INTRAVITAL DETERMINATION OF pH GRADIENT BETWEEN CYTOPLASM AND LYSOSOMES IN HUMAN FIBROBLASTS FROM NORMAL SUBJECTS AND PATIENTS WITH LYSOSOMAL STORAGE DISEASES

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Many lysosomal storage diseases have been shown to be based on a hereditary deficiency of one or other lysosomal hydrolase, or factors essential for the functioning of enzymes of this group. For example, the most numerous group of lysosomal storage diseases, the glycosidoses, is characterized by a wide range of defects involving glycosidases: reduced activity of these enzymes, disturbance of their intracellular transport into lysosomes, absence of protein stabilizers and activators essential for a definite organization and the functional activity of glycosidases [11]. Against the background of achievements in the identification of a particular lysosomal pathology, we have comparatively few data on biochemical parameters which are apparently not directly connected with the primary lysosomal enzyme defect, but which can play an important role in the regulation of intralysosomal processes. Parameters of this kind include, for example, the hydrogen ion concentration and the pH gradient between cytoplasm and lysosomes, thanks to which a nonequilibrium distribution of various low-molecular-weight organic compounds can be maintained throughout the cell, and which in turn, affects cell metabolism [4, 10, 15] and intercellular transport [9, 14]. The writers previously demonstrated a significant increase of pH in lysosomes of human fibroblasts during sucrose loading in vitro, a procedure which models to some degree lysosomal storage diseases [1].

The aim of this investigation was to study intralysosomal pH and also the pH gradient between cytoplasm and lysosomes in fibroblasts from normal human subjects and from patients with three typical lysosomal storage diseases, belonging to the glycosidosis group: mannosidosis (deficiency of acid α-D-mannosidase, EC 3.2.1.24), Fabry's disease (deficiency of ceramide-trihexoside-α-galactosidase, EC 3.2.1.22), and Krabbe's disease (deficiency of β-galactocerebrosidase, EC 3.2.1.46).

EXPERIMENTAL METHOD

Experiments were carried out on strains of human skin fibroblasts: normal embryonic (1075, 1214) and postnatal (1000, M-19), and also on pathological cells from patients with Fabry's (1026) and Krabbe's diseases (1018), and with mannosidosis (1136). All strains were obtained from the cell bank of the Institute of Medical Genetics, Academy of Medical Sciences of the USSR, and strain 19-19 was obtained from the Institute of Poliomyelitis and Virus Encephalitis. The measurements were made on the 8th day of the stationary phase of growth of the cells. Cells were grown on coverslips in penicillin flasks on Eagle's medium with the addition of 15% bovine serum, 0.3% glutamine, 50 U/ml kanamycin, and 10 mM HEPES buffer. The intralysosomal pH (pH_l) was determined by an absorption cytospectrophotometric method, with the aid of neutral red as the indicator dye [2]. The size of the lysosomes was estimated visually by comparison with the size of the measuring probe (diameter 1 μ, objective 100). The area of the lysosomes, expressed as the product of two diameters,

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TABLE 1. Value of Intralysosomal pH in Normal and Pathological Human Fibroblasts

Cells	pH_l	S_l, rel. units	ΔpH_{p-n}
Normal fibroblasts	6,36±0,06	1,76±0,22	—
Fibroblast in:			
Krabbe's disease	6,39±0,05	2,21±0,10	0,03
Fabry's disease	6,58±0,07	2,51±0,38	0,22
Mannosidosis	6,52±0,03	2,59±0,16	0,16

Legend. Here and in Table 2 mean weighted value of pH_l of normal fibroblasts of all strains studied is given (6.02; 6.44; 6.38; 6.33), ΔpH_{p-n}) deviation of pH_l in pathological cells relative to normal fibroblasts.

TABLE 2. Value of Intralysosomal pH in Lysosome Populations with about Equal Average Diameter of Normal and Pathological Human Fibroblasts

Cells	pH_l	S_l, rel. units	pH_{p-n}
Normal fibroblasts	6,42±0,05	2,62±0,03	—
Fibroblasts in:			
Krabbe's disease	6,42±0,05	2,60±0,01	0,00
Fabry's disease	6,60±0,05	2,63±0,03	0,18
Mannosidosis	6,51±0,03	2,61±0,01	0,09

was obtained in relative units. the error of the method is examined in detail in [6]. It depends on the value of pH_l, and it averages ±0.16 U. To observe the basic conditions of cytophotometry (the Bouguer Law), only those lysosomes whose size was larger than the measuring probe could be measured. For this reason, in normal fibroblasts not more than 10% of the total number of lysosomes was measured, and this led to exaggeration of the average pH_l. The writers showed previously that an increase in size of the lysosomes in cells in culture correlates with an increase in their pH [1, 2]. In pathological cells (except strain 1018) nearly all lysosomes could be measured, for as a rule their size increased during accumulation of nondegradable compounds, and for that reason the average pH corresponded sufficiently accurately to the real value. An artificial shift of pH_l toward the larger side for normal cells had the result that the increase in lysosomal pH revealed by this method during accumulation of undegradable compounds in them was always less than the true value. The pH of the cytoplasm (pH_c) was determined in individual cells with the aid of fluorescein diacetate [3]. The error of method did not exceed ±0.15 U. In each preparation pH was measured in no fewer than 20 cells and pH_l in 15-30 lysosomes. All the results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Fabry's and Krabbe's diseases belong to the group of sphingoglycoproteinoses, whereas mannosidosis belongs to the glycoproteinosis group. The hereditary deficiency of galactocerebrosidase in Krabbe's disease does not cause the accumulation of undegraded compounds, or an increase in size of the lysosomes of the skin fibroblasts, because these cells do not contain the substrate for the deficient enzyme. As a result the lysosomal compartment of the fibroblasts in Krabbe's disease does not differ from normal visually. In mannosidosis and Fabry's disease, intensive accumulation of incomplete hydrolysis products of macromolecules takes place in the skin fibroblasts (readily soluble oligosaccharides and insoluble heterogeneous compounds with predominance of ceramide-trihexoside respectively). In this case the dimensions of the lysosomes were appreciably increased, and in virtually all lysosomes the pH could be determined (Table 1). Measurement of the intralysosomal pH showed that in both normal and pathological fibroblasts an increase in size of the lysosomes correlated with an increase in their pH (Fig. 1). A similar relationship was found previously for human fibroblasts in model experiments with sucrose [1], and also for other types of cells in culture [7, 13].

TABLE 3. Values of pH_l and S_l in Heterogeneous and Homogeneous Lysosomes in Fabry's Disease and Mannosidosis

Disease	Average pH_l		Mean S_l, rel. units	
	homogeneous	heterogeneous	homogeneous	heterogeneous
Fabry's disease	6,34±0,06	6,78±0,08	2,00±0,20	3,85±0,60
Mannosidosis	6,45±0,02	6,67±0,03	2,54±0,12	2,69±0,12

TABLE 4. Values of mean pH_c and pH_l and of pH Gradient between Them in Normal and Pathological Fibroblasts

Cells	pH_c	pH_l	ΔpH_{c-l}
M-19	6,92±0,03	6,38±0,05	0,54:
Fibroblasts in:			
Krabbe's disease	6,97±0,07	6,39±0,05	0,58:
Fabry's disease	6,86±0,06	6,58±0,07	0,28:
Mannosidosis	6,98±0,06	6,52±0,03	0,46:

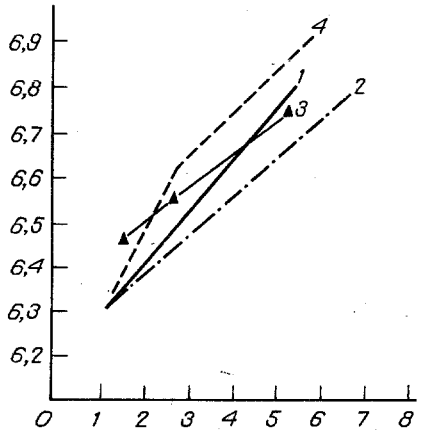

Fig. 1. pH as a function of size of lysosomes in normal and pathological fibroblasts. Abscissa, area of lysosomes (S_l, relative units); ordinate, intralysosomal pH (pH_l). 1) Normal human fibroblasts; pathological fibroblasts in: 2) Krabbe's disease, 3) mannosidosis, 4) Fabry's disease.

Comparison of the mean pH and size of the lysosomes in normal and pathological fibroblasts showed no visible increase in intralysosomal pH in Krabbe's disease, as would be expected. In two other strains of pathological cells whose lysosomes accumulated unhydrolyzed compounds, pH_l was increased on average by 0.2, to 6.58 ± 0.07 in Fabry's disease and 6.52 ± 0.03 in mannosidosis (Table 1). For lysosomes of the same size, in the first case did not differ, whereas in the other two cases it was higher (Table 2) than in normal fibroblasts (although this increase for cells in mannosidosis was not significant). The reason for this increase of pH is unknown but it can be postulated that accumulation of unhydrolyzed compounds may be accompanied by nonspecific inhibition of activity of intralysosomal macromolecules, including the proton pump, maintaining acidification of the intralysosomal medium [5, 8, 12, 15].

Lysosomes homogeneous and heterogeneous with respect to staining were found in pathological fibroblasts stained with neutral red. These lysosomes were particularly numerous in cells from the patient with Fabry's disease (Table 3). Lysosomes heterogeneous for staining were larger (3.85 ± 0.6 relative units) and their mean pH was 0.4 unit higher (6.73 ±

0.08) than in homogeneous organelles (2.00 ± 0.2 and 6.34 ± 0.06 relative units respectively). Lysosomes with a heterogeneous structure of their matrix also often had higher pH values than homogeneous organelles of the same size.

Measuring the pH of the cytoplasm in pathological and normal fibroblasts revealed no significant changes with respect to this parameter (Table 4). An increase of pH_l in the case of pathology, accompanied by relative constancy of pH of the cytoplasm, led to reduction of the pH gradient between these structures compared with normal. Lowering of the pH gradient between cytoplasm and lysosomes could be the cause of depression of functional activity of the cell also, as has been shown, for example, for the action of inhibitors of energy metabolism [2] and also for cystine transport from lysosomes [15].

Thus an increase in intralysosomal pH was found in pathological fibroblasts during accumulation of unhydrolyzed compounds in these organelles, accompanied by a marked decrease in the pH gradient between cytoplasm and lysosomes. An increase in pH of the lysosomes was observed both with an increase in size of these organelles and also in lysosomes of the same size, possibly due to reduction of work activity of the proton pump in the lysosomal membrane through nonspecific inhibition by an excess of accumulated compounds. An increase in pH of the lysosomes and decrease in the pH gradient between cytoplasm and lysosomes may lead to disturbance of the optimal conditions for functioning of lysosomal enzymes and, consequently, to delay and also, possibly, partial inhibition of intralysosomal hydrolysis.

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