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THE PERMEABILITY OF THE LYSOSOMAL MEMBRANE TO SMALL IONS

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Summary

The permeability of the lysosomal membrane to small anions and cations was studied at 37°C and pH 7.0 in a lysosomal-mitochondrial fraction isolated from the liver of untreated rats. The extent of osmotic lysis following ion influx was used as a measure of ion permeancy. In order to preserve electroneutrality, anion influx was coupled to an influx of K^+ in the presence of valinomycin, and cation influx was coupled to an efflux of H^+ using the protonophore 3-*tert*-butyl-5,2′-dichloro-4′-nitrosalicilylanilide. Lysosomal lysis was monitored by observing the loss of latency of two lysosomal hydrolases.

The order of permeability of the lysosomal membrane to anions was found to be $SCN^- > I^- > CH_3COO^- > Cl^- \approx HCO_3^- \approx P_i > SO_4^{2-}$ and that to cations $Cs^+ > K^+ > Na^+ > H^+$. These orders are largely in agreement with the lyotropic series of anions and cations.

The implications of these findings for the mechanism by means of which a low intralysosomal pH is produced and maintained are discussed.

Introduction

The lysosomal hydrolases have low pH optima [1] and it is now well-established that the intralysosomal matrix is acidic [see refs. 2—4]. Studies on the distribution of the weak base methylamine [5—8] have demonstrated a pH gradient across the lysosomal membrane of approximately 1 unit at physiological pH values. Several mechanisms have been proposed for the generation and maintenance of the lysosomal ΔpH. These include an ATP-driven H^+ pump [9], and a Donnan equilibrium resulting from the presence of non-diffusible anions at the lysosomal interior [10,11] and a selective permeability of the lysosomal membrane to cations [8]. A third mechanism has been proposed by Reijngoud

Abbreviations: S-13, 3-*tert*-butyl-5,2′-dichloro-4′-nitrosalicilylanilide; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid.

et al. [12]. This mechanism also invokes the presence of intralysosomal, negatively-charged groups (in equilibrium with their protonated forms), but it is suggested that H^+ influx or efflux (or OH^- efflux or influx, respectively) is accompanied by a stoichiometric movement of other ions, which prevents a build-up of a transmembrane electrical potential. Much controversy exists concerning which of these mechanisms applies to the in vivo situation.

In order to envisage how the low intralysosomal pH is produced in the cell, it is essential that there be more information than is at present available on the permeability of the lysosomal membrane to small ions under conditions similar to those in vivo. Up to now most studies on the ion permeability of the lysosomal membrane have been carried out under extremely unphysiological conditions. Henning [8] studied the relative permeability to cations of tritosomes, i.e. lysosomes prepared from rats injected with Triton WR-1339. In addition to perturbations to the membrane which might be caused by the detergent, the elevated cholesterol content of lysosomes prepared in this way [13] may alter the permeability properties of the lysosomal membrane considerably (see refs. 14—16). Furthermore, the investigations of Henning [8] were carried out at 0°C as were those of Goldman and Rottenberg [5] on the permeability of the lysosomal membrane to H^+ and K^+. At this temperature the permeability characteristics of the lysosomal membrane are quite different to those at 37°C (ref. 17; see also ref. 7).

In the study described here, the relative permeability of the unmodified lysosomal membrane to small ions at 37°C and at pH 7.0 has been examined. Relative ion permeability was determined by observing the extent of osmotic lysis * of lysosomes suspended in slightly hypertonic solutions of various salts. Lysosomal lysis was measured as release of latency of acid phosphatase and β-hexosaminidase, two intralysosomal hydrolases having a high degree of latency [18]. Observation of release of latency of lysosomal hydrolases has been used previously to study the permeability of the lysosomal membrane to carbohydrates [19], amino acids and small peptides [20] and other compounds (see Reijngoud and Tager [4] for a review). Using this technique, specific information concerning the permeability of the lysosomal membrane can be obtained using impure, but relatively unperturbed, lysosomal preparations.

In our study, the relative permeability of the lysosomal membrane to small anions was determined by using potassium salts in the presence or absence of the K^+-specific, electrogenic ionophore valinomycin and that to monovalent cations by using salts of the permeant anion iodide in the presence or absence of the protonophore 3-*tert*-butyl-5,2′-dichloro-4′-nitrosalicilylanilide (S-13).

Materials and Methods

Preparation of a lysosome-enriched fraction from rat liver. Male rats of the Wistar Albino Glaseo strain were killed by decapitation. Their livers were removed immediately and minced and washed with ice-cold 0.35 M-sucrose, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid (HEPES),

* The term lysis is used here to denote any extensive breakdown in the structural integrity of the lysosomal membrane.

pH 7.0. The resulting mixture was homogenized using a motor-driven, Teflon-in-glass homogeniser and the homogenate centrifuged at 4°C and 500 $\times g_{max}$ for 10 min in the 69404 rotor of an MSE Mistral 6L centrifuge. The supernatant was centrifuged for a further 20 min at 4°C and 18 000 $\times g_{max}$ as above. The resulting lysosome-enriched pellet was suspended in a small volume of ice-cold 0.35 M sucrose/10 mM HEPES, pH 7.0 and placed on ice until required. All experiments were carried out within 2 h of the rats being killed.

Measurement of free enzyme activity. Following incubation of the lysosomal fraction under the conditions described in the figure and table legends, 0.275 ml aliquots of the incubation mixture were assayed for acid phosphatase (EC 3.1.3.2) activity according to the method of Appelmans and de Duve [22], except that the aliquot was added to 0.475 ml of 132 mM sodium acetate/526 mM sucrose/84 mM disodium β-glycerophosphate, pH 5.0. Aliquots of 0.05 ml of the incubation mixture were assayed for β-hexosaminidase (EC 3.2.1.30) by the method of Rietra et al. [23], except that the aliquot was added to 0.25 ml of 100 mM sodium acetate/400 mM sucrose/5 mM *p*-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside, pH 5.0.

Total enzyme activities were determined as above, using suitably diluted samples of the lysosomal fraction which had been sonicated on ice at 4 μM peak to peak in an MSE sonicator. Sonication was for 1 min/ml of sample carried out in periods of 15 s with 45 s cooling.

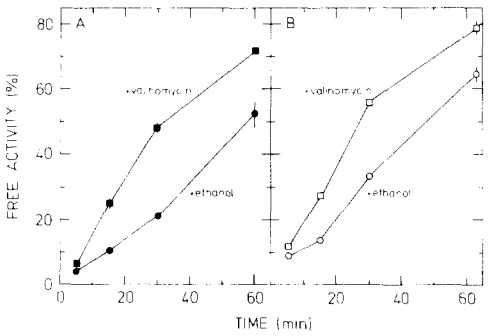
Protein was determined by the method of Yonetani [24]. Disodium β-glycerophosphate (Grade I) and valinomycin were purchased from Sigma Chemical Co. Ltd. (St. Louis, Mo., U.S.A.). *p*-Nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside was purchased from Koch-Light Laboratories Ltd., Colnbrook, U.K. S-13 was a gift from Dr. P. Hamm, Monsanto Co., St. Louis, U.S.A.

Results

1. Valinomycin-stimulated release of latency of lysosomal enzymes

The possibility of inducing entry of a permeant anion into lysosomes by generating an electrogenic K^+ influx was investigated by observing release of latency of enzymes in lysosomes incubated in KI medium in the presence and absence of the K^+-ionophore valinomycin. I^- was chosen as an anion which would probably be highly permeant across the lysosomal membrane, as it is in several other model and biological membranes [25—27], perhaps permeating in the form of I_3^- [28] or I_5^- [26], [28]. There was a time-dependent increase in the free activities of acid phosphatase and of β-hexosaminidase, when lysosomes were incubated in KI medium at 37°C and this was stimulated considerably by valinomycin (Fig. 1).

In this experiment and those reported below, the release of latency of β-hexosaminidase appears to be greater than that of acid phosphatase. It has been suggested that intralysosomal enzymes are unevenly distributed amongst lysosomes from parenchymal and non-parenchymal liver cells [29], [30]. Those lysosomes containing a higher proportion of β-hexosaminidase may be slightly less stable than the others, resulting in greater release of this enzyme than of acid phosphatase.



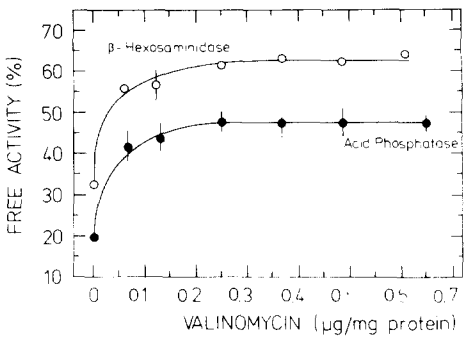


Fig. 1. Time course of latency of acid phosphatase and β-hexosaminidase in lysosomes supsended in KI medium. Two samples of the lysosomal suspension (1.45 ml, 100.5 mg protein) were added to two 5.8 ml aliquots of 200 mM KI/10 mM HEPES, pH 7.0, 15 μl 10 mg/ml valinomycin in ethanol was added to one sample and 15 μl of ethanol to the other. Both samples were incubated at 37°C. Periodically, samples of the ethanol- (●,○) and valinomycin- (■,□) containing mixtures were assayed for acid phosphatase (A) and β-hexosaminidase (B) activity as described in Materials and Methods. Results are the means and S.D. of three determinations. 100% acid phosphatase activity was 69.0 ± 3.7 nmol/min per mg protein. 100% β-hexosaminidase activity was 17.0 ± 0.3 nmol/min per mg protein.

Fig. 2. Concentration dependence of valinomycin-induced release of latency of lysosomal enzymes. The lysosomal suspension (0.5 ml, 20.5 mg protein) was added to 2 ml of 200 mM KI/10 mM HEPES, pH 7.0. Valinomycin was added from ethanolic solutions to give the concentrations indicated. The volume of ethanol was always adjusted to 15 μl. After 30 min incubation at 37°C, aliquots of the incubation mixture were assayed for acid phosphatase (●) and β-hexosaminidase (○) activity. Results are the means and S.D. of three determinations. 100% acid phosphatase activity was 56.9 ± 1.8 nmol/min per mg protein. 100% β-hexosaminidase activity was 19.7 ± 0.5 nmol/min per mg protein.

In the absence of valinomycin, the initial rate of latency release was lower than that observed subsequently. This latency release may have been due, at least in part, to non-osmotic lysosomal breakdown. A similar effect is seen at 37°C when lysosomes are suspended in a medium containing the impermeant solute sucrose [31]. Part of this initial latency release, however, may have been due to osmotic lysis following the slow influx of K^+ together with I^- (see below). Ethanol had no effect on enzyme release (results not shown). After 30 min incubation, the rate of enzyme release was approximately the same in the absence and presence of valinomycin. In all experiments reported below, latency release was measured after incubation for 30 min.

The dependence of latency release on valinomycin concentration is shown in Fig. 2. Release becomes saturated at approximately 0.25 μg valinomycin/mg protein. As shown in Fig. 3, valinomycin-induced latency release is strongly inhibited by high concentrations of sucrose.

The above results indicate that valinomycin-induced latency release occurs via osmotic lysis of lysosomes as a consequence of a coupled K^+/I^- influx.

2. Anion permeability of the lysosomal membrane

The observation of osmotic lysis of lysosomes resulting from a coupled K^+/anion influx, as discussed above, was extended to investigate the permeability of the lysosomal membrane to various other anions. Lysosomes were suspended in a number of potassium-salt media and the release of latency of acid phosphatase and β-hexosaminidase was measured in the presence and absence of a

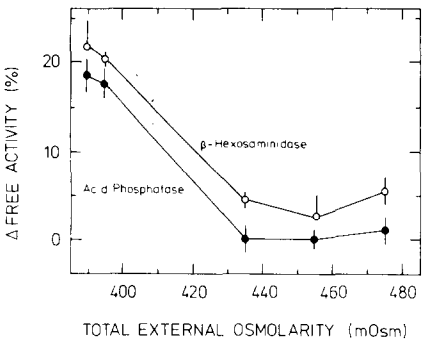


Fig. 3. Effect of external osmolarity on valinomycin-induced release of latency of lysosomal enzymes. To 1.6 ml of 200 mM KI/10 mM HEPES, pH 7.0 was added 0.4 ml (7.02 mg protein) of the lysosome-enriched fraction suspended in 10 mM HEPES, pH 7.0 and sufficient sucrose to give the final osmolarity indicated. After adding 13 μl of 1 mg/ml valinomycin, the mixture was incubated at 37°C for 30 min. Aliquots were then assayed for acid phosphatase (●) and β-hexosaminidase (○) activity. For each osmolarity the difference between the free activity in the presence and absence of valinomycin was calculated, and the mean and S.D. of 3 such pairs is shown. 100% acid phosphatase activity was 76.6 ± 5.2 nmol/min per mg protein. 100% β-hexosaminidase activity was 22.1 ± 0.5 nmol/min per mg protein.

saturating concentration of valinomycin, and in the presence of valinomycin and of a high concentration of sucrose (see Table I).

The release of latency in the absence of valinomycin varied according to the composition of the medium. This may reflect osmotic lysis due to permeation of the anions and a natural K^+ permeability (see section on cation permeability) or to differences in the chaotropic nature of the various anions.

The presence of valinomycin caused increased latency release in all salt media except sulphate and in all cases this increase was inhibited by a high concentration of sucrose, indicating that it occurred via osmotic lysis. No valinomycin-induced increase in latency release occurred in sucrose medium, excluding the possibility of any degradative effects of the ionophore on the lysosomal membrane.

In KSCN medium the background release of latency of β-hexosaminidase was very high and masked any possible effects of valinomycin. However, the background release of latency of acid phosphatase in this medium was lower and a clear valinomycin-induced increase in latency release can be seen. This variation in background latencies again suggests that the bulk of the β-hexosaminidase may be stored in lysosomes which are less stable, and therefore more subject to the chaotropic activities of SCN^-, than the remainder.

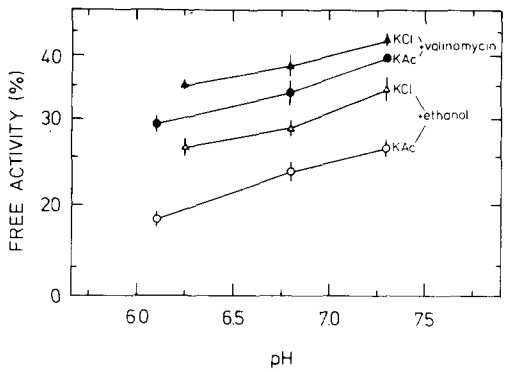
The effect of valinomycin on lysosomes in potassium acetate medium is of particular interest. Although the stimulation of osmotic lysis by valinomycin suggested that the swelling was the result of a coupled K^+/CH_3COO^- influx, the possibility existed that it was due to increased acetic acid influx, occurring as a consequence of a K^+/H^+ exchange and the subsequent increase in intralysosomal pH. This possibility was investigated by studying the effect of external pH on the release of latency of lysosomes suspended in KCl and in CH_3COOK media in the absence and presence of valinomycin. As shown in Fig. 4, lysis increased as the external pH was raised. The increase in lysis with

TABLE I

RELEASE OF LATENCY OF ACID PHOSPHATASE AND β-HEXOSAMINIDASE IN LYSOSOMES SUSPENDED IN VARIOUS POTASSIUM SALT MEDIA AND IN SUCROSE

To 1.2 ml of a solution containing 400 mosM of the solute indicated (buffered at pH 7.0 and prepared as described below) was added 0.3 ml of the lysosome-enriched fraction (7.27 mg protein) suspended in sucrose (350 mM or, in the high osmolarity incubations, 675 mM) and 10 mM HEPES, pH 7.0. After adding 10 μl of 1 mg/ml valinomycin or 10 μl of ethanol, the suspensions were incubated at 37°C for 30 min. Aliquots of the incubation mixture were then assayed for acid phosphatase and β-hexosaminidase activity. Results are the means and standard deviations of three determinations. The $KHCO_3$ solution contained 70 mM HEPES and was prepared and adjusted to pH 7.0 immediately before the experiment. The potassium acetate solution was prepared by adjusting 400 mM potassium acetate to pH 7.0 with 400 mM acetic acid and adding an equal volume of 20 mM HEPES, pH 7.0. The potassium phosphate solution was prepared by adjusting 400 mM KH_2PO_4 to pH 7.0 with 400 mM KOH and adding an equal volume of 20 mM HEPES, pH 7.0. The KI, KCl, K_2SO_4 and sucrose solutions contained 10 mM HEPES, pH 7.0.

Addition to incubation medium	Release of latency (%) after incubation of lysosome in media containing							
	KSCN	KI	CH_3COOK	$KHCO_3$	K_2HPO_4	KCl	K_2SO_4	Sucrose
Acid phosphatase								
Ethanol	50.2 ± 1.4	31.0 ± 1.9	28.0 ± 2.5	13.2 ± 0.8		16.6 ± 1.9	15.6 ± 1.7	11.8 ± 0.5
Valinomycin	84.8 ± 4.2	56.0 ± 1.0	37.5 ± 0.2	20.0 ± 0.4		23.5 ± 1.3	16.5 ± 2.0	11.1 ± 0.3
Valinomycin (high osmolarity)	51.4 ± 3.0	30.0 ± 3.3	19.0 ± 1.4	11.9 ± 0.2		13.0 ± 0.9	15.8 ± 2.6	10.3 ± 1.1
β-Hexosaminidase								
Ethanol	81.7 ± 3.4	46.5 ± 2.4	43.5 ± 1.8	28.7 ± 2.4	27.5 ± 1.4	31.9 ± 1.8	30.8 ± 1.7	27.0 ± 0.6
Valinomycin	88.9 ± 6.1	68.9 ± 3.1	53.7 ± 2.8	36.2 ± 1.8	35.1 ± 1.7	39.0 ± 1.2	31.8 ± 0.6	26.4 ± 1.0
Valinomycin (high osmolarity)	73.3 ± 1.8	34.4 ± 2.3	34.4 ± 2.2	27.2 ± 1.7	27.3 ± 0.8	29.7 ± 1.6	31.6 ± 1.1	25.6 ± 0.8
Total activity (nmol/min per mg protein)								
Acid phosphatase	52.2 ± 0.8	53.2 ± 1.3	53.3 ± 1.9	44.6 ± 0.3		53.4 ± 1.4	38.4 ± 2.0	53.2 ± 4.7
β-Hexosaminidase	16.5 ± 0.8	17.2 ± 0.9	16.0 ± 1.2	14.7 ± 0.91	15.9 ± 0.8	18.4 ± 1.1	16.0 ± 1.0	16.2 ± 0.7



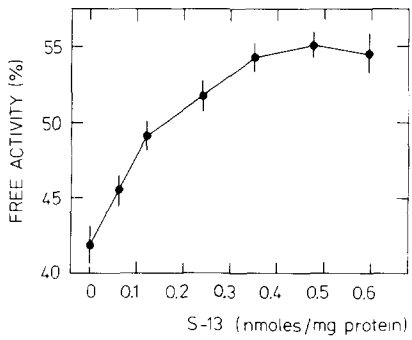


Fig. 4. Effect of pH of medium on the release of latency of acid phosphatase in lysosomes incubated in potassium acetate or KCl medium. The lysosomal suspension (0.25 ml, 17.5 mg protein) was added to 1 ml of 200 mM CH_3COOK/10 mM HEPES, or 200 mM KCl/10 mM HEPES, giving the pH indicated. After adding 4 μl 2.5 mg/ml valinomycin or 4 μl ethanol, the mixture was incubated at 37°C for 30 min. Aliquots from the CH_3COOK- (●,○) and KCl- (▲,△) containing mixtures were then assayed for acid phosphatase activity. (100% acid phosphatase activities were determined separately under each set of incubation conditions). Results are the means and S.D. of three determinations. Abbreviation: KAc, CH_3COOK.

Fig. 5. Stimulation by S-13 of valinomycin-induced release of latency of acid phosphatase in lysosomes suspended in KI medium. The lysosomal suspension (0.2 ml, 6.7 mg protein) was added to 0.8 ml of 200 mM KI/10 mM HEPES, pH 7.0. 10 μl of 1 mg/ml valinomycin was added followed by S-13 from an ethanolic solution to give the concentration indicated. The volume of ethanol was always adjusted to 20 μl. After 30 min incubation at 37°C, aliquots of the incubation mixture were assayed for acid phosphatase activity, 100% activity being 77.4 nmol/min per mg protein. Results are the means and S.D. of four determinations.

increasing pH in CH_3COOK medium would not occur if acetic acid were the only permeant species as the proportion of acetic acid decreases with increasing pH. The similar pH-dependence of valinomycin-induced lysis in both KCl and CH_3COOK media suggests that in both cases lysis occurred via the same mechanism, i.e. a K^+/anion influx. We conclude, therefore, that the osmotic lysis of lysosomes in CH_3COOK medium was indeed due to a K^+/CH_3COO^- influx.

The question arises of the relative permeability of the lysosomal membrane to CH_3COO^- and Cl^-. According to Fig. 4, the valinomycin-induced increase in latency release at pH 7.0 is significantly greater in CH_3COO^- than in Cl^- medium. In Table I however, the effect of valinomycin in CH_3COO^- medium is only slightly greater than that in Cl^- medium and the difference is within the experimental error. In Table II, are shown the results of four further experiments where valinomycin-induced latency release in CH_3COO^- and Cl^- media was examined. In all cases valinomycin caused a greater increase in latency release in CH_3COO^- than in Cl^- medium. We conclude from this and the data of Table I that the lysosomal membrane is permeable to the anions tested in the order $SCN^- > I^- > CH_3COO^- > Cl^- \approx HCO_3^- \approx P_i > SO_4^{2-}$. At pH 7.0, P_i is present almost completely as HPO_4^{2-} and $H_2PO_4^-$. It is not possible to say which of these is the permeant species.

3. Cation permeability of the lysosomal membrane

(i) Permeability to H^+. Of particular importance with regard to the mechanism of the maintenance of a low intralysosomal pH is the question of the permeability of the lysosomal membrane to H^+. To investigate this the effect

TABLE II

VALINOMYCIN-INDUCED RELEASE OF LATENCY OF LYSOSOMAL ENZYMES IN KCl AND CH_3-COOK MEDIA

The free activities of acid phosphatase and β-hexosaminidase were determined after incubation of lysosomes for 30 min at 37°C in 200 mM KCl/10 mM HEPES, pH 7.0 or in 200 mM CH_3COOK/10 mM HEPES, pH 7.0 in the presence of ethanol or valinomycin. The experimental conditions were as described in the legend to Table I, except that the samples of lysosome-enriched fractions contained 8.5—9.6 mg protein and were always suspended in 350 mM sucrose/10 mM HEPES, pH 7.0. For each experiment, the difference between the free enzyme activities in the presence or absence of valinomycin was calculated and the mean ± S.D. for three such pairs is shown.

Enzyme	Exp.	Valinomycin-induced release of latency (%)	
		CH_3COOK medium	KCl medium
Acid phosphatase	1	17.0 ± 3.1	5.3 ± 1.9
	2	14.2 ± 3.6	5.8 ± 2.1
β-Hexosaminidase	3	12.4 ± 2.0	8.1 ± 1.3
	4	15.1 ± 1.7	6.3 ± 3.4

was studied of the protonophore S-13 on the valinomycin-induced release of latency of acid phosphatase from lysosomes suspended in KI medium (Fig. 5). There was a 42% enzyme release in the presence of valinomycin alone and this increased in a concentration-dependent manner to a maximum of 55% on addition of S-13. This increase can be explained by an S-13 stimulated exchange of intralysosomal H^+ for K^+ in the medium, in the presence of valinomycin (cf. ref. 4). Since intralysosomal H^+ is in equilibrium with ionizable groups, the K^+/H^+ exchange causes an increase in intralysosomal osmolarity further to that

TABLE III

RELEASE OF LATENCY OF ACID PHOSPHATASE AND β-HEXOSAMINIDASE ON INCUBATING LYSOSOMES IN CESIUM, POTASSIUM, OR SODIUM IODIDE.

To 1.2 ml of a solution containing 360 mOsM of the salt indicated and 10 mM HEPES, pH 7.0, was added 0.3 ml of a lysosome-enriched fraction (23.5 mg protein) and 10 μl of ethanol or 10 μl of 400 μM S-13. After incubation for 30 min at 37°C, aliquots of the suspension were removed for measurement of acid phosphatase and β-hexosaminidase activity as described in Materials and Methods. Results are means and S.D. of three determinations.

Addition to incubation medium	Release of latency (%) after incubation of lysosomes in		
	CsI	KI	NaI
Acid phosphatase			
Ethanol	19.3 ± 1.0	17.5 ± 1.0	17.9 ± 1.0
S-13	27.5 ± 0.0	22.9 ± 0.8	20.6 ± 0.5
S-13 (high osmolarity)	20.9 ± 1.3	19.8 ± 1.1	19.0 ± 1.3
β-Hexosaminidase			
Ethanol	29.1 ± 0.2	26.4 ± 0.8	26.7 ± 0.8
S-13	39.2 ± 1.2	32.4 ± 0.6	29.5 ± 0.9
S-13 (high osmolarity)	30.0 ± 1.0	22.8 ± 1.0	28.6 ± 1.5
Total activity (nmol/min per mg protein)			
Acid phosphatase	63.2 ± 1.2	56.9 ± 2.8	57.8 ± 0.3
β-Hexosaminidase	24.2 ± 0.2	21.7 ± 0.5	27.0 ± 1.9

caused by K^+/I^- influx, leading to enhanced osmotic lysis. These data indicate therefore, that at 37°C and pH 7, the lysosomal membrane does provide some barrier to H^+ movement.

(ii) Permeability to Na^+, K^+ and Cs^+. In the experiment of Table III, the permeability of the lysosomal membrane to Cs^+, K^+ and Na^+ was compared by suspending lysosomes in 180 mM solutions of the iodide salts of these cations with and without S-13. In the absence of S-13, the release of latency of the two enzymes was slightly greater in CsI than in KI or NaI. Addition of S-13 brought about a further release of latency, this effect being most pronounced in CsI and least in NaI. In all media the effects of S-13 were inhibited by the presence of a high concentration of sucrose, indicating that release of latency occurred via osmotic lysis of the lysosomes. This increased lysis in the presence of S-13 can be attributed to an enhanced cation/H^+ exchange (cf. Fig. 5), and, in the presence of the protonophore, cation influx will be rate-limiting. Although the differences in the extent of release of latency in the presence of S-13 were small, they were significant, and indicate that the permeability of the lysosomal membrane to the cations is in the order $Cs^+ > K^+ > Na^+$. Furthermore, all three cations are more permeant than H^+.

Discussion

Using the ionophore valinomycin, it is possible to induce an electrogenic influx of K^+ across the lysosomal membrane thus favouring anion entry. This leads to osmotic lysis of the lysosomes and release of internal enzymes. The order of anion permeancy as measured in this way is $SCN^- > I^- > CH_3COO^- > Cl^- \approx P_i \approx HCO_3^- > SO_4^{2-}$. This order is mainly consistent with the lyotropic series of anions as determined by Voet [19]. The position of an ion in this series is dependent on its energy of hydration or, more specifically, the strength of its associated electrostatic field (with the ionic field strength increasing in the above series). The lyotropic series influences anion permeability in some (see refs. 27, 32, 33) but by no means all (see ref. 34) biological membranes. In the case of the lysosomal membrane, it is reasonable to conclude that the lyotropic series is the main determinant of anion permeability.

We consider it unlikely that the chaotropic nature of the various anions influenced the above order. Whilst chaotropic activities may have had some effect on background latency levels, the observation of valinomycin-induced osmotic lysis ensures that only permeability differences are being considered.

The observation of a higher permeability to CH_3COO^- than to Cl^- is a slight deviation from the lyotropic series, according to which Cl^- would be expected to be more permeant than CH_3COO^-. This may be due to the fact that CH_3COO^-, unlike the other anions tested, is organic and this might affect its permeability and lyotropic characteristics differently. The possibility that valinomycin-induced osmotic lysis of lysosomes suspended in acetate medium was not due to anion entry but occurred via influx of the acid subsequent to a K^+/H^+ exchange and a rise in intralysosomal pH, is excluded by two observations. First, there is a strong similarity between the effects of pH on valinomycin-induced loss of latency of acid phosphatase from lysosomes suspended in KCl and CH_3COOK medium (Fig. 4). Second, K^+ appears to be more

permeant across the lysosomal membrane than H^+, which makes it very unlikely that valinomycin would stimulate a K^+/H^+ exchange.

The permeability of the lysosomal membrane to cations at 37°C varies in the order, $Cs^+ > K^+ > Na^+$ and this is, again according to the lyotropic series of ions [21]. The order ($Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$) of ability of monovalent cations to exchange with intratritosomal H^+ at 0°C [8] is consistent with that observed here. However, contrary to the conclusion of Henning [8] that H^+ was anomalously the most permeant cation (H^+ has the highest ionic field strength [35]) we found H^+ to be the least permeant of the cations tested.

Davidson and Song [17] demonstrated that lysosomes suspended at 37°C in isotonic KCl medium are less stable than those suspended in NaCl medium, from which they concluded that the lysosomal membrane is more permeable to K^+ than to Na^+. Our results are in agreement with this conclusion and our demonstration of inhibition of S-13 induced latency release by high external sucrose concentration confirms that osmotic effects are being observed.

Verity and Brown [36] studied the release of latency of enzymes from lysosomes suspended in media containing KCl, $NH_4H_2PO_4$ and CH_3COONH_4. They saw slow latency release in KCl medium and concluded that KCl is relatively impermeant across the lysosomal membrane. However, they did not consider what the rate-limiting step in KCl entry is; according to our results it is K^+ movement. From their observation of a high latency release in $NH_4H_2PO_4$ and CH_3COONH_4 media, Verity and Brown [36] concluded that the lysosomal membrane is permeable to NH_4^+, CH_3COO^- and PO_4^{3-}. The latter conclusion is unjustified considering that the proportion of phosphate present as PO_4^{3-} at the pH they used (7.5) is approx. 0.0001% [37]. Furthermore their results are confused by the extensive lysis which they observed in both media within the first 5 min of incubation, probably resulting from the permeation of NH_3 and concentration of NH_4^+ at the acidic lysosomal interior [4].

We believe that the investigation reported here is the first extensive study of the permeability of the untreated lysosomal membrane to anions and cations at physiological temperature and pH. Furthermore, the above findings have important implications concerning the mechanism of production and maintenance of the low intralysosomal pH.

Henning [8] investigated the ability of various types of metal ion to raise the internal pH of tritosomes at 0°C by exchanging with internal H^+. He concluded that the lysosomal membrane was much more permeable to H^+ than to K^+ (or other monovalent cations) and that, on this basis, the lysosomal ΔpH could be explained by a Donnan equilibrium owing to the presence of impermeant, internal, negatively charged groups. Our results indicate, however, that the untreated lysosomal membrane at 37°C has a low permeability to H^+ and a higher permeability to K^+. The low proton permeability would clearly disfavour an equilibrium distribution of H^+. The main objection to a Donnan equilibrium mechanism, however, is presented by the permeability of the lysosomal membrane to K^+. In the cytosol the concentration of K^+ is >100 mM [38]. Consequently a Donnan distribution of H^+ leading to a ΔpH of 1 unit would necessitate that the intralysosomal K^+ concentration be >1 M. We consider it extremely unlikely therefore, that the low intralysosomal pH is maintained by a Donnan equilibrium under intracellular conditions.

Reijngoud et al. [12] have proposed that the low intralysosomal pH is maintained by the presence of impermeant negatively-charged groups (in equilibrium with their protonated forms) and by H^+ influx or efflux (or OH^- efflux or influx, respectively), which is accompanied by a stoichiometric movement of other ions to prevent the build-up of transmembrane charge gradients. The permeability of the lysosomal membrane to K^+ and H^+, however, also raises an objection to this mechanism, as the magnitude of a ΔpH produced in this way would be lowered by the exchange of cytosolic K^+ for intralysosomal H^+. Provided that the lysosomal lifetime were sufficiently short, the low H^+ permeability of the lysosomal membrane might limit the K^+/H^+ exchange effectively enough to prevent large changes in internal pH. The intralysosomal buffering capacity [39] might play an important role here. However, it has been suggested on the basis of studies on the turnover of lysosomal proteins that the lysosomal half-life is approximately 7 days [40]. If this is the case then some active mechanism to prevent the equilibration of K^+ and H^+ must be postulated. Energy-dependent proton pumps in the lysosomal membrane have been proposed [11,41] though there is no clear evidence for their existence (see refs. 2, 4, 8). Recently, a lysosomal membrane-bound ATPase has been reported [42]. However, the possibility that this ATPase activity is due to microsomal contamination of the lysosomal fraction has not been eliminated since no microsomal marker was measured. Nevertheless, the possibility that there is an ATP-dependent proton pump in the lysosomal membrane that brings about an exchange of intralysosomal K^+ for cytosolic H^+ (cf. the non-electrogenic ATPase of hog gastric mucosa [43,44]) must be investigated.

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