suggested that this will lead to improvements in the outpatient supervision and treatment of glaucoma patients.

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CHANGES IN THE LIVER OF RATS EXPOSED IN UTERO TO CHRONIC HYPOXIA

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Disturbance of cell division and an increase in the level of pathological mitoses (PM) may be the structural trace of disadaptation. Disturbance of cell division and elevation of the PM level in the corneal epithelium are observed under the influence of various experimental conditions: hypoxia, hypothermia, hyperthermia [3, 6-8]. The corneal epithelium is not actively involved in the process of adaptation. To evaluate the role of a raised PM level in the formation of the structural trace and morphological changes, it was decided to analyze disturbances of cell division in fetal and neonatal rat hepatocytes and also in month-old rats exposed to hypoxia during intrauterine development.

EXPERIMENTAL METHOD

On the 5th or 6th days of pregnancy famale mice were placed in an SKB-48 pressure chamber and raised to an altitude of 9000 m, corresponding to a pressure of 224 mm Hg. Every day the animals were exposed for 4 h from 9 a.m. to 1 p.m. for 7 days as described in [3]. Control animals were simply placed in the pressure chamber. The offspring of rats exposed during pregnancy to hypoxia and the control animals were divided into three groups. Group 1 comprised 104 fetuses at 16-17 days of intrauterine development (the 4th day after final exposure to hypoxial), Group 2 comprised 166 newborn rats, 2 days after birth (10th day after the final session of hypoxial), and Group 3 comprised 76 rats aged 80 days (40 days after the final session of hypoxia). The total body weight of the fetuses and newborn rats was determined, and after the animals had been humanely killed, the liver was removed and weighed. Pieces of tissue were fixed in Carnoy's fluid and histological sections were cut from them and subjected to systemic morphometric analysis [1]. The state of DNA synthesis was judged by autoradiography with ^{3}H-thymidine. For this purpose, pieces of liver measuring 0.2 mm^3 were incubated, immediately after sacrifice of the animals, in 5 ml of medium 199 for 60 min at 37°C with ^{3}H-thymidine (5 μCi in 1 ml, specific radioactivity 70 Ci/mmole). Autoradiographs were prepared by the method in [5]. The index of labeled nuclei of the hepatocytes (ILN) was determined after examination of at least 3000 nuclei. The labeling intensity (LI) was

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TABLE 1. Effect of Intrauterine Hypoxia and Changes in Body Weight and Morphometric Parameters of Liver in Offspring of Albino Rats

Group of animals	Time after final exposure to hypoxia, days	Body weight, g	Weight of liver, g	Relative area of		
				hepatocytes	blood vessels	foci or hematopoiesis
1-						
Control	4	4,2±0,3	0,13±0,005	74,0±2,3	19,8±1,9	6,2±1,0
Experimental		3,5±0,2*	0,16±0,01*	65,6±1,8*	7,3±0,9*	27,1±1,3*
2-						
Control	10	6,6±0,1	0,17±0,006	86,9±1,0	5,9±0,5	7,2±0,8
Experimental		5,6±0,08*	0,2±0,005*	78,8±1,1*	6,3±0,8	14,9±0,6*
3-						
Control	40	85,0±6,1	4,2±0,2	87,6±1,8	4,6±0,7	7,8±1,1
Experimental		70,0±3,5*	3,6±0,2*	92,1±3,5	4,4±0,3	3,5±0,4*

Note. Asterisk indicates statistically significant difference compared with control.

TABLE 2. Effect of Intrauterine Hypoxia on Hepatocyte Proliferation in Offspring of Albino Rats

Group of animals	Time after final exposure to hypoxia, days	MI, %	PM, %	ILN, %	LI
1-					
Control	4	16,2±1,3	2,9±0,4	10,0±1,2	21,7±2,5
Experimental		12,9±1,0*	11,0±8,8*	6,4±0,6*	20,6±2,5
2-					
Control	10	12,7±0,7	6,7±0,6	10,2±0,5	33,8±1,5
Experimental		9,1±0,5*	17,6±0,5*	6,7±0,5*	26,5±1,3*
3-					
Control	40	2,3±0,2	3,0±0,8	0,7±0,04	18,9±1,7
Experimental		5,4±0,4*	7,2±1,0*	1,1±0,09*	25,2±2,2*

Note. *$p < 0.05$: statistically significant difference compared with control.

expressed as the average number of grains of silver above nucleus, and ILN was expressed as a percentage. The mitotic index (MI) was determined on the basis of differential counting of mitotic figures, phase by phase, after examination of at least 3000 hepatocytes in Groups 1 and 2 and 8000 in Group 3. During identification of pathological mitoses (PM) [2] the number of PM was expressed as a percentage of the total number of mitoses.

EXPERIMENTAL RESULTS

Intrauterine hypoxia led to the development of hypotrophy of the fetus and newborn rats in all three grops of experiments (Table 1). The body weight of the animals remained lower than the control. The weight of the liver in the first and second experimental groups was significantly greater than in the control, but in Group 3, on the other hand, the weight of the liver of young rats exposed in utero to hypoxia remained significantly lower than the corresponding values for the intact group. The results of morphometric analysis help to explain the change in weight of the liver at different times after exposure to hypoxia. In the experiments of Group 1 there was a significant decrease in the area occupied by hepatocytes; meanwhile there was an increase in the area occupied by hematopoietic tissue, by 4.5 times. The area occupied by blood vessels decreased by 2.5 times.

In the experiments of Group 2, just as before there was an increase in the area occupied by hepatocytes, but the area of hematopoietic tissue was twice that observed in the control. However, the area of the blood vessels in this group of experiments did not differ from values for the intact group.

In the experiments of Group 3 there was a twofold increase in area of hematopoietic foci. Other parameters tested did not undergo significant changes. The hematopoietic function of the liver was at a level characteristic of the early period of its development, and at the moment of birth it was in the process of transition mainly to the medullary pathway of hematopoiesis. Enlargement of foci of hematopoiesis and a reduction in the area occupied by hepatocytes, on the account, was thus evidence of delayed development of the liver. It seems therefore correct to conclude that exposure to hypoxia causes stimulation of cell division in hematopoietic tissue [15].

The results of investigation of cell division provide some explanation of the reduction in the area occupied by hepatocytes (Table 2).

In the first and second experimental groups a significant reduction in MI was observed compared with the control values. In Group 3 (40 days after the end of the sessions of hypoxia) there was a more than twofold increase in MI compared with the control. Changes in MI observed in the experimental groups were determined by interphase processes. The results of autoradiographic analysis demonstrate that in the first and second experimental groups there was a decrease in the number of cells switching from the L_1- into the S-period. This was shown by a significant reduction of ILN by 1.5 times. No significant changes in LI, characterizing the velocity of DNA synthesis, were observed in Group 1. In the experiments of Group 2, however, 10 days after the end of exposure to hypoxia, there was a significant decrease in LI, i.e., besides a decrease in the number of cells starting the S-period, the velocity of DNA synthesis was reduced. In the experiments of Group 3 (40 days after the end of the sessions of hypoxia), besides a 1.5-fold increase in the number of DNA-synthesizing nuclei, there was an increase in the velocity of DNA synthesis. It must be pointed out, however, the intrauterine hypoxia led to elevation of the PM level in all three groups by 2.4-3.4 times compared with the control values. It is striking that, besides a general increase in the number of aberrant mitoses on account of colchicine-like forms, culminating as a rule in death of the cells, the content of "bridges" in the experimental groups was significantly increased. According to some views which have been expressed [2, 14], this form of pathology of mitosis can be reproduced in subsequent cell generations. The fact that the content of PM remained high 40 days after the final session of exposure to stress indicates that aberrant mitoses arise, not as a result of the direct harmful action of the extremal factor. Hypoxia possibly may induce lasting changes in metabolism, leading to a rise of the PM level [3, 9, 11]. Another probable mechanism is the transmission of pathology of mitosis to daughter cells. We regard these suggestions as being not alternative, but mutually complementary. It must be pointed out in particular that in the experiments of Group 3 a combination of a raised PM level and activation of DNA synthesis was observed. This combination can be evaluated as a manifestation of tissue dystrophy [7].

Disturbance of cell division of the hepatocytes under the influence of chronic intrauterine hypoxia in young rats provides to some extent a model of changes which we discovered previously in hepatocytes of fetuses whose mothers were subjected to a degree of risk amounting to more than 9 points during pregnancy [10]. Depression of DNA synthesis in the fetal hepatocytes, a decrease in MI, and an increase in the number of aberrant mitoses also were observed in these investigations. The view that the majority of unfavorable factors influencing the mother realized their action on the fetus through chronic intrauterine hypoxia confirms the appropriateness of the present experimental model of the clinical situation [12, 13]. The experimental data indicate that disturbance of cell division of the fetal hepatocytes under unfavorable conditions can last a long time and can participate in hepatobiliary pathology. It will be recalled that hepatobiliary pathology is observed on the largest scale in pediatric practice at the ages of 5-7 and 10-12 years [4]. The authors cited suggest that diseases commence much earlier. Clinical investigations and the results of the present experimental study indicate that a definite role in the formation of postnatal liver diseases may be played by chronic intrauterine hypoxia.

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HISTOCHEMICAL ANALYSIS OF ENZYMES OF TRANSPORT AND BIOENERGETICS OF RAT SMALL INTESTINAL ENTEROCYTES AFTER VAGOTOMY AND DIBUNOL ADMINISTRATION

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Activation of lipid peroxidation (LPO) in biological membranes plays a definite role in the pathogenesis of the neurodystrophic process developing in organs of the digestive system after vagotomy. The concentration of malonic dialdehyde, an end product of LPO, is increased in the liver of vagotomized rats [4]. It has also been shown that vagotomy leads to the development of hypoxia in the denervated organs (stomach, jejunum, liver) [13], and this is a powerful factor inducing LPO [10]. Meanwhile it has been demonstrated that vagotomy is accompanied by multiple disturbances, at varied depths, of the structure of cell membranes of the stomach, liver, pancreas, and intestine [3]. Disturbance of the compact and regular structure of the lipoprotein complexes of biomembranes leads as a rule to the activation of LPO [6]. The view that unphysiological intensification of LPO occurs in the digestive organs after vagotomy is supported indirectly by the fact that vitamin E has a positive effect on certain functional and morphological characteristics of hepatocyte mitochondria [5].

In this investigation an attempt was made, by pharmacological methods, to correct the postdenervation changes taking place in the epithelium of the small intestine, by the use of the phenolic antioxidant dibunul (2,6-di-tert-butyl-4-methylphenol). Activity of key enzymes of transport and bioenergetics, namely alkaline phosphatase (AlP) and succinate dehydrogenase (SDH), was chosen as the morphological criterion for evaluating the metabolic and functional state of the intestinal epithelial cells.

EXPERIMENTAL METHOD

Male albino rats (n = 53) weighing 180-210 g were used. Under anesthesia (150 mg Na γ-hydroxybutyrate + 0.6 mg diazepam (100 g body weight, respectively), bilateral subdiaphragmatic trunk vagotomy was performed on 30 rats and 15 rats served as the control. Starting with the 2nd day after the operation, half of the rats received dibunol intraperitoneally in a dose of 20 mg/kg, in a 3% solution of Tween-80. The rats were killed three at a time (intact, vagotomized, and vagotomized and receiving dibunol), 7, 14, and 30 days after vagotomy, and 6, 13, and 29 days, respectively, after the beginning of the course of injections. In a special series of experiments in order to estimate the effect of dibunol on the test parameters of the intact rats, the compound was injected in the same dose for 14 days. All the animals were deprived of food for 16-18 h before sacrifice. Fragments of the proximal part of the jejunum were frozen in liquid nitrogen and mounted in blocks. In the frozen sections AlP was detected by the method in [1] and SHD by the method in [8]. Photometry was carried out with the MTsFU-2MP scanning microscope-photometer at a wavelength of 546 nm. At least 15 scanning fields per section were tested photometrically (9 measuring points in each field); the size of the fields corresponded to the brush border (in the case of AlP) and the apical pole (SDH) of the enterocytes. The choice of cells for photometry was made randomly within the epithelium of the intestinal villi. The results of the measurements were subjected to statistical analysis with the aid of Strelkov's tables [11].

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