sisted for 30 min. Under these circumstances no changes in cardiac ejection or linear and volume velocity of the blood flow in the aorta were observed. The results of these experiments show that when the blood supply to the myocardium is assessed, some idea can be obtained of the values of the linear blood flow rate, especially at its average values, when the blood supply to the myocardium is assessed.

The investigations described above were carried out with the aid of transducers in which the piezoelectric element was set at an angle of 45° to the myocardial surface. The piezoelectric element probably works at the same angle to the surface of the coronary artery also, for usually the main branches of these vessels are arranged almost parallel to the myocardial surface. This makes it possible to measure the linear blood flow rate. With ultrasound of a frequency of 26.8 MHz the sensitivity of the doppler signal to linear velocity was 240 Hz/cm/sec. The resolving power of the frequency detector of the ultrasonic instrument under these circumstances was about 0.3 cm/sec. During working on the middle coronary artery the signal to noise ratio was usually 30-40 dB or higher, thus guaranteeing sufficient reliability of recording the shape of the coronary blood flow curve.

Thus the technique of high-frequency ultrasound, which possesses high sensitivity, guarantees reliable measurement of the coronary blood flow in rats. Its use can be particularly effective in combined investigations of the dynamics of cardiac activity, both in the healthy animal and if lesions of the cardiovascular system of various kinds are present.

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FUNCTIONAL STATE OF THE HEMATOPOIETIC SYSTEM IN DIFFERENT STAGES OF CCl_4-INDUCED LIVER FIBROSIS IN MICE

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It was shown previously that functional activity of the Kupffer cells is depressed in CCl_4-induced fibrosis in the liver [1-3]. Besides macrophages in other locations, the Kupffer cells play a most active role in the regulation of hematopoiesis, for they secrete GM-CSF [3, 4, 15], erythropoietin, IL-1, TNFα, and PGE I and PGE II. The transition from fibrous transformations into the irreversible form is associated with a decrease in the degree of mononucle-

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TABLE 1. Number of Cells in Peripheral Blood and Bone Marrow of Mice with CCl_4-Induced Fibrosis of the Liver (M ± m)

Type of cell	Group of animals		
	1	3	5
Leukocytes (total number) × 20/liter	6,8±0,43	9,0±0,81 $p<0,01$	8,2±0,54 $p<0,05$
Made up of			
Neutrophils	1,8±0,13	5,9±0,49 $p<0,001$	3,4±0,26 $p<0,001$
Monocytes	0,4±0,04	0,5±0,11	0,4±0,06
Lymphocytes	4,6±0,27	2,7±0,27 $p<0,001$	3,9±0,29 $p<0,01$
Erythrocytes, × 10/liter	7,6±0,51	7,2±0,70	6,9±0,54
Reticulocytes, × 10/liter	0,9±0,06	1,7±0,09 $p<0,001$	0,2±0,04 $p<0,001$
Myelokaryocytes, total number, × 10/femur	22,0±1,32	25,6±2,54	26,1±1,19
Immature neutrphilic granulocytes	4,2±0,30	7,6±0,47 $p<0,001$	6,4±0,52 $p<0,01$
Mature neutrophilic granulocytes	9,5±0,45	10,0±0,35	8,8±0,41
Monocytes/macrophages	0,6±0,03	0,9±0,05 $p<0,01$	1,8±0,07 $p<0,001$
Erythroid	4,6±0,15	3,9±0,30	2,6±0,17 $p<0,001$
Lymphoid	3,5±0,55 (5)	3,1±0,51 (5)	4,9±0,25 (5)

Legend. Values of p given by comparison with group 1. Number of animals shown between parentheses.

ar infiltration in the liver. By potentiating infiltration, in some cases fibrosis of the liver can be retarded [1]. It can be postulated that the development of lasting fibrosis of the liver is linked both with depression of the functions of the liver macrophages in situ and with disturbances of functions of the centers of hematopoiesis, where precursors of the effector cells of inflammation are formed.

The aim of this investigation was to study the state of hematopoiesis, including the colony-forming ability of bone marrow cells before and after stimulation in mice at different stages of CCl_4-induced fibrosis of the liver.

EXPERIMENTAL METHOD

Experiments were carried out on 30 male BALB/C mice Weighing 20-25 g. The animals were divided into five groups: 1) intact mice; 2) mice receiving vegetable oil in a sessional dose of 0.15 ml intraperitoneally twice a week for 2 weeks; 3) mice receiving a 20% oily solution of CCl_4 by the same schedule; 4 and 5) animals receiving vegetable oil and 20% oily solution of CCl_4 respectively for 16 weeks. To stimulate hematopoiesis, zymosan was injected intravenously in a dose of 100 mg/kg. The mice were killed 1 day after stimulation. The total number of cells in the blood and bone marrow was counted by means of a "Labor Skel" counter (Hungary). A differential count of the blood and bone marrow cells was carried out on films stained by the Giemsa method. To determine CFU, bone marrow cells were cultured in medium containing 0.80 of methylcellulose [8]. To stimulate growth of the erythroid colonies (CFU-E) the serum of BALB/c mice was used 24 h after removal of 30% of the circulating blood, whereas granulocytic-monocytic colonies (CFU-GM) were 48 h after intravenous injection of zymosan in a dose of 100 mg/kg. CFU-E were counted after 3 days, and CFU-GM after 7 days of culture of the cells in a humid chamber at 37°C and in 5% CO_2. Colony-stimulating activity (CSA) of the mouse serum was estimated on the basis of growth of CFU-GM after the addition of 0.15 ml of serum to 10^5 bone marrow cells from normal BALB/c mice. Interleukin-1-producing ability of the blood monocytes was determined before and after their stimulation by lipopolysaccharide (LPS) of *E. coli* (serotype 0111:B4) in vitro and was expressed in indices of stimulation of thymocyte proliferation [9]. The numerical results were subjected to statistical analysis by Student's t test.

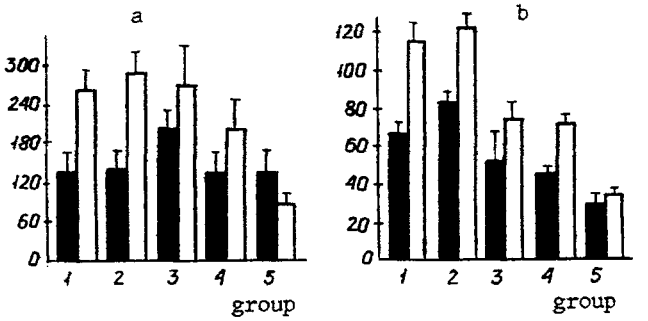

Fig. 1

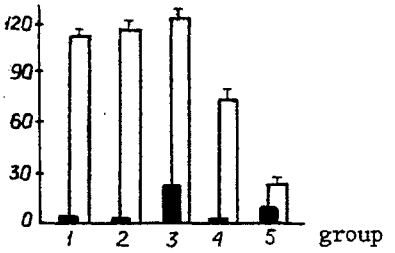

Fig. 2

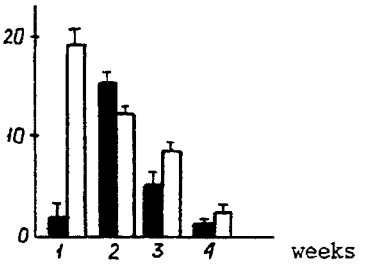

Fig. 3

Fig. 1. Effect of stimulation by zymosan on number of CFU-GM (a) and CFU-E (b) of mouse bone marrow in different experimental groups. Black columns – without stimulation, white columns – 24 h after stimulation by zymosan.

Fig. 2. Colony-stimulating activity of mouse blood serum in different phases of fibrosis of the liver. Black columns – spontaneous, white columns – 24 h after stimulation by zymosan.

Fig. 3. IL-1-producing activity of blood monocytes in different phases of fibrosis of the liver. Black columns – unstimulated, white columns – stimulated by LPS.

EXPERIMENTAL RESULTS

The cell composition of the peripheral blood and bone marrow is given in Table 1. It shows that in the acute phase of development of fibrosis (2 weeks) an absolute neutrophilia was present, with some reduction of the blood lymphocyte count. In the bone marrow, compared with the previous cell composition there was an increase in the number of immature neutrophilic granulocytes and monocytes/macrophages. When fibrosis became permanent (16 weeks) all these changes became less clear, except a further increase in the number of medullary monocytes/macrophages.

In normal BALB/c mice on average 129 ± 8.91 precursors of myelopoiesis and 59 ± 7.2 of erythropoiesis were identified in the bone marrow. The number of myeloid colonies formed by bone marrow cells 24 h after intravenous injection of zymosan was increased by 2.2 times (Fig. 1a), and the number of erythroid colonies by 2.1 times (Fig. 1b). Before stimulation with zymosan, CSA of the serum was virtually not determinable, but after stimulation the serum sharply intensified growth of CFU-GM of the bone marrow of normal mice (Fig. 2).

The number of CFU-GM 2 weeks after injection of oil (group 2) was virtually unchanged, whereas the number of CFU-E was 1.3 times greater than in intact mice. Meanwhile, after injection of zymosan the number of CFU-GM was increased by 2.1 times (Fig. 1a) and the number of CFU-E by 1.5 times (Fig. 1b). Serum CSA was absent before stimulation, but after stimulation it increased as in group 1 (Fig. 2). The number of CFU-GM 2 weeks after injection of CCl_4 was increased by 1.4 times compared with their number in the mice of groups 1 and 2. Meanwhile the number of CFU-E was about the same as in intact animals. In response to zymosan the number of CFU-GM and CFU-E was increased by 1.3 times (Fig. 1a, b). Unlike the animals of groups 1 and 2, in mice of group

3 serum CSA could be detected even before stimulation by zymosan. After injection of zymosan, CSA increased by 7.1 times (Fig. 2).

The bone marrow cells 16 weeks after continuous injection of oil (group 4) gave growth of rather fewer CFU-GM and CFU-E than in the normal state. After injection of zymosan their number rose by 1.3 and 1.4 times respectively (Fig. 1a, b). CSA of the serum before stimulation by zymosan was virtually undetectable, but after injection it was found regularly (Fig. 2).

The number of CFU-GM 16 weeks after continuous injection of CCl_4 (group 5) differed only a little from normal, whereas CFU-E was 1.9 times below normal. After injection of zymosan the number of CFU-GM not only failed to rise but, paradoxically, it fell by 1.4 times, whereas the number of CFU-E was virtually unchanged after stimulation (Fig. 1a, b). Before stimulation very low serum CSA was detected, but after injection of zymosan activity increased by only 2.3 times (Fig. 2).

Disinhibition of granulomonocytopoiesis was thus observed in the early phase of fibrosis of the liver. This was manifested as an increase in the number of blood neutrophils and also of immature neutrophilic granulocytes and monocytes/macrophages. At the same time, there was an increase in the number of colonies of granulomonocytic type formed by bone marrow cells. Functions of the erythroid branch during this period did not change significantly. In the presence of formed fibrosis in the liver the number of monocytes/macrophages in the bone marrow increased, whereas the number of CFU-GM decreased by 1.5 times compared with the early phase of fibrosis.

In the early phase of fibrosis the reaction of the hematopoietic system to zymosan was maintained: the number of CFU-GM in the stimulated mice rose by 1.3 times, the number of CFU-E by 1.9 times. Meanwhile, in the case of advanced fibrosis, the response to zymosan changed significantly: the number of CFU-GM not only did not increase in response to the stimulus, but actually decreased, whereas the number of erythroid colonies remained virtually unchanged.

In fibrosis not only the function of the resident macrophages, but also of their precursors – the blood monocytes – is inhibited [6, 7]. According to our data, in the early phase of fibrosis (2 weeks of CCl_4 injections) basal IL-1 secretion by monocytes increased sixfold, whereas in developed fibrosis, on the other hand, it was reduced by 1.5 times. Moreover, in response to LPS the monocytes intensified IL-1 production by only 1.3 times, compared with 6.8 times normally (Fig. 3). The writers showed previously that other parts of the mononuclear phagocyte system (MPS), namely pulmonary and splenic [10], respond weakly to a stimulus in fibrosis. These observations suggest that the weak, or at times paradoxical, response of hematopoiesis to zymosan in fibrosis may be due to general depression of the MPS. Confirmation of this view may be given by the low serum CSA level in response to injection of zymosan into mice with advanced fibrosis [9].

In fibrosis of the liver, as a rule an endotoxin accumulates in the blood, though normally it is neutralized by Kupffer cells [3]. Meanwhile, permanent endotoxemia may lead to depression of hematopoiesis: anemia, leukopenia, and reduction of the number of myeloid precursors due to disturbance of the functions of the specific hematopoietic microenvironment [5]. The possibility cannot be ruled out that this factor also plays an important role in those modifications of hematopoiesis which we observed in our animals with formed fibrosis of the liver.

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EXPERIMENTAL MODEL OF APNEUSIS AND PERIODIC BREATHING

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KEY WORDS: apneusis; periodic breathing; hydroxybutyrate

Pathological breathing develops in terminal states in the form of Cheyne-Stokes', Biot's, and Kussmaul's breathing, apneusis, or gasping. Many clinical and experimental cases of pathological types of breathing have been described [1, 2, 7]: deep hypoxia of varied origin, intoxication, head injuries, shock, asphyxia neonatorum, deep anesthesia, extensive blood loss, and division and cooling of the brain stem [3, 8, 9]. A common component of the different causes of pathological types of breathing is a disturbance of the central component of regulation of breathing, namely rhythmogenesis [5, 6]. The study of the mechanisms of pathological types of breathing have aroused the interest of experimenters (chiefly as a method of studying the causes of rhythmogenesis and the principles governing regulation of breathing) and clinicians (mainly concerned with a search for their effective treatment). However, the experimental study of this problem is difficult because of the lack of simple, reliable, and readily reproducible experimental models of pathological types of breathing. The known methods are as a rule laborious and very traumatic: surgical division and cooling of the brain stem and vagus nerves, arterial bloodletting of up to 50% of the total circulating blood volume [11-14]. There have been only sporadic studies in which several pathological types of breathing have been reproduced experimentally by administration of various drugs (pentobarbital, cyanides) [4, 10].

The aim of this investigation was to develop an experimental model of apneusis and periodic breathing by injecting hydroxybutyrate (HB) into cats.

EXPERIMENTAL METHOD

Experiments were carried out on 19 noninbred cats, male and female, weighing from 2 to 4 kg, anesthetized with pentobarbital (40 mg/kg, intraperitoneally). The rectal temperature was measured and maintained by means of an electric heater with an accuracy of 0.5% within the range from 37.5 to 38.5°C. Tracheotomy was performed at the level of the upper third of the trachea. A cannula connected to transducers for recording the parameters of respiration (respiration rate – RR, pneumotachogram) was introduced into the trachea. To record the intraesophageal pressure (IEP) a sterile catheter was introduced into the esophagus, with an elastic balloon filled with liquid, and joined to a venous pressure transducer, fitted on its tip. The arterial blood pressure (BP) was recorded by means of

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