Cells of the J774 mouse macrophage-like cell line possess organic anion transporters that transport fluorescent dyes such as Lucifer Yellow out of the cytoplasmic matrix of the cells; the dye is both sequestered in endosomes and secreted into the extracellular medium. Lucifer Yellow that is sequestered within endosomes is subsequently delivered to the lysosomal compartment. In the present studies we demonstrated that probenecid inhibited removal of Lucifer Yellow from the soluble cytoplasm and sequestration into membrane bound organelles by quantitating Lucifer Yellow fluorescence in both soluble and membrane-associated fractions of J774 cells. In addition, we examined the uptake of Lucifer Yellow into isolated subcellular organelles derived from J774 cells. Lucifer Yellow transport in the organelar fraction of J774 cell homogenates was temperature- and pH-dependent and did not require ATP. Subcellular organelles from J774 cells were fractionated into endosome- and lysosome-enriched fractions by Percoll density gradient centrifugation. Lucifer Yellow was preferentially taken up by vesicles of the endosome-enriched fraction, and this transport was inhibited by probenecid.

These studies provide direct evidence that probenecid inhibits Lucifer Yellow transport out of the cytoplasmic matrix and into cytoplasmic vacuoles in J774 cells and that organic anion transport in isolated organelles derived from J774 cells occurs preferentially in endosome, rather than in lysosome-enriched fractions; they suggest that Lucifer Yellow is carried across membranes via a secondary active transport process that requires proton symport or hydroxyl anion antiport.

Macrophages secrete a number of inflammatory mediators and metabolites. Some of these, such as leukotrienes, prostaglandins, glutathione, bilirubin, and lactate, are organic anions that must cross a cellular membrane in their exodus from the macrophage cytoplasmic matrix to the extracellular environment. The mechanisms by which these organic anions cross macrophage membranes are unknown. We have shown that mouse peritoneal macrophages and cells of the J774 mouse macrophage-like cell line transport water-soluble ionic fluorescent dyes such as Lucifer Yellow from the cells' cytoplasmic matrix. Although Lucifer Yellow is membrane-impermeant, it can be introduced into the cytoplasmic matrix of mouse macrophages or J774 cells by reversible permeabilization of their plasma membrane with ATP (1). Dye introduced into the cytoplasmic matrix by this method does not remain in this location: it is sequestered within endosomal vacuoles (2) and secreted into the extracellular medium (3). Some of the Lucifer Yellow sequestered within endosomal vacuoles is subsequently delivered to lysosomes. Lucifer Yellow sequestration and secretion appear to be active transport processes and are blocked by probenecid and sulfinpyrazone, compounds which inhibit organic anion transport in polarized epithelia (3). To define the pH and energy requirements of Lucifer Yellow transport and the subcellular location of the carriers that mediate transport of this dye, we measured Lucifer Yellow uptake into subcellular fractions of J774 cells that were enriched in membrane-bound organelles.

MATERIALS AND METHODS

Chemicals—ATP (special grade) was purchased from Boehringer Mannheim Biochemicals. Lucifer Yellow CH (tetrathium salt) was from Molecular Probes, Inc. (Eugene, OR), and probenecid was from Sigma.

Cells and Media—The J774 mouse macrophage-like cell line (4) was grown in spinner cultures in Dulbecco’s modified Eagle medium supplemented with 10% heat-inactivated (56°C, 30 min) bovine serum (D10). Cells were removed from the spinner cultures on the day of the experiment.

ATP Permeabilization—Lucifer Yellow (0.5 μg/ml) was introduced into the cytoplasm of J774 cells (1.2 × 10⁶/ml) by permeabilizing the cells with 5 mM ATP in D10 containing 0.5 mg/ml Lucifer Yellow at 37°C for 0 min as described (1). ATP permeabilization was terminated by adding a volume of 100 mM MgCl₂ sufficient to yield a final concentration of 5.8 mM Mg²⁺. The cells were washed twice in phosphate-buffered saline containing Ca²⁺ and Mg²⁺.

Subcellular Fractionation—J774 cells (1.5 × 10⁶) were washed in KCl buffer (150 mM KCl, 10 mM HEPES,¹ 1 mM MgCl₂, 1 mM EGTA, pH 7.4), resuspended in 10 ml of sucrose homogenization buffer (250 mM sucrose, 20 mM HEPES, 1 mM EGTA, 1 mM MgCl₂, pH 7.5) containing 1 mM dithiothreitol, 0.6 IU (trypsin inhibitor unit)/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride, placed in a stainless steel Dounce homogenizer, and homogenized until 85-90% of the cells were disrupted as determined by phase contrast microscopy. The nuclei and intact cells were pelleted by centrifugation at 300 × g for 10 min at 4°C. The postnuclear supernatant was either decanted and used for experiments directly or further fractionated by layering 9 ml of it on top of 30 ml of a solution containing 27% Percoll, 250 mM sucrose, and 20 mM HEPES (pH 7.5), and centrifuging the material in a Sorvall SS-34 rotor at 15,000 × g for 60 min at 4°C. The resulting gradient contained three distinct bands: two bands of light buoyant density which formed near the top of the gradient and a third band of greater buoyant density which formed near the bottom of the tube. The gradient was collected either in 1-ml aliquots

¹ The abbreviations used are: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, [ethylenbis(oxyethylenenitrilo)]tetracetate acid.
from the bottom of the tube using a Gilson microfractionator or by
directly harvesting each band by hand using a serological pipette. To
remove the Percoll from the gradient fractions they were centrifuged
at 100,000 x g for 60 min at 4 °C using a Beckman model SW 50.1
swinging bucket rotor on a Beckman model L5-65 ultracentrifuge.
The sedimentable organelles contained in each fraction formed a
layer immediately above the dense Percoll pellet and were removed
by pipette.

Quantification of Lucifer Yellow Uptake—Lucifer Yellow fluores-
cence was measured in a Perkin-Elmer 650-40 fluorescence spectro-
photometer at an excitation wavelength of 430 nm and an emission
wavelength of 540 nm as described (5). Dye standards were made in
0.05% Triton X-100. Lucifer Yellow uptake is expressed as nanograms
of Lucifer Yellow/mg of cell protein. Protein determinations were
performed either by a modification of the method of Lowry et al. (6)
or by the Coomassie Blue Method (7).

Electron Microscopy—Organelles in subcellular fractions of low
and high buoyant density were pelleted by centrifugation at 10,000 x
g in an Eppendorf microfuge. The pellets were fixed with 2% glutar-
alddehyde in 0.1 M cacodylate buffer with 4.5% sucrose and 0.45 mM
CaCl2 (pH 7.4), post-fixed in 1% osmium tetroxide in cacodylate
buffer (pH 7.4), stained en bloc with saturated uranyl acetate in water
for 20 min, and dehydrated in a graded series of ethanol solutions.
The pellets were oriented and embedded in Epon. Sections were cut
on an MT 6000 ultramicrotome to contain the full thickness of the
pellet, stained with uranyl acetate and lead citrate, and examined
from the top to the bottom of the pellet with a JEOL 1200 EX
electron microscope.

RESULTS

Probenecid Inhibits Secretion and Vacuolar Sequestration
of Lucifer Yellow in J774 Cells—We showed previously that
J774 cells both sequester Lucifer Yellow within endosomes
and secrete the dye into the extracellular medium and that
probenecid inhibits both sequestration and secretion of the
dye (2, 3). In the present studies, we have directly quantitated
dye sequestration by cellular organelles. To accomplish this,
J774 cells were loaded with Lucifer Yellow by ATP permea-
bilization, allowed to reseal, and incubated in Lucifer Yellow-
tree medium at 37 °C for varying intervals in the presence or
absence of 5 mM probenecid. The cells were homogenized and
the large membrane-bound organelles (“organellar fraction”) were
separated from the soluble portion (“cytosolic fraction”) of the
postnuclear supernatant by centrifugation at 20,000 X
g for 10 min. We then measured Lucifer Yellow fluorescence
associated with the total cell homogenate and with various
subcellular fractions.

Cells incubated in the absence of probenecid rapidly se-
creted Lucifer Yellow into the surrounding medium as dem-
onstrated by a rapid decrease in Lucifer Yellow content of the
cell homogenate, whereas cells incubated in the presence of
probenecid did not demonstrate a decrease in Lucifer Yellow
content (Fig. 1). The cytotoxic fractions of cells incubated in
the absence of probenecid also demonstrated a rapid decline
in Lucifer Yellow content at the same time that the organellar
fraction from these cells accumulated dye. In contrast, both
the cell homogenate and the cytotoxic fraction of cells incu-
bated in medium containing probenecid showed no change in
Lucifer Yellow content during this period; moreover, their
organellar fractions did not demonstrate an increase in Lu-
cifer Yellow content, even though at the times studied the
cytoplasm of the probenecid-treated cells contained a higher
concentration of dye than did the cytoplasm of cells incubated
in the absence of probenecid.

This experiment provided quantitative evidence that J774
cells simultaneously sequester Lucifer Yellow into membrane-
bound vacuoles and clear the dye from the cytoplasmic matrix
and that probenecid inhibits these processes. In addition, it
confirmed that Lucifer Yellow sequestering vacuoles could be
isolated from J774 cells without being disrupted. That pro-
benecid blocked Lucifer Yellow sequestration into the orga-
nellar fraction shows that dye uptake is not an artefact of the
homogenization procedure.

Lucifer Yellow Sequestration in Cell Homogenates Is Tem-
perature-dependent—Lucifer Yellow secretion from intact
cells is markedly reduced at 4 °C (3). To determine whether
isolated cellular organelles accumulate Lucifer Yellow and
whether this uptake is temperature-dependent, J774 cells were
homogenized, the nuclei were removed by centrifugation, and
the postnuclear supernatant was centrifuged at 20,000 X
g for 10 min at 4 °C to sediment large membrane bound vesicles.
These vesicles were incubated in KCl buffer containing 0.5
mg/ml Lucifer Yellow at 4 or 37 °C. The samples then were
centrifuged, and the Lucifer Yellow content of the sedimented
organelles was measured. The Lucifer Yellow content of samples
incubated at 37 °C increased with time (Fig. 2). The most
rapid increase in the uptake of Lucifer Yellow into vacuoles
occurred during the first 5–10 min, with a much slower
increase occurring thereafter. There was little Lucifer Yellow
uptake into the organelar fraction at 4 °C. The small amount
of Lucifer Yellow uptake that did occur at 4 °C exhibited
linear kinetics and did not reach the same level as that
observed at 37 °C, even after 120 min of incubation (Fig. 2).
This suggests that Lucifer Yellow uptake at 4 °C was due to
diffusion. The difference between the uptake that occurred at
37 °C and at 4 °C yielded an estimate of the amount of Lucifer
Yellow carried by specific membrane transporters (Fig. 2, broken
line).

We performed two kinds of experiments to confirm that
the Lucifer Yellow associated with sedimentable vesicles in
the studies described above was due to transport of Lucifer
Yellow into a membrane bound compartment and not merely

Fig. 1. Probenecid inhibits secretion of Lucifer Yellow
from the cytoplasmic matrix of J774 cells and sequestration
of Lucifer Yellow within cytoplasmic vacuoles. 1.5 x 10^6
J774 cells were loaded with Lucifer Yellow (LY) by ATP" permeabil-
ization at 37 °C. The cells were then incubated in Lucifer Yellow free medium
at 37 °C in the presence (broken lines, open markers) or absence (solid
lines, closed markers) of 5 mM probenecid. The cells were fractionated as
described, and Lucifer Yellow content was measured in the cell
homogenate, the cytosolic fraction, and the organellar fraction of the
postnuclear supernatant. The top panel shows the Lucifer Yellow content
of the homogenate (circles) and cytosolic fraction (squares). The bottom
panel shows Lucifer Yellow content of the organellar fraction.

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to membrane binding of the dye. First, we examined the effects of repeatedly freezing and thawing the samples on their retention of Lucifer Yellow. Vesicles derived from the postnuclear supernatant were incubated with Lucifer Yellow as described above and washed twice. One aliquot of the sedimented vesicles was solubilized in 2 ml of 0.05% Triton X-100, whereas a second aliquot was resuspended in KCl buffer, frozen in liquid nitrogen three times, and centrifuged at 20,000 x g for 10 min at 4 °C. The sedimented material was solubilized in 2 ml of 0.05% Triton X-100 and its Lucifer Yellow content compared with that of the vesicles pellet without being subjected to freezing and thawing. The Lucifer Yellow content of the vesicles subjected to repeated freeze-thaw cycles was reduced by 80% compared to the Lucifer Yellow content of the other samples. Second, we exposed membrane vesicles to hypotonic medium and quantitated the release of Lucifer Yellow from these vesicles. Vesicles derived from the postnuclear supernatant were incubated with Lucifer Yellow as above, washed at 4 °C, and resuspended either in KCl buffer or in dilute (1.5 mM) KCl buffer. We then centrifuged the vesicles and quantitated their Lucifer Yellow content. The Lucifer Yellow content of vesicles that were exposed to hypotonic medium was reduced by 76% compared to vesicles that had been resuspended in isotonic medium. These two experiments suggest that the bulk of the sequestered Lucifer Yellow was contained in membrane bound vesicles and was not merely bound to their membranes.

We also examined by fluorescence microscopy samples of the organellar fraction that had been incubated at 37 °C with Lucifer Yellow before and after freezing and thawing. Examination of the homogenate prior to freeze-thawing revealed numerous vesicular structures of heterogeneous size containing fluorescent dye (not shown). These fluorescent vesicles were not seen after freezing and thawing. This provided morphological confirmation that the Lucifer Yellow incorporated into the organellar fraction in cell homogenates was contained within membrane delimited compartments.

Lucifer Yellow Sequestration in Cell Homogenates Is pH-dependent—Lucifer Yellow efflux from intact J774 cells is inhibited by acidification of the extracellular medium, and is maximal when the cells are incubated in medium with pH ≤ 7.5 (3). To determine whether Lucifer Yellow transport into cytoplasmic vesicles is also pH-sensitive, we measured the effect of pH on Lucifer Yellow uptake into these organelles. The postnuclear supernatant of J774 cells was incubated in medium containing 0.5 mg/ml Lucifer Yellow at pH 6.2, 7.4, or 8.6 (Fig. 3). There was a marked increase in the quantity of Lucifer Yellow recovered in the organellar fraction as the pH was reduced. Vesicles incubated at pH 6.2 took up four times more Lucifer Yellow than did vesicles incubated at pH 7.4 and six times more dye than vesicles incubated at pH 8.6.

This result is consistent with the inhibitory effect of acidifying the extracellular medium on Lucifer Yellow efflux from intact cells. In both intact cells and isolated organelles Lucifer Yellow transport occurred in the same direction as the proton gradient. In intact cells incubated in alkaline medium this gradient is directed outward; lowering the external pH decreases this gradient and results in less Lucifer Yellow efflux from the cells. In vesicles isolated in neutral buffer and incubated in acidic medium, the pH gradient was directed inward; raising the pH in the medium surrounding the vesicles diminished the inward-directed proton gradient and also diminished Lucifer Yellow transport. This suggests that Lucifer Yellow transport in both the intact cell homogenate is dependent on H+ symport or OH− antiport.

To determine whether ATP stimulates Lucifer Yellow uptake into cytoplasmic vacuoles, the postnuclear supernatant from 2 x 10^6 J774 cells was centrifuged at 20,000 x g for 10 min at 4 °C, and the pelleted organelles were resuspended in KCl buffer. Aliquots of this suspension were incubated in KCl buffer containing 0.5 mg/ml Lucifer Yellow in the presence or absence of 1 mM MgATP for 10 min at 37 °C, centrifuged at 10,000 x g for 10 min at 4 °C, and the amount of Lucifer Yellow taken up into the sedimented organelles was assayed fluorometrically. An equal amount of Lucifer Yellow was incorporated into organelles incubated in the presence and absence of ATP, indicating that Lucifer Yellow transport into these vesicles did not require ATP.

Lucifer Yellow Transport in Endosome- and Lysosome-enriched Fractions—In intact J774 cells, Lucifer Yellow is first transported from the cytoplasmic matrix into a prelysosomal compartment and is subsequently transferred from this compartment to lysosomes (2). This prelysosomal compartment possesses many of the characteristics of endosomes. Lysosomes and endosomes can be separated from each other on Percoll density gradients because the buoyant density of lysosomes is greater than that of endosomes (8). To determine whether there are differences in Lucifer Yellow transport into endosomes and lysosomes, we separated the post nuclear supernatant fraction of J774 cells into endosome- and lysosome-enriched fractions and studied dye transport into the membrane bound organelles contained in each of them. The postnuclear supernatant of 1.5 x 10^6 J774 cells was layered
on a 27% Percoll density gradient and centrifuged for 60 min at 15,000 x g. The resulting endosome- and lysosome-enriched bands were collected and centrifuged for 60 min at 100,000 x g to sediment the Percoll. The organelles formed a layer on top of the Percoll pellet. They were resuspended in KCl buffer for further studies.

The morphological characteristics of the two fractions were examined by electron microscopy (Fig. 4). The less dense fraction (r = 1.04 g/ml) contained membrane-bound vacuoles with electron-lucent interiors that were heterogeneous in size. A few strips of rough endoplasmic reticulum were occasionally seen in this fraction. The fractions of greater density (r = 1.08 g/ml) consisted of a homogenous population of membrane-delimited structures possessing electron dense interiors characteristic of lysosomes.

In a separate experiment, the postnuclear supernatant from 1.5 x 10^6 J774 cells was layered on a 27% Percoll gradient and centrifuged for 60 min at 15,000 x g. One ml fractions were collected and each fraction was assayed for the presence of enzymatic markers of plasma membrane (alkaline phosphodiesterase I), mitochondria (cytochrome c oxidase), and lysosomes (β-glucuronidase) (Fig. 5). The peak of alkaline phosphodiesterase activity was located in the less dense fractions. Although a small proportion of the total β-glucuronidase activity was in these fractions as well, the major peak of β-glucuronidase activity was located in the fractions of greatest buoyant density and accounted for >90% of the total β-glucuronidase activity. Cytochrome c oxidase activity was distributed in a broad band throughout the fractions of intermediate buoyant density.

From the distribution of the enzyme markers and the morphological characteristics of the fractions observed by electron microscopy, we concluded that the lysosomes were contained in the fractions of greatest buoyant density ("lysosomal fractions") and that the plasma membrane derived and early endocytic vesicles were located in the fractions of least buoyant density ("endosome-enriched fractions"). These conclusions are consistent with the work of Galloway et al. (8).

Lucifer Yellow transport was studied in the endosome- and lysosome-enriched fractions of J774 cell homogenates. 1.5 x 10^6 J774 cells were homogenized and fractionated by Percoll density gradient centrifugation. The lysosome enriched and endosome enriched fractions were incubated in KCl buffer containing 0.5 mg/ml Lucifer Yellow (LY) at pH 7.5 and 37 °C. The organelles contained in these fractions were washed three times in cold KCl buffer by sedimentation, and their Lucifer Yellow content was assayed as described. □, endosome-enriched fraction; ■, lysosome-enriched fraction.

The buoyant density of vacuoles sequestering Lucifer Yellow also was assessed in another manner. J774 cells were homogenized and the postnuclear supernatant was incubated with 0.5 mg/ml Lucifer Yellow at 37 °C for 5 min. The membrane-bound organelles contained in the postnuclear supernatant were washed twice in KCl homogenization buffer by sedimentation, layered on a 27% Percoll density gradient, and centrifuged at 15,000 x g for 60 min, all at 4 °C. One-ml fractions were collected from the bottom of the tube, and the Percoll was removed from each sample by ultracentrifugation (100,000 x g x 60 min). The pellet from each sample was solubilized in 2 ml of 0.05% Triton X-100, and its Lucifer Yellow content was measured (Fig. 5). Almost all of the

FIG. 5. Lucifer Yellow is sequestered within vesicles of light buoyant density in J774 cell homogenates. The postnuclear supernatant of 5 x 10^6 J774 cells was collected and incubated in KCl buffer containing 0.5 mg/ml Lucifer Yellow at 37 °C for 5 min and then centrifuged on a 27% Percoll density gradient as described. The gradient fractions were assayed for enzymatic markers of plasma membranes (alkaline phosphodiesterase I, broken line and open circles) and of lysosomes (β-glucuronidase, solid line and open squares), and for Lucifer yellow (bars).

FIG. 6. Lucifer Yellow uptake into vesicles of the endosome- and lysosome-enriched fractions of J774 cell homogenates. 1.5 x 10^6 J774 cells were homogenized and fractionated by Percoll density gradient centrifugation. The lysosome-enriched and endosome-enriched fractions were incubated in KCl buffer containing 0.5 mg/ml Lucifer Yellow (LY) at pH 7.5 and 37 °C. The organelles contained in these fractions were washed three times in cold KCl buffer by sedimentation, and their Lucifer Yellow content was assayed as described. □, endosome-enriched fraction; ■, lysosome-enriched fraction.
Lucifer Yellow was contained within the fractions of lowest density, coincident with the alkaline phosphodiesterase activity.

Probenecid Inhibits Lucifer Yellow Transport in Endosome-enriched Subcellular Fractions—5 mM probenecid markedly inhibits Lucifer Yellow sequestration and secretion in intact cells (3). Studies described above using the postnuclear supernatant fraction of J774 cells demonstrated that Lucifer Yellow uptake was inhibited by 5 mM probenecid. We also assessed the effect of probenecid on the sequestration of Lucifer Yellow into the endosome-enriched fraction of J774 cell homogenates. The endosome-enriched fraction was collected and incubated with 0.5 mg/ml Lucifer Yellow at 37 °C in the presence or absence of 5 mM probenecid (pH 7.5) (Fig. 7). In the absence of probenecid, Lucifer Yellow uptake averaged 311 ± 82 ng of Lucifer Yellow/mg of protein at 5 min and 517 ± 77 ng of Lucifer Yellow/mg of protein at 15 min. In the presence of 5 mM probenecid, uptake at 5 min averaged 162 ± 44 ng of Lucifer Yellow/mg of protein and at 15 min, 293 ± 83 ng of Lucifer Yellow/mg of protein. This represents a 40% reduction in Lucifer Yellow transport in the presence of probenecid.

DISCUSSION

Organic anion transport has been studied primarily in polarized epithelia, including renal proximal tubular cells (9) and hepatocytes (10). In renal proximal tubular cells, organic anions such as urate and p-aminohippurate are transported across both the basolateral and apical plasma membranes of the cells, resulting in net secretion of these molecules (11). Several different organic anion transporters mediate these processes, and polarized cells may have different transport systems in their apical and basolateral membranes (9).

We have shown previously that Lucifer Yellow secretion from intact J774 cells is most efficient when cells are incubated in an alkaline medium. In the present experiments, dye sequestration into isolated cytoplasmic vesicles was most efficient when the medium in which they were suspended was acidic. These results are consistent with one another, since in both instances Lucifer Yellow is transported in the direction of the proton gradient. These findings suggest that Lucifer Yellow transport in J774 cells is a secondary active transport process involving either H+ symport or OH− antiport. Secondary active transport involving H+ symport or OH− antiport has been described in polarized cells. Blomstedt and Aronson (12) found that transport of urate and p-aminohippurate into dog renal microvillus membrane vesicles was stimulated by an inwardly directed proton gradient. Similarly, Blitzer et al. (13) demonstrated similar pH dependence for cholate uptake in vesicles derived from rat basolateral liver plasma membranes. Finally, Olsnes et al. (14) described a pH-regulated anion antiporter in Vero cells.

Lucifer Yellow is first sequestered into an early endocytic compartment and not into lysosomes in intact J774 cells. Similarly, isolated vesicles in the endosome-enriched fraction accumulated more dye than did isolated lysosomes. The reason for preferential uptake into endosomes is not clear. If the transmembrane proton gradient is a primary determinant of organic anion transport, then it is possible that Lucifer Yellow transport does not occur directly into lysosomes because of their low internal pH. This line of reasoning suggests that Lucifer Yellow sequestration into endosomes of intact cells must occur before the endosomes have had an opportunity to acidify (i.e. early endosomes). Schmid et al. (15) described similar pH dependence for Cholate uptake by isolated vesicles in the endosome-enriched fraction of 5774 cells. The endosome-enriched fraction was collected and incubated with 0.5 mg/ml Lucifer Yellow at 37 °C in the presence or absence of 5 mM probenecid (pH 7.5) (Fig. 7). In the absence of probenecid, Lucifer Yellow uptake averaged 311 ± 82 ng of Lucifer Yellow/mg of protein at 5 min and 517 ± 77 ng of Lucifer Yellow/mg of protein at 15 min. In the presence of 5 mM probenecid, uptake at 5 min averaged 162 ± 44 ng of Lucifer Yellow/mg of protein and at 15 min, 293 ± 83 ng of Lucifer Yellow/mg of protein. This represents a 40% reduction in Lucifer Yellow transport in the presence of probenecid.

It is possible also that the organic anion transporters are present in endosomes but not in lysosomes. The transporters may cycle between plasma membrane and endosomes or reside permanently in the endosomal compartment, but may never enter the lysosomal compartment. (The transferrin receptor exemplifies a plasma membrane protein that eschews lysosomes.) This is consistent with our suggestion that organic anion transporters are located in early endosomes.

The physiologic significance of the J774 organic anion transporter is not known. As noted previously, macrophages secrete or are involved in the metabolism of a wide variety of substances, many of which are organic anions. These include prostaglandins (16), leukotrienes (17), glutathione (18), lactate (19), and bilirubin (20). That prostaglandins and leukotrienes are major secretory products of macrophages is underscored by the fact that arachidonate comprises 25% of macrophage membrane fatty acids (16).

Probenecid-sensitive transporters mediate prostaglandin and leukotriene transport in other tissues. Prostaglandin release by renal proximal tubule cells is inhibited by probenecid as is leukotriene C4 transport across the choroid plexus (21). It is conceivable, therefore, that the organic anion transporter we have described in J774 macrophages is involved in the secretion of arachidonate metabolites by these cells.

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