testify that in rays of the same age kept under the same conditions the offspring differ significantly already in embryogeny in brain weight and in morphometric parameters of the state of the cortex, and that these differences are not leveled after birth. It may be assumed that the differences described relate to: 1) genetic differences of the animals studied; 2) individual differences of biochemical (including hormonal) parameters of the maternal blood; 3) local in utero differences in the conditions of development of embryos, including those from one litter.

REFERENCES

1. A. A. Volokhov, *Vestn. Akad. Med. Nauk SSSR*, №12, 49-53 (1978).
2. N. I. Dmitrieva and V. G. Kassil', *Arkh. Anat.*, **83**, № 9, 84-91 (1982).
3. N. I. Dmitrieva and Yu. S. Dmitriev, *Zh. Evol. Biokhim. Fiziol.*, **28**, № 4, 524 (1992).
4. V. N. Kornienko, V. I. Ozerova, and A. S. Petrukin, *Pediatriya*, № 3, 71-76 (1986).
5. B. Ya. Ryzhavskii and I. R. Eremenko, *Fiziol. Zh.*, **78**, № 5, 109-112 (1992).
6. V. M. Svetukhina, *Arkh. Anat.*, **42**, № 2, 524-530 (1962).
7. T. A. Tomberg, T. A. Tal'vik, and M. Kh.Yurisson, *et al.*, *Pediatriya*, № 9, 50-54 (1989).
8. M. V. Ugryumov, *Neuroendocrine Regulation in Ontogenesis* [in Russian], Moscow (1989).
9. R. V. Uchakina, O. A. Lebed'ko, and L. N. Nosova, *Current Topics in Pathology of Pregnancy and Infancy in the Far East* [in Russian], Novosibirsk (1989), pp.151-156.
10. S. Ciaroni, T. Cecchini, and P. Del. Yrando, *Acta Embryol. Morphol. Exp.*, **11**, №3, 171-180 (1990).
11. I. Danko and M. Zibrin, *Plzensky Lek. Sborn.*, Suppl. 63, 177-178 (1991).
12. J. Nagel and Schierdhorn, *J. Hirnforsch*, **16**, № 4,371-378 (1975).
13. A. J. Patel, R. Balazc, and A. L. Johnson, *J. Neurochem.*, **20**, № 4, 1151-1165 (1973).

Ultrastructural Changes in Liver Cells during Severe Iron-Deficiency Anemia

T. A. Ageeva, V. A. Shkurupii, T. I. Pospelova, and M. I. Loseva

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Iron-deficiency anemias are highly prevalent [2,4]. Progressive tissue hypoxia in this condition initiates membrane lipid peroxidation and lowers the level of antioxidant defense [1,11], leading to labilization of membranes, notably of lysosomal membranes [12]. This may be regarded as a risk factor associated with the use of membranotropic and lysosomotropic agents, including the iron-containing drugs [3] used in the treatment of iron-deficiency anemias. We thus considered it expedient to study the ultrastructural changes, particularly in lysosomes, occurring during iron deficiency in various cells of the liver, an organ which is highly sensitive to hypoxia and which participates in iron metabolism and in the clearance of administered lysosomotropic drugs.

Department of Pathoanatomy and Department of Hospital Therapy, Novosibirsk Medical Institute. (Presented by Yu. M. Borodin, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Blood was taken from the marginal vein of the ear every other day, 20 ml at a time, for 40-43 days in chinchila rabbits weighing 3.5 kg. The animals

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TABLE 1. Results of Hepatocyte Ultrastructure Morphometry, *Mean ± SEM*

Parameters	Control	Iron-deficiency anemia
Mitochondria:		
external membrane, S_v	0.9±0.0	0.8±0.0*
internal membrane, S_v	2.7±0.2	1.9±0.2*
N_v	0.3±0.0	0.3±0.0
Peroxisomes, V_v	1.3±0.2	0.9±0.1
GER, V_v	7.3±0.9	3.6±0.4*
S_v	1.6±0.2	1.3±0.1
Ribosomes:		
adhered, N_v	301±36	206±25*
free, N_v	308±36	230±34
Autophagosomes, autophagolysosomes, V_v	0.2±0.07	0.4±0.1
Lysosomal apparatus, V_v	0.5±0.11	1.0±0.2*

Note. Asterisk: reliable difference from control; S_v: membrane surface density, μ^2/μ^3; V_v: volumetric density (% of cytoplasm volume); N_v: numerical density (number per μ^3 of cytoplasm volume).

developed stable severe iron-deficiency anemia: hemoglobin 60-70 g/liter, serum iron 10.6±0.6 μmol/liter, total serum iron-binding capacity 126.2±2.6 μmol/liter. For labeling of Kupffer's cells a suspension of latex particles 1.1 μ in diameter was intravenously injected into animals in a dose of 0.1 ml/kg 1.5 h before sacrifice.

Liver samples for electron microscopy were fixed in 1% OsO_4 solution on a phosphate buffer, dehydrated in ascending grades of alcohol, and embedded in epon. Ultrathin slices were contrast-stained with uranil acetate and zink citrate. The hepatocyte cytoplasm (100 negatives per animal group), sinusoidal endotheliocytes (50 negatives per group), and Kupffer's cells (30 negatives per group) were photographed under a JEM/100S electron microscope. Endothelial and Kupffer's cells were differentiated and the morphometry thereof was performed as described previously [7]. The number of phagocytizing Kupffer's cells and of latex particles phagocytized by a single Kupffer's cell were counted on semithin epon slices stained with toluidin blue, as described previously [8]. Cell morphometry was carried out outside necrotic zones. Intact animals were controls. Each group consisted of 5 animals, and 5 blocks of organ samples per animal were prepared. Differences between compared mean values were considered reliable at $p<0.05$ (the Student test).

RESULTS

The area of the mitochondrial external membrane surface was reduced by 16%, and that of the internal membrane by 30% in hepatocytes of animals with iron-deficiency anemia (Table 1). The number of cristae in mitochondria was decreased, the matrix was clarified in some of them, while others looked more electron-dense. Cristae disappeared from these and electron-dense inclusions appeared. These changes are indicative of a deenergized state of mitochondria. The energy deficit in the cells under conditions of severe iron-deficiency anemia was evidently not compensated by peroxisomes because their number and volumetric share were unchanged (Table 1). The volume of granular endoplasmic reticulum (GER) was reduced twofold, while the surface area of its membranes was the same as in the control (Table 1). The number of free and, all the more so, of adhered ribosomes was reduced by 25 and 32%, respectively (Table 1). Evidently, this resulted from disengagement of ribosomes from the GER membranes, their reduced synthesis in the nucleus, and disintegration within autophagosomes. Autophagosomes and autophagolysosomes most frequently contained

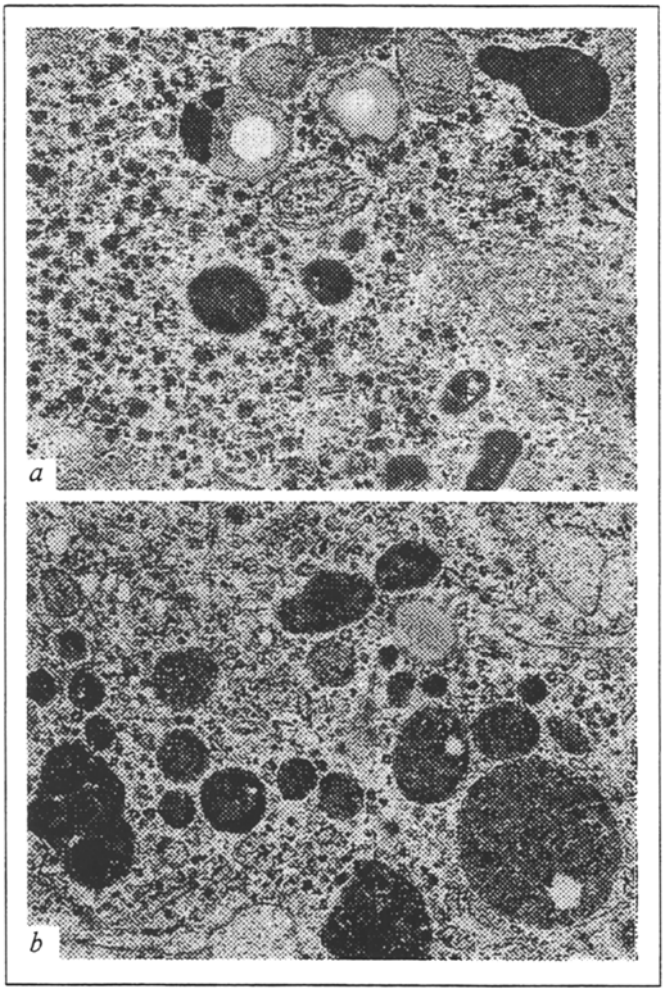

Fig. 1. Hypertrophy and hyperplasia of liver cell lysosomal apparatus in severe iron-deficiency anemia. *a*) hepatocyte, ×12.250; *b*) Kupffer's cell, arrow shows latex particle, ×14.700.

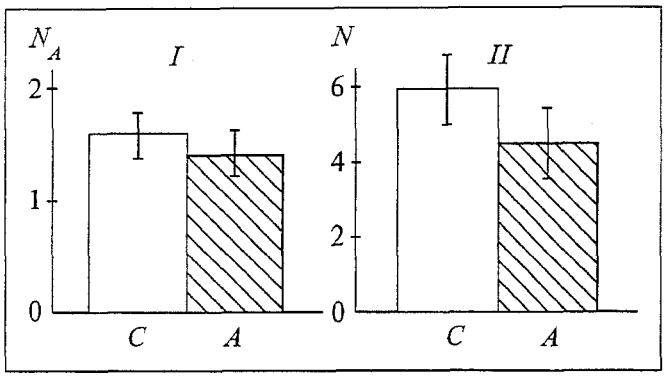

Fig. 2. Changes in numbers of Kupffer's cells and their phagocytic activity in rabbit liver. *I*) Kupffer's cell count per 10.000 μ² (N_A); *II*) number of latex particles phagocytized by a single Kupffer's cell (N); C: control; A: severe iron–deficiency anemia.

elements of GER, ribosomes, and glycogen. As a result, the volume of secondary lysosomes increased almost twofold (Table 1, Fig. 1, *a*); their membranes are more labile, this possibly enhancing diffusion of lysosomal hydrolases into the vascular bed [6].

A marked reduction of GER surface size and volume was observed in the endotheliocytes, this being associated with a reduction of the numbers of adhered and free ribosomes by 42 and 33%, respectively (Table 2). Endotheliocytes were visibly larger, the sinusoidal lining looked thickened, and the number of fenestrae was reduced, possibly due to edema. Similar changes have been observed in acute stress [9]. Lining detachment sites and necrotic endotheliocytes were found in sinusoids. These changes may be responsible for deterioration of liver parenchymatous cell trophism and are conductive to the development of destructive changes in hepatocytes.

TABLE 2. Results of Morphometry of Liver Sinusoidal Endothelial and Kupffer's cells, *Mean ± SEM*

Parameters	Control	Iron-deficiency anemia
Endotheliocytes		
Mitochondria:		
external membrane, S_V	0.6±0.1	0.6±0.1
internal membrane, S_V	1.2±0.2	1.0±0.1
GER,		
S_V	2.3±0.1	1.5±0.1*
V_V	10.4±1.3	6.8±0.9*
Ribosomes:		
adhered, N_A	23±2	13±2*
free, N_A	43±5	29±4*
Kuppfer's cells		
Mitochondria:		
external membrane, S_V	0.7±0.1	0.5±0.1
internal membrane, S_V	2.0±0.3	1.3±0.2*
GER,		
S_V	2.0±0.2	1.2±0.1*
V_V	9.6±1.6	5.9±0.9*
Ribosomes:		
adhered, N_A	23±3	14±2*
free, N_A	46±5	24±3*

Note. N_A: numerical density (number per μ² of cytoplasm section area); other notation as in Table 1.

Kupffer's cells of animals with iron-deficiency anemia were similarly increased in size mostly on account of hypertrophy and hyperplasia of the lysosomal system (Fig. 1, *b*). The numebr of Kupffer's cells phagocytizing red cells increased by 31%. In parallel with this, a decrease of granular reticulum surface and volumic densities by 38% and of the number of adhered and free ribosomes by 37 and 48.5%, respectively, was observed. The area of the mitochondrial internal membrane surface decreased by 36.5% (Table 2).

These shifts indicate that in Kupffer's cells, just as in hepatocytes and endotheliocytes, the mitochondrial energy-producing functions was reduced, as were synthetic processes geared toward meeting the cells' own requirements plus "export".

Dead Kupffer's cells were found in sinusoids of animals with iron-deficiency anemia. Monocytes and transitional forms between monocytes and Kupffer's cells were rather frequent, this evidently being a compensatory reaction to loss of a part of the sinusoidal cell population in the liver. This, and the reduced phagocytic activity of Kupffer's cells (Fig. 2), explains to a certain extent the lower resistance of patients with iron-deficiency anemias to various infections [5].

The total volume of the lysosomal apparatus in Kupffer's cells increased twofold, while in endotheliocytes in increased threefold (Fig. 3). The

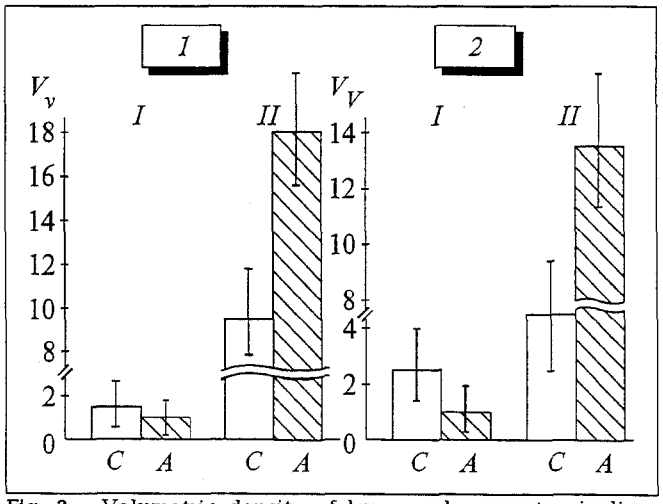

Fig. 3. Volumetric density of lysosomal apparatus in liver sinusoidal cells. *1*) sinusoidal endotheliocytes; *2*) Kupffer's cells; V_v: volumetric density (% of cytoplasmic volume); *I*) primary lysosomes; *II*) secondary lysosomes; other noteation as in Fig. 2.

growing volumetric share of secondary lysosomes in hepatocytes, endotheliocytes, and Kupffer's cells in iron-deficiency anemia creates a high background of lysosomal enzymes in the blood plasma [6] and is a risk factor during cell exposure to membranotropic and lysosomotropic agents; therefore, the use of lysosomotropic drugs containing iron ions characterized by prooxidant properties in the treatment of patients with iron-deficiency anemia [10] may exacerbate cell membrane damage and stimulate the development of destructive processes.

REFERENCES

1. A. A. Golovin, V. D. Konvai, and Yu. V. Red'kin, *Klin. Med.*, № 8, 64-66 (1989).
2. L. I. Idel'son, *Hypochromic Anemias* [in Russian], Moscow (1982).
3. T. A. Korolenko, *Biochemical Aspects of Lysosomotropism* [in Russian], Novosibirsk (1983).
4. V. I. Nikulicheva and F. S. Khusainova, *Gematol. Transfusiol.*, № 6, 20-22 (1983).
5. L. A. Panacheva, V. M. Sharaputo, and L. Yu. Zyubina, in: *Early Diagnosis, Prevention of, and Rehabilitation after Prevalent Diseases in Industrial Workers* [in Russian], Moscow (1989), pp. 109-112.
6. T. I. Pospelova, T. A. Ageeva, M. I. Loseva, *et al.*, *Gematol. Transfusiol*, № 9-10, 25-28 (1992).
7. V. A. Shkurupii and I. N. Indikova, *Tsitologiya*, № 3, 269-274 (1978).
8. V. A. Shkurupii and V.N. Gavrilin, *Ibid*, №5,537-542 (1987).
9. V. A. Shkurupii, *Liver Cells Ultrastructure in Stress* [in Russian], Novosibirsk (1989).
10. B. R. Bacon, R. S. Britton, and R. O'Heill, *Hepatology*, **9**, № 3, 398-404 (1989).
11. J. Boime, E. Smith, and F. E. Hunter, *Arch. Biochem.*, **139**, № 3, 425-443 (1970).
12. Y. Kalra, A. K. Chaudhary, K. L. Massey, and K. Prasad. *Molec. Cell Biochem.*, **94**, № 1, 1-8 (1990).

Morphological Criteria of Reduced Permeability and Trophism of Cirrhotically Changed Rat Liver and their Increase under the Influence of Pyrogenal and Pyridoxal Phosphate (Morphometry and Transmission and Scanning Electron Microscopy Findings)

G. M. Vakulin

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Lack of data on fine microcirculatory changes in the liver in cirrhosis [10,12] and on the pathogenetic role of hypoxic ultrastructural disorders dictates the need to seek the origin of the microcirculatory disorders occurring in the organ in this disease. Development of research in this field using morphometry and transmission and (especially) scanning electron microscopy is impeded by insufficient knowledge of drug effects on the permeability and trophism of the cirrhotically altered liver.

Central Research Laboratory, Novosibirsk Medical Institute (Presented by Yu. I. Borodin, Member of the Russian Academy of Medical Sciences)

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