Mechanism and Rate of Permeation of Cells by Polycyclic Aromatic Hydrocarbons*

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The principal mechanism of cellular uptake of benzo(a)pyrene and other polycyclic aromatic hydrocarbons (PAH) from lipoproteins into cells is spontaneous transfer through the aqueous phase (Plant, A. L., Benson, D. M., and Smith, L. C. (1985) J. Cell Biol. 100, 1295-1308). Cellular uptake of benzo(a)pyrene from low density lipoproteins followed first-order kinetics with a rate constant that was independent of the relative lipoprotein concentrations or cell number but which was 2 orders of magnitude smaller than the rate constant for benzo(a)pyrene desorption from low density lipoproteins. Moreover, identical rate constants for cellular uptake of benzo(a)pyrene and five other PAH from low density lipoproteins (Avigan, 1959; Shu and Nicols, 1979) and for benzo(a)pyrene transfer from these donor vehicles differed by 10-fold. When phosphatidylcholine vesicles containing benzo(a)pyrene and a nontransferable fluorescence quencher were mixed with cells in a stopped-flow system, two kinetic components were distinguished: a fast component with a rate constant corresponding to that measured for transfer of benzo(a)pyrene out of vesicles, followed by a much slower component, with a time course approximating that measured for cellular accumulation of benzo(a)pyrene by other techniques. Rate constants for desorption of a series of PAH which contained different number of aromatic rings from phosphatidylcholine vesicles differed over a 70-fold range. First-order rate constants for cell uptake of benzo(a)pyrene and five other PAH of different molecular sizes had the same 70-fold range of values, but were 2 orders of magnitude smaller than their respective rate constants for desorption from single bilayer vesicles. In addition, activation energies for cell uptake were essentially identical to the respective activation energies for desorption of PAH from phosphatidylcholine vesicles, confirming the mechanistic similarity of the two processes.

The passive uptake of molecules by cells has been investigated extensively, with most studies concerned with the transport of water-soluble non-electrolytes (Stein, 1967). The ratelimiting step in uptake of water-soluble substances by cells involves the absorption of the hydrophobic molecule by the external leaflet of the plasma membrane bilayer and diffusion through the membrane (Davison and Danielli, 1943). Increased hydrophobicity of molecules has been correlated with more rapid rates of uptake (Collander, 1954; Smulders and Wright, 1971; Wright and Pietsch, 1974; Bindslev and Wright, 1976). Whereas this correlation has been clearly demonstrated experimentally and is well founded theoretically (Stein, 1981), the range of substances previously examined has not included extremely hydrophobic molecules. Collander (1954) tested compounds with oil-water partition coefficients between 0.00003 and 0.3. Klocke et al. (1972) studied the rates of uptake of the 1,5-carbon fatty acids with ether-water partition coefficients between 0.1 and 100. Giorgi and Stein (1981) examined uptake rates for steroids with octanol:water partition coefficients as high as 100. Passive uptake of extremely hydrophobic fatty acids by cells has also been studied (Sallee and Dietschy, 1973; Sherrill and Dietschy, 1975). These studies have suggested that cellular uptake of large, moderately lipophilic solutes all encounter the same rate-limiting barrier at the plasma membrane.

Polycyclic aromatic hydrocarbons (PAH) are common environmental pollutants. The mechanism by which PAH enter cells and are distributed intracellularly has considerable importance since many of them are potent carcinogens. Benzo(a)pyrene, like other PAH, is very poorly soluble in water, ~10^{-10} M (Mackay and Shiu, 1977), and has an octanol: H2O partition coefficient of 5,000,000 (Mackay et al., 1980). In plasma, benzo(a)pyrene partitions readily into plasma lipoproteins (Avigan, 1959; Shu and Nichols, 1979). If the inferred relationship between hydrophobicity and uptake rate holds for these compounds, then their rate of entry into cells should be extremely rapid.

The entry of benzo(a)pyrene into cells from plasma lipoproteins has been shown to be a spontaneous, nonmediated transfer process (Remsen and Shireman, 1981; Plant et al., 1985). Benzo(a)pyrene uptake by cells does not involve lipoprotein endocytosis since uptake is independent of the class of lipoproteins with which benzo(a)pyrene is originally associated and occurs at identical rates with either live or HCHO-fixed cells (Plant et al., 1985). Entry of benzo(a)pyrene into living cells from low density lipoproteins (LDL) occurs equally well in the absence as in the presence of receptors for lipoproteins (Remsen and Shireman, 1981).

The mechanism for equilibration of many of the noncovalent lipoprotein components among the various lipoprotein classes is spontaneous transfer through the aqueous phase (Smith et al., 1978, 1983; Duckwitz-Peterlein and Moraal, 1978). This process involves desorption of a lipophil from the

* The abbreviations used are: PAH, polycyclic aromatic hydrocarbons; LDL, low density lipoproteins; HDL, high density lipoproteins; VLDL, very low density lipoproteins; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; DNP-DOA, N-(2,4 dinitrophenyl)- N-diocadecylamine.

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hydrophobic surface, diffusion through the aqueous phase, and readsorption into another hydrophobic compartment. Spontaneous transfer of lipophils among lipoproteins, model lipoproteins, and phospholipid vesicles has been studied extensively. The variables have been characteristics of the transferring species (Doody et al., 1980; Massey et al., 1982; Pownall et al., 1983; Plant et al., 1983; Phillips et al., 1980) and characteristics of the donor vehicle, such as lipid composition (Damer and Scherphof, 1981; Pownall et al., 1983) and vehicle size (Charlton et al., 1978; Charlton and Smith, 1982; Alm gren, 1980; Smith and Doody, 1981). Desorption of the molecule from a hydrophobic environment for dissolution in the aqueous phase is the rate-limiting step, whereas diffusion of the molecule and readsorption are extremely rapid.

Benzo(a)pyrene and other PAH transfer between phosphatidylcholine single bilayer vesicles and between LDL with half-times of several hundred milliseconds (Smith and Doody, 1981; Plant et al., 1983). By contrast, the rate constant for benzo(a)pyrene entry into cells is about 200-fold slower than the rate constant for desorption from LDL (Plant et al., 1985). Since the rate of uptake of benzo(a)pyrene by cells is limited by the rate of desorption from extracelluar donor lipoproteins or vesicles, it is of interest to identify the rate-limiting step in cellular uptake.

MATERIALS AND METHODS

Digital Imaging Fluorescence Microscopy—The digital imaging fluorescence microscopy system has been described (Benson et al., 1985). Briefly, the system consists of a Leitz Divit fluorescence microscope with a mercury arc excitation source, a Hamamatsu C-1000 silicon intensified target video camera, a Grinnell 274 image processor, and an LSI 11/23 minicomputer (Lab Dates) with 256K RAM and a 40 Mbyte hard disc. Living cells were maintained on the microscope stage in a humidified Bionique chamber (Corning, Lake Placid, NY) at 37°C in a 5% CO₂ atmosphere and were monitored for viability by time-lapse video recording of phase-dense lysosome movement (Willingham and Pastan, 1978). To initiate a kinetic experiment, medium containing benzo(a)pyrene labeled LDL or HDL was added to cells on the stage through ports in the chamber lid. Benzo(a)pyrene fluorescence intensity was quantified during 1-s exposures to excitation light with a 10-nm band-pass filter centered at 365 nm. Emitted light was observed with a filter that transmitted wavelengths greater than 400 nm. For each exposure, fluorescence intensity of each 0.065-μm² pixel in the field was digitized and assigned a value between 0 and 255. Neutral density filters were used to attenuate excitation light so that photo bleaching was eliminated (Benson et al., 1985). Cells treated with 2% HCHO were examined by digital fluorescence microscopy in a similar manner. Under the experimental conditions employed, only intracellular fluorescence was a significant contributor to the total fluorescence intensity of the images.

Cell Culture—Normal human fibroblasts and P388D1 murine macrophages were cultured as previously described (Plant et al., 1985). Fibroblasts were grown on 4.4-cm² No.1 glass coverslip culture dishes and transferred to the Bionique chamber for microscopy experiments. Other kinetic experiments were performed with suspensions of P388D1, murine macrophages which were cultured in RPMI 1640 medium and 10% fetal calf serum. P388D1 macrophages in dishes were suspended by rinsing with cold medium and collected by centrifugation. These cultures have been previously shown not to metabolize benzo(a)pyrene during these experiments (Plant et al., 1985).

Preparation of Lipoproteins, Vesicles, and Benzo(a)pyrene-labeled Solutions—Lipoproteins were prepared by ultracentrifugal flotation (Havel et al., 1955) to obtain VLDL (ρ < 1.006), LDL (ρ = 1.019–1.063), and HDL (ρ = 1.063–1.21). Lipoproteins were labeled with benzo(a)pyrene or [3H]-benzo(a)pyrene by drying organic solutions of benzo(a)pyrene on 200-μm glass beads and incubating the lipoprotein solution with the beads overnight. Benzo(a)pyrene content of LDL, VLDL, and HDL was determined by 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) single-walled vesicles containing 2 nM POPC and 60 μM N-(2,4-dinitrophenyl)-N,N-dioctadecylamine (DNPA), a fluorescence quencher (Plant et al., 1983), were prepared by mixing 60 μl of a 5-propanol solution containing all components into 1 ml of standard buffer (Baerli and Korn, 1973). Vesicles were centrifuged through Sephadex G-25 minicolumns to remove the solvent (Fry et al., 1978). Filter Kinetic Assays—For efflux experiments, cells were first incubated 20 min with [3H]-benzo(a)pyrene-labeled LDL. Cells were separated from the benzo(a)pyrene-labeled LDL by centrifugation, washed once in serum-free medium, and resuspended. Efflux was initiated by addition of unlabeled LDL. To initiate cellular uptake, [3H]-benzo(a)pyrene was added to 2 ml of RPMI 1640 medium or standard buffer containing 2 × 10⁶ P388D1 macrophages. Cell suspensions were stirred continually with a magnetic bar. Aliquots of the suspensions were removed at various times, added to 5 ml of standard buffer containing 5 mg ml⁻¹ bovine serum albumin, and rapidly filtered under reduced pressure through glass-fiber filters (Whatman). Cells and associated benzo(a)pyrene were trapped by the filters, which were counted in scintillation fluid without further processing. For filtration experiments using aqueous solutions of benzo(a)pyrene, benzo(a)pyrene amounts were estimated from the filter buffer solutions.

Fluorometric Kinetic Assays—P388D1 cells were suspended at a cell density of 1–3 × 10⁶ cells ml⁻¹ in either RPMI 1640 medium or standard buffer and stirred continuously in a temperature-regulated cuvette holder. Reactions were initiated by the addition of POPC vesicles containing 0.3 mol % PAH and 3 mol % DNPA. The increase in fluorescence intensity, which was the result of PAH transfer from quenched vesicles to cells, was measured in an SLM 8000 spectrophotometer, using the following excitation and emission wavelength settings, respectively, benzoperylene, 336 and 408 nm; 1,2,3,4-tetrabenzanthracene, 300 and 400 nm; benzo(a)pyrene, 385 and 406 nm; 9-phenanthrenecent, 387 and 420 nm; 3,4-benzophenanthrene, 318 and 410 nm; and pyrene, 338 and 350 nm.

Stopped-flow Experiments—Lipoproteins or vesicles containing PAH were mixed rapidly with vesicles containing DNPA-DNA in a Durrum-Gibso stopped-flow instrument described previously (Plant et al., 1983). Benzo(a)pyrene-labeled LDL (0.025 mg of protein ml⁻¹), VLDL (0.65 mg of protein ml⁻¹), and HDL (0.48 mg of protein ml⁻¹) were mixed with 2 mM POPC containing 60 μM DNPA-DOA. Other PAH, 6 μM in 0.2 mM POPC vesicles, were mixed with 2 mM POPC vesicles containing quencher. P388D1 cells (2 × 10⁶ cells ml⁻¹) were mixed with 0.2 mM POPC vesicles containing 6 μM benzo(a)pyrene and 60 μM DNPA-DOA. Mixing time of the instrument was about 4 ms. Fluorescence intensities measured at millisecond time intervals were stored in 2048 channels in a Biomation 805 waveform recorder. For all kinetic assays, cells were examined by microscopy after the reactions. There was no observable cell debris, and the cells appeared to be structurally intact after mixing.

Data Analysis—Stopped-flow data were analyzed on-line with parallel interface between an Apple II Plus microcomputer and the Biomation waveform recorder. All kinetic data were analyzed by nonlinear least squares regression with respect to a monoeponential function (Wentworth, 1965). The analytical program involved a reiterative procedure and stringent criteria for convergence. Results of the analysis were evaluated by comparative plots of data points and the fitted function. In addition, a rigorous statistical analysis was performed for every reaction, including standard deviation of points from the fitted line, standard deviations for rate constant, initial intensity and final intensity values, and covariance of errors in these parameters. Kinetic data which were not collected by stopped-flow methods were entered from the keyboard and analyzed with the same program.

RESULTS

Rates of Benzo(a)pyrene Desorption from Extracellular Donors—The rate constant for the entry of benzo(a)pyrene into cells from LDL was measured by three independent experimental methods (Plant et al., 1985) and was about 10⁵ times smaller than the rate constant for desorption of benzo(a)pyrene from LDL, which was determined by stopped-flow methods (Table I). Furthermore, only one rate constant for cell uptake of benzo(a)pyrene was obtained, even when the diameter of the donor vehicle was different. Rate constants

For further reading, please refer to the original publication cited in the text.
Prepared by a helpful assistant. The text is a scientific discussion on the uptake of benzo(a)pyrene by cells, focusing on the kinetics of this process and the role of lipoproteins in facilitating this uptake. The text includes tables and figures to illustrate the data and conclusions. The discussion addresses the potential mechanisms for this uptake, such as diffusion and collisional events, and how these processes are influenced by factors such as lipoprotein concentration and type.
transfer was initiated with 0.5 µg ml⁻¹ [G-³H]benzo(a)pyrene to the cell is significant, a diffusion barrier at the surface of the cell could conceivably limit diffusion of the LDL as described under "Materials and Methods." Time intervals and filtered as described under "Materials and Methods." Solid lines represent analysis of data with respect to a monoexponential function.

**TABLE III**

| Rate constants for benzo(a)pyrene uptake as a function of unlabeled LDL concentration |
|----------------------------------------|
| Rate constants were measured by the filtration protocol. The transfer was initiated with 0.5 µg ml⁻¹ [G-³H]benzo(a)pyrene-labeled LDL as described under "Materials and Methods." |

| Unlabeled LDL | k⁺, min⁻¹ |
|---------------|------------|
| µg ml⁻¹       |           |
| 0             | 0.22 ± 0.02|
| 5             | 0.19 ± 0.02|
| 50            | 0.20 ± 0.02|
| 500           | 0.21 ± 0.06|

FIG. 2. Uptake of benzo(a)pyrene by cells in the presence of increasing concentrations of unlabeled LDL. P388D1 macrophages (2 x 10⁶) were suspended in 2 ml of Tris, pH 7.4, containing 0.15 M NaCl and 0 (O), 5 (C), 50 (A), or 500 (G) µg ml⁻¹ unlabeled LDL and were stirred constantly at 21°C. At time 0, 0.5 µg ml⁻¹ LDL containing [G-³H]benzo(a)pyrene was added. Aliquots were taken at time intervals and filtered as described under "Materials and Methods." Solid lines represent analysis of data with respect to a monoexponential function.

**TABLE IV**

| Rate constants for efflux of benzo(a)pyrene from cells |
|---------------------------------------------------------|
| Rate constants were measured by the filtration protocol. The cells were previously labeled with [G-³H]benzo(a)pyrene as described under "Materials and Methods." |

| LDL acceptor | Cells | k⁻, min⁻¹ | Half-time |
|--------------|-------|-----------|-----------|
| µg ml⁻¹      | ml⁻¹  |           |           |
| 50           | 1 x 10⁸| 0.20 ± 0.02| 3.5       |
| 500          | 1 x 10⁸| 0.25 ± 0.01| 2.8       |
| 5000         | 1 x 10⁴| 0.24 ± 0.03| 2.9       |

ml⁻¹, which corresponded to 6 x 10¹², 6 x 10¹³, and 6 x 10¹⁴ acceptors, respectively. The measured rate constants were essentially identical to those measured for uptake, about 0.2 min⁻¹ (Table IV). The rate constants for efflux were also independent of extracellular lipoprotein concentrations. These data suggest that competitive uptake between extracellular LDL and the plasma membrane for benzo(a)pyrene is not the rate-limiting process in the entry of benzo(a)pyrene into cells.

**Absence of Boundary Water Layer Effects**—If the distance of separation of LDL and the plasma membrane must be micrometers before the probability of net transfer of benzo(a)pyrene to the cell is significant, a diffusion barrier at the surface of the cell could conceivably limit diffusion of the carrier macromolecules into close proximity with the cell. To test this possibility, the carrier macromolecule was omitted. Cells were mixed with aqueous solutions of [G-³H]benzo(a)pyrene (Fig. 3). When movement of benzo(a)pyrene was diffusion-limited, the rate constant for this reaction was identical to that obtained in the presence of LDL. Thus, the relatively slow rate constant for benzo(a)pyrene entry into cells was independent of the lipoprotein carrier and not related to possible effects of diffusion barriers on movement of either benzo(a)pyrene or LDL.

Benzo(a)pyrene uptake was examined directly by measuring the constant for transfer of benzo(a)pyrene from phosphatidylcholine vesicles to the plasma membrane of cells. POPC vesicles containing benzo(a)pyrene and the nonexchangeable fluorescence quencher DNP-DOA were mixed rapidly with P388D1 macrophages in a stopped-flow system (Fig. 4). When the reaction was followed at the faster time base of 5 ms/channel, two kinetic components were distinguished. A rapid increase that preceded the slow reaction occurred with a rate constant of 61 min⁻¹, about the same as the rate constant measured for transfer of benzo(a)pyrene between vesicles, 72 min⁻¹. The fast reaction contributed approximately 10% of the total increase in benzo(a)pyrene fluorescence. This quantity apparently represented benzo(a)pyrene associated with the plasma membrane-associated benzo(a)pyrene. When the increase in fluorescence intensity was followed at a time base of 100 ms/channel for approximately 3 min, a portion of a much slower reaction was observed. Therefore, the rate of transfer of benzo(a)pyrene to the plasma membranes of cells

![FIG. 2](image1.png)

![FIG. 3](image2.png)

![FIG. 4](image3.png)
is not determined by the rate at which benzo(a)pyrene desorbs from the donor vehicle and is apparently not impeded by a diffusion barrier surrounding the cells. Diffusion of the molecule through the aqueous phase and its readsorption by the acceptor vesicle are too fast to measure on a millisecond timescale (Charlton et al., 1976).

Activation Energies for Benzo(a)pyrene Transfer—The temperature dependence for uptake of benzo(a)pyrene by cells is shown in Fig. 5. The activation energies for uptake and efflux are 17.6 and 18.0 kcal mol⁻¹, respectively. The magnitude of the activation energy for uptake of benzo(a)pyrene by cells is similar to that obtained from stopped-flow measurement of spontaneous transfer of benzo(a)pyrene between single bilayer vesicles, 11.7 kcal mol⁻¹ (Table V). These activation energies correspond to those reported for diffusion of molecules through phospholipid bilayers (Davison and Danielli, 1943). Activation energies of about 4 kcal mol⁻¹ for fluorescence polarization of perylene in membranes suggest a relatively small temperature dependence for PAH movement in membranes (Rudy and Gitler, 1972). Therefore, diffusion of benzo(a)pyrene appears not to be the rate-limiting step for intervesicular transfer.

Effect of Molecular Size on PAH Transfer Rates—Rate constants for transfer of PAH between single bilayer vesicles are inversely proportional to their molecular surface areas (Plant et al., 1983). Increases in hydrophobic surface area of the transferring molecules and the decreased rate constants for intervesicular transfer are the consequence of reduced partitioning into the aqueous phase from the hydrocarbon domain. If a desorption process were to be rate-limiting in the cellular uptake of PAH, then an inverse relationship between molecular size of PAH and rate of cell uptake would be expected. A series of PAH of various molecular sizes were examined to compare the rate constants for spontaneous transfer between single bilayer model membrane vesicles and the rate constants for uptake of this series of PAH by cells. Fig. 6 showed a strong positive correlation between the rate constants measured in these two systems, although rate constants for cellular uptake were 2 orders of magnitude smaller than rate constants for intermembrane transfer. This observation confirmed that the rate-limiting step in PAH permeation of cells was a desorption process which was kinetically independent of desorption from the extracellular donor and that the rate constant for permeation of PAH was inversely proportional to the hydrophobicity of the compound.

**DISCUSSION**

A physiologically relevant mode of presentation of benzo(a)pyrene to cells is as a noncovalent component of lipoproteins rather than as microcrystalline dispersions (Kocan et al., 1983; Lakowicz et al., 1980). Studies in vivo (Grubbs and Moon, 1973) and in vitro (Shu and Nichols, 1981) have shown that dibenzanthracene and benzo(a)pyrene partition readily into plasma lipoproteins. Cell uptake of benzo(a)pyrene has been shown to occur independently of lipopro-

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**Table V**

*Comparison of rate constants and activation energies for transfer of PAH between vesicles and from vesicles to cells*

| PAH                   | Vesicles → Vesicles | Vesicles → Cells |
|-----------------------|---------------------|-----------------|
|                       | $k_d$, min⁻¹ | $E_d$, kcal mol⁻¹ | $k_u$, min⁻¹ | $E_u$, kcal mol⁻¹ |
| Benzoperylene         | 54             | 18.0            | 0.1           | 12.3               |
| 1,2,3,4-Dibenzanthracene | 96            | 17.0            | 0.2           | 15.0               |
| 3,4-Benzopyrene       | 174            | 11.7            | 1.0           | 17.5               |
| 9-Phenylanthracene    | 588            | 10.8            | 1.0           | 15.5               |
| 3,4-Benzophenanthrene | 990            | 7.0             | 1.8           | 11.3               |
| Pyrene                | 3702           | 10.2            | 9.0           | 12.0               |

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**Fig. 5.** Activation energy for cellular uptake of benzo(a)pyrene. Rate constants for uptake were determined by fluorometry with stirred suspensions of POPC, macrophages and POPC vesicles containing benzo(a)pyrene and the fluorescence quencher DNP-DOA. Excitation and emission wavelengths were 365 and 406 nm, respectively. The correlation coefficient was 0.999, and the activation energy was 17.6 kcal mol⁻¹.

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**Fig. 6.** Comparison of rate constants for transfer of PAH between POPC vesicles and for transfer from vesicles to cells. Rate constants were measured at 37°C by stopped-flow for transfer between vesicles (ordinate) and by spectrofluorometry for transfer into cells (abscissa). The compounds, in order of increasing rate constants, were benzoperylene (structure not shown), 1,2,3,4-dibenzanthracene, benzo(a)pyrene, 9-phenylanthracene, 3,4-benzophenanthrene, and pyrene. The correlation coefficient was 0.994, and the slope was 412.

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**Fig. 7.** Model for equilibrium distribution for benzo(a)pyrene between extracellular and intracellular lipid compartments. $k_{LP}$ is the rate constant for desorption from LDL; $k_{mem}$ is the rate constant for desorption from the plasma membrane; and $k_{LD}$ is the rate constant for desorption from a lipid droplet.
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tein endocytosis (Remsen and Shireman, 1981; Plant et al., 1985) and the size of the donor vehicle (Plant et al., 1985).

Fig. 7 depicts PAH uptake into cells as an equilibration process involving three compartments: the extracellular lipoprotein or vesicle, the cell plasma membrane, and the intracellular lipid membranes and compartments. The experimental results, taken together, indicate that the rate-determining step in the permeation of cells by PAH is transferred out of the plasma membrane and that \( k_{\text{mem}} \), the rate constant for desorption of the PAH from the plasma membrane, is the first-order rate constant that is measured experimentally. Since the rate of desorption of benzo(a)pyrene from extracellular LDL or vesicles is very fast, a steady state is rapidly achieved between benzo(a)pyrene in extracellular donors and benzo(a)pyrene in the plasma membrane. Steady state plasma membrane concentrations of benzo(a)pyrene are reached in milliseconds, with a rate constant which is determined by the rate constant for benzo(a)pyrene desorption out of the donor vehicle. Rate constants for benzo(a)pyrene permeation by cells are 2 orders of magnitude smaller than rate constants for desorption of benzo(a)pyrene from lipoproteins or single bilayer vesicles.

Differences in rate constants for desorption of lipophils from cell plasma membranes compared to POPC vesicles could in part be due to differences in lipid composition and packing constraints. Differences in rate constants for desorption of lipophils from model membranes have been measured as a function of matrix lipid composition (Damen and Scherphof, 1981; Poznansky and Czekanski, 1979; Phillips and Rothblat, 1985). However, the slow rate constant for transfer from cell plasma membranes is probably primarily due to the effect of the small radius of curvature of these membranes compared to smaller cells, vesicles, and lipoproteins. Rate constants for lipophil desorption from different lipoprotein classes have been found to be inversely proportional to the radius of the lipoprotein particles (Charlton and Smith, 1982; Almgren, 1980; Smith and Doody, 1981). Bojesen (1982) reported a 10-fold larger rate constant for transfer of cholesterol from plasma lipoproteins to red blood cells than from red blood cells to plasma. The rate constant for transfer of benzo(a)pyrene from red blood cell membranes to LDL (data not shown) is 1.07 min\(^{-1}\), or 7-fold faster than the rate constant for efflux from a fibroblast or P388D, macrophage. The diameter of red blood cells is about 5–10 \( \mu \)m, compared to diameters of about 30–50, 0.03, and 0.023 \( \mu \)m for P388D, macrophages, POPC vesicles, and LDL, respectively.

The passive transfer mechanism described here for cellular uptake of PAH is analogous to cellular uptake of cholesterol (Phillips and Rothblat, 1985) and fatty acids (Walter and Gutknecht, 1984). These data suggest that cellular uptake of all relatively soluble lipophils is dominated by a passive mechanism. The relatively slow rate of benzo(a)pyrene uptake by cells suggests that the permeation of tissues by PAH and other passively transferring lipophils may be extremely slow when many layers of cells are involved and depends on the content of intracellular lipid.

The observed inverse relationship between hydrophobicity for a series of PAH and their permeation into cells is directly opposed to conclusions drawn from cell permeation studies involving relatively water-soluble compounds. For water-soluble molecules, diffusion of the hydrophobic molecule across the hydrophobic interior of the plasma membrane is rate-limiting. By contrast, for a very lipophilic molecule, desorption from the interior surface of the plasma membrane is slow, compared to other events. Thus, rate constants for membrane permeation of a series of homologous compounds that differ in hydrophobicity should increase with increasing hydrophobicity of the permeant, but only to the point at which the insolubility of the molecule in the aqueous phase limits the rate of desorption of the permeant from the inner leaflet of the plasma membrane.

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Rothblat, J. (1985). However, the slow rate constant for transfer from cell plasma membranes is probably primarily due to the effect of the small radius of curvature of these membranes compared to smaller cells, vesicles, and lipoproteins. Rate constants for lipophil desorption from different lipoprotein classes have been found to be inversely proportional to the radius of the lipoprotein particles (Charlton and Smith, 1982; Almgren, 1980; Smith and Doody, 1981). Bojesen (1982) reported a 10-fold larger rate constant for transfer of cholesterol from plasma lipoproteins to red blood cells than from red blood cells to plasma. The rate constant for transfer of benzo(a)pyrene from red blood cell membranes to LDL (data not shown) is 1.07 min\(^{-1}\), or 7-fold faster than the rate constant for efflux from a fibroblast or P388D, macrophage. The diameter of red blood cells is about 5–10 \( \mu \)m, compared to diameters of about 30–50, 0.03, and 0.023 \( \mu \)m for P388D, macrophages, POPC vesicles, and LDL, respectively.

The passive transfer mechanism described here for cellular uptake of PAH is analogous to cellular uptake of cholesterol (Phillips and Rothblat, 1985) and fatty acids (Walter and Gutknecht, 1984). These data suggest that cellular uptake of all relatively soluble lipophils is dominated by a passive mechanism. The relatively slow rate of benzo(a)pyrene uptake by cells suggests that the permeation of tissues by PAH and other passively transferring lipophils may be extremely slow when many layers of cells are involved and depends on the content of intracellular lipid.

The observed inverse relationship between hydrophobicity for a series of PAH and their permeation into cells is directly opposed to conclusions drawn from cell permeation studies involving relatively water-soluble compounds. For water-soluble molecules, diffusion of the hydrophobic molecule across the hydrophobic interior of the plasma membrane is rate-limiting. By contrast, for a very lipophilic molecule, desorption from the interior surface of the plasma membrane is slow, compared to other events. Thus, rate constants for membrane permeation of a series of homologous compounds that differ in hydrophobicity should increase with increasing hydrophobicity of the permeant, but only to the point at which the insolubility of the molecule in the aqueous phase limits the rate of desorption of the permeant from the inner leaflet of the plasma membrane.