

Detection of Luteotropic and Mammatropic Activity in the Serum of Rats at Midpregnancy¹ (34287)

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Early workers first demonstrated a luteotropic factor in eighth to sixteenth day rat placental extracts and suggested that the fetal placenta was responsible for the activity (1). Subsequent studies showed a synergism of rat placental extracts with female sex steroids in inducing lobular-alveolar development of the mammary gland (2, 3). Others have determined that 12-day rat placental extracts have the greatest luteotropic and mammatropic activity (4, 5). Recent reports presented evidence for the placental origin of the luteotropic and mammatropic factor (called rat chorionic mammatropin or RCM) by demonstrating RCM in the fetal placenta and in the serum of day 12 pregnant hypophysectomized rats (6, 7). The purpose of the present study was to corroborate the finding of RCM activity in the serum of the pregnant rat.

Materials and Methods. The animals in this study were Sprague-Dawley rats obtained from Holtzman Inc., Madison, Wisconsin. Female rats 120 days old with known estrous cycle histories were used to demonstrate the luteotropic action of serum, whereas, male rats 30 days of age were used to indicate mammatropic action. Day one of pregnancy was defined as the day spermatozoa were found in the vaginal smear. Blood was obtained from animals under ether anesthesia by exsanguination from the dorsal aorta on day 6, 11, 12, 13, and 19 of pregnancy; blood from virgin, nonpregnant rats served as the control. Whole blood was initially allowed to clot at room temperature and

then stored at 5° until centrifugation some 12 hr later. The serum obtained from 6 animals was pooled into respective groups and stored at 5° until used.

Luteotropic action was demonstrated by the daily subcutaneous injection of 0.1–0.6 ml of serum starting on the day of vaginal cornification and continuing for 8 days. On the fourth day, the left uterine horn was traumatized on the antimesometrial side with a burred needle. On the eighth day the animal was sacrificed and examined for the presence of deciduomata. Both sides of the uterus were weighed and a 100 mg increase in the weight of the left horn when compared with the right was considered a positive deciduomata response. Vaginal smears were continued throughout the experiment. Mammatropic action was demonstrated by daily intraductal injection of 0.1 ml of serum plus 25 µg of prednisolone acetate into the left abdominal teat of the immature male rat; the right side served as the control gland. In previous studies Lyons (3) emphasized the importance of glucocorticoids for the initiation of secretory activity when mammatropin was present; no response was noted, however, when up to 100 µg of prednisolone acetate was administered alone. The injection regimen was continued for 3 days, and upon sacrifice the mammary glands were mounted and rated according to the method of Lyons and Johnson (8).

Results. The minimal effective dose (MED) of 12-day serum for inducing deciduomata formation was 0.5 ml; however, in doses of 0.1–0.4 ml there was an inhibition of estrus without deciduomata formation, (Table I). There was no response, *ie.*, no inhibition of estrus or deciduomata formation, with serum from nonpregnant or rats pregnant 6,

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TABLE I. The Influence of Serum from Rats at Various Stages of Pregnancy on the Estrous Cycle and Deciduomata Induction.

Pregnancy (days)	Vol of serum/day (ml)	No. of rats	Inhibition of estrus	Deciduomata formation
Control ^a	0.1 or 0.6	6	0/6	0/6
6	0.1 or 0.5	4	0/4	0/4
11	0.1 or 0.5	4	0/4	0/4
12	0.1 or 0.4	4	4/4	0/4
12	0.5	6	6/6	6/6
13	0.1 or 0.5	4	0/4	0/4
19	0.1 or 0.5	4	0/4	0/4

^a Nonpregnant.

11, 13, or 19 days. The MED of 12-day serum for demonstrating the mammatropic action was 0.1; minimal lobulo-alveolar development was observed in all animals whereas no secretory activity was noted (Table II). No mammatropic response was observed with serum either from nonpregnant animals or from rats pregnant 11 or 13 days.

Discussion. In a preliminary experiment (Cohen and Gala, unpublished) the injection of serum from 12-day pregnant rats appeared not to stimulate endogenous prolactin production because after cessation of serum treatment the rats became cyclic within 2-3 days (the serum was injected for 8 days and then stopped). Disruption of the estrous cycle and deciduomata formation appeared to be due to a luteotropic substance found in the serum of 12-day pregnant rats and confirmed the previous reports of Matthies who obtained similar results using hypophysectomized rats (6, 7). The higher MED needed for deciduomata formation as

compared to that needed for the inhibition of estrus is an interesting observation. An explanation of this observation, however, is not immediately obvious.

The possibility that the luteotropic response observed of serum obtained from 12-day pregnant rats was due to progesterone concentration of the serum appears remote. Yochim and De Feo (9) reported that 2 mg of progesterone/day must be administered in order to obtain maximal decidual response. Further, Wiest *et al.* (10) reported that serum from 12-day pregnant rats contained only 8 $\mu\text{g}/100$ ml of progesterone. Thus, even on the eight day injection regimen used here, less than 1 μg of total progesterone was administered with the serum. It appears then that the luteotropic response of 12-day pregnancy serum is in all probability due to RCM.

The mammatropic activity of serum from 12-day pregnant rats while demonstrating modest lobular development, did not induce secretory activity, in contrast to the findings of Matthies (7, 11). Whether this difference was due to the fact that Matthies used the Long-Evans rat and we used Sprague-Dawley is not known but is offered as a possibility. Further, greater volumes of 12-day rat serum were needed in our study to induce deciduomata formation than those reported previously (7, 11) and again may be due to strain differences. Although it is evident that there is a peak in RCM activity in the serum of pregnant rats, the mechanisms that bring this about are obscure.

Summary. Serum obtained from rats during various stages of pregnancy was assessed

TABLE II. Influence of Serum from Rats at Various Stages of Pregnancy on the Mammary Gland.

Pregnancy (days)	No. of rats ^b	Lobular-alveolar development	Secretory activity
Control ^a	4	0/4	0/4
11	4	0/4	0/4
12	6	6/6	0/6
13	4	0/4	0/4

^a Serum from nonpregnant animals.

^b All animals received intraductal administration of 0.1 ml of serum plus 25 μg of prednisolone acetate daily for 3 days.

for luteotropic and mammotropic activity. The data presented indicated that there was a marked increase in the activity of serum of rats pregnant 12 days but undetectable amounts were found in rats pregnant 11 or 13 days. There is reason to believe that this activity is of placental origin, but the physiologic significance and the mechanism of this surge remain to be elucidated.

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