human skin fibroblasts. Thus all types of cells studied were able to accumulate lipids when incubated with complexes containing LDL, FN, H, and G, although the ability of the cells to accumulate lipids depended on the type of cells. It must be emphasized that the greatest quantity of lipids was accumulated by cells of the subendothelial intima, i.e., by the layer of the vessel wall which undergoes pathological changes during atherogenesis. It can thus be tentatively suggested that components of the extracellular matrix play an important role in the formation of lipid-loaded foam cells, which accumulate in regions of the arterial wall affected by atherosclerosis.

Consequently components of the extracellular matrix can disturb LDL catabolism in human cells. During classical receptor binding LDL are quickly taken up by lysosomes and are hydrolyzed, and during endocytosis of insoluble LDL—H—FN—B complexes processes of LDL degradation are disturbed, probably due to ineffective uptake of LDL by lysosomes.

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SPECTROPHOTOMETRIC DETERMINATION OF OSMOTIC RESISTANCE OF LYSOSOMES ISOLATED FROM ANIMAL TISSUES

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KEY WORDS: lysosomes; osmotic resistance

The fact that lysosomes have a single semipermeable and closed membrane endows them with properties of an osmotic system [5]. Low-molecular-weight substances damage them by a greater degree than compounds with high molecular weight [3]. Realization of the osmotic properties of lysosomes is observed in various pathophysiological states and exposure to physicochemical factors. Reoxygenation of the anoxic heart [4], ischemia of the liver [6], and myocardium [9], and also overloading of lysosomes with lysosomotropic preparations [1] lead to vacuolar degeneration of these particles. This is the reason why determination of the osmotic resistance of lysosomes is important as a convenient and informative test when studying the dynamics of the state of the lysosomal apparatus of the cell in experiments conducted in the field of molecular biology, cellular biochemistry, biochemical pharmacology, and clinical medicine. However, quantitative estimation of the osmotic resistance of lysosomes is difficult because of the lack of a rapid and direct method of determining the structural integrity of these particles. This property is evaluated indirectly by determining the balance between different forms of activity of acid hydrolases, marker enzymes for these organelles [2, 7]. Nonsedimented activity of the enzyme as a fraction (in per cent) of total activity is regarded as a measure of integrity of the lysosomes. The disadvantages

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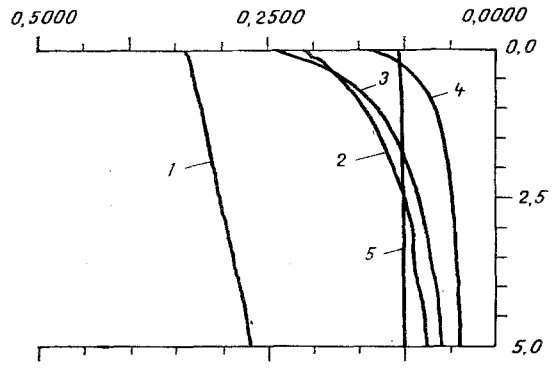

Fig. 1

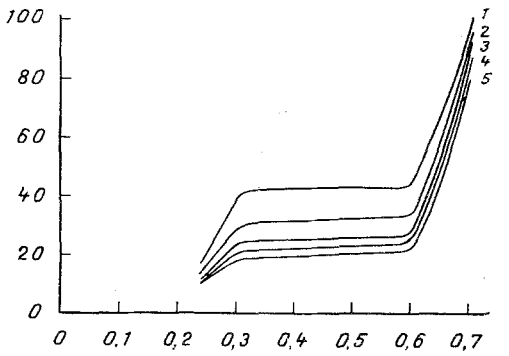

Fig. 2

Fig. 1. Original serial recording of changes in A_{520} of suspension of intact lysosomes depending on duration of incubation and molarity of sucrose solution. Abscissa, optical density (A_{520}) of lysosome suspension (in relative units); ordinate, duration of incubation (in min). 1-4) Molarity of sucrose solutions used as incubation medium was 0.7, 0.6, 0.3, and 0.25 M, respectively; 5; samples were incubated in distilled water.

Fig. 2. Dependence of optical density of suspension of intact lysosomes on molarity of sucrose solution at different times of incubation. Abscissa, sucrose concentration (in M); ordinate, percentage of initial value of A_{520}. 1-5) Samples were incubated for 1, 2, 3, 4, and 5 min, respectively.

of the method include the time-consuming and laborious nature of the analysis, the use of expensive reagents in short supply, and the low level of specificity of the determination. This last feature is due to the fact that a change in osmotic behavior of the medium may cause solubilization of membrane-bound hydrolases, without destruction of the lysosomes [2]. The results of calculation of the balance between forms of enzyme activity in this case will be distorted. Moreover, sensitivity to a change in osmotic pressure of the medium differs for different enzymes. This makes it difficult to compare results obtained with different acid hydrolases.

The aim of this investigation was to develop a method of assessment of osmotic resistance of isolated lysosomes, which is free from the disadvantages mentioned above.

EXPERIMENTAL METHOD

Intact noninbred male albino rats weighing 120-180 g were deprived of food for 48 h before the experiments. The liver was removed from decapitated animals, perfused on ice with ice-cold physiological saline (0.14 M NaCl), dried with filter paper, carefully freed from connective tissue, and weighed. A homogenate was prepared in a Potter-Elvehjem glass homogenizer with Teflon pestle (gap 0.21 mm) for 90 sec at 1200 rpm in 0.33 M sucrose (pH 7.4), containing 1 mM EDTA. An enriched lysosome fraction was obtained by differential centrifugation [8], resuspended in 0.7 M sucrose (pH 7.0) in the ratio of 1:0.2 (wet weight of tissue per volume) to a concentration of lysosomal protein of 5 mg/ml, kept at 0-4°C, and used as the original suspension.

The structural integrity of the lysosomes was judged by determining absorbance of the lysosomal suspension [3]. The original suspension was added to a solution of sucrose (pH 7.0), previously heated to 37°C, in the ratio of 1:100-150 (v/v) so that the initial extinction was about 0.4. This value was taken as 100% absorbance. The degree of destruction of the lysosomes corresponded to a fall of optical density of the samples, expressed as a percentage of this original value. Changes in extinction were recorded continuously during incubation for 5 min in a thermostated cuvette (diameter 1 cm) at 520 nm on a computerized recording spectrophotometer (Beckman, USA), programmed for the "Time drive" mode. The blind sample contained the same components with the exception of lysosomes. The rate of destruction of the lysosomes was measured as the rate of decrease of absorbance at 520 nm (ΔA_{520}/min), with operation programmed on the "Kinetics II" mode, and during incubation of the samples under the same conditions, but for 1 min. Sucrose solutions containing 1 mM EDTA were made up in phosphate-citrate buffer, whose pH was adjusted to 7.0 with concentrated NaOH solution. The molarity of the sucrose solutions varied from 0.7 to 0.25 M.

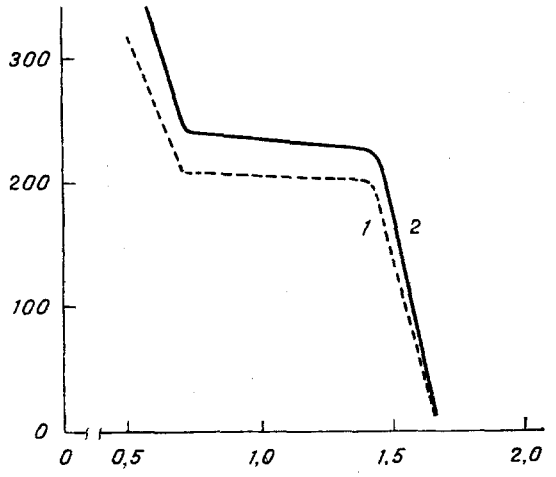

Fig. 3. Dependence of rate of destruction of isolated lysosomes on osmotic pressure of incubation medium and duration of keeping of original suspension on 0-4°C. Abscissa, value of the osmotic pressure of the incubated medium (in MP); ordinate, rate of reduction of A_{520} (in ΔA_{520}/min). Specimens were kept at 0-4°C for 16 (1) and 40 (2) hours.

EXPERIMENTAL RESULTS

The original graphic recording of changes in optical density of the lysosomal suspension during incubation in sucrose solutions of various concentrations for 5 min is illustrated in Fig. 1. Since the original values of A_{520} for the graphs obtained do not coincide, for convenience of analysis changes in A_{520} with time are expressed as percentages of the original value. It will also be clear from Fig. 1 that the process of disintegration of lysosomes under these conditions was linear in character with sucrose present in a concentration of 0.7 M. Reducing the sucrose concentration in the incubation medium sharply changed the character of this process, which became biphasic. Thus during the first 30 sec of incubation lysis of the lysosomes took place quickly, but later the rate diminished. The most significant changes in the structural integrity of the lysosomes were found when the concentration of the sucrose solution was 0.25 M. Under these conditions breakdown of the lysosomes almost reached its highest possible level (80%) in only 30 sec. Characteristically, the remaining curves all tended toward this limit (Fig. 1: 1, 2, and 3). Dependence of the degree of destruction of the lysosomes on molarity of the sucrose solution is shown in Fig. 2. It is complex in character. The degree of injury to the lysosomes remained constant between values of molarity of the sucrose solution from 0.6 to 0.3 M and depended only on the duration of incubation. The sharpest changes in resistance of lysosomes to the osmotic pressure of the incubation medium were found when the concentration of the sucrose solution fell from 0.7 to 0.6 and from 0.3 to 0.25 M. Characteristically, with lengthening of the duration of incubation, differences in the degree of damage to the lysosomes between sucrose concentrations of 0.6 and 0.25 M disappeared, and the value of this parameter tended to the upper limit of 90%. This rule could be seen more clearly in the dependence of the rate of lysosome destruction on the osmotic pressure of the incubation medium (Fig. 3). The maximal rate of destruction of the lysosomes occurred within a narrow range of pressure (from 1.6 to 1.4 MP), it remained virtually constant as the pressure fell from 1.4 to 0.7 MP, after which it again rose sharply. It will be clear from Fig. 3 that to calculate the initial rate of breakdown of the lysosomes, the original data shown in Fig. 1 can be used, for in the initial stage (up to 30 sec) this process followed a linear course in time, and the graph (Fig. 3: 1) had the same appearance as in the case of direct measurement of the rate of change of A_{520} by the "Kinetics II" program (Fig. 3: 2). The differences between these curves can be attributed only to different periods of keeping of the initial suspension at 0-4°C. After further incubation for 24 h at 0-4°C (Fig. 3: 2) the osmotic sensitivity of the lysosomes was increased throughout the range of osmotic pressure studied.

The dependence obtained, for osmotic resistance of isolated intact lysosomes, were thus relatively simple and sufficiently informative. For instance, it follows from Fig. 1 that after incubation of the lysosomal suspension for only 30 sec under these conditions, easily measurable changes in A_{520} could be obtained. Compared with the enzymic method, the total time of the analysis was reduced from 5 h to 30 min. Measurements can be made with the apparatus working on two different programs and the osmotic resistance of the lysosomes estimated as a percentage of the initial value of A_{520} (Figs. 1 and 2), or relative to the initial rate of destruction (Fig. 3). For comparative evaluation of the action of different physicochemical factors on the lysosomes in vivo and in vitro, the half-destruction time ($t_{1/2}$) is also an acceptable parameter. It can be calculated from the data in Fig. 1 as the time during which the number of lysosomes was reduced by half. The presence of a limiting value of the degree

of destruction of the lysosomes in Figs. 1 and 2 indicates that the residual value of A_{520} is determined by fragments of the lysosomal membranes. This also is proved by the addition of lysosomes to distilled water, which caused an instant drop of 70% in A_{520} from its initial value, but only 3% after incubation for 5 min (Fig. 1: 5). In this case the residual A_{520} was higher than in samples with sucrose (Fig. 1: 2-4), in view of the absence of any additional harmful action of the pH of the medium. On addition of Triton X-100 to the samples the value of A_{520} became 0. Consequently, the mechanism of destruction of the lysosomes on a change in concentration of the sucrose solution was osmotic and not detergent in nature. In the enzymic method the sensitivity of the lysosomes to a change in osmotic pressure of the medium was measured after preliminary treatment with H_2S at 20°C for 1 h, or after incubation for 15-30 min in 0.125 M sucrose or 0.12 M sucrose in 0.04 M Tris-acetate buffer (pH 7.4) [2]. It will be clear from Figs. 1 and 2 that under these conditions there were virtually no whole lysosomes. Consequently, in this particular case it was not the integrity of the lysosomes that was measured, but the individual sensitivity of the acid hydrolases to the solubilizing action of the osmotic pressure of the incubation medium. The method described above is free from this disadvantage.

Destruction of lysosomes in sucrose solutions with concentrations of between 0.7 and 0.25 M must be interpreted as the result of the combined action of three factors. The temperature of the incubation medium (37°C), the pH value of 7.0, and the concentration of the sucrose solution of below 0.3 M all have a labilizing influence. Under these conditions the osmolarity of the incubation medium above 0.3 M appears as a factor stabilizing the lysosomes. The fraction of these particles is characterized by a high degree of heterogeneity with respect to morphological and structural parameters. It can be tentatively suggested that within the concentration range of the sucrose solution from 0.7 to 0.6 M lysosomes most susceptible to a change in the osmotic pressure of the medium are destroyed. As Fig. 2 shows, these account for about 60% of the total number.

The lysosomal fraction was obtained by the traditional method of differential centrifugation. The diameter of the particles was 70-100 nm. Under these experimental conditions no sedimentation of lysosomes was observed.

The osmotic resistance of isolated lysosomes can be used as a parameter characterizing the dynamic structural-functional state of these particles and the degree of the damaging action of various physicochemical factors on them in vivo and in vitro. Both the change in A_{520} and ΔA_{520}/min, expressed as a percentage, and the value of $t_{1/2}$ can be used as a measure of this action. By varying the osmotic pressure of the incubation medium the force of the labilizing or stabilizing action of lysosomotropic substances and also the character of action (stabilizing or labilizing) of chemical preparations can be estimated.

Thus compared with the enzymic method, the method described above significantly shortens the time of analysis and the labor involved, it leads to economy in the use of expensive reagents, and it increases the specificity and universality of the determination.

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