

# LOCALIZATION OF GLYCOGEN IN THE HUMAN PLACENTA

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In a previous communication [3] we reported findings relating to the distribution of glycogen in the uterus and placenta of white rats. In the present research our aim was to investigate the content and distribution of glycogen in the human placenta during the early stages of its development.

There have been very few histochemical investigations of the carbohydrate metabolism of the human placenta. Wislocki and Dempsey [4], using Bauer's method, investigated the glycogen in 9 uteruses at the 8th-16th week of pregnancy. S. S. Kasab'yan [2], using Shabadash's method, investigated the glycogen in human placentas, but without taking into consideration the term of pregnancy. This author claims that before three months of pregnancy the villi and decidual tissue are rich in glycogen, but that later the glycogen content falls sharply. He found glycogen in large quantities in the cytotrophoblast (in 75% of cases) and in the syncytial layer (in more than 60% of cases). He found glycogen comparatively rarely in the stroma of the villi (in 20% of cases). A. P. Dyban [1], using the methods of Shabadash, Lillie, Shigimitsu and Kumamoto, detected glycogen in the human chorion at the 4th-10th week of pregnancy, and showed that glycogen is localized in the cytotrophoblast of the villi and of the chorial plate. In his opinion, the plasmoditrophoblast is devoid of glycogen.

## METHOD

The material studied consisted of the chorion and placenta from women at the 4th to the 40th week of pregnancy. The material was fixed within 2 hours of abortion, or premature or full-term labor. Pieces from the maternal and fetal surfaces of the placenta were excised. Fixation was carried out in a mixture of absolute alcohol and neutral formalin. The glycogen was determined by the Bauer-Feulgen method. In the preparation of the Feulgen's reagent and the rinsing fluid, sodium bisulfite was replaced by sodium metabisulfite (or sulfite).

Paraffin-wax sections were cut to a thickness of 6-8  $\mu$  and spread out in a drop of 4% chromic acid, which was applied to a glass slide, previously smeared with egg-white and glycerol. The dewaxed sections were taken through alcohols to 4% chromic acid, the latter being added, in the form of the anhydride, to 70°, 45°, and 30° alcohols. After treatment with 30° alcohol, the sections were rinsed in 3 changes of 4% chromic acid. Their staining was always controlled by means of phthalin. The nuclei of the cells were stained with hemalum, azure-2, methyl violet, methyl green and methylene blue, and also with malachite green. The best results were obtained with the last three stains. Since the cells did not have a uniform content of polysaccharide, we distinguished three degrees of glycogen content, and used the following terms to define them. The presence of glycogen in the cell in the form of solitary granules was designated as "traces," the presence of glycogen in the apical or basal portion of the cell only, or in the form of many granules scattered throughout the cell, as "little", and when the whole cell was filled with glycogen, we described this picture by the term "much" glycogen.

## RESULTS

At the fourth or fifth week of pregnancy the human chorion possesses secondary villi, which are beginning to undergo vascularization. The villi are covered with trophoblast, in which an inner cellular, and an outer syncytial, layer may be distinguished. We found glycogen in the stroma and trophoblast of the majority of villi, in the cell knots, the endothelium of the fetal vessels, the uterine epithelium, the epithelium and lumen of the glands, the decidual cells and the intercellular spaces at the site of destruction of the connective tissue of the uterine mucous membrane under the influence of the chorial syncytium.

In the cellular layer of the trophoblast there was much glycogen (see figure, a) and only in a few cells

was none present. In the stroma of the villi there was little glycogen. There was also little glycogen in the epithelium of the uterus; it was present in many cells of this epithelium, and was localized in the basal or apical part of these cells, sometimes forming a sharply outlined narrow band.

There was little glycogen in the large decidual cells; in the unchanged connective tissue of the stratum spongiosum between the uterine glands, glycogen was found rarely, in the form of traces. There was much glycogen in the epithelium of the altered glands; the bulk of the glycogen was localized usually in the apical divisions of the epithelial cells. There was also much glycogen in the lumen of these glands, where it was found in the form of freely lying droplets of different sizes. The largest quantity of glycogen was observed in the cell knots connected with the villi. Glycogen could be

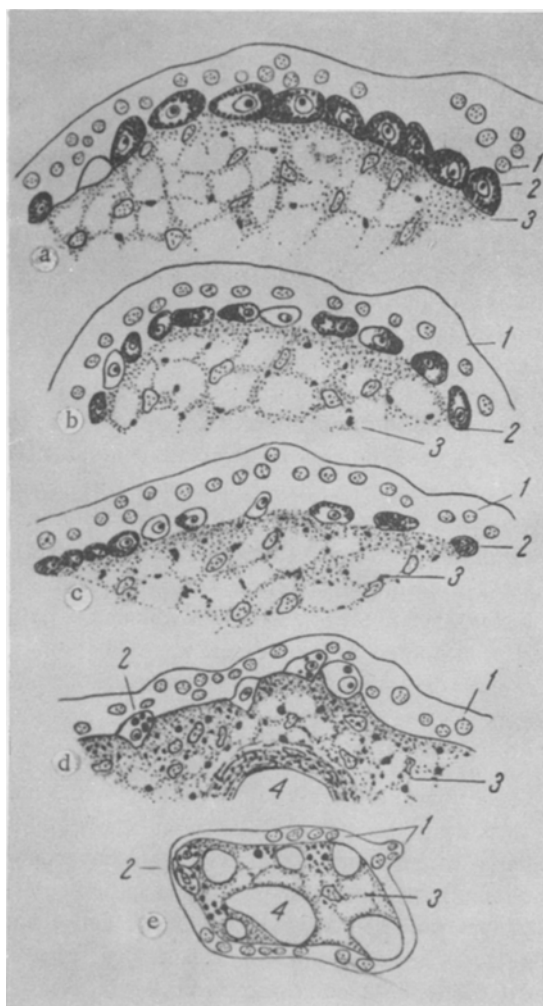
detected until the end of pregnancy in the endothelium of the fetal vessels and the leucocytes of the maternal blood.

At the 12th week the decidua basalis reaches its highest development, the villi become more ramified, and the cellular layer of the chorionic epithelium is slightly thinned. At this stage of development, as hitherto, there was much glycogen in the layer of Langhans, in the cells of which mitotic figures were sometimes encountered (see figure, b). In the stroma of the villi its amount was increased. In the decidual cells there was little glycogen, and much in the cell knots.

At the 16th week of pregnancy glycogen was detected in the cells of Langhans as before. There was much glycogen in the connective tissue of the villi (see figure, c). In the cell knots, often permeated with fibrinoid, there was also much glycogen. Cell knots, connected with villi, were encountered more frequently in the sections, and they were larger in size; mitoses were seen among the cells of the knot. They arose as a result of proliferation of the cells of Langhans; the nuclei of the syncytium divided by amitosis. Glycogen was found in the form of tiny granules in the walls of the umbilical vessels and in the Wharton's jelly of the umbilical cord.

At the 20th week of pregnancy there was little glycogen in the decidual cells. In the fibrinoid masses, light islands ("infarcts") could be seen in the degenerating stroma of the villi, in which many clumps and drops, often of large size, of glycogen were present. There was also much glycogen in the stroma of the normal villi. None whatsoever was found in the Kashchenko-Hofbauer cells. Cells of Langhans were found singly, and only in rare cases in the form of a continuous layer; mitoses were sometimes found in them. Glycogen was observed in these cells in the form of one or a few, often large, granules; in some cells it was completely absent (see figure, d). Glycogen was also found in the walls of the umbilical vessels; in the umbilical vein it spread slightly beyond the limits of the muscular layer. No glycogen was found in the remainder of the thickness of the umbilical cord.

At the 40th week of pregnancy glycogen was noted as traces in the cytoplasm of the few remaining decidual cells. The villi had little glycogen in their stroma and epithelium at their points of attachment to the decidua tissue. Many cell knots were penetrated by fibrinoid. Between the cells of the knots there was little glycogen, but much was present in the stroma of the villi with which these knots were connected. The "white infarcts" in the fibrinoid masses contained either little or much glycogen. In the connective tissue and walls of the vessels of the larger villi much glycogen was present. In occasional cases villi were found with cells of Langhans containing glycogen in the form of a few, sometimes large granules (see figure, e). In the



Distribution of glycogen (black dots) in the villi of the human chorion at the 4th-5th (a), 12th (b), 16th (c), 20th (d), and 40th (e) weeks of pregnancy. 1) Nuclei of trophoblastic syncytium; 2) cells of Langhans; 3) stroma of villi; 4) fetal vessels. Magnification: ocular 7 $\times$ ; objective 90 $\times$ .

leucocytes of the fetal blood glycogen was observed at the 20th, 32nd, 34th, 36th, and 40th week of pregnancy.

In conclusion it must be pointed out that glycogen was present throughout pregnancy in the ectodermal epithelium of the amnion and the mesenchymal cells of the chorionic plate, in the extraplacental chorionic, and also the amnion. Glycogen was also found in the form of tiny granules in the whole thickness of the umbilical cord until the 16th week, inclusive; at the 20th week of pregnancy it was present only in the wall of the umbilical vessels, with a very slight spread beyond the limits of the muscular layer of the umbilical vein. The cell knots contained much glycogen at the beginning of pregnancy; its total content here increased in the course of pregnancy, but in the last third of pregnancy, with the increase in the fibrinoid in the cell knots the quantity of glycogen diminished, and at the 40th week it was seen in small quantities only between the cells. In the stroma of the villi there was little glycogen at first, but its content later increased; in the second half of pregnancy hardly any glycogen was observed in the stroma of the smaller villi; it was present in large amounts in the stroma of the larger and main villi. In the cells of Langhans, glycogen was found throughout pregnancy; in these cells there was much glycogen at first, but later, with loss of the layer of Langhans, its content diminished. No glycogen was detected in the syncytial layer of the trophoblast throughout the whole of pregnancy, which is contrary to the observations of S. S. Kasab'yan [2], and in agreement with those of A. P. Dyban [1].

#### SUMMARY

Glycogen was determined by the Bauer-Feulgen method in the chorion and placenta of 4- to 40-weeks-

pregnant women. It could be detected through the whole pregnancy in the ectodermal epithelium of the amnion and mesenchymal cells of the chorion laeve, extraplacental chorion, and amnion. Glycogen was also found throughout the entire cross section of the umbilical cord up to the 16th week, inclusive; at the 20th week of pregnancy glycogen was determined only in the wall of the umbilical vessels, with an insignificant spread beyond the muscular layer of the umbilical vein. With the progress of pregnancy the amount of glycogen in the cellular nodules increased, but in the last third of pregnancy, with the rise of the fibrinoid content, the amount of glycogen in the cellular nodules began to drop; at the 40th week of pregnancy it could be detected only in small amounts between the cells. The glycogen level in the stroma of the villi was low at first, but rose later; large amounts of glycogen were found in the stroma of the larger and stem villi during the last half of pregnancy; it was almost entirely absent in the stroma of smaller villi. Glycogen was revealed in the Langhans' cells throughout the whole period of pregnancy, whereas in the syncytial layer of the trophoblast it was absent altogether.

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\*Original Russian pagination. See C. B. translation.