# MORPHOHISTOCHEMICAL INVESTIGATION OF THE EMBRYONIC LIVER AFTER $CCl_4$ ADMINISTRATION AT VARIOUS STAGES OF ONTOGENY

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The reaction of the embryonic rat liver 48 h after injection of $CCl_4$ into the mother on the 15th-22nd day of pregnancy was studied by morphohistochemical methods. A decrease in weight of the fetuses was observed after the 16th day of pregnancy; the relative weight of the fetuses of the experimental group diminished initially (15th-16th day) but later was appreciably greater than that of the control fetuses. An increase in free fat was found in the liver of the fetuses after the 16th day of intrauterine development. The appearance of glycogen in the cytoplasm of the hepatocytes was delayed and its amount decreased. Involution of the hematopoietic tissue was delayed. It was also shown that morphological changes in the embryonic liver increase toward the end of pregnancy and are much less pronounced than in the maternal liver.

A previous investigation [3] showed considerable changes in the morphohistochemical characteristics of the maternal liver during pregnancy, reflecting an increase in its functional activity. A parallel decrease was found in the severity of damage to the maternal liver tissue 48 h after administration of $CCl_4$.

Considering that the liver passes through phases of differentiation during histogenesis as the fetus becomes incorporated into the system of homeostasis, it was decided to investigate the response of the embryonic liver to injection of a hepatotropic poison at various stages of organogenesis. It will be recalled that there is no general agreement on the degree and character of injury to the embryonic liver [1, 2, 6, 11].

The object of the present investigation was to study the morphohistochemical characteristics of the embryonic rat liver after exposure to $CCl_4$ at different periods of antinatal development of the fetus.

## EXPERIMENTAL METHOD

Fetuses of 64 albino rats were investigated from the 15th to the 22nd day of pregnancy. The experimental group consisted of 34 pregnant rats receiving an injection of $CCl_4$ in a dose of 0.3 ml/100 g body weight 48 h before sacrifice. The control group consisted of 30 intact rats at the corresponding periods of pregnancy. The phases of the estrous cycle and the presence of spermatozoa in the genital tract were determined by the vaginal smear method [4]. The embryonic liver was fixed in alcohol–formalin and embedded in paraffin wax in the usual way. Some of the unfixed liver tissue was used for making frozen sections. After removal of the wax the sections were stained with hematoxylin-eosin, with gallocyanin for RNA, with PAS-reagent for neutral glycoproteins and glycogen, and by the method of Barrnett and Seligman for protein SH-groups. Acid and neutral lipids were detected with Sudan III and Sudan IV. Appropriate enzymic and chemical control tests were used [5]. The relative weight of the fetal liver was calcu-

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TABLE 1. Weight of Embryos and Relative Weight of Liver of Fetuses of Normal Animals and of Rats Receiving $CCl_4$ (M ± m)

| Day of embryogenesis | Weight of fetuses (in g) | | Relative weight of fetal liver (in mg) | |
|---|---|---|---|---|
| | control | experiment | control | experiment |
| 16th | 320,0±21,7 | 271,2±14,2<br>$P<0,1$ | 6,6±0,305 | 2,83±0,08<br>$P<0,001$ |
| 18th | 975,0±48,14 | 770,0±43,5<br>$P<0,02$ | 8,19±0,316 | 8,86±0,697<br>$P<0,05$ |
| 21st | 4058,0±237,2 | 3260,0±251,0<br>$P<0,001$ | 7,47±0,423 | 9,37±0,894<br>$P<0,05$ |
| 22nd | 5116,6±237,2 | 4320,0±298,0<br>$P<0,001$ | 5,87±0,554 | 7,37±0,458<br>$P<0,05$ |

lated per 100 mg weight of the fetus. The numerical results were subjected to statistical analysis by the Fisher–Student method.

## EXPERIMENTAL RESULTS

As Table 1 shows, the relative weight of the fetal liver reached a maximum on the 18th day of development. Microscopic examination of the liver of 15-17-day fetuses of the control group showed bands of epithelial cells with wide sinusoids between them. In the blood vessels and also between the vessel wall and liver cells were many hematopoietic cells, the number of which fell appreciably after the 20th day of development. Glycogen was found in the fetal liver histochemically on the 18th day of embryogenesis as tiny PAS-positive granules in the cytoplasm of individual hepatocytes, as a rule close to the blood vessels. On the 20th-21st day of development the glycogen granules filled the cytoplasm of most hepatocytes diffusely. Before birth, in some cases the glycogen content fell appreciably.

The fetuses of the rats receiving $CCl_4$ had a smaller body weight, especially on the 18th day of development. The relative weight of the fetal liver in the experimental group was reduced on the 15th-16th day of development, but later it was considerably higher than the control. It will be noted that if the poison was injected on the 12th-15th day of pregnancy death of the fetuses occurred in most cases.

No appreciable morphological changes could be found in the liver of the fetuses of intact rats and of rats poisoned with $CCl_4$ on the 15th-18th day of embryogenesis. However, on the 16th day, in the liver of the experimental group of embryos, the content of acid and neutral lipids was raised, reaching a maximum toward the end of fetal development. In the liver of fetuses of rats receiving $CCl_4$ groups of liver cells not containing nuclei were found after the 19th day, usually on the 20th-22nd day of development. The pale, structureless cytoplasm of these cells did not give histochemical reactions for glycogen, RNA, or SH-groups. No accumulation of leukocytes or of any type of connective tissue cells could be seen in the zones of the changed hepatocytes. Outside the foci of destruction, in the cytoplasm of many of the parenchymatous cells pale vacuoles with sudanophilic contents were detected.

From the 18th day of development an increase in the proliferative power of the hematopoietic tissue and a marked increase in the number of hematopoietic cells compared with the control fetuses were observed after the 18th day of development. It should be noted in particular that the appearance of glycogen in the liver of the fetuses of rats receiving $CCl_4$ was delayed and granules of it were not found until the 19th day. In most cases the glycogen content in the hepatocytes remained low until the end of embryonic development. The RNA content in the liver of the 15-19 day embryos of the experimental group was indistinguishable from the control, whereas on the 20th-21st day the intensity of staining of the cytoplasmic RNA was reduced somewhat. No difference was found in the content of protein SH-groups in the liver of the experimental and control fetuses.

Even at the end of pregnancy the severity of damage to the fetal liver was much less than to the maternal liver.

These investigations thus showed that administration of a hepatotropic poison to pregnant rats is not without its effect on the fetus. Lipid metabolism was most sensitive to the action of $CCl_4$, and changes were found in it after the 16th day of embryogenesis.

The delay in the synthesis and accumulation of glycogen in the hepatocytes observed in these experiments, which can be used to some extent as an indicator of the functional development of the liver tissue, deserves special attention. Glycogen synthesis in the fetal liver is known to be under the control of hor-

mones of the pituitary–adrenal system [7-9]. According to Vaillant [14], the appearance of glycogen in the hepatocytes of healthy embryos coincides in time with the onset of adrenocortical secretory activity. Injection of $CCl_4$ into adult animals leads to a decrease in the P-450 content in the microsomes of the adrenals [13]. Depression of adrenocortical function may perhaps take place in the fetus also, with the result that glycogenesis in the fetal hepatocytes is delayed and depressed. This hypothesis is confirmed indirectly by the increase in the number of hematopoietic cells in the fetal liver after administration of $CCl_4$, because involution of the hematopoietic tissue has been shown not to take place in fetuses from which the adrenals have been removed, but it is restored after injection of hydrocortisone.

It may be postulated that the great reduction in the intensity of the toxic effect of the poision on the fetal liver is due to the presence of a blood–placental barrier, although according to Bhattacharya [6], injection of $CCl_4$ into the fetus either subcutaneously or into the amniotic sac does not lead to any increase in its harmful action. Bearing in mind that it is not $CCl_4$ itself which has a harmful effect on the liver cells, but free radicals formed in the course of its metabolism [12], the relative resistance of the fetal liver may evidently be explained by the functional imperfection of the fetal hepatocytes and, in particular, of their detoxication systems. In that case the increase in the severity of damage to the hepatocytes during ontogeny after injection of $CCl_4$ would be linked with differentiation of the liver cells and with the appearance of systems able to metabolize the hepatotropic poison.

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