

# ADHESION OF PHOSPHOLIPID VESICLES TO CHINESE HAMSTER FIBROBLASTS

## Role of Cell Surface Proteins

RICHARD E. PAGANO and MASATOSHI TAKEICHI

From the Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210. Dr. Takeichi's present address is the Department of Biophysics, Kyoto University, Kyoto, Japan.

### ABSTRACT

The adhesion of artificially generated lipid membrane vesicles to Chinese hamster V79 fibroblasts in suspension was used as a model system for studying membrane interactions. Below their gel-liquid crystalline phase transition temperature, vesicles comprised of dipalmitoyl lecithin (DPL) or dimyristoyl lecithin (DML) adsorbed to the surfaces of EDTA-dissociated cells. These adherent vesicles could not be removed by repeated washings of the treated cells but could be released into the medium by treatment with trypsin. EM autoradiographic studies of cells treated with [ $^3\text{H}$ ]DML or [ $^3\text{H}$ ]DPL vesicles showed that most of the radioactive lipids were confined to the cell periphery. Scanning electron microscopy and fluorescence microscopy further confirmed the presence of adherent vesicles at the cell surface.

Adhesion of DML or DPL vesicles to EDTA-dissociated cells modified the lactoperoxidase-catalyzed iodination pattern of the cell surface proteins; the inhibition of labeling of two proteins with an  $\sim 60,000$ -dalton mol wt was particularly evident. Incubation of cells with  $^3\text{H}$ -lipid vesicles followed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis showed that some of the  $^3\text{H}$ -lipid migrated preferentially with these 60,000-mol wt proteins.

Studies of the temperature dependence of vesicle uptake and subsequent release by trypsin showed that DML or DPL vesicle adhesion to EDTA-dissociated cells increased with decreasing temperatures. In contrast, cells trypsinized before incubation with vesicles showed practically no temperature dependence of vesicle uptake.

These results suggest two pathways for adhesion of lipid vesicles to the cell surface—a temperature-sensitive one involving cell surface proteins, and a temperature-independent one. These findings are discussed in terms of current models for cell-cell interactions.

The biochemical and physical properties of cell surface membranes are of fundamental importance to our understanding of the complex phe-

nomena underlying cell-cell interactions. One approach toward elucidating these properties is to substitute a synthetic membrane surface of known

composition and structure for one of the natural membrane surfaces. Thus, in principle, it should be possible to isolate electrostatic, structural, and dynamic contributions to the interaction process by controlled and systematic variation of the chemical and physical properties of the synthetic membrane surface. This kind of approach to the study of membrane interactions has become possible in recent years by the development and characterization of model lipid dispersion systems whose molecular structure and properties closely approximate those of the lipid component of natural membranes. Detailed reviews on the formation and properties of the liquid crystal, liposome, or lipid vesicle system can be found elsewhere (3, 36).

Although the interaction of such vesicles with cells has been the subject of much recent intensive investigation, these studies have dealt in large part either with the fusion of phospholipid vesicles with cells, or with the modifications of cell behavior by lipid vesicle treatments (1, 4, 7, 9, 12, 17, 18, 20, 23-26, 29, 31, 33, 37, 38, 40). For example, previous studies from this laboratory utilizing Chinese hamster V79 cells (17, 25, 26) or mouse thymic lymphocytes (18) have demonstrated that vesicle-cell fusion, accompanied by a minor vesicle-cell lipid exchange component, is the major pathway of uptake for egg yolk lecithin (EYL) or dioleoyl lecithin (DOL) vesicles. It should be noted that the vesicles used in these studies had a "fluid" membrane character since their gel-liquid crystalline phase transition temperature ( $T_c$ ) is well below the temperatures ( $0^\circ$ - $40^\circ\text{C}$ ) over which the vesicle-cell interaction studies were carried out. The interaction of dimyristoyl lecithin (DML) vesicles with V79 cells was also studied and was found to exhibit an enhanced uptake at a temperature below its  $T_c$  (17), but its mechanism of uptake was not further explored.

In the present study, we show that vesicles of the appropriate molecular composition have a high affinity for the V79 cell surface but do not undergo vesicle-cell fusion. Thus, this system can be used to study membrane-membrane interactions in the manner described above. In particular, it is shown that unilamellar lipid vesicles ( $\sim 500$  Å diam) generated from DML ( $T_c \approx 20.9^\circ\text{C}$ , [22]) or DPL ( $T_c \approx 36.4^\circ\text{C}$ , [22]) are taken up at temperatures below their  $T_c$  by V79 cells in suspension primarily through the formation of a stable adsorption or adhesion of vesicles to the cell surface. It is further demonstrated that this vesicle-to-

cell adhesion depends on the presence of several cell surface proteins which have a high affinity for "rigid" vesicle lipids. A preliminary report of these findings appears elsewhere (28).

## MATERIALS AND METHODS

### *Cell Culture and Preparation of Single Cell Suspensions*

Chinese hamster V79 lung cell fibroblasts (35) were used in this study. Cells were grown on Falcon plastic tissue culture plates (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) in Eagle's minimum essential medium (MEM) supplemented with 15% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.), 0.2% lactalbumin hydrolysate (Grand Island Biological Co.) and 58.5 mg/liter L-glutamine.

Cell monolayers which had just attained a confluent state ( $\sim 2 \times 10^7$  cells per 140-mm diam culture dish) were used for preparing suspensions of single cells. Such cultures were usually obtained by inoculating  $1.2$ - $1.4 \times 10^4$  cells/cm<sup>2</sup> culture dish and incubating for 2 days at  $37^\circ\text{C}$  in an atmosphere of 5%  $\text{CO}_2$  in air. Overcrowded cultures failed to give a well-dispersed population of single cells and were avoided. EDTA-dissociated cells (E cells) were prepared as follows. Monolayer cultures were rinsed three times with Puck's  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free saline (CMF) (32), and incubated (10 ml/plate) with 1 mM EDTA in 10 mM Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid)-buffered  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free saline (HCMF; 8.0 g NaCl, 0.4 g KCl, 0.09 g  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 1 g dextrose, 2.38 g Hepes, and 4.8 ml 1 M NaOH in 1,000 ml  $\text{H}_2\text{O}$ , pH 7.4) for 15 min at  $37^\circ\text{C}$  on a gyratory shaker operating at 100 rpm. The detached cells were centrifuged (200 g, 4 min,  $4^\circ\text{C}$ ), washed twice in CMF, and finally suspended in HCMF. For trypsinized cells (T cells), monolayer cultures were dissociated with EDTA as described above and subsequently washed twice in CMF containing 1 mM  $\text{Ca}^{2+}$ . The washed cells were then suspended in 0.01% crystalline trypsin (type I, Sigma Chemical Co., St. Louis, Mo.) in HCMF containing 1 mM  $\text{Ca}^{2+}$  ( $10$  ml/ $2 \times 10^7$  cells) and incubated for 10 min at  $37^\circ\text{C}$ . Cells were then centrifuged and resuspended in CMF containing 0.1% soybean trypsin inhibitor (type I-S, Sigma Chemical Co.). The cells were washed two more times in CMF and finally suspended in HCMF. The viability of trypsinized cells, assessed by trypan blue dye exclusion, was not significantly different from that of E cells ( $>90\%$ ).

BALB/c 3T3 cells, used in EM autoradiographic control experiments (see below), were grown in Dulbecco's modified Eagle's medium with 10% calf serum in an atmosphere of 5%  $\text{CO}_2$  in air at  $37^\circ\text{C}$ . Cultures were incubated overnight with washed (39, 41) latex beads (2  $\mu\text{m}$  diam; Dow Chemical Co., Midland, Mich.), and subsequently washed free of unincorporated beads with Puck's saline before use.

### *Lipids, Lipid Purity, and Lipid Vesicles*

Dimyristoyl lecithin (DML), dipalmitoyl lecithin (DPL), di- $^{14}\text{C}$ myristoyl lecithin ( $^{14}\text{C}$ DML), and di- $^3\text{H}$ palmitoyl lecithin ( $^3\text{H}$ DPL) were synthesized as described elsewhere (17).  $^3\text{H}$ DML and  $^3\text{H}$ DPL of high specific activity ( $>5$  Ci/mmol) were prepared by catalytic hydrogenation of the appropriate precursors with tritium gas as described (17). To ensure complete hydrogenation of the samples, the radioactive products were further hydrogenated with  $\text{H}_2$  gas and fresh catalyst. Both the unlabeled and radiolabeled DML and DPL samples were purified in an identical manner by gradient elution of silicic acid columns using chloroform and methanol as the eluting solvents. The unlabeled samples exhibited a single spot on thin-layer chromatography (TLC;  $\geq 500$   $\mu\text{g}$  applied/spot). The radiolabeled samples were further analyzed for purity as described below. All lipids were stored under  $\text{N}_2$  at  $-15^\circ\text{C}$  and periodically examined for purity.

TLC analysis of the purified  $^3\text{H}$ DML,  $^{14}\text{C}$ DML, and  $^3\text{H}$ DPL samples was carried out using silica gel H TLC plates ( $5 \times 20$  cm;  $\geq 10^4$  dpm applied/plate) developed with chloroform:methanol:7 M ammonium hydroxide (230:90:15, by volume) or chloroform:methanol:acetic acid:water (25:15:4:2, by volume) as the developing solvent systems. The developed chromatograms were air dried and scraped into 5-mm wide zones along the entire length of the TLC plate using a manual TLC zonal scraping device (Analabs Inc., North Haven, Conn.). The silica gel from each zone was subsequently assayed for radioactivity by liquid scintillation counting. This analysis revealed that virtually all of the radioactivity ( $>99\%$ ) chromatographed with an  $R_f$  value of known lecithin standards, using either developing solvent system. No radioactivity was detected in the region of the chromatogram corresponding to the positions of free fatty acids or lysolecithin.

The methyl esters derived from the radioactive DML and DPL samples were subjected to gas-liquid chromatographic analysis using a Hewlett-Packard Co. (Palo Alto, Calif.) 5711A gas chromatograph equipped with an effluent splitter and a gas fraction collector (Packard Instrument Co., Inc., Downers Grove, Ill.). All the radioactivity ( $>99\%$ ) eluted as methyl- $^3\text{H}$ - or  $^{14}\text{C}$ myristate and methyl- $^3\text{H}$ palmitate, respectively.

Unilamellar lipid vesicles were prepared by sonication (15, 17) in HCMF and used immediately. All sonications were carried out in an argon atmosphere under the following conditions: DPL (30 min;  $45^\circ\text{C}$ ) and DML (30 min;  $40^\circ\text{C}$ ). To test for lipid degradation which might result during sonication (13), aliquots of freshly prepared  $^{14}\text{C}$ DML,  $^3\text{H}$ DML, or  $^3\text{H}$ DPL vesicles were lyophilized and dissolved in chloroform:methanol (1:1). When these samples were subjected to TLC analysis ( $\sim 10^4$  dpm applied/TLC plate) as described above, no radioactivity was found migrating as free fatty acid or lysolecithin. This finding obtained with radioactive DML and DPL is in agreement with other reports (16, 38) showing

no detectable degradation of lecithins following a similar sonication procedure.

### *Vesicle Uptake*

Freshly prepared cells were incubated in HCMF with radiolabeled vesicle suspensions for different time intervals. Incubation conditions were chosen such that the applied vesicles were present in great excess with respect to the number of cells ( $4 \times 10^6$  cells/ml; 1 mg vesicle lipid/ml;  $>10^7$  vesicles/cell). Incubations were stopped by washing the cells three times with chilled HCMF. Each washing was carried out in a new test tube to eliminate high background counts due to the nonspecific adsorption of vesicles to the glassware. The final cell pellet was air-dried, solubilized in 10% Triton X-100, and counted in a liquid scintillation counter. Duplicate or triplicate measurements were made and the variation was within  $\pm 5\%$ .

### *Electron Microscopy*

For scanning electron microscopy, cell suspensions in HCMF were plated onto clean glass cover slips (5 min,  $22^\circ\text{C}$ ) and fixed (30 min,  $22^\circ\text{C}$ ) with 3% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.1). The cultures were rinsed twice with HCMF and then fixed (15 min,  $22^\circ\text{C}$ ) in 1%  $\text{OsO}_4$  dissolved in 0.3 M cacodylate buffer. After a rinse in distilled water, the preparations were dehydrated with increasing concentrations of ethanol. The ethanol was then replaced with acetone. The latter was replaced by liquid  $\text{CO}_2$  and the samples were dried in a Sorvall Critical Point Drying System (DuPont Instruments, Sorvall Operations, Newtown, Conn.). Specimens were coated with gold and examined in a Cambridge stereo scan microscope operating at 30 kV.

EM autoradiographic studies employing  $^3\text{H}$ DML ( $\sim 40$  mCi/mmol) or  $^3\text{H}$ DPL ( $\sim 3$  mCi/mmol) vesicles were carried out as previously described (8, 17, 27). In control experiments, cells incubated (1 h;  $37^\circ\text{C}$ ) with  $^3\text{H}$ DPL were washed three times in HCMF, and plated onto cultures of bead-labeled 3T3 cells. After a brief co-incubation at  $37^\circ\text{C}$ , the cultures were fixed in glutaraldehyde, postfixated in  $\text{OsO}_4$ , and processed for EM autoradiography as above. Electron micrographs were obtained with a JEOL 100S microscope operating at 80 kV.

### *Fluorescence Microscopy*

DPL vesicles containing trapped 6-carboxyfluorescein (6-CF; Eastman Organic Chemicals, Div., Eastman Kodak Co., Rochester, N. Y.) were prepared according to the method of Weinstein et al. (37, 38) with modifications. Briefly, DPL was suspended in HCMF containing 20 mM 6-CF and sonicated as described above. The 6-CF-containing vesicles were then separated from untrapped dye by chromatography on Sephadex G-25 ( $2.5 \times 30$  cm), using HCMF as the eluting buffer. Cells were incubated (1 mg vesicle lipid/ml;  $4 \times 10^6$  cells/ml) with 6-CF-containing vesicles, washed three times in HCMF,

and examined in a Zeiss fluorescence microscope utilizing epi-illumination. Exciting light from a 200-W mercury lamp was first passed through a heat filter and then through an appropriate combination of interference and barrier filters to give maximum brightness for fluorescein.

### Iodination of Cells

Cell surface labeling was performed as previously described (19, 30) with minor modifications. After incubation (1 h, 2°C) with vesicles, the cells were washed three times in HCMF to ensure complete removal of excess lipid and suspended in chilled HCMF with 10 mM glucose at a concentration of  $4 \times 10^6$  cells/ml. Na<sup>125</sup>I (5 mCi/0.1 ml, carrier free, New England Nuclear, Boston, Mass.) was added at a final concentration of 200 or 400  $\mu$ Ci/ml, followed by lactoperoxidase (B grade, Calbiochem, San Diego, Calif.) and glucose oxidase (type V, Sigma Chemical Co.) at final concentrations of 20  $\mu$ g/ml and 0.2 U/ml, respectively. The reaction mixture was incubated at 5°C for 7 min with occasional swirling, and the cells were sedimented and washed four to five times with HCMF. Cell viability was not affected by iodination.

### Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was carried out following the method of Laemmli (21), with modifications. The separation gel solution consisted of 7.5% (or 15%) acrylamide, 0.37 M Tris-HCl (pH 8.8), 0.2% *N,N'*-methylene-bis-acrylamide and 0.1% SDS. The gel was polymerized in a slab 1 mm thick  $\times$  17 cm in length by the addition of 0.05% (vol/vol) TEMED (*N,N,N',N'*-tetramethylethylenediamine) and 0.05% (wt/vol) ammonium persulfate. The stacking gel contained 3% (or 5%) acrylamide, 0.125 M Tris-HCl (pH 6.8), 0.36% *N,N'*-methylene-bis-acrylamide, 0.1% SDS and was polymerized under fluorescent light with 0.1% TEMED and 0.16% riboflavin. The electrode buffer (pH 8.5) contained 6 g Tris, 28.8 g glycine, and 1 g SDS in 1,000 ml. Cells were dissolved in 0.125 M Tris-HCl (pH 6.8), 1% SDS, 10% glycerol, 2 mM phenylmethylsulfonylfluoride, and 0.001% bromphenol blue. Molecular weight markers were obtained from the following sources:  $\beta$ -galactosidase, catalase, ovalbumin and cytochrome-c (Sigma Chemical Co.); BSA (Armour Pharmaceutical Co., Chicago, Ill.) and Con A (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.). After adding 5-10% 2-mercaptoethanol, the samples were placed in boiling water for 2 min. Electrophoresis was carried out at 11 mA until the bromphenol blue marker reached a distance of 13 cm from the top of the separation gel. <sup>125</sup>I-labeled gels were stained with 0.025% Coomassie Blue in 25% 2-propanol and 10% acetic acid and destained with 5% ethanol and 10% acetic acid. For <sup>3</sup>H-lipid-containing

gels, staining was carried out overnight in 0.1% Coomassie Blue in 15% TCA. Destaining was done with 10% acetic acid followed by replacement with a dimethyl sulfoxide solution containing the scintillator 2,5-diphenylloxazole (PPO) (6). Gels were dried under vacuum and autoradiographed with X-ray film (type NS, Eastman Kodak Co.).

Extraction of <sup>3</sup>H-lipids from polyacrylamide gel slices was performed by the method of Bligh and Dyer (5). The extracted lipids were separated by TLC, and the radioactivity in individual spots was determined as described above.

## RESULTS

### EDTA-Dissociated Cells

Typical uptake data for EDTA-dissociated cells incubated with DPL vesicles are shown in Fig. 1. At 37°C, constant levels of uptake are reached after 5-10 min, while at 2°C slightly longer incubations are required for apparent saturation. At all times, the absolute amounts of DPL uptake at 2°C are two to two and a half times greater than that at 37°C. Kinetics similar to those shown in Fig. 1 are observed with DML, but its uptake is always less than when DPL is used (Table I). Preincubation of cells (30 min, 37°C) with 5 mM NaN<sub>3</sub> and 50 mM deoxyglucose has no effect on DPL uptake but reduces DML uptake at 37°C to about 80% of control values (Table I). The presence of 1 mM Ca<sup>2+</sup> and 1 mM Mg<sup>2+</sup> during the vesicle-cell incubation results in a slight (~5%) enhancement of vesicle uptake.

The exogenous phospholipid which becomes cell associated upon incubation with DML or DPL

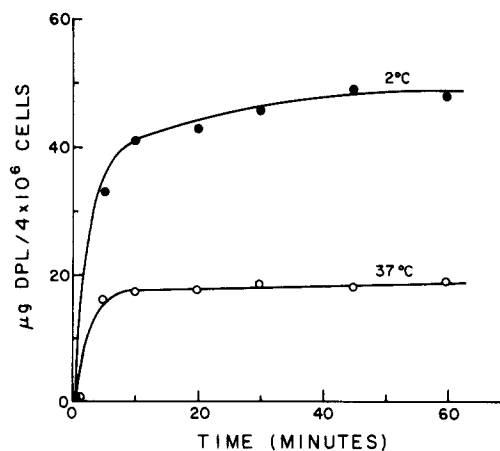


FIGURE 1 Uptake of [<sup>3</sup>H]DPL vesicles by EDTA-dissociated cells at 2° and 37°C.

TABLE I  
Comparison of DPL and DML Uptake by EDTA-dissociated and Trypsinized V-79 Fibroblasts in Suspension

Cell type	Temperature of vesicle incubation (°C)	Lecithin uptake*, µg/4 × 10 <sup>6</sup> cells/h			
		Control		With metabolic inhibitors‡	
		DML	DPL	DML	DPL
EDTA-dissociated	2	18.0	50.0	96	110
	37	6.5	20.1	80	110
Trypsinized	2	7.5	19.3	105	109
	37	7.0	12.1	100	99

\* All experiments were carried out in duplicate or triplicate. Variation was within ±5%.

‡ Cells were preincubated with 5 mM NaN<sub>3</sub> and 50 mM 2-deoxyglucose for 30 min at 37°C before incubation with vesicles. The metabolic inhibitors were also present during vesicle-cell incubations.

vesicles can be released by treatment with trypsin. Cells were incubated for 1 h with DPL or DML vesicles and subsequently washed (three times) free of excess vesicles. Aliquots of the cells were then subjected to a 10-min incubation with an 0.01% solution of crystalline trypsin (1 ml/4 × 10<sup>6</sup> cells) at 22°C. The reaction was stopped by addition of a 10-fold excess of trypsin inhibitor, and centrifugation of the cells. Analysis of the cell pellet and supernate revealed that varying amounts (~0–60%) of the cell-associated DPL or DML can be released into the medium by trypsin, depending on the temperature at which the initial vesicle-cell incubation is carried out (Table II). Prolonged incubation with trypsin (up to 30 min) does not further reduce the amounts of cell-associated DML or DPL. All of the radioactivity released by trypsin is chloroform-methanol extractable and chromatographs as lecithin. Spontaneous release of [<sup>3</sup>H]DPL or DML from cells in the absence of trypsin is negligible. The release of DPL or DML cannot be induced with heat-inactivated trypsin or with trypsin inhibitor alone.

Incubation of cells with DML or DPL vesicles has a significant effect on the susceptibility of certain cell surface proteins to lactoperoxidase-catalyzed iodination. Fig. 2 A–C presents autoradiographs of the SDS-polyacrylamide (7.5%) gel electrophoresis patterns obtained for the proteins from <sup>125</sup>I-labeled control cells (Fig. 2 A), and for cells preincubated for 1 h at 2°C with DPL (Fig. 2 B) or DML (Fig. 2 C) vesicles. Although many of the radiolabeled bands are unaffected by vesicle

treatments, the extent of <sup>125</sup>I-labeling in several regions (I–V; Fig. 2) of the gel was obviously different for control vs. DPL or DML vesicle treatments. A prominent difference is in region III of the gels in which it is seen that two bands, corresponding to an ~6,000 dalton mol wt, are heavily labeled in control cells, and apparently unlabeled in the vesicle-treated cases. In contrast, the labeling in regions I, II, IV, and V of the gels appears to be enhanced for DPL and DML treatments when compared to the control. Coomassie Blue-staining patterns of the gels corresponding to Fig. 2 A–C were virtually identical.

EDTA-dissociated cells were also incubated with [<sup>3</sup>H]DML vesicles for 1 h at 2°C, and the cellular proteins from washed cells were solubilized with SDS and subjected to polyacrylamide gel electrophoresis. Fig. 2 E, G shows the fluorographic patterns obtained in this experiment with 7.5 and 15% polyacrylamide gels. While most of the <sup>3</sup>H-radioactivity migrates at the front of the gel, presumably as SDS-DML micelles, a number of radioactive bands also appear throughout the gel. In particular, it is noteworthy that two specific bands (Fig. 2 E, G, arrows) are heavily labeled with tritium, and that the position of these bands corresponds to the position of the two protein bands whose <sup>125</sup>I-labeling was inhibited by vesicle treatments (Fig. 2 B, C). All of the <sup>3</sup>H-cpm in these bands are chloroform-methanol extractable. More than 75% of the radioactivity chromatographs as lecithin, the rest as lysolecithin and free fatty acid.

TABLE II  
Release of Cell-Associated DPL and DML Vesicles from Cells by Trypsinization\*

Vesicle type	Temperature of vesicle incubation (°C)	Vesicle radioactivity released from cells %
DML	2	40–50
	15	29–42
	32	5–12
	37	1–2
DPL	2	55–62
	20	49–57
	37	3–9

\* E cells were incubated with unilamellar lipid vesicles for 1 h at indicated temperature, washed, and treated with 0.01% trypsin for 10 min at 22°C. Measurements represent the range of values found in four experiments.

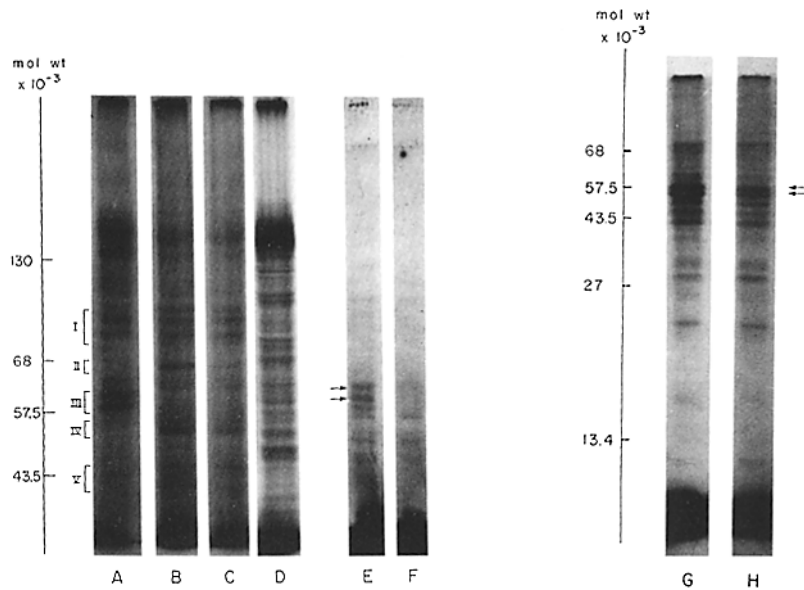


FIGURE 2 SDS-polyacrylamide gel electrophoresis patterns obtained from control and vesicle-treated cells. Autoradiographs are for the  $^{125}\text{I}$ -labeled proteins from (A) EDTA-dissociated cells (E cells), (B) DPL-treated E cells, (C) DML-treated E cells, and (D) trypsin-treated cells (T cells). Fluorograms are for (E, G) E cells and (F, H) T cells treated with  $[^3\text{H}]\text{DML}$  vesicles. All vesicle treatments were carried out for 1 h at  $2^\circ\text{C}$ . (A-F) 7.5% gels; (G, H) 15% gels. The positions of the molecular weight markers are shown:  $\beta$ -galactosidase (130,000); BSA (68,000); catalase (57,500); ovalbumin (43,500); Con A (27,000); and cytochrome *c* (13,400).

EM autoradiographs of cells treated with  $[^3\text{H}]\text{DML}$  or  $[^3\text{H}]\text{DPL}$  vesicles at  $2^\circ\text{C}$  show heavy labeling of the cell surface as seen in Fig. 3*a* for a DML treatment. Incubation of cells with either vesicle type at  $37^\circ\text{C}$  for 1 h results in a grain distribution similar to that previously reported by this laboratory (17) using radioactive dioleoyl lecithin vesicles — namely, most of the label appears to be in the cytoplasm of the treated cells with little in the nucleus and relatively small amounts associated with the surface membrane (Fig. 3*b*). Analysis of the lipids extracted from treated cells immediately after incubation with vesicles shows that more than 90% of the radioactivity chromatographs as lecithin, thus indicating that the grains seen in the autoradiographs are from the exogenously introduced lecithin, and not some degradation product.

To rule out the possibility that the radiolabeled phospholipids might be redistributed by the organic solvents used in EM specimen preparation, the following additional control was carried out. Cells labeled in suspension with  $[^3\text{H}]\text{DPL}$  (1 h;  $37^\circ\text{C}$ ) were washed three times and plated onto cultures of latex bead-labeled 3T3 cells. Latex

bead labeling was used to permit positive identification of the unlabeled cell type (3T3) at the EM level. After a brief co-incubation of the cells at  $37^\circ\text{C}$ , to permit attachment of the radiolabeled cells to the culture dish, the mixed cultures were fixed and processed for EM autoradiography as usual. EM autoradiographs of this control show no transfer of label from  $[^3\text{H}]\text{DPL}$ -labeled cells to the nonradioactive, bead-labeled 3T3 cells. Typical results both for a pair of cells separated by several microns and for a pair of cells in close apposition are seen in Fig. 3*c*.

Fig. 4 shows a typical scanning electron micrograph of an EDTA-dissociated cell in the absence of vesicle treatment. Numerous projections and attachments to the glass substrate are visible (arrows; Fig. 4*a*) 5 min after plating of the cells. Microvilli also appear to be extended away from the cell body. At high magnification the regions of the cell surface between projections appear smooth (Fig. 4*b*). In contrast, cells treated with DPL vesicles for 1 h at  $2^\circ\text{C}$  differ from untreated cells in several respects. Few microvilli extend outward from the cell surface into the bathing medium (Fig. 4*c*), but rather they appear to be

held lengthwise on the cell body. Furthermore, no cellular projections extending onto the glass coverslip are seen. At higher magnification (Fig. 4*d*) the cell surface has a rough or bumpy appearance compared to controls (Fig. 4*b*).

#### *Trypsin-Treated Cells*

The interactions of DML and DPL vesicles with trypsinized cells were also studied. Depending on the temperature of vesicle-cell incubation, these cells incorporate  $\sim 1.5$ – $2.5 \times$  less DML or DPL than nontrypsinized cells (Table I). In agreement with this finding, EM autoradiography shows much heavier surface accumulation of [ $^3\text{H}$ ]DML in EDTA-dissociated than in trypsin-treated cells (not shown). Similar results were obtained using [ $^3\text{H}$ ]DPL vesicles.

The iodination pattern in trypsin-treated cells (Fig. 2*D*) is not significantly affected by vesicle treatments. Furthermore, the two protein bands (Fig. 2*E*, *G*) that are heavily labeled with [ $^3\text{H}$ ]DML in the case of EDTA-dissociated cells are only lightly labeled (Fig. 2*F*, *H*) in the case of trypsin-treated cells.

Scanning electron micrographs of trypsin-treated cells (Fig. 5) were qualitatively similar to those of EDTA-dissociated cells (Fig. 4). Incubation with DPL vesicles results in both a reduction in the number of microvilli projecting away from the cell (Fig. 5*c*) and a rough appearance of the cell surface (Fig. 5*d*). The degree of roughness, however, is obviously less than for DPL treatments of EDTA-dissociated cells (Fig. 4*d*). Scanning electron micrographs of DML-treated cells are not significantly different from those of the DPL treatments shown in Fig. 4 and 5.

#### *Fluorescence Microscopy*

Cells treated at low temperatures with DPL vesicles containing 6-CF show a bright ring of fluorescence (Fig. 6*a*, *c*) surrounding most of the cells in the field of observation. The intensity of this fluorescence is greater with EDTA-dissociated (Fig. 6*a*) than trypsin-treated cells (arrows, Fig. 6*c*). When cells are incubated with fluorescent vesicles at 37°C, an even distribution of dye is seen throughout the cell, with only slight variations in intensity from cell to cell (Fig. 6*b*, *d*). No significant differences in fluorescence intensity are seen between EDTA-dissociated and trypsin-treated cells at 37°C. Very little fluorescence is seen in control populations of cells treated with DPL vesicles containing no 6-CF (Fig. 6*e*), or

with cells incubated with a solution of vesicles and untrapped dye.

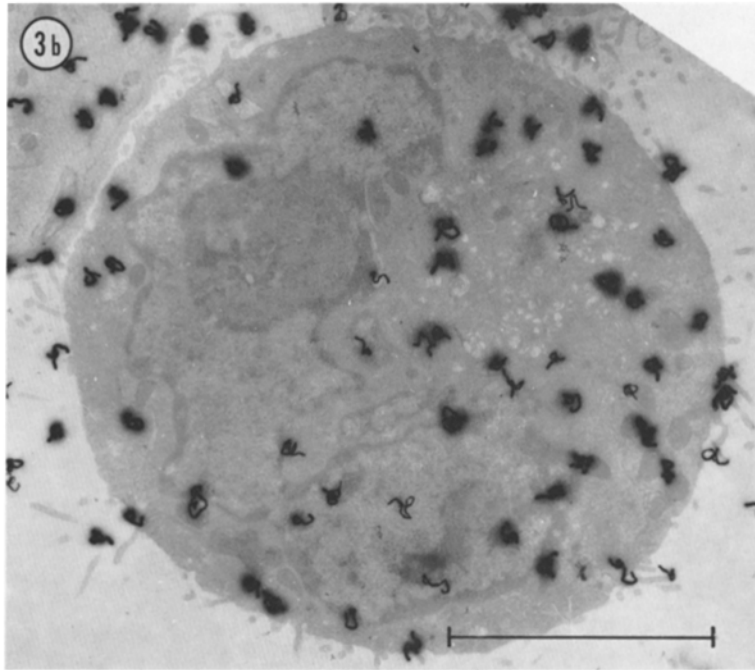
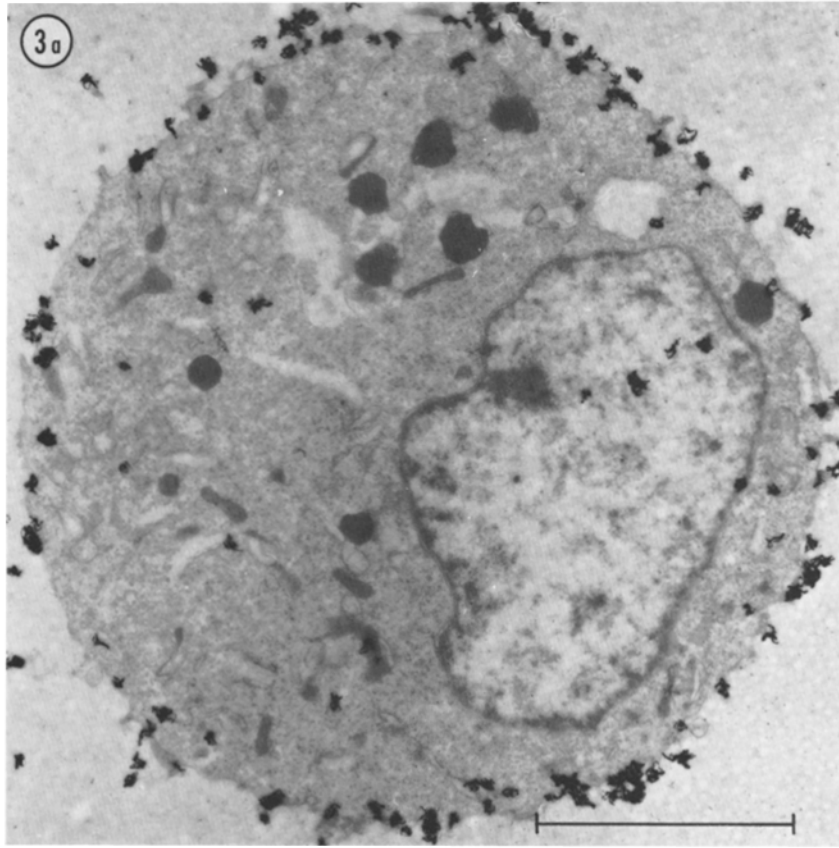
#### *Temperature Dependence of Vesicle Uptake*

The temperature dependence of DML and DPL vesicle uptake by EDTA-dissociated and trypsin-treated cells is shown in Fig. 7. Cells were incubated for 1 h at the indicated temperature, washed, and assayed for the uptake of exogenous lecithin. For EDTA-dissociated cells, DML uptake is nearly constant between 20° and 40°C, but increases markedly below about 19°–20°C. For DPL, vesicle uptake increases with decreasing temperatures over the entire range of temperatures examined. The rate of increase is greater, however, between 40° and 30°C than between 30° and 0°C. Treatment of cells with trypsin (10 min, 37°C) before incubation with vesicles markedly alters the temperature dependence of vesicle uptake. In the case of DML, the uptake between 0° and 39°C becomes nearly temperature independent. For DPL, trypsinization markedly reduces vesicle uptake at low temperatures, resulting in an uptake profile with little temperature dependence. Prolonged trypsinization of cells (up to 30 min) does not further modify vesicle uptake.

#### DISCUSSION

Although this communication deals primarily with the adhesion pathway for vesicle uptake found at temperatures below the  $T_c$  of DML or DPL, it should be noted that above this temperature a different mechanism of uptake is indicated. Thus, significant amounts of cell-associated DML or DPL can be released by trypsin when vesicle-cell incubations are carried out below the vesicle  $T_c$ , but only negligible amounts are released following incubations above this temperature (Table II). The notion of different mechanisms of vesicle uptake above and below the vesicle  $T_c$  is also supported by our EM autoradiographic studies (Fig. 3) showing markedly different distributions of silver grains over cells treated with [ $^3\text{H}$ ]DML at 2° vs. 37°C. On the basis of our previous studies with V79 cells using “fluid” EYL or DOL vesicles (17, 25, 26), it seems likely that the mechanism of DML and DPL uptake above their  $T_c$  involves vesicle fusion with the cell surface. The finding of a uniform fluorescence in cells treated at 37°C with DPL vesicles containing 6-CF (Fig. 6*b*, *d*) is also consistent with a fusion mechanism (37, 38) above the vesicle  $T_c$ .

The uptake of DML or DPL vesicles described





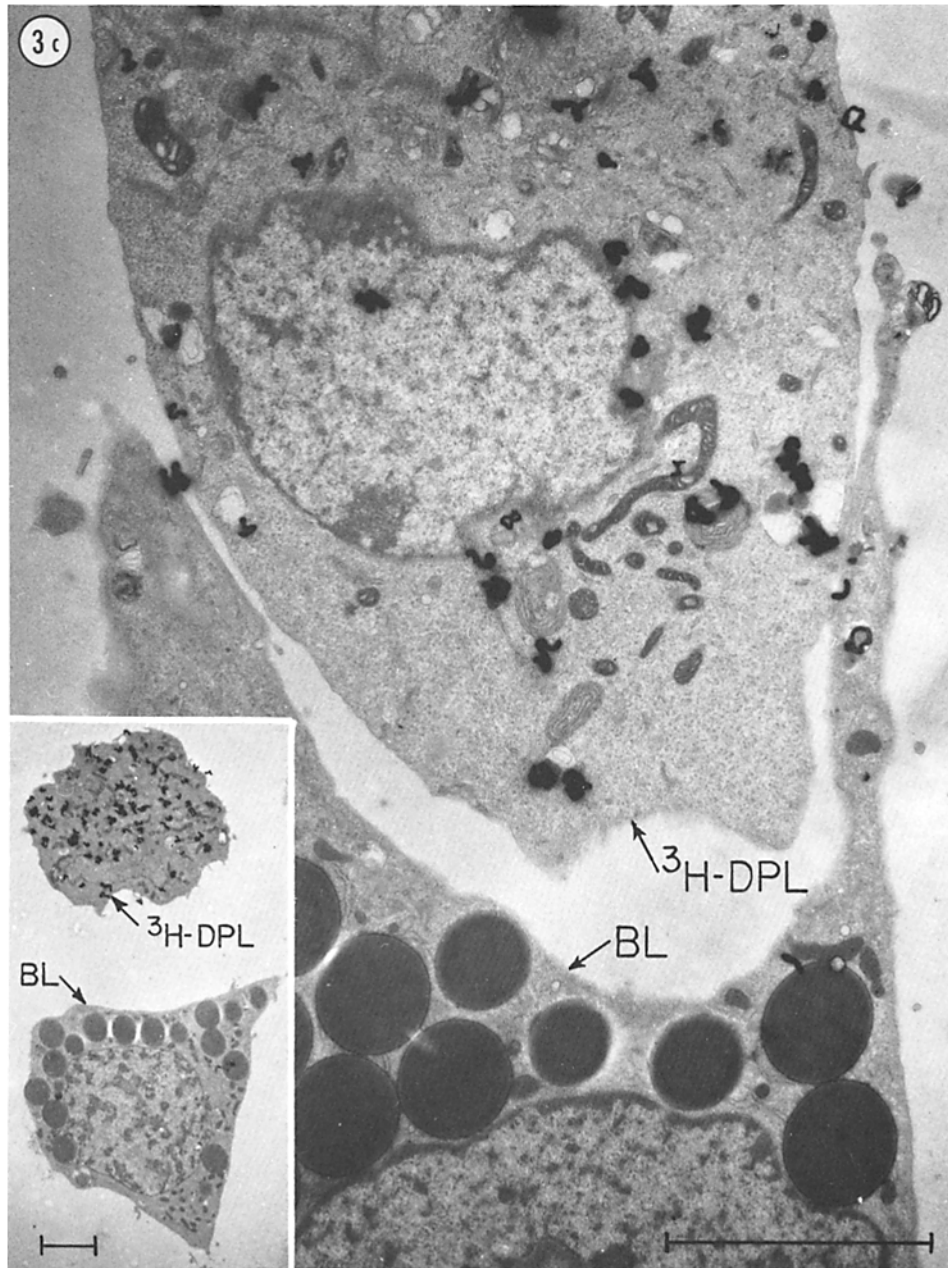


FIGURE 3 Electron microscope autoradiographs of EDTA-dissociated cells incubated with [ $^3\text{H}$ ]DML vesicles for 1 h at (a) 2°C and (b) 37°C. To rule out redistribution of radiolabeled lipids during processing of samples for electron microscopy, fibroblasts labeled with [ $^3\text{H}$ ]DPL (1 h at 37°C) were washed and plated onto bead-labeled (BL) cultures of BALB/c 3T3 cells and processed for EM autoradiography (c). No transfer of  $^3\text{H}$ -lipid was observed. Bars, 5  $\mu\text{m}$ .

in this communication is not the result of a significant contribution of an endocytotic pathway since saturation levels of uptake are reached after only 5–10 min of incubation at 37°C (Fig. 1). If vesicle

uptake involves an active process, then incubation for times >5 min should increase the uptake. Additionally, involvement of an active cellular process requires a significant increase in uptake

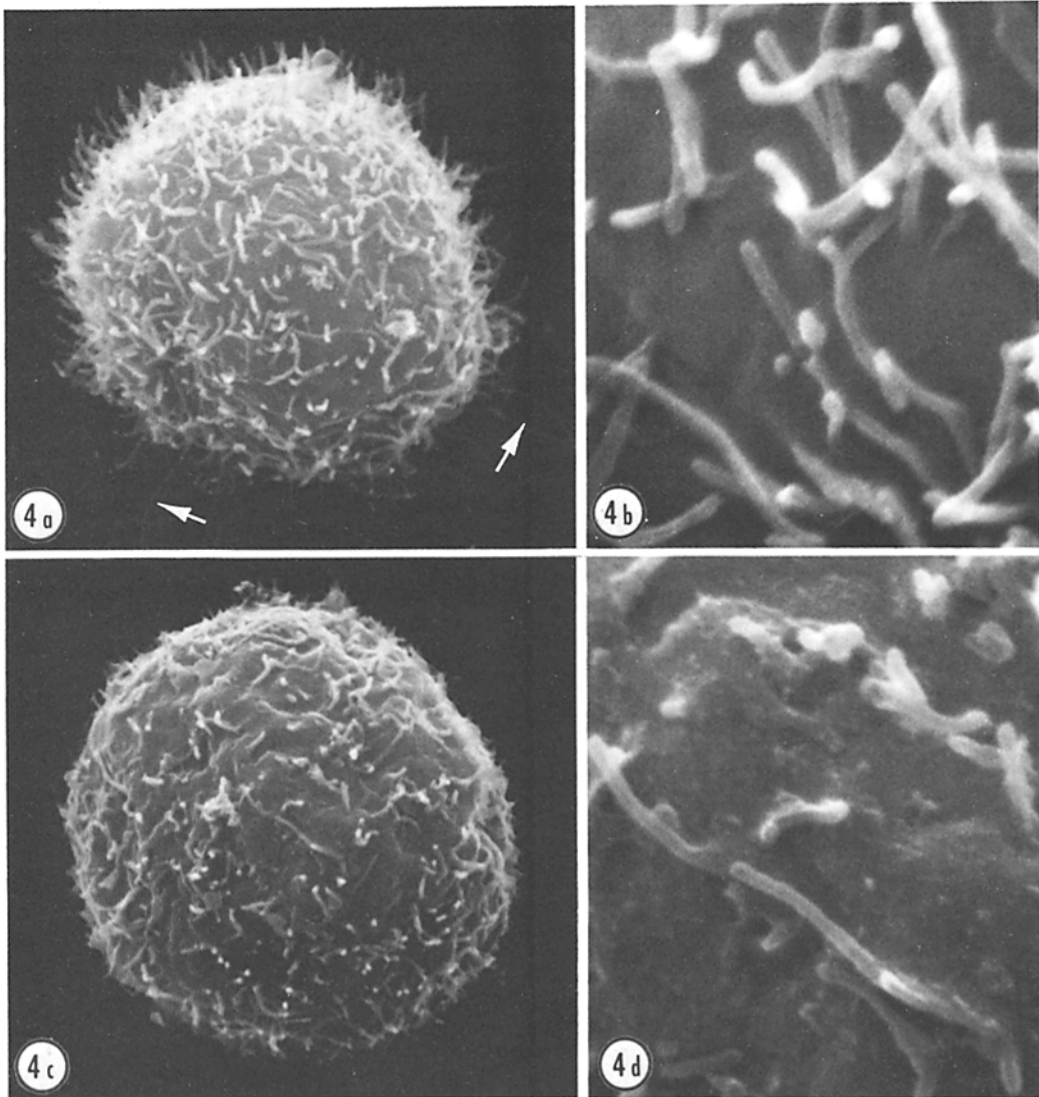


FIGURE 4 Scanning electron micrographs of EDTA-dissociated cells. (*a* and *b*) untreated controls; (*c* and *d*) DPL-treated (1 h at 2°C). Arrows in Fig. 4*a* indicate typical sites of attachment to glass substratum. Low magnification =  $\times 7,000$ ; high magnification =  $\times 28,000$ .

with increasing temperature. However, in every case, DML or DPL uptake was either greater at lower temperatures or showed no significant temperature effect (Fig. 7). Finally, pretreatment of cells with combined inhibitors of respiration and glycolysis (Table I) has virtually no effect on DPL uptake and results in only a small inhibition ( $\sim 20\%$ ) of DML uptake at 37°C.

Our finding of a relative insensitivity in DML and DPL uptake in the presence of combined metabolic inhibitors (Table I) appears to conflict

with the recent results of Poste and Papahadjopoulos (31), who showed that such treatments almost completely inhibit endocytosis of lecithin vesicles in another cell type (BALB/c 3T3). In addition, they reported an enhanced vesicle uptake with increasing temperatures of vesicle-cell incubation, while we have observed just the opposite (Fig. 7). However, a direct comparison of their results with those presented here is not possible because of differences in a number of variables, e.g. cell type, conditions of vesicle-cell incu-

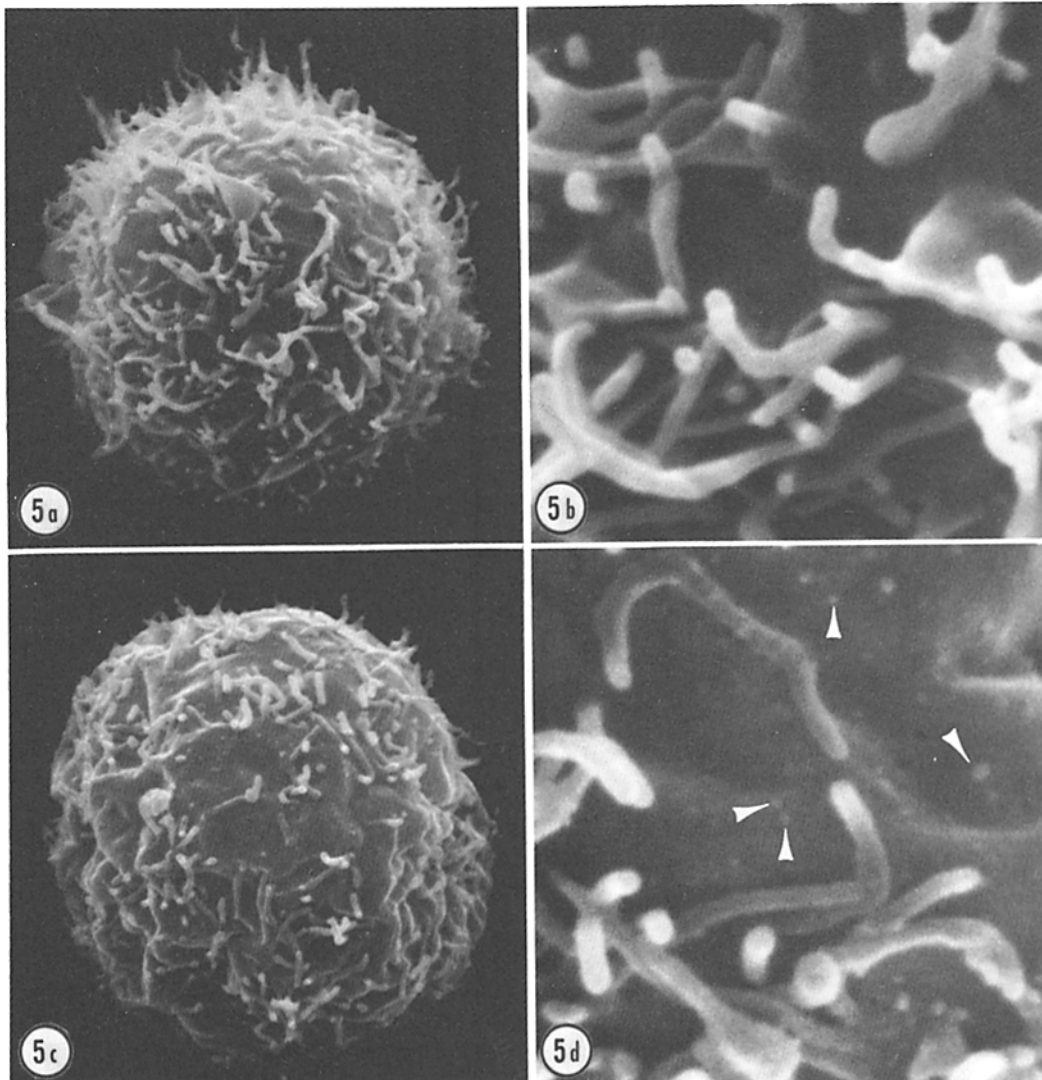


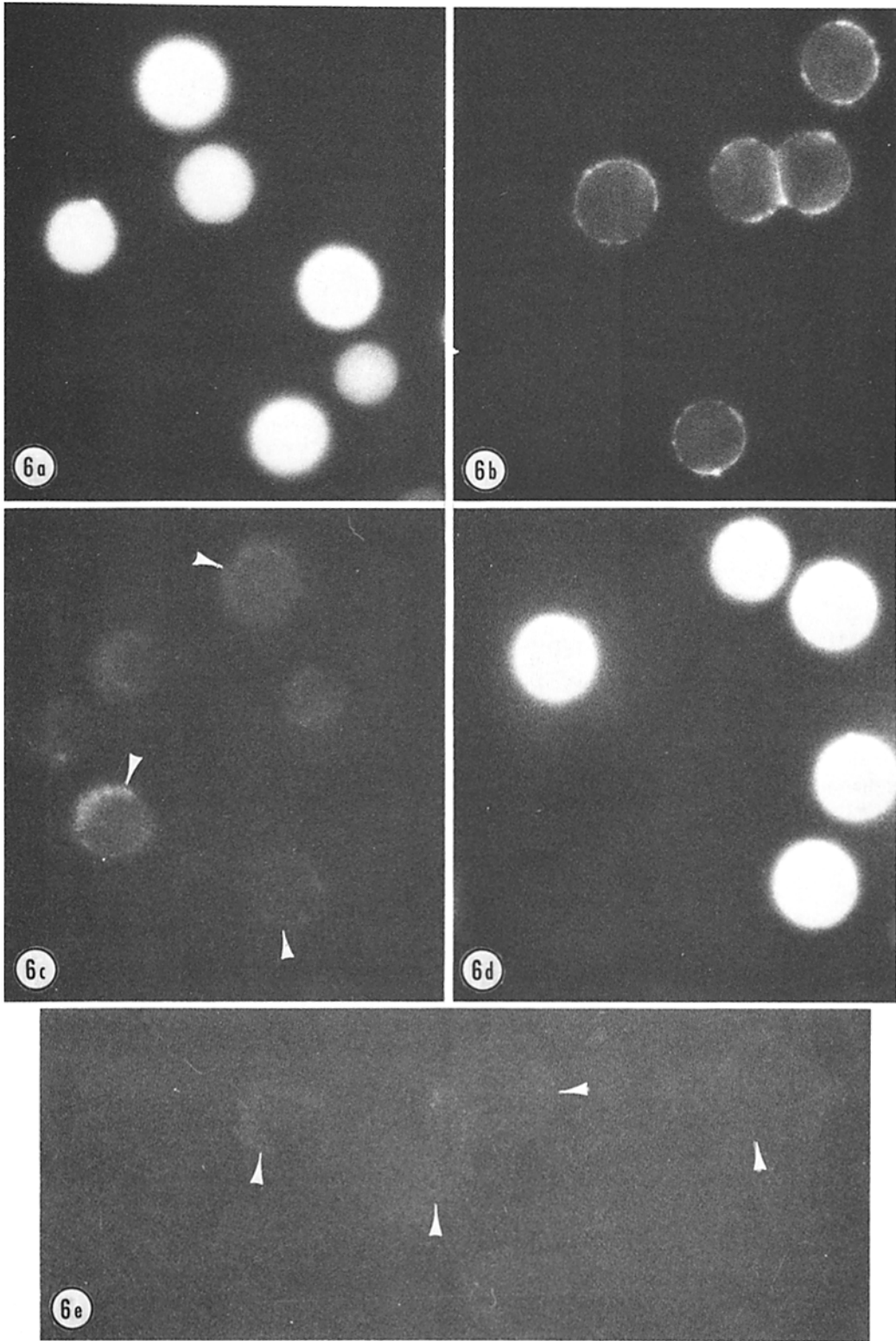
FIGURE 5 Scanning electron micrographs of trypsin-treated cells. (*a* and *b*) untreated controls; (*c* and *d*) DPL-vesicle treated (1 h at 2°C). Several spherical structures (~300–1,000 Å diam) resulting from vesicle treatment are shown at arrows in Fig. 5*d*. Low magnification = × 7,000; high magnification = × 28,000.

bation, and composition of the lipid vesicles that were used. In particular, we wish to emphasize the last parameter, since, in the present study, vesicles were formed only with lecithins containing homogeneous acyl chains.

*Evidence for Vesicle-Cell Adsorption ( $T < T_c$ )*

The findings reported here support a vesicle-to-cell adsorption process at low temperature in both

EDTA-dissociated and trypsin-treated cells. Scanning electron micrographs of V79 cells (Fig. 4*d*, 5*d*) reveal a characteristic bumpy surface after vesicle treatment. The size of such “bumps” ranges from ~300 to 1,000 Å, and is consistent with the adsorption of DPL or DML vesicles to the cell surface. DPL treatments of cells seem to hold microvilli to the cell body along their extended length, preventing their full extension into the bathing medium and inhibiting spreading of the cells onto a glass substrate. This could be



**FIGURE 6** Fluorescence micrographs of cells treated with DPL vesicles containing 6-carboxyfluorescein. Incubations were carried out for 30 min at 2° or 37°C as follows. (a) E cells, 2°C; (b) E cells, 37°C; (c) T cells, 2°C; and (d) T cells, 37°C. In control populations of cells (at arrows) incubated (30 min, 2°C) with DPL vesicles containing no trapped dye, very little fluorescence was seen (e).

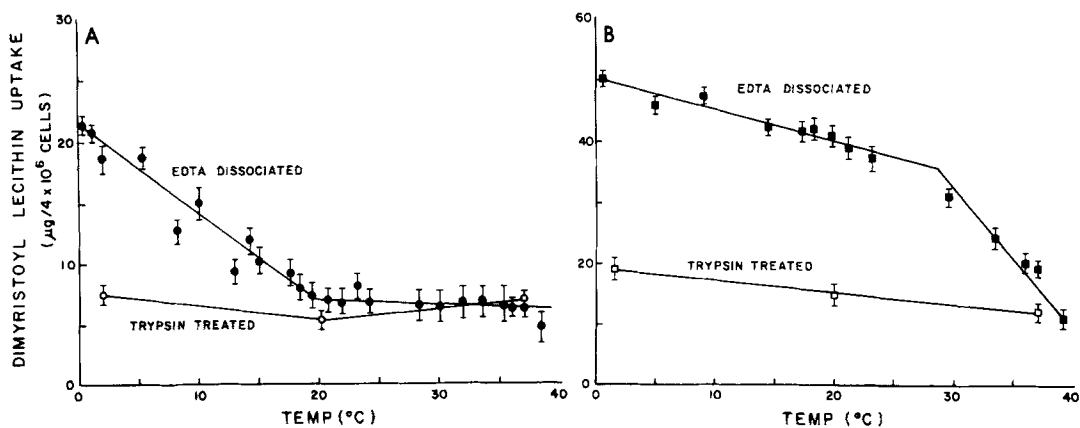


FIGURE 7 Temperature dependence of vesicle uptake by EDTA-dissociated and trypsin-treated cells. (A) DML and (B) DPL.

explained if the adsorbed vesicles act as a bridge, binding different regions of the cell surface together. EM autoradiographs further showed an accumulation (~90%) of [<sup>3</sup>H]DML (Fig. 3a) or [<sup>3</sup>H]DPL at the cell periphery after a low temperature incubation, suggesting that the adsorbed particles seen in Figs. 4 and 5 represent applied vesicles. Finally, fluorescence microscopy of cells treated with vesicles containing the water-soluble fluorescent dye, 6-CF, show an intense ring of fluorescence at their periphery (Fig. 6a, c). Such a pattern of fluorescence is additional evidence for a vesicle-to-cell adsorption process as the dominant pathway of uptake at temperatures below the vesicle  $T_c$ .

#### Mode of Vesicle-to-Cell Adhesion

Our data suggest the importance of cell surface proteins in the vesicle-to-cell adsorption phenomenon discussed above. The finding that uptake of vesicles by EDTA-dissociated cells is always greater than that by its trypsinized counterpart suggests that a trypsin-sensitive material is required for the greater binding of vesicles to the cell surface. The significant release of DPL or DML from the cell surface by trypsin (Table II) is also consistent with the involvement of a cell surface protein moiety in vesicle binding. Scanning electron micrographs of cells (Figs. 4d, 5d) show that the number of vesicles bound to the nonmicrovillous regions of EDTA-dissociated cell surfaces is much greater than in trypsinized cells. This suggests that the lower uptake of vesicles in trypsin-treated cells is not simply due to a reduction in the cell surface area available for vesicle-to-cell adhe-

sion which could arise from subtle differences in the diameter and number of microvilli in the two cell types.

DML or DPL vesicle pretreatments of EDTA-dissociated cells modified significantly the lactoperoxidase-catalyzed iodination of some of the cell surface proteins. The modifications in gel patterns shown in Fig. 2 could result from (a) altered mobility of proteins in the gel due to the presence of tightly bound exogenously supplied DML or DPL lipids which were not completely removed (2, 14) from the cellular proteins by SDS solubilization and/or (b) a modified accessibility of some of the cell surface proteins to <sup>125</sup>I labeling by lactoperoxidase. While we cannot completely exclude the first possibility at this time, the absence of heavily labeled [<sup>3</sup>H]DML bands in regions I, II, IV, and V of the fluorograph shown in Fig. 2E suggests that the enhanced iodinated bands seen in those regions after vesicle treatment (Fig. 2A-C) are not due to an anomalous migration of proteins in gels resulting from their incomplete delipidation. Our finding of an apparent inhibition of <sup>125</sup>I labeling of the ~60,000-dalton mol wt cell surface proteins in the presence of adherent vesicles (Fig. 2A vs. 2B, C) is consistent with explanation (b) above. Thus, incubation of cells with [<sup>3</sup>H]DML vesicles followed by SDS polyacrylamide gel electrophoresis and fluorography showed that the [<sup>3</sup>H]DML, although associated with a number of protein bands, was strongly associated with the two proteins whose iodination is inhibited by vesicle pretreatments of cells (arrows; Fig. 2E, G). Furthermore, this association was considerably weakened or nearly absent (Fig. 2F, H) in trypsin-treated cells which also exhibit a greatly reduced

DML or DPL vesicle-to-cell adhesion (Table I; Figs. 4, 5). Thus, we conclude that vesicle binding to the surface of EDTA-dissociated cells involves a number of cell surface proteins, several of which have a high affinity for vesicle lipid and are protected from iodination as a result of vesicle binding.

The adsorption of DML and DPL vesicles to cells may also involve a trypsin-insensitive protein or a nonprotein component of the cell surface. We suggest that vesicle adhesion to trypsin-treated cells may involve primarily such nonprotein regions of the cell surface, whereas adhesion to EDTA-dissociated cells involves both protein and nonprotein elements. In agreement with this idea is our finding that DML or DPL vesicle treatments of cells affects the iodination pattern in EDTA-dissociated but not trypsinized cells. Since the binding of DML or DPL vesicles to EDTA-dissociated cells at low temperatures is much greater than to trypsin-treated cells, adsorption to surface proteins probably dominates the uptake process at this temperature. With increasing temperatures, the levels of vesicle uptake by the two cell types approach one another (Fig. 7), suggesting that adsorption to the nonprotein part prevails. We speculate that this nonprotein part of the cell surface involved in vesicle uptake may represent accessible regions of plasma membrane lipids.

In the present study, we have shown that below their  $T_c$ , DML and DPL lipid vesicles adhere mostly to a trypsin-sensitive material on the surface of EDTA-dissociated cells. Our findings suggest a possible pathway for intercellular adhesion involving both protein and lipid. According to such a scheme, trypsin-sensitive materials on one cell surface, which have the ability to combine with some of the exposed lipids from the plasmalemma of another cell, result in the adhesion of two surfaces. By analogy to our finding that only "solid" vesicles such as DML or DPL below their  $T_c$  form stable adhesions to cells, it is suggested that the lipids in those regions of plasma membrane involved in cell-to-cell adhesions are highly specialized, probably containing relatively high proportions of "rigid" or saturated acyl chains compared to the plasma membrane as a whole. Such a model differs from current ideas on the molecular basis for intercellular adhesion of cells which emphasize either specific interactions between cell surface proteins and/or carbohydrates of contacting cells, or interactions between the plasma membrane lipid bilayers of adjacent cells

(10, 11, 34). The proposed model is not intended to explain specific interactions between cells, however, the present findings do suggest that lipid and protein interactions between contacting cells may be important in stabilizing specific membrane contacts once formed.

We thank Doctors Steve Roth, Malcom Steinberg, and Alex Sandra for critically reading this manuscript. We also wish to thank Dr. Weinstein and associates for making available a preprint of their studies using 6-CF vesicles before publication, and Dr. John Rash for use of the critical point drying apparatus. We gratefully acknowledge the expert technical assistance of Ms. Elaine Asch and Mr. W. Duncan. The scanning electron microscope time for this project was supported by the University of Maryland Center for Materials Research, Department of Mechanical Engineering, and Electron Microscope Facility, College Park, Md.

This investigation was supported by the Carnegie Institution of Washington and the National Institutes of Health Research grant GM 22942. M. Takeichi was the recipient of a fellowship from the Whitehall Foundation.

Received for publication 13 December 1976, and in revised form 18 April 1977.

## REFERENCES

1. ALDERSON, J. C. E., and C. GREEN. 1975. Enrichment of lymphocytes with cholesterol and its effect on lymphocyte activation. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **52**:208-211.
2. AWASTHI, Y. C., T. F. CHUANG, T. W. KEENAN, and F. L. CRANE. 1971. Tightly bound cardiolipin in cytochrome oxidase. *Biochim. Biophys. Acta.* **226**:42-52.
3. BANGHAM, A. D. 1972. Lipid bilayers and biomembranes. *Annu. Rev. Biochem.* **41**:753-776.
4. BATZRI, S., and E. D. KORN. 1975. Interaction of phospholipid vesicles with cells. Endocytosis and fusion as alternate mechanisms for the uptake of lipid-soluble and water-soluble molecules. *J. Cell Biol.* **66**:621-634.
5. BLIGH, E. G., and W. J. DYER. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**:911-917.
6. BONNER, W. M., and R. A. LASKEY. 1974. A film detection method for tritium labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46**:83-88.
7. BRUCKDORFER, K. R., R. A. DEMEL, J. DEGIER, and L. L. M. VAN DEENEN. 1969. The effect of partial replacements of membrane cholesterol by other steroids on the osmotic fragility and glycerol permeability of erythrocytes. *Biochim. Biophys. Acta.* **183**:334-345.
8. CARO, L. G., R. P. VAN TUBERGEN, and J. O.

- KOLB. 1962. High-resolution autoradiography. I. Methods. *J. Cell Biol.* **15**:173-188.
9. CHEN, J. S., A. DEL FA, A. DiLUZIO, and P. CALISSANO. 1976. Liposome-induced morphological differentiation of murine neuroblastoma. *Nature (Lond.)*. **263**:604-606.
  10. CURTIS, A. S. G. 1967. The Cell Surface: Its Molecular Role in Morphogenesis. Academic Press, Inc., New York. 405 pp.
  11. CURTIS, A. S. G., C. CHANDLER, and N. PICTON. 1975. Cell surface lipids and adhesion. III. The effects on cell adhesion of changes in plasmalemmal lipids. *J. Cell Sci.* **18**:375-384.
  12. GRANT, C. W. M., and H. M. McCONNELL. 1973. Fusion of phospholipid vesicles with viable *Acholeplasma laidlawii*. *Proc. Natl. Acad. Sci. U. S. A.* **70**:1238-1240.
  13. HAUSER, H. O. 1971. The effect of ultrasonic irradiation on the chemical structure of egg lecithin. *Biochem. Biophys. Res. Commun.* **45**:1049-1055.
  14. HELENIUS, A., and K. SIMONS. 1975. Solubilization of membranes by detergents. *Biochim. Biophys. Acta.* **415**:29-79.
  15. HUANG, C. 1969. Studies on phosphatidylcholine vesicles. Formation and physical characteristics. *Biochemistry.* **8**:344-352.
  16. HUANG, C., and J. P. CHARLTON. 1972. Studies on phosphatidyl choline molecules before and after ultrasonic and gel filtration treatments. *Biochem. Biophys. Res. Commun.* **46**:1660-1666.
  17. HUANG, L., and R. E. PAGANO. 1975. Interactions of phospholipid vesicles with cultured mammalian cells. I. Characteristics of uptake. *J. Cell Biol.* **67**:38-48.
  18. HUANG, L., K. OZATO, and R. E. PAGANO. 1976. Interaction of phospholipid vesicles with mouse thymocytes *in vitro*. *Fed. Proc.* **35**:1703. (Abstr.)
  19. HUBBARD, A. L., and Z. A. COHN. 1972. The enzymatic iodination of the red cell membrane. *J. Cell Biol.* **55**:390-405.
  20. INBAR, M., and M. SHINITZKY. 1974. Increase of cholesterol level in the surface membrane of lymphoma cells and inhibitory effect on ascites tumor development. *Proc. Natl. Acad. Sci. U. S. A.* **71**:2128-2130.
  21. LAEMMLI, U. K. 1970. Cleavage of structural protein during the assembly of the head of Bacteriophage T4. *Nature (Lond.)*. **227**:680-685.
  22. LENTZ, B. R., Y. BARENHOLTZ, and T. E. THOMPSON. 1976. Fluorescence depolarization studies of phase transitions and fluidity in phospholipid bilayers. I. Single component phosphatidyl choline liposomes. *Biochemistry.* **15**:4521-4528.
  23. MAGEE, W. E., C. W. GOFF, J. SCHOKNECHT, M. D. SMITH, and K. CHERIAN. 1974. The interaction of cationic liposomes containing entrapped horseradish peroxidase with cells in culture. *J. Cell Biol.* **63**:492-504.
  24. MARTIN, F. J., and R. C. MACDONALD. 1976. Lipid vesicle-cell interactions. III. Introduction of a new antigenic determinant into erythrocyte membranes. *J. Cell Biol.* **70**:515-526.
  25. PAGANO, R. E., and L. HUANG. 1975. Interaction of phospholipid vesicles with cultured mammalian cells. II. Studies of mechanism. *J. Cell Biol.* **67**:49-60.
  26. PAGANO, R. E., L. HUANG, and C. WEY. 1974. Interaction of phospholipid vesicles with cultured mammalian cells. *Nature (Lond.)*. **252**:166-167.
  27. PAGANO, R. E., K. OZATO, and J. M. RUYSSCHAERT. 1977. Intracellular distribution of lipophilic fluorescent probes in mammalian cells. *Biochim. Biophys. Acta.* **465**:661-666.
  28. PAGANO, R. E., and M. TAKEICHI. 1977. Adhesion of phospholipid vesicles to Chinese hamster fibroblasts: role of cell surface lipid and protein constituents. *Biophys. J.* **17**:165a. (Abstr.)
  29. PAPAHAJIOPOULOS, D., G. POSTE, and B. E. SCHAEFFER. 1973. Fusion of mammalian cells by unilamellar lipid vesicles: influence of lipid surface charge, fluidity and cholesterol. *Biochim. Biophys. Acta.* **323**:23-42.
  30. PHILLIPS, D. R., and M. MORRISON. 1971. Exposed protein on the intact human erythrocyte. *Biochemistry.* **10**:1766-1771.
  31. POSTE, G., and D. PAPAHAJIOPOULOS. 1976. Lipid vesicles as carriers for introducing materials into cultured cells: influence of vesicle lipid composition on mechanism(s) of vesicle incorporation into cells. *Proc. Natl. Acad. Sci. U. S. A.* **73**:1603-1607.
  32. PUCK, T. T., S. J. CIECIURA, and A. ROBINSON. 1958. Genetics of somatic mammalian cells. III. Long-term cultivation of euploid cells from human and animal subjects. *J. Exp. Med.* **108**:945-956.
  33. RAHMAN, Y.-E., and B. J. WRIGHT. 1975. Liposomes containing chelating agents. Cellular penetration and a possible mechanism of metal removal. *J. Cell Biol.* **65**:112-122.
  34. ROTH, S. 1973. A molecular model for cell interactions. *Q. Rev. Biol.* **48**:541-563.
  35. STAMBROOK, P. J., and J. E. SISKEN. 1972. Induced changes in the rates of uridine-<sup>3</sup>H uptake and incorporation during the G<sub>1</sub> and S periods of synchronized Chinese hamster cells. *J. Cell Biol.* **52**:514-525.
  36. THOMPSON, T. E., and F. A. HENN. 1970. Experimental phospholipid model membranes. In Membranes of Mitochondria and Chloroplasts. E. Racker, editor. Van Nostrand Reinhold Co., New York. 1-52.
  37. WEINSTEIN, J. N., S. YOSHIKAMI, P. HENKART, R. BLUMENTHAL, and W. A. HAGINS. 1976. Transfer of a fluorescent dye from lipid vesicles to cells. *Biophys. J.* **16**:104a. (Abstr.)
  38. WEINSTEIN, J. N., S. YOSHIKAMI, P. HENKART, R. BLUMENTHAL, and W. A. HAGINS. 1977. Lipo-

- some-cell interaction: transfer and intracellular release of a trapped fluorescent marker. *Science (Wash. D. C.)*. **195**:489-492.
39. WEISMAN, R. A., and E. D. KORN. 1966. Uptake of fatty acids by *Acanthamoeba*. *Biochim. Biophys. Acta*. **116**:229-242.
40. WEISSMANN, G., D. BLOOMGARDEN, R. KAPLAN, C. COHEN, S. HOFFSTEIN, J. COLLINS, A. GOTTLIEB, and D. NAGLE. 1975. A general method for the introduction of enzymes by means of immunoglobulin-coated liposomes into lysosomes of deficient cells. *Proc. Natl. Acad. Sci. U. S. A.* **72**:88-92.
41. WETZEL, M. G., and E. D. KORN. 1969. Phagocytosis of latex beads by *Acanthamoeba castellanii* (Neff). III. Isolation of the phagocytic vesicles and their membranes. *J. Cell Biol.* **43**:90-104.