Reparative Regeneration of Rat Fetal Liver after Partial Hepatectomy

A. V. Elchaninov and G. B. Bolshakova

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A reproducible model of fetal liver regeneration was created. Resection of 20% liver was carried out in rat fetuses on day 17 of prenatal development. The organ weight was restored after 2 days at the expense of an increase in hepatocyte mitotic activity; cell hypertrophy was minor. After recovery, the cell composition of the operated liver did not differ from the control, *i.e.* the regeneration was organotypical.

Key Words: *regeneration; liver; prenatal period; proliferation; hepatocyte hypertrophy*

Numerous experimental data on mammalian organ regeneration in animals of different age were accumulated by the present time [1,2]. However, the reparation during the prenatal period remains little studied. Regeneration of the myocardium and skin in rat fetuses is best studied [2,9]. No data on the compensatory growth of fetal parenchymatous growth were presented, because of methodological difficulties of operations on mammalian fetuses and postoperative complications [5]. Since liver regeneration after partial hepatectomy is a classical model of regeneration [1], study of mammalian fetal liver reparation is an important problem of fundamental biology and medical practice (in view of development of prenatal surgery) [6].

We studied liver regeneration in rat fetuses and evaluated the impact of hepatocyte division and hypertrophy for the organ weight restoration. This task implied the creation of a reproducible model of rat fetal liver regeneration after partial hepatectomy.

MATERIALS AND METHODS

Partial hepatectomy was carried out in fetuses of albino rats on pregnancy day 17, the first term when the operation was feasible. The uterine wall and fetal skin in the right subcostal area were dissected and a fragment of the right lobe of the liver was resected. The resection volume was about 20%. Intact newborn rats from the same litter served as the control. The animals were narcotized with ether and sacrificed 3 and 6 hours and 1, 2, 3, 7, 10, 14, 25, and 60 days after the intervention (pregnant females were narcotized while the fetuses were developing in utero and after delivery rat pups were narcotized). The livers were weighed, fixed in Carnoy solution, and processed by the standard histological methods. Paraffin sections (5-7 μ) were stained with hematoxylin and poststained with eosin and by the method of Mallory. Experimental and control groups consisted of 7-10 animals.

Cell composition of fetal liver was evaluated by measuring volume density of its main constituents (hepatocytes and hemopoietic cells). Morphometric analysis was carried out by the "fields" method using a grid of 10 mobile nodes shifted along the entire area in several sections (200 test points per liver) at the light microscope magnification ×1500.

Hepatocyte proliferation was evaluated by estimation of their mitotic index (MI), expressed in promille (‰). Mitoses were counted per 6000 cells for each animal.

The nucleus/cytoplasm ratio in hepatocyte was studied in smear impressions of the liver fixed in me-

Laboratory of Growth and Development, Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** elchandrey@yandex.ru. A. V. Elchaninov

thylene blue and stained with hematoxylin and eosin. The areas of the nuclei and cytoplasms were measured in 100 cells of each animal.

The MI and volume densities were compared and the mean arithmetic and standard deviation were calculated by the formula for fractions. The 95% confidence intervals for fractions were calculated by the φ test. Two selected lobes were compared using z test.

Comparison of the groups by liver weight and areas of hepatocytes and their nuclei were carried out using Student's *t* test in case of normal distribution of the data and Mann–Whitney test in other cases. The data were analyzed using Microsoft Excel 2003 and Sigma Stat 3.5 (Systal Software Inc.) software. The 5% level of significance was selected.

RESULTS

The weight of the liver in intact rat fetus on day 17 of prenatal development is 127.1±5.9 mg. Liver weight recovery after resection is completed during the prenatal period. Two days after partial hepatectomy, the absolute weight and liver/body weight proportion in the fetuses virtually did not differ from the control, and later the time course of liver growth was the same in the two groups (Table 1). Histological analysis of the resection area showed no signs of tissue growth from the wound surface. The formation of the cicatrix in the necrotic focus started from day 3 postoperation.

Hepatocytes of control rat fetuses occupied 38.9±1.3% liver volume on day 17 of prenatal development (Table 2).

Hemopoietic cells located in the sinusoids between the trabeculas occupied 44.4±1.3% of the organ, which corresponded to published data [4].

Three hours after the operation, the volume density of hepatocytes and hemopoietic cells in the resected liver did not differ from the control and hence, no appreciable edema or reduction of hemopoietic cell count developed. After 2 days, when liver weight recovered, the hepatocyte/hemopoietic cell proportion

TABLE 1. Liver Weight in Experimental and Control Rats during Different Periods Postoperation (*M±m*)

Day after operation	Liver weight, mg		Liver to body weight, %	
	control	experiment	control	experiment
1	182.6±6.6	153.7±8.3*	9.1±0.3	7.8±0.2*
2	239.8±23.8	233.9±18.8	8.4±0.3	7.4±0.4
3	344.4±21.8	332.1±14.0	7.8±0.3	7.1±0.3
7	417.1±12.8	370.1±23.0	4.0±0.2	4.4±0.3
10	430.0±33.3	385.0±23.0	–	–
14	507.1±30.3	484.3±29.2	–	–
25	1648.6±142.1	1677.1±105.3	–	–
60	11,500.0±651.0	10,500.0±522.0	5.8±0.2	5.9±0.5

Note. Here and in Table 2: *$p<0.05$ compared to the corresponding control.

TABLE 2. Volume Density of Structural Components of the Liver after Partial Hepatectomy during Prenatal Period (%, *M±m)*

Time after operation	Hepatocytes		Hemopoietic cells	
	control	experiment	control	experiment
3 h	38.9±1.3	40.5±1.3	44.4±1.3	44.1±1.3
1 day	36.4±1.3	48.9±1.3*	47.2±1.3	41.2±1.3*
2 days	59.6±1.3	59.1±1.3	33.0±1.3	32.9±1.3
3 days	67.2±1.3	71.1±1.2*	23.7±1.1	19.1±1.0*
7 days	79.8±1.1	80.7±1.1	9.6±0.8	9.0±0.8

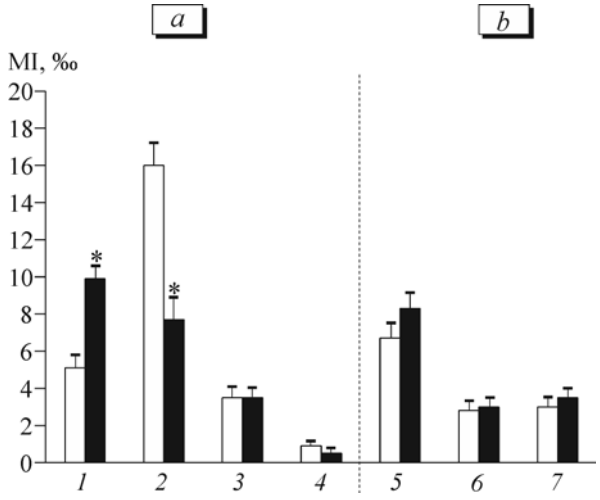

Fig. 1. Mitotic activity of hepatocytes after partial hepatectomy carried out in rat fetuses on day 17 of prenatal development. Light bars: control; dark bars: experiment. Abscissa (numerator: age (in days), denominator: period postoperation): *a*) prenatal period: *1*) 17/6 h; *2*) 18/1 day; *3*) 19/2 days; *4*) 21/3 days; *b*) postnatal period: *5*) 3/7 days; *6*) 6/10 days; *7*) 10/14 days. **p*<0.05 compared to the corresponding control.

in experiment and control was virtually the same, in other words, the regeneration was organotypical.

The hemopoietic function of the liver gradually ceases in operated on and control animals with aging (Table 2), which is characteristic of normal development of the organ during the late prenatal and postnatal periods [4].

Intact fetal hepatocytes intensely divide on day 17 of prenatal development, their MI being 9.8±0.47‰ (Fig. 1). It decreases 6 h after surgery in the experimental group, presumably because of operation stress (Fig. 1). After 24 h, MI increases significantly in comparison with age-matched control. All mitotic phases were observed in regenerating fetal liver (Fig. 2), which fact indicated the increase in the absolute count of hepatocytes. Increase in the volume density of hepatocytes in resected liver during this period confirmed this hypothesis (Fig. 1, Table 2).

Starting from gestation day 19 (day 2 postoperation), mitotic activity of hepatocytes in experimental and control animals decreased. During week 1 after birth, MI increased again and then gradually decreased

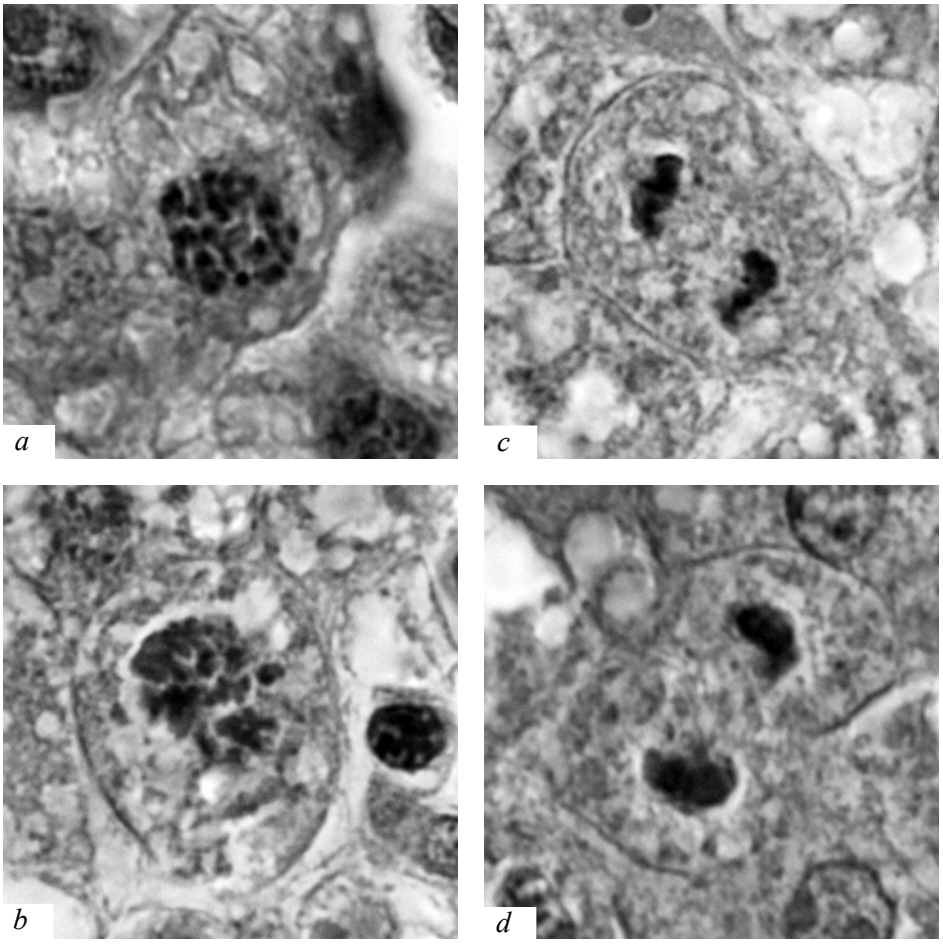

Fig. 2. Hepatocyte mitosis phases in regenerating rat fetal liver. *a*) prophase; *b*) metaphase; *c*) anaphase; *d*) telophase. Hematoxylin and eosin staining, ×1500.

with age. This time course of hepatocyte MI is characteristic of normal development of the liver [3,7].

Study of the nucleus/cytoplasm ratio of mononuclear hepatocytes showed that the volume of hepatocytes in experiment and control varied from 281.1±2.0 to 440.9±6.9 μ^2 during all periods of the study. Hence, cell hypertrophy in fetuses is inessential for liver regeneration. Binuclear hepatocytes as an indirect sign of polyploidization were detected in smear impressions of the liver of experimental and control animals only 25 days after resection (day 21 after birth) in about the same quantities.

Hence, during the prenatal period regeneration hypertrophy of the liver is observed [1], but in contrast to the postnatal period, this regeneration is due to cell proliferation, but not hypertrophy.

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