found no changes whatsover. Neonatal androgenization increases pituitary lactotropic activity [7,8].

The fact that the STH level in the adenohypophysis of neonatally androgenized rats is unchanged is evidence of the selective action of TP on the regulation of secretion of gonadotropic hormones. The change in gonadotropic activity can evidently be explained by a decrease in the tonic inhibitory influence of the hypothalamic catecholamines of LTH secretion and of the stimulating effect on secretion of lutenizing hormone.

LITERATURE CITED

1. V. N. Babichev, Probel. Éndokrinol., No. 3, 120 (1969).
2. F. P. Martynenko and T. O. Sakhno, Ukr. Biokhim. Zh., No. 1, 122 (1975).
3. A. G. Reznikov, in: The Mechanism of Action of Hormones [in Russian], Kiev (1975), p. 88.
4. J. B. Ansell and M. F. Beeson, Analyt. Biochem., 23, 196 (1968).
5. C. A. Barraclough and R. A. Gorski, Endocrinology, 68, 68 (1961).
6. M. Hyyppa and U. K. Rinne, Acta Endocrinol. (Copenhagen), 66, 317 (1971).
7. R. S. Mallampati and D. C. Johnson, J. Endocrinol., 59, 209 (1973).
8. R. S. Mallampati and D. C. Johnson, Neuroendocrinology, 9, 255 (1974).

EFFECT OF INJURY TO THE MATERNAL LIVER ON REACTIVE CHANGES IN THE LIVER OF THE YOUNG

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The liver of young rats aged 30 days was studied spectrocytophotometrically (content of glycogen, amino acids, RNA, and DNA) and morphometrically (size of the nuclei and nucleoli, mitotic index) 48 h after intragastric administration of CCl_4. Toxic hepatitis had been produced in the mothers of these rats before pregnancy. The results indicate that previous hepatitis in the mother not only affected the morphological nature and histochemical properities of the liver of the progeny, but also led to considerable changes in the response of the hepatocytes to administration of the poison, for the harmful effect of the hepatotoxin was increased.

KEY WORDS: injury to the liver by CCl_4; toxic hepatitis; hepatocytes; changes in liver of the progeny.

Diseases of the liver, especially infectious hepatitis, occupy an important place among the causes of intrauterine pathology of the fetus and may be the cause of delay in the general development of the child and its predisposition to various diseases [5]. Diseases of the liver are possible in the postnatal period in a child whose mother had hepatitis shortly before or during the preceding pregnancy [6]. Experiments have shown [2,3] that degenerative changes characterized by extensive vacuolation of the cytoplasm of the hepatocytes, signs of balloon degeneration, increasing processes of destruction of the cell nuclei, and so on, arise in young rats whose mothers had been poisoned with α-methylstyrene. Triple administration of CCl_4 to rats causes physical underdevelopment and delay in sexual maturation of young rats, in which the first pregnancy is late to occur and runs a pathological course [4].

In the investigation described below the morphological and histochemical reaction of the liver of young rats aged 30 days to administration of a hepatotoxin was studied. Before pregnancy toxic hepatitis had been produced in the mothers of these young rats (three injections of CCl_4, each of 0.3 ml/100 g body weight, as a

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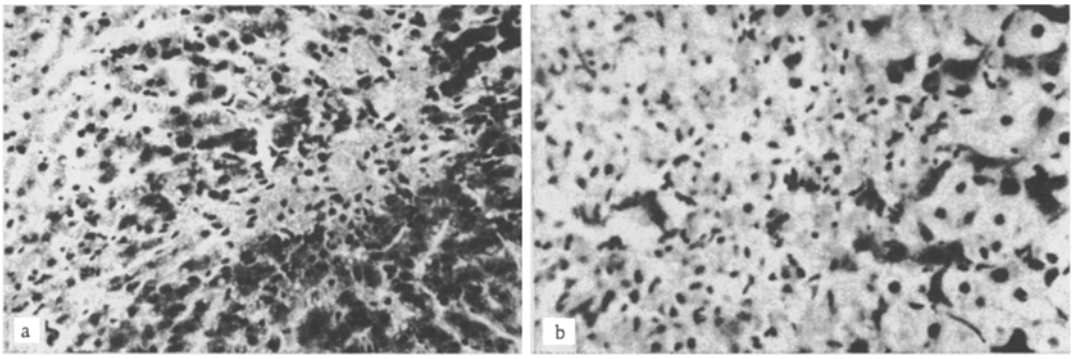

Fig. 1. Liver of control (a) and experimental (b) young rats 48 h after intragastric administration of CCl_4. Hematoxylin–eosin, 200×.

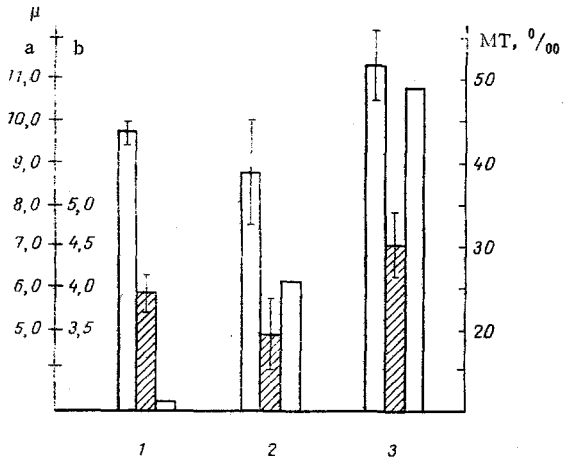

Fig. 2. Changes in size of nuclei (a) and nucleoli (b) and in mitotic index (c) of hepatocytes of animals of experimental and control groups: 1) intact, 2) control, 3) experimental rats. Ordinate: a) size of nuclei, b) size of nucleoli, in conventional units, c) mitotic index, in promille (on right).

50% oily solution, at intervals of 2 days) [9].

EXPERIMENTAL METHOD

Altogether 21 livers of rats aged 30 days were studied 48 h after intraventricular administration of CCl_4 (0.2 ml of a 50% oily solution/100 g body weight); the mothers (nine) of these young rats had previously had toxic hepatitis. The livers of 22 young rats poisoned with CCl_4, but whose mothers were intact, served as the control.

After fixation in alcohol–formalin and Carnoy's fluid the liver was embedded in paraffin wax in the usual way. The dewaxed sections were stained with hematoxylin–eosin and impregnated with silver by Foot's method. Glycogen and neutral glycoproteins were revealed by the PAS reaction, α-amino acids by ninhydrin and Schiff's reagent, SH-amino acids by treatment with DDD as described by Barnett and Seligman, basic and acidic proteins with Fast Green, RNA with gallocyanin by Einarson's method, and DNA by the Feulgen reaction with hydrolysis in hot HCl. The following substances were determined quantitatively in the hepatocytes by a two-wave method on a probe spectrocytophotometer: glycogen and α-amino acids [9], SH-amino acids and RNA [8], and DNA [7]. The area of the nuclei and nucleoli of the hepatocytes was measured with an ocular micrometer along the greatest diameter with a magnification of: objective 90×, ocular 15×. The number of dividing hepatocytes was counted in 6000 cells and expressed in promille.

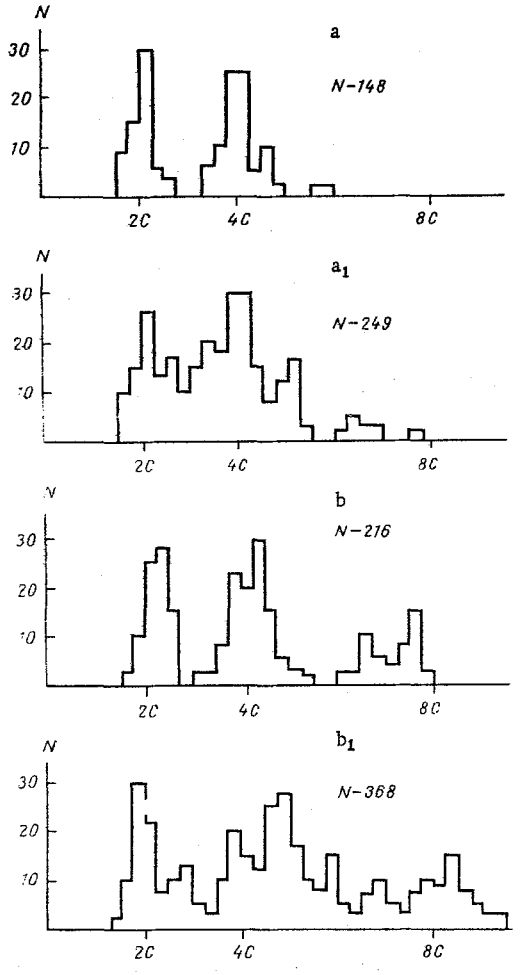

Fig. 3. Histograms of DNA content in hepatocyte nuclei: a) intact young rats aged 30 days; a_1) the same young rats 48 h after injection of CCl_4; b) intact rats whose mothers had previously had toxic hepatitis; b_1) the same young rats 48 h after administration of CCl_4. Ordinate, frequency of occurrence; abscissa, DNA content in ploidy units.

EXPERIMENTAL RESULTS

Administration of CCl_4 to young rats aged 30 days, in whose mothers toxic hepatitis had been produced before pregnancy, led after 48 h to the development of severe damage to the liver tissue, expressed as a marked enlargement of the zone of necrotic degenerating cells (Fig. 1). The area of damage to the hepatocytes amounted to half, or sometimes the greater part of the liver lobule. Elsewhere many cells with signs of hydropic degeneration and cloudy swelling were seen. The glycogen content in the cytoplasm of all liver cells of the rats of the experimental group was sharply reduced, whereas in the control animals glycogen disappeared only from the cytoplasm of the necrotic hepatocytes located around the central vein (Fig. 1). The reaction for protein showed a decrease in the intensity of staining in the zone of the dying cells and in same of the hepatocytes from the middle and peripheral zone. A marked increase in the intensity of staining was found in liver cells in the periportal zones. A similar pattern was found on histochemical demonstration of RNA.

Measurement of the nucleoli in the hepatocytes revealed a significant increase in their size 48 h after administration of CCl_4 to the rats of the experimental group (Fig. 2). Changes in the diameter of the nuclei and in the number of mitotically dividing cells were in the same direction.

The results of cytospectrophotometric investigation of the DNA content in the hepatocyte nuclei of the control animals showed an increase in the population of nuclei with a tetraploid DNA content and the appearance of cells of the diploid series, in various phases of the synthetic period (Fig. 3). Meanwhile administration of CCl_4 to young rats whose mothers had previously had toxic hepatitis caused an increase in the number of synthesizing nuclei with the polyploid DNA content, in excess of 4c and 8c (Fig. 3). Before poisoning, a higher percentage of polyploid cells was found in the liver of these animals than in the liver of the intact young rats.

Impregnation of the sections with silver by Foot's method showed differences in the characteristics of the argyrophilic "skeleton" of the liver: In the control group the fibers were delicate, thin, short, and twisted, whereas in the experimental group they were much longer, coarser, and frequently loosely arranged.

The facts described above indicate that previous toxic hepatitis in the mother rat not only was reflected in the morphology and histochemical properties of the liver of the progeny, but also led to changes in the response of that organ to subsequent administration of the hepatotropic poison, the harmful action of CCl_4 was potentiated, despite an increase in the number of mitotically dividing cells and of nuclei with a polyploid DNA content. The appearance of the latter may perhaps reflect functional exhaustion of the liver because of its earlier intrauterine development and differentiation [2,3].

During pregnancy there is thus close interconnection between the organs and systems of the mother and the fetoplacental complex. Normal development and differentiation of the organs and tissues of the embryo take place only if the parameters of the endocrine organs and of organs directly participating in the maintenance of fetal vital activity are kept within physiological limits for pregnancy. Dysfunction of the homonymous organ in the fetus arises as a result of a disturbance of normal relations within the mother – placenta – fetus system and of disturbance of the correlation between interactions of the affected organ and the organism as a whole after birth, i.e., compensatory changes taking place in the developing fetus to enable it to exist and develop in the antenatal period are the cause of the pathological disturbances after birth.

LITERATURE CITED

1. R. I. Gritsyuk, Pediatriya, No. 6, 59 (1970).
2. K. S. Lobyntsev, L. V. Zakharova, Yu. I. Savchenkov, et al., in: Problems in the Toxicology and Hygiene of Synthetic Rubber Production [in Russian], Voronezh (1967), pp. 63-64.
3. K. S. Lobyntsev, Yu. I. Savchenkov, V. P. Tereshchenko, et al., in: Proceedings of an All-Union Symposium on the Study of the Effect of Chemicals on the Young Organism and Aspects of Age Toxicology [in Russian], Moscow (1969), pp. 140-143.
4. N. Z. Maisuradze, Ya. S. Tsgareli, and M. A. Kinkladze, Transactions of the Research Institute of the Physiology and Pathology of Women [in Russian], Vol. 5, Tbilisi (1969), pp. 179-185.
5. Kh. Murzaleiva and G. Kusainova, Pregnancy and Botkin's Disease [in Russian], Alma-Ata (1965).
6. E. N. Ter-Grigorova and V. S. Ter-Grigorov, Congenital Virus Hepatitis [in Russian], Moscow (1967).
7. M. Mendelsohn, J. Biophys. Biochem. Cytol., 4, 407 (1958).
8. L. Pevzner and E.-D. Sandergene, Acta Histochem. (Jena), 39, 101 (1971).
9. J.-M. Vernier and N. Daugeras, Histochemie, 32, 307 (1972).