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Fusion of lipid vesicles with ascites tumor cells and their lipid-depleted variants. Studies with radioactive- and fluorescent-labeled vesicles

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Cultured ascites tumor cells and their lipid-depleted variants, which contained 35–40% less membrane phospholipid and cholesterol, were used for fusion experiments with unilamellar lipid vesicles which were between 300 and 600 nm in diameter. Vesicle–cell interaction was followed by tracer studies using vesicles double-labeled in the lipid moiety, by vesicle-encapsulated [^{3}H]dextran, and by measurements of energy transfer between *N*-(10-[1-pyrene]decanoyl)sphingomyelin-labeled vesicles and α-parinaric acid-labeled cells in the presence of poly(ethylene glycol) (PEG) as fusogen. The reaction rates measured with the radiolabeled vesicles were found to follow patterns similar to those obtained with the resonance energy transfer assay. This latter method revealed a vesicle–cell membrane fusion reaction, which was substantiated by radiolabeling the internal cellular compartment after treatment of the cells with [^{3}H]dextran-encapsulated vesicles as shown by electron microscopic autoradiography on semi-thin sections. Endocytosis as a reaction mechanism can be excluded, since no energy transfer was observed at 25°C in the absence of PEG. Investigations of vesicle bilayer order and fluidity on vesicle–cell interaction revealed optimal reactivity, with intermediate fluidity corresponding to cholesterol/phospholipid ratios between 0.7 and 1.0 and fluorescence depolarization (P) values of 0.18 and 0.21. Lipid depletion decreased the reaction velocity between cells and vesicles by about 20%, exhibiting V values of 33.2 μmol/min, as compared to the control of 41.4 μmol/min determined for 10^7 cells. The affinity constants for vesicle lipid were affected only slightly with K_m values of 0.195 mM (0.210 mM). The activation energies for the reaction were calculated to give values of $E_A = 22.44$ kJ/mol for the control and of $E_A = 20.4$ kJ/mol for the modified cells. These data indicate that the decrease in membrane lipid content apparently has no major influence on the extent of the interaction.

Introduction

Membrane fusion is a complex phenomenon of both fundamental and practical importance [1]. A number of models have been developed, supported by some experimental evidence, to describe this membrane–membrane interaction [2,3]. How-

Abbreviations: PEG, poly(ethylene glycol); PBS, phosphate-buffered saline; C/P, cholesterol to phospholipid; PC, phosphatidylcholine; lysoPC, lysophosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine.

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ever, the initial reaction to induce the fusion process involving some kind of rearrangement of the bilayer is still unknown. It is assumed that the major energy barriers which have to be overcome in membrane fusion are electrostatic repulsion and the water shell bound by the polar headgroups of the lipid molecules. To bind and remove the water from the surface of the membranes is thought to be the main action of the known fusogenic chemicals, like PEG and others [4–10].

There are several mechanisms of interaction between liposomes and cells which are being discussed; stable adsorption, endocytosis, fusion and lipid transfer [11]. An ability to differentiate between these mechanisms is critical for many applications. Fluorescence energy transfer offers several advantages for this purpose [12,13]. A number of different pairs of fluorophors have been applied both in liposome–liposome [14,15] and liposome–cell fusion experiments [16]. In this study we have taken ascites tumor cells labeled with α-parinaric acid [17], which is easily taken up by these cells, and pyrene-labeled lipid vesicles in addition to radioactive-labeled vesicles to investigate the mechanism of vesicle–cell interactions.

Recently, Roos and Choppin [18,19] have selected stable cell mutants of mouse fibroblasts which differed in their fatty acid composition, and have compared them in their fusion properties. Their initial observation that fatty acids may play an important role in the fusion process could not be confirmed upon further investigation of their system. In a previous study we have described the preparation and properties of lipid-depleted ascited tumor cells, obtained under cell culture conditions [20]. With the notion that these modified cells may be more suitable for fusion with lipid vesicles because of their greater demand for lipids, we have used these cells in our interaction experiments and have compared them with the non-modified cells. Surprisingly, we have found a 20% lower reaction velocity between lipid-depleted cells and vesicles but rather similar K_m values and activation energies in both cell variants. Therefore, we think that electrostatic repulsion and hydration of the membranes are mainly determining the fusion reaction, more than the lipid content of the membranes. Preliminary data have recently been presented [21].

Materials and Methods

Materials

PC (egg yolk), lysoPC and cholesterol were obtained from Serva (Heidelberg) and [^{3}H]cholesterol and [^{14}C]PC from New England Nuclear (Dreieich). Percoll, Sephacryl S-1000 and Sepharose CL-4B were purchased from Pharmacia-LKB (Freiburg), α-parinaric acid from P-L Biochemicals, *N*-(10-[1-pyrene]decanoylsphingomyelin from Sigma (Munich) and 4-hydroxy-*m*-phenylenediammonium dichloride from Merck (Darmstadt).

Cell Cultures

Ascites tumor cells, ELT Bonn [22], were cultivated in minimum essential medium with Earle's salt and 10% horse serum. Cell cultures were seeded at an initial cell density of about 10^5 cells per ml using sterile 75-cm^2 Corning tissue culture flasks. The medium was changed every 2nd day by centrifugation of the cells, and cell densities were determined with the aid of a Neubauer hemocytometer. Viability not lower than 98% under our growth conditions was checked by the Trypan blue exclusion test. To obtain lipid-depleted cells, the culture cells were transferred after one washing step into delipidated serum medium at a cell density of about $2 \cdot 10^5$ cells per ml, and kept under these conditions for at least 24 h. The lipid-depletion procedure did not affect the protein content of the cells which were characterized by Percoll density gradient fractionation. Further details of the characterization of these cells are described elsewhere [20].

Plasma membrane preparation

Cells were disrupted after a swelling treatment in hypotonic salt solution according to Mamaril et al. [23]. A nuclear-free $12000 \times g$ pellet was prepared from the cell homogenate by differential centrifugation which was further fractionated by gel-chromatography on Sephacryl S-1000 to yield purified plasma membranes [24]. Protein was determined according to Lowry et al. [25].

Vesicle preparation

The lipid vesicles were prepared by a combination of ultrasonic treatment [26] and membrane

filtration [27]. Amounts of 16.2 mg egg-yolk PC, 7 mg cholesterol and 1.8 mg lysoPC (in the case of radioactively labeled vesicles 0.07 μCi [^{14}C]PC/mg of PC and 2 μCi [^{3}H]cholesterol/mg of cholesterol were added) were dissolved in chloroform/methanol (2:1) and transferred into a reaction vessel, the solvent was evaporated to obtain a thin lipid film, then 10 ml PBS (pH 7.4) added and the mixture was sonicated at 20°C for 5 min. The milky suspension was subsequently filtered through a 0.45 μm filter and then centrifuged at 100000 × g for 30 min to precipitate amorphous lipid material and some metal from the sonicator. The almost clear solution was analyzed by gel filtration on Sepharose CL-4B, monitored both by turbidity and radioactivity measurements. Generally two peaks were obtained, a major one appearing in the exclusion volume, and a minor one with a distribution constant (K_d) of 0.34 [28]. The overall yield of vesicle material was about 25%. Pyrene-sphingomyelin-labeled vesicles were made by mixing 1 mol% of *N*-10-[1-pyrene]decanoylsphingomyelin with the lipid solution prior to sonication and filtration. The vesicle preparations were quantified by determination of the cholesterol and phospholipid content.

Electron microscopy

The liposomes were visualized by the negative staining procedure with potassium phosphotungstate yielding unilamellar vesicles with a diameter between 300 and 600 m (peak 1), and a diameter between 100 and 300 nm (peak 2). Although no difference in reactivity with ascites cells was observed between the two vesicle populations generally the larger vesicles were used for the interaction experiments.

Tracer studies

Amounts of 5 · 10^6 cells and 50 μg vesicle lipid suspended in 100 μl PBS (without Ca^{2+} and Mg^{2+}) (pH 7.4) were mixed with 400 μl 56.3% PEG 4000 solution (final PEG concentration 45%), and incubated at 25°C for 10 min. Viability tests at the end of the reaction yielded about 25% stained cells, as determined by the Tryptan blue exclusion test. After addition of 1.5 ml 0.9% NaCl solution the cells were pelleted and washed once with 2 ml of the same solution which proved to remove all non-tightly bound radioactivity satisfactorily. The cells were then resuspended and taken for radioactivity countings.

0.4 ml aliquots of the cell suspensions were mixed with 1 ml of protosol and kept at room temperature overnight. The samples were then neutralized with 50 μl glacial acetic acid, taken up in 10 ml Aquasol and counted in a Marck III (Nuclear, Chicago) scintillation counter.

Labeling of cells with α-parinaric acid

The sodium salt of α-parinaric acid, dissolved in 80% ethanol, was added either as free and or as BSA (1%) complex to the culture cells to give a final concentration of 100 μg parinaric acid per 5 · 10^5 cells per ml of culture medium. Uptake of the acid usually reached the plateau-phase after 4 h, and the cells were harvested thereafter, or in some experiments they were labeled for 24 h, then the pellets were washed once with 0.9% NaCl solution, resuspended and used for the fluorescence energy transfer experiments. The amount of bound α-parinaric acid was determined by measuring the absorbance at 320 nm, and by using the molar absorption coefficient $\epsilon = 73000$ for the calculation. The control cells revealed values of 2.36 nmol and the lipid-depleted cells of 2.03 nmol α-parinaric acid bound per 10^6 cells. Preparation of the plasma membranes of parinaric acid-labeled cells and analysis of the lipid extracts by thin-layer chromatography showed that most of the acid in this membrane fraction was bound to PC and PE.

Lipid analyses and fluorescence anisotropy measurements

Plasma membrane and vesicle cholesterol were analyzed by GLC [29] and phospholipid by phosphorus analysis [30] and by the procedure of Raheja et al. [31]. Fluorescence polarization and intensity were obtained by measurements of the fluorescence intensities detected through a polarizer oriented parallel and perpendicular to the direction of polarization of the excitation beam. Further details of the procedure have been described elsewhere [20]. The calculation of the fluorescence polarization was made according to Shinitzky and Inbar [32].

Resonance energy transfer assay

Aliquots of $2 \cdot 10^6$ α-parinaric acid-labeled cells suspended in PBS (pH 7.4) were incubated at 25°C for 10 min in the presence of pyrene-labeled vesicles (between 100 and 700 μg vesicle-lipid) and 45% PEG 4000 in a final volume of 1 ml. The reaction was stopped by adding 3 ml cold PBS. The cells were pelleted by centrifugation, washed once with 1 ml PBS, resuspended in the same buffer solution and taken for the fluorescence measurements using a Perkin-Elmer MPF4 fluorescence spectrophotometer. The samples were excited at 297 nm (slit width 7–10 nm), which is the excitation maximum of α-parinaraic acid-labeled cells, and the emission spectra were recorded between 310 and 450 nm (slit width 9–10 nm). The pyrene-labeled vesicles exhibited two emission maxima at 375 and 397 nm. As controls, the complete reaction mixture was either kept at 4°C or the sample was incubated at 25°C in the absence of PEG. In both cases, no or only very little energy transfer between α-parinaric acid and pyrene was measured.

Electron microscopic autoradiography

Interaction of ascites cells with [^{3}H]dextran-encapsulated vesicles in the presence of PEG was performed as described previously. In the control experiment the cells were treated with free, i.e., non-vesicle-encapsulated, [^{3}H]dextran. The cells were then fixed simultaneously in 2% glutaraldehyde and 1% OsO_4 dissolved in 0.1 M cacodylate buffer (pH 7.2) at 0°C for 30 min, according to Franke et al. [33]. After washing in cold cacodylate buffer several times, the cells were postfixed in 1% OsO_4 dissolved in 0.1 M cacodylate buffer (pH 7.2) at 4°C for 30 min. The material was dehydrated in graded acetone at room temperature and embedded in an epoxy mixture according to Spurr [34].

During dehydration the specimen were stained for 30 min with 1% uranyl acetate in 70% acetone. Semi-thin sections of 0.25 and 0.5 μm mounted on grids were covered with a thin film of Ilford L-4 emulsion using an expanded wire loop. These semi-thin sections were preferred for the autoradiography as compared with normal ultra-thin sections, because they contained a larger amount of incorporated [^{3}H]dextran. After exposure of 6–8 weeks at 4°C, the autoradiographs were developed for 1.5 min at room temperature with 1.125 g of 4-hydroxy-*m*-phenylenediammonium dichloride and 4.5 g of anhydrous sodium sulfite, diluted to a total volume of 250 ml with redistilled water with addition of 2 ml of a 10% (w/v) potassium bromide solution. After washing with aqua bidest., the specimens were fixed for 5 min with a commercially available fixative which is normally used for negative films. Additionally, the preservation of the cellular structures was reported by viewing ultra-thin sections which were poststained with lead citrate (cf. Fig. 4c). This staining step was omitted for the autoradiographs to avoid a too strong contrast of the semi-thin sections. The samples were viewed in a Siemens Elmiskop 102 at 100 kV.

Results

The control- and lipid-depleted cells, which on a Percoll gradient equilibrated at different densities, 1.058 vs. 1.076 g/cm^3, were obtained by published procedures [20]. The phospholipid and cholesterol composition of the plasma membranes of these cells is shown in Table I, revealing a 35–40% reduction of both constituents in the lipid-depleted cells. These cells were used to study the kinetics of vesicle–cell interaction employing radioactive- as well as fluorescent-labeled vesicles.

TABLE I

CONTENT OF FREE CHOLESTEROL, LIPID-PHOSPHORUS, THE *C/P* MOLAR RATIO AND THE FLUORESCENCE DEPOLARIZATION VALUES (*P*) OF THE PLASMA MEMBRANES OF CONTROL AND LIPID-DEPLETED CELLS

	Control	Lipid-depleted
Cholesterol/protein (μg/mg)	20.9 ± 4.1 [a] (8) [b]	12.8 ± 4.4 (61.2%) [c] (4)
Phospholipid/protein (μg/mg)	170.2 ± 50 (6)	112.0 ± 10 (65.8%) (6)
C/P (molar)	0.22 ± 0.06 (6)	0.17 ± 0.02 (5)
P (37°C)	0.291	0.314

[a] The data represent the mean ± S.D.
[b] Number of experiments with duplicate analyses.
[c] Percent value of the control.

TABLE II

INTERACTION OF LIPID VESICLES (C/P = 0.75) LABELED WITH [^{3}H]CHOLESTEROL AND [^{14}C]PC WITH ASCITES CELLS AT 25°C IN THE PRESENCE OF INCREASING PEG 4000 CONCENTRATIONS

The data represent the mean ±S.D. of 2–6 experiments.

PEG 4000 (concn. in %)	Cell-bound radioactivity (% of total activity applied)	
	[^{3}H]cholesterol	[^{14}C]PC
0	1.40±0.62	1.22±0.7
5	2.50±0.10	2.30±0.2
10	7.45±1.0	7.24±0.7
20	10.42±1.9	10.46±1.6
30	10.21±1.7	10.60±1.5
40	17.74±4.2	18.14±2.5
45	21.35±2.3	21.35±1.9

Kinetics of vesicle–cell interactions

Initial studies on the influence of PEG upon cell viability revealed a rather high cytotoxicity of this fusogen at a concentration of 45%, and a reaction temperature of 37°C amounting to about 40% of stained cells as shown by the Trypan blue exclusion test. All vesicle–cell interactions were therefore performed at 25°C, a temperature at which the cytotoxic PEG effect could be drastically reduced (10%). The effect of the amount of PEG is shown in Table II, revealing increasing interactions with the highest rate at 45% of final PEG concentration. Also other PEGs with molecular weights of 1500 and 6000 were tested, but with no significant differences in their fusion capacities. The influence of vesicle concentration on their interaction both with control and lipid-depleted cells is shown in Fig. 1. Measurements of resonance energy transfer between pyrene-labeled vesicles and α-parinaric acid-labeled cells are shown in Fig. 1A, indicating a higher reactivity of the control cells. This was also observed with radioactive-labeled vesicles, which is shown in Fig. 1B. From these data, V and K_m were calculated for both cell variants (Fig. 2), exhibiting V values of 41.4 (33.2) μmol/min and K_m values of 0.195 (0.211) mM for the control and lipid-depleted cells. The inset of Fig. 2 gives the corresponding activation energies. Although the reaction velocity of the lipid-depleted cells was reduced by about

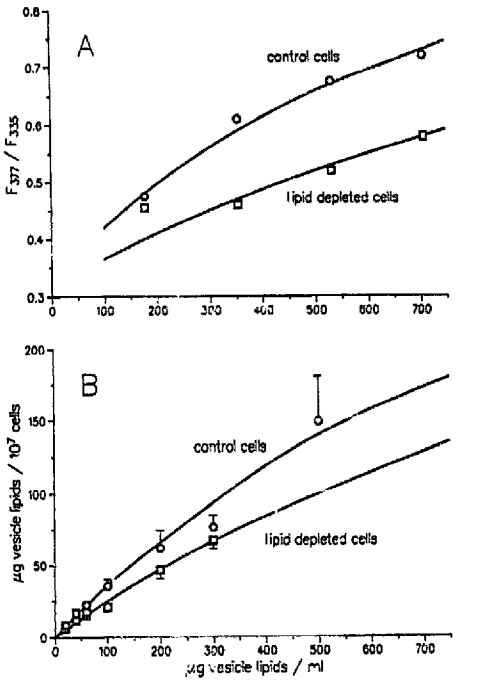

Fig. 1. Vesicle–cell interaction in relation to the vesicle concentration. (A) Assay of resonance energy transfer between α-parinaric acid-labeled cells and pyrenesphingomyelin-labeled vesicles; (B) transfer of ^{3}H/^{14}C radioactivity from vesicles to ascites cells. Details of the incubation conditions are described under Materials and Methods.

20%, K_m and E_a were only marginally affected in these cells.

Influence of vesicle bilayer order parameter on vesicle–cell interaction

Lipid vesicles without cholesterol and with increasing cholesterol mole fractions were prepared and their fusion properties were investigated (Table III). Vesicles with a C/P ratio between 0.72 and 1.0 and P values of 0.18 and 0.21 proved to be the best suitable for this purpose. The data in Table III further indicate nearly identical labeling of the cells, both with cholesterol and phospholipid, which precludes a selective molecular exchange mechanism of single vesicle components.

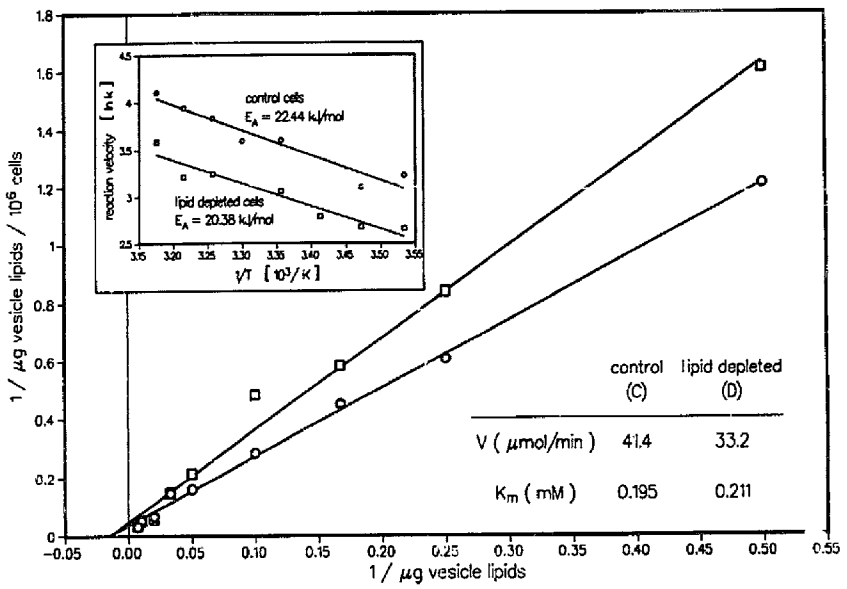

Fig. 2. Lineweaver-Burk plots of double radioactive-labeled vesicles interacting with ascites cells and their lipid-depleted variants. Inset: Arrhenius plots, from which the activation energies were calculated.

TABLE III

INTERACTION OF LIPID VESICLES OF INCREASING *C/P* RATIOS LABELED WITH [^{3}H]CHOLESTEROL AND [^{14}C]PC WITH ASCITES CELLS AT 25°C IN THE PRESENCE OF 45% PEG

Data represent means ± S.D. of three experiments.

Vesicle *C/P* ratio	Cell-bound radioactivity (% of total activity applied)	
	[^{3}H]cholesterol	[^{14}]PC
0	–	25.0 ± 6.2
0.48	33.8 ± 7.2	29.4 ± 2.6
0.72	41.0 ± 7.5	42.8 ± 5.0
0.95	41.0 ± 7.5	40.0 ± 7.5
1.45	31.3 ± 4.1	29.7 ± 4.1

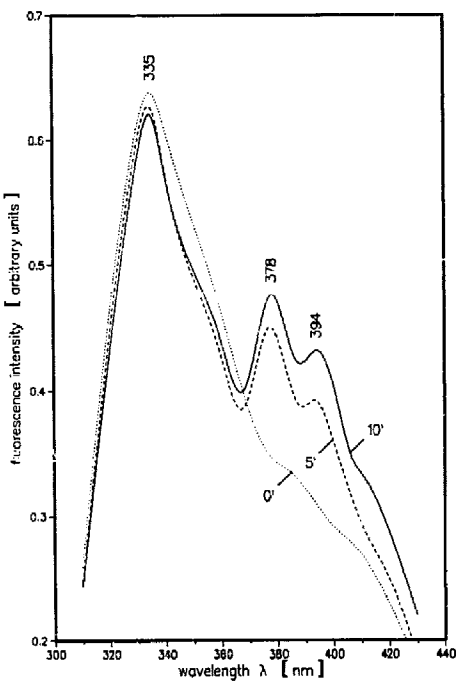

Fig. 3. Time-dependence of resonance energy transfer between *N*-(10-[1-pyrene]decanoylsphingomyelin-labeled vesicles and α-parinaric acid-labeled cells. Emission spectra of the control (no incubation ·····), and of the reaction products after 5 min (- - - - - -) and 10 min (———) of reaction time at 25°C. The reaction mixtures contained about 700 μg (0.82 μmol) of vesible lipid in 1 ml.

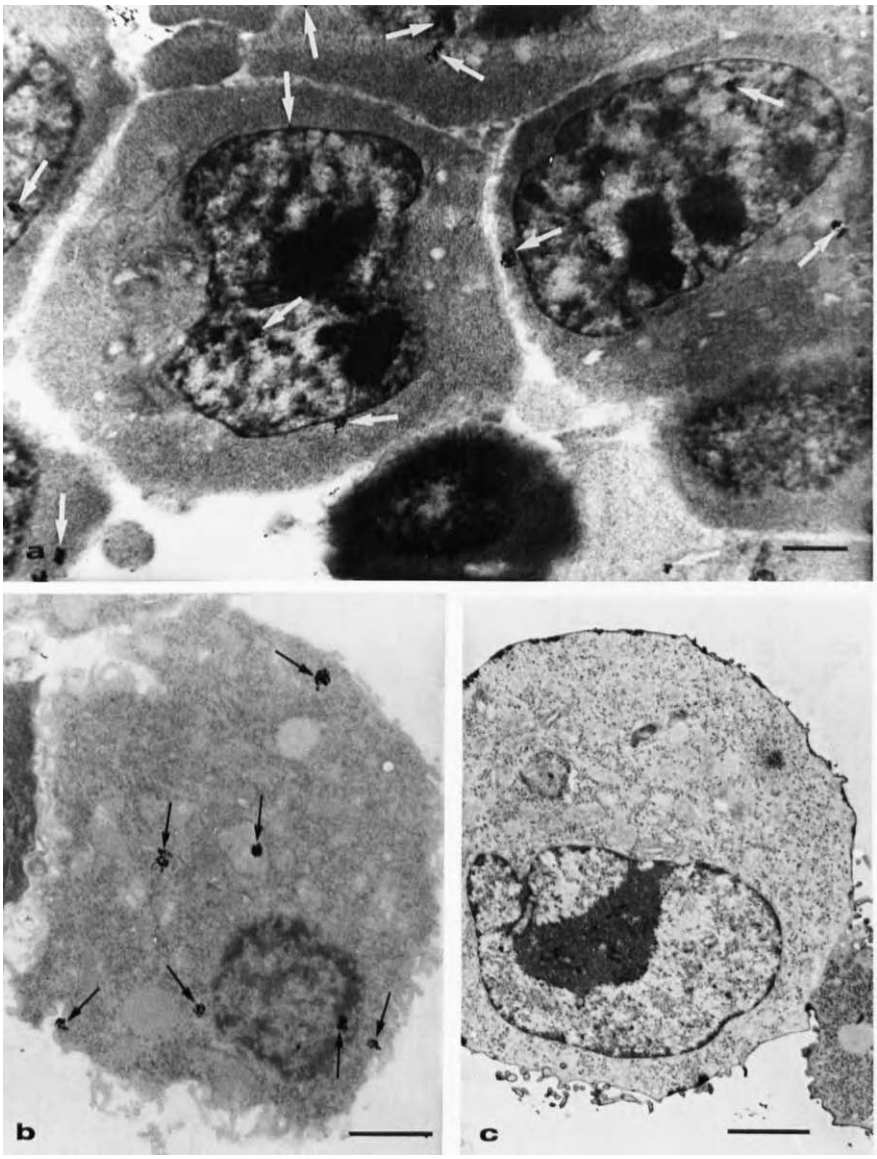

Fig. 4. Electron microscopic autoradiography of [^{3}H]dextran-encapsulated vesicles fused with ascites tumor cells. (a) Semi-thin section (0.5 μm) of some ascites cells (overview). Radioactive reaction products (arrows) are visible in all cells. (b) Semi-thin section (0.25 μm) of an entire cell. The autoradiographic grains (arrows) can be seen over the cytoplasm and the nucleus. (c) Ultra-thin section (about 80 nm) of an ascites cell (control) which was additionally poststained with lead citrate. Here the plasma membrane of the intact cell is clearly visible. Control specimens have been incubated in the presence of free [^{3}H]dextran. No reaction products can be seen. Magnifications are indicated with a bar, representing 2 μm.

Resonance energy transfer measurements

For a further study of the reaction mechanism between vesicles and cells we have labeled the ascites cells with α-parinaric acid, and the vesicles with pyrene covalently bound to sphingomyelin. Since the excitation spectra of both fluorophores show practically no overlap (spectra not shown), they proved suitable for these transfer measurements. Time-dependent energy transfer is shown in Fig. 3, indicating the appearance of pyrene emission bands in the α-parinaric acid emission spectrum. The zero-time spectrum corresponds to a reaction mixture kept at 4°C during incubation, or was incubated at 25°C in the absence of PEG. Vesicle concentration-dependent resonance energy transfer has previously been shown (Fig. 1A). No energy transfer was observed at 25°C in the absence of PEG. Data on energy transfer efficiency measurements, which were conducted with double fluorescent-labeled vesicles, could not be evaluated due to the disturbing side-effects of PEG.

Electron microscopic autoradiography

For the demonstration of the mixing between the vesicle and intracellular aqueous compartment, we have used [^{3}H]dextran encapsulated into vesicles for the experiment. Analyses of the reaction products were carried out by electron microscopic autoradiography on semi-thin sections shown in Fig. 4. Silver grains can be observed within the cytoplasm in regions free of membrane structures. Labeling is also observed in the nucleus indicating that non-encapsulated dextran molecules have penetrated the nuclear envelope, possibly via the nuclear pore complex. In a series of control experiments with PEG, empty vesicles and free [^{3}H]dextran either alone or in various combinations, some intracellular radioactivity was observed. This amounted to about 25–30% of the radioactivity obtained with [^{3}H]dextran-loaded vesicles, suggesting that this labeling background results from the fraction of dead cells becoming permeable to this compound. In summary, it can be concluded that the major amount of [^{3}H]dextran must have been incorporated into the intact cells by a vesicle-mediated process and not by trapping or other means.

Discussion

The interaction of lipid vesicles with cultured cells and their use as carriers for drugs and macromolecules has been a matter of intensive investigation throughout the last several years [35,36]. Most of the information available on the mechanisms involved in the interaction and uptake of lipid vesicles by cells has come from experiments in which vesicles containing radiolabeled phospholipids in the vesicle membrane were incubated with cells under defined conditions and the amount of cell-associated radioactivity was measured. We in our study have prepared double-labeled vesicles with [^{3}H]cholesterol and [^{14}C]PC and have studied their interaction with ascites tumor cells. Since, as our results indicate, both radiolabels were associated with the cells in equal amounts, we conclude that an interaction of entire vesicles with the cells must have occurred and not an exchange reaction between a particular vesicle lipid and the plasma membrane of the cells. It is known that exchange rates are much lower for phospholipids than for cholesterol [36–39].

The most widely discussed mechanisms for liposome–cell interactions are fusion induced by chemicals and endocytosis. In a number of investigations it has been shown that in the presence of PEG, the predominant interaction is fusion [4–10,40] requiring only a short reaction time. The effect of PEG to induce fusion was attributed to its ability to exert a high osmotic pressure and to dehydrate the system [10], resulting in a pronounced bilayer destabilization. Endocytosis as a mechanism for vesicle–cell interactions has been described for systems in which no PEG was used [11,41]. Unless an endocytotic process in the presence of PEG is postulated, our data are compatible with a fusion reaction, particularly since no intimate contact between the two membrane systems is required for endocytosis.

Fusion between lipid vesicles and cells was shown by resonance energy transfer experiments in the presence of PEG as fusogen, and also by labeling the intracellular compartment with dextran which had been encapsulated into the vesicles. Differences in the molecular weight of various

PEGs did not affect this process to any major extent. We have also tested calcium as fusogen in combination with PS-containing vesicles. Under our conditions calcium induced mainly a vesicle-cell adsorption, since most of the radiolabel was lost from the cells upon density-gradient fractionation of the reaction product. It has, however, to be emphasized that our vesicles contained only 10% PS of total phospholipid, in contrast to other reports where pure PS-vesicles have been used in the Ca^{2+}-induced fusion experiments [42]. On the other hand, there seems to be some dispute with respect to the significance of the PS-Ca^{2+}-system as a model for biological membrane fusion [43]. Our results on the effect of Ca^{2+} are basically explained by a study made by Sundler et al. [44], who found that vesicles containing 70 mol% PC do not fuse even at high Ca^{2+} concentration. To increase the fusogenic properties of our vesicles, we have added 10% lysoPC to the vesicle lipid composition [45]. The effect of cholesterol on the Ca^{2+}-induced aggregation and fusion of PS-cholesterol vesicles has recently been investigated [46].

The interaction experiments with the lipid-depleted cells revealed no increase in reactivity, as anticipated, but a relatively small decrease of the reaction velocity, which can be explained by a lower membrane fluidity of these cells (Table I). This interpretation would be compatible with the cholesterol effect on the fusion capacity of our vesicles, which was found to be optimal at intermediate P values between 0.18 and 0.21. Nevertheless, the differences in fusion properties between control and lipid-depleted cells are not large enough to consider the lipid content of these cells to be of major importance in the fusion process. There are some reports in the literature to support the inhibitory action of vesicle cholesterol above 30 mol% in the fusion reaction [47,48]. In contrast, Kirby et al. [49] have shown that the stability of liposomes in plasma can be considerably improved by incorporating high proportions of cholesterol into the bilayer, knowing that the stability of the liposomes is an important prerequisite for their effective use as drug carriers under in vivo conditions. The importance of membrane fluidity with respect to its fusion properties was recently questioned by studies on mouse fibroblasts with altered membrane fatty acid compositions, which did not reveal any correlation between fluidity and the control of cell fusion [18,19].

The temperature-dependence of vesicle–cell interaction has been documented previously [50], and the activation energies determined in our fusion reaction both for the control and lipid-depleted cells were found to be almost identical to the Arrhenius coefficient for myoblast fusion [51], and about one-half of the activation energy determined for an exchange process occurring through an aqueous interphase [52], indicating again that lipid exchange between the membranes can be excluded. Comparing the fusion capacity of our vesicles with the Ca^{2+}-induced interaction of acidic liposomes with red blood cells [53], we found values which were about two orders of magnitude higher. Resonance energy transfer as a measure for fusion processes is considered to be the most reliable method in this field. Our system employing ·parinaric acid and pyrene-labeled sphingomyelin has not been used before in this combination. Presently, experiments are under way to use this system for the introduction of biologically active molecules into the cells and the study of their effect on cellular function.

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