EFFECT OF MEMBRANOUS STRUCTURES OF THE KUPFFER CELLS ON BLOOD CLOTTING

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The effect of subcellular fractions of the endothelial Kupffer cells on blood clotting was studied. Cells were obtained from the rabbit liver by magnetic fractionation and the subcellular structures were then isolated from them by differential centrifugation. The inner membranous structures were found to carry thromboplastic activity. No correlation was found between the blood-clotting potential of the intracellular structures of the endothelial cells and the level of their cathepsin C activity.

KEY WORDS: Kupffer cells; subcellular fractions; thromboplastic activity; cathepsin C; succinate dehydrogenase.

After destruction of the endothelium of the blood vessels soluble enzymes enter the blood stream along with intracellular membranous structures, with which the tissue thromboplastin activity is closely bound [1]. However, it was not clear until recently which of the subcellular fractions carries thromboplastic activity. It has been shown [5-7] that microsomes isolated from the lungs, spleen, kidneys, and liver were the most active as regards their effect on blood clotting in vitro. The mitochondria and nuclei also accelerated blood clotting.

Since the most important role in the development of hemostatic changes after injury to blood vessels is played by their endothelial lining, it was decided to study the thromboplastic properties and distribution of certain enzymes in the subcellular fractions of the endothelial Kupffer cells.

EXPERIMENTAL METHOD

Experiments were carried out on 16 rabbits of both sexes weighing 1.7-2.4 kg. The endothelial Kupffer cells were obtained as described previously [2] by magnetic fractionation in 0.25 M sucrose solution with 0.01 M EDTA at pH 7.4. A cell homogenate was obtained in a Potter–Elvehjem homogenizer with Teflon pestle and the subsequent fractionation was carried out by differential centrifugation [11] in a solution of sucrose in Tris-HCl buffer, pH 7.4. The nuclear fractions were isolated at 600-800 g for 10 min. The fraction of "heavy" mitochondria, or the lysosomal-mitochondrial fraction, at 11,700-12,000 g for 35 min. The last supernatant fraction, containing some of the lysosomes, microsomes, and residues of the endoplasmic reticulum, was filtered through a VUFS ultrafilter with average pore size of 0.25 μ to remove membrane material. All operations were carried out at 0-2°C. Parallel with the subfractionation of the endothelial cells, analogous fractions were isolated from hepatocytes and from a whole liver homogenate.

The purity of the resulting fractions was verified by determining the activity of "marker" enzymes – succinate dehydrogenase [15], acid phosphatase [9], and cathepsin C [10]. The protein content was determined by Lowry's method [13]. The total and relative activity of the enzymes was determined [12]. The thromboplastic activity of the subcellular fractions was judged from the quickening of the clotting time of platelet-free rabbit plasma. The results were subjected to statistical analysis by Fisher's method [8].

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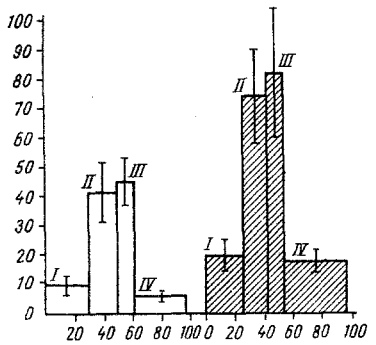

Fig. 1

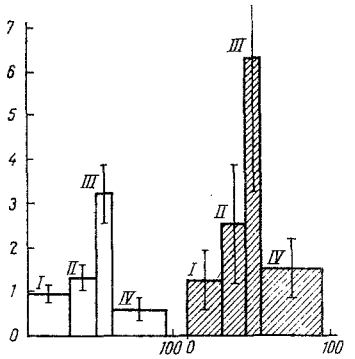

Fig. 2

Fig. 1. Distribution of succinate dehydrogenase activity in subcellular fractions of Kupffer cells (shaded columns) and hepatocytes (unshaded columns): I) nuclear fraction; II) "heavy" mitochondria; III) "light" mitochondria; IV) supernatant fluid. Abscissa, protein content (in % of its content in whole homogenate); ordinate, enzyme activity (in units/mg protein).

Fig. 2. Distribution of acid phosphatase activity in subcellular fractions of Kupffer cells (shaded columns) and in hepatocytes (unshaded columns). Legend as in Fig. 1.

EXPERIMENTAL RESULTS AND DISCUSSION

The investigation showed that the membranous subcellular structures isolated from whole liver homogenate had considerable thromboplastic activity even if the fractions were diluted 1 : 500 or more. The fractions of "heavy" and "light" mitochondria and the supernatant fluid were most active. The cell matrix, obtained by ultrafiltration, did not shorten the plasma recalcification time.

The specific activity of the enzymes studied in the subcellular fractions of the Kupffer cells was 1.5-2 times higher than the activity of the same fractions of the hepatocytes (Figs. 1 and 2). Succinate dehydrogenase activity was completely absent from the ultrastructure of the homogenate of the hepatocytes and Kupffer cells and only in two experiments were traces of phosphatase activity present, evidence of the high degree of purification from mitochondria and lysosomes obtained by ultrafiltration.

The differences discovered in the enzyme activity must evidently be explained by the participation of the Kupffer cells in phagocytosis, as a result of which the level of certain metabolic processes in the endothelial cells was higher than in the hepatocytes.

TABLE 1. Thromboplastic Activity and Cathepsin C Activity in Subcellular Fractions of Kupffer Cells and Hepatocytes (M ± m)

Test object	Kupffer cells		Heptatocytes	
	thromboplastic activity (in sec)	cathepsin C activity (in mmoles NH_3/mg protein	thromboplastic activity (in sec)	cathepsin C activity (in mmoles NH_3/mg protein)
Cell homogenate	78,7±4,85	0,027±0,007	75,5±3,9	0,014±0,012
Nuclei	138,0±4,36	0,015±0,006	141,5±9,56	0,010±0,005
"Heavy" mitochondria	66,5±5,21	0,030±0,010	74,2±17,6	0,013±0,006
"Light" mitochondria	71,0±9,59	0,018±0,010	70,0±8,22	0,010±0,007
Supernatant	90,1±18,6	0,033±0,010	67,7±12,9	0,024±0,004
Ultrafiltrate of supernatant	Absent	0,168±0,063	159,5±21,2	0,072±0,051
Control	171,4±4,67	—	171,4±4,67	—

Meanwhile the distribution and magnitude of the thromboplastic activity in the organelles of the Kupffer cells did not differ significantly from these parameters in the subcellular fractions of the hepatocytes (Table 1).

It will be clear from Table 1 that the clotting power of whole homogenate of both endothelial cells and hepatocytes was lower than the activity of the mitochondrial fractions. Meanwhile the cell matrix not only did not accelerate plasma recalcification but, in some cases, it actually retarded it compared with the control. This fact agrees fully with the view that procoagulant and anticoagulant substances exist in the cells. During fractionation the membranous material and, consequently, the thromboplastic principle were concentrated and at the same time were freed from inhibitory agents.

Comparison of the results of investigation of the blood clotting potential of the subcellular fractions with the activity of cathepsin C which, according to Purcell and Barnhart [14], activates prothrombin revealed no correlation. For instance, the ultrafiltrate with the highest cathepsin C activity had virtually no thromboplastic properties. The absence of parallel between clotting and proteolytic activity has also been observed by other workers [3, 4].

The results of these experiments thus showed that the subcellular organelles of the endothelial Kupffer cells possess thromboplastic activity which is concentrated to the highest degree in the membranous structures. The presence of cathepsin C has essentially no effect on blood clotting.

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