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UPTAKE OF PERSISTENT ENVIRONMENTAL CHEMICALS BY CULTURED HUMAN CELLS

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Abstract—Uptake of the persistent environmental chemicals 2,2′,4,4′,5,5′-hexachlorobiphenyl and 1,1,1-trichloro-2,2-di-(4-chlorophenyl)ethane (the insecticide DDT) by Chang liver cells, an established human cell line, has been investigated. Monolayer cells were incubated with culture medium to which the lipophilic model compounds had been added.

The time course of uptake of either compound was biphasic, reaching equilibrium after about 5 hr of incubation. The ratio of DDT:hexachlorobiphenyl uptake was dependent on the presence of serum proteins. Increasing concentrations of serum proteins in the culture medium progressively inhibited uptake.

Efflux from the cells was not entirely reversible: 10–20% of the chemicals were not released. Uptake was a linear function of the external concentration of the compounds. Absorptive binding to the outer cell plasma membrane could be determined by removing bound chemicals with fetal calf serum ("back exchange"). With this method, temperature-dependent translocation through the cell plasma membrane could directly be demonstrated. The effect of low temperature as well as the influence of metabolic inhibitors point out the contribution of energy-driven uptake pathways. Demonstration of LDL receptor-like binding protein on Chang liver cells facilitated estimation of the role of receptor-mediated uptake. This route of uptake proved to be of minor importance only, as was transport of the protein-bound chemicals via fluid pinocytosis.

The results demonstrate that cellular endocytosis of plasma membrane-bound chemicals constitutes a major uptake pathway for lipophilic chemicals.

Much information is available on the interaction of persistent organic chemicals with intact cells, subcellular organelles and with a large number of cellular enzymes. On the other hand, comparatively little attention has been paid to the question of transport through the cell plasma membrane and the intracellular distribution of such compounds. Given the example of DDT,‡ the following observations may illustrate how time-dependent transport processes to variable length precede the biological effect. Narahashi and Haas [1] had observed that lobster nerve membrane potentials were changed within 20 min of DDT exposure. A quicker biological response (within a few seconds) was elicited upon direct application of the insecticide onto the outer hair walls of the moth *Antheraea polyphema* [2]. In contrast, much longer periods of exposure were required in cell culture systems. Effects on the membrane potential of Chang liver cells did not become clearly evident until 7 hr of incubation [3]; effects on specific membrane proteins of these cells even required up to 48 hr of cell exposure [4, 5]. Recently, 15–40 hr of exposure time to hexachlorobiphenyl of rat adipose tissue slices were required to establish uptake equilibrium [6].

We found it worthwhile to examine more closely potential routes of uptake under cell culture conditions. We focused on the interaction of the two widespread lipophilic environmental pollutants 2,2′,4,4′,5,5′-hexachlorobiphenyl (6-CB), congener to the polychlorinated biphenyls, and of the insecticide DDT with Chang liver cells, an established cell line of human origin. Growth of the cells in monolayer culture is supported by the addition of 8% of fetal calf serum to the culture medium. Binding studies of 6-CB and DDT to human blood proteins had previously shown that all the major serum protein fractions bind and exchange these chemicals within a wide concentration range [7–15]. Thus, we rather had to deal with how binding of these chemicals to serum proteins would interfere with rate and extent of uptake by cultured monolayer cells. After describing general parameters of uptake, such as dependence on time and concentration, progressive inhibition of uptake with the concentration of serum proteins will be shown. Furthermore, an attempt will be made to discriminate more clearly adsorptive binding of such compounds to the outer cell plasma membrane from their subsequent transport into the cells. After binding and concentration in the cell plasma membrane, uptake en route of cellular endocytosis will be demonstrated. Finally, alternative transport pathways such as receptor-mediated endocytosis and fluid pinocytosis will be examined; these

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‡ Abbreviations used: DDT, 1,1,1-trichloro-2,2-di-(4-chlorophenyl)ethane; 6-CB, 2,2′,4,4′,5,5′-hexachlorobiphenyl; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; BSA, bovine serum albumin.

transport routes proved to be of minor importance only.

EXPERIMENTAL SECTION

Chemicals and materials

2,2',4,4',5,5'-Hexachlorobiphenyl was purchased from Promochem (Wesel); the insecticide DDT was from Riedel deHaen (Seelze). (^{14}C)2,2',4,4',5,5'-Hexachlorobiphenyl (0.3 GBq/mmol = 8,2 mCi/mmol) was from New England Nuclear (Dreieich), whereas (^{14}C)DDT (3,1 GBq/mmol = 85 mCi/mmol) and carrier-free (^{125}I)NaI (74 MBq/μg iodine = 2 mCi) were from Amersham Buchler (Braunschweig). Dansylcadaverin, Colchicine, Cytochalasin B, Cycloheximide, Monensin, Nigericin and A-23187 were purchased from Sigma (Taufkirchen); 2,4-Dinitrophenol and NaN_3 were from Merck (Darmstadt). Bovine serum albumin (essentially fatty acid free) was purchased from Sigma (Taufkirchen). High density (HDL) and low density lipoprotein (LDL) fractions were isolated from newborn calf serum [16]. Chemicals and plastic ware for routine cell culture were supplied by Biochrom (Berlin) and Greiner (Nürtingen), respectively.

Cell culture

Chang liver cells (American Type Culture Collection, No. CCL 13) were obtained through Gibco-Europe (Karlsruhe). Stock cultures were grown in monolayer in Dulbecco's modified Eagle's medium (DMEM) supplemented with glutamine (2 mM), $NaHCO_3$ (45 mM) and 8% of fetal calf serum (FCS) in an atmosphere of 95% air/5% CO_2 at 37° in a humidified incubator.

Uptake experiments (standard procedure)

The cells were plated in plastic culture dishes (10 cm dia.) at a density of 2×10^6 cells/dish on the day preceding the experiment. The radioactively labeled environmental chemicals 6-CB and DDT were dissolved in a minimum amount of dimethylsulfoxide or ethanol; aliquots were pipetted into experimental culture medium (DMEM + 8% FCS) to the final concentration required (standard: 20 μM). Equilibration was attained by incubation overnight at 37° in a humidified incubator. Samples of this medium were counted for radioactivity to check for even distribution of dissolved chemicals. The concentration of the solvents in DMEM never exceeded 1% of dimethylsulfoxide or 0.05% of ethanol. For the experiments, the cell culture medium was replaced with 10 ml of this medium and the cells were incubated at 37° as long as indicated (standard: 5 hr). Incubation was stopped by aspiration of the medium and subsequent rinsing of the cells with 3×10 ml of ice-cold Dulbecco's phosphate-buffered saline containing 2 mM of EDTA (PBS/EDTA). The cells were dissolved in 1 ml of 0.5 N NaOH and samples were removed for estimation of cellular protein [17] and of radioactivity by liquid scintillation counting.

Adsorptive binding of the compounds to the outer cell plasma membrane was determined by the method of "back exchange" with fetal calf serum [18, 19]. To this, exposure of cells to the experimental culture medium was stopped by aspiration of the medium. The cells were then treated at 4° as follows: (i) rinsing with 3×1 ml of cold PBS/EDTA; (ii) brief exposure to 3×1 ml of cold fetal calf serum to remove the cell surface adsorbed compounds by back exchange; (iii) rinsing with PBS/EDTA as above.

To study uptake from serum-free medium, the lipophilic chemicals were dispersed in DMEM by intensive sonification (Branson sonifer, 3×1 min at 80 W). Counting of sample radioactivity of the clear culture medium assured an even distribution of the dispersed chemicals for the time of the experiments. The theoretical concentrations thus obtained were 8 μM of 6-CB and 15 μM of DDT. The physical form of the compounds in the medium (e.g. formation of microcrystals and/or micelles) was not checked. Interference of possibly sedimenting material with uptake measurements was checked by control experiments:

Volume of aqueous phase (ml)	Addition of FCS	Uptake of 6-CB (μg/mg protein)
3	–	1
12	–	2
3	+	3
12	+	10

Thus, uptake was for some reason dependent on the volume of the incubation medium. Since the presence of fetal calf serum proteins did not change this result, sedimentation could not interfere with uptake. Nevertheless, care was taken to carry out all experiments with an identical volume.

Binding of 6-CB and DDT to serum protein fractions

Bovine serum albumin (BSA). Serum-free DMEM was supplemented with BSA to the desired concentration (cf. Fig. 3B). Solutions of the environmental chemicals were pipetted into the stirred culture medium to the final concentration of 20 μM. Further use was as described above.

Low density lipoprotein (LDL). LDL, isolated from newborn calf serum [16], was incubated in culture medium (DMEM, 1.4 μg LDL/ml) at 37° for 1 hr with 0.22 μg 6-CB/ml or 0.04 μg DDT/ml, respectively. Since even low speed centrifugation caused a significant loss of LDL, the preparation was used without further separation.

High density lipoprotein (HDL). The same procedure as above was followed, but with 5 μg HDL/ml.

Uptake of serum proteins by the cells

Uptake of albumin (BSA), high density lipoprotein (HDL) and low density lipoprotein (LDL) by monolayer Chang liver cells was measured using the radioiodinated proteins. BSA was iodinated by means of the chloramine T method [20], and HDL and LDL were labeled following the modified iodine monochloride methods [21, 22]. The labeled proteins were separated from excess iodination reagents on small columns of Sephadex G-25. The protein containing fractions of the void volume were combined and dialyzed against 0.14 M NaCl. The specific

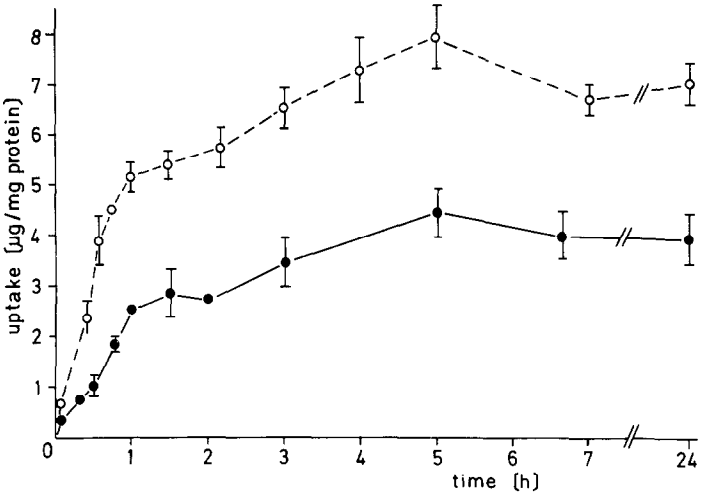

Fig. 1. Time course of 6-CB and DDT uptake from complete culture medium. Monolayer cells were incubated for the indicated times with complete culture medium (DMEM + 8% FCS) and 6-CB (●——●, 20 µM) or DDT (○---○, 20 µM). Points are the mean of three experiments (±SD).

Table 1. Effect of temperature on membrane translocation of DDT

Line	Temperature of 1st incubation (°C)	2nd incubation (°C)	Uptake of DDT (µg/mg protein)
1	37	37	1.40 ± 0.10
2	4	4	0.39 ± 0.10
3	4	37	0.60 ± 0.05

Monolayer cells were incubated at the indicated temperatures for one hour with complete culture medium containing 20 µM of DDT (1st incubation). Thereafter, the medium was aspirated and the cells were rinsed with 3 × 10 ml of ice-cold PBS/EDTA. Incubation was continued for 1 hr without addition of fresh medium (2nd incubation). After brief treatment of the cells with 3 × 1 ml of FCS for back exchange of membrane-bound DDT, the residual, non-exchangeable pool of DDT in the cells was measured.

activities obtained were 1600×10^6 cpm/mg BSA, 994×10^6 cpm/mg HDL protein, and 421×10^6 cpm/mg LDL protein. Cells were incubated at 37° for 5 hr with DMEM containing the iodinated proteins as indicated in Table 1. Incubation was stopped by aspiration of the medium; subsequent analysis was as above.

RESULTS AND DISCUSSION

Uptake characteristics

Chang liver cells were attached on plastic dishes and cultured in monolayer with complete culture medium (DMEM + 8% of fetal calf serum). For the experiments, this medium was supplemented with the lipophilic chemicals which were uniformly distributed in the aqueous solution as a result of their binding to serum proteins.

As shown in Fig. 1, the time course of uptake is

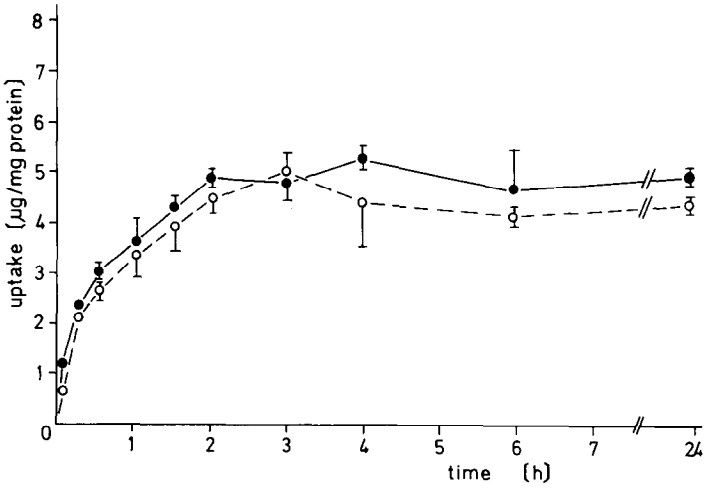

Fig. 2. Time course of 6-CB and DDT uptake from serum-free culture medium. Monolayer cells were incubated for the indicated times with serum-free culture medium (DMEM) and 6-CB (●——●, 8 µM) or DDT (○---○, 15 µM); each compound was dispersed in DMEM by sonification as described in the Experimental Section. Points are the mean of three experiments (±SD).

composed of different phases: a fast initial phase (up to 1 hr) is followed by a slower uptake phase until final equilibrium is reached after 5 hr of cell exposure. Albeit subject to some variation, the biphasic time course could reflect the sequential events of initial binding of the lipophilic compounds to the cell surface followed by a slower translocation through the plasma membrane. With time progressing, efflux of the metabolically stable compounds may also contribute to the slow-down of uptake rates. Quantitatively, cellular uptake of DDT at equilibrium was nearly twice as much as that of 6-CB.

Uptake from culture medium deprived of serum proteins is shown in Fig. 2. After intensive sonification, the maximum possible concentrations in the aqueous culture medium were 8 μM of 6-CB and 15 μM of DDT, respectively. Under these conditions, the time course of uptake from the optically clear serum-free medium is not significantly altered (cf. Fig. 2 with Fig. 1), as is the extent of DDT uptake. However, the extent of 6-CB uptake at equilibrium is increased, about 4-fold with respect to DDT and to the lower external 6-CB concentration. Apparently, serum proteins limit the extent of uptake as a consequence of binding the individual lipophilic compounds with differing affinity (see below, Fig. 3B). As a consequence, the partition ratio of these chemicals between the aqueous phase and the cell monolayer is changed; the *n*-octanol/water partition coefficients of 6-CB and DDT (both of which range from 6.2 to 6.4, refs. 23 and 24) make uptake of 6-CB and DDT quantitatively comparable primarily in serum-free medium.

Uptake as a function of the culture medium protein concentration is shown in Fig. 3. The first serum increments still promote uptake, presumably by increasing the effective aqueous concentration of the lipophilic compounds. But protein concentrations exceeding 0.15 mg/ml progressively inhibit uptake of either compound (Fig. 3, panel A); at the highest possible concentration (33 mg FCS/ml = undiluted fetal calf serum) uptake is nearly abolished. the stronger binding affinity of 6-CB to albumin bears relevance only with this protein as the sole carrier protein in the culture medium (panel B). Then, the uptake inhibition curve of 6-CB is shifted to the left

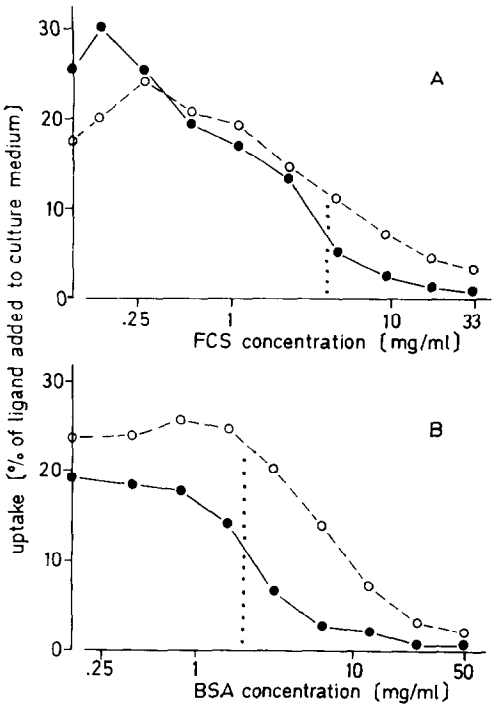

Fig. 3. Uptake of 6-CB and DDT as a function of the external protein concentration. Monolayer cells were incubated under standard conditions with 6-CB (●——●) or DDT (○---○) and with the indicated concentrations of fetal calf serum (FCS, panel A) or bovine serum albumin (BSA, panel B). The dotted lines indicate the protein concentration of FCS and BSA in standard culture medium (DMEM + 8% FCS).

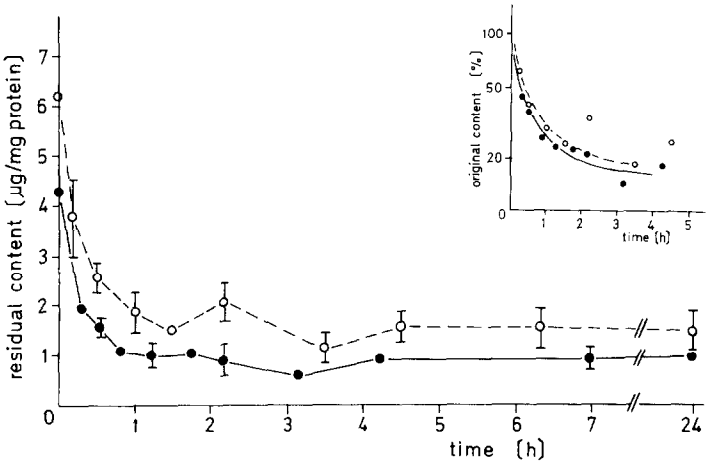

Fig. 4. Release after uptake (reversibility). Monolayer cells had been preincubated for 20 hr under standard conditions with 6-CB (●——●) or DDT (○---○). At time "zero", the medium was replaced with fresh medium lacking the environmental chemicals. At the times indicated, the medium was aspirated and the cells were analyzed for the residual content of the chemicals. Points are the mean of three experiments (±SD). Inset: plot of log percentage original cellular content vs time.

of the corresponding DDT curve by a factor of 4; this figure correlates favorably with the results of an *in vitro* binding test (data not shown). The shift is not observed with fetal calf serum proteins (panel A). Thus, the binding affinity of lipophilic compounds to serum albumin is not the only limiting factor of the extent of cellular uptake.

For studying reversibility, monolayer cells had been equilibrated overnight with 20 μM of the compounds. Efflux from the cells was initiated by replacement of the medium with fresh medium lacking the environmental chemicals. As shown in Fig. 4, uptake is largely reversible; but a distinct fraction of 10–20% of either compound was retained by the cells and proved refractory to even repeated medium changes. The inset shows the non-linear kinetics of efflux. The intracellular compartments which irreversibly sequester part of these persistent compounds are not yet known.

Figure 5 shows that uptake is a linear function of concentration within the entire concentration range examined (DDT: 2–100 μM; 6-CB: 5–70 μM); saturation of uptake equilibrium could not be approached. With 6-CB, concentrations exceeding 70 μM were not used because of beginning cell deterioration. Linearity of uptake was also preserved by cells which had been preloaded overnight by exposure to culture medium containing 20 μM of the unlabeled compounds (data not shown). In any case, there is no indication of specific, saturable, carrier-dependent transport processes.

From the linear relation between concentration of the chemicals and uptake, an enrichment factor can be estimated. Based on a mean cell volume of 1800 μm^3 (for comparison: Mouse L cells have 1765 μm^3, ref. 25), and on the experimentally measured ratio of protein to cell number (1 mg cell protein = 1.9×10^6 cells), an uptake value of 4.5 μg of 6-CB and 10 μg of DDT per 3.5 mm^3 cells can be derived at the concentration of 20 μM (cf. Fig. 5). Since a volume of 3.5 mm^3 of culture medium contains 0.025 μg of 6-CB and DDT, an enrichment factor of 180 for 6-CB and 400 for DDT can be estimated.

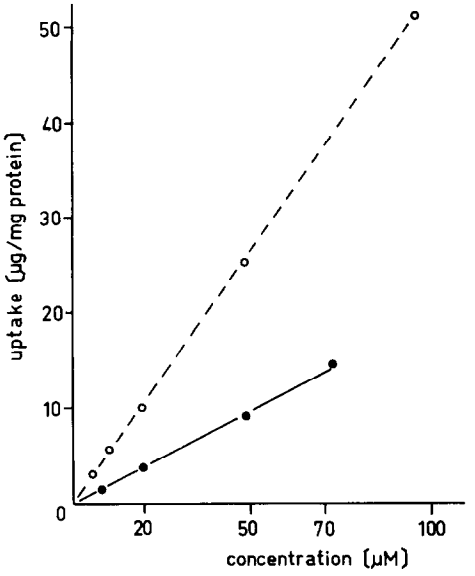

Fig. 5. Uptake of 6-CB and DDT as a function of concentration. Monolayer cells were incubated under standard conditions, but with the indicated concentrations of 6-CB (●——●) or DDT (○---○). The result of one experiment (out of two) is shown.

Binding to the cell plasma membrane and endocytosis

Before transport into the cells, any substance must interact with the cell plasma membrane. Consequently, the extent of adsorptive binding of the model chemicals to the cell surface was studied by adapting the method of "back exchange" [18, 19]. To this, the lipophilic chemicals, reversibly bound to the outer cell surface, were removed by washing the cells with fetal calf serum. The results of this "back exchange" experiment are shown in Fig. 6 (panel A: 6-CB; panel B: DDT). The upper curves (curves 1) reproduce the time course of total uptake, that is the amount of the chemicals remaining with the cell monolayer after incubation at 37° and rinsing with buffer. The middle curves (curves 2) show the residual cell-associated fractions after back exchange with cold serum. The difference of curves 1 and 2 gives then the exchangeable pool, i.e. the compounds bound to the outer cell plasma membrane and removable by serum; the percentage of this pool was

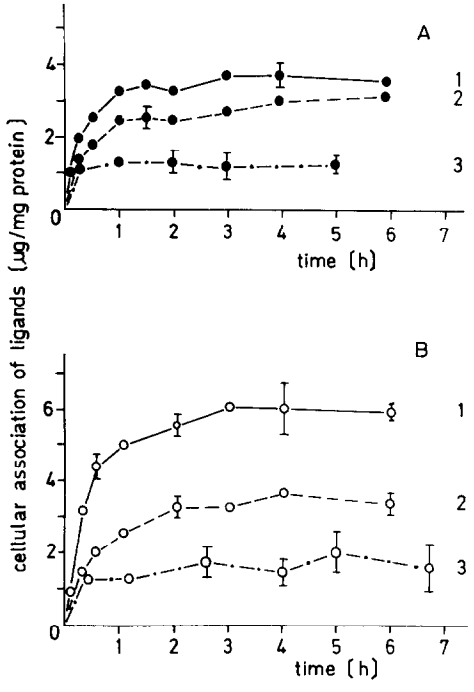

Fig. 6. Estimation of adsorptive binding. Monolayer cells were incubated under standard conditions at two different temperatures of 37° (curves 1 + 2) and 4° (curves 3). The time course of cell association is shown for 6-CB (panel A) and DDT (panel B). Curves 1 (——): uptake after the standard wash procedure with cold PBS/EDTA; curves 2 (----): residual uptake (tightly cell-bound fraction) after back exchange with cold FCS; curves 3 (·—·—·): same, but after incubation at 4°. For clarity, error bars (mean ± SD of three experiments) are shown only when largely exceeding the size of the graphical dots.

25 ± 5% of 6-CB and 45 ± 5% of DDT. The lower curves (curves 3) give the residual cell content after incubation at 4° and back exchange with serum as above. Since uptake by endocytosis is blocked at this low temperature [25] and the exchangeable pool has been removed by serum treatment, the residual cell content indicates uptake by energy-independent routes (passive diffusion). The percentage contribution of this pathway is 35 ± 5% of 6-CB and 25 ± 5% of DDT. The difference of curves 2 and 3 would then indicate temperature-dependent uptake pathways such as constitutive endocytosis which comprises 40 ± 5% of 6-CB and 30 ± 5% of DDT.

The translocation of DDT from the outer surface of the cell plasma membrane to intracellular compartments, inaccessible to back exchange, can best be demonstrated by the effect of temperature. Monolayer cells were exposed to culture medium containing 20 μM of DDT at 4° to block endocytosis. The medium was removed after 1 hr and incubation continued at the elevated temperature of 37° to initiate energy-dependent translocation (endocytosis) of the insecticide through the plasma membrane. As shown in Table 1, low temperature incubation indeed renders a large fraction (70%) of the total cell-associated insecticide exchangeable by fetal calf serum (cf. line 2 vs line 1); this percentage indicates the outer cell plasma membrane-bound fraction of the insecticide. After removal of the culture medium, warming the cells to 37° initiated translocation from the outer membrane binding sites across the plasma membrane (line 3). Endocytosis steps in again and increases the non-exchangeable intracellular pool of DDT (i.e. increase of uptake). The translocation of the insecticide DDT, as shown in this experiment, is reminiscent of the translocation of the fluorescent lipid C_6-N-(4-nitro-benzo-2-oxa-1,3-diazole) phosphatidyl ethanolamine in fibroblast cells [26].

The influence of a number of metabolic inhibitors is listed in Table 2. As anticipated, the endocytic inhibitors colchicine and cytochalasine B as well as the Ca^{2+}- ionophore A-23187 (also known to inhibit internalization of the LDL receptor, ref. 27) decrease uptake of 6-CB and DDT. The inhibition range of 30–40% compares favorably with the similar percentage of endocytosis obtained from the data in Fig. 6. Inhibitors such as the K^+-ionophor Nigericin and the Na^+-ionophor Monensin, which interrupt recycling of LDL receptors in human fibroblasts [28, 29], as well as dansylcadaverin, a specific blocker of polypeptide hormone-mediated receptor endocytosis [30], did not impair uptake. Dissipation of the membrane potential by the respiration uncoupler 2,4-dinitrophenol and inhibition of protein biosynthesis by cycloheximide even proved beneficial to uptake for reasons not further investigated.

Fluid pinocytosis

Monolayer cells continuously incorporate extracellular fluid by a constitutive process called fluid pinocytosis [31, 32]. Pinocytic uptake is estimated by measuring cell ingestion of non-metabolizable marker substances such as the polysaccharide inulin with the molecular weight of 6000 [33]. When uptake data are expressed as ingested volume/time, the resulting index number is independent from concentration.

The results of pinocytic uptake experiments, which included also the serum lipoprotein fractions HDL and LDL, are compiled in Table 3. Constitutive fluid pinocytosis is indicated by the figure of inulin (line 1). Uptake of albumin (line 2) and specific uptake of HDL (lines 7 vs 8) exceed but slightly the extent of fluid pinocytosis, showing that there are no specific uptake mechanisms for these proteins. In contrast, the rather high index number of LDL (line 9) could reveal LDL receptor-mediated endocytosis [34]. The high index numbers of the protein-bound chemicals 6-CB and DDT indicate the existence of additional uptake mechanisms with much greater capacity than fluid pinocytosis (lines 3–6, 10, 11). The possible involvement of LDL receptor-mediated endocytosis (line 11) was checked by experiment; the results (see below) rule out any significant contribution of this uptake route.

Receptor-mediated endocytosis

Transport of serum cholesterol into human fibroblast cells is primarily accomplished by the route of LDL receptor-mediated endocytosis. The detection of a highly specific LDL receptor like binding protein on Chang liver cells was of great advantage in examining this potential uptake mechanism. As shown in

Table 2. Effect of metabolic inhibitors on uptake

Inhibitor	Effect on	Inhibitor Concentration (μM)	Uptake (% of control) 6-CB	DDT	N
Dansylcadaverin	Receptor endocytosis	20	94	92	1
Colchicine	Microtubular structure	1	75 ± 2	59 ± 5	7
Cytochalasine B	Microfilament structure	10	66 ± 5	64 ± 8	5
Cycloheximide	Protein biosynthesis	1000	151 ± 40	149 ± 60	5
Monensin	Na^+ transport	50	101	94	1
Nigericin	K^+ transport	8	96	96	1
A-23187	Ca^{2+} Transport	2	71	66	1
2,4-Dinitrophenol	Respiration, membrane potential	1000	149	111	1
NaN_3	Respiration	1000	108	107	1

Monolayer cells were incubated at 37° under standard conditions with the inhibitor in question at the concentrations indicated. Control = 100% denotes uptake under identical conditions without inhibitor. The results of N experiments are given ±SD.

Table 3. Uptake of 6-CB and DDT en route of fluid pinocytosis

Line	Compound	Concentration (μg/ml)	Pinocytic Index (μl medium/ mg protein/hr)
1	Inulin	10	6
2	BSA	2000–5000	20
3	6-CB, bound to BSA	7.2	525
4	6-CB, bound to FCS	7.2	504
5	DDT, bound to BSA	7.1	759
6	DDT, bound to FCS	7.1	915
7	HDL	5	58
8	HDL + FCS	320	18
9	LDL	1.4	986
10	6-CB, bound to LDL	0.22	476
11	DDT, bound to LDL	0.04	1395

Uptake of the compounds listed was measured as described in the Experimental Section. The uptake data obtained were then calculated as to indicate the degree of fluid pinocytosis [33]. The figures of one experiment in paralell are tabulated. Index range of N additional experiments: line 4, 344–1409 (N = 3); line 5, 755–626 (N = 3); line 6, 539–915 (N = 5).

Fig. 7, specific and saturable binding of the lipoproteins HDL and LDL to Chang liver cells could be detected. Moreover, LDL receptor biosynthesis can be included several-fold in cultured fibroblast cells upon incubation of the cells with lipoprotein-deficient serum [34]. As shown in Table 4, induction of LDL receptor binding in Chang liver cells was possible after 48 hr of incubation with lipoprotein-deficient serum. In clear contrast to the 6-fold induction of LDL receptor binding and internalization, uptake of the LDL-bound chemicals is virtually unchanged; minor deviations from control values do not correlate with induction. Thus, even specific cell membrane receptor-like binding proteins do not promote uptake of lipophilic chemicals, bound to the corresponding carrier proteins. Likewise, cholesterol uptake in cultured fibroblasts was shown to

Table 4. Uptake of LDL-bound 6-CB and DDT by cells previously exposed to lipoprotein-deficient serum

Substance	Uptake (−fold of control) 12 hr preincubation	48 hr preincubation
LDL	3.19 ± 0.30	6.25 ± 2.11
6-CB	0.91 ± 0.13	0.67 ± 0.04
DDT	1.57 ± 0.35	1.47 ± 0.62

Monolayer cells had been exposed to culture medium (DMEM) supplemented with lipoprotein-deficient serum (32 mg protein/ml, equivalent to 8% FCS). After preincubation for 12 hr and 48 hr, the medium was replaced with DMEM containing LDL-bound 6-CB or DDT, respectively. After further incubation at 37° for 5 hr, uptake of LDL, 6-CB and DDT was measured. Control = 100% denotes uptake under standard conditions. Data are the mean of three experiments (±SD).

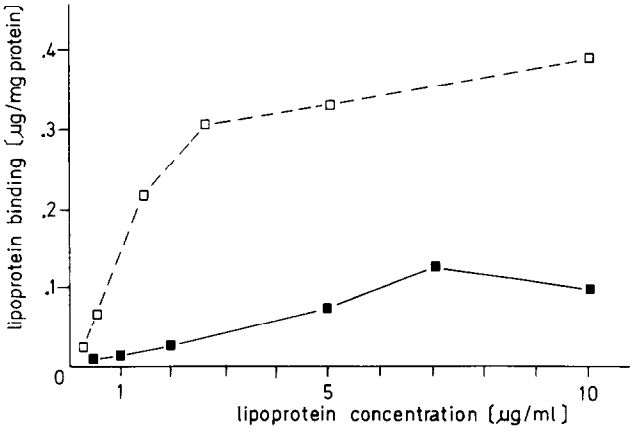

Fig. 7. Binding of the lipoproteins HDL and LDL. Monolayer cells were incubated at 4° with culture medium (DMEM) containing the radioiodinated lipoproteins HDL (■—■) or LDL □---□) at the indicated concentrations. Specific binding, i.e. the difference of lipoprotein binding in the absence (=total binding) and in the presence (=unspecific binding) of 8% FCS, is plotted vs. the lipoprotein concentration. Specific binding amounted to 75–80% of total binding.

eventually bypass the established LDL receptor-mediated endocytic pathway [35].

Concluding remarks

Uptake of lipophilic environmental chemicals by human cells, cultured in monolayer, is a process more complex than anticipated from "conventional" uptake studies with, for example, cells in buffered suspension. Even under equilibrium conditions of uptake, a considerable fraction of the compounds remains associated with the cell plasma membrane. It appears that internalization of membrane-bound chemicals occurs inevitably during constitutive membrane endocytosis. Since the chemicals are concentrated in the lipid-rich plasma membrane, a major gate of entry into the cell is through membrane endocytosis. The percentage of uptake and distribution under equilibrium conditions can be estimated as follows:

Parameter	Percentage for 6-CB	DDT
Binding to the cell plasma membrane	25	45
Uptake by passive diffusion	35	25
Uptake by endocytosis	40	30

On the other hand, transport pathways such as fluid pinocytosis and receptor-mediated endocytosis appear but of minor importance.

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