EFFECT OF SEX AND THE PHASE OF THE ESTROUS CYCLE ON INTENSITY OF INJURY AND REPAIR PROCESSES IN THE LIVER OF RATS WITH ACUTE CARBON TETRACHLORIDE POISONING

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The pattern of injury and repair in the liver of Wistar rats depending on sex, the phase of the estrous cycle, and also under the conditions of deficiency of female sex hormones and after injection of β-estradiol into ovariectomized rats was studied by morphometric, histo chemical, and electron-microscopic methods. Structural disturbances caused by CCl_4 were found to be increased and reparative reactions inhibited in the liver of females both during the period of a natural increase in the blood estrogen concentration and under the influence of exogenous estradiol, and ovariectomy also had a protective effect. In males, structural changes in the liver were more marked than in females with a low blood estrogen level and differed only a little from those in females during the period of increased secretion of sex steroids.

KEY WORDS: toxic hepatitis; sex steroids; injury to and repair of cells; ultrastructure.

Steroid hormones, as powerful regulators of biochemical processes in cells, can modify the response of organs and tissues, especially the liver, to pathogenic action [1, 8, 9].

The study of hormonal effects on the reactivity of organs is interesting, and sex differences in the endocrine balance as well as hormonal changes during the sex cycle in females can be used for this purpose. It has been shown [12, 14] that the blood estrogen concentration in rats starts to rise on the last day of diestrus, reaches a maximum in the middle of proestrus, and returns to its original level at the end of this phase (Fig. 1).

The object of the present investigation was to compare the processes of injury and repair in male and female rats with toxic hepatitis during the periods of maximal and minimal secretion of estrogens and also during a deficiency of these steroids and after injection of β-estradiol.

EXPERIMENTAL METHOD

Wistar rats (males and females) weighing 170-230 g were used. The scheme of the experiment and the groups of animals are shown in Fig. 1 and Table 1. To identify the phases of the estrous cycle vaginal smears were examined cytologically [2]. A deficiency of sex steroids was created by bilateral ovariectomy; β-estradiol (oily solution) was injected subcutaneously after CCl_4 poisoning in a dose of 600 units/100 g body weight. CCl_4 was injected into the stomach through a tube as a 50% oily solution in a dose of 0.3 ml CCl_4/100 g body weight. The animals were decapitated 48 h after poisoning.

Pieces of liver were fixed in 10% neutral formalin and in Carnoy's and Lilly's fluids. Paraffin sections were stained with hematoxylin–eosin, nucleic acids were demonstrated with gallocyanin–chrome alum, glycogen by the PAS reaction, and lipids in unfixed liver sections were stained with Sudan III and IV [4]. The extent of necrosis was assessed quantitatively by the method of counting points lying above necrotic and intact hepatocytes, using a special ocular insertion [7]. By means of the screw-operated AM-9-4 ocular micrometer, the diameters of all nucleoli in 100 liver nuclei were determined and their area of cross section calculated by the equation for a circle. Mitoses were counted in 5000-15,000 hepatocytes. The numerical results were sub-

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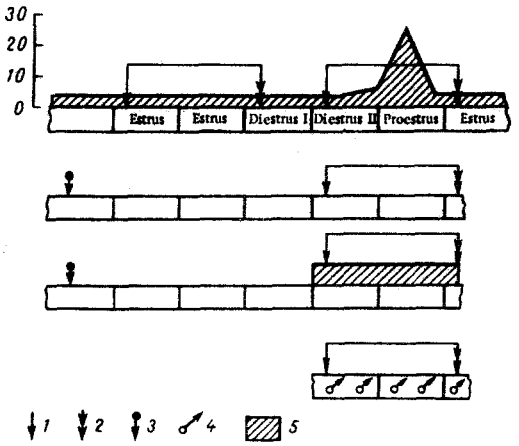

Fig. 1. Scheme of experiment. 1) Injection of CCl_4; 2) sacrifice of animals; 3) ovariectomy; 4) male rats; 5) injection of β-estradiol. Abscissa, duration of experiment and phases of estrous cycle (in days); ordinate, concentration of β-estradiol in peripheral blood plasma by the Brown–Grantsca method [3] (in mg/ml).

TABLE 1. Changes in Hepatic Parenchyma of Rats 48 h after Administration of CCl_4 (M ± m)

Group of animals	Number of necrotic hepatocytes, %	Total area of cross-section of nucleoli in nucleus, μ^2	Mitotic index, ‰
1. Female rats in period of maximal estrogen secretion (8)	70±5	4,3±0,2	3,9±1,4
2. Female rats in period of minimal estrogen secretion (10)	44±4	7,3±0,1	24,0±1,9
3. Male rats (10)	61±4	7,2±0,1	9,0±3,2
4. Ovariectomized rats (8)	20±2	6,3±0,3	38,0±1,4
5. Ovariectomized rats + β-estradiol (8)	31±4	5,8±0,4	21,0±2,2
P_{1-2}	<0,001	<0,001	<0,001
P_{2-3}	<0,01	—	<0,001
P_{2-4}	<0,001	<0,001	<0,001
P_{1-3}	—	<0,001	—
P_{4-5}	<0,05	—	<0,001

Legend. Number of animals given in parentheses.

jected to statistical analysis by Student's criterion. Pieces of liver for electron microscopy were fixed in buffered OsO_4 solution and embedded in Epon-812. Sections cut on the LKB-III ultratome were stained with uranyl acetate and then with lead citrate and examined in the JEM-7A electron microscope.

EXPERIMENTAL RESULTS

The highest mortality, about 70%, was observed in the group of female rats poisoned with CCl_4 during the period of maximal secretion of sex steroids. The highest percentage of necrotic centrolobular hepatocytes was found in the liver of the surviving animals (Table 1). The liver cells at the periphery and, to some extent, at the center of the lobules had no glycogen and little RNA in their cytoplasm, which contained droplets of fat. In most nuclei there was one small nucleolus (Table 1). An electron-transparent hyaloplasm was observed in many hepatocytes, with vacuoles of grossly dilated cisterns of granular endoplasmic reticulum (GER) and single attached ribosomes, clusters of smooth membranes large lipid drops, mitochondria with disorganization of their typical structure, and occasional autophagosomes with membranous material were visible (Fig. 2a). The picture described is evidence of advanced destructive changes in the liver tissue with inhibition of intracellular repair processes in the hepatocytes during the period of maximal concentration of estrogens in the blood. The proliferative ability of the hepatic parenchyma of the rats was low under these conditions (Table 1).

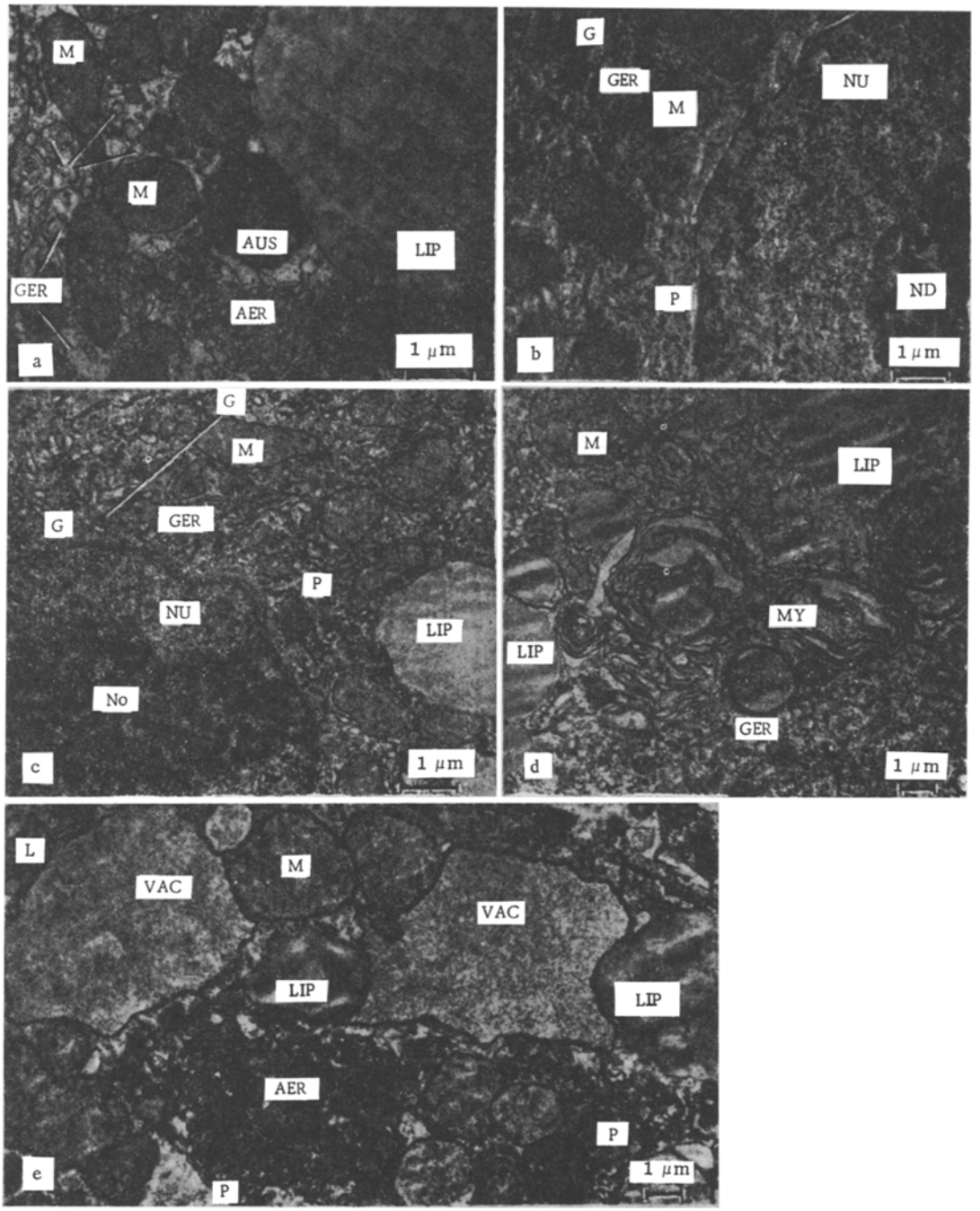

Fig. 2. Ultrastructural changes in hepatocytes of rats with acute CCl_4 poisoning depending on sex and phase of estrous cycle: a) part of a hepatocyte, showing structural changes, from a female rat poisoned with CCl_4 during period of maximal secretion of sex steroids (15,000×); b) part of hepatocyte of female rat poisoned with CCl_4 at period of low estrogen secretion, showing signs of restoration of ultrastructure (9000×); c) part of hepatocyte of ovariectomized rat poisoned with CCl_4, with ultrastructure preserved (7500×); d) part of hepatocyte of ovariectomized rat poisoned with CCl_4 after injection of β-estradiol (6000×); e) part of hepatocyte of male rat with ultrastructural disturbances caused by CCl_4 poisoning (6000×). AUS) autosome; AER) agranular endoplasmic reticulum; VAC) vacuoles; G) glycogen; GER) granular endoplasmic reticulum; L) lysosome; LIP) lipid drop; M) mitochondrion; MY) myelin-like inclusion; P) polysomes; NU) nucleus; NO) nucleolus.

In the liver of the rats receiving CCl_4 at a time of low estrogen secretion the structural disturbances of the parenchyma were not as severe as those observed in the liver cells of the animals of the previous series.

The number of necrotic hepatocytes was much smaller (Table 1). In the middle and peripheral zones of the lobule, besides cells characterized by marked destructive changes (vacuolation of GER, disorganization of mitochondria, abundance of lipid droplets), hepatocytes were frequently found with cytoplasm staining intensively for RNA, corresponding to the accumulations of GER with large numbers of attached ribosomes, and also polysomes, detected by electron microscopy (Fig. 2b). The appearance of glycogen granules in the zones of the Golgi complex and predominance of small primary lysosomes over secondary were typical. These features are evidence of the high working activity of the cells under conditions of injury and they could serve the purpose of intracellular regeneration [3, 5, 6]. The mitotic activity of the hepatocytes (Table 1) is evidence of a high rate of renewal of the injured tissue.

In ovariectomized animals the hepatotoxic effect of CCl_4 was reduced still more (Table 1). The presence of lipid drops in the cytoplasm was characteristic of those hepatocytes which remained; no other disturbances of cell structure were found. Under the conditions of a deficit of sex steroids the manifestations of intracellular regeneration were less marked than in rats with intact gonads at a time of low estrogen secretion, as shown by the smaller size of the nucleoli (Table 1) and their segregation, and by the smaller numbers of polysomes and primary lysosomes, and also of glycogen granules in the zones of the Golgi complex (Fig. 2c). The mitotic activity of the liver tissue under these experimental conditions was considerably increased (Table 1)

Injection of β-estradiol into ovariectomized rats intensified the structural disturbances of the hepatocytes produced by CCl_4 (Table 1). Besides the marked lipid infiltration in the cytoplasm of the hepatocytes, absence of glycogen granules and a reduction in the intensity of staining for RNA were observed; electron-microscopy revealed vacuolation and fragmentation of the GER, the almost complete disappearance of attached and free ribosomes, destruction of the mitochondria, accumulation of lamellar concentric membranes (Fig. 2d), and increased segregation of nucleolar material. Signs of inhibition of intracellular repair processes were combined with inhibition of cell proliferation (Table 1).

The increased intensity of the structural disturbances and inhibition of repair processes in the liver of the females observed both during the period of natural elevation of the blood estrogen level and under the influence of exogenous estradiol, and also the protective effect of ovariectomy suggest that female sex steroids are a factor which aggravate the course of the pathological changes in the liver. A similar conclusion was drawn by Yatsenko [10], who studied liver function in women with virus hepatitis in the course of the menstrual cycle.

In males structural disturbances of the liver parenchyma caused by CCl_4 were similar in severity to those observed in the liver of females poisoned during the period of maximal secretion of hormones (Table 1). Among the features described above, one which attracted attention was the large number of balloon cells, characterized electron-microscopically by well-marked vacuolation of GER (Fig. 2e). Meanwhile, at the periphery of the hepatic lobules cells with the structural manifestations of intracellular regeneration noted above were frequently found; a moderate increase in mitotic activity also was observed (Table 1).

The liver of males, characterized by a different endocrine balance and, in particular, by a high level of secretion of androgens, thus occupies an intermediate position between the liver of females in the different phases of the sex cycle, and this may account for the contradictory nature of results obtained by some workers [11, 13] who compared the resistance of males and females to CCl_4 without allowance for the phase of the sex cycle. Since the period of basal secretion of estrogens in rats accounts for about four-fifths of the sex cycle, it can be concluded that for the greater part of reproductive life females are more resistant to the action of CCl_4 than males.

LITERATURE CITED

1. S. G. Dobrovol'skaya, N. I. Tsirel'nikov, and G. S. Yakobson, Byull. Éksp. Biol. Med., No. 2, 114 (1973).
2. Ya. M. Kabak, A Manual of Practical Endocrinology [in Russian], Moscow (1968).
3. A. E. Kondakova, T. A. Korolenko, G. M. Vakulin, et al., Tsitologiya, No. 11, 1382 (1973).
4. A. G. E. Pearse, Histochemistry, Theoretical and Applied [Russian translation], Moscow (1962).
5. E. de Robertis et al., Cell Biology [Russian translation], Moscow (1973).
6. D. S. Sarkisov and B. V. Vtyurin, Electron Microscopy of Destructuve and Regenerative Intracellular Processes [in Russian], Moscow (1967).
7. S. B. Stefanov, Tsitologiya, 16, 1439 (1974).
8. G. S. Yakobson, "On the role of corticosteroid hormones in the pathogenesis of toxic hepatitis and cirrhosis of the liver," Doctoral Dissertation, Novosibirsk (1971).
9. G. S. Yakobson, in: Regulation of Processes of Regeneration and Cell Division (Proceedings of a Symposium) [in Russian], Moscow (1977), p. 68.

10. L. A. Yatsenko, "Clinical features distinguishing the course of virus hepatitis in women and the functional characteristics of the adrenal cortex and gonads," Author's Abstract of Doctoral Dissertation, Kishinev (1969).
11. S. A. Bengmark and R. Olsson, Pathol. Microbiol. (Basel), 27, 167 (1964).
12. K. Brown-Grant et al., J. Endocrinol., 48, 295 (1970).
13. U. G. Chaturvedi, Indian J. Med. Sci., 23, 374 (1969).
14. C. Dupon and M. M. Kim. J. Endocrinol., 59, 653 (1973).
15. I. H. Luft, J. Biophys. Biochem. Cytol., 9, 409 (1961).

PROLIFERATION OF THE EXOCRINE AND ENDOCRINE PORTIONS OF THE PANCREAS AFTER ITS RESECTION

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After resection of the pancreas (about 40% of the weight of the organ) in (CBA × C57BL/6) hybrid mice weighing 20-28 g of the regenerative ability of the organ was found to be weak. After 21 days of the experiment no appreciable recovery of the weight of the organ had taken place. An increase in proliferative activity (the number of mitoses and the diurnal fraction of cells labeled with [^{3}H]thymidine were counted) was transient in character and did not extend to all the organ. The greatest number of labeled nuclei in the epithelium of the acini and islets was found in the region near to the site of injury, where the tissue of the organ was a little edematous. In areas not far from the wound surface but remaining unchanged the number of labeled cells was increased only in the early period after the operation. In regions of the organ remote from the site of injury (the duodenal loop) the number of labeled cells in the islets and acini was the same as in the control. The number of labeled cells in the islets was greater than in the acini.

KEY WORDS: mouse pancreas; islets and acini; proliferation; autoradiography.

Proliferative processes in the pancreas after resection of the organ have been inadequately studied. Most investigations have been conducted on rats and their object has been to study proliferation only of the exocrine part of the organ [1, 4, 7, 10, 12, 14]. Only in isolated investigations, also conducted on rats, has the important role of proliferative processes in the pancreatic islets been demonstrated [8, 13]. A few other investigations in this field made use of [^{3}H]thymidine [5, 6], but unfortunately they did not take into account the diurnal rhythm of cell division. The dependence of proliferative activity of the tissue of the gland remaining after resection on its remoteness from the site of injury has virtually not been studied.

The object of this investigation was to make a more detailed study of the proliferative activity of the epithelium of islets and acini of the pancreas after resection of the organ. The daily fraction of cells taking part in proliferation was determined. The index of labeled and dividing cells was calculated in three zones of the pancreas located at different distances from the site of trauma.

EXPERIMENTAL METHOD

Experiments were carried out on hybrid (CBA × C57BL/6) male mice with a mean weight of 20 and 28 g. About 40% of the tissue of the pancreas was removed in the experimental animals. Intact mice served as the control. The animals were decapitated at 10 a.m. on the 3rd, 4th, 5th, 7th, 16th, and 21st days after the operation, 6-8 animals at each time. All the animals were given intraperitoneal injections of [^{3}H]thymidine five times in the course of the 24-h period (at noon, 5 and 10 p.m., and 5 and 8 a.m.) in a dose of 0.25 μCi/g body weight. The specific activity of the isotope was 1.4 Ci/mmole. The pancreas was fixed in Bouin's fluid. Paraffin sections 4 μ thick were cut. The sections were coated with type M (NIKFI) emulsion and exposed for 45

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