Glycogen Storage during Last Part of Pregnancy in Uterus, Liver and Heart of Rats

The highest content of glycogen in the non-pregnant rat uterus is found at proestrus[1]. At diestrus, uterine glycogen is at its lowest value[1]. Recent investigations have demonstrated that glycogen is localized primarily in the myometrium[2,3]. The carbohydrate metabolism in the rat uterus during late pregnancy appears to be very slightly investigated, and little is known regarding the biochemical events in the uterus during parturition and postparturition. The regulation of glycolysis, glycogen synthesis and its degradation during early pregnancy was elaborately studied by GREENSTREET and FOTHERBY[4]. Most of the recent papers have shown the modifying effects of repeated injections of estradiol and progesterone on uterine glycogen but no systematic study has been performed to determine the daily variations in glycogen content during the last part of pregnancy[5,6]. This paper describes an investigation of the glycogen storage in the rat uterus from the 18th day of pregnancy to 24 h post-parturition. The glycogen concentration of the liver and heart during the entire observation period was also determined to find out any possible relationship or transport to the uterus.

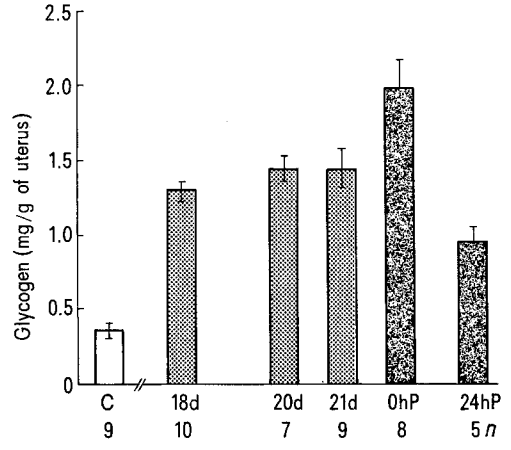

Fig. 1. The evolution of glycogen storage in the uterus of non-pregnant (C) and pregnant rats. 18 d, 20 d, 21 d, days of pregnancy; 0 h P and 24 h P, 0 h and 24 h after birth; *n*, number of rats utilized in each group. Statistically: C differs from 18 d, 20 d, 21 d, 0 h P 24 h P ($P < 0.001$). 18 d differs from 0 h P ($P < 0{,}005$). 21 d differs from 0 h P ($P < 0{,}005$). 24 h P differs from 0 h P ($P < 0.001$).

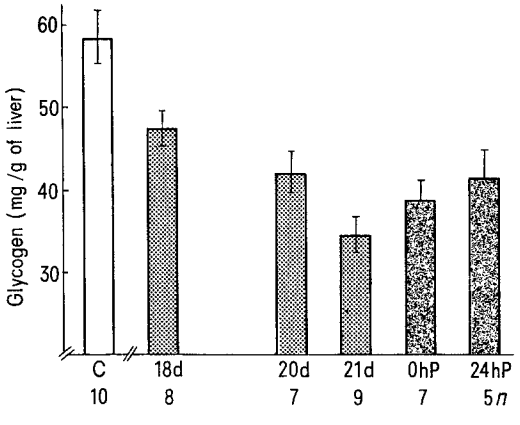

Fig. 2. Gestational changes in the concentration of hepatic glycogen in rats. C, non-pregnant rats; 18 d, 20 d, 21 d, days of pregnancy; 0 h P and 24 h P, 0 and 24 h after parturition; *n*, number of animals used. Statistical differences between different groups are given below: C and 18 d ($P < 0.02$). C and 21 d ($P < 0.001$). 21 d and 20 d ($P < 0.05$). 18 d and 21 d ($P < 0.001$).

Materials and methods. Albino female rats of Sherman strain, weighing 250 ± 50 g, were used throughout the determination period. The age of the rats ranged from 12 to 14 weeks. They were housed at a constant temperature of 22 °C with exposures to natural light and darkness during the months of January to April. The animals were maintained on commercial laboratory food ad libitum. The rats were made pregnant by keeping one male with seven females in a cage from 19.00 h to 09.00 h once only. The fertilization was supposed to occur at 02.00 h. This was taken to be an approximation of the beginning of pregnancy. The females were palped 14 days later, and if positive at 02.00 h, they were considered $14^1/_2$ days pregnant. Under normal conditions parturition occurred during 21st and 22nd day of pregnancy. On each specified day of pregnancy, the animals were killed by neck fracture and their liver, heart and uterus were excised immediately. The organs were rinsed in ice-cold 0.9% KCl, blotted dry on filtre paper and cut in small pieces in a watchglass placed on crushed ice. About one gram of each organ was transferred to a pyrex assay tube containing 1 ml of 60% KOH.

Assay of glycogen. Glycogen was extracted according to the principle of SLOSSE by dissolving the tissues in 60% KOH and its further conversion to glucose[7]. For each assay about 1 g of the liver, heart or uterus was taken, cut into small pieces and transferred to a screw cap assay tube containing 1 ml of 60% KOH. The tubes were incubated in a water-bath at 100 °C for 3 hours. At the end of the incubation, 4 ml distilled water was added to each sample and the tubes were cooled. To accelerate precipitation, 10% in volume of 20% sodium acetate and 10 ml of ethyl alcohol 95° was added to each tube. The tubes were placed in a freezer at −30 °C for 1 h. At the end of precipitation, the tubes were shaken mechanically for 10 min, centrifuged at a moderate speed for 5 min and the supernatant was discarded. The precipitate was washed with 10 ml of 60% ethyl alcohol and supernatant was discarded after centrifugation. The washing with 60% alcohol was performed 3 times. Final washing was made with 10 ml of 95% alcohol. The precipitate was dried in a water-bath at 100 °C. When dry, 10 ml of hot distilled water and 0.67 ml of concentrated HCl (d: 1.175) were added. The tubes were closed firmly and incubated for 1 h at 100 °C for conversion of glycogen to glucose. After incubation, the contents of the tubes were neutralized with 60% KOH and pH was adjusted to 7 with the help of a glass electrode. The volume of each tube was adjusted to 15 ml and the contents of the tube were assayed for glucose by the method of glucose

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oxidase[8,9]. The details of calculations are given elsewhere[8]. The results are expressed in mg glycogen/g organ weight. Statistical differences are calculated with Fisher's Student *t*-test. The mean values are expressed with standard errors.

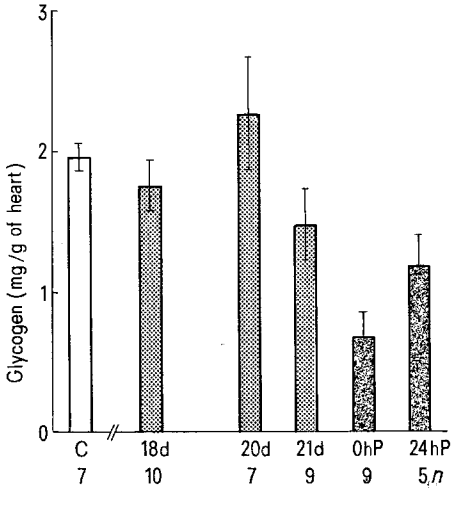
Results. Figure 1 shows the glycogen content of the uterus in non-pregnant and pregnant rats. The mean value for uterine glycogen in non-pregnant rats was very low. On the 18th day of pregnancy an important increase of 358% of the cntrol value was observed. This increase remained nearly stable during 20th and 21st day of pregnancy. At 0 h of parturition, the uterine glycogen demonstrated further increase and reached its maximum value (544% higher than the controls). Afterwards glycogen content of uterus started to decline. At 24 h post-parturition the mean value was 48.1% lower than the value of 0 h parturition, but it was still 266% higher than the controls.

The changes in hepatic glycogen during pregnancy, parturition and post-parturition are illustrated in Figure 2. The non-pregnant rats show the highest content of glycogen in the liver. On 18th, 20th and 21st days of pregnancy, respective declines of 18.5%, 28% and 41% as compared with non-pregnant liver glycogen were observed. On the 21st day of pregnancy, hepatic glycogen was at its lowest value. At parturition and 24 h post-parturition progressive increases in liver glycogen occured.

Figure 3 provides the evolution of glycogen in the rat heart from the 18th day of pregnancy to 24 h post-parturition. On the 18th and 20th day of pregnancy, only slight variations in cardiac glycogen were observed as compared to the control group. There was an important decline on the 21st day of gestation (36.4% lower than the non-pregnant rats). At 0 h parturition glycogen in the heart diminished to the lowest level (70.3% lower than the control group) but this decline did not last very long, as 24 h after parturition cardiac glycogen again started to increase (205% of 0 h parturition).

Discussion. The results of the present investigation demonstrate progressive increases in the content of uterine glycogen on the 18th, 20th and 21st day of pregnancy. At the same time hepatic glycogen stores declined significantly. These variations suggest an active transport of hepatic glycogen to the uterus which is most active during last part of pregnancy and parturition. Our present results could be compared with previous studies on the metabolism of glucose and changes in enzymes of glycogen synthesis and metabolism in the rat uterus during early pregnancy[10–12]. The latter authors found systematic increases in glycogen concentration of the uterus during 2 to 7 days of pregnancy, but the maximum increase in uterine glucose occurred on the 3rd day of pregnancy. The activity of glucose-6-phosphatase and glycogen synthetase-D also showed markedly important increases during the first 12 days of gestation[12]. The elevated glycogen content of the uterus, as well as the liver, at 0 h parturition also suggests concomitant rise in the activities of all the forms of glycogen synthetase during this period. After parturition, the amount of glycogen in the uterus and the liver started to return to the non-pregnant values.

Another explanation for the modifications in glycogen stores can be gathered from our previous findings on the excretion, release and storage of adrenaline, noradrenaline and their metabolites during late pregnancy[13–15]. We observed highly significant increases in plasma, urine and tissue catecholamines from 18th day of pregnancy to 24 h post-parturition. Therefore any modifications in sympathetic nervous activity could be supposed directly to affect glycogen storage. Cardiac glycogen acted like liver glycogen, which also declined markedly during 21st day of pregnancy and 0 h parturition suggesting that glycogen from this organ also served as the source of energy for uterus. It could also be considered that the decrease in glycogen content of the heart from 21st day of pregnancy to 0 h parturition is a consequence of an increase in the mechanical activity of the heart which supplies more blood to the contracting uterus during this interval of pregnancy and parturition. Although it is still hard to specify the exact interpretation for modified glycogen storage of different organs, a possible explanation is that the activities of the enzymes for glycogen synthesis and degradation (glycogen synthetase and phosphorylase) are changed in relation to each other. Recently ovarian hormones have been shown to increase glycogen synthetase[16–18] and phosphorylase[19–21]. The modifications



← Fig. 3. The glycogen content of rat heart during late pregnancy, parturition and post-parturition. C, control rats; 18d, 20d, 21d, days of pregnancy; 0 h P and 24 h P, h after birth; *n*, number of cases for each group. Statistical differences between different groups: C and 21d ($P < 0.02$). C and 0 h P ($P < 0.005$). 20d and 21 d ($P < 0.02$). 20d and 0 h P ($P < 0.001$). 0 h P and 24 h P ($P < 0.05$).

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in hormonal state induced by the pregnancy could be considered an important factor for variations in tissue glycogen. The observed variations in glycogen concentration of the uterus support the suggestion that an active transport of hepatic and cardiac glycogen to the uterus exists during late pregnancy.

Résumé. Ce travail décrit l'évolution du contenu en glycogène de l'utérus, du foie et du cœur chez des rattes en fin de gestation, au moment de la parturition et en post-partum. Le glycogène de l'utérus augmente de façon très significative du 18e jour de gestation jusqu'au moment de la parturition. Au contraire le glycogène hépatique diminue du 18e au 21e jour de gestation. Le contenu en glycogène du cœur décroit brutalement du 20e jour de gestation à 0 h post-partum. Dès 24 h post-partum les réserves en glycogène de chacun des organes amorcent un retour vers les valeurs témoins. Les résultats suggèrent qu'il existe un déplacement du glycogène hépatique vers l'utérus pendant la dernière partie de la gestation.

SIMONE PARVEZ and H. PARVEZ

University of Paris XI, Center of Orsay, Endocrinology Laboratory, F-91405 Orsay-91 (France),
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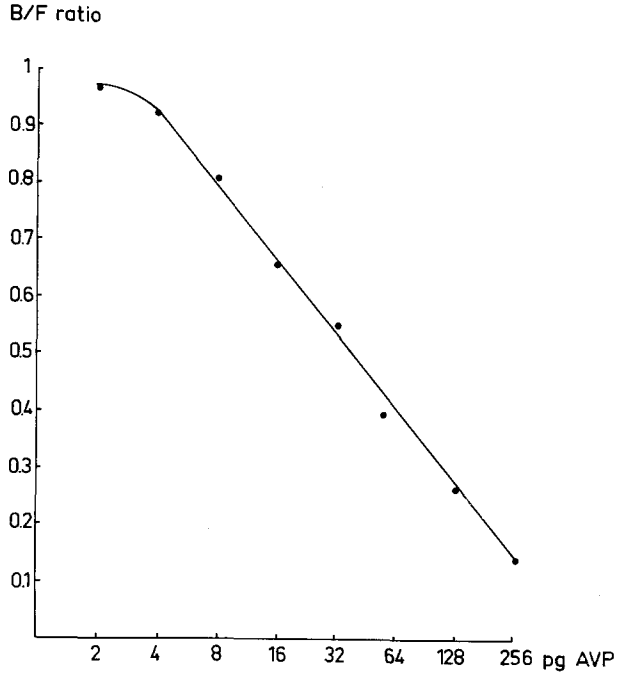
A Radioimmunoassay of Vasopressin. A Note on Pituitary Vasopressin Content in Brattleboro Rats

A recently developed radioimmunoassay of vasopressin was tested for its sensitivity and reliability by determining the amount of vasopressin in posterior pituitaries of rats with hypothalamic hereditary diabetes insipidus (D. I.) (Brattleboro strain). Posterior pituitary vasopressin content of heterozygous D.I. rats has been reported[1], but little data on pituitary vasopressin content of homozygous D.I. rats exist. Therefore, the amount of vasopressin in posterior pituitaries of homozygous and heterozygous rats of the Brattleboro strain was assessed by both radioimmunoassay and bioassay.

Antibodies against synthetic arginine-8-vasopressin (AVP; 300 IU/mg, Ferring) were produced as described by SKOWSKY and FISHER[2]. AVP was coupled to thyroglobulin by ethylcarbodiimide and the product was emulsified in Freund's complete adjuvant and injected i.m. into young adult New Zealand rabbits (1.5–3.0 kg).



Standard curve of arginine-8-vasopressin (AVP) showing inhibition of binding of ^{125}I-AVP by antibody at a final dilution of 1:60,000 in the presence of serial dilutions of unlabelled AVP. The bound/free ratio of ^{125}I-AVP is plotted against the concentration of standard AVP.

An initial dose of antigen, corresponding to 0.4 mg AVP was given, followed by a booster with half this amount of antigen every 3 weeks. The first antiserum was collected 18 weeks after the initial injection.

AVP was labelled with Na^{125}I (Amersham) using chloramine T as oxydant. The reaction was stopped by addition of human serum. Separation of ^{125}I-AVP, from labelled albumin and unlabelled AVP, was accomplished by passage through a Sephadex G 25 fine column (1.0 × 10.0 cm) equilibrated with 0.2 *M* acetic acid. This tracer with a specific activity of 200 μCi/μg was stored in small portions at −20 °C. A Veronal buffer (pH 8.0) was used as diluent[3].

Standard amounts of AVP ranging from 1 pg to 128 pg were used for the calibration curve (Figure), which included antibody blank and diluent blank. The final dilution of the antibody was 1:60.000. The total volume was 110 μl/tube. The incubation time was 48 h at 4 °C. Ammonium sulphate was used for the separation of bound and free ^{125}I-AVP. The detection limit in buffer was 2 pg AVP. Similar standard curves for AVP were obtained, ranging from 2 to 128 pg over a number of assays, and also the same detection threshold of 1–3 pg was found, indicating a high degree of reproducibility. Concerning cross reactivity with related peptides, the detection thresholds of oxytocin and of lysine-8-vasopressin (LVP) were ca. 60 pg. Standard curves with these peptides did not run parallel to the AVP curve.

The pressor activity of the posterior pituitary extracts was estimated by the blood pressure bioassay in anesthetized rats pretreated with phenoxybenzamine (10 mg/kg) as described by DEKANSKI[4].

Homozygous and heterozygous male rats of the Brattleboro strain, bred under SPF conditions (TNO, Zeist) were killed by decapitation. The pituitaries were removed from the sella, the posterior lobes separated from the anterior lobes, weighed, homogenized in 2.1 ml diluent buffer and stored at −20 °C until testing. Aliquots of pituitary homogenates of homozygous and heterozygous rats were tested for pressor activity in a 2 × 2 bioassay. In 3 posterior pituitary homogenates of homozygous D.I.

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