COMPOSITION OF THE POPULATION OF LYSOSOMAL STRUCTURES SEDIMENTED DURING DIFFERENTIAL CENTRIFUGATION OF MOUSE LIVER HOMOGENATES

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UDC 616-008.922.1-08

KEY WORDS: mouse liver; stereometry; ultrastructures; acid hydrolases.

Differential centrifugation of liver homogenates [4, 9] enables subcellular structures precipitated in accordance with their sedimentation properties to be investigated. The morphological and functional composition and the cellular origin of lysosomal structures sedimented with nuclear, mitochondrial, and microsomal fractions have received the least study.

Accordingly, in the present investigation the radii of the lysosomes of parenchymatous and sinusoidal liver cells were calculated and an attempt made to describe the theoretically possible composition of the population of lysosomal structures sedimented with the granular fractions during differential centrifugation of liver homogenate.

EXPERIMENTAL METHOD

Samples of liver from male C57BL mice aged 2 months were taken from the margin of the left lobe of the liver, fixed in 1% OsO_4 solution, and embedded in Epon. Semithin sections (1 μ thick) were cut from blocks prepared for electron microscopy and stained with toluidine blue. In accordance with the known principles of stereometry, a scheme of morphometric investigation of the material was drawn up for the purposes indicated above. In semithin sections and under a magnification of 945, the relative total values of parenchymatous and sinusoidal liver cells were determined by means of a test grid with 25 nodal points. By using nucleo-cytoplasmic ratios the fractions occupied in liver sections by the cytoplasm of these cells were calculated. Because of the small size of the sinusoidal cells, this ratio for them was calculated from electron micrographs. The ratio between the total volumes of the cytoplasms of cells of the two types of tissue in the liver were then calculated. To determine the total area of the hepatocytes, required for morphometric measurements ensuring that the representation of the cells of the two tissues corresponded to that actually existing in the organ, the total area of the cytoplasms of randomly selected sinusoidal cells was multiplied by the coefficient obtained previously. Volumes of lysosomal structures in parenchymatous and sinusoidal liver cells were determined on electron micrographs. The areas of sections of the lysosomal structures were then measured by means of a point lattice system. The step of the test system, equal to the mean diameter of the smallest lysosomes (±15%, error of its representativeness) was determined experimentally by measuring the diameters of the small lysosomes in cells of different types under a magnification of 60,000. All clearly identifiable lysosomal structures with distinct outlines of their limiting membranes were

TABLE 1. Distribution of Lysosomal Structures in Mouse Liver Cells (M ± m)

Recorded parameters of structures	Hepatocytes			Sinusoidal cells		
	1	2	3	1	2	3
Total volume of structures of one type, %	41,0	52,5	6,5	41,8	30,5	27,7
Radii of structures of one type, μ	0,226±0,014	0,288±0,022	0,399±0,015	0,299±0,023	0,415±0 039	1,080±0,269

Legend. 1) Primary lysosomes, 2) secondary lysosomes, early stage, 3) secondary lysosomes, late stage.

Central Research Laboratory, Novosibirsk Medical Institute, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 10, pp. 420-422, October, 1981. Original article submitted March 18, 1981.

0007-4888/81/9210-1341$07.50

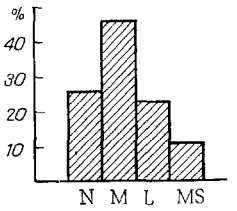

Fig. 1. Volume of lysosomal structures of different sizes in population of lysosomes of mouse liver cells studied (in % of total volume of lysosomal structures studied in all liver cells). N) Nuclear fraction (from 0.490 to 1.920 μ; M) mitochondrial fraction (from 0.470 to 0.254 μ); L) lysosomal fraction (from 0.192 to 0.235 μ); MS) microsomal fraction (from 0.096 to 0.166 μ).

analyzed (structures cut tangentially were excluded). By approximating the cross-sections of the structures as areas of circles, their radii and volumes were calculated. By multiplying the volumes by frequencies of structures of different sizes, separated into groups based on features of their structural-functional state and cell category, their contribution to the total volume of lysosomal structures in per cent was determined: within cells of one type, within a population of cells of different types, and within a population of structures put together purely on the basis of their structural-functional state.

The preparative and analytical procedures followed the previous description [1]. The $P < 0.05$ level of significance was adopted for differences between means (Student's t-test).

EXPERIMENTAL RESULTS

The ratio of the volumes of the cytoplasms of the parenchymatous and sinusoidal cells in the mouse liver is 11.6. The total area of sections through the cytoplasm of the sinusoidal cells which was analyzed was 2095 μ^2. Consequently, by multiplying this value by the ratio given above, the area (24,302 μ^2) of the sections through the cytoplasm of hepatocytes, which was necessary for the morphometric investigations, was calculated. Lysosomal structures in the hepatocytes occupied 0.82 ± 0.14%, and in sinusoidal cells 6.86 ± 0.80% on the volumes of their cytoplasms. It was calculated that the lysosomal structures of hepatocytes accounts for 58.0 ± 0.03% of the total volume of the lysosomes of all types of cells investigated, whereas sinusoidal cells accounted for 42.0 ± 0.14%. These figures agree with data in the literature [2] obtained in experiments on rat liver. The study of lysosomal structures in parenchymatous and sinusoidal cells of mouse liver yielded results (Table 1) which show that in the course of "vital activity" changes in structural and functional properties of the lysosomes from primary to secondary are associated with an increase in their size. The presence of macrophages in the sinusoidal cells evidently is responsible for the high percentage of large secondary lysosomes of heterophagous origin in this group of cells (Table 1).

The distribution of subcellular structures on differential centrifugation is based on differences in their sedimentation properties, which are mainly determined by particle size and also by density and shape [9]. Knowing the viscosity and density of the medium and assuming that subcellular particles in a fluid will be close to spherical in shape, in agreement with data in [7, 8] the radii of particles in different fractions sedimented during differential centrifugation can be calculated. The calculations showed that with a partition factor F = 600g particles with a radius of over 0.47 μ (nuclear fraction) will be sedimented, at F = 3300g particles with a radius of over 0.24 μ (the heavy mitochondrial or mitochondrial fraction), at F = 10,000g particles with a radius of over 0.18 μ (light mitochondrial or lysosomal fraction), and at F = 100,000g particles with a radius of over 0.04 μ

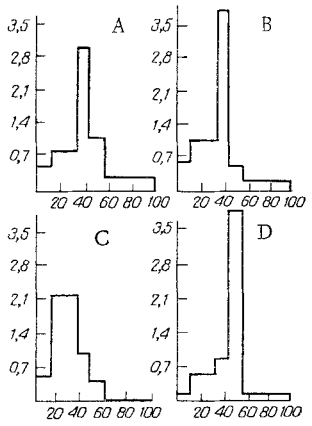

Fig. 2

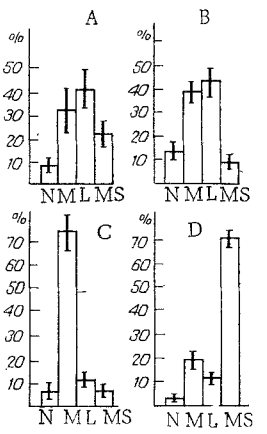

Fig. 3

Fig. 2. Intracellular distribution of marker enzymes in mouse liver. Abscissa, protein content in fraction (in % of total protein content in homogenate); ordinate, specific enzyme activity (in relative units). Order of fractions: nuclear, mitochondrial, lysosomal, cytosol. A) Acid phosphatase; B) cathespin D; C) succinate dehydrogenase; D) glucose-6-phosphatase.

Fig. 3. Distribution of activity of marker enzymes in mouse liver among granular fractions. Ordinate, activity of enzymes in separate fractions (in % of total activity of enzymes in granular fractions). Remainder of legend as to Figs. 1 and 2.

(microsomal fraction). The calculated radii (by the morphometric method) of the lysosomal structures of all liver cells tested were distributed between limits of 1.92 and 0.096 μ, figures similar to those [3] obtained with rats. This series of structures was divided into groups depending on the lengths of their radii (Fig. 1). These groups will subsequently be called fractions, the boundaries of which in this particular series were defined by the writers previously.

The results of biochemical investigation showed the presence of acid phosphatase and cathepsin D activity in all granular fractions of mouse liver homogenates (Figs. 2 and 3). Evidently this is due to the heterogeneous composition of the lysosomal structures in granular fractions, a theoretical model of which is shown in Table 2. According to the morphometric data (Table 2), lysosomal structures of sinusoidal cells accounted for 88.3% in the nuclear "fraction." This could probably be due to the higher content of cathepsin D in this fraction than of acid phosphatase (see Figs. 2a, b and 3a, b). In the microsomal fraction (see Figs. 2a, b and 3a, b) the higher content of acid phosphatase than of cathepsin D evidently may have been connected with the supposedly greater volume of primary lysosomes of hepatocytes in this fraction (Table 2), which according to data in the literature [5, 6, 10] has the highest acid phosphatase activity. The greatest volume of primary lysosomes of sinusoidal cells in the mitochondrial fraction (Table 2) could evidently be responsible for the rather higher content of cathepsin D than of acid phosphatase in this fraction (see Figs. 2a, b and 3a, b). Analysis of the composition of the lysosomal fraction showed disparity between the morphometric data (the least volume in it occupied by lysosomes of sinusoidal cells) and the biochemical data (the highest content of cathepsin D), which contradicts results obtained for the other fractions described above, so that further study is evidently required.

In conclusion, it must be pointed out that with a decrease in size of the lysosomal structures (Table 2) the total volume of lysosomes of auto- and heterophagous types in the fractions decreases. In hepatocytes, with a decrease in size of the lysosomal structures the total volume of both primary and secondary lysosomes in the fractions increased (Table 2). This relationship was not found in the sinusoidal cells, evidently because of the heterogenous composition of the sinusoidal cells.

TABLE 2. Results of Morphometry of Lysosomal Structures of Mouse Liver Cells (in % of total volume of lysosomal structures in "fraction")

Fraction	Radius of structures, μ	Hepatocytes				Sinusoidal cells				Total volume of secondary lysosomes in fraction
		1	2	3	Total 1, 2, 3	1	2	3	Total 1, 2, 3	
N	1,920—0,490	—	—	11,7	11,7	25,1	16,9	46,3	88,3	74,9
M	0,470—0,254	8,2	49,1	—	57,3	33,4	9,3	—	42,7	58,4
L	0,235—0,192	32,5	40,6	6,3	79,4	12,5	8,1	—	20,6	55,0
MS	0,166—0,096	43,1	27,8	1,2	72,1	24,1	3,8	—	27,9	32,8

Legend. 1) Primary lysosomes, 2) secondary lysosomes of auto- and heterophagous type, early stage, 3) secondary lysosomes of auto- and heterophagus type, late stage. N) Nuclear fraction, M) mitochondrial fraction, L) lysosomal fraction, MS) microsomal fraction.

LITERATURE CITED

1. A. B. Pupyshev, A. E. Malygin, O. R. Grek, et al., Farmakol. Toksikol., No. 3, 294 (1979).
2. A. Blouin, R. P. Bolender, and E. R. Weibel, J. Cell Biol., 72, 441 (1977).
3. M. Davies, in: Lysosomes in Biology and Pathology, Vol. 4, Amsterdam (1975), p. 305.
4. C. De Duve, B. C. Pressman, R. Gianetto, et al., Biochem. J., 60, 604 (1955).
5. D. L. Knook and E. C. Sleyster, Exp. Cell Res., 99, 444 (1976).
6. D. L. Knook and E. C. Sleyster, Biochem. Biophys. Res. Commun., 96, 250 (1980).
7. A. K. Schachman, in: Ultracentrifugation in Biochemistry, Academic Press, New York (1959), p. 214.
8. W. C. Schneider, J. Biol. Chem., 176, 259 (1948).
9. T. J. C. Van Berkel, J. K. Kruijt, and J. F. Koster, Eur. J. Biochem., 58, 145 (1975).
10. E. R. Weibel, W. Stauble, H. R. Gnagi, et al., J. Cell Biol., 42, 68 (1969).

LIPID PEROXIDATION IN THE GRAFT AND RECIPIENT'S TISSUES UNDER DIFFERENT CONDITIONS OF TRANSPLANTATION

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UDC 616.6.02-08:[612.397.2-06:612.262

KEY WORDS: skin graft, auto- and homografting, malonic dialdehyde, chemiluminescence.

When tissues such as glands of internal secretion, skin and, in particular, large whole organs are transplanted, a lasting clinical effect, in the shape of true survival of autografts, or lengthening of primary survival of homografts, is observed only if they retain their viability. In tissues with no circulation and also in isolated tissues surviving *in vitro*, incompletely oxidized products of glycolysis and ions of metals of variable valency accumulate, changes take place in the composition and structure of phospholipids, and the antioxidative activity of lipids is reduced, all of which may contribute to an increase in the intensity of lipid peroxidation (LPO) [1, 2, 4, 5, 7, 11, 12]. Accumulation of endogenuous products of LPO has been shown to lead to marked inhibition of functional activity of isolated tissues, thus indicating the important role of the intensity of LPO in viability [6].

During transplantation tissues again find themselves under unfavorable conditions — until their circulation is restored they are in an ischemic state. In addition, the graft sur-

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 10, pp. 422-424, October, 1981. Original article submitted February 30, 1981.

0007-4888/81/9210-1344$07.50