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EVIDENCE THAT DESFERRIOXAMINE CANNOT ENTER CELLS BY PASSIVE DIFFUSION

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Abstract—Accumulation of [^{14}C]desferrioxamine by rat visceral yolk sac *in vitro* has been compared with that of [^{14}C]sucrose, a probe for fluid-phase pinocytosis. Kinetic parameters for both substrates are closely similar, as are the effects of inhibitors. It is concluded from these data, and from theoretical considerations, that desferrioxamine cannot enter cells other than by pinocytosis and that, once internalized, it will remain in the lysosomes. The results indicate the need for a re-evaluation of the pharmacokinetic mechanisms traditionally accepted for the drug's ability to deplete iron from cells and tissues.

Desferrioxamine [1] is the only iron chelator of proven clinical benefit in the treatment of iron overload. When administered by continuous subcutaneous infusion, it can lead to iron excretion in the urine in amounts exceeding 15 mg/day. The drug is not without disadvantages, however: it is ineffective by mouth, and it has a short plasma half-life, owing to rapid renal excretion of the free form as well as of the iron-complex.

It is widely assumed that desferrioxamine has access to several intracellular sites when used clinically for the treatment of iron overload or for studying iron-mediated radical generation in cell culture systems. However, recent studies [2] on the ability of desferrioxamine and some hydroxypyridones to induce the oxidation of haemoglobin to methaemoglobin in intact erythrocytes indicated that desferrioxamine was not crossing the plasma membrane.

Strongly hydrophilic organic compounds cannot cross plasma membranes by passive diffusion, and enter cells by only two mechanisms, namely by pinocytosis and on porters. Where no porter exists for the solute in question, entry is solely by pinocytosis. Sucrose is such a solute: it is captured by non-specific fluid-phase pinocytosis, a mechanism that leads to its transfer into the lysosomes. Since sucrose hydrolysis by the lysosomal glycosidases proceeds at a negligible rate, and since the lysosome membrane is impermeable to sucrose, the disaccharide accumulates in and can lead to the distention of the lysosome compartment [3]. The rate of accumulation of radioactivity by cells incubated in the presence of [^{3}H]- or [^{14}C]sucrose is an accepted method for measuring the cells' capacity for fluid-phase pinocytosis [4, 5].

In the absence of direct evidence, it might be expected that cells would handle desferrioxamine much as they handle sucrose. The availability to us of a sample of [^{14}C]desferrioxamine has permitted an experimental examination of this proposition. As test system we have used the 17.5-day rat visceral yolk sac maintained in organ culture. This system has been used extensively for quantitative studies on both fluid-phase and adsorptive pinocytosis, owing to the remarkably reproducible results that it yields [6].

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MATERIALS AND METHODS

[^{14}C]Desferrioxamine (0.37 μCi/μmol) was a gift from Ciba Geigy. [^{14}C]Sucrose was from Amersham International, and colchicine was from Sigma.

Uptake of ^{14}C-labelled solutes by 17.5-day rat visceral yolk sacs was measured as described by Roberts *et al.* [7]. Yolk sacs were incubated individually in Erlenmeyer flasks containing 10 mL medium 199 and the ^{14}C-labelled solute. After the appropriate time-interval, yolk sacs were removed and washed three times in ice-cold 1% (w/v) NaCl, before processing for scintillation counting and protein estimation [8].

RESULTS

Figure 1 shows the results of an experiment in which 17.5-day visceral yolk sacs were incubated for periods up to 4 h in the presence of [^{14}C]sucrose. Uptake of radioactivity, expressed as a clearance, appears to be linear over this period, permitting the calculation of an uptake rate. In four identical experiments, the average uptake rate, or Endocytic Index [9], was 3.39 ± 0.25 (SD)μL per mg yolk sac protein per hour, a value that may be compared with 2.04 ± 0.21, the uptake rate reported by Roberts *et al.* [7].

Figure 1 also shows that uptake of [^{14}C]sucrose is profoundly inhibited by either a low incubation temperature (4°) or the presence of colchicine (10 μg/mL). A similar inhibition of uptake of ^{125}I-labelled polyvinylpyrrolidone, another marker of fluid-phase pinocytosis in the visceral yolk sac, was reported by Duncan and Lloyd [10].

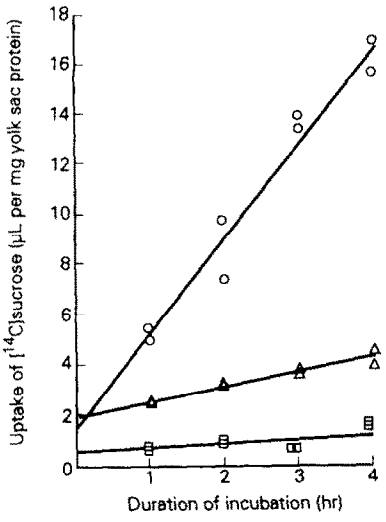

Fig. 1. Uptake of [^{14}C]sucrose by 17.5-day rat visceral yolk sacs in culture, at 37° (○), at 4° (□), and at 37° in the presence of 10 μg colchicine/mL (△). Each point represents a single yolk sac.

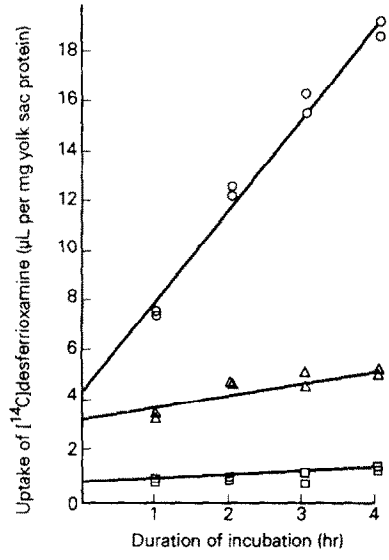

Fig. 2. Uptake of [^{14}C]desferrioxamine by 17.5-day rat visceral yolk sacs in culture, at 37° (○), at 4° (□), and at 37° in the presence of 10 μg colchicine/mL (△). Each point represents a single yolk sac.

Figure 2 shows the results of experiments in which yolk sacs were incubated with [^{14}C]desferrioxamine. Again uptake was linear with time, the mean rate in five experiments being 3.23 ± 0.60 μL/mg protein/hr. Low temperature or the presence of colchicine was inhibitory.

DISCUSSION

As explained in the introduction, the rate of uptake of [^{14}C]sucrose is an accepted measure of fluid-phase pinocytosis in mammalian cells. We now report that, using the rat visceral yolk sac as test system, [^{14}C]sucrose and [^{14}C]desferrioxamine are similarly accumulated in a linear fashion over an extended incubation period, and moreover that the rates of accumulation of sucrose and desferrioxamine are identical. The concordance of these rates is compelling evidence that the same mechanism, namely pinocytosis, is responsible for the uptake of both substances; also that uptake of desferrioxamine, like that of sucrose, is by fluid-phase (i.e. not adsorptive) pinocytosis. The essentially identical effects of inhibitors on the uptake of the two substrates provides additional evidence for uptake by pinocytosis.

These results indicate that, if desferrioxamine can enter the visceral yolk sac cells by simple permeation, its entry thus is quantitatively insignificant as compared with the molecule's uptake by pinocytosis. This should not be a surprising conclusion: desferrioxamine has a very low lipid solubility: its oil:water partition coefficient is 0.01 [11], a value too low to be compatible with passive diffusion across plasma membranes [12].

The intracellular fate of [^{14}C]desferrioxamine following pinocytosis is not directly indicated by our experiments, but there is every reason to suppose that it reaches the lysosomes and stays there. As explained above, this is the fate of sucrose. Recent evidence concerning the ability of solutes to cross the lysosome membrane by passive diffusion [13] leaves little doubt that desferrioxamine would be non-permeant. Certainly the linearity of uptake with time provides good evidence that pinocytosed [^{14}C]desferrioxamine remains within the cell and is not returned to the culture medium.

To what extent can our conclusions be extrapolated to mammalian cells other than those of the rat visceral yolk sac? Cell types differ little in their plasma membranes' capacity for passive diffusion [12]. Therefore, unless a particular cell type possesses a porter for desferrioxamine in its plasma membrane, pinocytosis will constitute the drug's only route into that cell. Furthermore, unless a cell's lysosome membrane has a desferrioxamine porter, pinocytosed desferrioxamine will not reach any intracellular sites other than the endosomal and lysosomal vacuolar apparatus. We know of no evidence for carrier-mediated translocation of desferrioxamine across cellular membranes. As indicated in the introduction, Rice-Evans *et al.* [2] have concluded that desferrioxamine does not enter human erythrocytes. Our findings are compatible with this conclusion, since the human erythrocyte is a cell in which pinocytosis does not take place.

We urge a re-evaluation of the traditional view that desferrioxamine's pharmacological effects are dependent on the drug's penetration into cells.

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