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RELATIONSHIP BETWEEN MEDIUM pH AND THAT OF THE LYSOSOMAL MATRIX AS STUDIED BY TWO INDEPENDENT METHODS

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SUMMARY

1. The method of estimating the intralysosomal pH by measuring the distribution of [^{14}C]methylamine in lysosomes isolated from the livers of Triton WR 1339-treated rats has been critically examined.

2. In lysed lysosomes, methylamine is bound to the membrane fragments, but this binding can be completely suppressed by increasing the concentration of monovalent cations in the medium.

3. In intact lysosomes, the binding of [^{14}C]methylamine is only partly inhibited by monovalent cations at 25 °C.

4. The accumulation of [^{14}C]methylamine in intact lysosomes is progressively inhibited as the concentration of methylamine is increased. A similar inhibition of [^{14}C]methylamine accumulation is obtained with NH_4Cl.

5. Similar values for the intralysosomal pH were obtained from measurements of the distribution of methylamine, dimethylamine and trimethylamine, which are accumulated in the lysosomes, and of 5,5-dimethyloxazolidinedione-2,4, which is excluded.

6. The breakdown of endocytosed ^{125}I-labelled bovine serum albumin by intact isolated lysosomes is much less sensitive to the pH of the medium than the breakdown of added protein by lysed lysosomes.

7. The intralysosomal pH has been estimated by comparing the rate of breakdown of endocytosed ^{125}I-labelled albumin in intact lysosomes as a function of medium pH with that of added ^{125}I-labelled albumin by lysed lysosomes at different pH values. The values obtained agree well with those calculated from the distribution of [^{14}C]methylamine.

8. Methylamine and NH_4Cl inhibit the breakdown of ^{125}I-labelled albumin in intact lysosomes, particularly at high medium pH, but have no effect on the breakdown by lysed lysosomes.

Abbreviations: DMO, 5,5-dimethyloxazolidinedione-2,4; MES, 2-(*N*-morpholino)ethane sulphonic acid; MOPS, morpholinopropane sulphonic acid.

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9. It is concluded that a pH difference across the lysosomal membrane (more acidic inside than outside) is maintained by the presence of indiffusible negatively charged groups within the lysosomes, and by the permeation across the lysosomal membrane of protons together with permeant anions (or of OH^- in exchange for anions).

INTRODUCTION

The function of the lysosomal apparatus is to bring about the degradation of macromolecules and cell fragments of intra- and extracellular origin by hydrolases with an acid pH optimum [1]. From this general statement two main characteristics of the lysosomes can be deduced. Firstly, the pH inside the organelles must be low enough to allow the hydrolases to function efficiently, and the pH must be maintained at this low value. Secondly, the lysosomal membrane must be relatively permeable to the low molecular weight products of hydrolysis, so that the intralysosomal osmolarity does not increase unduly and lead to disruption of the organelles.

Several studies have been described in which attempts have been made to assess the magnitude of the intralysosomal pH and to determine the mechanism by which it is regulated. Changes in colour of indicator dyes bound to an endocytosable carrier have been used to estimate the intralysosomal pH with the help of a calibration curve [2–5]. Reijngoud and Tager [6] and Goldman and Rottenberg [7] have estimated the intralysosomal pH by measuring the distribution of the lipid-soluble weak base methylamine across the lysosomal membrane (see also ref. 8). Finally, information on the intralysosomal pH has also been obtained by studying the effect of the extralysosomal pH on an intralysosomal degradation process [9, 10].

When using the distribution of a radioactively labelled lipid-soluble weak base (or acid) to estimate the pH within a membrane-bound vesicle, the Henderson-Hasselbach relation is used, and a number of assumptions are made, particularly about the activity coefficients of the species involved both within and without the vesicle, and about binding of the base or acid (see refs. 11 and 12 for a discussion of the principles and limitations of the method). Since it is of importance to check whether these assumptions are valid in the case of the lysosomal system, we have approached the problem of the estimation of the intralysosomal pH in the following way.

Firstly, we have calculated the intralysosomal pH from the distribution of a number of weak bases and the distribution of a weak acid. Secondly, we have studied the factors influencing the adsorption of the indicators to the lysosomal membrane. Finally, we have used an entirely different method to estimate the intralysosomal pH. We have measured the rate of intralysosomal degradation of endocytosed denatured ^{125}I-labelled albumin (as described by Mego and coworkers [9, 10]) as a function of extralysosomal pH, and compared these rates with those obtained using lysed lysosomes. A measure of the relative intralysosomal pH could then be obtained by extrapolation. Both methods give very similar results.

MATERIALS AND METHODS

Materials. ^3H_2O, [^{14}C]methylamine, [^{14}C]dimethylamine, [^{14}C]trimethylamine, 5,5-[^{14}C]dimethyloxazolidinedione-2,4 (DMO) and [^{14}C]sucrose were obtained from the Radiochemical Centre, Amersham, England, and ^{125}I from Philips Duphar, Petten, The Netherlands. The bovine serum albumin (Fraction V) was from Sigma Chemical Co., St. Louis, U.S.A., and the Triton WR 1339 from Rohm and Haas, Philadelphia, U.S.A.

Isolation of lysosomes. Lysosomes were isolated by the flotation method of Trouet [13], as described by Kussendrager et al. [14], from livers of Triton WR 1339-treated rats.

Determination of the distribution of radioactive substances and calculation of the intralysosomal pH. The distribution of labelled compounds was determined exactly as described by Reijngoud and Tager [7] for intact lysosomes. The temperature of incubation was 25 °C and the amount of lysosomal protein used was 1–3 mg/ml per incubation.

When the distribution in lysosomal membrane fragments was measured, the intact lysosomes were allowed to burst by preincubation in water before the other constituents of the medium were added. After incubating the lysosomes for 1 min, the incubation medium was centrifuged at 24 000 $\times g_{max}$ for 5 min in a MSE 6L centrifuge. The further treatment of supernatant and pellet was the same as in the case of intact lysosomes. Both with intact and with lysed lysosomes, the sucrose space was determined by carrying out parallel incubations in the presence of [^{14}C]sucrose and ^3H_2O.

The inside pH was calculated from the distribution of the ^{14}C-labelled weak bases as described previously [7] and from the distribution of the 5,5-[^{14}C]dimethyloxazolidinedione-2,4 by using the following formula [12]:

$$pH_{in} = pK_a + \log\left[\frac{[A]_{in}}{[A]_{out}}\left(1 + 10^{pH_{out} - pK_a}\right) - 1\right]$$

in which pK_a = negative logarithm of the dissociation constant of DMO, $[A]_{in}/[A]_{out}$ = the accumulation factor of the acid corrected for adhering water, pH_{in} is the intralysosomal pH and pH_{out} is the pH of the medium. The pK_a for DMO was taken as 6.39 [12]. The sucrose-impermeable space was 0.37±0.10 of the total space (18 determinations).

Determination of the breakdown of denatured ^{125}I-labelled bovine serum albumin in intact and by broken lysosomes. Denatured bovine serum albumin, labelled with ^{125}I according to the method of Greenwood et al. [15] and denatured by the method of Mego et al. [16], was injected into rats 30 min prior to decapitation. A large granule fraction was isolated from the liver following the method described by Mego and McQueen [17]. The final pellet was suspended in 0.25 M sucrose.

Except when otherwise stated the incubation medium (final volume 5 ml) consisted of 0.25 M sucrose, 25 mM 2-(*N*-morpholino)-ethane sulfonic acid (MES), 25 mM morpholinopropane sulphonic acid (MOPS), and 50 mM mercaptoethanol, adjusted to the indicated pH with unneutralized Tris. In most experiments, 1.7 mM $MgCl_2$ was also present. The temperature of the incubation was 37 °C; before and after the experiment the pH of the incubation medium was measured. At several time intervals samples of 1 ml were taken and added to 1 ml 10 % trichloroacetic acid.

After spinning down the protein, the acid-soluble radioactivity in a sample of the supernatant was measured in Bray's solution in a Nuclear-Chicago Isocap 300 scintillation counter.

The rate of hydrolysis in intact lysosomes was not linear with time, but declined after about 10–15 min (see e.g. Fig. 5 in ref. 20; see also [9, 10]). In the studies reported below, hydrolysis in the initial 5, 6 or 10 min was used in calculating the rates.

When a lysed lysosomal preparation was tested for its protein-degrading activity the following procedure was used.

A lysed large granule fraction was isolated as described above, with two exceptions: uninjected rats were used and the final fraction was suspended in water and sonicated in a MSE sonic disintegrator for ten 30-s periods at 22 kHz (2 μm peak to peak). The suspension was kept in an ice-water bath. The incubation medium was the same as that used with intact lysosomes, except that 1.7 mM $MgCl_2$ was always present and a tracer amount of ^{125}I-labelled bovine serum albumin was added. The further procedure for measuring the activity was the same as above. The rate of hydrolysis of ^{125}I-labelled albumin was linear for at least 30 min.

RESULTS

In Table I the results are summarized of an experiment in which the accumulation of methylamine in intact isolated lysosomes was followed in time. It is clear that the accumulation is at equilibrium within 45 s. We have therefore chosen 1 min as the time of incubation in order to ensure that equilibrium has been reached.

In order to determine if binding of methylamine to the lysosomes occurs, we used membranes of lysed lysosomes as a model. Since there is no sucrose-impermeable space in this case (the accumulation factor for sucrose was 1), any binding observed must be to membrane sites exposed to the medium.

In Fig. 1 the effect of KCl on the accumulation of methylamine by a lysosomal membrane pellet is shown. The binding of methylamine to these membrane fragments

TABLE I

TIME COURSE OF [^{14}C]METHYLAMINE ACCUMULATION BY RAT LIVER LYSOSOMES

Rat liver lysosomes from Triton WR 1339-treated rats were incubated at 25 °C in a medium (final volume 6 ml) containing 20 mM Tris/acetate (pH 8.5), 130 mM KCl, 1 mM EDTA, ^3H_2O and [^{14}C]methylamine. In parallel experiments [^{14}C]sucrose was added instead of [^{14}C]methylamine. At the times indicated, samples were removed for the determination of radioactivity in the pellets as described in the text.

Time (s)	[^{14}C]Methylamine accumulation factor in osmotic space
15	46.6
30	53.0
45	55.3
60	49.8
90	45.2
120	46.7

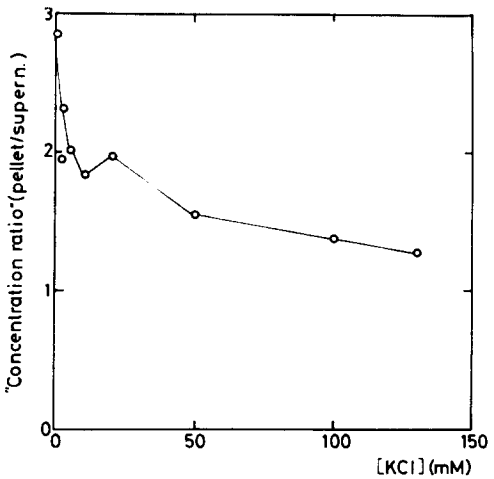

Fig. 1. Effect of KCl on binding of methylamine to lysed lysosomal membranes. Rat liver lysosomes from Triton WR 1339-treated rats were incubated for 2 min in water prior to the addition of the other constituents of the medium. The composition of the final medium (final volume 1 ml) was 10 mM Tris · HCl (pH 7.5), KCl at the concentrations indicated, mannitol to bring the final osmolarity to 120 mosM, ^3H_2O and either [^{14}C]methylamine or [^{14}C]sucrose. The incubation temperature was 25 °C and the incubation time after the pretreatment in water was 1 min.

which occurs at low salt concentrations declined as the ionic strength was increased. At about 130 mM KCl the concentration ratio of methylamine was about 1, which means that the concentration in the pellet (membranes and adhering water) was the same as in the medium. Thus no binding to membranes occurs in high salt medium.

In Fig. 2 the effect of increasing concentrations of unlabelled methylamine on the binding of this compound by lysed lysosomes in two types of medium is shown. When one compares the effect of methylamine concentration on the absorption of methylamine in a mannitol-containing medium (Fig. 2) with the results of Fig. 1, it becomes clear that the membrane contains binding sites with about the same affinity for methylamine (presumably the protonated form) and K^+. This can also be deduced

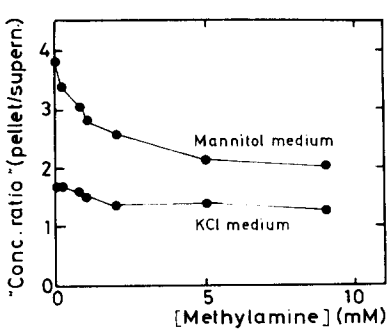

Fig. 2. Effect of methylamine concentration on the binding of [^{14}C]methylamine by lysed rat liver lysosomes. The experiment was performed as described in Fig. 1. The final medium contained 130 mM KCl or 250 mM mannitol, and 10 mM Tris · HCl (pH 7.5), methylamine at the indicated concentrations, ^3H_2O and either [^{14}C]methylamine or [^{14}C]sucrose.

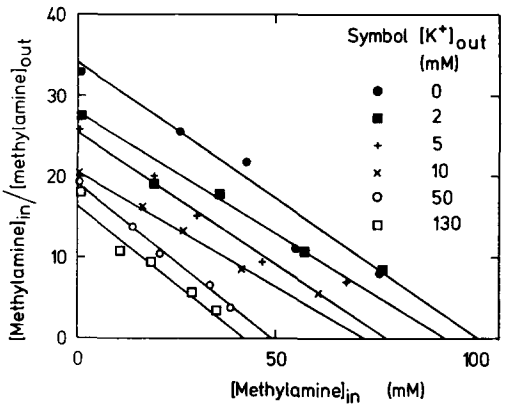

Fig. 3. Influence of methylamine concentration on [^{14}C]methylamine accumulation at different concentrations of K^+ in intact rat liver lysosomes. Rat liver lysosomes from Triton WR 1339-treated rats were incubated at 25 °C in a medium containing mannitol and KCl to give a final osmolarity of 250 mosM, 10 mM Tris/acetate (pH 7.5), methylamine at different concentrations, ^3H_2O and either [^{14}C]methylamine or [^{14}C]sucrose. The incubation volume was 1 ml and the incubation time 1 min. The concentrations of K^+ used are indicated in the figure.

from the lack of effect of increasing concentrations of methylamine on the accumulation factor in a high salt medium. In our opinion the adsorption of either methylamine or K^+ reflects electrostatic interaction of the cations with negatively charged groups on the membrane.

The adsorption also occurs in intact lysosomes. In Fig. 3 the effect of different concentrations of KCl on the adsorption of methylamine is shown in the form of a Scatchard plot. It appears that in intact lysosomes only one kind of binding site exists for methylamine. Furthermore, K^+ competes in a non-competitive way with the methylamine. The dissociation constant for methylamine (or apparent 'K_m') remains fairly constant, but the total number of binding sites decreases (from 103 mM at 0 mM KCl to 43 mM at 130 mM KCl in this experiment). This type of inhibition (or competition) for binding sites can be explained if we assume the following model for

TABLE II

EFFECTS OF SALTS ON CALCULATED ΔpH ACROSS THE LYSOSOMAL MEMBRANE

Lysosomes were isolated from Triton WR 1339-treated rats and incubated with 20 mM Tris · HCl (pH 7.5) and 240 mM sucrose or 130 mM salt. In addition, the reaction mixture contained ^3H_2O and either [^{14}C]methylamine or [^{14}C]sucrose. Temperature 25 °C.

Medium	ΔpH
Sucrose	1.69
Tris · HCl	1.39
LiCl	1.40
NaCl	1.39
KCl	1.26
RbCl	1.24
CsCl	1.20
Choline chloride	1.45

the lysosomes. There is only one kind of binding site with an equal or nearly equal affinity for both K^+ and the protonated form of methylamine. Of these binding sites those on the outside of the membrane are available to both cations, whereas the sites on the inside of the lysosomes are available to methylamine only.

Table II shows the effect of different cations at 130 mM on ΔpH, as calculated from the distribution of methylamine, in intact lysosomes. Two conclusions may be drawn. Firstly, the cations had only a small effect on ΔpH, indicating that they can not permeate freely through the lysosomal membrane. Secondly, the effectiveness of the inorganic cations in decreasing ΔpH ($Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$) is according to the radius of the hydrated ion [18]. The smaller the radius the greater the inhibition of methylamine accumulation.

From these results the conclusion seems justified that methylamine accumulation in lysosomes is brought about by a mechanism in which electrostatic interaction with the excess of negatively charged groups in the lysosomes plays an important role. Furthermore, the negatively charged groups within the lysosome will play an important role in determining the magnitude of the pH difference across the lysosomal membrane; the acid-base equilibria of these groups will determine the ratio charged to uncharged base or acid, as used in the Henderson-Hasselbach equation.

Table III shows that the inside pH calculated from the measured distribution of three weak bases, namely methylamine, dimethylamine and trimethylamine at different pH values of the medium are approximately the same, indicating that the interaction of all three bases with the lysosomes is similar. In Table IV the same type of comparison is made but now between the weak base methylamine and the weak acid DMO. Whereas methylamine accumulates in the lysosomes, DMO is excluded at pH values of 6.5 or higher. With both indicators a similar inside pH could be calculated. However, since DMO is excluded the determination of the inside pH is not as accurate as when methylamine is used.

In Fig. 4 all our data on the influence of the pH of the medium on the intralysosomal pH, calculated from the methylamine accumulation data, are summarized. In a salt-poor medium the pH_{in} rose from 4.53 ± 0.28 at a pH_{out} of 5.0 to 6.61 ± 0.61 at

TABLE III

CALCULATION OF INTRALYSOSOMAL pH FROM DISTRIBUTION OF DIFFERENT AMINES

Lysosomes from Triton WR 1339-treated rats were incubated in a medium containing 250 mM mannitol, ^3H_2O, either ^{14}C-labelled base or [^{14}C]sucrose and 25 mM MES, 25 mM MOPS and Tris to bring the pH to the value indicated. Final volume, 1 ml. Temperature 20 °C.

pH_{out}	pH_{in} calculated from distribution of		
	Methylamine	Dimethylamine	Trimethylamine
5.0	4.59	4.97	4.91
6.0	5.41	5.71	5.13
6.5	5.64	5.91	5.35
7.0	5.98	6.12	5.76
7.5	6.33	6.33	6.55
8.0	6.52	6.55	6.24
8.5	6.83	6.95	6.85

TABLE IV

CALCULATION OF INTRALYSOSOMAL pH FROM DISTRIBUTION OF DMO AND METHYLAMINE

Rat liver lysosomes isolated from rats treated with Triton WR 1339 were incubated in a medium containing 250 mM mannitol, 25 mM MES, 25 mM MOPS, Tris to bring the pH to the value shown, and either [^{14}C]sucrose, [^{14}C]DMO or [^{14}C]methylamine. The accumulation factor was obtained by correcting the concentration ratio pellet/supernatant for the sucrose permeable space.

pH_{out}	Concentration ratio pellet/supernatant		Accumulation factor (f)		pH_{in} calculated from (f) for	
	DMO	Methylamine	DMO	Methylamine	DMO	Methylamine
5.0	1.18	1.85	1.39	2.85	5.92	4.96
6.0	1.06	2.56	1.13	4.39	6.15	5.36
6.5	0.86	4.65	0.70	8.93	6.20	5.54
7.0	0.70	5.86	0.35	11.57	6.44	5.94
7.5	0.60	9.20	0.13	18.03	6.36	6.22

a pH_{out} of 8.5. In a salt medium the inside pH was about 0.3 unit higher than that at the corresponding pH_{out} in a salt-poor medium.

In Fig. 5 the influence of methylamine and of NH_4Cl on the ΔpH is shown. Both compounds decrease the pH difference in a concentration-dependent manner.

If we assume that the distribution of methylamine can be used as an indicator of the inside pH, and that the variations seen are a real indication of the variations in intralysosomal conditions, the difference must also be reflected by intralysosomal processes, the rates of which also depend on the inside pH. We chose as an intralysosomal process the degradation within the lysosomes of endocytosed ^{125}I-labelled, denatured bovine serum albumin.

The effect of the pH of the medium on ^{125}I-labelled bovine serum albumin degradation by a lysed large granule fraction and the breakdown of endocytosed ^{125}I-

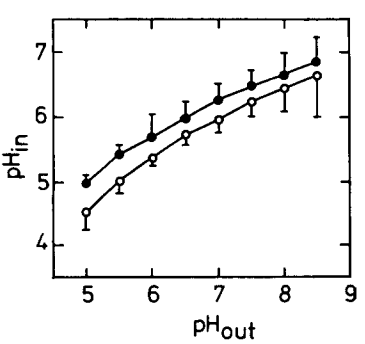

Fig. 4. Effect of medium pH on the intralysosomal pH in a KCl- (● — ●) and in a mannitol- (○ — ○) containing medium. The vertical bars indicate the standard deviation (n = 5).

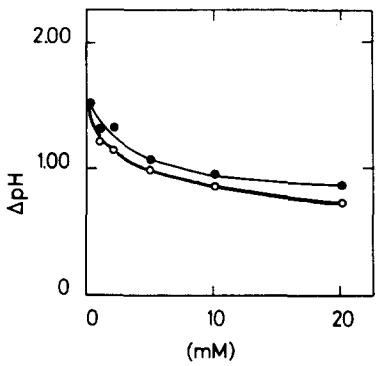

Fig. 5. Effect of NH_4Cl and methylamine on ΔpH. Rat liver lysosomes from Triton WR 1339-treated rats were incubated at 25 °C, in a medium containing 250 mM mannitol, 10 mM MES, 10 mM MOPS, sufficient Tris to bring the pH to 7.5, ^3H_2O, either [^{14}C]methylamine or [^{14}C]sucrose and the concentrations of NH_4Cl or methylamine shown. The incubation volume was 1 ml, the incubation time 1 min. ● — ●, methylamine; ○ - - ○, NH_4Cl.

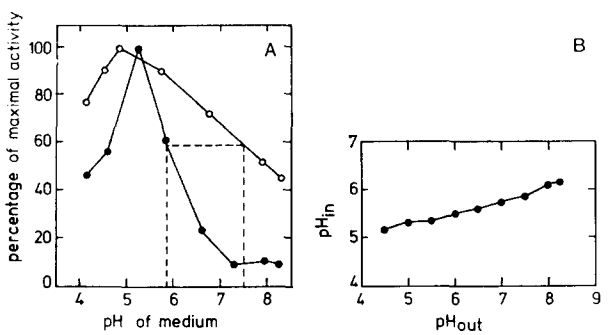

Fig. 6. Effect of medium pH on hydrolysis of ^{125}I-labelled albumin by broken and in intact lysosomes. In A the lysed preparation (● — ●) was obtained by sonicating a large granule fraction from rat liver as described in Materials and Methods. The intact preparation (○ — ○) was isolated from rats injected 30 min previously with ^{125}I-labelled albumin. The incubation time was 6 min (intact lysosomes) or 20 min (lysed lysosomes). For other conditions, see text. In B the intralysosomal pH as a function of the medium pH, calculated from the experimental data in A, is shown.

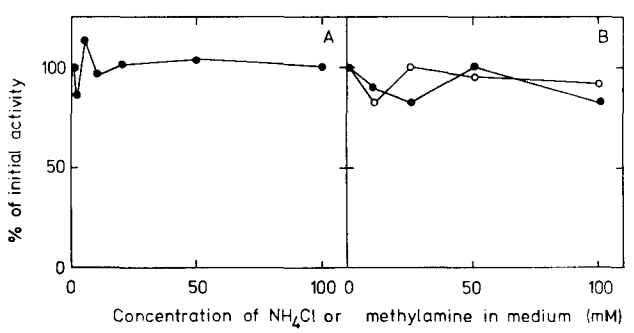

Fig. 7. Effect of NH_4Cl or methylamine on hydrolysis of ^{125}I-labelled albumin by broken lysosomes. The experiment was performed as described in Fig. 6A, in a medium containing 200 mM sucrose, 20 mM MES, 20 mM MOPS, Tris to bring the pH to the indicated value, and either methylamine (● — ●) or NH_4Cl (○ - - ○) at the indicated concentrations. (A) pH 6.2, (B) pH 7.4.

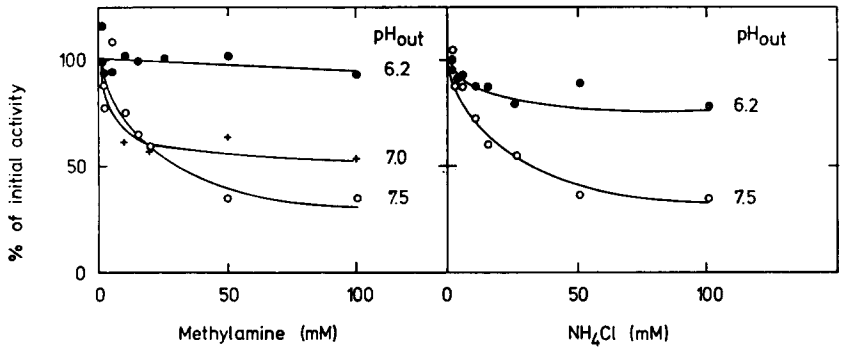

Fig. 8. Effect of NH_4Cl or methylamine on hydrolysis of endocytosed ^{125}I-labelled albumin in intact lysosomes at different pH values of the medium. A large granule fraction isolated from the liver of a rat injected with ^{125}I-labelled albumin 30 min prior to decapitation was incubated in a medium containing 200 mM sucrose, 20 mM MES, 20 mM MOPS, Tris to bring the pH to the value indicated, and NH_4Cl (left) or methylamine (right) at the concentrations indicated.

labelled albumin in intact lysosomes is shown in Fig. 6A. Relative activities are plotted, and normalized in such a way that the activity at the pH optimum is taken as 100, which makes comparison possible between the two curves. It appears from these two plots that in the intact lysosomes the rate of degradation is protected against the influence of the medium pH, when we compare this with broken lysosomes. It is also clear that the outside pH still influences the rate of degradation. Fig. 6B shows the intralysosomal pH calculated from the data of Fig. 6A. Thus with this method as well it can be shown that the lysosomal matrix responds to the pH of the surrounding medium. At all pH values no decrease in latency was found in these media after 5 min of incubation.

Since in the experiments using methylamine as a pH indicator NH_4Cl, and methylamine itself, decreased the inside pH (Fig. 5), we studied the influence of these substances on ^{125}I-labelled albumin breakdown. Methylamine has hardly any effect on the hydrolysis of ^{125}I-labelled albumin by lysed lysosomes at a pH of 6.2 (Fig. 7A) or 7.4 (Fig. 7B). Similarly NH_4Cl had no effect at pH 7.4 (Fig. 7B). The effect of NH_4Cl at pH 6.2 was not tested.

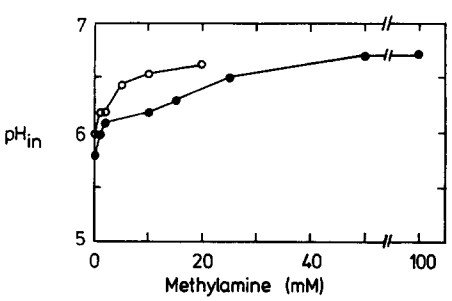

Fig. 9. Comparison between the effect of methylamine on the intralysosomal pH calculated from the distribution of methylamine (○—○) or from the rate of hydrolysis of endocytosed ^{125}I-labelled albumin (●—●). For conditions, see text.

The effect of methylamine and NH_4Cl on the degradation of endocytosed albumin in intact lysosomes is shown in Figs. 8A and 8B. In this case a clear-cut influence was found. At pH 6.2 there was only a slight inhibition; this was more pronounced in the case of NH_4Cl than of methylamine. At higher pH values of the medium a greater inhibition of the degradation process was found, which was dependent on the concentration of NH_4Cl and methylamine present.

DISCUSSION

The calculation of the intralysosomal pH on the basis of distribution data of lipid-soluble weak acids or bases is based on several assumptions allowing one to apply the Henderson-Hasselbach equation on both sides of the membrane. Waddell and Bates [11] have extensively discussed the validity of these assumptions particularly in the light of the current theories of the meaning of pH. They conclude that the distribution data of weak lipid-soluble acids or bases provide a measure of the pH inside the membrane-bound vesicle, and not the concentration of H^+. It represents a relative quantity which is operationally meaningful. It is for this reason that we studied in addition an intralysosomal process to see whether the quantity calculated from the distribution of methylamine is physiologically significant.

Calculations of the intralysosomal pH from measurements of the distribution of a weak base (or acid) across the lysosomal membrane show that the pH within the organelle responds to that of the medium. The response of an intralysosomal process (the hydrolysis of endocytosed ^{125}I-labelled albumin) to extralysosomal pH suggests that the estimation of the relative intralysosomal pH from the distribution of methylamine is of physiological significance. Calculation of the intralysosomal pH based on measurements of the rate of hydrolysis of ^{125}I-labelled albumin in intact and lysed lysosomes gave results similar to those obtained from the distribution of the weak base. However, it should be pointed out that when the extralysosomal pH is 5 or lower, both methods are too inaccurate to give reliable results. In this respect it is of interest to note that inhibition of lysosomal processes in vitro by a high pH of the medium has also been demonstrated in vivo; when human skin fibroblasts are cultured in a medium of pH 8.0, the hydrolysis of mucopolysaccharides is much lower than that at pH 6.8 [23].

Furthermore, Seglen [24, 25] has found that ammonia inhibits protein degradation in isolated rat liver parenchymal cells. This inhibition could be due to an accumulation of ammonia within the lysosomes (see discussion following ref. 25; cf. ref. 26).

This conclusion implies that the lysosomal membrane must be permeable to protons (or OH^-), and the question arises of the mechanism of the permeation. Goldman and Rottenberg [7] and Henning [8] propose that the lysosomal membrane is permeable to both protons and monovalent cations, and that these ions are distributed across the lysosomal membrane according to a Donnan equilibrium. We [19] have shown that while this is the case at 0 °C, the temperature at which Goldman and Rottenberg [7] and Henning [8] carried out their measurements, the lysosomal membrane is impermeable to monovalent cations at 25 or 37 °C. Since electroneutrality must be maintained, and since protons cannot exchange with monovalent cations at 25 or 37 °C unless an ionophore is added (see ref. 19 and the accompanying paper [20]), the mechanism of proton transport must involve co-transport with a permeant anion (or exchange of OH^- for permeant anions).

From the experiments on the effect of monovalent cations on the accumulation of methylamine, it can be concluded that a part of the accumulation is due to electrostatic interactions with easily accessible negatively charged groups, presumably on the outside of the lysosomal membrane. That part of the accumulation not affected by monovalent cations is presumably due to negatively charged groups on the inside of the lysosomal membrane. As shown in the accompanying paper [20], these groups can be made accessible to monovalent cations by adding ionophores (see also ref. 19).

The effect of methylamine or ammonia on the intralysosomal pH can be regarded as a titration of the negatively charged groups and of undissociated groups in acid-base equilibrium with them. Indeed, the lysosomes have a considerable buffering capacity [21]. This titration is formally very similar to the binding of monovalent cations and of protonated methylamine by electrostatic interaction with negatively charged groups in lysed lysosomes, and can be compared with the ion-exchange properties of the lysosomal membrane described by Henning et al. [22].

Thus the presence of the negatively charged groups within the lysosomes ensures that the micro-environment of the lysosomal hydrolases is kept at a pH lower than that of the extralysosomal environment. This occurs only if the lysosomal membrane is intact. In lysed lysosomes, the rate of hydrolytic processes, for instance the degradation of ^{125}I-labelled albumin, is lower than that in intact lysosomes at a medium pH greater than about 6.

The results presented in this paper lead us to conclude that the distribution of methylamine can be used as an accurate means of calculating the intralysosomal pH, provided that the lysosomes are suspended in a salt medium in order to prevent binding of the base to negatively charged groups on the outside of the lysosomal membrane. For instance, we have recently found that the accumulation of the weak base chloroquine by isolated lysosomes, is accompanied by a decrease in the accumulation of [^{14}C]methylamine [21]. If the intralysosomal pH is calculated from the latter, the accumulation of chloroquine can be almost quantitatively accounted for by the increase in pH that occurs and the buffering capacity of the lysosomes.

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