Ultrastructure of Rat Liver Cells after Exhausting Physical Training

M. M. Kalashnikova, T. P. Seene, and O. V. Smirnova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 126, No. 7, pp. 101-104, July, 1998
Original article submitted July 7, 1997

In rats, running of the maximum intensity caused death of some hepatocytes, an increase in the number of phagosomes in Kuppfer cells, and the emergence of connective tissue fibers in the space of Disse. Ultrastructural investigation of hepatocytes showed delayed release of bile products into bile capillaries, decrease in glycogen content, increase in the number of mitochondria (many of them were divided by the cristae), and irregular distribution of ribosomes in the rough endoplasmic reticulum. Accumulation of erythrocytes in the sinusoids, fragments of dead hepatocytes, Kuppfer cells with numerous phagosomes, and connective tissue fibers in the space of Disse were observed in rat liver after exhausting swimming. Study of hepatocyte ultrastructure revealed intense protein synthesis (as evidenced by increased number of ribosomes and unchanged mitochondria and cisternae of the rough endoplasmic reticulum), separation of cytoplasmic fragments with ribosomes into sinusoids, absence of glycogen, and lipid accumulation.

Key Words: *liver; hepatocyte; physical load; clasmatosis*

While investigating the effect of the maximum physical load on the organism, it is important not only to assess biochemical and morphological alterations in the muscles [6-10], but also to characterize the response of the liver, a vitally important internal organ. We studied the ultrastructure of rat liver after exhausting running and swimming.

MATERIALS AND METHODS

Experiments were performed on 12 male Wistar rats (10 experimental and 2 control) weighing 200-300 g. Five rats were trained by running in a treadmill at a speed of 95 m/min, which is regarded as the maximum load [6], for 6 weeks: 20 sec running, 15 min rest, 8 times every day, 5 times every week, and 2 times during the last two-week period. Five rats were trained by exhausting swimming for 2 weeks: the 1st day for 5 h, the 2nd day for 7 h, the 3rd day for 9 h, the 4th-10th days for 7-10 h at water temperature 33°C. The liver was fixed 12 h after the last training session [9]. The rats had free access to food and water [6]. Pieces of liver were fixed in 2.5% glutaraldehyde in S-collidine buffer (pH 7.2-7.4) and postfixed with OsO_4 in the same buffer. Ultrathin sections were stained by the method of Reynolds and examined in a JEM-100C electron microscope.

Laboratory of Evolutionary Histology, Institute of Problems of Ecology and Evolution Russian Academy of Sciences, Moscow

RESULTS

Exhausting running had no effect on the body weight of rats as compared with the control. Ultrastructural examination showed that some hepatocytes died and their fragments were found in the space of Disse. Kuppfer cells were filled with phagosomes. Collagen fibers were present in the space of Disse and in the zone of dying hepatocytes. Hepatocytes differed in ultrastructure and number of mitochondria: hepatocytes with mitochondria that did not differ from normal mitochondria in ultrastructure and number,

0007-4888/98/0007-0731$20.00

hepatocytes with increased number of mitochondria with numerous cristae in them and with swollen mitochondria with clarified matrix, hepatocytes with numerous mitochondria the majority of which had clarified matrix and were divided by the cristae, in some mitochondria the cristae separated unchanged part of the mitochondrion from a clarified part without cristae. In all hepatocytes, the cisternae of rough endoplasmic reticulum (RER) surrounded the mitochondria; however, ribosomes were not always evenly distributed on the RER. The Golgi complex consisted of one or two flat cisternae and several vacuoles with the contents of various density. The vacuoles were located near bile capillaries. Smooth endoplasmic reticulum was located at the sinusoidal pole of pole of hepatocytes. The number of peroxisomes was increased only in hepatocytes containing numerous mitochondria with clarified matrix. The glycogen content of hepatocytes was markedly decreased; some hepatocytes had no glycogen. Numerous very small particles (presumably lipids) were observed in the sinusoids. These particles were also seen in the cavities of irregular shape formed as a result of invagination of the plasma membrane and its separation into the hepatocyte cytoplasm together with the contents of the sinusoid. When these cavities were located far from the plasma membrane, they were surrounded by mitochondria. The nuclei of all functioning hepatocytes contained nucleoli with loose contents and homogeneous chromatin. The nuclear membrane was undulated. The occurrence of binuclear hepatocytes was high; the nuclei in them were located close to each other, suggesting a recent cell division.

Exhausting running leads to death of numerous hepatocytes. Dead cells were replaced by collagen fibers, which were also observed in the space of Disse. As a result, the architectonics of liver lobules was impaired, as well as the exchange between hepatocytes and blood. We believe that polymorphism reflects the reaction of hepatocytes to increased physical load and the varieties in the rhythm of functioning and degree of adaptation according to the law of alternating activity of functioning units [4,5]. It can be suggested that the reaction of hepatocytes with unchanged mitochondria is weaker than that of hepatocytes with numerous mitochondria, including those with clarified matrix. An increase in the number of mitochondria points to intensification of hepatocytic function; however, the presence of mitochondria with clarified matrix implies various intensity of mitochondrial function. Hepatocytes with great number of mitochondria, the majority of which (or all) had clarified matrix, occasional cristae, or were divided by the cristae, indicate the maximum functional activity at the borderline with dystrophy. The fact that in all hepatocytes the mitochondria were surrounded by the cisternae of RER with unevenly distributed ribosomes indicates production and export of protein; however, clarified matrix and reduced number of cristae in the mitochondria testify to the maximum functional tension at the edge of dystrophy. Shrunk bile capillaries and reduced Golgi apparatus with the contents of various density suggests normal synthesis of bile products and impairment of their release. Intense function of the nuclei (hetechromatin is absent), alternating activity of hepatocyte and their organelles,

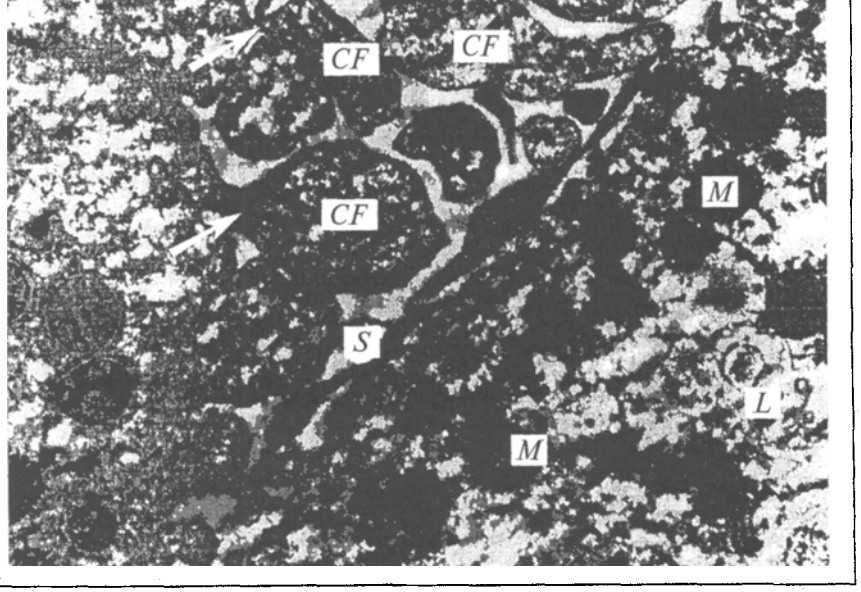

Fig. 1. Cytoplasmic fragments separating from hepatocyte (arrow) into the sinusoid. *CF)* cytoplasmic fragment; *M)* mitochondrion; *L)* lipid drop; *S)* sinusoid, ×8200.

increased number of lysosomes in Kuppfer cells, collagen fibers in the sinusoids, and depleted glycogen stores suggest that the liver cannot sustain exhausting physical load for long time. Comparison of the ultrastructure of hepatocytes after considerable physical load(running at a speed of 35 m/min for 6 weeks) with that after the maximum load (running at a speed of 95 m/min for 6 weeks) showed that the mitochondria are most seriously affected and glycogen disappears [7]. After 6 weeks the liver adapts to running at 35 m/min [2] and fails to adapt to running at 95 m/min, as evidenced by abnormal ultrastructure of the mitochondria in the majority of hepatocytes.

In rats subjected to exhausting swimming the ratio of liver mass to the body mass was higher than in the controls. Numerous dead hepatocytes were observed. The sinusoids were widened and filled with erythrocytes and fragments of dead hepatocytes. Collagen fibers were located in the spaces of Disse. Kuppfer cells were enlarged and had numerous phagosomes sometimes containing erythrocytes. The majority of hepatocytes were one nucleus with one or two nucleoli with a loose contents. The nuclei were often located at the nuclear membrane. The heterochomatin content was very low. Heterochromatin was observed as a very thin layer at the nuclear membrane and as small dense bodies in the karyoplasm. The pores in the nuclear membrane were widened. Ovoid or spheric mitochondria with dense matrix and small number of cristae were observed. They contacted with RER cisternae. Hepatocytes contained numerous free ribosomes. The Golgi apparatus was located in the bile capillary zone and consisted of one or two flattened cisternae and several vacuoles containing vesicles or flakes. Occasional cytoregresomes were observed near bile capillaries. The occurrence of peroxisomes was very low. Hepatocytes practically did not contain glycogen, while their content of membrane-free lipid drops was high. Pronounced clasmatosis of cytoplasmic fragments with ribosomes into sinusoids was documented (Fig. 1).

Thus, exhausting swimming causes death of hepatocytes and widening and plethora of sinusoids. This may account for the increase in liver mass. Accumulation of collagen fibers in the space of Disse impairs the exchange between hepatocytes and blood. Ultrastructural observations indicate that the majority of hepatocytes produce proteins which are released into sinusoids by clasmatosis of cytoplasmic fragments with ribosomes. We believe that this phenomenon is of a compensatory nature, since about 6.5% protein is utilized in the muscles during exhausting swimming [7,9]. Clasmatosis occurs when rapid release of some compounds into peripheral blood is necessary [1,3]. We think that the increase in the number of lipid drops in hepatocytes is also a compensatory reaction. Lipids are supplied from fatty tissue and are used as an energy substrate in the absence of glycogen.

REFERENCES

1. M. M. Kalashnikova, *Byull. Eksp. Biol. Med.*, **100**, No. 9, 355-358 (1985).
2. M. M. Kalashnikova, T. P. Seene, and O. V. Smirnova, *Ibid.*, **108**, No. 9, 283-285 (1989).
3. A. N. Klimov and V. A. Nagornev, *Ibid.*, **122**, No. 9, 268-270 (1996).
4. G. N. Kryzhanovskii, In: *Biological Rhythms and Compensation of Impaired Functions* [in Russian], Moscow, pp. 20-34 (1973.
5. D. S. Sarkisov, *Ibid.*, pp. 35-46.
6. T. P. Seene, R. A. Masso, and K. P. Alev, *Fiziol. Zh.*, **66**, No. 3, 354-361 (1980).
7. T. P. Seene, M. M. Umnova, and I, L. Novoselova, *Functional Micromorphology of Skeletal Muscles* [in Russian], Tartu, (1989).
8. P. Kaasik, K. Alev, and T. Seene, *Scand. J. Lab. Animal Sci.*, **23**, No. 1, 35-39 (1996).
9. T. Seene, M. Umnova, K. Alev, *et al.* in: *The Way to Win*, Eds. J. T. Viitasala *et al.*, Helsinki (1995), pp. 187-189.
10. T. Seene, M. Umnova, K. Alev, *et al. J. Muscle Res. Cell Motil.*, 17, No. 1, (1997).