THE UPTAKE AND INTRACELLULAR ACCUMULATION OF AMINOGLYCOSIDE ANTIBIOTICS IN LYSOSOMES OF CULTURED RAT FIBROBLASTS

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Abstract—The uptake and intracellular localization of aminoglycoside antibiotics: streptomycin, dihydrostreptomycin, gentamicin, kanamycin and amikacin, has been studied in cultured rat embryo fibroblasts, using cell fractionation techniques and microbiological assay. On a volume basis, aminoglycosides are accumulated 2 to 5-fold by fibroblasts, over a wide range of external concentrations. Accumulation proceeds slowly, and stable intracellular contents of drugs are obtained only after 4 days of incubation. After differential or isopycnic centrifugation of cell homogenates, the antibiotics are found consistently associated with lysosomal acid hydrolases, and clearly dissociate from marker constituents of mitochondria, plasma membrane, endoplasmic reticulum or peroxisomes. This indicates that aminoglycosides enter cells, but are localized exclusively in lysosomes. We suggest that the relative inefficiency of aminoglycoside antibiotics to act against intracellular bacteria is due (1) to the low pH prevailing in lysosomes, which depress the antibacterial activity of these drugs and defeat their accumulation in those organelles; (2) the lack of accumulation of these drugs in other subcellular structures, like phagosomes, which may harbour intracellular bacteria.

The uptake and accumulation of aminoglycoside antibiotics into mammalian cells is a subject of much controversy. Using cells cultured or maintained in vitro, a number of investigators showed that streptomycin is almost inactive against a variety of habitual or occasional intracellular bacteria, including Brucella [1-4], Mycobacterium tuberculosis [5-7], Salmonella [8-9], Staphylococcus aureus [10-13]. Similar data were obtained for gentamicin [14] and kanamycin [15]. On basis of these results, it was concluded that aminoglycosides do not enter cells; this conclusion was reinforced by the observation that intracellular bacteria do not display a metabolic behavior that would make them less sensitive to antibiotics [16].

The lack of activity of streptomycin on intracellular bacteria is however not always complete as evidenced in infected macrophages incubated long enough in presence of the drug [17,18]. Prompt inhibition of bacterial growth has also been demonstrated in fibroblasts infected by Salmonella typhosa [19], and in phagocytic cells harbouring Pasteurella tularensis [20,21], Shigella [22] or Salmonella enteritidis [23].

The first direct evidence that streptomycin enter cells was given by Eksenplanogow [24] who observed that antibiotic activity could be recovered from macrophages incubated in presence of streptomycin. Using [3H]dihydrostreptomycin, Bonventre and Imhoff [25] showed that this antibiotic was actually accumulated by cultured macrophages and BHK cells. However, the intracellular localization of the antibiotics was not studied and no explanation could be given for the conflicting data regarding their intracellular activity.

We report a quantitative study of the uptake and the subcellular localization of streptomycin—and its derivative dihydrostreptomycin—, gentamicin, kanamycin and amikacin in cultured fibroblasts. Preliminary reports have appeared in abstracts [26,27] or in review articles [28-30].

MATERIAL AND METHODS

Microbiological assays of aminoglycosides. Aminoglycosides were assayed by the "cylinder plate" method with Bacillus subtilis as test organism [31]. Triton X-100 (to a final concentration of 0.1%) was added to samples containing cells or cell extracts, to achieve complete dissolution.

The effect of fibroblast proteins (up to 2 mg/ml), calf serum (1%), cell culture medium or sucrose (up to 0.3 M) on the assay was carefully investigated and found negligible.

Bacillus subtilis (ATCC 6633) was grown on Agar medium No. 1, and kept as a spore suspension.

In one instance, a turbidimetric assay, using Klebsiella pneumoniae (ATCC 10031) was used [31]. K. pneumoniae was maintained on agar medium No. 7 and transferred to Bacto dextrose broth for test purpose.

Cell culture and harvest. Cells were obtained from trypsinized rat embryo carcasses and grown in a semi-synthetic medium, as previously described in detail [32]. Cells were used after the 2nd or 3rd subculture. Under those conditions, the culture consisted mostly of an homogeneous population of fibroblast-like cells, as observed in the light or electron microscope [32];
no other cell types, including macrophages or muscle cells could be clearly recognized.

Experiments were made on confluent cells, i.e. when increase in the cell protein content of the culture was no longer significant; the cell density was then about $7.5 \times 10^5$ cells (or 90 $\mu$g of cell protein) per cm$^2$ of growing area [32].

For cell uptake studies, the cell sheet was carefully washed with phosphate-buffered saline (PBS) to remove any trace of culture medium, and dispersed in a solution of Triton X-100 0.1% in distilled water, by vigorous mechanical shaking.

Cell fractionation. For fractionation experiments, cells were detached from glass with 0.5 mM EDTA in PBS, homogenized and subjected to differential or isopycnic centrifugation, exactly as previously described [32].

A lysosomal fraction was isolated from the livers of rats injected with Triton WR-1339 [33]; a liver microsomal fraction was obtained by differential centrifugation [34]; this latter fraction displays aryl hydroxylase activity in presence of NADPH [35].

Biochemical assays. Methods of assay of enzymes and RNA are those described by us previously [32]. Lysosomal cathepsin B was measured with $\alpha$-N-benzoyl-$\beta$-arginine-2-naphthylamide [36, 37].

Uptake of aminoglycosides. Confluent fibroblasts were cultured during 4 days in presence of aminoglycosides—streptomycin, gentamicin, kanamycin and amikacin—at concentrations up to 1 mg/ml. Under our experimental conditions aminoglycosides were not appreciably inactivated. As shown in Fig. 1, there is a fairly linear relationship between the extracellular concentration in aminoglycoside and the intracellular antibiotic activity. For an external drug concentration of 0.5 mg/ml, intracellular contents range from 5 $\mu$g/mg of cell protein for streptomycin up to 13 $\mu$g/mg of cell protein for gentamicin.

Fig. 1. Cellular accumulation of gentamicin, amikacin, streptomycin or kanamycin. Confluent cells were incubated 4 days in presence of drug, at the concentrations indicated in abscissa. The cell protein content remained stable throughout the experiment. Mean value (+ S.D.) of three independent experiments.
Fig. 2. Kinetics of accumulation of streptomycin, gentamicin and kanamycin. Cells were grown to confluency and antibiotic was then added to the culture medium. Left: cells incubated with streptomycin at 350 μg/ml (●), 650 μg/ml (■) or 1,350 μg/ml (○). Right: cells incubated with gentamicin (●) or kanamycin (□) both at 350 μg/ml. During the course of the experiments, no significant change in cell protein content was noticed.

Fig. 3. Release of streptomycin from fibroblasts. Cells were grown in presence of antibiotic at 350 μg/ml (●), 650 μg/ml (△) or 1350 μg/ml (○) for 5 days and had reached confluency. After transfer into medium without streptomycin, the cellular content in antibiotic was measured at the intervals of time indicated in abscissa. Mean value (± S.D.) of 3 independent experiments. Insert: same results represented in semi-logarithmic coordinates; each point is the amount of antibiotic recovered in cells, as percentage of the amount accumulated during the first period of culture. During the experiment no important change in the cell protein content of the cultures was noticed.
Fig. 4. Influence of calf serum concentration on the uptake of streptomycin. Cells were grown in medium containing 10% calf serum, until they had reached confluency. This medium was then replaced by medium supplemented with 500 µg/ml streptomycin and containing 2% (○), 6% (▲), 12% (▼) or 24% (●) of calf serum. Uptake of antibiotic was recorded during the next 4 days. The protein content of the different cultures were very similar.

Fig. 5. Fractionation of an homogenate of fibroblasts by differential centrifugation. Cells were incubated 4 days in presence of streptomycin (100 µg/ml) and the homogenate was fractionated into 5 fractions: N, M, L, P and S. These are represented by blocks ordered in the same sequence over the abscissa, where they span a length proportional to their protein content. The ordinate (height of the blocks) gives the relative specific activity (or amount for RNA), which is the percentage of activity recovered in each fraction over the percentage of protein of the same fraction. The surface area of each block is thus proportional to the percentage of activity found in each fraction. Percentages relate to the sum of activities found in all the fractions. Recoveries from the original homogenate ranged between 89-105 per cent for fibroblasts constituents and was 92 per cent for the antibiotic activity.
cin at different external concentrations, and in presence of gentamicin or kanamycin, both at 350 μg/ml. Uptake of aminoglycosides proceeds at a slow rate, and more than 4 days are required before a stable intracellular content is reached. The rate of uptake of streptomycin is roughly proportional to its concentration. The cell content in antibiotic increases in a constant fashion during the first 3 days and then reaches a plateau value. A very similar kinetics of uptake is observed for kanamycin and gentamicin (Fig. 2) and was also found for dihydrostreptomycin and amikacin.

**Release of streptomycin.** Confluent cells incubated in presence of streptomycin at three different concentrations during 5 days, were washed and transferred into streptomycin-free medium. We investigated the subsequent loss of streptomycin from cells, by collecting them at various times after transfer and measuring their antibiotic and protein content (Fig. 3). The streptomycin content of the cells decreases in a roughly exponential fashion; the rate of loss is proportional to the intracellular content of drug and the “half-life” of intracellular streptomycin was found to be approximately 1.7 days.

**Influence of serum on accumulation of aminoglycosides.** The influence of serum concentration on the uptake of aminoglycosides by fibroblasts was investigated since streptomycin and gentamicin bind to some extent to serum proteins; such binding has not been observed for kanamycin. Cells were subcultivated in normal medium, and transferred after confluency in a medium containing streptomycin, gentamicin or kanamycin (500 μg/ml) and calf serum at increasing concentrations. The uptake of antibiotics was recorded during the next 5 days. The results for streptomycin (Fig. 4) indicate that serum exerts a negative effect on both rate and level of accumulation of this antibiotic. This level (expressed as the ratio between the cell and medium concentrations) decreases from 6 to 1.6 when serum concentration rises from 2 to 24% of cell protein, when the serum concentration rose from 2 to 24%.

By equilibrium dialysis we investigated directly the extent of the binding of streptomycin (500 μg/ml) to serum proteins under our culture conditions. We found that, even at a serum concentration of 24% in culture medium, less than 3 per cent of streptomycin is bound. By contrast, in the test homogenate, streptomycin distributes essentially like RNA and the distribution profile of the antibiotic is largely different from that of N-acetyl-β-glucosaminidase and thapsigargin. As could be expected from the data of Fig. 2, the amount of streptomycin detected in the homogenates increased markedly. In every instance, the density distribution patterns of antibiotic activity and N-acetyl-β-glucosaminidase were found very similar to each other. In a control experiment, no antibiotic activity could be detected in fractions obtained by isopycnic centrifugation from fibroblasts cultivated in the absence of streptomycin.

In four other experiments, fibroblasts were incubated 4 days in presence of 200 μg/ml of dihydrostreptomycin, amikacin, gentamicin or kanamycin, and fractionated by isopycnic centrifugation. The median equilibrium density of the various constituents assayed are given in Table 1. In Fig. 7, are presented the distribution patterns of each of the antibiotics studied, along with those of the acid hydrolases N-acetyl-β-glucosaminidase and thapsigargin B in the amikacin experiment. Within each experiment, these patterns are very similar to each other: they present very close median and modal density values and display similar shapes. From one experiment to the other, some variations are observed, but affect similarly the patterns of the hydrolases and of the antibiotic.

**Distribution of streptomycin added to an homogenate.** It was important to establish whether the typical distribution observed for streptomycin and other aminoglycosides in Figs. 5–7, did not reflect merely a fractionation artifact like adsorption of the antibiotic onto a given subcellular structure during homogenization of the cells. An homogenate (= test) was therefore prepared from cells cultivated in the absence of antibiotic, and streptomycin was added to it immediately afterwards, to a final concentration of 1.5 μg/mg of cell protein. In parallel, a second homogenate (= control) was prepared from cells cultivated 4 days in presence of streptomycin (100 μg/ml). Both homogenates were fractionated by differential centrifugation, according to a simplified scheme which yielded only 2 fractions: NML and PS (Fig. 8). In the control homogenate, the distribution patterns of N-acetyl-β-glucosaminidase and streptomycin are largely similar; by contrast, in the test homogenate, streptomycin distributes essentially like RNA and the distribution profile of the antibiotic is largely dissociated from that of N-acetyl-β-glucosaminidase.

**Sensitivity of streptomycin to metabolic alteration.** Sensitivity of streptomycin (50 μg/ml) to fibroblast constituents (2 mg of protein/ml) was investigated by incubating the antibiotic at 37° in presence of lyzed homogenates at pH 5.0 and 7.0, under sterile conditions. No significant inactivation of streptomycin occurred for incubation periods up to 48 hr.

These experiments were repeated using lysosomal and microsomal fractions, obtained from rat liver. No inactivation of streptomycin (50 μg/ml) was observed
Fig. 6. Fractionation of cytoplasmic extracts of fibroblasts by density equilibration in linear sucrose gradients. Cells were incubated 4 days in presence of streptomycin 100 µg/ml. The graphs show the mean results (± S.D.) of three independent experiments. These results are presented as normalized histograms of density distribution of constituents or activities [42]. The abscissa is the density span of the gradients divided in 15 sections of equal density increment (0.0133) from 1.07 to 1.27 g/cm³.

The ordinate is the frequency of constituents or activities in each section (Q/ΣQΔρ, where Q is the amount of constituent or activity present within the section, ΣQ the sum of the amounts found in all subfractions and Δρ the density increment of the section). The surface of each section of the diagram gives the fractional amount of constituent or activity equilibrating within the section. Solid blocks on each side of the distribution patterns represent material recovered at densities below 1.07 or above 1.27 g/cm³ and are arbitrarily constructed over the spans 1.05–1.07 and 1.27–1.30 g/cm³. Complementary data are presented in Table 1.

either (1) after incubation during 48 hr and at 37°C in presence of a purified lysosomal fraction (0.75 mg of protein/ml), at pH 4, 5 or 6; or (2) after incubation up to 6 hr at 37°C with 5 mg of protein of a microsomal fraction, in presence of MgCl₂ 5 mM, MnCl₂ 25 µM and a system producing NADPH, made of glucose 6-phosphate 5 mM, NADP 0.5 mM, NADH 0.5 mM and 1 unit/ml of glucose 6-phosphate dehydrogenase.

Activity of streptomycin and other aminoglycosides at acidic pH. Although stable in a wide range of pH, aminoglycosides are microbiologically less active at acid than at neutral or alkaline pH [43]. This effect of pH was investigated quantitatively with Klebsiella pneumoniae, by measuring the ID₅₀ of streptomycin (concentration reducing the growth rate to 50 per cent of that observed in absence of antibiotic, [31], at pH 5, 6, 7 or 8.

Results of two independent experiments are presented in Table 2: a drop of pH from 8 to 5 results in an almost 100-fold increase of the ID₅₀ of streptomycin. Similar observations were made for gentamicin and amikacin.

DISCUSSION

Experiments reported in this paper show that streptomycin and four other aminoglycoside antibiotics penetrate slowly into cultured fibroblasts where they accumulate under an active form. At steady state, the ratio of intracellular to extracellular concentration varies between 2 for streptomycin and 5 for gentamicin. This concentration ratio is comparable to that found by Bonventre and Imhoff [25] for [³H]dihydrostreptomycin in macrophages and BHK cells.

Intracellular localization of aminoglycosides. Differential and isopycnic centrifugation allows satisfactory analytical separation of the main subcellular com-
Table 1. Median densities (g/cm³) of subcellular constituents and antibiotic activity after equilibration of cytoplasmic extracts in sucrose-H₂O gradients

<table>
<thead>
<tr>
<th>Constituent or antibiotic activity*</th>
<th>Streptomycin† (100 µg/ml)</th>
<th>Dihydrostr.</th>
<th>Amikacin 200 µg/ml</th>
<th>Gentamicin</th>
<th>Kanamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5′Nucleotidase</td>
<td>1.148 ± 0.009</td>
<td>1.152</td>
<td>1.141</td>
<td>1.148</td>
<td>1.151</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td>1.163 ± 0.004</td>
<td>1.167</td>
<td>1.159</td>
<td>1.164</td>
<td>1.166</td>
</tr>
<tr>
<td>NADH: cyt. c reductase</td>
<td>1.164 ± 0.005</td>
<td>—</td>
<td>—</td>
<td>1.167</td>
<td>1.163</td>
</tr>
<tr>
<td>RNA</td>
<td>1.153 ± 0.007</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Protein</td>
<td>1.151 ± 0.007</td>
<td>1.159</td>
<td>1.154</td>
<td>1.161</td>
<td>1.161</td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>1.197 ± 0.003</td>
<td>1.198</td>
<td>—</td>
<td>1.195</td>
<td>1.211</td>
</tr>
<tr>
<td>Cathepsin B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>1.204 ± 0.009</td>
<td>1.210</td>
<td>1.201</td>
<td>1.189</td>
<td>1.212</td>
</tr>
<tr>
<td>Antibiotic activity</td>
<td>1.205 ± 0.007</td>
<td>1.205</td>
<td>1.198</td>
<td>1.185</td>
<td>1.208</td>
</tr>
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</table>

* Recoveries of constituents or antibiotic activity amongst the fractions ranged between 72 and 117 per cent of the amount of activity found in the cytoplasmic extract.
† These values are similar to those obtained on confluent fibroblasts, cultured in a medium containing 100 µg/ml streptomycin and 100 units/ml penicillin, as reported previously [32].

Components of cultured fibroblasts [32, 44], namely mitochondria (cytochrome oxidase), plasma membranes and related structures (5′ nucleotidase), endoplasmic reticulum (partially NADH: cytochrome c-reductase and RNA), lysosomes (N-acetyl-β-glucosaminidase, cathepsin D and B) and peroxisome-like structures (catalase).

When homogenates or cytoplasmic extracts of fibroblasts, incubated with aminoglycosides, are fractionated by differential or isopycnic centrifugation, we

Fig. 7. Distribution patterns of acid hydrolases (glucosaminidase = N-acetyl-β-glucosaminidase) and of antibiotic activities after fractionation of cytoplasmic extracts of fibroblasts by density equilibration. Cells were incubated 4 days in presence of 200 µg/ml of dihydrostreptomycin (A), amikacin (B), gentamicin (C) or kanamycin (D). Results are presented as in Fig. 7. Complementary data are given in Table 1.
Fig. 8. Distribution patterns of antibiotic activity and of N-acetyl-β-glucosaminidase of two homogenates of fibroblasts fractionated by differential centrifugation. The homogenates were divided into two subfractions: NML—obtained by centrifuging the homogenate at 25,000 r.p.m. in rotor Ti 50 (Beckman Instruments), for 6 min 42 sec ($W = 2.5 \times 10^9 \text{ rad}^{-2} \text{sec}^{-1}$ [36])—and a supernatant PS. Left: fibroblasts were cultivated in the absence of antibiotic and streptomycin was added to the homogenate, immediately after homogenization of cells; Right: fibroblasts were cultivated 4 days in presence of streptomycin (100 μg/ml). The streptomycin contents of both homogenates were similar (â 1.5 μg/mg of cell protein). Results are presented as in Fig. 6.

Table 2.

<table>
<thead>
<tr>
<th>pH</th>
<th>5.0</th>
<th>6.0</th>
<th>7.0</th>
<th>8.0</th>
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<tr>
<td>Expt 1</td>
<td>46.4</td>
<td>9.26</td>
<td>1.17</td>
<td>0.50</td>
</tr>
<tr>
<td>Expt 2</td>
<td>63.1</td>
<td>12.6</td>
<td>1.71</td>
<td>0.68</td>
</tr>
</tbody>
</table>

observe a constant association between antibiotic activity and acid hydrolases. The distribution patterns of the antibiotic activities are clearly distinct from those of the other cell constituents assayed in this work. This indicates strongly that the intracellular aminoglycosides are largely confined to lysosomes in the homogenates or cytoplasmic extracts of fibroblasts. These experiments were performed at antibiotic concentrations recommended for routine use in culture media [45]. Since lysosomes of rat embryo fibroblasts cultured under these conditions, occupy about 3 per cent of the cell volume [46], the concentration ratio of the antibiotics between the lysosomal milieu and the culture fluid in the steady state, varies between 60 and 150 depending of the drug.

The association of aminoglycosides with lysosomes does not result of a mere adsorption of the intracellular antibiotics onto these organelles during the homogenization in 0.25 M sucrose (Fig. 8).

By comparison with the other aminoglycides, gentamicin displays a smaller buoyant density and provokes a small but distinct shift of the density pattern of N-acetyl-β-glucosaminidase towards lighter fractions; this will be further investigated.

The mechanism of intracellular penetration and of lysosomal accumulation of aminoglycosides. Intracellular penetration and intralysosomal accumulation of aminoglycosides may occur by endocytosis or by permeation through membranes, followed by trapping in lysosomes [29].

Endocytosis. Endocytosis allows the uptake and intralysosomal accumulation of substances of low molecular weight such as xylose, mannitol, sucrose or peptides [47–53]. For aminoglycosides, endocytosis would have to be of the fluid [54] or non selective [29]—kind, in view of the linear dependence of rate of uptake on concentration. To account by such a mechanism for the initial rates of uptake observed with streptomycin and kanamycin (Fig. 2) we must assume a daily pinocytic intake of 3–3.5 μl/mg cell protein, or 60–70 per cent of the cell volume. This is an acceptable value in the light of the morphometric studies of Steinman et al. [55] on L cells, and of our own estimates on rat fibroblasts (P. Tulkens and Y. J. Schneider, unpublished results).

Not all observations, however, fit with this hypothesis. On one hand, the rate of uptake of gentamicin is three times that of streptomycin: on the other hand, macrophages take up dihydrostreptomycin [25] at a rate comparable to that observed here for fibroblasts, although their pinocytic rate is undoubtedly much higher [55]; no difference is either observed in uptake of streptomycin between phagocytozing and non-phagocytozing macrophages [71].

It is also difficult to explain the discharge of streptomycin from lysosomes (Fig. 3). This reflects probably a release and not a catabolism, since streptomycin resists lysosomal and microsomal enzymes under conditions in which these enzymes are active [35, 56–58]; moreover no catabolic pathway has been uncovered for streptomycin in mammalian cells [43, 59]. This discharge could however be explained by our recent observations indicating the existence of a rapid shuttle of membrane elements, most probably small vesicles, between plasma membrane and lysosomes [60, 61].

Permeation and intralysosomal trapping. Lysosomes constitute a very acid compartment within the cell...
Antibiotics in lysosomes of cultured rat fibroblasts

Charges, probably sialic acid residues [63-65]; they consequently concentrate a variety of basic compounds [66-68]. The theory of this process has been examined elsewhere [29]. It rests on the assumption that uncharged molecules (if sufficiently lipophilic) move readily, and their protonated forms very slowly if at all, across biological membranes. The steady state level depends on various molecular properties of the drug, and on the difference between extra- and intra-lysosomal pH. The kinetics of accumulation is compatible with such a mechanism, and so is the slow rate of entry of streptomycin which due to its 2, 6-diamino-l, l-dicarboxylic structure must have strong basic guanidinium groups [69] must have concentrations in lysosomes. If trapping of aminoglycosides in lysosomes is a therapeutically self-limiting process, the localization of aminoglycosides in lysosomes can be explained by lowering the concentration of the permeant form. On the other hand, it is surprising that a vanishingly small concentration of uncharged peritrichous bacteria; they ought to be confirmed on other cell types, [24, 25]. Our own equilibrium dialysis experiments failed to disclose any significant binding even of streptomycin.

In conclusion, the relative contribution of permeation and of endocytosis-exocytosis on the movements of aminoglycoside antibiotics from medium into lysosomes and back must remain an untested question, which stumbles on what appear to be mutual contradictions between experimental findings. Pharmacological significance of intralysosomal accumulation of aminoglycosides. Our studies have been performed on cells that do not often harbour bacteria; they ought to be confirmed on other cell types, like macrophages or polymorphonuclear leukocytes that are highly susceptible to intracellular infection. Our results may however explain the relative inefficiency of aminoglycoside antibiotics against intracellular bacteria. Aminoglycosides are accumulated by cells, but are localized predominantly in lysosomes. The exact pH within lysosomes, in cells, is unknown, but all available data, including most recent ones [62] point to values between 4 and 5; the activity of aminoglycosides will therefore be severely impaired (Table 2, [15, 72, 73]) despite their concentration in lysosomes. If trapping of aminoglycosides in lysosomes results from the acidity of these organelles, intralysosomal concentration is a therapeutically self-defeating process. The localization of aminoglycosides in lysosomes will also explain their poor activity on those bacteria which manage to keep away from lysosomes, but sojourn in phagosomes that do not fuse with lysosomes [74]. The low rate of uptake of aminoglycosides by fibroblasts, or by other cell types [24, 25] may also explain their inefficiency, especially towards rapidly dividing bacteria, which may kill the host cell before sufficient accumulation of the drug might occur.

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