## ADAPTIVE MECHANISMS OF THE ALBINO RAT

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PLACENTA TO DEHYDRATION

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Mechanisms of adaptation of the albino rat placenta to maternal dehydration as a result of restriction of the water intake were investigated. In the dehydration experiments nonpregnant animals survived for 6-7 days and pregnant animals for 10-11 days. Compared with the controls, the placenta of the experimental animals showed considerable thinning of the labyrinthine bands, increased permeability of the ground substance of the connective tissue and of the endothelial-trophoblastic substances, and a high rate of protein synthesis in the syncytiotrophoblast. These changes are evidence of the high adaptive powers of a provisional organ such as the placenta.

Disturbance of fetal development by the action of pathogenic agents (high temperature, cooling, anoxia, x rays, drugs, starvation) on pregnant animals, inducing changes in the newly formed placenta, has frequently been discussed in the literature [1, 3, 5-7].

The investigation described below is one of a series to examine the general problem of the mechanisms of adaptation of the mother-placenta-fetus system to changes in the external environment. An environmental factor which is very important for development of the embryo is the water and salt balance of the mother. This problem is of great practical importance where man is concerned for it is connected with the development of obstetrical diseases such as the toxemias of pregnancy.

The object of this investigation was to study mechanisms of adaptation of the albino rat placenta to maternal dehydration.

## EXPERIMENTAL METHOD

The development of the chorioallantoic placenta of the albino rat was studied under normal conditions and during feeding on dry food only. Noninbred albino rats weighing 180-200 g were used. The pregnant animals were divided into two groups: 1) control, receiving the ordinary animal house diet; 2) animals (12) with a restricted water intake, receiving only dry bread to eat.

The experiment began on the 7th day of pregnancy, i.e., at the time of placentation. Nonpregnant rats were found to be more sensitive to dehydration than pregnant; they died on the 7th day after the experiments began. Pregnant animals receiving dry food only died after 17 days, i.e., on the 10th-11th day after the beginning of the experiment.

The animals were killed by decapitation on the 10th, 11th, 12th, 13th, 14th, 15th, 16th, and 17th days of pregnancy. The material was fixed in 10% neutral and acid formalin and in Carnoy's mixture. Paraffin sections, 5-7  $\mu$  in thickness, were stained by the usual histological methods, by the PAS reaction, and with toluidine blue at various pH values, and with Hale's reagent with the corresponding controls for detection of mucopolysaccharides. Sections were stained for proteins with bromphenol blue, with ninhydrin and Schiff's reagent, and by tetrazonium coupling; nucleic acids were studied by staining by Brachet's method.

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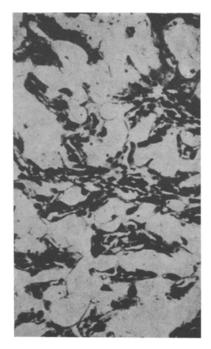


Fig. 1

Fig. 2

Fig. 1. Area of labyrinth of albino rat placenta in control animal at 17th day of pregnancy; toluidine blue at pH 4.2,  $250 \times$ .

Fig. 2. Area of labyrinth of albino rat placenta at 17th day of pregnancy. Maternal dehydration. Toluidine blue  $250 \times$ .

## EXPERIMENTAL RESULTS

On the 10th day of development the elements of the future fetal placenta consist of the allantois and structures of the trophoblast which are not connected together. The inner surface of the ectoplacental cone consists of small, compactly arranged cells of the trophoblast, from which wide bands are formed in the direction of the maternal tissues. In the outer layer of the rudimentary placenta the trophoblastic cells are large, with branching processes, arranged as a loose network. Comparison of the patterns of development of the placentas of the control and experimental animals at this period of pregnancy showed no morphological differences.

From the 11th day of pregnancy a sharp decrease in the thickness and number of the trophoblastic elements concerned in the formation of the choricallantoic placenta was observed in the experimental animals. The cytotrophoblast around the large fetal vessels consisted of small, indefinitely shaped cells arranged in two or three layers.

On the 12th day of pregnancy the differences between the development of the placentas of the experimental and control animals were more clearly marked. In the normal rats the labyrinth of the placenta was present in rudimentary form, and the zona spongiosa was in the process of formation. The bands of the labyrinth were wide, and many of the trophoblastic cells were in a state of mitosis. Fetal blood vessels were located inside the bands, and maternal lacunae outside them. In the experimental animals no clear line could yet be drawn between the zona spongiosa and the labyrinthine zone. Most of the fetal part of the placenta consisted of maternal lacunae, and the fetal vessels surrounded by the thin cytotrophoblast occupied a relatively small area compared with the dilated maternal lacunae.

From the 13th to the 15th days of pregnancy, growth and development of the newly formed structures took place in the placenta, and by the 17th day in the normal animals the folded structure of the labyrinth was clearly marked, with its dense network of fetal vessels separated from the maternal lacunae by the trophoblast and by occasional cells of the cytotrophoblast (Fig. 1). The trophoblastic cells in the zona spongiosa were large and surrounded by extensive islands of cells containing glycogen.

In the experimental animals the folded structure of the labyrinth was clearly marked only in the zone surrounding the large fetal vessels; toward the zona spongiosa the folding diminished, and most of the labyrinth in that area consisted of maternal lacunae; the fetal vessels were filled with erythroblasts and surrounded by the thin syncytiotrophoblast (Fig. 2). Optimal conditions were thus provided for metabolic exchange between the fetus and mother.

The results of the histochemical tests for glycogen in the placenta of albino rats kept on the normal animal house diet were in full agreement with the observations of Sharov [11]. In the control animals after the 11th day the zones of glycogen-containing cells in the newly formed maternal part of the placenta were clearly distinguishable; in the experimental animals, solitary decidual cells with infrequent, tiny, crimson granules were found in the zona spongiosa. Not until the 12th to the 14th day of development were small (compared with normal) areas of glycogen-containing cells clearly distinguishable in the intermediate zone of the placenta. During the same period crimson granules appeared in the wall of the fetal placental vessels of the chorionic plate. Not until the 15th day of pregnancy were glycogen granules found in the syncytiotrophoblast, but they were fewer than in the control animals. As pregnancy continued, gradual accumulation of glycogen took place in the placental labyrinth and in its outer part bordering on the zona spongiosa, but there was less of it than in the control.

Staining the placenta with buffered solutions of toluidine blue gave the following results. From the 11th to the 14th days of pregnancy in both the experimental and control series  $\beta$ - and  $\gamma$ -metachromatic granules accumulated at pH 3.6-3.8 and, in particular, at pH 4.2-4.4 in the cytoplasm of the giant cells, in the interstitial substance between them, in the ground substance of the connective tissue of the chorionic plate, and along the course of the larger fetal vessels.

With an increase in the duration of pregnancy the picture changed. Whereas in the control series acid mucopolysaccharides accumulated in the perivascular connective tissue and the permeability of these structures was reduced [10], in the experimental animals the content of high-polymer compounds decreased and the  $\beta$ - and  $\gamma$ -metachromasia disappeared; i.e., the permeability of these structures was increased. Similar changes were revealed by Hale's method with the appropriate controls.

In connection with these problems it was interesting to study the role of the albino rat placenta in protein metabolism, for many workers have concluded that the placenta can synthesize specific protein components independently, and these could possibly enter the fetal blood stream [2, 5, 8, 12].

The synthesis and content of RNA in the cell correlate with the intensity of protein synthesis [4].

Histochemical analysis of protein substances in the placentas of the experimental rats showed that the intensity of their staining for protein was slightly less than in the placentas of the control animals. Similar results were obtained by staining for RNA.

The dehydration experiments thus demonstrated thinning of the labyrinthine bands of the placenta, thereby ensuring optimal conditions for metabolic exchange between the fetus and mother; an increase in the permeability of the ground substance of the perivascular connective tissue, and a high intensity of synthesis of proteins required for the developing embryo in the syncytiotrophoblast. These observations are evidence of the high adaptive powers of a provisional organ such as the placenta.

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