MORPHOLOGY AND PATHOMORPHOLOGY

HISTOCHEMICAL STUDY OF THE GLYCOGEN CONTENT OF THE UTERUS AND PLACENTA OF WHITE RATS AT DIFFERENT STAGES OF GESTATION

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Our earlier histochemical studies [4] revealed certain regularities in the distribution of acid and alkaline phosphatase activities of uterine and placental tissues of white rats at different stages of pregnancy. The placenta of these animals is distinguished by its very high phosphatase activity, manifested by the high metabolic level prevailing in this tissue. The highest activities were found in the trophoblast and in its membrane surrounding the fetal vessels, and which is in contact with the maternal blood lacunae.

Bearing in mind the connection between carbohydrate metabolism and phosphatase activity, we undertook a study of the content and histotopography of glycogen in the uterus and placenta of rats at various stages of pregnancy, using histochemical methods for this purpose.

We have been able to trace only a very few papers dealing with the histochemical study of carbohydrate metabolism in the placenta. S. Kasab'ian [2] applied Shabadash's method to the investigation of glycogen content of the human placenta at various stages of pregnancy (1 to 10 months), and found a high glycogen content of the villi during the first three months, followed by an abrupt fall. The same was found for the decidual tissues. From his data, it appears that the process of glycogen storage proceeds most actively in the trophoblast. The active part played by the trophoblast in the carbohydrate metabolism of the ovum has been shown by Goldman [6], who was able, using Best's carmine method, to demonstrate the presence of glycogen in the trophoblastic syncytium of rat placenta. Wislocki, Deane, and Dempsey [10, 5] were not able to confirm this finding. These authors applied the Bauer-Feulgen, the Best carmine, and the Wislocki silver-staining methods [9] to the study of the content and the distribution of glycogen in the placentae of guinea pigs, white mice, rabbits, jerboas, and rats, without, unfortunately, sufficiently taking into account the stage of gestation at which the tissues were examined. These authors were able to detect glycogen only in the mononuclear giant cells of rodent trophoblast tissue, and could not find it in the trophoblastic syncytium. Other workers, apart from those cited above, have been unable to detect glycogen in the trophoblastic syncytium.

EXPERIMENTAL METHODS*

Our experimental material consisted of uteri and placentae taken from 18 white rats. The tissues were taken from nonpregnant animals, and from gravid rats on the 6th, 10th, 15th, 17th, 18th, and 19th days of pregnancy, and were fixed in a mixture of 9 parts of absolute alcohol and 1 part of neutralized formalin. Glycogen was stained by the Bauer-Feulgen procedure. We used sodium sulfite instead of sodium bisulfite for the

*The experimental material was supplied by Prof. P. G. Svetlov.

preparation of Feulgen's reagent and of the washing fluid. Paraffin tissue sections 6-8 μ in thickness, were smoothed down in a drop of reagent (20 g of potassium dichromate and 5 g of chromic acid in 1 liter of distilled water) on a slide previously treated with albumin-glycerol mixture. Some of the sections were straightened out in a drop of distilled water, but this did not give equally good results. The sections were cleared in the usual way, except that chromic anhydride was added to the 45% alcohol. The staining of the sections was always checked by ptyalin treatment.

EXPERIMENTAL RESULTS

Glycogen was found in traces in the non-gravid myometrium, in the apical parts of the epithelial cells of some of the endometrial glands, and in their secretion.

Glycogen could not be detected in the uterus on the 6th day of gestation, when implantation of the ova had not yet taken place [3], or was in progress. Only traces of glycogen could be seen in the myometrium.

By the 10th day, when the elements of the future placenta, assuring the further development of the fetus, had already been laid down, the uterus contained considerable amounts of glycogen. It was particularly abundant at the mesometrial side of the proliferating endometrium. The glycogen is seen in the form of grains and granules of different sizes in the cytoplasm of cells of the decidual tissues located between the larger lacunae

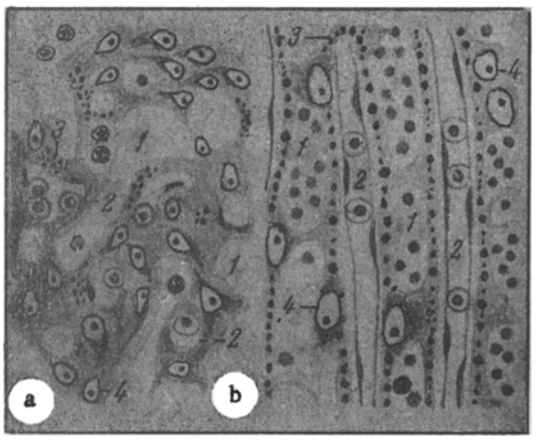

Fig. 1. Sections of white rat placentae (a) of the 15th, (b) of the 19th day of gestation: 1) subchorial blood space; 2) fetal capillaries; 3) glycogen in the trophoblastic syncytium (black spots); 4) syncytial nuclei. Magnification: Ocular 7 ×, objective 40×.

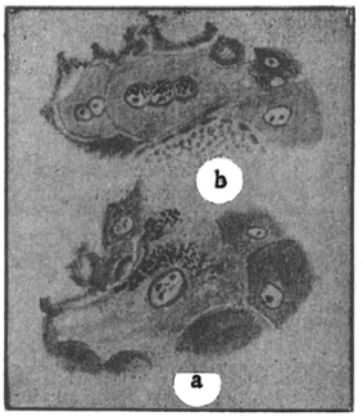

Fig. 2. Glycogen (black spots) in giant mono- and polynuclear cells of the trophoblast of the placentae of white rats, (a) on the 15th, (b) on the 19th day of gestation. Magnification: ocular 7×, objective 20×.

distributed around the ectoplacental cone. Its amount diminishes as it approaches the muscularis and the margin of the uterine cavity, where it can no longer be perceived. Only traces of glycogen were seen in the maternal epithelium, particularly over the fetal sac, where the cells are stretched considerably, and in the muscle layer. It was found in considerable amount in certain cells of the ectoplacental cone, which is derived from the trophoblast. Many of the cells did not contain glycogen, or showed only traces of it. Glycogen was not seen in the cells of the ectoplacental cone which were in a state of mitotic division.

The placenta is fully formed by the 15th day of gestation. At this stage, and thereafter up to full term, glycogen is absent from the allantoic mesenchyme zone of the placenta. Only in very rare cases can it be seen in the trophoblastic syncytium of the subchorial blood spaces, as minute droplets or granules (Fig. 1, a). Islets of maternal tissue consisting of more than 10 cells, as well as isolated maternal cells found in the blood spaces, are rich in glycogen. It is absent from the trophoblast of the boundary zone along the narrow lacunae of maternal blood, but is present in large amounts in the so-called "glycogen cells", situated in large groups between the lacunae. Many of these cells do not, however, contain any glycogen. Glycogen is visible in the form of aggregates of different sizes, in many of the giant cells (Fig. 2, a). It is concentrated in the peripheral cyto-

plasm of the cells, or in the perinuclear region, and only rarely is a giant cell uniformly packed with glycogen. Some of the giant cells contain a dark brown pigment, in the form of short, rod-shaped particles; this appears to be hemosiderin [11]. Such cells contain finely granular glycogen. Glycogen is distributed irregularly through the maternal part of the placenta. Large conglomerates of glycogen are present in the cells contiguous with the giant cell zone, where the larger venous sinuses are situated. Less glycogen is present, mostly in the form of granules and droplets of different sizes, toward the line of future separation of the placenta. Some parts of the maternal placenta do not contain any glycogen. Large globular cells, with a granular cytoplasm, are to be encountered among trophoblast cells forming the lining of the lumina of the venous sinuses; these cells are rich in glycogen. These cells, which often possess two nuclei, sometimes protrude into the lumen of the sinus, where they are separated from the maternal blood by a layer of attenuated endothelium. Granular glycogen-containing cells may be seen in other sections in the lumen of the venous sinuses. Glycogen-loaded granular cells, with one or two nuclei, are found in the greatest number around the blood vessels of the maternal placenta, and are even more numerous in the extraplacental part of the decidua basalis, which is the chief site of their formation. Single glycogen-loaded cells are to be found in the subchorial blood spaces, to which they were carried by the blood stream.

The granular cells, which were first described in rats by Gerard [6] in 1925, probably then disintegrate. Evidence in support of this is afforded by the presence among such cells seen in the lacunae of weakly staining forms, with barely distinguishable outlines. Moreover, in agreement with the observations of other authors [7], we found granules of free glycogen in the blood of the venous sinuses. There are no clear indications as to the origin and functions of the granular cells. Endocrine, phagocytic, and trophic functions have been ascribed to them [6]. According to some workers, these cells originate from fibroblasts, while others believe that they originate from muscle or decidual cells, or that they are hypertrophied endothelial cells.

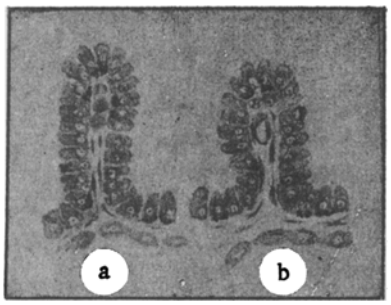

Fig. 3. Section of the wall of the yolk sac of (a) a 15-day, (b) a 19-day white rat fetus. Glycogen is seen as black spots in the entodermal epithelium. Magnification: ocular 7x, objective 20x.

In our opinion, these cells are of connective tissue origin, since on the 6th day of gestation cells containing acidophilic granules are to be found in the lamina propria, in the vicinity of the muscular coat, and it is probable that it is from these that the glycogen-rich, globular, granular cells originate. Glycogen is present in small amount in the myometrium. It is absent from the cells of the walls of the yolk sac, or is present in traces or small amounts only (Fig. 3, a).

The placenta achieves its highest degree of development on the 17th day of gestation. at this stage, the islets of maternal tissue found in the placental labyrinth zone are loaded with glycogen. The glycogen content of the attenuated syncytial trophoblast rises. It is distributed as very fine granules throughout the whole of the labyrinth zone. Its distribution in the other elements of the placenta remains similar to that found on the 15th day of pregnancy. In the giant cells, the glycogen is frequently concentrated in one particular part of the cell. The glycogen content of the maternal placenta falls. It may be found in the walls of the umbilical artery up to the termination of pregnancy, and in smaller amount in the umbilical vein. Glycogen is absent, or present only in traces, in the folded part of the wall of the yolk sac. It is absent from many cells. It is concentrated mostly in the basal, less frequently in the apical, regions of entodermal cells. It is absent from the cells of the smooth part of the wall of the yolk sac.

The glycogen content of the syncytial trophoblast of the labyrinth continues to rise during the 18th and 19th days (see Fig. 1, b), both the number and the dimensions of the granules increasing. Isolated conglomerates of glycogen may even be seen on the 19th day. A considerable part of the glycogen cells of the boundary zone have undergone degeneration at this stage. Glycogen is to be found in small amount in the trophoblast cells of certain regions of this zone. Little glycogen can be seen in the cytoplasm of the giant cells, being sometimes concentrated at the nuclear membrane (see Fig. 2, b). The glycogen content of the decidua basalis

remains high, being mostly localized in the granular cells. There is little glycogen in the epithelium of the folded parts of the wall of the yolk sac, being situated in the basal parts of the cells; many cells do not contain any (see Fig. 3, b). The glycogen content of the maternal epithelium is low.

In general, accumulation of glycogen is seen to vary parallelly with the phosphatase activity, and in particular the acid phosphatase activity, of a given region of the placenta and uterus; this is in accordance with the findings of other authors [8].

Both glycogen and acid phosphatase make their appearance in the rat uterus toward the 10th day of pregnancy. With formation of the placenta, glycogen is initially found in large amount in its maternal portion, but the content falls as pregnancy proceeds. Alkaline phosphatase is absent during the entire period of gestation, while acid phosphatase activity, which is high initially, falls as pregnancy proceeds.

The glycogen content of the decidua basalis also appears to be correlated with the acid phosphatase activity. As pregnancy advances, both of these factors show a parallel rise.

Glycogen is to be found throughout pregnancy in the giant cells of the trophoblast, both mono- and polynuclear. These cells show a low alkaline, but a high acid phosphatase activity. Both alkaline and acid phosphatase activities were found to be very high in the syncytial trophoblast of the labyrinth. Glycogen makes its appearance here from the earliest stages of formation of the placenta, and its persists, in gradually increasing amount, as pregnancy proceeds. The vitelline placenta plays scarcely any part in the carbohydrate metabolism of the fetus. The most active part is played by the trophoblast, which has also been shown by Z. P. Zhemkova [1] to be very actively concerned in protein metabolism. It thus appears that embryonic development is fulfilled largely as a result of the various activities of the placental trophoblast.

SUMMARY

Histochemical studies of the distribution of glycogen in sections of the placenta and the uterus of white rats were made, using the Bauer-Feulgen technique, on material from the 6th, 10th, 15th, 17th, 18th, and 19th days of pregnancy. Only traces of glycogen were found in the non-gravid uterus. During pregnancy there is a correlation between the phosphatase, in particular the acid phosphatase, activities of uterine and placental tissues and their glycogen contents. A gradual rise is seen in the glycogen content of the decidua basalis, the syncytial trophoblast of the labyrinths, and the giant cells of the trophoblast, viz., in those components of the fetal adnexa which play the most important part in the carbohydrate metabolism of the embryo. Only traces of glycogen are to be seen in the vitelline placenta.

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