Protection Effect of Polyhydroxyl Compounds on Heart Lysosomal Structures

Sucrose in isotonic concentration with regard to red cells (0.25 *M*) is ordinarily used in the preparation of tissue homogenates and in the fractionation of subcellular particles, due to the protecting effect of the sugar on the morphological and biochemical properties of cell structures. The protection does not depend only on the isotonicity, since salt solutions, isotonic for red cells, are not capable of exerting the same protection. This difference between non-electrolyte and electrolyte solutes with regard to the protecting effect upon subcellular structures is clearly evident in the study of latency of lysosomal enzymes[1,2].

In an attempt to investigate whether this property is specific for sucrose, we have compared its protective effect on heart lysosomes with that of other polyhydroxyl compounds. In particular we have established the lowest concentration of polyol capable of maintaining the catalytic latency of the lysosomal enzymes at the highest degree, best preserving the integrity of the structures with which the enzymes are associated.

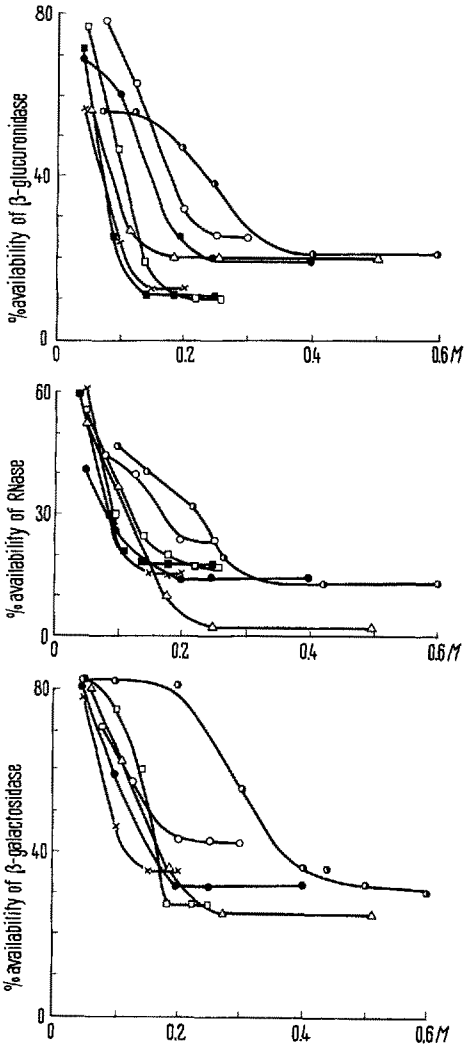
Beef heart lysosomes, prepared according to ROMEO et al.[3] ($M_1 + M_2$ fractions), have been washed with and resuspended in 0.4 *M* glucose (Erba, Italy), 0.4 *M* glucosamine (Sigma, USA), 0.25 *M* saccharose (Erba), 0.2 *M* raffinose (Erba), 0.25 *M* inositol (Merck, Germany) or 0.4–0.8 *M* glycerol (Erba), as well as in mixed solutions of glucose, fructose (Merck) and galactose (Merck), at a final concentration of 0.2 *M* for each hexose. The availability of β-glucuronidase (EC 3.2.1.31), acid ribonuclease (EC 2.7.7.17) and β-galactosidase (EC 3.2.1.23) to their substrates was then recorded at different concentrations of the polyols in the enzyme assays[3]. The availability values are calculated from the ratio between the free and the total activity[3], the latter being measured in the presence of 0.16% Triton X-100.

Experiments have shown that the availability of the acid hydrolases decreases as the concentration of the polyhydroxyl compounds increases in the enzyme assays (see Figure). As reported in the Table, the limits of the concentration range at which the minimal enzyme availability is reached are highest for the monosaccharide and lowest for the trisaccharide, whereas the disaccharide, the amino sugar and inositol occupy intermediate positions. For the mixtures of monosaccharides, the sum of the hexose concentrations coincides with that of glucose alone. However, the concentration of each monosaccharide is about that of the disaccharide if the mixture is formed by 2 hexoses, and is that of the trisaccharide if the mixture is formed by 3 hexoses. Experiments carried out with glycerol, at concentrations as high as 1.2 *M* in the enzyme assays, have shown that this polyol had a small, if any, protective effect[4].

Range of concentrations of polyhydroxyl compounds in the enzyme assays at which the lowest enzyme availability is reached (see Figure)

Glucose	0.38–0.44 *M*	Glucosamine	≤ 0.2 *M*
Glucose + fructose (each)	0.19–0.22 *M*	Saccharose	0.22–0.26 *M*
Glucose + fructose + galactose (each)	0.12–0.14 *M*	Raffinose	0.12–0.15 *M*
Inositol	0.20–0.24 *M*		

Thus, except for glycerol, all the polyhydroxyl compounds tested exert a stabilizing effect on lysosome structure, which may be explained as arising from a simple phenomenon of osmotic compensation. Since, however, for the saccharides tested there is a striking inverse relationship between the number of hydroxyl groups in the molecule and the concentration at which maximal protection occurs, a direct influence of the hydrophylic



Variation of availability of β-glucuronidase, acid ribonuclease and β-galactosidase to their substrates as a function of the concentration of polyhydroxyl compounds in the enzyme assays. The activity of the 3 hydrolases has been measured as reported by ROMEO et al.[3]. –◑– glucose; –△– saccharose; –×– raffinose; –□– glucose + fructose; –■– glucose + fructose + galactose; –○– glucosamine; –●– inositol.

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[4] The experiments with glycerol were complicated by the fact that the total activity of β-glucuronidase was increased and that of ribonuclease and β-galactosidase was decreased,

groups of the polyols on the membrane lipoproteins[3] cannot be ruled out. Deviations from this relationship are found for inositol and glucosamine, which, however, differ from the other polyols used in that the former bears only secondary hydroxyl groups and the latter has an additional even more polar group.

The fact that glycerol fails to protect the lysosomal structures, at least at the concentrations tested, may be due to its ability to penetrate rapidly into a lipoprotein membrane[5]. In fact, the tonicity of a solution for a membrane-limited organelle is determined by the degree of permeability of the membrane to the solute molecule. On the other hand, it is well known that sucrose, the most common polyol used to stabilize intracellular structures, does penetrate into a lipoprotein membrane, e.g. that of the mitochondrion[6]. One may then assume that also the other polyols penetrate into a lipoprotein membrane, their 'lipid-solubility' being the main factor responsible for the rate of their diffusion across the membrane[7]. If this is the case, an alternative explanation of our data would then be that the number and the type of polar groups in the polyol molecules would regulate the rate of penetration of the different solutes into the lysosome membrane and consequently determine, for each polyol, the concentration at which the highest protection of the lysosomal structures occurs[8].

Résumé. L'effet protecteur de la glucose, de la saccharose et de la raffinose et d'autres composés hydroxylés (glycérol, glucosamine et inositol) sur les lysosomes de cœur de bœuf a été étudié en suivant la variation de la latence catalytique des hydrolases acides (β-glucuronidase, ribonucléase et β-galactosidase). La plus faible activité catalytique libre (le maximum de protection sur la membrane lysosomale) a été observée à différentes concentrations des polyoles, la plus basse étant celle de la raffinose, la plus élevée celle de la glucose et entre les deux celle de la saccharose, de la glucosamine et de l'inositol. Le glycérol ne donne aucune protection dans les conditions expérimentales indiquées dans l'article.

D. ROMEO, N. STAGNI
and M. C. PUGLIARELLO

Istituto di Chimica Biologica dell'Università di Trieste (Italy), 28th November 1966.

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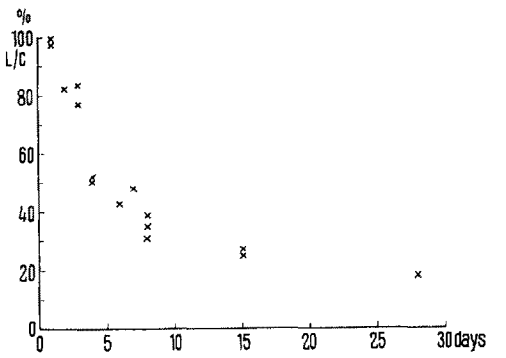
Secretory Responses and Choline Acetylase of the Rat's Submaxillary Gland After Duct Ligation

Ligation of the duct of the rat's submaxillary gland causes a marked glandular atrophy[1]. Histologically the decrease in the size of the gland has been found to correspond to an atrophy of the acini while the tubules are less affected[2]. In the present investigation, the secretory function of the duct-ligatured submaxillary gland of rats was determined by measuring the secretory responses to sialagogue drugs. This seemed to be of particular interest since the tubules are very likely of great importance for the production of saliva (see OHLIN[3]). In addition, the effect of duct ligation on the parasympathetic neurones of the gland was studied by estimation of the activity of choline acetylase. The acetylcholine-synthesizing enzyme is localized in the cholinergic fibres of salivary glands[4].

28 female rats weighing about 200 g were used. The right submaxillary duct was ligatured in all rats. In 15 rats the secretory responses to sialagogue agents were determined and in 13 the activity of choline acetylase.

To study the secretory responses, the rats were anaesthetized with chloralose (100 mg/kg) i.v. after preliminary ether. The submaxillary ducts were cannulated with fine glass cannulae giving about 100 drops out of 1 ml of distilled water. The amount of saliva secreted appearing at the tip of the cannula was noted after i.v. injections of 20 μg/kg adrenalin, 10 μg/kg methacholine, and pilocarpine given in increasing doses of from 50–1000 μg/kg every 30–60 sec until the maximal secretory rate was reached. The secretory responses were studied 1 day to 4 weeks (see Figure) after the duct was ligatured. At the end of the experiments, the position of the ligature was controlled and the submaxillary glands were dissected, carefully cleaned and weighed.

The activity of choline acetylase in the submaxillary gland was determined 1 week after duct ligation, as previously described[4]. The enzyme activity was estimated in 2 groups of rats; 1 group contained pooled glands from 6 animals and the other from 7. The activity is expressed as total activity, μg acetylcholine/h/gland, and concentration, μg acetylcholine/h/g acetone powder.



The weight of the rat's submaxillary gland after duct ligation (= L) in % of that of the control gland (= C) at different time intervals.

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