ON THE "GLYCOGEN FUNCTION" OF THE MYOMETRIUM IN PREGNANCY

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Translated from Byulleten Eksperimental' noi Biologii i Meditsiny Vol. 49, No. 4, pp. 101-104, April, 1960
Original article submitted March 12, 1959

In a preceeding investigation [1] it was shown that with the advent of pregnancy there is a gradual increase in the amount of glycogen in the muscles of the uterus, attaining a maximum at the time of birth. The physiological significance and the mechanism of regulation of this process remained unelucidated, however, since it was not successfully connected with changes in the contractile activity of the myometrium [2].

In subsequent investigations [3] it was shown that in the regulation of the "glycogen function" of the myometrium essential importance is apparently held by impulses from the fetal side, developing in the uterine cavity. Thus, it was shown that if animals (female rabbits) in which the fallopian tube on one side was ligated, became pregnant, as a result of which the fetuses developed in only one horn of the uterus, then in the mucosal and muscular layers of this "empty" horn glycogen was not accumulated, at the same time that its quantity in the horn containing the fetuses was able to reach 4-5% in relation to the dry weight of the tissue [1, 3].

The data of these experiments provided a basis for postulating that the process of glycogen accumulation in the myometrium during pregnancy holds some sort of meaning in the development of trophics of the intrauterine fetus.

The task of the present investigation involved further study on the question of the possible physiological importance of the "glycogen function" of the myometrium and its regulatory mechanisms.

METHOD

The experiments were carried out on female rabbits. The peritoneal cavity was opened under novocaine anesthesia. The horn of the uterus was quickly detached and on a refrigerated frosted glass opened lengthwise along the mesometrial side. The fetuses and placentae were removed, and the amputated periplacental portions of the horn of the uterus were frozen with dry ice to a firm state. The mucosal layer was separated from the frozen "disks" of tissue by means of a scalpel. The solid portions of uterine muscle were immersed in a warm solution of potassium hydroxide, after which the amount of glycogen in them was determined by the method of Pflueger. The result of each investigation was presented as the arithmetic mean of 2-3 parallel determinations.

For the investigation of the rate of separation of the tissue glycogen during its incubation the unfrozen portion of uterine tissue was suspended in a glass container, 50 ml in volume, with a ground stopper. On the bottom of the container there was filter paper moistened with water. In this moist chamber the tissue was kept in an incubator at 37° for 1 hour. The tissue was then frozen, treated according to the description above, and the amount of glycogen in the muscular layer, remaining after an hour of incubation, determined. The intensity of the separation of glycogen during the incubation was expressed in percent of its original amount. In a number of the experiments after the incubation the amount of glycogen in the tissue increased somewhat. It is necessary to postulate that in those cases a technical error took place in the separation of the mucosal layer.

The operation of removing one or several fetuses was performed under novocaine anesthesia, or, rarely, under total ether narcosis. The fetus was carefully removed through a small longitudinal incision in the wall of the horn on the antimesometrial side, along with its sacs and all the perifetal waters. A ligature was placed about the umbilical cord of fertilized eggs and drawn tightly. The fertilized egg was removed, the end of the umbilical cord together with the ligature embedded in the cavity of the uterus, and the wound closed with a continuous suture. The rapid and careful performance of the operation permitted removing one or several fetuses, without causing exfoliation of their placentae and without disrupting the normal development of the remaining fetuses in the intact fetal receptacles of the operated and unoperated horns of the uterus.

RESULTS

The results of several of the experiments, pertaining to 14-20 days of pregnancy, are presented in Fig. 1. These experiments showed that in the interlocular portions, i.e., in the portions of the myometrium between the isolated fetal receptacles, there was contained practically the same amount of glycogen as in the myometrium of the non-pregnant animals (about 100 mg %), while in

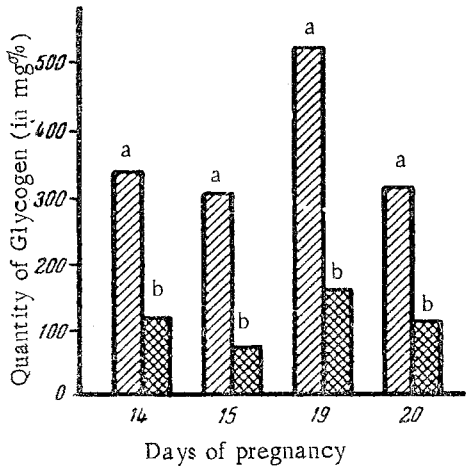

Fig. 1. The concentration of glycogen in the different portions of uterine muscle depending on the duration of pregnancy. a) In the periplacental portions; b) in the interlocular portions.

the myometrium of the fetal receptacles of the same uterine horn glycogen was contained in quantitites several times greater. It must be noted that such irregularity in the distribution of glycogen is found in animals with a multiple pregnancy. In pregnant women there are no marked differences in the concentration of glycogen of the uterine muscles as compared to the muscle of the lower segment [2].

The data presented in Fig. 1 confirms the already stated contention that the accumulation of glycogen in the myometrium during pregnancy is related, apparently, to the life-activity of the fetus developing in the cavity of the uterus. It was essential to obtain, as far as possible, direct indications of the presence of such a relationship. For this purpose, 1-2 fetuses were removed from one horn of the uterus on the 14th to 19th day of pregnancy. On the 18th to 20th day of pregnancy, using novocaine anesthesia, the peritoneal cavity was opened and portions of the intact and operated uterine horns were taken for determinations of the amount of glycogen in the myometrium. The portions of the uterus corresponding to the site of removal fetuses were easily discovered by the traces of the suture lines (the same incision was cut to take tissue for the investigation). Signs of inflammation or adhesion were not detected in a single instance.

The results of the investigation performed are presented in the table. They show that in the myometrium of the portions of the horn in which fetuses were removed there is a lower concentration of glycogen than in the myometrium of the intact horn containing fetuses. However, it is clearly seen that the fewer the days passed between the removal of a fetus and the determination of the glycogen, the smaller this difference: It was completely absent after an interval of 3 days, it was insignificant after an interval of 4-5 days, and it was markedly manifested after an interval of 14-15 days. It is very probable that this is related to the presence or absence

The Concentration of Glycogen in the Myometrium of the Intact and Operated Horn Following the Removal of Fetuses

Animal No.	Removal of the fetus (day of pregnancy)	Glycogen determination (day of pregnancy)	Day after operation	Concentration of glycogen (in mg%) intact horn	Concentration of glycogen (in mg%) operated horn
1	15-th	18-th	3-rd	233	236
2	14-th	18-th	4-th	279	257
3	17-th	21-st	4-th	363	326
4	18-th	23-rd	5-th	274	250
5	19-th	24-th	5-th	336	262
6	16-th	30-th	14-th	1 138	167
7	15-th	30-th	15-th	964	463

of the "living" functioning placenta. The experiments showed that if the investigation was carried out 5-6 days after the operation, the placenta in the portions of the myometrium between the fetal receptacles from which a fetus was removed remained thickly implanted in the uterine wall and retained the usual "living" appearance. If the investigation was carried out 14-15 days after the operation, the placenta was shown to be freely moveable in the cavity of the uterus, and considerably altered in regard to size, color, and consistency. Not excluded, however, is the possibility that the importance is held not by the presence or absence or a functioning placenta, but the interval of time elapsed from the instant of removal of the fetus: After 3-5 days the absence of a fetus may still not express itself, while after 14-15 days it is expressed in a completely defined fashion. In any case, the data presented in the table shows that the fertilized egg, developing in the uterine cavity, regulates the "glycogen function" of the myometrium.

In the process of investigating the concentration of glycogen in the uterus it was discovered that in definite functional states of the uterus the glycogen contained in its muscular layer was characterized by unusual stability: It either did not separate or it separated to an insignificant degree after prolonged storage of the tissue. This phenomenon was studied in a series of experiments in which the intensity of separation of the glycogen during incubation of the isolated rabbit uterine tissue was determined. Since the original quantity of glycogen in one experiment was sometimes 10-15 times greater than in another, it was necessary, in special experiments, to clarify whether or not the detected difference in the intensity of separation of the glycogen depended on the difference in its original quantity. These experiments showed that there was no direct dependency between the intensity of separation of the glycogen and the original amount of glycogen in the tissue.

Fourteen experiments were set up on non-pregnant rabbits. Of these, in 11, after one hour of incubation, no

glycogen remained in the uterine muscles – it separated completely. Only in 3 trials was there still discovered about 1/3 of the original quantity. After a duration of pregnancy of from 12 to 17 days (5 experiments) after 1 hour of incubation an average of 62 ± 2.5% of the glycogen was broken down in the muscles of the uterus. In none of the experiments was the glycogen completely broken down, as was observed in the myometrium of the non-pregnant uterus. After a pregnancy of from 18 to 28 days (12 experiments) after 1 hour of incubation an overage of 17 ± 5.3% of the original quantity of glycogen was separated from the muscles of the uterus, while in 5 out of the 12 trials its quantity practically did not change throughout the time of incubation. Since the difference between the mean proportions characterizing the separation of the glycogen in the myometrium of the isolated non-pregnant uterine tissue in the first and second half was shown to be statistically valid, it is possible to consider it proven that with the advent of pregnancy and the increase in its duration, the "stability" of the glycogen in the myometrium increases.

At the very end of pregnancy, for 1-2 days before parturition, separation of the glycogen during the period of incubation was practically absent in 5 out of the 6 trials. In only one trial did the amount of glycogen decrease after 1 hour of incubation by 18%. Thus, at the end of pregnancy there is observed a further increase in the "stability" of the glycogen in the myometrium. In 5 experiments the intensity of separation of the glycogen in the uterine muscles of the rabbits was determined at the time of parturition, and in 18 experiments, in rabbits during the first 48 hours after parturition. The data obtained is subjected to analysis with difficulty, in view of the great scattering of the values. It is possible to postulate that this is related to the rapid and difficultly measured changes in the functional state of the uterine muscles during parturition and shortly after parturition. In any case, in the post-partum period the separation of glycogen after incubation of the uterine muscle is already marked by absolute clarity, attaining an average of 39 ± 6.3% and consisting, in isolated experiments, of even 54-74% of the original values.

Figure 2 affords the opportunity of comparing the data obtained in these experiments. It shows how significantly and quickly the "stability" of the myometrial glycogen increases along with the development of the pregnancy.

It is appropriate to mention Wertheimer's investigation [4] at this time, which showed that the glycogen in the liver of the fetus and in the placenta also possesses a high degree of stability: It does not separate in the presence of such powerful glycogen-mobilizing actions as the introduction of adrenalin, hunger, or generalized chilling of the organism, as well as in the presence of tissue autolysis.

The results presented in the present experimental investigation show that in the regulation of the "glycogen

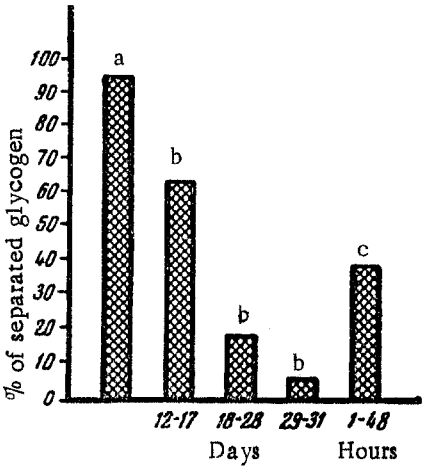

Fig. 2. The intensity of separation of the glycogen during incubation of the myometrium (mean data). a) Outside pregnancy; b) during pregnancy (indicated days of the pregnancy); c) after parturition (indicated hours).

function" of the myometrium an essential role is played by impulses associated with the life activity of the fertilized egg developing in the uterine cavity, the nature of which is still not elucidated.

The character of the distribution of the glycogen in the myometrium, and specifically its accumulation in large quantities in the fetal receptacles and its almost complete absence in the interlocular portions of the uterus, point to the influence of these impulses on the metabolism of the myometrium localized within the boundaries of one uterine horn or even within the boundaries of one fetal receptacle.

Apparently, it may be considered proven that such a clearly expressed physiological reaction of the myometrium to pregnancy as the accumulation in it of large amounts of glycogen is regulated by the fetus developing in the uterine cavity. This makes highly probable the hypothesis that the physiological importance of this "glycogen function" of the myometrium is connected with the necessities of the developing fetus and, apparently, with its trophics.

SUMMARY

Investigation was made relative to the physiological significance of the "glycogen function" of myometrium in pregnancy and of the mechanisms regulating it. The amount of glycogen in the sections of myometrium lying between individual fetuses was found to be same as in those of non-gravid animals. The glycogen level in myometrium directly bordering on the fetuses was several times greater. The glycogen level in the myometrium of the uterine horn from which the fetuses were removed 3-5-15 days prior to the investigation was lower than in the myometrium of the intact horn containing fetuses. With the development of pregnancy there is a growing "resistance" of the myometrial glycogen during its incubation in a thermostat at 37°C. This "resistance" appears to be

maximal near the term of pregnancy. The data obtained conforms with the results of previous investigations. It shows that in the control of the "glycogen function" of myometrium an important role is played by the impulses appearing in connection with the vital activity of the fetus and that the physiological significance of this myometrial function is assocated with the requirements of the developing fetus, and probably with its trophics.

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