10. P. Germroth, W. K. Schwerdtfeger, and E. H. Buhl, Brain Res., **494**, 187 (1989).
11. A. Maranto, Science, **217**, 953 (1982).
12. J. H. Sandell and R. H. Masland, J. Histochem. Cytochem., **36**, No. 5, 555 (1988).
13. F. G. Wouterlood, B. Jorritsma-Bynam, and P. H. Goede, J. Neurosci. Meth., **33**, 207 (1990).

CELL ULTRASTRUCTURE IN THE PYGMY SHREW

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KEY WORDS: liver; hepatocyte; physical exercise

In connection with the study of the fine structure of rat hepatocytes engaged in intensive physical exercise (running) under experimental conditions [4] it is interesting to compare data thus obtained with ultrastructure of the hepatocytes of animals which normally are characterized by high motor activity. Pygmy shrews are small animals feeding on insects, their larvae, earthworms and seeds [1]. In their search for food they are on the move for a large part of the day, and consume two to four times as much food as their body weight [1, 8]. It has been shown experimentally that the interval between feeding is 15 min for the common shrew and 10 min for the pygmy shrew [6].

EXPERIMENTAL METHOD

The ultrastructure of the liver cells of juvenile female pygmy shrews (*Sorex minutissimus*), the lesser shrew (*Sorex minutus*), and a juvenile female common shrew (*Sorex araneus*), caught in July in the Yenisei taiga (Mirnyi settlement), was studied. Pieces of liver were fixed in a 2.5% solution of glutaraldehyde in S-collidine buffer, pH 7.2-7.4, and post fixed with OsO_4. The material was embedded in Epson and sections, stained by Reynolds' method, were examined in the JEM-100C electron microscope. The relative volumes of the organelles and inclusions were studied by means of a random-step grid [7]. Since the ultrastructure of the liver cells of the three species of shrew studied is basically similar, we give a description and the results of counting done on the hepatocytes of the pygmy shrew. For instance, the relative volume of mitochondria in the liver of the pygmy shrew was 35.6 ± 0.20 conventional unit, of the rough endoplasmic reticulum 6.35 ± 0.06, the smooth endoplasmic reticulum 4.05 ± 0.03, the Golgi complex 4.25 ± 0.04, secondary lysosomes 1.35 ± 0.03, with autophagosome 0.4 ± 0.05, peroxisomes 2.8 ± 0.03, and glycogen 8.75 ± 0.14.

Both mono- and binuclear hepatocytes were seen. The nuclear membrane formed numerous pores. There was little heterochromatin and it was localized along the nuclear membrane. A quite large nucleolus lay in the karyoplasm. The numerous round and oval mitochondria with moderately dense matrix were conspicuous (Fig. 1). Very many cristae were present in the matrix, and many mitochondria were dividing along the cristae. Each mitochondrion was surrounded by a small, flat cistern of the rough endoplasmic reticulum (RER). A few vesicular profiles of the smooth endoplasmic reticulum (SER) were located in the cytoplasm. The Golgi complex was small and consisted of one or two flat cisterns and tiny vesicles. Secondary lysosomes with finely granular contents were seen in the zone of the Golgi complex. Peroxisomes were quite numerous, measured 0.4-0.6, and did not contain a nucleoid. The biliary capillaries were closed. Many ribosomes were present. There was no fat in the cytoplasm, and small concentrations of glycogen were not found in every hepatocyte. Small autophagous vacuoles containing mitochondria or myelin figures were seen.

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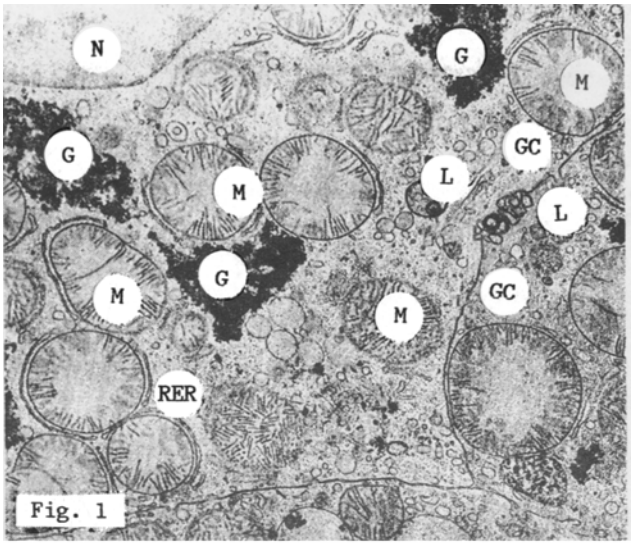

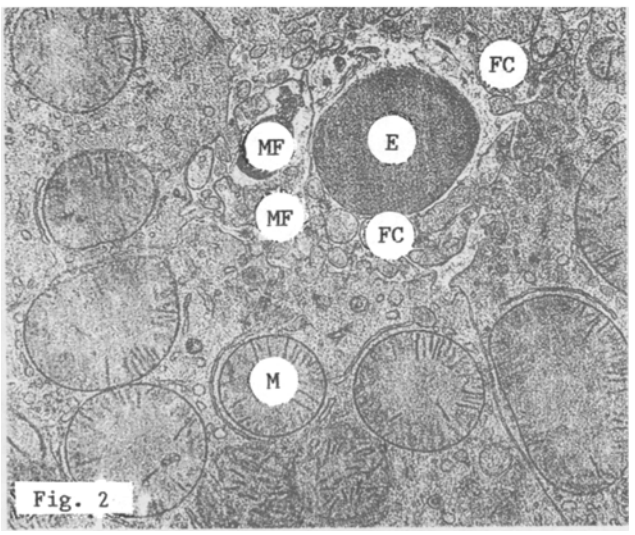

Fig. 1. Hepatocyte of *Sorex minutissimus*. N) Nucleus; M) mitochondrion; GC) Golgi complex; L) lysosome; RER) rough endoplasmic reticulum; G) glycogen. 10,000×.

Fig. 2. Sinusoid in liver of *S. minutissimus* with erythrocyte (E), myelin figures (MF), and fragments of cytoplasm of a hepatocyte (FC) in its lumen. Remainder of legend as to Fig. 1. 15,000×.

A characteristic feature of the pygmy shrew liver is that all sinusoids, even the smallest, were open, and as a rule erythrocytes were visible in their lumen (Fig. 2), which is extremely rare in other animals. The cell membrane at the sinusoidal pole formed numerous microvilli, and also club-shaped outgrowths, which later evidently became detached into the lumen of the sinusoid, for numerous fragments of cytoplasm surrounded by a membrane and containing finely granular material could be seen there. Between the microvilli and in the lumen of the sinusoid structures resembling myelin figures could be seen, and these also were found in the cytoplasm of the hepatocytes. In the sinusoids we found freely lying cells of macrophagal type with large phagosomes in their cytoplasm. Their cell membrane formed long outgrowths and deep invaginations, into which the myelin figures entered, i.e., phagocytosis evidently took place. The sinusoidal cells of the liver had their usual structure. The elongated nucleus contained a thick layer of heterochromatin at the periphery. Their cytoplasm contained large mitochondria, a few profiles of the RER and SER, a Golgi complex, and individual phagosomes.

The investigation showed that the hepatocytes of *S. minutissimus* function intensively. The numerous mitochondria, and the abundance of cristae in them point to a high level of energy metabolism [5]. A similar picture was observed in the liver of the hummingbird, which, like the pygmy shrew, exhibits high motor activity [10]. The close topographic contact of virtually every mitochondrion with a cistern of the RER indicates rapid protein synthesis "for export." The formation of club-shaped outgrowths of the cytoplasm on the sinusoidal surface of the hepatocyte and their subsequent clasmatosis into the lumen of the sinusoid are evidence of intensive secretion of the synthesized products into the blood stream, which we have already noted previously [3]. The myelin figures which we observed in the cytoplasm of the hepatocytes and in the sinusoids are evidently the result of digestion of dying fragments of cytoplasm. The fact that macrophages in the lumen of the sinusoid contain myelin figures which we found both in sinusoids and in hepatocytes, indicates that intensive removal of undigested parts of the cytoplasm takes place not only through the biliary capillary, but also through the blood stream. The small size of the Golgi complex, and the fact that it contained no dilated portions do not indicate that accumulation of many bile products took place in it, and the presence of small vesicles between the biliary capillary and Golgi complex indicates only transient secretion of products of the bile, evidently frequently and in small amounts, by way of adaptation to the frequency and small size of the meals consumed by the pygmy shrew. The open sinusoids, and the presence of erythrocytes even in the smallest sinusoids, indicate a good blood and oxygen supply to each hepatocyte, further evidence of the high metabolic activity of the liver and confirming the physiological data showing high oxygen consumption of the pygmy shrew [2].

If the ultrastructure of the liver cells of the pygmy shrew, for which high mobility is the natural physiological state, is compared with the ultrastructure of the rat liver while carrying out physical exercise by running on a treadmill [4], both similarities and differences can be seen. In both cases the number of mitochondria in the hepatocytes is increased (many dividing along the cristae), but they are more numerous in the pygmy shrew [4]. Significantly more cristae are present in each mitochondrion. For instance, whereas in rats trained for 1.5 months, we counted from 25 to 50 cristae per section through one mitochondrion, in the pygmy shrew they numbered between 30 and 94; they were also considerably more extensive. In trained rats the biliary capillaries are closed, and before them the Golgi complex is saturated with products of the bile, evidence that secretion of the bile products is inhibited. In the pygmy shrew the biliary capillary is closed, and the small size of the Golgi complex and the tiny vesicles between them suggest that bile products are secreted frequently and in small portions. A point of similarity is that the liver of trained rats and of the pygmy shrew contains a quite large number of peroxisomes, which besides their role in protein and lipid metabolism and in synthesis of products of the bile [9], also contain catalase and thus contribute to the higher nonspecific resistance of the liver and of the animal as a whole. Incidentally, quite large autophagosomes, whose contents are secreted into a biliary capillary, are found in trained rats, whereas in the pygmy shrew myelin figures are secreted also into the sinusoid, where they are ingested by macrophages, and also into the biliary capillary.

Whereas in rats during training to run on a treadmill adaptation of the liver to intensive motor activity took place, in the pygmy shrew under physiological conditions adaptation took place to two factors (the frequent small meals and high motor activity), as a result of which the secretion of bile products was not delayed in the pygmy shrew liver, unlike in the rat liver.

The study of liver cell ultrastructure under conditions of high physiological mobility showed that ways of adaptation of the liver to loading under physiological conditions and experimentally are basically similar, although the degree of adaptation under physiological conditions is higher (absence of large autophagous vacuoles, higher energy capacity of the mitochondria, better oxygen supply of the hepatocytes).

LITERATURE CITED

1. V. A. Dolgov, Shrews of the Old World [in Russian], Moscow (1985).
2. É. V. Ivanter, T. V. Ivanter, and I. L. Tumanov, Adaptive Features of Small Mammals: Ecological, Morphological, and Physiological Aspects [in Russian], Leningrad (1985)
3. M. M. Kalashnikova, Bull. Éksp. Bill. Med., No. 9, 355 (1985).
4. M. M. Kalashnikova, T. P. Sääne, and O. V. Smirnova, Bull. Éksp. Bill. Med., No. 9, 283 (1989).
5. A. Lehninger, The Mitochondrion [Russian translation], Moscow (1966).
6. V. E. Sokilov and G. N. Kuznetsov, Circadian Rhythm of Mammalian Activity: Cytological and Ecological Aspects [in Russian], Moscow (1978).

7. S. B. Stefanov, Tsitologiya, **16**, No. 11, 1439 (1974).
8. N. V. Tupikova, Zoo. Zh., **28**, No. 5, 561 (1949).
9. B. V. Kate, Stand. J. Clint Lab. Invest., **49**, 1 (1989).
10. R. K. Suarez, R. W. Brownsey, W. Vogel, et al., Am. J. Physiol., **255**, R699 (1988).

SUPPRESSION OF EPTHELIAL CELL PROLIFERATION IN MICE BY SPLENOCYTES FROM UNILATERALLY SIALADENECTOMIZED SYNGENEIC DONORS

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KEY WORDS: partial hepatectomy; unilateral sialadenectomy; inhibition of proliferation; regeneration information

Splenocytes of the regenerating spleen suppress proliferation of cells of a different histological type in syngeneic recipients [3]. This property of splenocytes coincides in time with their ability to inhibit several immune reactions [4].

To identify the type of lymphoid cells involved in regulating the level of proliferation in nonlymphoid organs and to ascertain any common features of its mechanism for cells of any histological type, the best course to follow is to choose an experimental model in which splenocytes from the undamaged spleen would possess suppressor properties relative to lymphocytes. We considered that an operation on the spleen itself would induce a combination of changes in the properties of its cells, in which their suppressor properties could be the result of many causes, not merely an increase in T-lymphocyte activity.

In the investigation described below the effect on proliferation of hepatocytes of the regenerating liver and of corneal epitheliocytes of the recipients of splenocytes after unilateral sialadenectomy (USE) in mice, which is accompanied by inhibition of the immune response [4], was studied. Both the tissue systems chosen have a high level of proliferation, so that the effect of inhibition of cell division could be distinguished more clearly.

EXPERIMENTAL METHOD

Altogether 395 male CBA mice weighing 18-20 g and obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used. USE on the future donors (removal of the submandibular and sublingual glands en bloc) and partial hepatectomy (removal of one-quarter of the liver tissue) on the future recipients were carried out under ether anesthesia. The animals were killed by cervical dislocation. The donors were killed 17, 48, 72, 144, and 168 h after the operation (the donor interval). A suspension of spleen cells was prepared by the method described previously [2]. The suspension was washed twice and centrifuged for 7 and 5 min at 1000 rpm and at 4°C. After determination of the viability of the lymphoid cells they were injected into the retro-orbital sinus in a dose of $7 \cdot 10^6$/ml of medium 199 for each recipient immediately after the operation. The recipients were killed mainly 48 h, but in certain cases 24 and 72 h after transfer (the recipient interval). Partially hepatectomized and intact recipients, which received the same dose of splenocytes either of intact donors (ID) or of donors undergoing a mock operation (MOD), served as the control. Each group consisted of 7-10 mice. Mitotic activity (MA) was determined in paraffin sections through the liver, 5 μ thick, stained with hematoxylin and eosin; the number of dividing cells was counted in 6000 hepatocytes and in total preparations of the cornea of the intact

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