Proc. Natl. Acad. Sci. USA
Vol. 78, No. 3, pp. 1685–1689, March 1981
Cell Biology

Use of lectins and polyethylene glycol for fusion of glycolipid-containing liposomes with eukaryotic cells

(fluorescence/fluorescent analogs/microinjection/photobleaching)

FRANCIS SZOKA*†, KARL-ERIC MAGNUSSON‡§, JOHN WOJCIESZYN‡¶, YU HOU‡, ZENON DERZKO‡¶, AND KENNETH JACOBSON‡¶

*Department of Physiology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, Massachusetts 02111; and ‡Department of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, New York 14263

Communicated by David Harker, November 17, 1980

ABSTRACT **Efficient fusion of phospholipid vesicles with monolayer cultures of eukaryotic cells was accomplished by attaching glycolipid-containing vesicles to the cell surface by using a lectin displaying binding for both the cell surface and the glycolipid, followed by treatment with polyethylene glycol. Fusion was inferred from the transfer of fluorescent lipid analog probes embedded in the vesicle membrane over the entire cell surface and of fluoresceinated proteins from the aqueous space of the vesicle to the cytoplasm of the cell. Fluorescence recovery after photobleaching showed that both the injected membrane and the cytoplasmic markers were mobile. Two different lectin–glycolipid combinations [*Ricinus communis* agglutinin I-lactosylcerebroside and concanavalin A-tetradecyl- (or hexadecyl-) maltobionamide] were used to promote attachment of lipid vesicles before polyethylene glycol-induced fusion with BG-9 human fibroblasts, NIL-8M2 hamster cells, or L-929 mouse cells. In the absence of lectin or polyethylene glycol, fusion was negligible. However, when both the lectin and the glycol were used, a dramatic increase in the transfer of both vesicle membrane and aqueous space markers from the liposomes to the cells occurred.**

The study of the dynamics of protein and nucleic acid structure and function in living cells has been advanced by the development of techniques for microinjecting exogeneous macromolecules into the cytoplasm of cells. The direct microinjection of molecules from micropipettes is a well-established technique (1–3); however, it requires skill and practice and the number of cells that can be injected is limited. Erythrocyte-mediated microinjection has been described by a number of groups (4–6), but the size of the molecules that can be loaded into the erythrocyte is restricted (for review, see ref. 7) and fusion results in the simultaneous transfer of the lipids and proteins of the erythrocyte into the recipient cell. Liposomes have also been used to deliver macromolecules and supramolecular assemblies into cells (8–10) and offer the advantage that only the defined lipid species from the liposomes are introduced into the recipient cell. However, the efficiency of liposome-mediated transfer into many cell types is low (11).

Recently, a highly efficient erythrocyte microinjection method using lectin-mediated binding was introduced by Mercer *et al.* (12). We have used this strategy to increase the efficiency of liposome-mediated transfer. Thus, a lectin having affinity for both target cell surface receptors and an appropriate glycolipid included in the liposome bilayer was used to specifically bind the liposomes to the cell surface. Subsequent addition of polyethylene glycol brought about an efficient fusion of the bound liposomes with the cells, leading to the high-efficiency transfer of lipid analogs from the vesicle to the plasma membrane and of encapsulated water-soluble compounds into the cytoplasm of the cells.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Chemicals. *N*-Stearoyldihydrolactocerebroside (LC), fluorescein isothiocyanate, and maltose were obtained from Sigma. Tetradecylamine was purchased from Aldrich. Carboxyfluorescein was obtained from Eastman Kodak and repurified as described (13). Concanavalin A was obtained from Pharmacia. *Ricinus communis* agglutinin, type I (RCA-I) was a product of E.Y. Laboratories (San Mateo, CA). The initial supply of tetradecylmaltobionamide (TM) was a generous gift of I. J. Goldstein. Subsequently, TM and hexadecylmaltobionamide (HM) were synthesized according to a published procedure (14). Polyethylene glycols were obtained from Polysciences (Warrington, PA) or from Union Carbide (New York).

The fluorescent lipid analogs—3,3′-dihexadecylindocarbocyanine (diI-C_{16}{3}) and 3,3′-dioctadecylindocarbocyanine (diI-C_{18}{3}) were the generous gift of A. S. Waggoner. The source, purity, and storage of the phospholipids used in this study have been reported (11).

Bovine serum albumin was a product of Miles. It was labeled with fluorescein as described (2).

Preparation of Liposomes. Unilamellar large liposomes were prepared by the reverse phase evaporation method (15) and had the following compositions: LC/phosphatidylglycerol (PtdGro)/egg yolk phosphatidylcholine (PtdCho)/cholesterol (Chol), 1:0.5:8.5:8 (these are termed LC vesicles); LC/PtdGro/PtdCho, 1:0.5:8.5; TM (or HM)/PtdGro/PtdCho/Chol, 1.5:0.5:4.5:3.5; TM (or HM)/PtdGro/PtdCho, 1.5:0.5:8.0. The glycolipids did not dissolve in diethyl ether. Therefore a diethyl ether/chloroform (1:1) mixture was used (15). These liposomes had a tendency to aggregate. To reduce this, the vesicles were extruded through a 0.4-μm polycarbonate membrane immediately after formation and dialyzed in Dulbecco's phosphate-

Abbreviations: Chol, cholesterol; diI-C_{16}{3}; 3,3′-dihexadecylindocarbocyanine; diI-C_{18}{3}; 3,3′-dioctadecylindocarbocyanine; PtdCho, egg yolk phosphatidylcholine; P_i/NaCl, phosphate-buffered saline; P_i/NaCl/Mg^{2+}/Ca^{2+}, phosphate-buffered saline containing Ca^{2+} and Mg^{2+}; HM, hexadecylmaltobionamide; LC, *N*-stearoyldihydrolactocerebroside; PtdGro, phosphatidylglycerol; RCA-I, *Ricinus communis* agglutinin I; TM, tetradecylmaltobionamide.

† To whom reprint requests should be addressed at present address: School of Pharmacy, University of California, San Francisco, CA 94143.

§ Permanent address: Department of Medical Microbiology, University of Linkoping, S-581 85 Linkoping, Sweden.

¶ Present address: Laboratories for Cell Biology, Department of Anatomy, University of North Carolina, Chapel Hill, NC 27514.

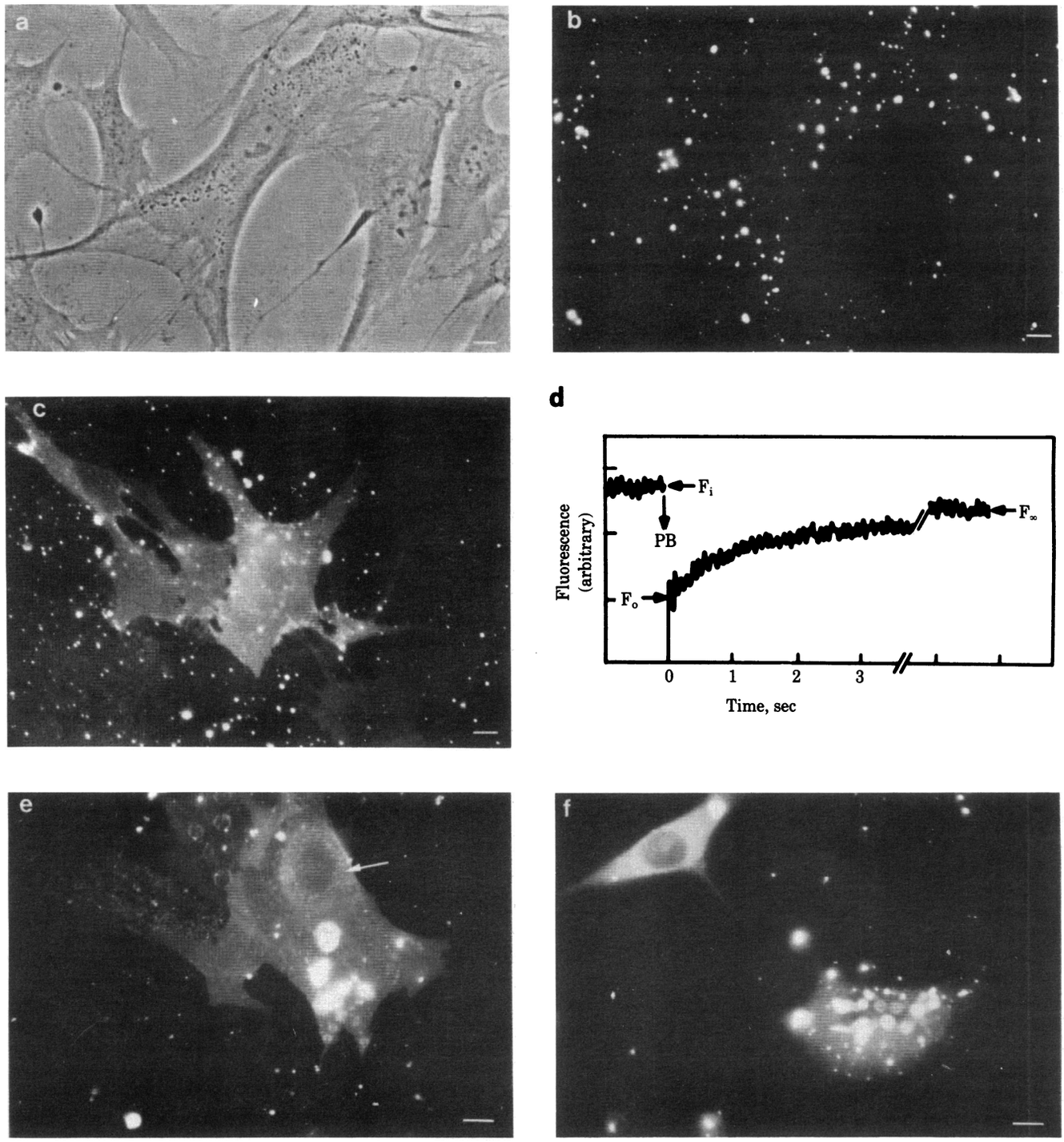

FIG. 1. (*a*) Phase-contrast image of BG-9 cells after adsorption of LC vesicles labeled with diI-C_{16}{3} at 0.25 mol %, as mediated by RCA-I (20 μg/ml) at 22°C, but before addition of polyethylene glycol. (*b*) Fluorescence image corresponding to *a*. (*c*) Fluorescence image of BG-9 cells after complete fusion protocol with diI-C_{16}{3}-labeled vesicles as in *a*. (*d*) Typical kinetics of fluorescence recovery after photobleaching at 22°C for BG-9 cell. Fusion protocol as in *c*; diI-C_{16}{3} is the fluorophore; $1/e^2$ spot size of the focused laser beam is about 3.4 μm (×25 objective used); and the apparent D is 8.8×10^{-9} cm^2/sec. (*e*) Fluorescence image of NIL-8M2 cells after fusion protocol with diI-C_{16}{3}-labeled LC-vesicles as in *a*. (*f*) Fluorescence image of L-929 cells after fusion protocol with LC-vesicles prepared with entrapped labeled albumin.

buffered saline (no Ca^{2+} or Mg^{2+}) (P_i/NaCl) for 2 hr before separation of encapsulated from nonencapsulated material.

diI-C_{16}{3} or diI-C_{18}{3}, when added, were at a final mol % of 0.25. Fluoresceinated albumin was added in P_i/NaCl (100 mg/ml); encapsulated albumin was separated from nonencapsulated by gel filtration on Sepharose 2B using P_i/NaCl or by flotation on Ficoll gradients (10).

Sonicated unilamellar vesicles of the same composition as the above, including 0.25 mol % diI-C_{16}{3} or diI-C_{18}{3}, were prepared as described (11) in P_i/NaCl at 10 μmol/ml.

Cell Culture. Cells were as described (11, 16). Subconfluent cultures were used, usually 24 hr after plating. Cell viability was routinely 99% as determined by trypan blue exclusion.

Fusion Procedure. The cells were washed 5 times with Dulbecco's phosphate-buffered saline containing Ca^{2+} and Mg^{2+} (P_i/NaCl/Mg^{2+}/Ca^{2+}). Then, 1 ml of the saline was placed on the cultures and RCA-I was added at 10 μg/ml. The cultures were incubated at 22°C (room temperature) for 5 min and then

Table 1. Effect of temperature on fusion efficiency of different cell lines

Temp, °C	Cells fused, %			
	BG-9*		NIL-8M2	
	diI-C_{18}{3}	Labeled albumin	diI-C_{16}{3}†	diI-C_{18}{3}*
37	50	49	86	ND
22	32	33	43	45
0–4	13	9	32	35

Unilamellar liposomes of composition LC/PtdGro/PtdCho/Chol (1:0.5:8.5:8) were fused by using RCA-I at 20 μg/ml and 45% (wt/wt) 4000-dalton polyethylene glycol. ND, not done.
* Large liposomes prepared by reverse phase evaporation method.
† Small liposomes prepared by sonication method and of identical composition to large.

200 nmol of the liposome preparation was added and incubation was continued for 30 min. The cells were washed 3 times with P_i/NaCl and 1 ml of a polyethylene glycol solution at 45% (wt/wt) was added for 1 min. Next, 2 ml of P_i/NaCl was added and mixed gently by swirling the dish, and incubation was continued for 10 min. Then the cells were washed 6 times with P_i/NaCl/Mg^{2+}/Ca^{2+}, put on ice, and examined by fluorescence microscopy. After the fusion protocol, short-term cell viability was determined on treated cells by placing tissue culture medium/fetal calf serum on the cells and determining trypan blue exclusion at 0 time and after 4 and 24 hr.

Fluorescence Quantitation of Cell-Associated Lipid. Fluorimetric quantitation of the fluorescent probe associated with the cells was as described (11). The quantitation was done on cells that were removed from the substrate by trypsinization and washed once with P_i/NaCl.

Fluorescence Microscopy and Studies of Fluorescence Recovery After Photobleaching. Fluorescence microscopy (11, 16) was used to evaluate the fusion efficiency, which is defined as the percentage of cells in the culture into which either fluorescent membrane or aqueous markers had been transferred. The criterion for fusion was that the lipid probes gave a uniform staining pattern that was distinct from the punctate pattern and any background fluorescence, and the transfer of water-soluble macromolecules to the cytoplasm had to be uniform and the probe excluded from the nucleus. Cells were photographed with Ektachrome 400 film; for weaker images, the film was push processed to an effective speed of 800 ASA.

RESULTS

Evidence for transfer

The phase contrast and fluorescence images of a BG-9 cell incubated with RCA-I and diI-C_{16}{3}-containing LC-vesicles are shown in Fig. 1 *a* and *b*, respectively. The fluorescence is punctate due to aggregation of fluorescent vesicles on the cell surface, and no diffuse fluorescence can be detected. After polyethylene glycol treatment and washing, however, the staining pattern is composed of both a uniform stain that we attribute to probe introduced from liposomes that have fused with the cell and some punctate areas due to liposomes that, although attached, have not fused. This occurs in the BG-9 cells (Fig. 1*c*), NIL-8M2 cells (Fig. 1*e*), and other cell types examined. Perinuclear intracellular staining can be observed (thick arrow, Fig. 1*e* after longer incubations. Following polyethylene glycol treatment, if a cell such as shown in Fig. 1*c*, is photobleached, recovery is rapid (Fig. 1*d*) with a diffusion constant (*D*) that reflects the mobility of the dyes in cell surface membranes (11, 17). In a typical experiment, *D* was $8.5 \pm 2.3 \times 10^{-9}$ cm^2/sec (mean ± SEM for five measurements). In general, fractional recovery ranged from 40% to 90%, depending on the combination of vesicle, lectin, and cell type, and provided an estimate of the amount of fused lipid relative to the total that had been absorbed. The lack of complete recovery is consistent with the images (Fig. 1 *c*, *e*, and *f*), which show a varying amount of punctate fluorescence coexisting with a more uniform staining pattern.

Transfer of water-soluble macromolecular markers entrapped in the aqueous space of the liposome to the target cell also occurs, as seen by the entry of fluoresceinisothiocyanate-labeled albumin into L-929 cells after the same fusion protocol (Fig. 1*f*). This photograph shows a cell (upper corner) that has the expected image for intracellular staining, including nuclear exclusion, and another cell (central) that has a combination of bright vesicle aggregates on a diffuse background of presumed cytoplasmic staining. Interestingly, this fluorescence also recovered substantially (unpublished results). The estimates of fusion efficiency were nearly the same when, in parallel experiments, membrane probes, diI-C_{16}{3} or diI-C_{18}{3}, or a vesicle aqueous-contents probe, labeled albumin, were used (see Tables 1, 2, and 3).

We found that, only in the presence of lectin, did vesicles appreciably bind to the cells and to the substrate and, only in the presence of both lectin and polyethylene glycol, did substantial fusion occur. Before fusion, the lactosyl (LC)-liposomes could be removed by competition with lactose and the maltobionamide liposomes could be removed by competition with α-methylmannoside but, after adding polyethylene glycol, the remaining punctate fluorescence could not be removed by incubation with the haptenic sugars. To substantiate the observation on binding, we found that, at an initial lipid concentration of 200 nmol/ml and an RCA-I concentration of 10 μg/ml, 2.5–3.0 nmol of lipid per 10^6 cells were associated with BG-9 cells in the presence of lectin, and 0.8–1.0 nmol per 10^6 cells were associated in the absence of lectin, as determined by measuring cell-associated fluorescence of diI-C_{16}{3} after the cells had been detached from the substrate by trypsinization.

Table 2. Effect of RCA-I concentration on fusion efficiency of different cell lines: Parallel experiments

RCA-I, μg/ml	Cells fused, %					
	BG-9		NIL-8M2		L-929	
	diI-C_{18}{3}	Labeled albumin	diI-C_{16}{3}	diI-C_{18}{3}	diI-C_{18}{3}	Labeled albumin
20	53	52	71	74	80–90	70
10	39	44	54	42	80	74
2	41	37	9	0	80	82
1	ND	33	ND	ND	50	41
0.2	ND	20	ND	ND	0	19
0	0	ND	0	0	ND	3

Liposomes of composition LC/PtdGro/PtdCho/Chol (1:0.5:8.5:8) were fused by using 45% (wt/wt) 4000-dalton polyethylene glycol at 22°C. Parallel single experiments were carried out with the vesicle membrane and the aqueous marker. ND, not done.

Table 3. Effect of RCA-I concentration on fusion efficiency of different cell lines: Aggregate data

	Cells fused, % ± SEM					
	BG-9		NIL-8M2		L-929	
RCA-I, μg/ml	diI-C_{18}(3) or diI-C_{16}(3)	Labeled albumin	diI-C_{18}(3) or diI-C_{16}(3)	Labeled albumin	diI-C_{18}(3) or diI-C_{16}(3)	Labeled albumin
20	50 ± 3 (12)	52 ± 2 (2)	53 ± 9 (3)	54 (1)	74 ± 6 (2)	70 (1)
10	51 ± 7 (5)	44 ± 5 (4)	48 ± 6 (2)	ND	69 ± 4 (4)	69 ± 2 (3)

Liposomes of composition LC/PtdGro/PtdCho/Chol (1:0.5:8.5:8) were fused by using 45% (wt/wt) 4000-dalton polyethylene glycol at 22°C. Data are mean (±SEM) of the number of experiments given in parentheses. ND, not done.

We also found that, after polyethylene glycol treatment and the subsequent washes, the lectin-treated cells retained 0.5–1.0 nmol of lipid per 10^6 cells and, in the absence of lectin, only 0.1–0.2 nmol of lipid per 10^6 cells remained cell associated; however, this measurement does not distinguish between associated label (which remains with the vesicle) and that which is actually transferred to the plasma membrane.

Factors affecting transfer

Incubation Temperature. The fusion procedure showed a pronounced temperature dependence (Table 1). At 4°C punctate binding was predominant, and at 37°C some internalization of vesicles (or probes or both) occurred. Optimization was performed at room temperature for convenience and to delay internalization of the lipid label.

Lectin Concentration. The degree of fusion depended on the lectin concentration at constant liposome concentration and composition (Tables 2 and 3). Fusion efficiency (percentage of cells fused) did not decrease much as the RCA-I concentration decreased from 20 to 10–2 μg/ml but then dropped off sharply.

Cells. Different cell lines showed somewhat different susceptibility to transfer of the markers (see Tables 2 and 3). Twenty-four hours after subculture, L-929 cells were more efficiently fused with vesicles at a given lectin concentration than were BG-9 or NIL-8M2 cells. Cells approaching confluence, in general, were not as sensitive to the fusion protocol but mild trypsinization (0.001% for 1 min at 22°C) increased their ability to fuse with liposomes.

Polyethylene Glycol Size and Concentration. Independent of the cell line, 1500- to 4000-dalton polyethylene glycol solutions (45% wt/wt) were the most effective (Table 4) and so were routinely used in the fusion experiments. Both 6000- and 20,000-dalton solutions became increasingly viscous and were therefore difficult to dilute and wash away from cultures. Concentrations ranging from 37.5% to 50% (wt/wt) were similar in inducing fusion with vesicles (data not shown); however, 50% polyethylene glycol detached a substantial number of cells from substrate, and 30% was only about one-third as efficient in producing fusion.

Other Lectin–Glycolipid Combinations. When using concanavalin A or maltobionamide vesicles, a combination of punctate and diffuse fluorescence was observed on individual cells. Mild treatment of the cells with trypsin (0.001%) before the fusion protocol decreased the punctate component and increased the percentage of cells fused (data not shown). This combination appears to be more complex, as evidenced by the need for trypsinization, and further optimization is needed.

Other Considerations. In several parallel experiments, fusion efficiency did not depend on whether the lipid had been prepared by reverse phase evaporation or sonication (see Table 1), nor was any pronounced effect of lipid composition observed. The presence or absence of Ca^{2+} or Mg^{2+} did not affect fusion efficiency. To avoid liposomal aggregation and loading the cells with divalent cations during the transfer process, P_i/NaCl was used in the preparation of liposomes. Polyethylene glycol was diluted within 1 min after addition to minimize cellular toxicity. In general, arranging the ratio of glycolipid to lectins to give visible aggregation of vesicles appeared to correlate with efficient fusion.

Cell Viability. The percentage of cells excluding trypan blue was studied in BG-9 cells immediately after the experiment and at 4 and 24 hr (Table 5). More than 90% of the cells excluded trypan blue 4 hr after fusion, and the cell morphology appeared normal. However, after 24 hr, only 40–50% of the cells excluded the dye. RCA-I showed only minimal toxicity on the cells after 24 hr (see Table 5). The lack of long-term viability may be due to the particular lectin–glycolipid combination used or to the large amount of lipid fused into the plasma membrane. To avoid this problem, other lectin–glycolipid combinations and lower concentrations of vesicles could be used to introduce molecules into the cell.

Table 4. Effect of molecular weight of polyethylene glycol on fusion efficiency of different cell lines: Parallel experiments

	Cells fused, %		
	BG-9	L-929	
$M_r \times 10^{-3}$	Labeled albumin	diI-C_{16}(3)	Labeled albumin
20	ND	ND	48
6	36	40	61
4	42	67	66
3.4	56	60	68
1.5	59	ND	74
0.6	17	25	33
0.4	10	8	0

Unilamellar liposomes of composition LC/PtdGro/PtdCho/Chol (1:0.5:8.5:8) prepared by the reverse phase evaporation method were fused by using RCA-I at 10 μg/ml and 45% (wt/wt) polyethylene glycol at 22°C. ND, not done.

Table 5. Viability of BG-9 cells after fusion protocol

	Viability, % ± SEM		
Cell treatment	0 hr	4 hr	24 hr
None	95	95 ± 5 (2)	100 ± 1 (2)
RCA-I (2 μg/ml) alone	89	92	83
Fusion protocol*	94 ± 2 (3)	91 ± 3 (3)	44 ± 3 (3)

Liposomes of composition LC/PtdGro/PtdCho/Chol (1:0.5:8.5:8) prepared by the reverse phase evaporation method and containing 0.25 mol % diI-C_{16}(3). Fusion was with 45% (wt/wt) 4000-dalton polyethylene glycol. Data are mean (±SEM) of the number of experiments given in parentheses.

* Fusion efficiency was similar to that given in Table 2 for RCA-I at 2 μg/ml.

DISCUSSION

The use of liposomes to bypass the permeability barrier of cells and organelles by a fusion mechanism has been proposed by a number of investigators (8, 18, 19). However, for many cell types, surface adsorption, endocytosis, and lipid exchange appear to be the predominant modes of liposome–cell interaction (for review, see ref. 8) and fusion appears to be a minor pathway (11). As a result, it has been difficult to use liposomes for introducing large quantities of molecules into cells. The protocol described here overcomes this limitation by promoting more efficient transfer of liposomal membrane as well as of aqueous space markers from the vesicle to the target cell. Our data suggest that the transfer event is a fusion process in which the vesicle membrane is introduced into the plasma membrane and the aqueous contents are emptied into the cytoplasm. The efficiency of this system depends on both the presence of the appropriate glycolipid, containing the liposome–lectin combination, and subsequent treatment of the cell–liposome complex with polyethylene glycol. In the absence or at suboptimal concentrations of any one of the components, the fusion efficiency was dramatically reduced.

A working model to describe the lectin-mediated polyethylene glycol-induced liposome cell fusion system is that first the lectin attaches the liposomes to the cell surface glycoproteins and glycolipids. It has been shown that appropriate lectins mediate the aggregation of various glycolipid- or glycoprotein-containing liposomes (20–23). Lectins have also previously been shown to enhance the association of glycoprotein-containing liposomes to erythrocytes (22) and of glycoproteins to eukaryotic cells (24, 25). It appears that polyethylene glycol treatment somehow creates exposed areas of plasma membrane lipids by segregation of membrane proteins and then perturbs the bilayer structure to allow closely opposed bilayers to fuse (26). Lectins enhance the fusion process either by promoting vesicle binding or by assisting in aggregation of membrane glycoproteins (or both), thereby exposing additional regions where liposomes can become closely apposed to the plasma membrane (27). Lectins could also destabilize the opposed bilayers and render them more susceptible to fusion (28).

Although we have used lectins and glycolipids, other chemical bridging techniques, such as antigen–antibody, avidin–biotin, and ligand–endogenous receptor, may also be capable of providing the necessary geometrical relationship between the liposome and the plasma membrane that leads to fusion in the presence of polyethylene glycol. It is also conceivable that centrifugal or electrical fields could be used to drive the vesicles onto the cell's surface, thus forming this critical relationship physically. In addition, other treatments [e.g., pH (29), other polyalcohols (10), or polymers] may be effective in other cell lines in promoting vesicle uptake or membrane fusion.

Regardless of the mechanism involved, this system brings about an efficient fusion of liposomes with eukaryotic cells. We have found that, with fluoresceinated albumin, 10^6–10^7 molecules can be introduced per cell (unpublished observation), which is in the range of that found with the erythrocyte microinjection technique (7). The principle advantage of this system over the erythrocyte-mediated microinjection method is that it can deliver larger macromolecules, such as mRNA (9) and DNA (10), in a well-defined lipid carrier (30). Each new cell system will, however, involve some degree of additional optimization regarding selection and concentration of the appropriate lectin and glycolipid so as to maximize fusion efficiency and cell viability. The system described here complements direct microinjection for introducing exogenous molecules into cells.

We gratefully acknowledge helpful discussions with Dr. R. Schlegel in the early phases of this study and Ms. Diane Millholland for expert technical assistance. We thank Dr. I. J. Goldstein for the initial supply of tetradecylmaltobionamide and Dr. A. S. Waggoner for the carbocyanine fluorescent probes. This investigation was supported by National Cancer Institute Grant CA16743 and the Charlton Fund, Tufts University. K.J. is an Established Investigator of the American Heart Association. K.-E.M. is the recipient of a U.S. Public Health Service International Fogarty Fellowship.

1. Socolar, S. J. & Lowenstein, W. R. (1979) in *Methods in Membrane Biology*, ed. Korn, E. D. (Plenum, New York), Vol. 10, pp. 123–179.
2. Stacey, D. W. & Allfrey, V. G. (1977) *J. Cell Biol.* **75**, 807–817.
3. Graessmann, M. & Graessmann, A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 366–370.
4. Furusawa, M., Nishimura, T., Yamaizumi, M. & Okada, Y. (1974) *Nature (London)* **249**, 449–450.
5. Loyter, A., Zakai, N. & Kulka, R. (1975) *J. Cell Biol.* **66**, 292–304.
6. Schlegel, R. A. & Rechsteiner, M. C. (1975) *Cell* **5**, 371–379.
7. Schlegel, R. A. & Rechsteiner, M. C. (1978) *Methods Cell Biol.* **20**, 341–354.
8. Kimelberg, H. K. & Mayhew, E. G. (1978) *Crit. Rev. Toxicol.* **6**, 25–78.
9. Wilson, F., Papahadjopoulos, D. & Taber, R. T. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3471–3475.
10. Fraley, R., Subramani, S., Berg, P. & Papahadjopoulos, D. (1980) *J. Biol. Chem.* **255**, 10431–10435.
11. Szoka, F. C., Jacobson, K., Derzko, D. & Papahadjopoulos, D. (1980) *Biochim. Biophys. Acta* **600**, 1–18.
12. Mercer, W. E., Terefinko, D. J. & Schlegel, R. A. (1979) *Cell Biol. Int. Rep.* **3**, 265–270.
13. Szoka, F. C., Jacobson, K. & Papahadjopoulos, D. (1979) *Biochim. Biophys. Acta* **551**, 295–303.
14. Williams, T. J., Plessas, N. R. & Goldstein, I. J. (1978) *Carbohydr. Res.* **67**, C1–C3.
15. Szoka, F., Jr. & Papahadjopoulos, D. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4194–4198.
16. Jacobson, K., Derzko, Z., Wu, E.-S., Hou, Y. & Poste, G. (1977) *J. Supramol. Struct.* **5**, 565–576.
17. Schlessinger, J., Axelrod, D., Koppel, D. E., Webb, W. W. & Elson, E. L. (1977) *Science* **195**, 307–309.
18. Papahadjopoulos, D., Poste, G. & Mayhew, E. (1974) *Biochim. Biophys. Acta* **363**, 404–418.
19. Pagano, R. E., Huang, L. & Wey, C. (1974) *Nature (London)* **252**, 166–167.
20. Surolia, A., Bachhawat, B. K. & Podder, S. K. (1975) *Nature (London)* **257**, 802–804.
21. Redwood, W. R., Jansons, V. K. & Patel, P. C. (1975) *Biochim. Biophys. Acta* **406**, 347–361.
22. Juliano, R. L. & Stamp, D. (1976) *Nature (London)* **261**, 235–237.
23. Curatolo, W., Yau, A. O., Small, D. M. & Sears, P. (1978) *Biochemistry* **17**, 5740–5744.
24. Gonzalez-Noriega, A. & Sly, W. S. (1978) *Biochem. Biophys. Res. Commun.* **85**, 174–182.
25. Juliano, R. L., Moore, M. R., Callahan, J. W. & Lowden, J. A. (1979) *Exp. Cell. Res.* **120**, 63–72.
26. Knutton, S. & Pasternak, C. A. (1979) *Trends Biochem. Sci.* **4**, 220–223.
27. Schlegel, R. A. & Mercer, W. E. (1980) in *Introduction of Macromolecules into Viable Mammalian Cells*, eds. Baserga, R., Croce, C. & Rovera, G. (Liss, New York), pp. 145–155.
28. van der Bosch, J. & McConnell, H. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4409–4413.
29. Schneider, H., Lemaster, J. J., Hochli, M. & Hackenbrock, C. R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 442–446.
30. Szoka, F., Jr. & Papahadjopoulos, D. (1980) *Annu. Rev. Biophys. Bioeng.* **9**, 467–508.