The results suggest that preparations obtained from the liver of C57BL mice at different temperatures include macromolecular adhesive factors which differ from one another. The discovery of adhesive activity in preparations obtained at 4°C on a model of the intact liver and the absence of any biological effect on models of the perfused liver or in a hepatocyte suspension indicate that their action is effected only when the cell surface is intact and, consequently, adhesive factors contained in them are located at the periphery of the cell surface. Since preparations obtained at 20°C exhibited adhesive activity on models of the intact and perfused liver, but had no action on adhesion of hepatocytes in suspension, it can be postulated that the adhesive factors present in them are semiintegral components of the plasma membrane.

The results of this comparative study of adhesively active preparations of C57BL mouse liver and preparations obtained in the same way from the CBA mouse liver indicate a disturbance of adhesive interactions between liver cells in CBA mice, which are evidently connected with changes in the properties of components of the cell surface, involved in adhesive processes.

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CHANGES IN LYSOSOME COMPOSITION IN HEPATOCYTES CULTURED IN VITRO AS A MODEL OF PATHOLOGICAL STATES

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Destructive processes in cells largely involve the functioning of an extremely heterogeneous class of cell organelles, namely lysosomes, but much is unknown about the regulation of their activity. Much research has been published to show that lysosomal hydrolases are released into the cell cytosol in various pathological states [3]. There is morphological evidence of the development of autophagy and an increase in the number of lysosomes in cells during pathology [7, 8]. However, it is not clear what morphological changes in lysosomes ought to reflect this process of acid hydrolase release. A model for studying processes of anoxia, ischemia, substrate starvation, and rehabilitation, using a primary monolayer culture of hepatocytes from 3-day-old rats was developed previously [1]. It was shown on this model that incubation of hepatocytes in Hanks' buffered salt solution for 20 min leads to an increase in nonsedimented activity of acid phosphatase, the marker enzyme of lysosomes. Reproduction of anoxia in Hanks' solution for 1 h potentiates this effect, and subsequent rehabilitation for 1 h restores normal values.

The above model was used in the present investigation to study changes in the composition of the lysosomal population in hepatocytes by light microscopy.

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TABLE 1. Number of Secondary Lysosomes in Culture of Hepatocytes under Different Pathological Conditions

Conditions to which exposed	Mean number of secondary lysosomes in hepatocytes	Mean number of secondary lysosomes in hepatocytes in original culture (control)
Normoxia in Hanks" solution, 37°C, 20 min	11,79±1,26*	15,03±0,93
Anoxia in Hanks' solution, 37°C, 1 h	6,22±2,48	
Rehabilitation for 1 h after anoxia in Hanks' solution	6,93±2,01	7,48±2,31
Growth medium, +4°C, 30 min	11,03±1,36*	17,08±2,05

Legend. *$p < 0.05$ compared with control level.

TABLE 2. Phagocytosis in Culture of Hepatocytes under Various Pathological Conditions

Conditions to which exposed	Number of cells with phagosomes, %	Number of phagosomes in cells
Original culture	9,6±2,4	1—3
Normoxia in Hanks' solution, 37°C, 20 min	11,95±3,55	1—4
Anoxia in Hanks' solution,	7,6±3,4	4—7
Rehabilitation for 1 h after anoxia in Hanks' solution	15,4±2,3	4—8

EXPERIMENTAL METHOD

Experiments were conducted on a monolayer culture of hepatocytes from 3-day-old rats on the 4th day of culture [1]. The cell cultures were grown on coverslips in Petri dishes. Before the beginning of the experiment, the coverslips with cells grown on them were rinsed in Hanks' solution and placed in a Petri dish (with the cells uppermost) containing this same solution, buffered with 10 mM HEPES pH 7.2 (37°C, 20 min). To produce a model of anoxia, open Petri dishes, prepared as described above, were placed in a gas-flow chamber at 37°C, through which nitrogen was passed for 1 h under constant pressure. In the model of rehabilitation after anoxia, the Hanks' solution was replaced by ordinary growth medium and the cells were placed for 1 h in a CO_2 incubator at 37°C. Control cultures were incubated in growth medium: medium 199 containing 10 mM HEPES, pH 7.2, 200 U/ml penicillin, 2 mM glutamine, 5% embryonic calf serum, in a normal atmosphere at 37°C. To detect lysosomes in the hepatocytes the method of intravital staining of the lysosomes with neutral red was used [5]. The number of lysosomes measuring 1-3 μ in the hepatocytes was counted. The size of the lysosomes was estimated visually by comparison with a cell measuring gauge. Lysosomes of this kind were conventionally described as secondary. Additionally, the number of larger lysosomes, over 3 μ in diameter, and taken to be phagosomes, was estimated in the hepatocytes. The lysosomes were counted visually on an MBI-6 microscope with magnification of 900. The number of lysosomes in each preparation was counted in at least 60 cells. In each experiment two or three coverslips were counted and the results added together. To characterize the original culture, a control was set up in each case. In each experiment coverslips with cells grown in one Petri dish were used. The results were analyzed to determine changes in the mean number of secondary lysosomes and phagosomes per cell and also the distribution of cells in the population with respect to the number of lysosomes 1-3 μ in diameter. Standard biometric algorithms [6] were used to form a variance series and to divide the primary data into classes. The maximal number of secondary lysosomes in the hepatocytes, according to our data, did not exceed 40 and there were six classes. The first class included cells with between 0 and 5 secondary lysosomes, the second class — between 6 and 11, etc. The results were subjected to statistical analysis by Student's test. Each experiment was repeated 5-6 times with cells from different seedings.

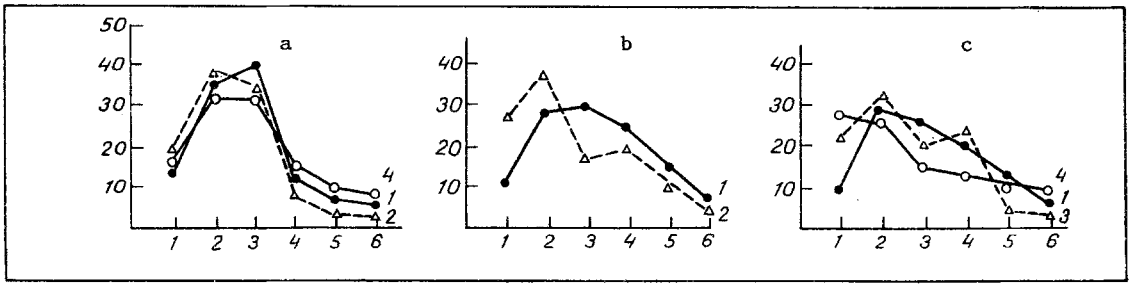

Fig. 1. Distribution of hepatocytes in cell population in culture with respect to number of secondary lysosomes. a) Normal culture grown on three different coverslips, taken from the same seeding in the same Petri dish, b) model of substrate starvation in Hanks' solution (37°C, 20 min). 1) Original culture, 2) exposure to experimental conditions, 3) anoxia in Hanks' solution (1 h, 37°C), 4) rehabilitation under original conditions (1 h). C) Model of anoxia in Hanks' solution followed by rehabilitation. Abscissa, Nos. of classes; ordinate, percentage of cells having the number of secondary lysosomes characteristic of the given class.

EXPERIMENTAL RESULTS

Lysosomes discovered with the aid of neutral red in hepatocytes from 3-day-old rats, after 4 days of culture in vitro, constituted a heterogeneous class of particles, distributed either around the nucleus or on one side of it. These were mainly small lysosomes under 1 μ in diameter. They were difficult to count, but there were about 40-60 per cell. The number of lysosomes over 1 μ in diameter was much less, on average from 4 to 20 per cell, although in some hepatocytes they numbered 40.

The mean number of lysosomes per cell and the distribution of hepatocytes in the population on the basis of the number of secondary lysosomes, for each hepatocyte culture grown from the same seeding on coverslips in the same Petri dish, did not change over a limited period of time (Fig. 1a).

Incubation of the hepatocyte culture in Hanks' solution for 20 min at 37°C led to a significant decrease in the mean number of secondary lysosomes per cell (Table 1) and to a change in the distribution of hepatocytes in the culture based on the number of secondary lysosomes (Fig. 1b). There was an increase in the relative proportion of cells with few secondary lysosomes, i.e., the number of secondary lysosomes was reduced in a considerable proportion of the hepatocytes.

During 1 h of anoxia in Hanks' solution and during 1 h of subsequent rehabilitation, no significant changes were found in the average number of secondary lysosomes compared with the control (Table 1). There was likewise no change in the distribution of hepatocytes with respect to the number of secondary lysosomes (Fig. 1c). There was an increase in the number of phagosomes in the cells, and after rehabilitation there was a tendency for the number of cells in the population with phagosomes to rise (Table 2).

An increase in the number of phagosomes in cells in various pathological states is known [11]. However, on incubation in Hanks' solution for 20 min the mean number of secondary lysosomes in the hepatocytes fell and a tendency was observed for the relative proportion of the cells containing fewer secondary lysosomes to increase. The decrease in the number of secondary lysosomes may have been due to their fusion with the formation of phagosomes, or to fragmentation of the secondary lysosomes into smaller structures. It is unlikely that the process of phagosome formation plays the principal role here, because there was no appreciable increase in the number of phagosomes in the hepatocytes or in the relative number of hepatocytes containing phagosomes (Table 2). The most likely explanation is a process leading to reduction in the size of the lysosomes. Few data could be found in the literature on this matter. Frolov [7] notes a decrease in the number of aryl sulfatase granules in sections through the rabbit heart after 10 and 20 min of acute focal cardiac ischemia. Kristev, studying the aftereffects of starvation, concludes that the initial phase of activation of the lysosomal apparatus consists of an increase in the number of primary lysosomes [4]. For endocytic cells, to which the hepatocytes belong, Dean [2] suggests the existence of recycling of the endocytic membrane through fragmentation of secondary lysosomes after fusion of endocytotic vesicles with the primary lysosome.

A series of publications describing the function of tubular lysosomes in macrophages has recently appeared [9, 14]. A particular feature of these lysosomes is that, under conditions of *c*AMP deficiency in the cells and depolarization of the microtubules, they disintegrate into microvesicles. It is suggested that structures of this kind may exist in other cells also, but as a result of the low temperature during processing of the material and the conditions of fixation, they break up into microvesicles.

The role of microtubules in lysosomal function has been stated in the literature [12]. On the other hand, we know that cooling cell cultures to +4°C for 15-30 min leads to destruction of the microtubules [10]. In the present investigation we studied the effect of cold on the lysosomal composition in the hepatocytes, assuming that lysosomal function is directly connected with the state of the microtubular system. After keeping hepatocyte cultures in normal growth medium for 30 min at +4°C the mean number of secondary lysosomes in the hepatocytes fell significantly below the control value (Table 1). We observed a similar effect when growth medium was replaced by Hanks' solution. The possibility cannot be ruled out that short-term treatment with Hanks' solution leads to disassembly of the microtubular system on account of a transient fall in the intracellular *c*AMP concentration, increasing the life span of the microtubules and improving their mechanical properties [13]. The result of this process is fragmentation of the relatively large secondary lysosomes into small vesicles. With longer exposure to Hanks' solution, and more especially under rehabilitation conditions, a compensatory increase in the *c*AMP concentration is possible, with consequent stabilization of the microtubular system that maintains the level of function of secondary lysosomes and phagosomes.

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