sectomized dog is kept on a growth hormone regimen, involves other factors besides direct growth hormone inhibition of insulin action.

*Summary.* Using $C^{14}$ glucose to label the body glucose pool, it was found that growth hormone treatment for 4-5 days in the hypophysectomized dog, resulted in increased rate of glucose release by the liver and plasma glucose uptake by the tissues. The plasma glucose concentrations were also increased. The significance of these findings is discussed.

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## Effects of Prolactin on Duration of Pregnancy, Viability of Young and Lactation in Rats. (23073)

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Several investigators have demonstrated that prolactin is luteotrophic in the rat. Evans *et al.*(1) reported that prolactin was the only anterior pituitary hormone capable of stimulating luteal function in hypophysectomized rats, while Desclin(2) and Mayer and Canivenc(3) found that it prolonged luteal function in normal cycling rats. Cutuly(4) showed that prolactin maintained early pregnancy in rats mated and then hypophysectomized, either before or after implantation of the ovum. However, Mayer and Klein(5,6) reported that prolactin failed to prolong gestation in intact rats when injected beginning on the 14th or 19th day of pregnancy, and continuing until or beyond the normal day of parturition. They also found that prolactin did not prolong the life of corpora lutea of pregnant rats following removal of uterine horns, presumably because the luteotrophic influence of the placentae was removed. They concluded that pituitary prolactin does not exert a luteotrophic action in rats during the latter half of pregnancy.

The present experiments were undertaken to determine the effects of several levels of purified prolactin on duration of gestation, functional activity of corpora lutea and initiation of lactation in rats. In general, the results show that the prolactin preparation used by us prolonged pregnancy and maintained functional activity of the corpora lutea, without preventing initiation of lactation.

*Methods.* One hundred and twenty-four

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TABLE I. Effects of Prolactin on Duration of Pregnancy and Viability of Young in Rats.

| Group and No. of rats | Treatment | Day of parturition 21 | 22 | 23 | 24 | 25 | Avg No. of young Alive | Still-born | Litters retained in uterus |
|---|---|---|---|---|---|---|---|---|---|
| (1) 39 | Controls, no treatment | 32 | 7 | | | | 8.5 | .3 | 0 |
| (2) 10 | .25 mg prolactin daily 16-20th day of preg. | | 9 | | | | 7.9 | .0 | 1 |
| (3) 10 | *Idem* 25th | 1 | 5 | 3 | | | 6.2 | .3 | 1 |
| (4) 15 | .5 mg prolactin daily 16-20th day of preg. | | 6 | 3 | 4 | | 3.7 | 2.4 | 2 |
| (5) 5 | *Idem* 25th | | 3 | | | | 3.8 | 1.0 | 2 |
| (6) 10 | 1 mg prolactin daily 16-20th day of preg. | 3 | 1 | 4 | | | 2.8 | 1.9 | 2 |
| (7) 10 | *Idem* 25th | | 1 | | 2 | 2 | 1.0 | 1.0 | 5 |
| (8) 5 | 4 mg prolactin daily 16-20th day of preg. | | | | | | .0 | .0 | 5 |
| (9) 5 | 1 mg prolactin daily 16-20th day of preg. Ovariectomy 19th day preg. | 5 | | | | | 6.0 | 1.2 | 0 |
| (10) 5 | 1 mg prolactin daily 16-20th day of preg. Ovariectomy 20th day preg. | | 5 | | | | 7.1 | .7 | 0 |
| (11) 10 | 7.5 mg progesterone daily 16-20th day of preg. | | | 1 | 4 | 5 | .0 | 8.5 | 0 |

pregnant albino rats, originally of Wistar strain, were used. They weighed from 165 to 220 g each, and were housed in air-conditioned quarters with Purina laboratory chow and drinking water available at all times. The day sperm were found in the vagina was considered the first day of pregnancy. With the exception of 4 groups of untreated pregnant rats, which served as controls, and one which received progesterone‡ all other groups were injected subcutaneously with .25, .50, 1.0 or 4.0 mg of prolactin§ daily from 16th through 20th days of pregnancy. In 3 groups, prolactin injections were continued through 25th day. The day of parturition was recorded for each rat, as well as the number of young born alive, stillborn or dead *in utero*. Attempts were made to express milk from the nipples beginning the 21st day. At the end of 26 days, all rats which had not dropped their young were killed and mammary glands and ovaries removed for gross and histological examination.

*Results.* Thirty-two of 39 control rats (Group 1) dropped their young on 21st day, and 7 on 22nd day. An average of 8.5 young were born alive and 0.3 young were born dead to each mother. When 0.25 mg of prolactin was injected from the 16th to 20th day of gestation (Group 2), 9 of 10 rats were delivered of offspring on the 22nd day and one failed to be delivered even by the 25th day. The same daily amount of prolactin given through the 25th day (Group 3) extended the average duration of pregnancy, and fewer living young were born per rat (Table I).

Injections of 0.5 mg (Groups 4 and 5) or 1 mg of prolactin daily (Groups 6 and 7) further prolonged gestation and increased the number born dead or retained *in utero*. Treatment with prolactin to the 25th day was more effective in these respects than when terminated on the 20th day. When 4 mg of prolactin was injected (Group 8), none of the rats was delivered of young by 26th day and all fetuses died *in utero*. Prolongation of gestation beyond the 23rd day resulted in death of most young.

Groups 9 and 10 were ovariectomized on the 19th or 20th day of gestation after receiving 1 mg of prolactin daily from 16th through 20th day of pregnancy. These rats were delivered 2 days after ovariectomy and most of the young were born alive. When 7.5 mg of progesterone was injected from 16th through 20th day of pregnancy (Group

‡ The crystalline progesterone was provided through the courtesy of Dr. J. O. Reed of Foundation Laboratories, New York.

§ We are indebted to Dr. A. Borman of Endocrine Research Section, Squibb Institute, New Brunswick, N. J., for prolactin, which was stated to contain approximately 15 I.U./mg.

11), the time of parturition was delayed and all young were born dead.

Secretion by mammary glands was initiated in almost all mother rats by the 21st day, regardless of how much prolactin they were given, but the amount of lactation appeared to be less in mothers with extended pregnancy than in those whose pregnancy was terminated. The corpora lutea of ovaries of prolactin-treated rats were large, well vascularized and were markedly predominant over follicular cells.

*Discussion.* Results of these experiments are believed to demonstrate that prolactin extends pregnancy in rats under our conditions, and that this depends upon dose of hormone and duration of treatment. The largest dose, 4 mg daily, was most effective in inhibiting parturition, while .25 mg daily had almost no effect on duration of pregnancy. When the period of gestation was extended by prolactin, fewer young were born alive and a greater number were born dead or retained *in utero*.

Since prolactin failed to prolong pregnancy in rats which had their ovaries removed just prior to expected parturition, it is clear that the pituitary hormone acts to extend gestation via its stimulation of the ovary. Moreover, since it has been demonstrated that injections of crystalline progesterone will extend gestation (Group 11), it appears that the prolactin action is to stimulate corpora lutea and increase secretion of systemic progesterone. Histological examination of the ovaries revealed large and extensive corpora lutea.

Lactation was initiated in prolactin-treated rats about the 21st day after fertilization, regardless of whether young were expelled or retained in the uterus. However, degree of secretion was usually greater in mammary glands of mothers which dropped their young than in those which retained them. This supports the view that antagonism between mammary growth and lactation during pregnancy is relative and both can proceed simultaneously(7,8). Earlier workers had reported that injections of crude prolactin extracts into pregnant rats resulted in initiation of lactation only after abortion or resorption of the embryos(9).

It is difficult to account for differences between results reported here and those of Mayer and Klein(5,6). They injected rats with 30 to 90 I.U. of prolactin daily for 5 to 10 days beginning on the 14th or 19th day of pregnancy, while we injected approximately 3.7 to 60 I.U. of prolactin daily for 5 to 10 days beginning on the 16th day of gestation. It is possible that factors other than prolactin in the preparations used influenced the results. The exact nature of the factor in rat placentae which can maintain gestation in hypophysectomized rats after midpregnancy is unknown, but Canivenc and Mayer (10) suggested that it is identical biologically with prolactin since it induced proliferation of the pigeon crop gland.

*Summary.* 1. Injections of approximately 3.7 to 60 I.U. of prolactin daily for 5 to 10 days, beginning on the 16th day of gestation, prolonged pregnancy in rats. The largest doses and longer treatment were most effective in preventing parturition, resulting in death of most of the young when pregnancy was extended beyond 23 days. 2. Prolactin failed to prolong pregnancy in rats ovariectomized on the 19th or 20th day of pregnancy, while injections of progesterone into intact rats extended pregnancy. These results as well as histological examination of corpora lutea, suggest that prolactin acts by extending functional activity of corpora lutea of pregnancy. 3. Lactation was usually initiated in prolactin-injected rats by the 21st day of gestation, independent of the occurrence of parturition. However, milk secretion was not as marked in rats which failed to be delivered of their young as in parturient rats.

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## Effects of Hyaluronidase and Cortisone on Connective Tissue Studied Electrometrically.* (23074)

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The ground substance of connective tissue behaves in many respects as heterogeneous colloid consisting of negatively charged aggregates. Of these, mucoproteins form a prominent component(1). The physico-chemical state of the ground substance is modulated by variations in internal environment and through reversible action of certain hormones and enzymes(2-5). An example of reversible behavior, reported in this paper, is the resynthesis of connective tissue colloids following disaggregation induced by hyaluronidase.

Many of the properties of the ground substance have been related to colloidal charge density, which can be determined electrometrically by measurement of liquid junction potentials(1-3). The electrometric method was used in this investigation to study disaggregation and reformation of connective tissue colloids in normal and cortisone treated rabbits after injection of hyaluronidase. The rate at which this occurs is believed to reflect a synthetic activity of cells. The results suggest that this procedure may be useful as a test of biologic resiliency of connective tissue.

*Material and methods.* Liquid junction potentials were measured serially on 22 albino rabbits, approximately 3 months of age. Eight of these animals received two 5 mg intramuscular injections of cortisone acetate (Merck) 48 and 24 hours prior to time of analysis. In 4 of these rabbits, electrometric determinations were also made before cortisone was administered. The animals were anesthetized by an intraperitoneal injection of 3% sodium pentobarbital. A 20-gauge one-half inch hypodermic needle was then inserted into the dermal layer of the shaved anterior abdominal wall. A reference junction was formed by placing a similar needle under the skin of the left hind leg.† After the desired salt solutions were instilled, the needles were connected to small syringe barrels filled with saturated KCl in agar which, in turn, were joined by saturated KCl bridges to calomel half cells. A Leeds-Northrop type K-2 potentiometer and galvanometer completed the circuit, which may be represented as follows:

| Hg | $Hg_2Cl_2$ | KCl (sat.) | NaCl (.15M) | Subcut. tissue — (refer.) |
|---|---|---|---|---|

| — Subcut. tissue (test site) | NaCl (I or II) | KCl (sat.) | $Hg_2Cl_2$ | Hg |
|---|---|---|---|---|

A baseline potential (E) was measured with 0.15 M NaCl (solution I) at both junctions. In each instance final value was based on the average of 3 consecutive readings taken at approximately 30-second intervals, and agreeing within one millivolt. The isotonic saline was then withdrawn from the experimental needle (abdomen) by aspiration with a 27-gauge needle, replaced by solution II, a 1/10

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† The reference junction may also be conveniently made by placing the shaved ear in a beaker of isotonic NaCl.