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Changes in Uterine Sialic Acid and Glycogen During Early Pregnancy in the Rat

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Changes in uterine sialic acid and glycogen were studied in rats during days 1–6 of pregnancy. There was a sharp decline in uterine glycogen following mating, and thereafter increased gradually to reach a peak level on day 4 (4 PM). Coinciding with the entry of blastocysts into the uterus [between day 4 (10 PM) and day 5 (10 AM)] uterine glycogen decreased to a low level which was maintained up to 4 PM on day 6 of pregnancy. Uterine sialic acid was maximal on day 0 (proestrus, 10 PM) and was maintained at this level up to day 1 of pregnancy. Thereafter, uterine sialic acid concentration declined gradually to the lowest level by day 4 (9 AM). There was a sharp increase in uterine sialic acid between 4 and 10 PM on day 4, and was followed by a marked decline between day 4 (10 PM) and day 5 (9 AM); it increased again on the evening of day 5 and attained significantly higher levels by day 6 (4 PM). The fluctuations in uterine sialic acid and glycogen during early pregnancy appear to be a sequel to the fluctuating levels of estrogens during proestrus, estrus, and early pregnancy. A possible role for sialic acid in attachment of blastocysts to the uterus is postulated.

INTRODUCTION

Implantation in the rat is the resultant of an orderly sequence of changes in the blastocyst and uterus initiated by the action of estrogen and progesterone. Shelesnyak and his co-workers postulated the existance of an "estrogen surge" (Shelesnyak, 1960; Shelesnyak *et al.* 1963) which occurs on the afternoon of day 4 post-coitum as a principal event in the initiation of the implantation process. Zeilmaker (1963); Finn and Martin (1967); DeFeo (1967); Miller *et al.* (1968) have questioned the validity of this hypothesis. The present studies were designed to examine the changes that occur in the uterus during the first 6 days of pregnancy using uterine glycogen and sialic acid as parameters and to determine if they are related, in any way, to the "estrogen surge" concept of Shelesnyak.

MATERIALS AND METHODS

Colony bred, adult, virgin female rats of the Holtzman strain, ranging in weight from 180 to 250 g, were used. They were maintained under uniform husbandry conditions with a constant temperature of 25 ± 1°C and lighting schedule of 14 hr light (0600–2000 hr) and 10 hr darkness (2000–0600 hr). They were fed a balanced diet and were provided with supply of tap water for drinking. Estrous cycles were followed by the examination of vaginal smears taken every morning and only those animals which had shown at least two regular 5-day cycles were used for the study. Autopsy schedule and designation of groups are given in Figs. 1 and 2.

A group of animals was autopsied at 10 PM on the night of proestrus (PO, unmated). Another group of females in proestrus were allowed to mate and were autopsied immediately following mating (PO, mated). Uteri were quickly removed; and water, seminal fluid, and spermatozoa were removed by pressing between filter papers. In other groups, females in proestrus were caged overnight with males of proved fertility. Mating was confirmed by the presence of spermatozoa in the vaginal smear the following morning which was designated as day 1 of pregnancy.

The animals were killed with an overdose of ether; and blood was quickly collected from the heart by puncture. The serum was hydrolyzed following the method of Hudson *et al.* (1965). Sialic acid was estimated in the hydrolyzed sample following the procedure of Warren (1959). The uteri were quickly dissected free of fat and weighed to the nearest milligram on a torsion balance. One horn was used for the estimation of sialic acid and the other horn for estimation

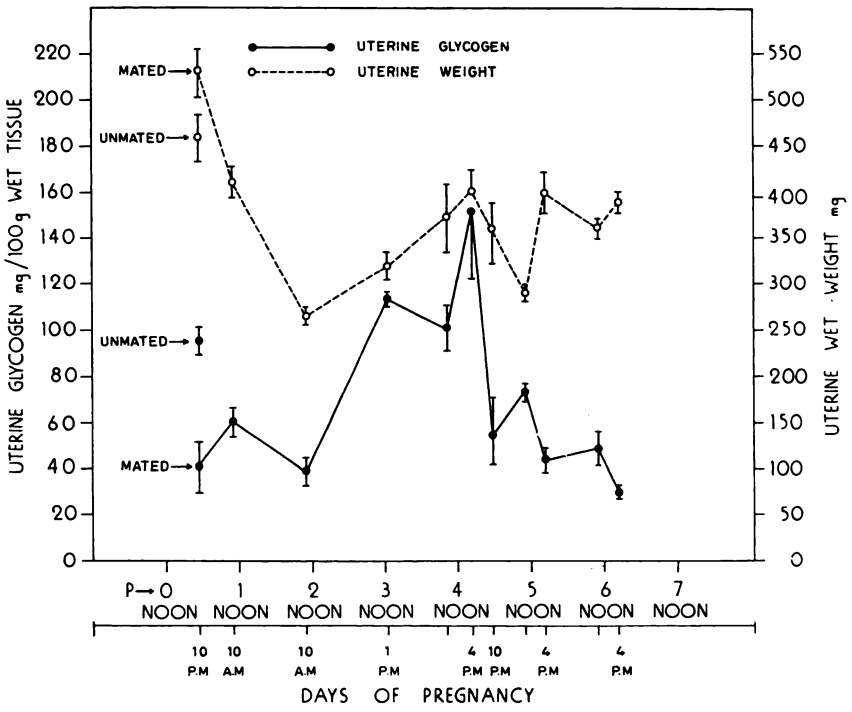

FIG. 1. Changes in uterine weight and glycogen during early pregnancy.

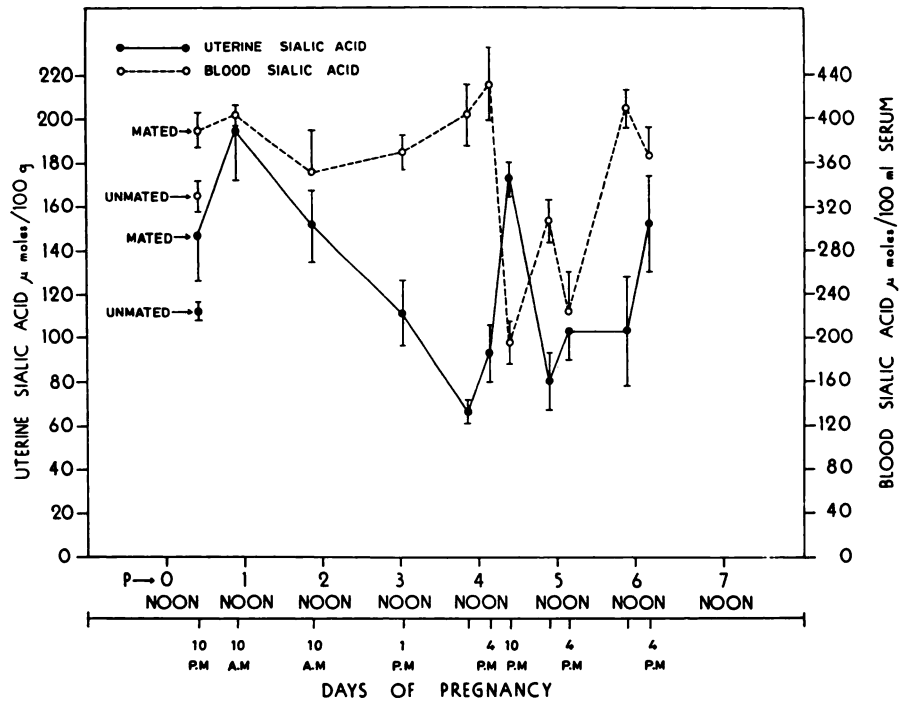

FIG. 2. Changes in sialic acid in uterus and serum during early pregnancy.

of glycogen. For the estimation of sialic acid, the uterine horn was homogenized in cold 0.1 N sulfuric acid and the homogenates were hydrolyzed for 1 hr at 80°C. Sialic acid was estimated in the supernatants according to the procedure of Warren (1959); appropriate precautions were taken as discussed by Rajalakshmi *et al.* (1969). For the estimation of glycogen, the uteri were hydrolyzed with hot 30% KOH; glycogen was precipitated by the method of Good *et al.* (1933) and estimated following the method of Montgomery (1957). The results were analyzed using Student's *t* test.

RESULTS

Uterine Weight

The uterine weight showed considerable fluctuations (Fig. 1). It was at its maximum in mated and unmated rats in proestrus and was maintained up to day 1 of pregnancy. However, a sharp and significant decrease in uterine weight was observed on day 2 (P_2; $p < 0.01$) followed by a slight, statistically insignificant increase ($p > 0.05$) on day 3 (P_3) and on day 4 (9 AM). The uterine weight increased significantly on day 4 (4 PM) ($p < 0.02$) and was maintained till 10 PM of the same day. However, uterine weight increased significantly ($p < 0.01$) on day 5 morning (P_5, 10 AM) when compared to weights on day 4 (4 PM) but was not significantly different from that on day 4 (10 PM) ($p > 0.05$). Thereafter, uterine weight increased significantly ($p < 0.01$) on day 5 (4 PM) and was maintained at this level till day 6.

Uterine Glycogen

The changes in uterine glycogen during early pregnancy are shown in Fig. 1. The concentration of glycogen in unmated rats in proestrus (PO, unmated) was 95.3 mg/100 g; within 15 min after mating (PO, mated), the uterine glycogen concentration decreased significantly ($p < 0.01$) from the unmated levels (PO, unmated). A transient but significant increase ($p < 0.05$) in uterine glycogen occurred on day 1 and declined to proestrus (mated) levels (PO, mated) on day 2 of pregnancy ($p < 0.01$). Thereafter, the concentration of uterine glycogen increased gradually to reach a peak on day 4 (4 PM). There was, however, a slight, statistically insignificant decrease ($p > 0.05$) on day 4 (9 AM). Glycogen levels showed a steep decrease between 4 and 10 PM on day 4 ($p < 0.001$). On the morning of day 5 (P_5, 10 AM), uterine glycogen concentration increased significantly ($p < 0.001$); however, glycogen levels declined gradually from day 5 (4 PM) to reach the lowest levels on day 6 (4 PM) ($p < 0.05$).

Uterine and Serum Sialic Acid

The changes in levels of uterine and blood sialic acid during the preimplantation period are shown in Fig. 2. The content of uterine sialic acid in unmated rats in proestrus was 0.256 ± 0.05 μmoles/horn (PO, unmated). The uterine sialic acid concentration increased in proestrus rats within 15 min of mating (PO, mated, $p < 0.05$) and continued to rise till day 1 of pregnancy ($p < 0.05$). Thereafter, uterine sialic acid concentration declined gradually reaching the lowest levels on day 4 (9 AM) ($p < 0.01$). The concentration of uterine sialic acid increased gradually from day 4 (4 PM) ($p < 0.05$) to reach a peak by 10 PM on day 4 ($p < 0.001$), to a level which was comparable to that on day 1 (P_1) of pregnancy. On the morning of day 5 (P_5, 10 AM) uterine sialic acid concentration declined sharply; it increased again on the evening of day 5 (P_5, 4 PM; $p > 0.05$) and attained significantly higher levels by day 6 (4 PM) ($p < 0.02$) at a time when the blastocysts were attached. The content of uterine sialic acid followed a pattern similar to that of concentration during days 0–6.

Serum sialic acid levels showed a significant increase following mating ($p < 0.05$). Though a slight but insignificant decrease was observed on day 2 ($p > 0.05$), serum sialic acid levels gradually increased to reach a peak on day 4 (10 AM); it remained at the same level till the afternoon on day 4

(P_4, 4 PM), and declined markedly on day 4 (10 PM) ($p < 0.001$). Thereafter, serum levels of sialic acid continued to rise except for an insignificant drop on day 5 (4 PM) and reached peak levels by day 6 (10 AM).

DISCUSSION

It has been clearly established that estrogen is necessary for implantation of the blastocysts in the rat (Cochrane and Meyer, 1957; Psychoyos, 1967). Estrogen evokes a series of time-dependent changes in nucleic acid and protein synthesis in the uterus and blastocysts during delayed implantation (Prasad *et al.*, 1966; Dass *et al.*, 1969; Mohla and Prasad, 1971). The "estrogen surge" concept of Shelesnyak (1960) and Shelesnyak *et al.* (1963) envisages the release of estrogen on day 4 postcoitum as a sequel to the action of LH. The hypothesis is based on the recurrence of the estrous cycle during pregnancy and the "surge" of estrogen coincides with the proestrus surge of estrogen that would occur had the rat not become pregnant. The secretion of estrogen during the estrous cycle is not a discrete discharge from the ovary but increases gradually throughout the night of diestrus to reach a peak lasting from 1000 to 2000 hr on the day of proestrus; likewise, during early pregnancy estrogen levels in the ovarian venous blood increase gradually to reach a lesser peak on day 4 (Yoshinaga *et al.*, 1969; Shaikh, 1971). These authors suggested that the peak of estrogen on day 4 confirms the estrogen surge theory of Shelesnyak. Since the increase in levels of estrogen appears to be gradual, we prefer to refer to it as an estrogen intervention rather than as a surge.

Sequential changes in several biochemical parameters occurring during days 1–4 of pregnancy may be considered in relation to the patterns of estrogen secretion. RNA synthesis reaches high levels on day 3 postcoitum in rats (Mohla *et al.*, 1970; Heald and O'Grady, 1970) and in the mouse (Miller *et al.*, 1968). Similarly, uptake of [^{3}H]phenylalanine and [^{3}H]leucine becomes pronounced by day 3 of pregnancy (Mohla *et al.*, 1970; Reid and Heald, 1970). Finn and Martin (1967) observed a large number of mitoses on day 3 of pregnancy in the luminal and glandular epithelium in the mouse while Mohla *et al.* (1970) demonstrated maximal DNA synthesis in the uterine epithelium of rat on day 2 of pregnancy. These results show that certain synthetic activities in the uterus reach high levels by day 3, at least 24 hr prior to the estrogen surge on day 4. It is likely that these changes, which constitute the priming phase of the preimplantation period, are triggered by the release of estrogen on the night of proestrus (2000–2300 hr) and estrus (1500 hr).

The varying patterns of uterine glycogen and sialic acid during early pregnancy are also of interest. Uterine glycogen decreases immediately following mating (Fig. 1, mated and unmated) which may be due to the utilization of glycogen for enhanced uterine contractions during this period. The gradual increase in uterine glycogen from day 1 to day 4 (4 PM) may be due to the synergistic action of rising levels of progesterone (Wiest *et al.*, 1968) and estrogen (Yoshinaga *et al.*, 1969). Coinciding with the entry of blastocysts into the uterus between day 4 (10 PM) and day 5 (10 AM), there was a sharp decline in uterine glycogen which continued up to day 6, the significance of which is not clear. It is interesting to note that histochemically demonstrable uterine glycogen occurs much later in time sequence than that determined biochemically (Christie, 1966; Foster *et al.*, 1963; Rosenbaum and Goolsby, 1957).

Fluctuating levels of uterine sialic acid follow an interesting pattern which bears a close relation to the changing pattern of estrogen during proestrus and early pregnancy. Levels of sialic acid were maximal at two distinct time intervals; one on day 1 of pregnancy which follows the peak of pro-

estrous estrogen and another on day 4 at 10 PM following the estrogen intervention on the afternoon of the same day. Sialic acid is a sensitive parameter of estrogen action (Rajalakshmi *et al.*, 1969). Yoshinaga *et al.* (1969) demonstrated that the proestrous estrogen secretion rate is three times higher than that during day 4 of pregnancy. However, the level of uterine sialic acid was approximately the same during the two periods and may represent maximal response of the uterus to estrogen. Our unpublished observations show that in immature rats uterine sialic acid showed a dose-dependent increase up to 0.02 μg of estrogen; with further increase in the dose of estrogen there was no additional increase in uterine sialic acid. The decreasing levels of sialic acid from day 1 onwards till 9 AM on day 4 may be due to the interaction between increasing levels of progesterone (Wiest *et al.*, 1968) and estrogen. The increasing levels of sialic acid between day 6, 10 AM and 4 PM may be due to reciprocal reaction between the blastocyst and uterus during the critical phase of attachment. Rajalakshmi *et al.* (1969) showed that, following a single administration of estrogen to rats undergoing experimentally induced delay of implantation, uterine sialic acid was maximally increased by 6 hr and remained at a lower plateau for up to 48 hr, similar to the condition seen in the intact, pregnant rat between day 4 (10 PM) and day 6.

Is there any physiological significance to these changes in uterine sialic acid? Sialic acid is part of a complex of sialomucoproteins which form the mucus of the uterus and vagina (Coppola and Ball, 1966; Carlborg, 1969). It is tempting to postulate a role for uterine sialic acid in relation to implantation. In our colony of rats, blastocysts reach the uterus between day 4 (10 PM) and day 5 (9 AM) (Mohla *et al.*, 1970). Sialic acid reaches a peak by day 4 (10 PM) and remains at a plateau at a lower level from day 5 morning till day 6 (10 AM). It is during this period that the blastocysts are spaced and attach to the uterine epithelium. Interference with the sialomucoproteins by treatment with neuraminidase on days 1–5 or days 5–7 results in failure of blastocyst attachment and implantation in mice (Gasic and Gasic, 1971). The increase in sialic acid on day 6 between 10 AM and 4 PM may, thus, be a biochemical event which is related to the attachment of the blastocysts. Attachment of rabbit blastocysts has been studied by Boving (1963) who has postulated that the sialomucoproteins around the blastocyst become sticky as a result of changes in the alkalinity of the blastocyst fluid. Similar studies have not been made with rat blastocysts but are under investigation.

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