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ACCUMULATION OF WEAK BASES IN RELATION TO INTRALYSOSOMAL pH IN CULTURED HUMAN SKIN FIBROBLASTS

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Summary

The volume of the lysosomal compartment in cultured human skin fibroblasts was estimated from the distribution between the cells and the medium of tracer amounts of labelled methylamine and chloroquine, which accumulate in the lysosomes, 2,2-dimethyloxazolidine-2,4-dione, which accumulates in the soluble cytoplasmic compartment relative to the lysosomes, and sucrose, which is excluded by the cells. In a foetal fibroblast line, the fractional volume of the lysosomal compartment was 0.044 ± 0.007 (n = 8). In fibroblasts from a patient with the I-cell disease, the fractional volume was 0.15.

The fractional volume of the lysosomal compartment was used to calculate the intralysosomal pH from the accumulation of the weak bases in the cells. The mean value obtained was 5.29 ± 0.04 (n = 8).

In fibroblasts incubated with various concentrations of chloroquine, the fractional volume of the lysosomal compartment and the accumulation of chloroquine in the cells were used to calculate the concentration of chloroquine in the lysosomes. The intralysosomal concentration increased from 3 to 114 mM as the extracellular concentration increased from 1 to 100 μM. Concomitantly, the intralysosomal pH increased from 5.3 in the absence of chloroquine to 5.9 in the presence of 100 μM chloroquine. A similar increase in intralysosomal pH could be calculated in fibroblasts incubated with different concentrations of ammonia.

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Abbreviations: Mes, 2-(*N*-morpholino)ethanesulphonic acid; Mops, 4-morpholinopropanesulphonic acid; DMO, 5,5-dimethyloxazolidine-2,4-dione.

Introduction

The degradation of macromolecules in the lysosomes is catalysed by the hydrolytic enzymes which have an acid pH optimum and exhibit little, if any, activity at neutral pH [1,2]. This observation led Coffey and de Duve [3] to propose that the intralysosomal pH must be low. Studies on the distribution of the weak base methylamine [4—7] have demonstrated a pH difference across the lysosomal membrane of approx. 1 unit at physiological pH values. More recently, Ohkuma and Poole [8], using a fluorescent probe, calculated that the ΔpH across the lysosomal membrane in macrophages is almost 3 units (for reviews concerning the intralysosomal pH see Refs. 9—13).

De Duve and coworkers [14,15] have introduced the term 'lysosomotropic agents' for extracellular compounds which are taken up by lysosomes and affect lysosomal functions. Lysosomotropic agents include weak bases like chloroquine, which accumulate in the lysosomes due to the low pH within these organelles (see Refs. 8, 14—18). Reijngoud and Tager [12,19] have shown that chloroquine is accumulated in isolated lysosomes.

The uptake of weak bases gives rise to several effects. Poole et al. [8,17,18] and Reijngoud and Tager [19] found that the accumulation of chloroquine in the lysosomal interior brings about a rise in the intralysosomal pH. Poole and coworkers [16—18] have observed that extensive vacuolation of cultured cells occurs after chloroquine treatment.

It has been shown that addition of chloroquine to the culture medium of human skin fibroblasts or macrophages leads to a decrease in the intracellular level of some lysosomal enzymes [20—22], a concomitant increase in the extracellular levels of these enzymes [20—23] and an inhibition of pinocytosis of enzymes [22,24,25] (see also Refs. 26 and 27). It has been suggested that these effects are due to an impairment of receptor recycling, possibly caused by an increase in intralysosomal pH [22,25]. That receptor recycling does, indeed, occur has been indicated by the studies of Tulkens and coworkers [28—31] on fibroblasts and those of Ashwell and Steer [32] on hepatocytes.

In addition to the indirect effects discussed above, it has been found that some lysosomal enzymes are directly inhibited by chloroquine [16,21,33—35].

It has recently become clear that cultured human skin fibroblasts contain a receptor specific for a phosphomannosyl recognition marker on acid hydrolases, enabling the latter to be delivered to the lysosomes [36—42]. Gonzalez Noriega et al. [22] have shown that the binding of acid hydrolases to the receptor is strongly pH dependent.

Since many of the effects of chloroquine described above may be related to changes in intralysosomal pH, we have developed a method for calculating the pH within lysosomes in intact cultured human skin fibroblasts. The method is described in this paper. In addition, the concentration of chloroquine within the lysosomes as a function of that in the medium has been calculated. The results have been reported in part in a preliminary form [35].

Materials and Methods

Cell lines and culture conditions. The fibroblasts used in this study were a control line derived from a foetus and one derived from a patient with I-cell

disease. The former line (FC) was obtained from Mr. N. Leschot (Department of Anthropogenetics, University of Amsterdam) and the latter (I 363) from Professor J. Leroy (Department of Genetics, University of Antwerp). Fibroblasts were grown in Ham's F10 medium (Flow Laboratories) supplemented with 15% heat-inactivated foetal calf serum (Boehringer, Mannheim), 16 mM $NaHCO_3$, penicillin (250 U/ml) and streptomycin (250 μg/ml) as described by de Groot et al. [43]. The solutions of chloroquine were sterilized by passing them through a Millipore filter (0.22 μm) before addition to the medium.

Measurement of distribution of radioactive methylamine, chloroquine, 5,5-dimethyloxazolidine-2,4-dione (DMO) and sucrose between the extracellular space and the fibroblasts. After trypsin treatment, the cells were suspended in Hanks' balanced salt solution plus 10% foetal calf serum. After centrifugation in a Homef centrifuge (low speed), the pellet was resuspended in Hanks' solution containing 1.67 mM $NaHCO_3$.

The incubation of fibroblasts (about 1 mg protein/ml) in ^3H_2O and either [^{14}C]methylamine, [^{14}C]chloroquine, [^{14}C]DMO or [^{14}C]sucrose was performed in Hanks' solution containing 1.67 mM $NaHCO_3$ buffered with 50 mM Mes/Mops in an equimolar ratio and sufficient Tris to bring the pH to the indicated value. The final volume of the incubations was 1 ml.

After incubation for the indicated time at 37°C, the cells were separated from the medium by centrifugation for 4 min in an Eppendorf centrifuge (Model 3200) run at full speed and the ^{14}C and ^{3}H radioactivity in pellet and supernatant were determined as described [5]. Corrections for adhering water were made as described [44].

Protein assay. Protein was estimated according to the method of Lowry et al. [45].

Chemicals. Chloroquine (disphosphate salt) was obtained from Sigma Chemical Co. (St. Louis, U.S.A.), [^{14}C]chloroquine from New England Nuclear Chemicals GmbH (Boston, U.S.A.) and [^{14}C]methylamine, [^{14}C]sucrose, [^{14}C]DMO and ^3H_2O from the Radiochemical Centre (Amersham, U.K.).

Theoretical

For a multi-compartment system like a fibroblast cell, the distribution of a weak base or acid is given by the relation

$$f_{tot} = p_1 f_1 + p_2 f_2 + p_3 f_3 + \ldots + p_n f_n \quad (1)$$

i.e.

$$f_{tot} = \sum_{i=1}^{n} p_i f_i \quad (2)$$

where f_{tot} = total accumulation factor of the weak base or acid, p_i = volume fraction of compartment i, and f_i = accumulation factor of the weak base or acid in compartment i (see Eqn. 11).

In our calculations, we consider the fibroblast cell as a two-compartment system containing a heterogeneous lysosomal compartment which is relatively acidic and a cytoplasmic compartment with a neutral pH. We assume that

methylamine and chloroquine will mainly accumulate in the lysosomal interior and that DMO will accumulate in the cytoplasmic compartment. We are aware that mitochondria will also accumulate DMO, but in the fibroblast lines we used the activity of cytochrome *c* oxidase or succinate dehydrogenase is very low (results not shown; see also Ref. 46), so that the fractional volume of the mitochondria in the cells will be low in comparison to that of the soluble cytoplasm (see Ref. 46) and the contribution of the mitochondrial compartment can be neglected in our calculations. Since the nuclear membrane is freely permeable to charged molecules (see e.g. Ref. 47), the nuclear compartment can be considered as part of the cytoplasm.

Using these assumptions, the following equations can be derived for the accumulation of the weak bases methylamine and chloroquine.

$$f_{tot_{MA}} = f_{c_{MA}} \cdot f_{l_{MA}} \cdot p + f_{c_{MA}} \cdot (1 - p) \quad (3)\ *$$

$$f_{tot_{CQ}} = f_{c_{CQ}} \cdot f_{l_{CQ}} \cdot p + f_{c_{CQ}} \cdot (1 - p) \quad (4)\ *$$

where MA = methylamine, CQ = chloroquine, p = volume fraction of lysosomes, c = cytoplasmic compartment, and l = lysosomal compartment.

Since chloroquine is dibasic, accumulation of the base across the plasma membrane, which separates two compartments with a small pH difference, can be neglected compared with the accumulation across the lysosomal membrane, which separates compartments with a large pH difference. Thus Eqn. 4 can be simplified to

$$f_{tot_{CQ}} = f_{c_{CQ}} \cdot f_{l_{CQ}} \cdot p \quad (5)\ *$$

Since methylamine has one basic group and chloroquine has two, the following relationships will exist (see Ref. 48 for a derivation and Refs. 13 and 19 for experimental evidence).

$$(f_{l_{MA}})^2 = f_{l_{CQ}} \quad (6a)$$

$$(f_{c_{MA}})^2 = f_{c_{CQ}} \quad (6b)$$

From Eqns. 3, 5, 6a and 6b, the following relationship can be derived:

$$f_{tot_{MA}} = \sqrt{p \cdot f_{tot_{CQ}}} + (1 - p) \cdot f_{c_{MA}} \quad (7)$$

$f_{tot_{MA}}$ and $f_{tot_{CQ}}$ can be measured from the accumulation of methylamine and chloroquine. $f_{c_{MA}}$ can be calculated from the distribution of DMO as follows.

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* In this equation, the lysosomal fraction is described in one term. Formally, the term $f_{l_j} \cdot p$ should be replaced by

$$\sum_{j=1}^{n} f_{l_{ji}} \cdot p_j ,$$

where p_j represents the volume fraction of each part of the heterogeneous lysosomal population and

$$\sum_{j=1}^{n} p_j$$

the total volume fraction of the lysosomal population.

The distribution of the weak acid, DMO, is given by

$$f_{tot_{DMO}} = f_{c_{DMO}} \cdot f_{l_{DMO}} \cdot p + (1 - p) f_{c_{DMO}} \quad (8)$$

Since DMO will not accumulate in the lysosomes this equation can be simplified to

$$f_{tot_{DMO}} = f_{c_{DMO}} \quad (9)$$

The distribution of DMO can thus be used to calculate the pH of the cytoplasmic compartment and, consequently, the accumulation factor for methylamine in the cytoplasm ($f_{c_{MA}}$). Using labelled DMO, methylamine, chloroquine and sucrose, the distribution factors (r) for these compounds can be measured as follows:

$$r_i = \frac{\text{dpm of indicator in the pellet}}{\text{dpm of indicator in the supernatant}} \quad (10)$$

From the r values, the accumulation factor f can be calculated:

$$f_i = \frac{r_i - r_{sucrose}}{1 - r_{sucrose}} \quad (11)$$

In isolated lysosomes, the intralysosomal pH can be calculated as follows (see Ref. 5 for a derivation).

$$pH_{in} = pH_{out} - \log f_{base} \quad (12)$$

In fibroblasts, where the fractional volume of the lysosomes is p, the intralysosomal pH can be calculated as follows (see Ref. 49):

$$pH_{in} = pH_{out} - \log f_{l_{base}} \cdot \frac{1}{p} \quad (13)$$

where $f_{l_{base}}$ is calculated from Eqn. 3 after using the calculated p (Eqn. 7).
If necessary, DMO and chloroquine accumulation were corrected for undissociated acid or base as described by Waddell and Butler [50].

We must stress that, except in the experiment shown in Table II, tracer amounts of the labelled indicators and/or short incubation times were used, so that it was unlikely that vacuolation of the type described by Poole and coworkers [16—18] had occurred.

Results

Time course of the uptake of chloroquine, methylamine and DMO

The distribution of [^{14}C]chloroquine, [^{14}C]methylamine, [^{14}C]DMO or [^{14}C]sucrose between medium and cells as a function of the time of incubation is shown in Table I. It is clear that complete equilibration of DMO and sucrose occurs within 2 min and that a longer time is required for equilibration of methylamine and chloroquine. When the reciprocal value of the accumulation factor for methylamine and chloroquine was plotted against the reciprocal value of the incubation time, a linear relationship was obtained. In analogy with a Lineweaver-Burk plot for enzyme kinetic data, the intercept on the $1/t$ axis can be assumed to be equal to $-1/t_{1/2}$, where $t_{1/2}$ represents the time

TABLE I

TIME COURSE OF ACCUMULATION OF INDICATOR COMPOUNDS IN HUMAN SKIN FIBROBLASTS

Fibroblasts were incubated for the indicated time intervals at 37°C in 1 ml of a medium containing Hanks' solution, 1.67 mM $NaHCO_3$, 50 mM Tris, sufficient Mes and Mops in equimolar ratio to bring the pH to 7.4, ^3H_2O and either [^{14}C]methylamine (1.80 μM; 0.1 μCi), [^{14}C]chloroquine (0.83 μM; 0.025 μCi), [^{14}C]DMO (1.70 μM, 0.1 μCi) or [^{14}C]sucrose (0.32 μM; 0.2 μCi). For further experimental details, see Materials and Methods.

Incubation time	f_{MA}	f_{CQ}	f_{DMO}	$r_{sucrose}$
2	2.90	94.7	1.25	0.48
4	4.02	174.3	1.06	0.47
8	5.63	231.2	1.17	0.48
12	6.46	290.8	1.29	0.45
24	7.25	360.0	1.17	0.53
			$\bar{r}_{sucrose}$ =	0.48

needed to reach half maximal accumulation. Fig. 1 shows that the time needed for half maximal accumulation is 3.8 min for methylamine and 8.3 min for chloroquine. Accordingly, the incubation times chosen for the accumulation experiments described below were 12 min for methylamine and 24 min for chloroquine. Longer incubation times were avoided since they led to reattachment of the fibroblasts to the incubation vessels.

Lack of binding of chloroquine, methylamine and DMO to fibroblasts

An essential assumption in the theoretical considerations is that the indicator compounds do not bind appreciably to cell constituents. Several investigators [51—53,56] have shown that binding of methylamine and DMO is negligible and that these compounds accumulate within compartments according to a pH gradient. Evidence that chloroquine does not bind aspecifically is provided by the results of sucrose-density fractionation experiments with the post-nuclear fraction of homogenates of chloroquine-treated fibroblasts [16] or with whole

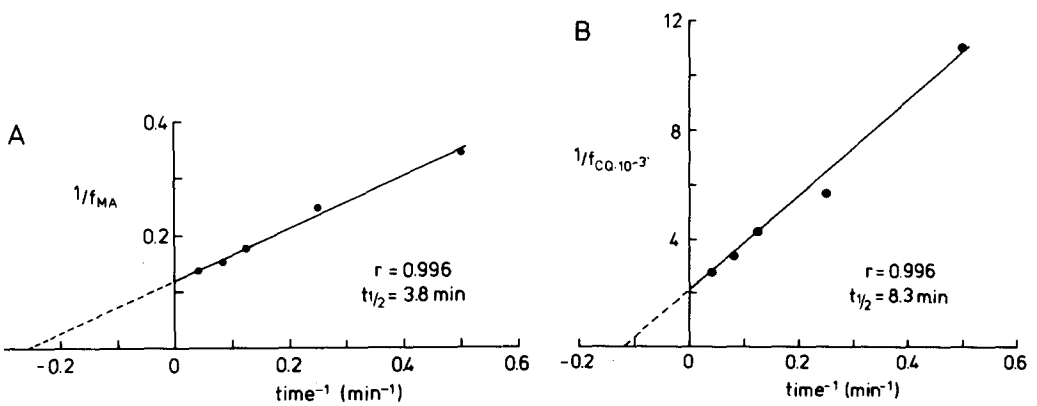

Fig. 1. Double-reciprocal plot of the time course of accumulation of methylamine (A) and chloroquine (B) in human skin fibroblasts. For experimental details, see Table I.

homogenates (including the nuclear fraction) of hepatoma cells [54] and fibroblasts (Hollemans, M., Oude Elferink, R. and Strijland, A., unpublished observations). Furthermore, Reijngoud [13] showed that in isolated lysosomes, chloroquine equilibrates across the lysosomal membrane according to the pH difference. Di Donato et al. [55] have concluded that chloroquine binds to the plasma membranes. However, this conclusion is based on the observation that part of the chloroquine accumulated by cells is released after incubation for 10 min with trypsin; this treatment may have led to a redistribution of the base between cells and medium, or to cell damage.

The following observations indicate that binding of the indicator compounds was negligible under our experimental conditions as well. In Table II, results are presented of an experiment in which the chloroquine concentration in the medium was varied. If binding had occurred, the percentage of chloroquine accumulated would have decreased as the concentration of chloroquine was increased, due to saturation of binding sites. However, it is clear that the percentage accumulation of chloroquine by the cells remained relatively constant irrespective of the concentration of the base in the medium. Another indication that aspecific binding of chloroquine to cellular constituents is negligible is given by the fact that cells lysed with Triton X-100 do not accumulate chloroquine (results not shown). Furthermore, the ionophore nigericin, which brings about an exchange of K^+ for H^+, dissipates the accumulation of chloroquine and methylamine almost completely (results not shown). The latter observation indicates that methylamine and chloroquine equilibrate according to a pH gradient, presumably that existing between the medium and the lysosomes.

Effect of pH of medium

If methylamine and chloroquine distribute according to the pH gradient across the lysosomal membrane, the medium pH can be expected to influence the accumulation of chloroquine and methylamine in a way similar to that seen in isolated lysosomes [13]. Furthermore, if DMO is an indicator for the cyto-

TABLE II

INFLUENCE OF ADDITION OF VARIOUS AMOUNTS OF CHLOROQUINE TO THE CULTURE MEDIUM ON THE PERCENTAGE CHLOROQUINE ACCUMULATED IN THE CELL

Fibroblasts were cultured as described in Materials and Methods for 22 h in the presence of the indicated amounts of chloroquine (a mixture of unlabelled and [^{14}C]chloroquine). After 22 h, the cells were harvested and the amount of chloroquine in the cells and the culture medium was determined.

[Chloroquine] (μM)	Radioactivity (cpm $\times 10^{-3}$)			Recovery (%)	Percent accumulated in cells
	added at $t = 0$	in medium at $t = 22$ h	in cells at $t = 22$ h		
5	82	65	19	102	23
10	170	123	39	95	24
15	250	179	110	116	38
25	421	292	64	85	18
40	76	49	19	89	28
60	106	68	28	91	29
80	141	95	33	91	26

TABLE III

INFLUENCE OF MEDIUM pH ON THE ACCUMULATION OF METHYLAMINE, CHLOROQUINE AND DMO

Fibroblasts were incubated at 37°C in 1 ml of a medium containing Hanks' solution, 1.67 mM $NaHCO_3$, 50 mM Mes/Mops/Tris at the indicated pH, ^3H_2O and either [^{14}C]methylamine, [^{14}C]chloroquine, [^{14}C]DMO or [^{14}C]sucrose. The incubations with methylamine, DMO and sucrose were stopped after 12 min and those with chloroquine after 24 min. For further experimental details, see Materials and Methods.

Medium pH	f_{MA}	f_{CQ}	f_{DMO}
5.2	0.72	1.32	1.72
6.0	0.73	4.58	2.00
6.8	1.68	50.82	1.90
7.3	3.88	310.1	1.64
7.8	7.63	1272	1.16
8.1	9.32	2275	—

plasmic pH, the distribution of DMO too will be influenced by the medium pH [56]. The results of the experiment described in Table III show that this is, indeed, the case; as the medium pH increased, the accumulation of methylamine and especially that of chloroquine increased very greatly, whereas that of DMO decreased slightly.

pH difference between medium and lysosomes in cultured fibroblasts

The control experiments described above indicate that the system using [^{14}C]methylamine, [^{14}C]chloroquine, [^{14}C]DMO and [^{14}C]sucrose can be used to calculate the lysosomal volume fraction and the intralysosomal pH in whole fibroblasts.

Using the data of Table III and Eqn. 13, the pH difference between the lysosomes and the medium was calculated as a function of the medium pH. As shown in Fig. 2, the ΔpH decreased as the pH of the medium was lowered from 8.1 to 6.0 (cf. Ref. 13). Extrapolation of these values indicate that the ΔpH would be zero at a medium pH of 4.4.

Fractional volume of lysosomes in fibroblasts

In Table IV the results of eight experiments are shown in which, p, the fractional volume of the lysosomes, was measured. The values obtained in normal

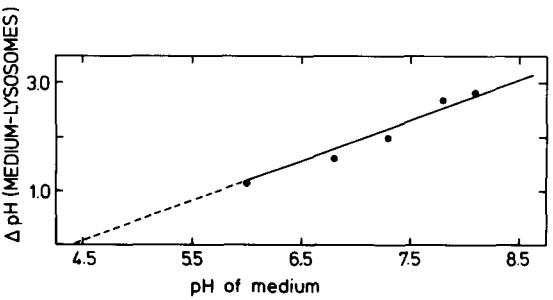

Fig. 2. Effect of medium pH on the pH difference between lysosomes and medium. For experimental details, see Table III.

TABLE IV

MEASUREMENTS OF THE FRACTIONAL VOLUME OF LYSOSOMES AND THE INTRALYSOSOMAL pH IN FOETAL HUMAN SKIN FIBROBLASTS

For experimental details, see Materials and Methods. p, fractional volume of lysosomes.

Expt.	pH of medium	p	pH in lysosomes
1	7.40	0.051	5.43
2	7.37	0.020	5.28
3	6.95	0.054	5.32
4	6.96	0.025	5.16
5	6.94	0.022	5.20
6	6.86	0.043	5.22
7	6.95	0.055	5.26
8	7.07	0.082	5.46
	Mean ± S.E.M:	0.044 ± 0.007	5.29 ± 0.04

foetal fibroblasts varied between 0.02 and 0.08 with a mean of 0.04. In fibroblasts from a patient with the I-cell disease, the p value was 0.15, i.e. 15% of the total cell volume was due to lysosomes. The intralysosomal pH in all experiments shown in Table IV was fairly constant (5.29 ± 0.04).

Accumulation of chloroquine in lysosomes

In the experiment of Table V, fibroblasts were incubated in media with different concentrations of chloroquine in the presence of [^{14}C]chloroquine, [^{14}C]methylamine, [^{14}C]DMO or [^{14}C]sucrose. From the accumulation of the indicators, the fractional volume of the lysosomes was calculated using Eqn. 7. The intralysosomal pH and the intralysosomal chloroquine concentration were calculated using Eqns. 7, 9, 11 and 13. It is clear that the concentration of chloroquine in the lysosomes was extremely high (about 110 mM when 100 μM chloroquine was added to the medium). Furthermore, we observed a slight but significant increase in the intralysosomal pH, from about 5.4 in the absence

TABLE V

ACCUMULATION OF CHLOROQUINE IN FIBROBLASTS AND EFFECT ON INTRALYSOSOMAL pH

The pH of the medium was 7.4. For other experimental details, see text. Abbreviations: CQ, chloroquine; f, accumulation factor; L, lysosomes; MA, methylamine; p, fractional volume of lysosomes.

CQ_{medium} (μM)		p	f_{MA}	f_{CQ}	pH_{in}	$[CQ]_L$ (mM)
$t = 0$	$t = 12$ min					
0	0	—	6.3	—	5.38	—
1	0.31	0.049	5.8	434	5.43	3
2	0.59	0.052	5.9	432	5.42	5
5	1.66	0.043	5.5	446	5.45	15
10	3.55	0.046	5.4	400	5.51	28
25	12.23	0.051	4.3	225	5.57	55
50	32.98	0.056	3.4	106	5.70	70
100	73.94	0.040	2.6	77	5.85	114
		Mean: 0.049				

TABLE VI

EFFECT OF NH_4Cl ON THE INTRALYSOSOMAL pH

For experimental details, see Table I. Abbreviations: CQ, chloroquine; MA, methylamine.

$[NH_4Cl]$added (mM)	p	f_{MA}	f_{CQ}	pH_{in}
0	0.020	3.38	309.76	5.28
5	0.027	2.15	61.37	5.69
10	0.018	1.85	33.29	5.74
25	0.022	1.69	16.83	5.93

of chloroquine to about 5.9 when 100 μM chloroquine was added to the medium. In the experiment of Table VI, the influence of NH_4Cl on the accumulation of trace amounts of labelled chloroquine, methylamine and DMO, and consequently on the intralysosomal pH was tested. Without NH_4Cl the intralysosomal pH was about 5.3 and in the presence of 25 mM NH_4Cl, the pH increased to a value of 5.9. Thus, the effect of 25 mM NH_4Cl on the intralysosomal pH is comparable to that of 100 μM chloroquine, reflecting the difference in valency between the two bases.

Discussion

The intralysosomal pH measured using the method described in this paper is in good agreement with that observed in isolated lysosomes [5,12], but is higher than that measured in a different type of cell (macrophages) using a different method [8].

The fractional volume of lysosomes in normal fibroblasts was calculated to be between 0.02 and 0.08, and this value is in agreement with those reported in the literature [57]. The value of p tended to increase with time after confluence (results not shown). In fibroblasts of a patient with the I-cell disease, in which extensive vacuolation occurs, a value of 0.15 was found.

Addition of chloroquine or NH_4Cl gives rise to a slight but significant increase in the intralysosomal pH. It has been proposed by Gonzalez-Noriega et al. [22] and by Tietze et al. [25] that chloroquine (or other weak bases) inhibit the recycling of lysosomal enzyme receptors. Gonzalez-Noriega et al. [22] showed that the binding of enzyme to the phosphomannosyl receptor of human skin fibroblasts is very pH sensitive; at pH 5.0 less than 20% β-glucuronidase was bound, whereas at pH 6.0 almost 80% of the enzyme was bound. These investigators [22] suggest that the addition of amines gives rise to an increase of the intralysosomal pH above the pH which favors dissociation of enzyme from the receptors. In this way the endoplasmic reticulum and Golgi membrane become depleted of free receptors and consequently newly synthesized acid hydrolases are not recognized and are secreted. Our measurements of the intralysosomal pH in human skin fibroblasts indicate a rise in pH which could, indeed, play an important role in receptor enzyme binding (see Fig. 7 of Ref. 22). It is of importance to note that we do not observe a pH rise of the magnitude of that reported by Ohkuma and Poole [8] in macrophages upon

addition of chloroquine or NH_4Cl. We have not been able to use their method [8] in fibroblasts, since insufficient fluorescent dextran was endocytosed to be able to carry out spectral measurements (results not shown).

Finally, we should stress that the measurements of the intralysosomal pH and of the accumulation of chloroquine in fibroblasts described in this paper do not provide information about the mechanisms involved in generating and maintaining a low intralysosomal pH. Our results can be explained equally well either by an energy-dependent proton pump [8,58,59] or by a Donnan-type equilibrium [4—7].

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