extracts used by Wiesner and Sheard.

The important result shown by the tabulated data is that under prolactin the maternal instinct was developed in several cases. This development was of high degree in 5 of 10 tests. The fuller expression of the instinct appeared at 6 days after beginning prolactin dosage in 1 case, at 7 days in 1 case, at 8 days in 2 cases, and at 18 days in another. Retrieving or cuddling occurred between the sixth and tenth day in all of these 5 cases. In 2 additional cases cuddling occurred at 8 and 9 days, and in still another case significant nest-building was shown at 5 days. In 2 cases there was complete failure of prolactin to induce a recognizable aspect of maternal behavior. Of the 4 control rats 3 showed no maternal behavior; the fourth case, whose peculiar previous history has been noted, first showed some maternal behavior after 17 days of dosage. One of the 4 rats (No. 224) injected with F.S.H. had an earlier history of prolactin injection and showed maternal behavior on the 15th day of this F.S.H. dosage; another, No. 230, was accidentally injected once (at 14th day) with prolactin, and 14 hours afterwards began retrieving young; No. 233 was perhaps a total failure, and No. 262 began showing significant nest-building on the 17th day of F.S.H. injection.

The rats which responded poorly or not at all were retested (Series I A). After repriming their ovaries the control rats and

those of the F.S.H. group which gave no response (or only an attenuated response) while members of Series I, promptly gave a full or an increased response when later injected with prolactin. Similarly those rats of Series I which had failed to respond to prolactin dosage were reprimed and later injected with an *unfractionated* pituitary extract (10 mg. daily) whose prolactin content was at least 1 unit per mg. To the 13th day of such injection no rat that failed to respond to prolactin had responded to the administration of the several pituitary hormones.

Summary. In virgin rats aged 67-81 days, with ovaries stimulated by Prolan or F.S.H. administration during 5 days, the injection of purified and previously heated prolactin induced maternal behavior in 6 of 10 rats after 6-10 injections. Previous conditioning by concaveation was limited to a single 12-hour period in 4 of these rats. The maternal instinct, in somewhat varying degrees, was exhibited by all these rats while the daily subcutaneous injections were in progress. Six of 7 rats of the control and F.S.H. injected groups, all showing little or no maternal behavior during a 25-day test, later developed or greatly accentuated their maternal behavior after 1 to 8 days treatment with prolactin. Four rats which showed no maternal behavior under prolactin also later failed to show such behavior after repriming and 13 days of administration of unfractionated pituitary extract. Prolactin is the anterior pituitary hormone specifically concerned in the activation of maternal behavior in virgin rats.

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Effect of Dietary Fats on Growth and Composition of Tumors.

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While many studies of the effect of diet on tumor growth have been made, few deal with the quality of the dietary fat and its effect on the lipid content of the tumor. Sugiura and Benedict^{1, 2} found that oral administration of 10% cod liver, olive, linseed and chaulmoogra oils had no influence upon susceptibility to and growth of

¹ Sugiura, K., and Benedict, S. R., *J. Cancer Research*, 1930, **14**, 311.

² Sugiura, K., and Benedict, S. R., *J. Cancer Research*, 1925, **9**, 204.