EFFECT OF CARBON TETRACHLORIDE ON MORPHOLOGY AND HISTOCHEMISTRY OF THE LIVER IN PREGNANT RATS

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The liver of intact pregnant rats was investigated morphometrically and histochemically after administration of carbon tetrachloride. On the 11th-14th day of normal pregnancy mitotic activity and the relative weight of the liver reached a maximum; after the 7th day the glycogen content was reduced in the hepatocytes and after the 15th day the RNA content was increased. The results indicate differences in the level of functional stress on the organ in the course of pregnancy. With the development of pregnancy the degree of damage sustained by the liver tissue of the rats through the action of CCl_4 was reduced; the increase in the mitotic index was directly proportional and the increase in the relative weight of the liver was inversely proportional to the values of these indices at the time of administration of the poison.

Pregnancy makes special demands on the liver connected with the need for providing for the energetic and metabolic requirements of the developing fetus, with the need for neutralizing and excreting the metabolic products of the fetus, and with the increase in the blood levels of various hormones, notably steroids, for the binding, inactivation, and elimination of which the liver is largely responsible [7, 4]. The functional stress on the liver is reflected to some degree in its structural and histochemical features [2, 4, 8, 11, 15].

The characteristic morphological and functional state of the liver tissue during pregnancy suggests that the organ reacts to harmful factors in specific ways. However, the data in the literature on this question are extremely contradictory [5, 9, 14, 18].

The object of the investigation described below was to study the morphological characteristics of the liver at different periods of pregnancy (from the 5th to the 21st day) after administration of CCl_4.

EXPERIMENTAL METHOD

Experiments were carried out on 102 noninbred female albino rats weighing initially 230-260 g. The experimental groups consisted of nonpregnant (16) and pregnant (48) rats which received CCl_4 48 h before sacrifice. The control groups contained intact nonpregnant (six) and pregnant (32) animals. The phases of the estrous cycle and the presence of spermatozoa in the genital tract were determined by the vaginal smear method [1].

The CCl_4 was injected into the stomach through a tube as a 50% oily solution in a dose of 0.3 ml pure CCl_4/100 g body weight. Pieces of liver were fixed in 10% formalin, in Carnoy's fluid, and in alcohol-formalin and embedded in paraffin wax in the usual way. Some of the liver tissue was used for the preparation of frozen sections in a cryostat. After dewaxing the sections were stained with hematoxylin and eosin, with Schiff's reagent by McManus's method, with bromphenol blue and ninhydrin-with-Schiff's reagent [6], and with toluidine blue at pH 4.2 [12]. Free lipids were determined by staining with Sudan III and IV [6]. The appropriate enzyme and chemical controls were performed at the same time. The relative weight of the liver was calculated per 100 g body weight, while the mitotic index was determined by counting the number

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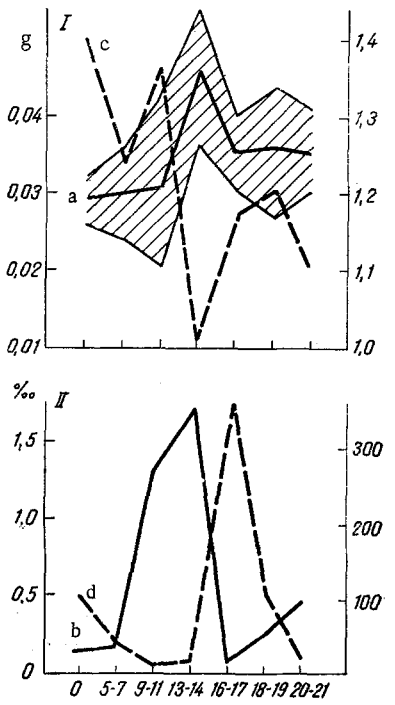

Fig. 1. Dynamics of weight of liver (I) and mitotic activity of hepatocytes (II) of pregnant rats receiving CCl_4: a and b) during normal pregnancy; c) ratio between weight of liver of pregnant rats receiving CCl_4 and weight of liver of control pregnant rats at corresponding times of pregnancy; d) ratio between mitotic index of hepatocytes of pregnant rats receiving CCl_4 and mitotic index of control pregnant rats at corresponding times of pregnancy. Abscissa, day of pregnancy; ordinate: I) on left – weight of liver of control pregnant rats (in g/g body weight), on right – coefficient of change in weight of liver of pregnant rats 48 h after administration of CCl_4 in relative units; II) on left – mitotic index of hepatocytes of control pregnant rats (in ‰), on right – coefficient of change of mitotic index of hepatocytes of pregnant rats 48 h after administration of CCl_4 in relative units.

of mitoses in 6000 hepatocytes and was expressed per thousand cells. The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The mortality among the pregnant animals from CCl_4 was 9.8% compared with 52.5% for the nonpregnant rats.

The relative weight of the rat liver rose sharply on the 13th-14th day of pregnancy, after which it fell and remained at about the same level, slightly higher than initially, until parturition. Administration of CCl_4 (Fig. 1, I) considerably increased the relative weight of the liver of the nonpregnant and also of the pregnant rats in the early stages, but to a lesser degree in the final stages of pregnancy, whereas administration of the poison on the 11th-12th day caused no increase in the relative weight of the liver.

The mitotic activity (Fig. 1, II) in the liver cells of the intact rats also rose significantly from the 11th to the 14th day of pregnancy. After administration of CCl_4 the increase in the mitotic index (Fig. 1d) was directly proportional to its value at the time the poison was given: administration of the hepatic poison during the time of maximal mitotic activity (on the 13th-14th day) led to the largest rise in the mitotic index after 2 days, whereas if CCl_4 was given on the 7th-11th or the 16th-17th day the degree of increase in the mitotic activity of the hepatocytes was much smaller.

Morphological examination of the liver of the pregnant animals in some cases revealed hepatocytes containing lipid vacuoles in their cytoplasm and enlarged Kupffer cells with eosinophilic cytoplasm. From the 7th day of pregnancy there was a progressive decrease in the glycogen content in the cytoplasm of the liver cells located in the peripheral zone of the lobules. Before parturition the glycogen disappeared completely from the hepatocytes of the peripheral zones partly from the central zones. The RNA content in the hepatocytes of the pregnant rats increased from the 15th day and reached a maximum at the end of pregnancy.

A picture of necrobiosis of the hepatocytes as the result of acidophilic degeneration was found 48 h after administration of CCl_4 in the central zones of the lobules in the liver of the nonpregnant rats. The zone of necrobiosis was usually infiltrated by connective-tissue cells of the lymphoid and histiocytic series. Pale swollen liver cells with pycnotic nuclei, called ballons because of their large size and structureless cytoplasm [16], were found in the central zone. The peripheral zones of the hepatic lobule contained very large hepatocytes with pale cytoplasm of a cellular texture as the result of its diffuse filling with lipid vacuoles. In most cases the hepatocytes of all zones of the lobule had lost all their glycogen. RNA remained chiefly in the hepatocytes of the peripheral zone as pale blue, finely dispersed granules, evenly distributed in the cytoplasm between the lipid vacuoles. Staining for total proteins showed an intensive, homogeneous reaction in the necrotic masses. Proteins were found in the cytoplasm of the hepatocytes in the peripheral zone in the form of a delicate network around the lipid droplets.

After administration of the poison to the pregnant animals the zones of necrobiosis decreased in size from the 7th day as pregnancy developed and the signs of balloon degeneration disappeared.

On the 20th-21st day small central lobular foci of necrosis were found in individual hepatic lobules, where they appeared as a few structureless remnants of liver tissue profusely infiltrated with connective-tissue cells; the cytoplasm of most hepatocytes contained numerous lipid vacuoles. With the course of pregnancy the predominant form of lesion of the liver tissue after exposure to CCl_4 was thus diffuse fatty degeneration.

In the early stages of pregnancy glycogen persisted in the liver of the animals in a few hepatocytes in the peripheral zone of the lobules; on the 13th-18th day numerous glycogen granules were found in nearly all the hepatocytes of the central and peripheral zones; on the 20th-21st day glycogen was found only in individual cells. The RNA content in the cytoplasm of the liver cells of animals receiving CCl_4 was significantly higher than in the nonpregnant animals as early as on the 7th day of pregnancy.

After the 9th day of pregnancy the content and character of distribution of the proteins began to change. In most cases the necrotic masses no longer showed deeply stained protein substances. The protein content outside the zones of necrosis in the hepatocytes was appreciably increased.

Very probably the peaks of mitotic activity and of the relative weight of the liver of the pregnant rats discovered in these experiments were due to the high functional load at the end of the period of formation of the definitive fetal organs, for during development and differentiation of the embryonic liver this organ begins to participate in the maintenance of fetal homeostasis [3, 17].

Fluctuations in the mitotic index and relative weight of the liver of the pregnant rats, in conjunction with the results of histochemical analysis of glycogen and RNA thus demonstrate variations in the level of hepatic function during pregnancy.

The functional stress on the maternal liver during pregnancy is evidently regulated by the "pregnancy dominant" with which is associated a certain level of activity of various hormones, notably steroids, the blood level of which rises in the course of pregnancy. Before parturition the excretion of estrogens is almost 1000 times higher than initially [10]. The hormones or their metabolites, undergoing conversions in the liver, can actively influence the enzyme systems of the liver cells [7]. In addition, estrogens are considered to be capable of stimulating the activity of the reticulo-endothelial system, as a result of which phagocytosis in the rat liver is increased by 3-6 times in the presence of estrogens [13, 14]. This fact may possibly explain the increased rate of lysis of the necrotic masses and the consequent negative results of histochemical tests for protein in the zones of central lobular necrosis in the liver of the pregnant animals.

The action of CCl_4 on the liver of pregnant rats thus takes place under special conditions when, as the results of these experiments show, during the progress of pregnancy the degree of damage to the liver tissue is reduced and proliferation of the hepatocytes is stimulated, as shown by the high RNA content and increased mitotic activity.

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