

Cytoplasmic Vacuolation of Mouse Peritoneal Macrophages and the Uptake into Lysosomes of Weakly Basic Substances

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ABSTRACT With few exceptions, weakly basic compounds that are sufficiently lipophilic in their neutral forms and sufficiently hydrophilic in their protonated forms accumulate in lysosomes. When the concentration within the lysosomes becomes sufficiently high, osmotic swelling occurs. The cells then take on a vacuolated appearance. The concentrations at which different weak bases cause lysosomal vacuolation vary over almost three orders of magnitude. For any particular weak base, it is the concentration of the neutral form that determines the extent of uptake and the degree of vacuolation. Chloroquine is anomalous in that concentrations $> \sim 30 \mu\text{M}$ cause less uptake and less vacuolation than do lower concentrations.

It has been found that the treatment of cells with a variety of chemical compounds leads to the formation, in the cytoplasm, of many large vacuoles (3, 20, 22, 23). Many of these substances, the best known being neutral red, are weak bases. De Duve et al. (7) have proposed a quantitative theory to account for the accumulation, in lysosomes, of weakly basic substances and the formation of vacuoles. This theory is the logical consequence of three assumptions. First, that the plasma and lysosomal membranes are highly permeable to the neutral forms of weak bases. Second, that these same membranes are impermeable, or very slightly permeable, to the protonated forms of the weak bases. And third, that the pH inside the lysosomes is considerably lower than it is outside the lysosomes. These assumptions have the following consequences. First, that weak bases will be trapped by protonation inside lysosomes and accumulate there. And, second, that when the concentration of the base inside the lysosomes approaches isotonicity, water will enter osmotically and the lysosomes will swell to form large vacuoles.

At the time this theory was proposed we had no quantitative measure of the pH in lysosomes. Recently we have devised a technique to measure this parameter in mouse peritoneal macrophages (13) and have discovered that the pH rises in the presence of weak bases, a possibility not considered in the original theory (7).

In this paper we will examine, in some detail, the ability of

a number of weak bases to induce vacuolation in mouse peritoneal macrophages and the kinetics of uptake of these bases into the cells. In subsequent papers we will explore, in more detail, the pH changes that occur in the lysosomes of mouse peritoneal macrophages exposed to weak bases, and the effect of these compounds on lysosomal protein degradation (18).

MATERIALS AND METHODS

Cell Culture

Mouse peritoneal macrophages were isolated by the method of Cohn and Benson (6) from NCS strain mice. They were cultured in Dulbecco's modified Eagle's minimum essential medium (12) at pH 7.6 (unless otherwise indicated), containing 20% fetal calf serum, 25 $\mu\text{g}/\text{ml}$ Gentamicin, and 2.5 $\mu\text{g}/\text{ml}$ Fungizone in glass Leighton tubes, either on the tube surface or on cover slips, in an atmosphere of 5% CO_2 in air.

Morphology

Cells cultured on cover slips, after various treatments, were washed with cold Hanks' solution. They were then fixed with cold 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.0, for 20 min. The cover slips were then washed with cold buffer and mounted on microscope slides in this buffer. They were sealed with nail polish and observed under phase contrast.

Measurement of Uptake Into Cells

Cells, cultured on the surfaces of Leighton tubes, were exposed to various media for various times as indicated below. In the measurement of the uptake of tritiated atropine, tritiated propranolol, and chloroquine, we used cells prelabeled with [^{14}C]leucine and we included 100 μM of [^{14}C]sucrose in the incubation

This paper is appearing without final revision due to the untimely death of Dr. Brian Poole.

medium (except for chloroquine uptake). The cells, after exposure to medium containing the base in question, were drained of medium and then the cells and residual medium were dissolved in 0.1 M NaOH-0.4% Na deoxycholate. Tritium and total trichloroacetic acid (TCA)-soluble ^{14}C were measured. From the TCA-soluble ^{14}C we calculated the amount of medium contamination and from the TCA-insoluble ^{14}C we calculated the amount of cell protein. As can be seen (see Fig. 5) sucrose uptake into cells is so little as to be negligible under these conditions. Chloroquine was measured fluorometrically, and residual medium was measured by the absorbance of phenol red at 550 nm. For studies of the uptake of [^{14}C]methylamine, tritiated sucrose was included in the medium. The cells, prelabeled with tritiated leucine, were drained of medium and extracted twice with 2 ml of 5% TCA. The residue was then dissolved in 0.1 M NaOH-0.4% Na deoxycholate. The [^{14}C]methylamine and tritiated sucrose were measured directly by scintillation counting of the TCA extract. In the short-term uptake studies (see insert in Fig. 6) the cells were incubated in serum-free medium and protein was measured by the automated Lowry procedure (11). For the measurement of [^{14}C]sucrose uptake the washed cells were dissolved directly in 0.1 M NaOH-0.4% Na deoxycholate and the ^{14}C was determined by scintillation counting. Because the uptake of sucrose was so small compared with the concentration in the medium, a blank was necessary. Cells were exposed to [^{14}C]sucrose at 0°C and the value determined in this way was subtracted from the value obtained at 37°C. Protein was measured by the automated Lowry method (11). Results are expressed as nanomoles per milligram cell protein and converted to cellular concentration on the assumption that the cells contain 5 μl of water/mg protein. The intralysosomal concentrations are, of course, much higher.

RESULTS

Vacuole Formation

Fig. 1 shows the appearance of cells exposed for 2 h to a number of compounds in the medium. Cells exposed to 10 mM trimethylamine (Fig. 1*b*), 500 μM amantadine (Fig. 1*c*), and 100 μM propranolol (Fig. 1*d*) showed clearly the formation of vacuoles, as did cells exposed to 80 mM sucrose (Fig. 1*h*). Cells exposed to 1 mM tributylamine (Fig. 1*e*), 10 mM aniline (Fig. 1*f*), or 10 mM Tris (Fig. 1*g*) did not show vacuoles.

Fig. 2 shows cells exposed to methylamine under various conditions. At a concentration of 10 mM in medium of standard pH (7.6), there was extensive vacuolation (Fig. 2*a*). When the medium pH was lowered to 7.0, the vacuolation was reduced (Fig. 2*b*), and when the pH was lowered still further to 6.6 there was almost no vacuolation (Fig. 2*c*). Such was the case also when the medium pH was maintained at 7.6 but the concentration of methylamine was lowered by a factor of 10 (Fig. 2*d*).

Fig. 3 shows that a similar dramatic reduction in the extent of vacuolation was observed in cells exposed to atropine at a concentration of 500 μM when the pH of the medium was lowered from 7.6 (Fig. 3*a*) to 7.0 (Fig. 3*c*). In the case of atropine, the reduction of medium concentration by a factor of only 5 (at constant medium pH) caused a dramatic reduction in the degree of vacuolation (Fig. 3*b*). Interestingly, when cells were exposed simultaneously to atropine, at a concentration that causes extensive vacuolation (Fig. 3*a*), and to 1 mM tributylamine (which, alone, causes no vacuoles [Fig. 1*e*]), the vacuolation was suppressed completely (Fig. 3*d*).

The results with chloroquine (Fig. 4) were strikingly different. At a concentration of 100 μM in a medium of standard pH (7.6), chloroquine caused clear vacuolation (Fig. 4*a*). However, when the chloroquine concentration was reduced to 30 μM (Fig. 4*b*) or when the pH was reduced to 7.0 (Fig. 4*c*) the vacuolation became much more extensive. When the pH of the medium was reduced further to 6.6, vacuolation was abolished (Fig. 4*d*).

None of the treatments illustrated above (Figs. 1-4) caused significant decrease in the ability of the cells to exclude trypan blue.

Table I shows a list of the weakly basic compounds that we

have tested and found to be vacuologenic along with an indication of the medium concentration required. All the effective compounds have pKs above neutrality (with the exception of neutral red). There is a general tendency for compounds with higher molecular weights to cause vacuolation at lower concentrations, but there are numerous exceptions.

Table II lists compounds that did not produce vacuoles at any concentration tested. We will return to the contents of this table in the Discussion.

Uptake of Compounds into Cells

Fig. 5 shows the time-course of uptake of three weak bases and of sucrose. Although the medium concentration of atropine was five times higher than that of propranolol, the uptake curves were remarkably similar in their time-course and in the amounts actually taken up by the cells. Initial uptake was somewhat more rapid in the case of propranolol (even though the concentration in the medium was lower) but both compounds showed a progressive decrease with time in the rate of uptake. The kinetics of uptake of chloroquine were similar to those of atropine and propranolol in that the initial uptake was rapid and the uptake rate slowed progressively, but after 1 h the cells sometimes suffered a slight decrease in their chloroquine content. The kinetics of uptake of sucrose were completely different from those seen for the other compounds. Uptake was progressive and showed no tendency to decline in rate over a period of 2 h.

Fig. 6 shows the results of a more detailed study of the rate of uptake of methylamine into cells from media containing this compound at various concentrations. At 10 mM, a concentration that causes extensive vacuolation (Fig. 2*a*), the kinetics of uptake were similar to those of atropine and propranolol, although the amounts taken up were somewhat higher. At lower concentrations of methylamine in the medium, less was taken up into the cells and the initial, very rapid phase of uptake was over sooner.

Fig. 7 shows the relationship between the amount of methylamine taken up into cells and its concentration in the medium. At concentrations below ~ 3 mM, uptake was linearly dependent on the medium concentration. At higher medium concentrations, relatively less methylamine was taken up. Thus the concentration ratio of cells-to-medium was $\sim 17:1$ at low concentrations but had fallen below 10:1 when the concentration of methylamine in the medium reached 10 mM.

Fig. 8 shows the results of a similar experiment performed with atropine. Here a very different result was obtained. The relative uptake of atropine increased with increasing concentration in the medium so that, by 500 μM external concentration, the concentration ratio of cells-to-medium was about twice what it had been at low concentration. (Similar results were obtained in a study of eserine uptake, but these data are not presented here because of doubts about the stability of this compound in aqueous solution.)

Fig. 9 shows the results of a similar experiment with chloroquine. In striking contrast to the results shown in Figs. 7 and 8, where more drug was taken up as the medium concentration increased, there was an optimal external concentration (~ 20 μM) for the uptake of chloroquine. At low concentrations the concentration ratio of cells-to-medium was $\sim 7,000:1$ but it dropped precipitously at higher concentrations.

The pH dependence of uptake is of great interest because a change in pH brings about a change in the ratio of the free base to the protonated form. Fig. 10 shows the pH dependence

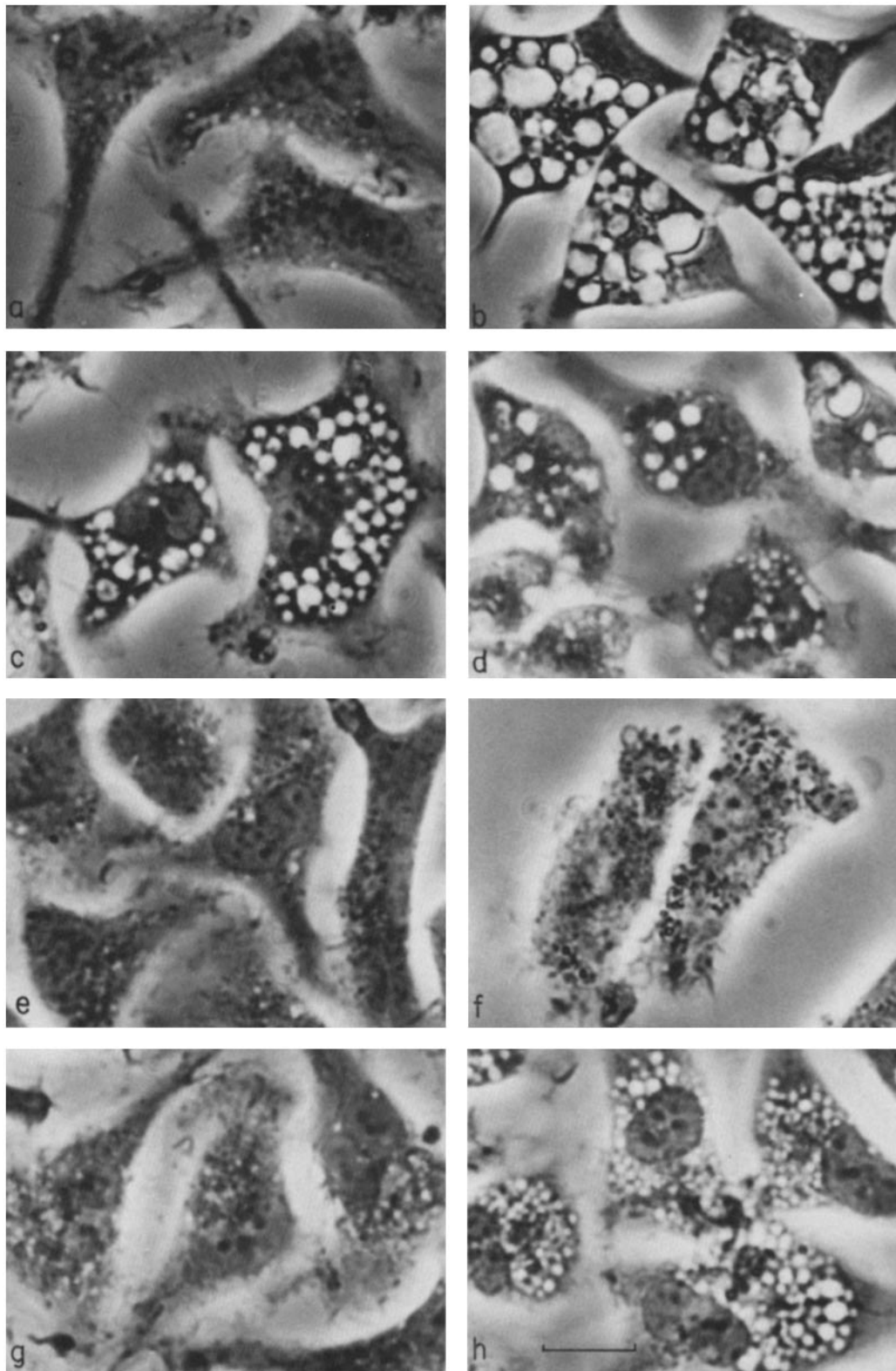


FIGURE 1 Macrophages were exposed for 2 h to medium containing the compounds indicated below. (a) control; (b) 10 mM trimethylamine; (c) 500 μ M amantadine; (d) 100 μ M propranolol; (e) 1 mM tributylamine; (f) 10 mM aniline; (g) 10 mM Tris; (h) 80 mM sucrose.

of the uptake of atropine and methylamine. As the pH of the medium increased from 6.6 to 7.6 the amount of atropine in the cells increased by a factor of \sim 25. At pH 7.6 the addition of 1 mM tributylamine inhibited uptake severely. With meth-

ylamine we found greater uptake at higher pH but the factor is only \sim 5. With chloroquine (Fig. 11) the picture was completely different. As the medium pH increased from 6.6 to 7.0, the chloroquine uptake increased by a factor of \sim 20. However,

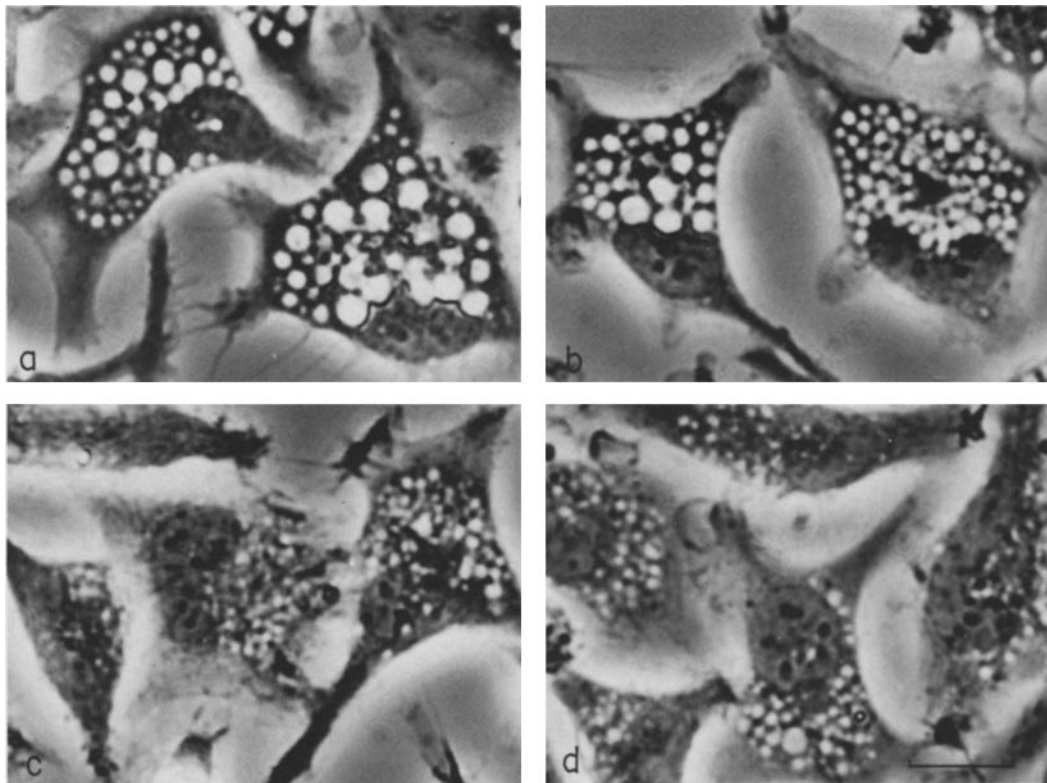


FIGURE 2 Macrophages were exposed for 2 h to medium at the indicated pH containing methylamine at the indicated concentration. (a) 10 mM, pH 7.6; (b) 10 mM, pH 7; (c) 10 mM, pH 6.6; (d) 1 mM, pH 7.6.

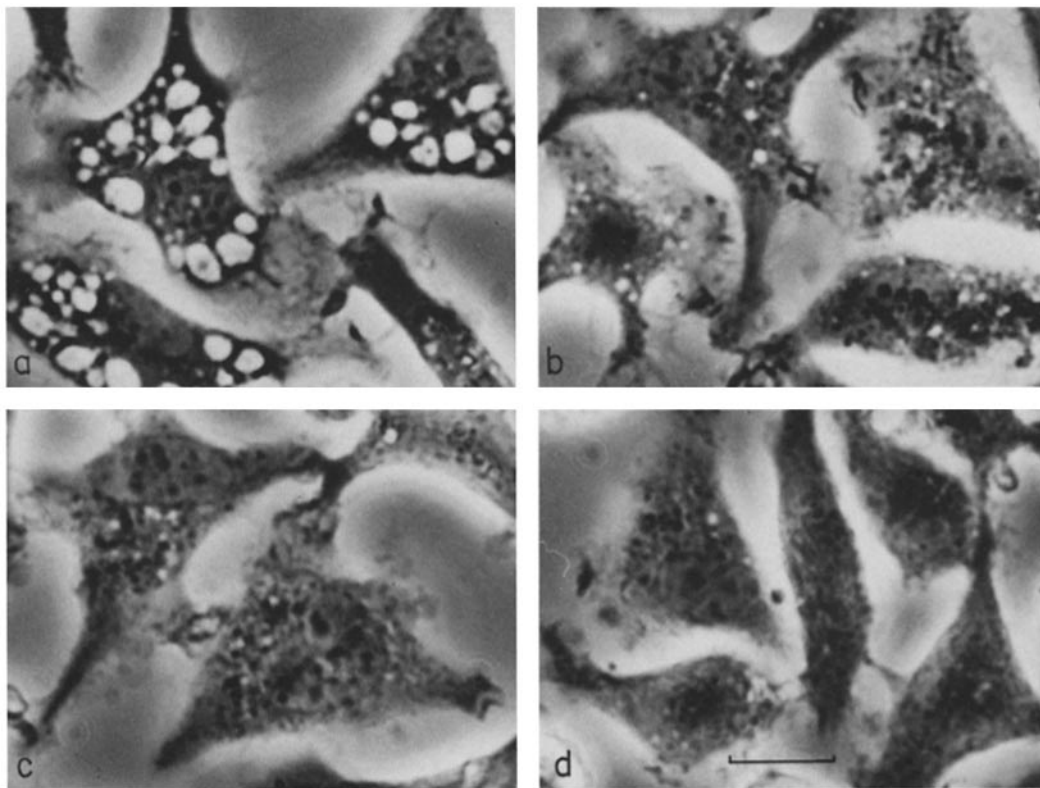


FIGURE 3 Macrophages were exposed for 2 h to medium at the indicated pH containing atropine at the indicated concentration. (a) 500 μ M, pH 7.6; (b) 100 μ M, pH 7.6; (c) 500 μ M, pH 7.0; (d) 500 μ M, pH 7.6, plus 1 mM tributylamine.

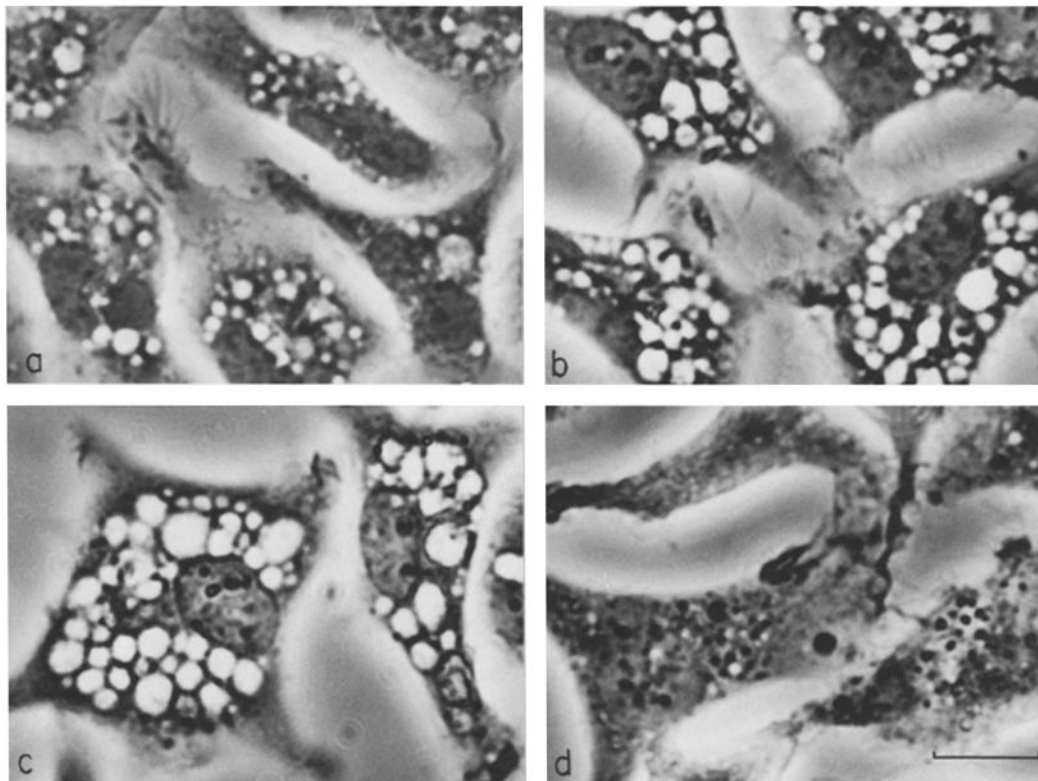


FIGURE 4 Macrophages were exposed for 2 h to medium at the indicated pH containing the indicated concentration of chloroquine. (a) 100 μ M, pH 7.6; (b) 30 μ M, pH 7.6; (c) 100 μ M, 7.0; (d) 100 μ M, pH 6.6.

when the pH of the medium was further increased to 7.6, the uptake of chloroquine was reduced drastically.

Table III shows the results of an experiment in which the effects of various substances on the cellular uptake of methylamine was measured. With the exception of chloroquine (which at this concentration inhibits its own uptake, see Fig. 9), those weakly basic substances that induce vacuolation at relatively low concentrations stimulated the uptake of methylamine. Those weakly basic substances that induce vacuolation only at higher concentrations inhibited methylamine uptake. Tributylamine, which causes no vacuolation itself, inhibited methylamine uptake as it did that of atropine (Fig. 10). The two acidic ionophores tested, X537A and nigericin, inhibited methylamine uptake, as did the uncouplers, CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) and dinitrophenol. Table IV shows the results of another experiment in which the effects of sucrose and concanavalin A on methylamine uptake were measured. Concanavalin A has been shown to produce vacuoles in macrophages (8, 9). Both of these compounds that increase the vacuolar space increased methylamine uptake.

DISCUSSION

As outlined in the Introduction, the uptake and concentration of weak bases in lysosomes can be understood as a consequence of the low pH inside lysosomes and the much greater permeability of the lysosomal membrane to the free bases as contrasted to the protonated bases. All the experimental results reported in this paper are consistent with this theory. However, we have shown that different weak bases show different properties in their interaction with the lysosomes of living cells. We have presented here no direct evidence concerning the nature of the mechanism that maintains the acidity within lysosomes. Our

previous results (13) have shown that this acidity is most probably the consequence of an active process of sequestration of protons within lysosomes, most likely some form of "proton pump."

One point we have established as a consequence of the experimental results reported in this paper is that the formation of cytoplasmic vacuoles in cells exposed to weakly basic substances in the medium is accompanied, in every case studied, by a large uptake into the cells of the weak base in question. Fig. 12 shows a summary plot of base uptake against our vacuolation score. Although the assessment of the vacuolation score involved a certain subjective element, it seems certain that we have been able to order the degree of vacuolation caused by the various treatments from none (0) to very extensive (3). It is noteworthy that the close correlation between uptake of a compound and vacuolation holds also in the case of the two compounds, chloroquine and atropine, that showed somewhat anomalous uptake behavior. Higher concentrations of chloroquine, in the form of the free base, resulted in reduced uptake (Figs. 9 and 11) and reduced vacuolation (Fig. 4). A small increase in the concentration of atropine caused a disproportionately higher increase in uptake (Fig. 8) and a very dramatic increase in vacuolation (Fig. 3). These findings provide confirmation for the hypothesis (7) that the increase in lysosomal volume that accompanies treatment of cells by weak bases, at appropriate concentrations, is the consequence of osmotic uptake of water into these organelles. The intralysosomal localization of chloroquine (22) and of neutral red (5) has been demonstrated directly by tissue fractionation. Moreover, the decreased buoyant densities observed for the treated lysosomes are completely consistent with a higher water content in these organelles. Finnin et al. (10) have shown that an increase in the osmotic potential of the medium will reduce the

TABLE I
Basic Substances That Cause Vacuolation

| Concentration | Compound | pK _a (s) | Refer- ence | mol wt | Degree of vacuolation at indicated concentration, mM | | | |
|---------------|----------------------------|---------------------|----------------|--------|---|-----|-----|-----|
| | | | | | 10 | 1 | 0.5 | 0.1 |
| 10-30 μM | Chloroquine | 8.1, 10.2 | (16) | 320 | | | | 2 |
| 100 μM | Neutral red | 6.5 | (16) | 252 | | | 3 | 2 |
| | Quinine | 4.1, 8.5 | (16) | 324 | | | | 3-2 |
| | Propranolol | 9.0 | (1) | 259 | | | | 3-2 |
| 0.5-1 mM | Lidocaine | 8.1 | (21) | 234 | | 3 | NT | 0 |
| | Eserine | 8.1 | (16) | 275 | | 3 | NT | 0 |
| | Procaine | 8.9 | (16) | 236 | | 3 | NT | 0 |
| | N,N-dimethyl-benzylamine | 8.9 | (16) | 135 | | 3 | NT | NT |
| | 4-Aminopyridine | 9.1 | (16) | 94 | | 3 | NT | 0 |
| | 4-Aminoquinoline | 9.4 | (16) | 158 | | | 3 | 0 |
| | Ephedrine | 9.6 | (16) | 165 | | | 3 | 0 |
| | 4-Dimethylaminopyridine | 9.7 | (16) | 122 | | 3 | NT | 0 |
| | Atropine | 9.9 | (16) | 289 | | | 3 | 0 |
| | Amantadine | 10.7 | (17) | 151 | | | 3 | 0 |
| | Mecamylamine | 11.4 | (2) | 167 | | | 3 | 0 |
| 1-10 mM | Imidazole | 7.1 | (16) | 68 | 3-2 | NT | | |
| | Pilocarpine | 7.1 | (16) | 194 | 3 | NT | | |
| | Nicotine | 3.1, 8.0 | (16) | 162 | 3-2 | NT | | |
| | Morpholine | 8.4 | (16) | 87 | 2 | NT | | |
| | Tetramethylethylenediamine | 5.7, 9.1 | (16) | 116 | 3 | NT | | |
| | Ammonia | 9.2 | (4) | 17 | 2 | 1-0 | | |
| | Trimethylamine | 9.8 | (16) | 59 | 3 | 1 | | |
| | Piperazine | 5.6, 9.8 | (16) | 86 | 2-1 | 0 | ↑ | |
| | Ethylenediamine | 6.8, 9.9 | (16) | 88 | 2-1 | 0 | NT | |
| | Putrescine | 9.4, 10.8 | (16) | 88 | 1 | 0 | ↓ | |
| | Methylamine | 10.6 | (16) | 31 | 3 | 1 | | |
| | Dimethylamine | 10.8 | (16) | 45 | 3 | 1 | | |
| | Ethylamine | 10.7 | (16) | 45 | 3 | 1-0 | | |
| | t-Butylamine | 10.7 | (16) | 73 | 3-2 | 1-0 | | |
| | Triethylamine | 10.7 | (16) | 101 | 2 | 0 | | |
| | Diethylamine | 11.0 | (16) | 73 | 3-2 | 0 | | |
| | Cadaverine | 7.1, 10.3 | (16) | 102 | 1-0 | 0 | | |
| | Piperidine | 11.1 | (16) | 85 | 1 | 0 | | |

Macrophages were exposed to the compound in question for 2 h. The degree of vacuolation was assessed on the scale; 0: none, 1: small, 2: medium, 3: large, NT: not tested. The meaning of these scores can be determined by the examination of Figs. 1-4. Commas between pK values indicate multiple dissociation. Most pK values refer to 25°C.

size of the vacuoles produced in transformed epithelial cells by exposure to procaine.

Another observation has been made in these studies and by previous workers. The external concentration of weak base required to elicit vacuolation with concomitant uptake of base into the cells varies over at least two orders of magnitude depending on the weak base in question. If uptake into lysosomes and vacuolation were the simple consequence of the basic properties of these compounds, as suggested by Reijngoud and Tager (19), then the only parameters necessary to describe the behavior of the bases would be their pK's and their concentrations. A glance at Table I shows that the situation is considerably more complex.

The pKs listed in Table I were found in the literature and correspond to dilute solutions of the bases, usually at 25°C. It could be that at 37°C, and at concentrations attained within lysosomes, the true pKs would be different. All the bases that caused vacuolation have pKs above neutrality, with the exception of neutral red. We hypothesized that this compound, the first discovered and probably the best known of the lysosomotropic agents might have a higher pK under physiological conditions. However, the preliminary measurements we have

made fail to substantiate the hypothesis, so neutral red remains an interesting exception to the rule.

As shown in Table II, we have found a number of weak bases that do not cause vacuolation even at a concentration of 10 mM. These have been divided into four groups. The compounds in the first group are lipophilic even in their protonated forms so that the concentration gradient of protonated base across the lysosomal membrane may be dissipated by back diffusion through the membrane. This would tend to short circuit the proton pump. Indeed, tributylamine has been shown to inhibit the uptake of methylamine (Table III) and atropine (Fig. 10). The compounds in group 2 have pKs below neutrality and the pH gradient across the lysosomal membrane would not result in extensive differences in the degree of protonation of the bases inside and outside the lysosomes (see reference 7). The compounds in group 3 are relatively hydrophilic even in their neutral forms and they probably would not permeate easily through membranes. The failure of the compounds in group 4 to cause vacuolation may be due to their toxicity.

With the few exceptions mentioned above, the occurrence or nonoccurrence of vacuolation after exposure to the many compounds listed in Tables I and II can be explained in a simple

TABLE II
Basic Substances That Do Not Cause Vacuolation at
Concentrations Up to 10 mM

| Group | Compound | pK _a (s) | Refer- ence | mol wt |
|-------|---------------------------------------|---------------------|----------------|-----------|
| 1 | S-Collidine | 7.4 | (16) | 121 |
| | Benzylamine | 9.3 | (16) | 107 |
| | Tributylamine | 9.9 | (16) | 185 |
| 2 | β-naphthylamine | 4.2 | (16) | 143 |
| | Aniline | 4.6 | (16) | 93 |
| | Pyridine | 5.2 | (16) | 79 |
| | N,N-Diethylamine | 6.6 | (16) | 149 |
| | Triethanolamine | 7.8 | (16) | 149 |
| 3 | Tris (hydroxymethyl)- aminomethane | 8.1 | (16) | 121 |
| | | 6.1, 9.9 | (16) | 111 |
| | Histamine | 8.3, 9.8, | (15) | 145 |
| | Spermidine | 10.9 | (14) | 202 |
| | Spermine | 8.0, 8.9, | (16) | 59 |
| | Guanidine | 10.0, 10.9 | | |
| 4 | Chlorpromazine | 9.3 | (16) | 319 |
| | 9-Aminoacridine | 10.0 | (16) | 194 |
| | Quinacrine | 7.7, 10.2 | (16) | 400 |

Experimental conditions and notation as for Table I.

way. However, all we have performed here is a screening. Many of these compounds have important pharmacological effects, and our simple interpretation may well be inadequate to explain fully the effects of some of these compounds.

The third point that we have verified by these experiments is that the concentration of the free form of the weak bases is the factor that determines the extent of uptake into cells and the parallel extent of vacuolation. This is shown by the experiments in which we varied the pH of the medium while holding constant the total concentration of base. As the pH is raised, the concentration of free base increases and we see more extensive vacuolation and more uptake of the bases into the cells.

An exception to the general rule that higher concentrations of free base cause more vacuolation and more uptake occurs in the case of chloroquine. Here we still see a close parallel between vacuolation and uptake, but at higher concentrations of free chloroquine base (whether as a consequence of higher

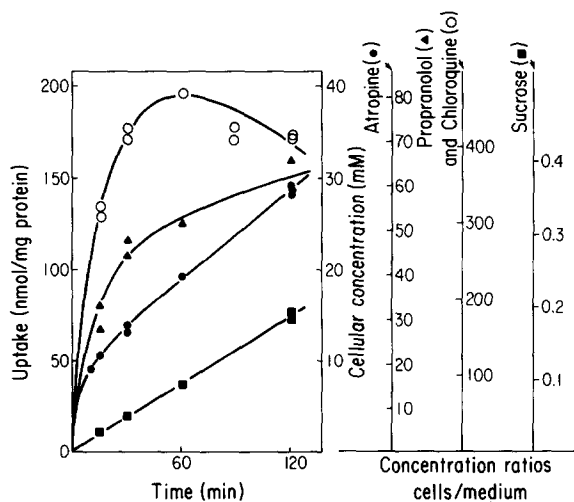


FIGURE 5 The time-course of uptake into macrophages of various substances from the medium. 500 μM atropine (●); 100 μM propranolol (▲); 100 μM chloroquine (○); 80 mM sucrose (■).

total chloroquine concentration or of higher medium pH) we have observed less uptake and less vacuolation. It would appear that higher concentrations of chloroquine may exert some toxic effect that results in an inhibition of the lysosomal uptake of this compound and of other weakly basic substances. Thus we see in Table III that chloroquine, alone among the bases that cause vacuolation at low concentrations, inhibits the uptake of methylamine.

The extent to which a weak base will be taken up into lysosomes depends on two parameters: the pH difference between the intralysosomal space and the medium, and the volume of the intralysosomal space. The most probable explanation of the results presented in Tables III and IV, where some vacuologenic bases increased methylamine uptake and others decreased it, is that those compounds that cause vacuolation at low concentration do so with less perturbation of lysosomal pH than do those basic substances that cause vacu-

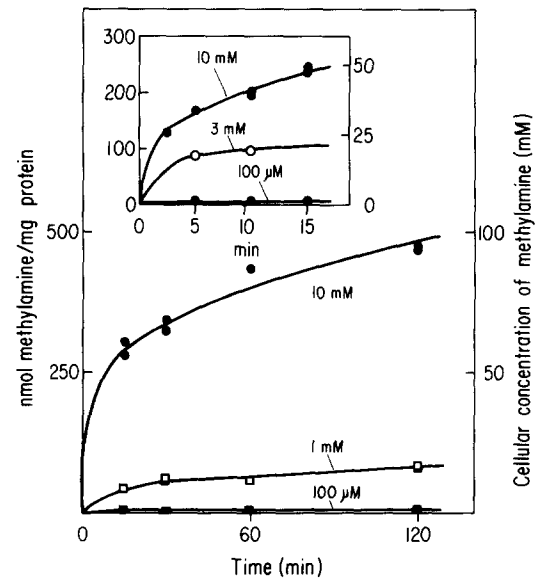


FIGURE 6 The time-course of uptake of methylamine. The medium contained methylamine at the concentrations indicated in the figure itself.

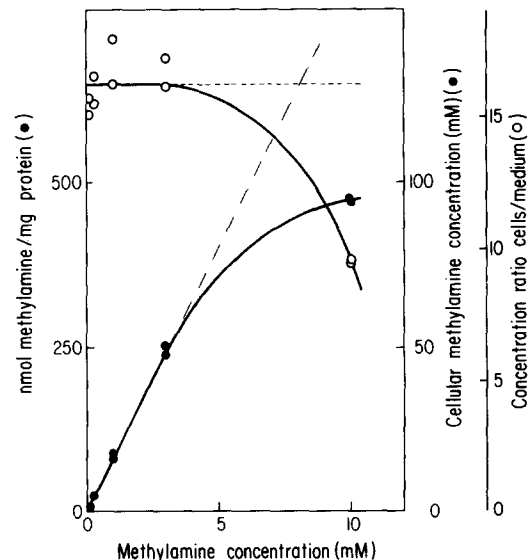


FIGURE 7 The concentration dependence of the uptake of methylamine from the medium after 2 h exposure.

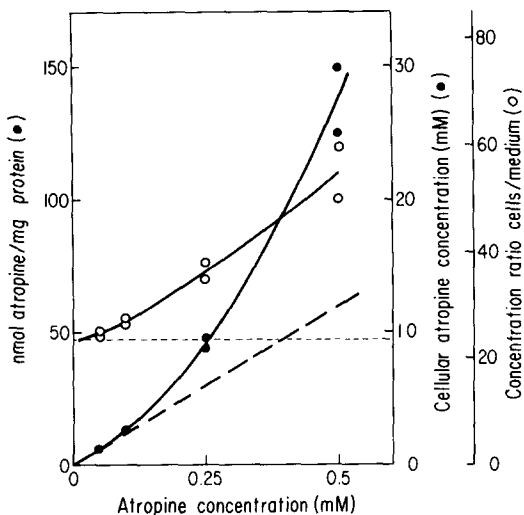


FIGURE 8 The concentration dependence of the uptake of atropine from the medium after 2 h exposure.

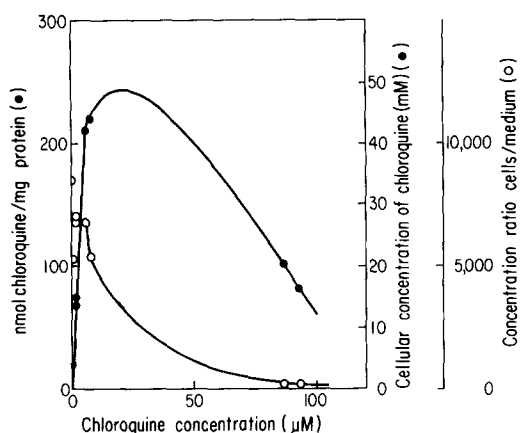


FIGURE 9 The concentration dependence of the uptake of chloroquine from the medium after 2 h exposure. The open circles (○) refer to the concentration ratios indicated on the right-hand ordinate scale. The solid circles (●) refer to the uptake indicated on the left-hand ordinate scale.

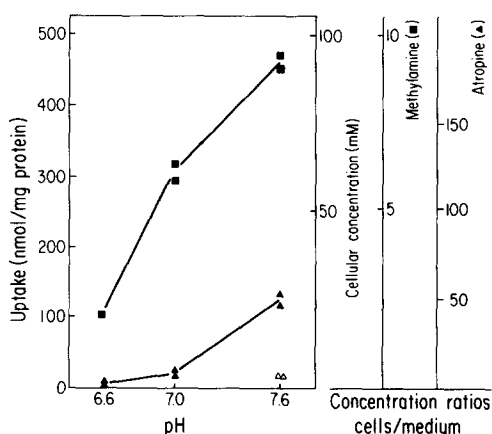


FIGURE 10 The pH dependence of the uptake from the medium of atropine and of methylamine in 2 h. 500 μ M atropine (▲); 10 mM methylamine (■); 500 μ M atropine + 1 mM tributylamine (△).

olation only at higher concentrations. Thus, more methylamine can be taken up into the swollen vacuolar system even though the intralysosomal pH has increased somewhat. We have shown previously (13) that nigericin and X537A raise the

intralysosomal pH. This effect explains the inhibitory effect of these compounds on methylamine uptake. In a subsequent paper (18) we will show that CCCP has a similar effect on intralysosomal pH.

Many of the compounds studied here have important biological and pharmacological effects. It remains possible that the vacuologenic effects of some of the compounds studied here depend on a mechanism other than simple concentration within lysosomes. We will defer a discussion of the relevance of their interactions with lysosomes to these effects until we

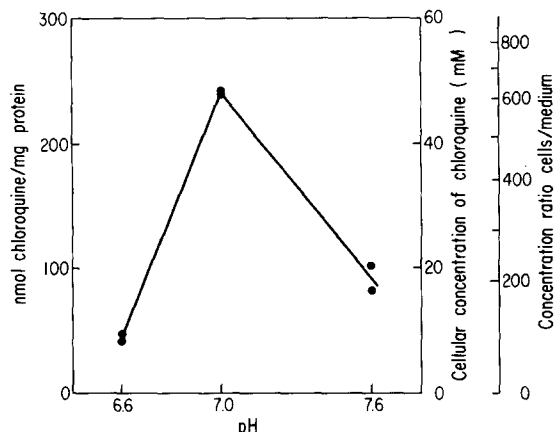


FIGURE 11 The pH dependence of the uptake of chloroquine in 2 h from medium containing 100 μ M chloroquine.

TABLE III
Effect of Various Substances on the Uptake of Methylamine

| Compound | Concentration | Uptake %* | Comments |
|--------------------|---------------|-----------|-----------------------------|
| Chloroquine | 100 μ M | 37 | |
| Quinine | 100 μ M | 133 | Basic, vacuolating |
| Propranolol | 100 μ M | 139 | |
| Amantadine | 500 μ M | 146 | |
| Procaine | 1 mM | 161 | |
| Eserine | 1 mM | 180 | |
| NH ₄ Cl | 10 mM | 54 | |
| Methylamine | 10 mM | 75 | |
| Tributylamine | 1 mM | 59 | Basic, inhibits vacuolation |
| Nigericin | 5 μ g/ml | 51 | Acidic ionophores |
| X537A | 20 μ g/ml | 41 | |
| CCCP | 200 μ M | 68 | Uncouplers |
| Dinitrophenol | 10 mM | 82 | |

The measurements were made after 2 h of incubation. The methylamine concentration in the medium was 50 μ M. Control uptake was 3.5 nmol/mg protein.

* Percentage of control.

TABLE IV
Effect of Other Vacuolating Agents on the Uptake of Methylamine

| Compound | Concentration | Methylamine Uptake %* |
|----------------|----------------|-----------------------|
| Sucrose | 80 mM | 132 |
| Concanavalin A | 100 μ g/ml | 178 |

Experimental conditions as in Table III. Control uptake was 2.7 nmol/mg protein.

* Percentage of control.

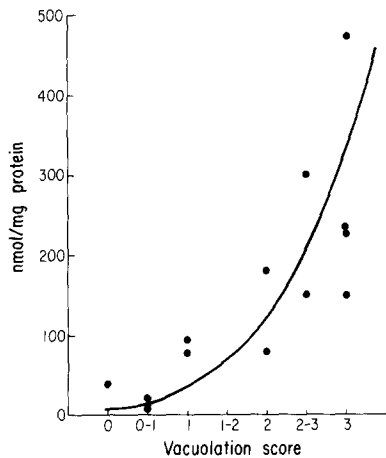


FIGURE 12 Correlation between uptake of compounds and vacuolation score. Data from previous figures.

have examined, in subsequent papers, their effects on intralysosomal pH and on intralysosomal protein degradation. We have performed these studies on mouse peritoneal macrophages because we can measure the intralysosomal pH in these cells. Our own experience with other cell types and scattered reports in the literature provide no indication that other cell types would not respond in a very similar manner.

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