

Contact-induced Redistribution of Specific Membrane Components: Local Accumulation and Development of Adhesion

Michael A. McCloskey and Mu-ming Poo*

Department of Physiology and Biophysics, University of California, Irvine, California 92717; *Section of Molecular Neurobiology, Yale University School of Medicine, New Haven, Connecticut 06510

Abstract. We have used a model system to explore the importance of long-range lateral diffusion of membrane proteins in specific membrane-membrane adhesion. Single, cell-size phospholipid vesicles containing a dinitrophenyl (DNP)-lipid hapten were maneuvered into contact with rat basophilic leukemia (RBL) cells carrying fluorescent anti-DNP IgE in their cell-surface Fc ϵ receptors. Upon cell-vesicle contact the antibody molecules underwent a marked lateral redistribution, accumulating at the site of contact and becoming significantly depleted from noncontacting membrane. As assayed with a micropipette suction method, there was a time-dependent increase in the strength of cell-vesicle adhesion. This development of adhesion paralleled the kinetics of accumulation of the adhesion-mediating antibody molecules at the zone of membrane-membrane contact. Both adhesion and redistribution were absolutely dependent upon a specific interaction of the IgE with the hapten: No redistribution occurred when vesicles lacking the DNP

hapten were pushed against IgE-armed RBL cells, and on cells bearing a 1:1 mixture of nonimmune rat IgE and anti-DNP mouse IgE, only the latter underwent redistribution. Vesicles containing DNP-lipids bound to RBL cells carrying anti-DNP IgE but not to cells carrying nonimmune rat IgE. Measurable nonspecific binding did not develop even after 15 min of pushing DNP-bearing vesicles against RBL cells sensitized with nonimmune IgE. Neither redistribution nor adhesion was blocked by metabolic poisons such as NaN₃ and NaF. Both redistribution and adhesion occurred in plasma membrane blebs previously shown to lack cytoskeletal filaments. The above observations are consistent with contact-induced redistribution of the IgE being a result of passive diffusion-mediated trapping rather than active cellular responses. Thus, long-range diffusion of specific proteins can in some cases contribute to the formation of stable adhesion between membranes.

TISSUE generation and regeneration must involve selective cell-cell or cell-substrate recognition. In vertebrates, chemical defense mechanisms also require highly specific recognition between cells of the immune system. These and other kinds of selective cell-cell interactions probably are mediated by the formation of bonds between special molecules on the surface of juxtaposed cells. Indeed, the chemical nature of some of the surface molecules that mediate cell-cell adhesion is beginning to be defined (see references 11 and 14 for reviews).

Development of selective adhesion, that is, an increase in the number of specific bonds between membranes, may depend upon lateral mobility of the bond-forming species in the plane of the membrane. Once two membranes have made contact, complementary molecules on opposing membranes must find each other, and this can depend upon their short-range diffusion within the zone of contact. Such dependence of adhesion on local diffusion is most apparent when the adhesion-mediating molecules are present at low lateral densities (18).

Lateral diffusion may also contribute to cell-cell or mem-

brane-membrane adhesion in a different way. If the bonding molecules are free to move over long distances within the membrane, in theory they should concentrate at the site of contact, thereby increasing the total strength of adhesion. This recruitment of bonds due to long-range diffusion will also be most pronounced at low lateral densities of the bonding species. As pointed out by Bell et al. (6), there are situations in which one might expect that diffusion-mediated trapping of the bonding species would be a prerequisite for the formation of stable membrane-membrane contacts. Although this reasoning appeals to logic, an experimental system is yet to be devised in which both adhesion and redistribution can be monitored directly and the relationship between them studied.

The aim of the present work is to develop a model system with well-defined membrane-membrane interactions in which both redistribution and adhesion can be measured in real time. The system chosen consists of rat basophilic leukemia (RBL)¹ cells armed with anti-dinitrophenyl (DNP) IgE and

1. *Abbreviations used in this paper:* DNP, dinitrophenyl; DOPC, dioleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; FITC, fluorescein isothiocyanate; RBL, rat basophilic leukemia; TNP, trinitrophenyl.

cell-size phospholipid vesicles bearing DNP-lipid haptens. The IgE is tightly bound to Fc ϵ receptors which are present at a lateral density of a few times $10^{10}/\text{cm}^2$ in the plasma membrane, and it mediates binding of the cells to DNP groups on the vesicle surface. Changes in the lateral distribution of IgE are followed by spot microfluorimetry, and adhesion strength is measured in single cell-vesicle pairs with an aspiration micropipette method.

Materials and Methods

Cells

The 2H3 subline of RBL IV cells (3) was grown, passaged, and harvested as described earlier (21). EDTA-eluted cells were suspended at $1-5 \times 10^6/\text{ml}$ in cell buffer (116 mM NaCl, 5.4 mM KCl, 2 mM CaCl $_2$, 1.5 mM MgCl $_2$, 1 mM Na-inorganic P, 0.1% glucose, 25 mM Hepes, pH 7.25, 0.05% bovine serum albumin [BSA]) containing 10–20 $\mu\text{g}/\text{ml}$ of fluorescent IgE conjugate. After 25–45 min at room temperature the cells were pelleted in an Eppendorf microcentrifuge (Brinkmann Instruments Co., Westbury, NY), rinsed twice in cell buffer, suspended in cell buffer, and kept on ice for 0–4 h before use.

Antibodies

IgE from the IR162 immunocytoma (4) was purified from ascitic fluid given to us by Dr. Henry Metzger. Mouse monoclonal anti-2,4-DNP IgE from the H1DNP- ϵ -26.82 hybridoma (19) was affinity purified from ascites fluid supplied by Dr. Fu-tong Liu. The fluorescent conjugates were fluorescence labeled and purified essentially as described before (21).

DNP-Lipid Haptens

We found that RBL cells armed with anti-DNP IgE failed to bind to lipid vesicles containing the lipid hapten N- ϵ -DNP-amino-caproylphosphatidylethanolamine, independent of the solid-fluid state of the vesicle membrane. Balakrishnan et al. got the same result with a different monoclonal anti-DNP IgE (2). We therefore synthesized another DNP-lipid hapten with a longer and more hydrophilic head group. 75 mg mixed beef brain gangliosides (Supelco, Inc., Bellefonte, PA) was dispersed in 22 ml 0.1 M Na acetate, 0.15 M NaCl, pH 5.6, and cooled on ice. NaIO $_4$ (4.5 ml, 0.2 M) was added with stirring, and the foil-covered reaction vessel was kept on ice for 10 min. This treatment causes fairly specific oxidation of the C $_3$ side chain of sialic acids to an aldehyde (30). Ethylene glycol (5.85 ml) was then added, and the reaction mixture was dialyzed at 4°C for 36 h against PBS and for another 2 d against distilled water. The oxidized gangliosides were lyophilized (63 mg recovered) and dissolved in 35 ml of a buffer containing 3.65 g hexanediamine and 5.58 g succinic acid, pH 6.0. After 30 min at room temperature 660 mg NaBCNH $_3$ was added in 5 ml 0.25 M Na succinate (7, 27). The pH was kept at 6.0 with NaOH for 48 h at room temperature, at which point another portion of reducing agent was added. 2 d later the reaction mix was acidified to pH 3.0 and left for 3 h to reduce any remaining aldehyde. The mixture was brought to pH 7.2 by addition of NaOH on ice and then dialyzed against distilled water, NaCl, and distilled water again. After lyophilization ~45 mg white powder was obtained. Thin-layer chromatography in chloroform-methanol-water (60:40:90, vol/vol) revealed one major ninhydrin-positive, α -naphthol-positive band at the origin. The primary amino-containing gangliosides were dissolved in 50 ml water containing 1 g NaHCO $_3$, and 100 ml ethanol containing 1 ml 2,4-dinitrofluorobenzene was then mixed with the aqueous solution. The inhomogeneous mix was protected from light and stirred for 3 h at room temperature. Solvent was then removed on a rotary evaporator at 40°C, and the residue was dissolved in chloroform-methanol (1:1, vol/vol). Insoluble material, mostly NaHCO $_3$, was spun down in a centrifuge. Thin-layer chromatography of the supernate now showed several α -naphthol-positive bands with mobilities slightly greater than those of unmodified gangliosides. The DNP-gangliosides were purified further by column chromatography on silica gel, using increasing amounts of methanol in CHCl $_3$ and finally CHCl $_3$ -MeOH-H $_2$ O (55:45:10) to elute the lipid hapten. The above procedure probably introduced ~1 DNP group per sialic acid residue. DNP was quantitated by its absorbance at 362 nm in CHCl $_3$ -MeOH (1:1), assuming a molar absorptivity (ϵ) of 2.0×10^4 liters/mol-cm, the average of the ϵ values for DNP- γ -aminobutyric acid and DNP-aminocaproylphosphatidylethanolamine in the same solvent.

Haptenated-Polyacrylamide Beads

2,4-DNP and 2,4,6-trinitrophenyl (TNP) groups were attached to the polymer backbone of spherical polyacrylamide beads (P2, Bio-Rad Laboratories, Burlin-

game, CA) via spacers of different lengths. The native beads were functionalized by displacement of the amide nitrogen with either hydrazine or ethylenediamine, as described by Inman (17). A portion of each preparation was reacted with fluorodinitrobenzene in ethanolic NaHCO $_3$ (26) or alternatively with trinitrobenzenesulfonate in aqueous borax, and the remainder was reacted exhaustively with succinic anhydride at pH 4.0. The reaction was repeated until no color remained with the beads upon treatment with trinitrobenzenesulfonate. The succinylated beads were then coupled with either hydrazine or ethylenediamine, using a water soluble carbodiimide in each case. DNP or TNP groups were then introduced as above. Beads were thus produced with DNP or TNP groups attached 2–12 atoms away from the polymer chain. Hapten densities were varied by control of the duration of the reaction, and although the final extent of haptentation was not quantitated, the depth of color of the beads gave a qualitative indication as to the relative amounts of hapten present.

Lipid Vesicles

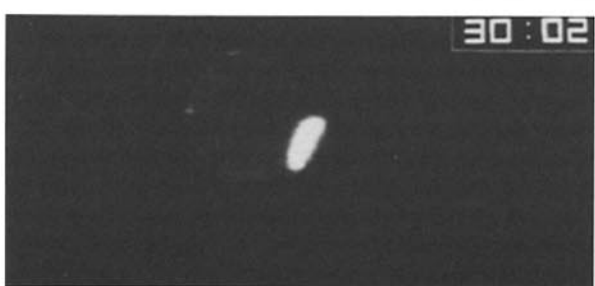
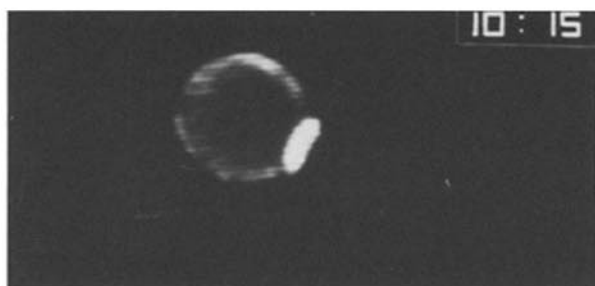
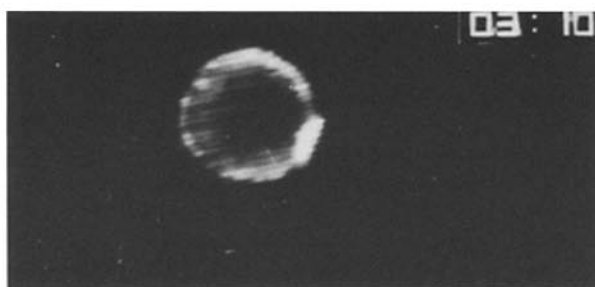
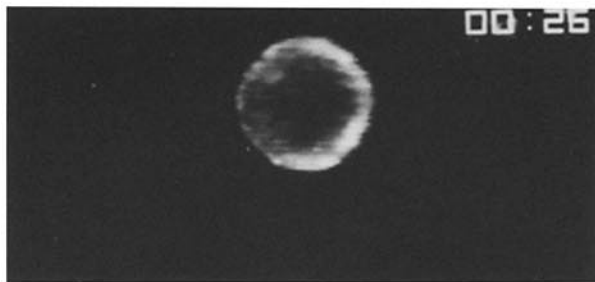
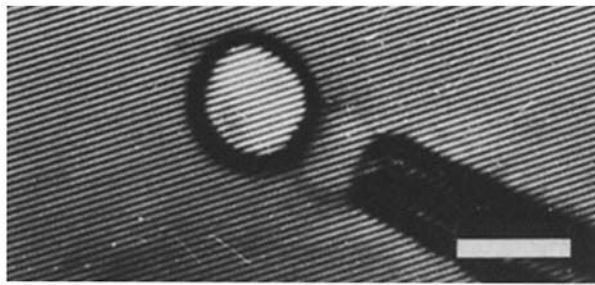
Dipalmitoylphosphatidylcholine (DPPC) and dioleoylphosphatidylcholine (DOPC) were from Sigma Chemical Co. (St. Louis, MO). Large oligolamellar phospholipid vesicles were formed by the procedure of Reeves and Dowben (25). Stock solutions of the host lipid and DNP-gangliosides were mixed to yield the appropriate mole ratios, and excess solvent was removed on the rotovap. The lipid (2.5 μmol) was then dissolved in 3 ml CHCl $_3$ -MeOH (1:1), and the solution was applied to a 17-cm-diam glass petri plate heated to 50°C. The plate was rocked to form as even a lipid film as possible while the solvent evaporated. Aqueous sucrose (0.325 M) was then added carefully to cover the film, and the petri plate was incubated overnight at $\geq 10^\circ\text{C}$ above the transition temperature of the host lipid. The vesicles were diluted 1:1 with 0.325 M glucose and recovered by centrifugation at 12,000 g for 30 min at 4°C. Freeze-fracture electron micrographs revealed vesicle populations with mostly from one to three bilayers per vesicle (McCloskey, M., unpublished result).

Single-Cell Adhesion Measurements

We determined the kinetics of development of adhesion in single vesicle-cell pairs in the following way. RBL cells armed with the appropriate IgE antibody were immobilized by being allowed to bind for 10 min to one end of the top surface of a cover slip chamber (No. 3298-S72, A. H. Thomas Co., Philadelphia, PA) in cell buffer with azide (CBA) (10 mM Hepes, pH 7.3, 28 mM NaN $_3$, 1 mM Na-inorganic P, 109 mM NaCl, 5.4 mM KCl, 0.1% glucose, 0.05% BSA). (Azide was included in all experiments, both to block active processes and to keep the cells round. With no or low azide the cells tend to flatten out on the substrate during the experiment, thus complicating interpretation of the fluorescence measurements.) The coverslip chamber was held on the stage of an IM 35 inverted microscope (Carl Zeiss, Inc., Thornwood, New York), and cells were observed through a 40 \times water immersion planapo objective. A small drop of vesicle suspension in either sucrose or cell buffer with azide was then added to the opposite end of the chamber so that random binding of vesicles to cells was partially prevented. A fire-polished micropipette with an average tip diameter of ~3.8 μm was then submerged beneath the bathing solution, and a defined suction pressure of $\sim 8 \times 10^{-5}$ dyn/ μm^2 was applied by means of a micrometer controlled water balance similar to that previously described (12). The micropipette was positioned next to a vesicle with the aid of a pneumatic micromanipulator (model C, de Fonbrune, Laboratory Products Inc., Boston, MA), and the captured vesicle was pushed directly against a cell. The entire procedure was followed on a video monitor (VM173-U, Hitachi, Ltd, Tokyo) and recorded on a $\frac{3}{4}$ in. format video cassette recorder (Panasonic Industrial Co., Secaucus, NJ). The video camera (TC1005, RCA, Lancaster, PA) was fixed to one eyepiece receptacle of the trinocular head. The time elapsed since vesicle-cell contact was displayed on the video monitor and recorded. For each cell-vesicle pair, at a defined time the micropipette was slowly withdrawn along the normal to the "plane" of membrane-membrane contact. Depending on the duration of contact, when the pipette was withdrawn either the cell-vesicle junction came apart or the pipette-vesicle seal ruptured, leaving the vesicle stuck to the cell. We scored the former as nonadherent and the latter as adherent pairs. The average diameter of RBL cells chosen for the measurements was 12.9 ± 1.1 μm , and the average diameter of the vesicles we chose was 6.3 ± 1.0 μm ($n = 103$).

Kinetics of IgE Redistribution

We followed the redistribution of cell-bound anti-DNP IgE to the site of vesicle-cell contact both qualitatively (video recording) and quantitatively. RBL cells were sensitized with the appropriate fluorescent IgE conjugate and then bound to the coverslip chamber as above. Vesicles were positioned against cells with a micropipette as described in the above paragraph. Video recording of the



fluorescence image was done with a silicon intensified target camera (RCA 1030/H). For quantitative recording, we replaced the video cameras with a microfluorimeter (Zeiss PM1) that we have previously used to measure lateral diffusion rates with postfield relaxation (21). We alternated the alignment of the measuring spot (4- μ m diam) from directly over the zone of membrane-membrane contact to directly over a noncontact zone, and measured the fluorescence intensity in these two regions as a function of time after contact. The cell-free background was subtracted and the intensities were normalized by dividing each by the time zero intensity. On control cells, the average drop in fluorescence intensity due to photobleaching by the exciting light was 5% of the initial value after 6 measurements (25 min) and 9% after 11 measurements (50 min). This is small as compared with the changes we observed due to receptor redistribution and thus would not have affected the results significantly.

Light and Electron Microscopy

Fluorescence, bright-field, and Nomarski differential interference contrast micrographs were taken both directly through the microscope with a 35-mm camera and from video records on the TV monitor. For direct fluorescence micrographs we used Kodak high speed recording film (No. 2475, Eastman Kodak Co., Rochester, NY), exposing for 20–80 s and push processing in DK-50 for 8 min. Other photographs were taken with Kodak Tri-X film.

For scanning electron microscopy, RBL cells were grown as a monolayer on 12-mm-diam round glass coverslips, sensitized with anti-DNP IgE, and incubated for 1 h with vesicles containing DNP-gangliosides. The cell-vesicle conjugates were fixed for 1 h at room temperature with 2% glutaraldehyde (Ted Pella, Inc., Tustin, CA) in 0.15 M Na cacodylate (pH 7.2) + 0.15 M sucrose. The coverslips were rinsed with 0.15 M Na cacodylate (pH 7.2) and then incubated for 60 min in 1% OsO₄ in the same buffer. After being rinsed in Na cacodylate the cell monolayers were dehydrated in a graded series of acetone-water mixtures and critical point dried in a CO₂ critical point drier (Ladd Research Industries, Inc., Burlington, VT). After being sputter coated with Au-Pd in a Hummer 2 (Technics, Inc., Alexandria, VA) the preparations were examined at 5 kV at a working distance of 5 mm in a Hitachi S500 scanning electron microscope.

Formation of Plasmalemma Blebs

Plasma membrane blebs were formed as described by Holowka and Baird (16). In brief, cell monolayers on round glass coverslips were incubated in a cell buffer containing 2 mM N-ethylmaleimide for 30 min at 37°C. The coverslips were then placed in the abovementioned adhesion chambers in cell buffer with azide.

Results

Contact-induced Redistribution of IgE

As shown in Fig. 1, when single vesicles bearing the DNP-lipid hapten were pushed against anti-DNP-armed RBL cells, the IgE underwent a marked lateral redistribution, accumulating at the site of contact and becoming very depleted elsewhere. The time course of accumulation for this particular cell-vesicle pair was similar to that of most others observed, being nearly complete within 30 min. The fluorescent IgE rarely failed to redistribute when a vesicle was pushed against the cell. This may relate to vesicle heterogeneity or immobile or internalized IgE. Regardless, a large majority of experiments yielded substantial accumulation of IgE at the contact zone.

Selectivity. Contact-induced lateral redistribution of the IgE

Figure 1. Contact-induced redistribution of anti-DNP IgE on a single RBL cell. Bright field and fluorescence images of RBL cell bearing Texas Red-mouse IgE (anti-DNP). At time 0, the cell was brought into contact with a DPPC vesicle containing 1 mol% DNP-ganglioside; time elapsed since contact is shown at top right of each panel. Note the marked depletion of IgE from noncontact regions and its accumulation at the zone of contact. Qualitatively, redistribution is nearly complete within 30 min on this cell-vesicle pair. Bar, 10 μ m.

was highly selective. Shown in Fig. 2 are fluorescence micrographs of RBL cells sensitized with a mole ratio of $\sim 1:1$ of Texas Red-labeled anti-DNP IgE and fluorescein isothiocyanate-labeled nonimmune rat IgE. When haptenated lipid vesicles were pushed against these cells, only the antibody with affinity for the hapten underwent redistribution. Thus, even if the redistribution were driven by active cellular responses, these processes selected only those IgE receptors carrying anti-

DNP IgE. Although an interaction of the IgE with DNP was required for IgE localization, a lipid bilayer was not an essential component of the target structure. As shown in Fig. 3, when polyacrylamide beads containing covalently bound DNP groups were pushed against RBL cells, the cell-bound IgE again accumulated at the cell-bead junction. As with the DNP-lipid hapten, however (see Materials and Methods), the exact structure of the linker arm was important. Beads with

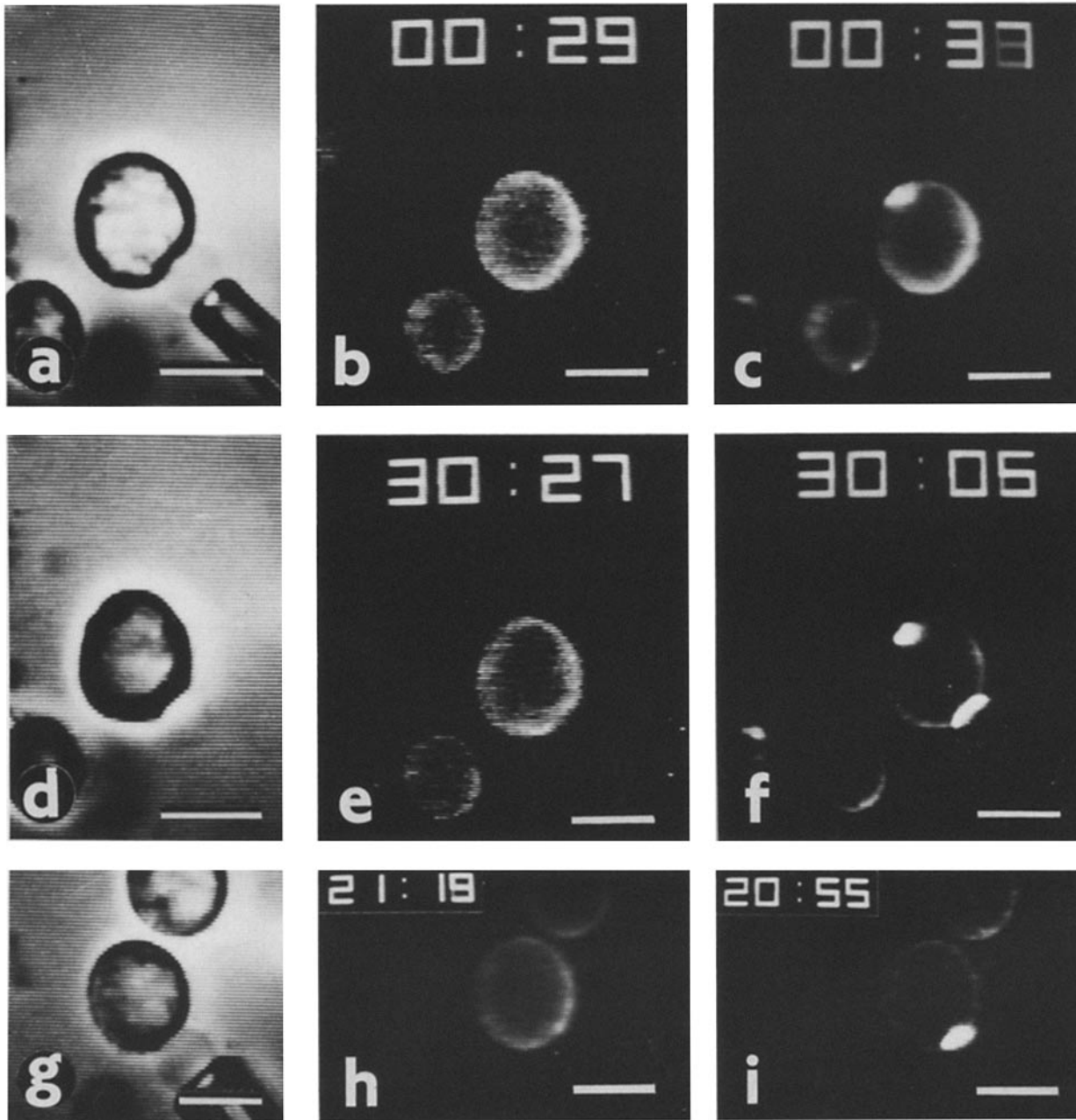


Figure 2. Contact-induced redistribution of IgE is selective. DPPC vesicles containing 1 mol% DNP-ganglioside were brought into contact with RBL cells bearing a mole ratio of $\sim 1:1$ of FITC-rat IgE (no affinity for DNP) and Texas Red-mouse IgE (anti-DNP). Only the latter collected at the site of contact. In *a-f* one cell-vesicle pair is shown and in *g-i* a separate pair is shown. (*a-c*) Bright field and fluorescence images of a cell just after contact with vesicle. (*d-f*) Bright field and fluorescence images 30 min after contact. (*b* and *e*) FITC fluorescence. (*c* and *f*) Texas Red fluorescence. No redistribution of FITC-IgE has occurred. The bright patch in *c* and *f* (upper left of cell) is due to an adventitious vesicle that had previously bound to the cell. (*g-i*) Another pair of RBL cells labeled as above, 20 min after contact with vesicle. (*g*) Bright field image. (*h*) 21 min 18 s after contact, the FITC fluorescence is still uniformly distributed. (*i*) 20 min 55 s after contact, Texas Red fluorescence is markedly nonuniform. Bars, 10 μm .

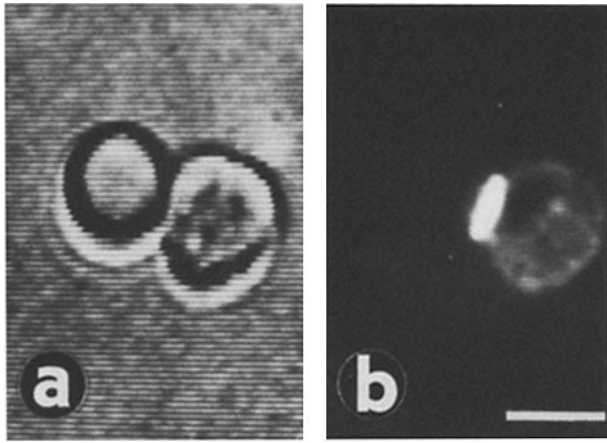


Figure 3. Redistribution of IgE occurs when a DNP-derivatized polyacrylamide bead is pushed against an anti-DNP IgE-armed cell. 30 min at room temperature were allowed for accumulation. Bar, 10 μ m.

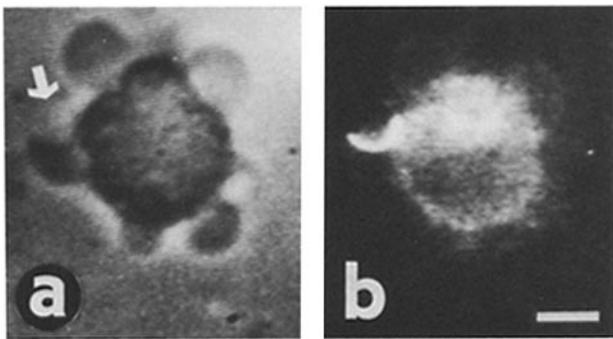


Figure 4. Redistribution of IgE occurs in plasma membrane blebs. RBL cells growing in monolayer culture on a cover glass were treated for 30 min with cell buffer containing 2 mM *N*-ethylmaleimide; this treatment causes the formation of large plasma membrane blebs that are not subtended by cytoskeletal filaments, and in which the IgE-Fc ϵ receptor complex diffuses much faster than it does in the surrounding plasma membrane. Arrow points to the top surface of a vesicle that had bound to bleb 15 min before recording. Note upward-pointing arc of fluorescence in membrane bleb. Bar, 5 μ m.

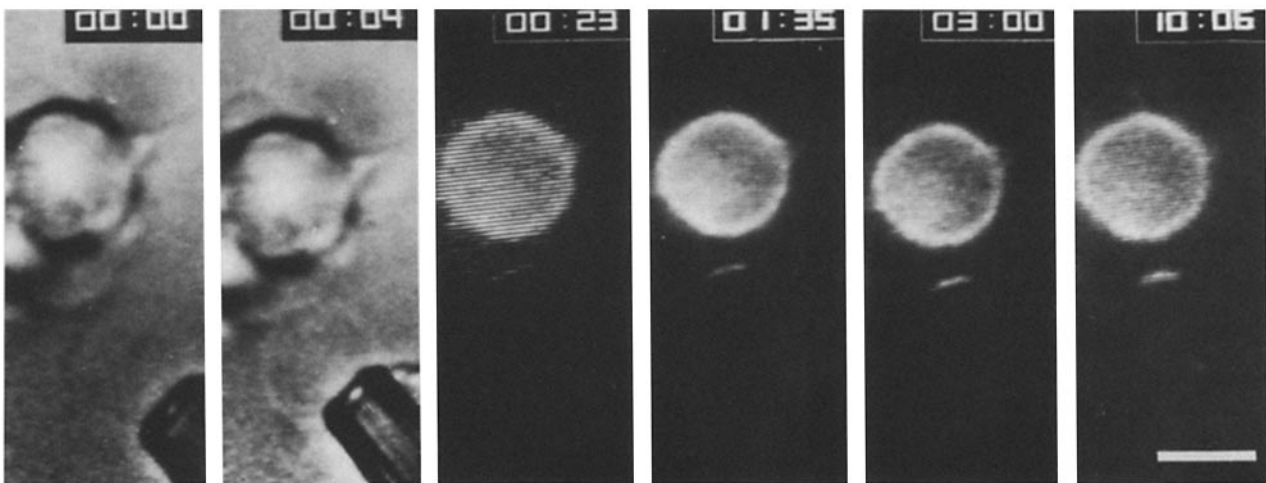


Figure 5. Time course of redistribution in a bleb. The time elapsed after contact is shown in the upper right of each photograph. The two bright field images are before and 4 s after pushing a vesicle against a bleb. DPPC vesicle contained 1 mol% DNP-ganglioside. Fluorescence images at successive times after contact show rapid redistribution of Texas Red-mouse IgE (anti-DNP). Bar, 10 μ m.

DNP or TNP groups attached directly to a hydrazido or an aminoethyl arm (two- or four-atom spacer) failed to bind to cells or induce redistribution, irrespective of the degree of haptentation. Beads with DNP groups on a 12-atom spacer from the polymer backbone bound avidly to IgE-armed cells and induced marked accumulation of the IgE. Beads containing TNP-groups on a 10-atom spacer bound well when the hapten density was high, i.e., when the bead color was deep magenta, but only poorly when the TNP was at lower density, i.e., when they were orange.

Energy Independence. Contact-induced redistribution of the IgE was seemingly independent of metabolic energy. All of the experiments were conducted with a cell buffer containing 28 mM sodium azide, a concentration sufficient to prevent cell spreading on the glass and more than enough to prevent active processes such as capping. The inclusion of 10 mM NaF also failed to block the redistribution. (In all experiments the tonicity was kept at \sim 310 mosM.)

Cytoskeletal Noninvolvement. Redistribution of the IgE apparently did not require an intact cytoskeleton, as it occurred in “blistered” regions of the membrane that had been induced to separate from the subtending cytoskeleton. Fig. 4 shows an RBL cell with several bulbous protrusions of plasma membrane that formed when the cell was incubated in a cell buffer containing 2 mM *N*-ethylmaleimide (see Materials and Methods). This treatment causes the formation of large plasma membrane blebs that reportedly are not subtended by cytoskeletal filaments, and in which the IgE-Fc ϵ receptor complex diffuses much faster than it does in the surrounding plasma membrane (16, 29). Beneath the arrow is a vesicle containing the DNP-lipid hapten, 15 min after binding to the plasma membrane bleb. Note the marked accumulation of fluorescence at the site of contact. Fig. 5 gives an indication of the time course of IgE redistribution within a bleb after contact with a haptentated vesicle. In general, the bleb experiment indicates that not only does redistribution occur within blebs, but it occurs at a rate faster than in the normal membrane. This is consistent with the greater diffusion coefficient of the IgE receptor complex in blebs than in normal plasma membrane (29).

Kinetics of Depletion and Accumulation

We used microfluorimetry to quantitate the rate of contact-induced redistribution on several cell-vesicle pairs. Shown in Fig. 6 are records from four cells, giving both the rates of accumulation of IgE at the site of contact and the rates of depletion from noncontact sites on the same cell. There was substantial variation from cell to cell, as expected from the previously observed variation in diffusion coefficients of the IgE-Fc ϵ receptor complex (21), as well as from differences in cell and vesicle radii and the extent of cell-vesicle contact. Despite the variation in rates from cell to cell, note the rough correlation between the speed and extent of depletion and of accumulation on any given cell. One does not expect the steady state fluorescence intensity per unit area to decrease in the noncontact zone as much as it increases in the zone of contact, due to the smaller size of the latter. The salient result is that the bulk of the IgE redistribution occurred within 15–30 min.

Study of Cell-Vesicle Adhesion

We assessed cell-vesicle adhesion at the population level by qualitative microscopic observations and at the single cell level by semiquantitative measurements with a suction micropipette. In the latter measurements we adjusted the hydrostatic pressure within the pipette by means of the micrometer and a jack beneath the water column. We monitored the movement of suspended vesicles into or out of the pipette tip to find the equilibrium point. The water column was then

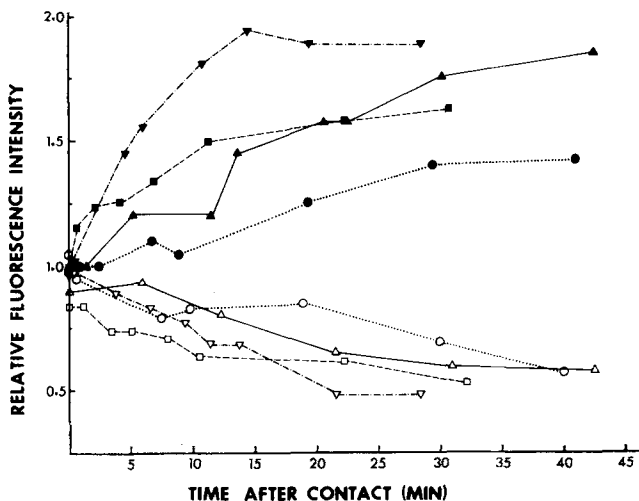


Figure 6. Kinetics of redistribution on four cell-vesicle pairs. The measuring spot of a microfluorimeter was positioned directly over the contact zone and then over a noncontact region of the plasma membrane at various times after pushing a vesicle against an anti-DNP-armed RBL cell. Fluorescence from the noncontact zone was monitored at 90° to the "plane" of contact. Fluorescence intensity at contact site is given by solid triangles, squares, and circles, and the open symbols show fluorescence over noncontact sites on corresponding cells. Both values at each time point for each cell have been normalized by dividing through by the time zero intensity for that cell. On these cells, the time course of redistribution is essentially complete within 15–45 min. Variation in rates can be attributed to different vesicle sizes, cell sizes, and diffusion coefficients of IgE-receptor complexes. Apparently, the rate and extent of accumulation are correlated with the rate and extent of depletion on these four cells.

lowered 8 cm beneath the pipette tip, producing a pressure difference across the pipette tip of $\sim 8 \times 10^{-5}$ dyn/ μm^2 . This pressure difference was then used to pull a vesicle off of the floor of the coverslip chamber and hold it at the tip of the micropipette. The vesicle was then pushed against a cell, and after a certain period of contact the pipette was slowly withdrawn along the normal to the zone of cell-vesicle contact.

In all cases ($n = 103$) the vesicles either came off of the pipette or off of the cell; never did the cells come loose from the glass coverslip. Fig. 7 illustrates four different trials of the experiment, i.e., four different cells and four different vesicles. If the separation was attempted after 30 s or 2 min of contact, the vesicles usually separated from the cells, as shown in Fig. 7, *a* and *b*, whereas if the separation was attempted after 15 min the vesicles usually failed to detach (Fig. 7*d*). Fig. 8 illustrates a case in which at one time point (10 min) both separation and attachment occurred frequently. Thus, for one defined period of contact the vesicles can behave differently, indicating the probabilistic nature of the phenomenon.

From measurements on many individual cell-vesicle pairs (not illustrated in Fig. 7 or 8), at different times after contact, we constructed the graph given in Fig. 9. There is a definite increase in the percentage of cell-vesicle pairs that remain stuck as a function of time after contact, indicating an increase in the average strength of cell-vesicle adhesion. All of these measurements were performed at a fixed aspiration pressure of $\sim 8 \times 10^{-5}$ dyn/ μm^2 and using a group of six different pipettes of $\sim 3.8 \mu\text{m}$ average tip opening. The average diameter of the 103 cells was $12.9 \mu\text{m}$, the average vesicle diameter was $6.3 \mu\text{m}$, and the linear extent (approximate diameter) of the contact zone averaged $3.6 \mu\text{m}$. Note that the increase of adhesiveness occurred within a period of ~ 15 min, as did the most dramatic increase in concentration of the IgE at the zone of membrane-membrane contact (Figs. 1 and 6).

Specificity of Cell-Vesicle Binding. As was the IgE redistribution, cell-vesicle binding was very specific, i.e., dependent upon a specific interaction of the DNP hapten with the anti-DNP antibodies. DNP-containing vesicles simply did not adhere to RBL cells bearing only nonimmune rat IgE on their surface, even when pushed into contact for up to 15 min (bottom curve in Fig. 9). If extracellular calcium or magnesium is necessary for binding in this system the required levels must be quite low, as neither metal was purposely included in the buffer used for our adhesion experiments.

Morphology of Contact Zone

In the original experiments with DNP-polyacrylamide beads, fluorescence microscopy showed that IgE from the RBL cell plasma membrane was often distributed in an arc-like profile pointing away from the cell, and occasionally nearly completely surrounding the bead. This also proved to be true in subsequent experiments with solid phase (DPPC) lipid vesicles, as shown in Fig. 10*a*. In contrast, fluid phase lipid vesicles (DOPC) partially engulfed the cell in many cases (Fig. 10*b*). That the arcuate fluorescence profiles around the DPPC vesicles correspond to IgE in the plasma membrane of the RBL cell and not to IgE or proteolytic fragments of the IgE that have been released from the cell (32) is suggested in the scanning electron micrographs shown in Figs. 11 and 12. Fig. 11 shows the collar-like projections that surround solid-phase vesicles bound to the cells via IgE-DNP interactions. (The

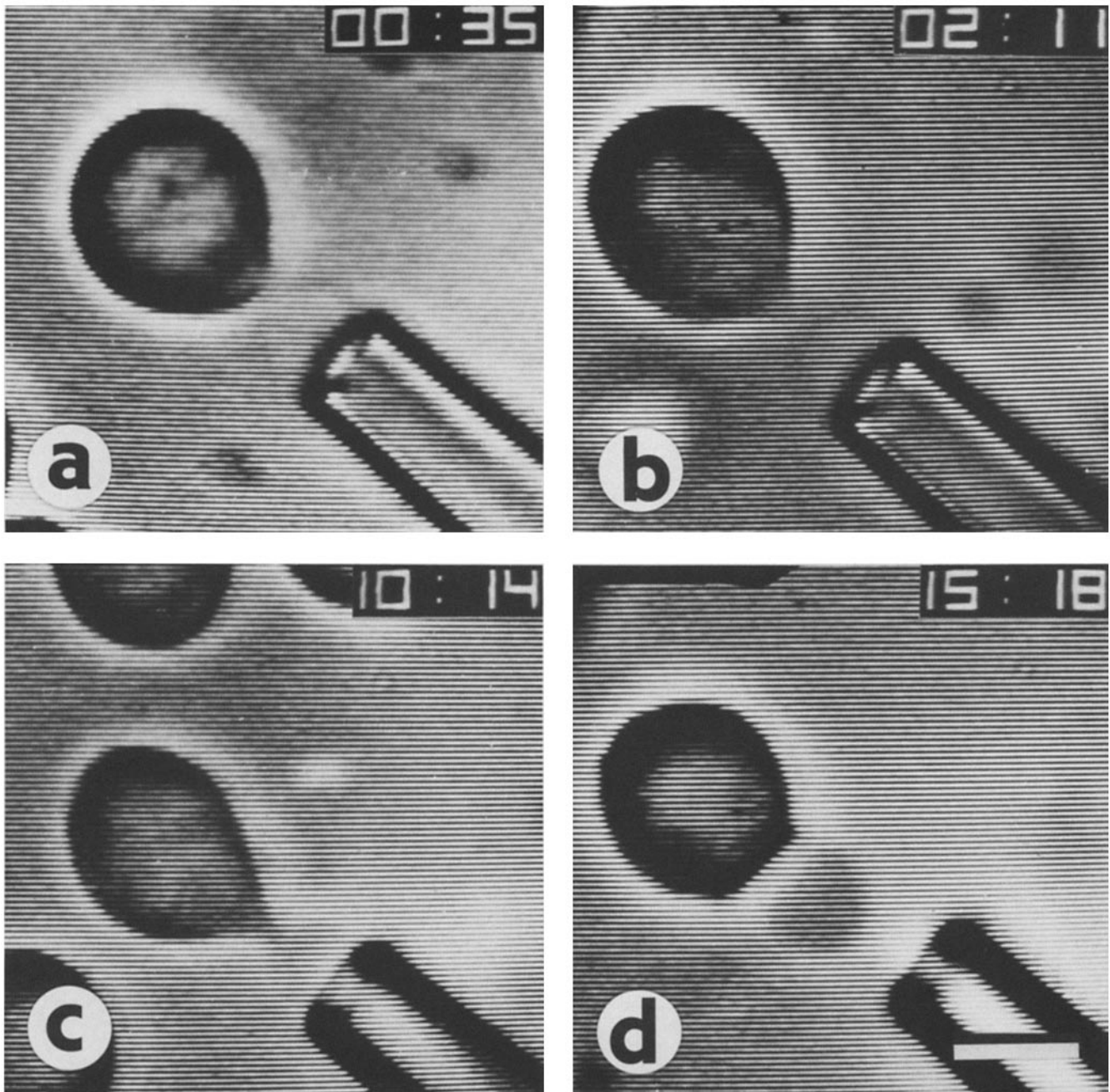


Figure 7. Micropipette suction method for adhesion measurement on four different cells. A vesicle is pushed into contact with cell at time zero, and at a defined time after that the pipette is slowly and steadily withdrawn until the vesicle either comes loose from the pipette or from the cell. Shown here are four separate cells; pipette withdrawal was begun 30 s, and 2, 10, and 15 min in a–d, respectively. Note that as the duration of contact increases, there is an increased distension of cells just before cell-vesicle or pipette-vesicle rupture. Bar, 10 μ m.

vesicle membrane has been dissolved by the dehydration and fixation protocol.) Scanning electron micrographs of RBL cells bound to fluid vesicles are shown in Fig. 12; note the much flatter appearance of the cell surface at the site of contact with vesicles.

Discussion

Contact-induced redistribution of specific membrane components is a process with several interesting biological implications (20). It is a potential means for controlling membrane

topography via cell-cell interactions, as exemplified by the localization of neurotransmitter receptors at sites of nerve-muscle contact (1, 23). In certain situations, it may also serve to enhance the strength of selective adhesion between membranes, as studied in the present experiments. Diffusion-mediated trapping due to the formation of bonds between specific components on juxtaposed membranes is about the simplest scheme that can explain contact-induced accumulation. The main thrust of this paper is the demonstration that highly selective reorganization of membrane components can be achieved, apparently without the expenditure of metabolic

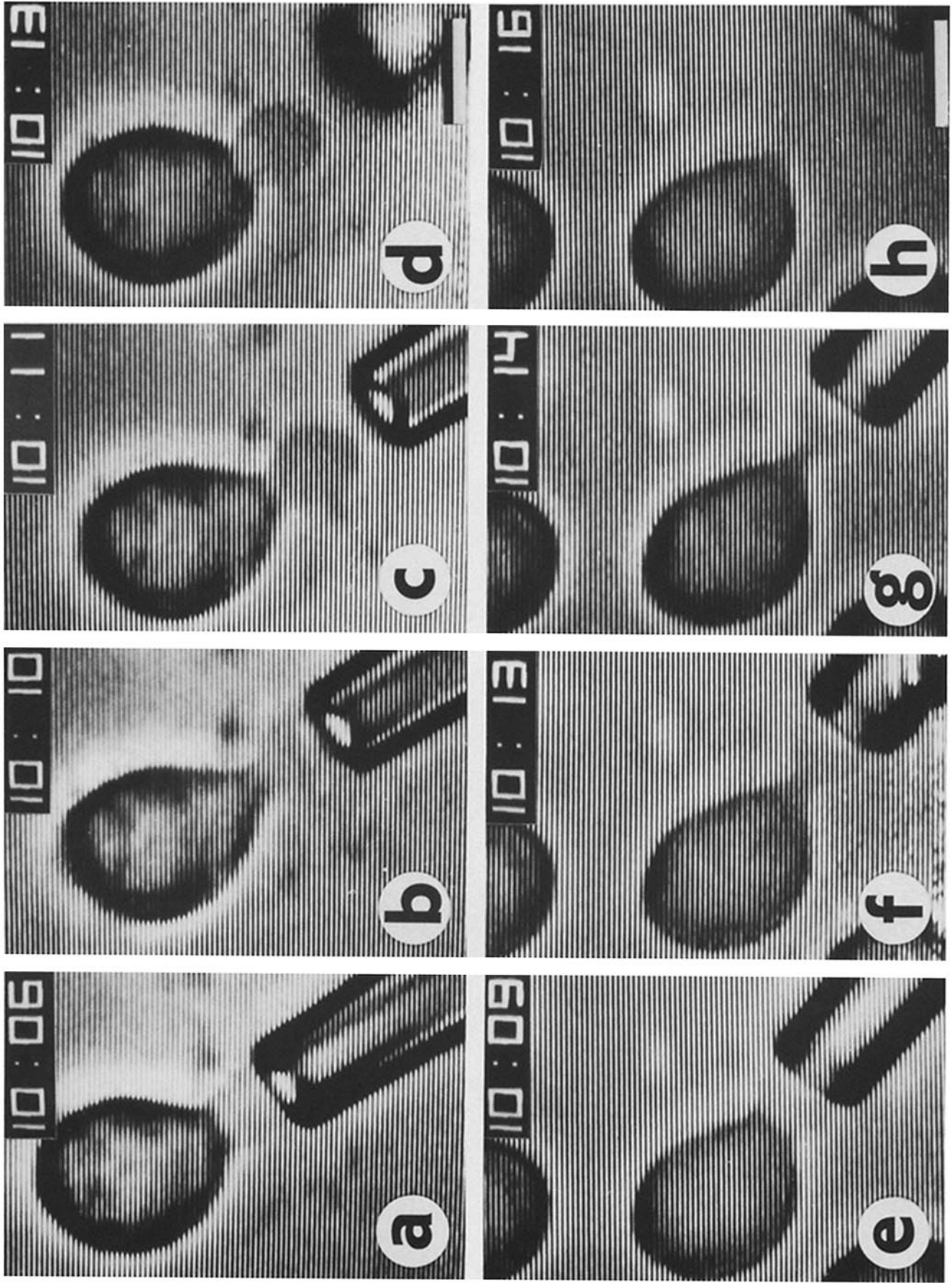


Figure 8. Time sequence of pipette withdrawal from two different cells. Pipette withdrawal was initiated at 10 min in both cases. *a-d* show vesicle remaining stuck to cell and *e-h* show vesicle remaining with pipette after coming loose from the cell. Note the relaxation of cells from their distended shape after rupture of the cell-vesicle or pipette-vesicle bond. Bar, 10 μm .

energy and without elaborate schemes involving cytoskeleton-directed motion.

Previous work has shown that contact between two cells or between a cell and a substrate can elicit marked accumulation of membrane components at the position of contact (1, 10, 22, 28, 31). What is unique about our study is that we followed the redistribution of a homogeneous molecular species as it

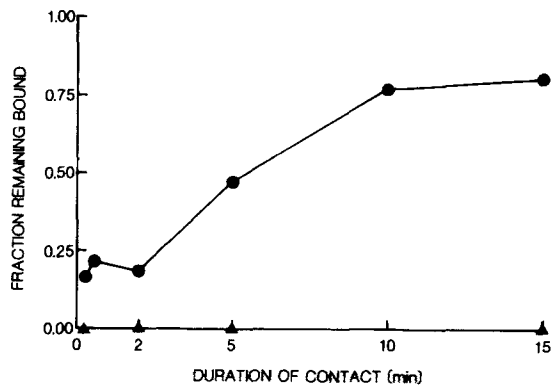


Figure 9. Kinetics of development of adhesion. The percentage of vesicles remaining stuck to cells upon pipette withdrawal is shown as a function of time after initial cell-vesicle contact. An identical suction pressure of $\sim 8 \times 10^{-5}$ dyn/ μm^2 was applied in each case. The upper curve is for cells sensitized with anti-DNP IgE and the lower curve is for cells sensitized with nonimmune rat IgE. 10–27 cell-vesicle pairs were examined at each time. The average pipette tip diameter (opening) was $3.8 \pm 0.2 \mu\text{m}$ SD ($n = 6$), the average cell diameter was $12.9 \pm 1.1 \mu\text{m}$ SD ($n = 103$), the average vesicle diameter was $6.3 \pm 1.0 \mu\text{m}$ SD ($n = 103$), and the average diameter of the contact zone was $3.6 \pm 0.5 \mu\text{m}$ SD ($n = 103$).

occurred and showed that it was a passive phenomenon. Weis et al. also observed redistribution of anti-DNP IgE on RBL cells bound to phospholipid monolayers containing DNP-lipids, but the specificity of redistribution was not explored (32).

Weis et al. emphasized the potential for increases in the local concentration of IgE receptors to trigger basophil degranulation without the formation of discrete molecular bridges between receptors (32). We are concerned here with the possibility that diffusion-mediated trapping of specific molecules is involved in the formation of stable adhesion sites between membranes. Although this idea is not new and its theoretical basis is uncomplicated (5, 9), no experiments have critically tested it. Capo et al. studied concanavalin A-mediated adhesion of rat thymocytes and concluded on the basis of a mathematical analysis that the concanavalin A had become concentrated at the zones of adherence between cells (8). However, they made no attempt to verify this conclusion by actually visualizing or directly quantifying the concanavalin A distribution. Weigel observed a marked accumulation of asialoglycoprotein receptors at the site of contact of hepatocytes with polyacrylamide substrates containing covalently bound galactose (31). He also found a systematic dependence of adhesion strength on the concentration of galactose in the gel. For lack of a suitable fluorescent probe or single-cell adhesion assay, Weigel could not follow redistribution of the receptor in real time or correlate the extent of redistribution with the adhesion strength.

The parallel increase in adhesion strength and local antibody concentration that we found in the present study provides the first direct experimental evidence for the supposition

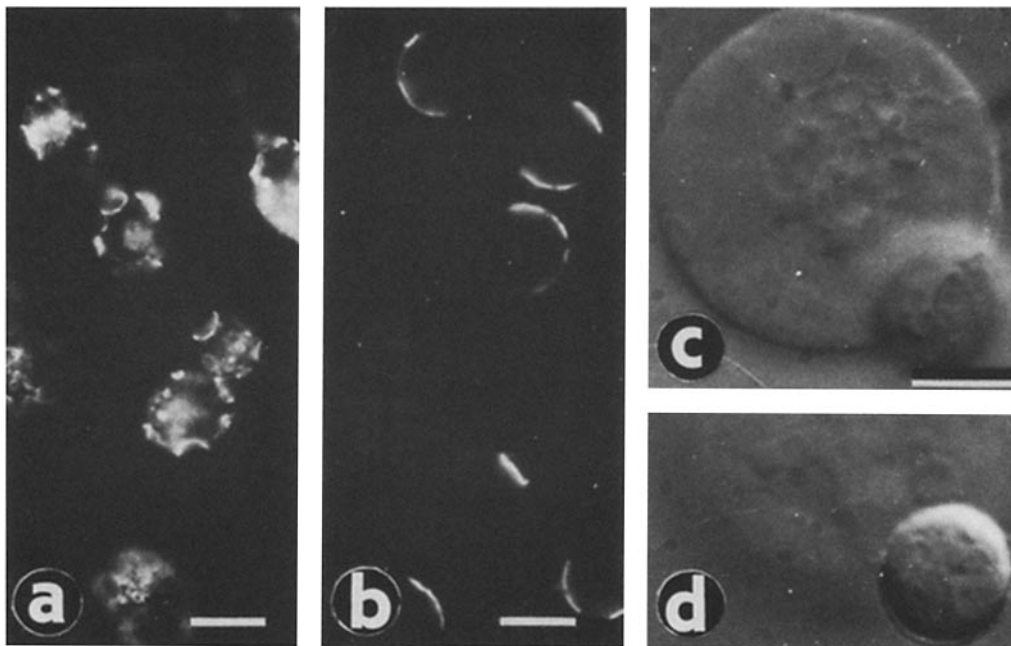
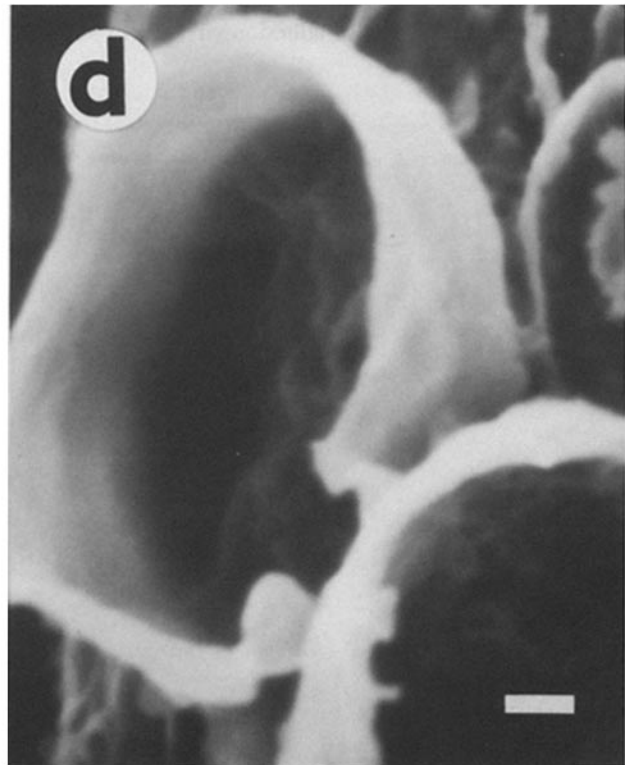
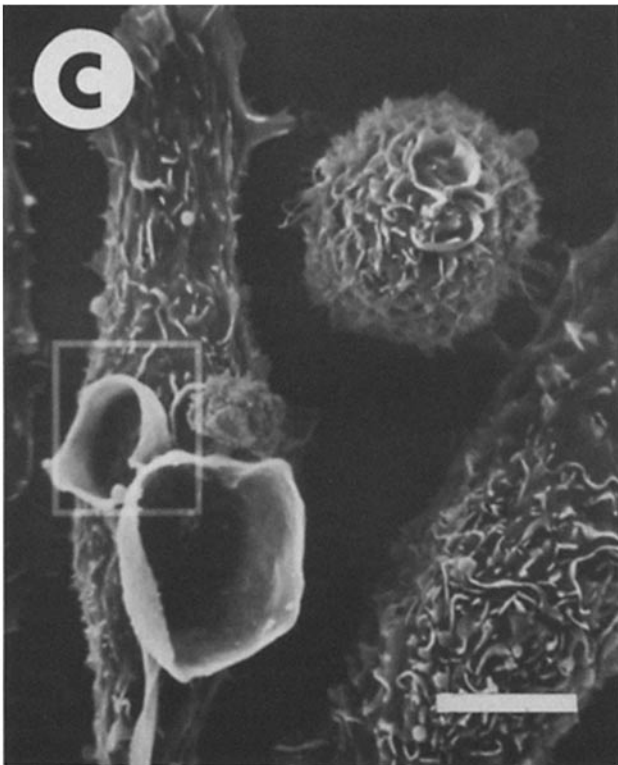
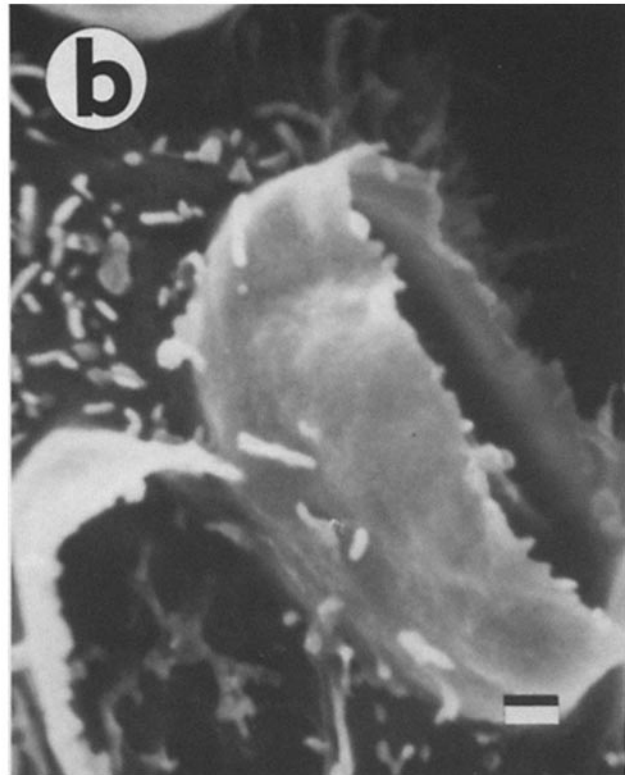
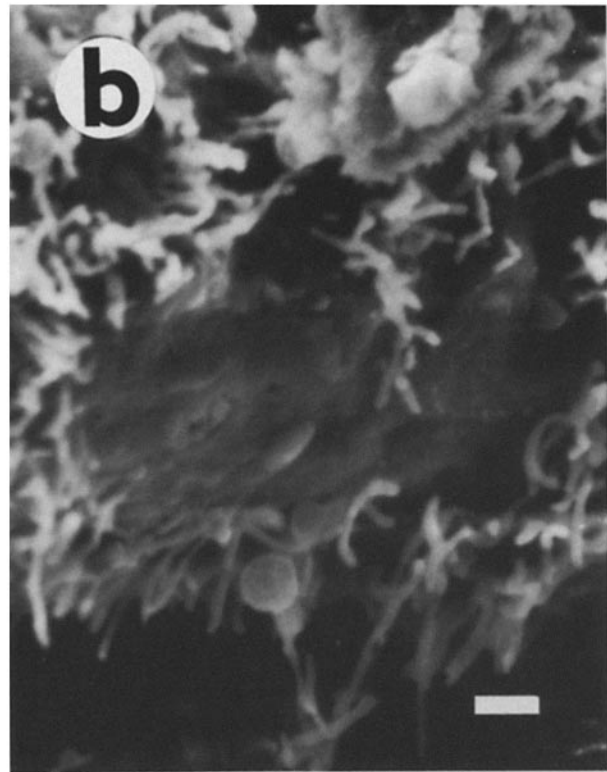
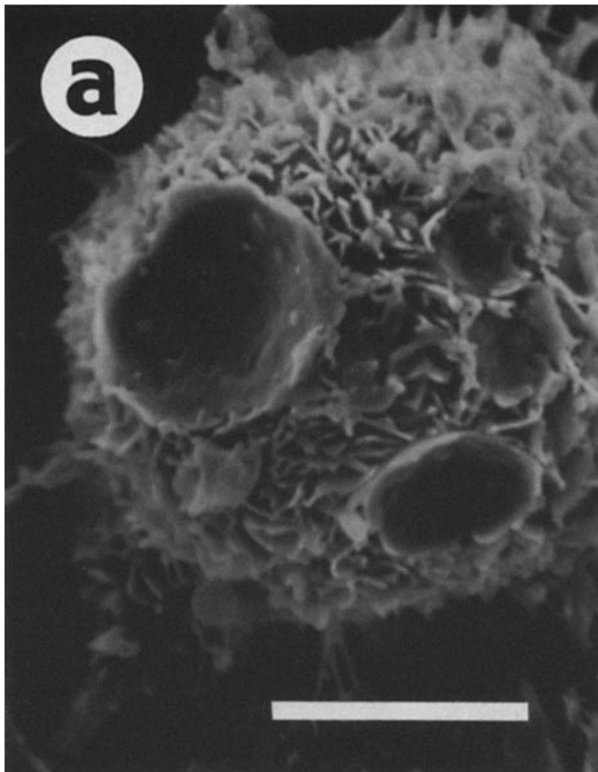


Figure 10. Solid-fluid state of the vesicle membrane influences the shape of the cell-vesicle contact zone. (a and b) Fluorescence micrographs of RBL cells labeled with Texas Red-mouse IgE (anti-DNP); after binding of either solid DPPC vesicles (a) or fluid DOPC vesicles (b). In a arcuate profiles extend away from cell surface, indicating that the cells have spread partially around the solid vesicles. (Blurry regions are due to bound vesicles that are out of the plane of focus). As indicated in b, the fluid vesicles behave in an opposite fashion to the solid vesicles, flattening out on the cell surface. Here the bright fluorescence delimits zones of contact that conform to the cell curvature rather than to the original spherical vesicle shape. Shown in c and d are Nomarski differential interference contrast micrographs of a large DOPC vesicle that has partially engulfed a cell to which it is bound. Two focal planes are shown. In c the edge of vesicle is visible on surface of cell. Bars, 10 μm .



Figures 11 and 12. (Above and opposite) SEM photos showing structure of cell surface after binding of either fluid (Fig. 12) or solid vesicles (Fig. 11). Cells have been fixed with glutaraldehyde and dehydrated with acetone; bookkeeping experiments with radioactively labeled vesicles indicated that >99% of the synthetic lipid was removed by this protocol. Craterlike collars of plasma membrane demark regions formerly occupied by vesicles. Note that fluid vesicles leave a much flatter imprint than solid vesicles. Bars: Fig. 11 (a and c) 5 μm ; (b and d) 0.5 μm ; Fig. 12 (a) 5 μm ; (b) 0.5 μm .



that long-range diffusion of specific proteins can strengthen cell-cell adhesion. Unfortunately, the system in present form does not allow an estimate of adhesion strength in absolute units, i.e., ergs per square centimeter. One would like to have such an estimate to compare with the adhesion strength calculated from the area of contact, the number density of IgE molecules at the site of contact, and the intrinsic energy of interaction between DNP and anti-DNP IgE. Such an equilibrium measurement is needed to prove unequivocally that the increase in adhesion strength is due solely to an increase in bond density.² Evans' method (12) for extracting interfacial energy densities by analyzing the shape deformation of adhering membranes does not seem immediately applicable, because as noted above the surface of the RBL cell is quite rough. It is conceivable that his analysis could be modified to suit the particulars of this system, but it may be simpler to modify the system to suit the analysis. Using the N-ethylmaleimide procedure (16), one can obtain plasma membrane vesicles from IgE-sensitized RBL cells, some of which are several microns in diameter, and most of which have a fairly smooth surface.

One complication in calculating the number of bonds between membranes concerns the area and morphology of the contact zone. Initial contact of a vesicle with an RBL cell must be with the tips or edges of the numerous surface projections that dot the cell. Such focal contacts were actually visualized by Weis et al. in their experiments using evanescent radiation to selectively excite fluorescence from IgE at the

2. Our kinetic measurements yield relative adhesion strength. We found qualitatively that suction pressures $>8 \times 10^{-5}$ dyn/ μm^2 made vesicle removal more probable and vice versa. Thus, the magnitude of the pressure differential across the pipette tip can influence the apparent kinetics of development of adhesion. Our choice of a pressure that is too low to dislodge the cells from the substrate seemed to us to be within the bounds biological relevance.

lipid monolayer-RBL cell junction (32). On the other hand, after 30 min of contact the cell and vesicle membranes have become smoothly appressed, as shown in Figs. 11 and 12. The total area of membrane-membrane contact thus increases within the same time frame that the local antibody concentration does. Further experiments using the plasma membrane vesicles described above may help to sort out the relative contributions of these two factors to the total adhesion strength.

Whether whole cells or plasma membrane vesicles are used, the present system does offer several advantages as a simple paradigm for direct, albeit qualitative, studies of specific membrane-membrane adhesion. With the micropipette technique one can control precisely the time zero for measuring the kinetics of adhesion and redistribution. This is virtually impossible to do with binding measurements made on cell populations, such as the currently popular aggregation assays. One can also study adhesion between selected cell types in a heterogeneous population. With minor improvements such as that mentioned above, one may be able to evaluate quantitatively a recent thermodynamic theory of specific cell-cell adhesion, which is strictly valid only for single cell-cell pairs (6). Several important parameters in this theory can be readily controlled with the lipid vesicle-RBL vesicle system. The density of specific antibody on the plasmalemma can be varied systematically by mixing rat and mouse IgE in the appropriate ratios before binding to the cells; the density of hapten in the lipid vesicle, its lateral mobility, and the deformability of the vesicle membrane can also be changed easily.

The above theory predicts that given sufficient membrane deformability, passive engulfment of one cell (or target vesicle) will result if the membrane-membrane adhesion is strong enough. Evans and Buxbaum have previously observed passive engulfment of small plasma membrane vesicles by eryth-

rocytes (13), and Haywood showed that liposomes containing gangliosides will passively engulf Sendai virus particles (15). When area constraints limit the extent of engulfment, the shape of the cell-vesicle contact zone is still predicted to depend upon the relative deformabilities of the cell and vesicle. The marked difference we observed in the shape of the cell-vesicle contact zone for solid vs. fluid vesicles (Figs. 10–12) is probably explicable in terms of differences in the bending resistance of the two types of artificial membrane. Whatever the explanation, it is one of the most clear cut effects of lipid fluidity on cell-vesicle interaction of which we are aware.

Finally, we noted a marked depletion of IgE-Fc receptor complexes from the noncontact regions of the cell. This depletion was most pronounced on cells that had been touched by a few hapten-bearing vesicles. This suggests that the noncontact region will become less able to adhere to other vesicles as a result of previous contact. In other words, contact-dependent local accumulation of specific molecules provides a lateral signaling mechanism to the noncontact region that contact has been made. By regulating the amount of a specific adhesion molecule on its surface a cell could thus regulate the number of permissible contacts. Such mechanisms for counting contacts may operate in the development of neuronal connections (24).

We are grateful to Dr. Evan Evans of the University of British Columbia (Vancouver) for introducing us to the use of suction micropipettes for making adhesion measurements. We are also indebted to Dr. Reuben Siraganian of the National Institutes of Health (NIH) for providing us with the 2H3 subline of RBL IV cells, to Dr. Fu-Tong Liu of the Medical Biology Research Institute (La Jolla, CA) for a sample of ascites fluid containing the anti-DNP IgE, and to Dr. Henry Metzger of the NIH for ascites fluid containing the IR162 rat IgE.

This work was supported by a grant from the NIH to Dr. McCloskey (GM 35901) and grants to Dr. Poo from the NIH (GM 30666) and the National Science Foundation (BNS-8543366).

Received for publication 23 September 1985, and in revised form 10 January 1986.

References

- Anderson, M. J., and M. W. Cohen. 1977. Nerve-induced and spontaneous redistribution of acetylcholine receptors on cultured muscle cells. *J. Physiol. (Lond.)* 268:757–773.
- Balakrishnan, K., S. Q. Mehdi, and H. M. McConnell. 1982. Availability of dinitrophenylated lipid haptens for specific antibody binding depends on the physical properties of host bilayer membranes. *J. Biol. Chem.* 257:6434–6439.
- Barsumian, E. L., C. Isersky, M. G. Petrino, and R. P. Siraganian. 1981. IgE induced histamine release from rat basophilic leukemia cell lines: isolation of releasing and nonreleasing clones. *Eur. J. Immunol.* 11:317–323.
- Bazin, H., P. Querinjean, A. Beckers, J. F. Heremans, and F. Dessy. 1974. Transplantable immunoglobulin-secreting tumours in rats. IV. Sixty-three IgE secreting immunocytoma tumours. *Immunology* 26:713–723.
- Bell, G. I. 1978. Models for specific adhesion of cells to cells. A theoretical framework for adhesion mediated by reversible bonds between cell surface molecules. *Science (Wash. DC)* 200:618–627.
- Bell, G. I., M. Dembo, and P. Bongrand. 1984. Cell adhesion. Competition between nonspecific repulsion and specific bonding. *Biophys. J.* 45:1051–1064.
- Borch, R. F., M. D. Bernstein, and H. D. Durst. 1971. The cyanohydroborate anion as a selective reducing agent. *J. Am. Chem. Soc.* 93:2897–2904.
- Capo, C., F. Garrouste, A.-M. Benoliel, P. Bongrand, A. Ryter, and G. I. Bell. 1982. Concanavalin-A-mediated thymocyte agglutination: a model for a quantitative study of cell adhesion. *J. Cell Sci.* 56:21–48.
- Chao, N.-m., S. H. Young, and M.-m. Poo. 1981. Localization of cell membrane components by surface diffusion into a "trap." *Biophys. J.* 36:139–153.
- Chow, I., and M.-m. Poo. 1982. Redistribution of cell surface receptors induced by cell-cell contact. *J. Cell Biol.* 95:510–518.
- Edelman, G. M. 1983. Cell adhesion molecules. *Science (Wash. DC)* 219:450–457.
- Evans, E. A. 1980. Minimum energy analysis of membrane deformation applied to pipet aspiration and surface adhesion of red blood cells. *Biophys. J.* 30:265–284.
- Evans, E., and K. Buxbaum. 1981. Affinity of red blood cell membrane for particle surfaces measured by the extent of particle encapsulation. *Biophys. J.* 34:1–12.
- Frazier, W., and L. Glaser. 1979. Surface components and cell recognition. *Annu. Rev. Biochem.* 48:491–523.
- Haywood, A. M. 1975. "Phagocytosis" of Sendai virus by model membranes. *J. Gen. Virol.* 29:63–68.
- Holowka, D., and B. Baird. 1983. Structural studies on the membrane-bound immunoglobulin E-receptor complex. I. Characterization of large plasma membrane vesicles from rat basophilic leukemia cells and insertion of amphipathic fluorescent probes. *Biochemistry* 22:3466–3474.
- Inman, J. K. 1974. Covalent linkage of functional groups, ligands, and proteins to polyacrylamide beads. *Methods Enzymol.* 34:30–58.
- Lewis, J. T., D. G. Hafeman, and H. M. McConnell. 1980. Kinetics of antibody-dependent binding of haptened phospholipid vesicles to a macrophage-related cell line. *Biochemistry* 19:5376–5386.
- Liu, F.-t., J. W. Bohn, E. L. Ferry, H. Yamamoto, L. A. Molinaro, L. A. Sherman, N. R. Klinman, and D. H. Katz. 1980. Monoclonal dinitrophenyl specific murine IgE antibody: preparation, isolation, and characterization. *J. Immunol.* 124:2728–2737.
- McCloskey, M. A., and M.-m. Poo. 1984. Protein diffusion in cell membranes: some biological implications. *Int. Rev. Cytol.* 87:19–81.
- McCloskey, M. A., Z.-y. Liu, and M.-m. Poo. 1984. Lateral electromigration and diffusion of Fcε receptors on rat basophilic leukemia cells: effects of IgE binding. *J. Cell Biol.* 99:778–787.
- Peng, H. B., P.-c. Cheng, and P. W. Luther. 1981. Formation of ACh receptor clusters by positively charged latex beads. *Nature (Lond.)* 292:831–834.
- Poo, M.-m. 1982. Rapid lateral diffusion of acetylcholine receptors in the embryonic muscle cell membrane. *Nature (Lond.)* 295:332–334.
- Poo, M.-m. 1985. Mobility and localization of proteins in excitable membranes. *Annu. Rev. Neurosci.* 8:369–406.
- Reeves, J. P., and R. M. Dowben. 1969. Formation and properties of thin-walled phospholipid vesicles. *J. Cell. Physiol.* 73:49–60.
- Schroeder, W. A., and J. LeGette. A study of the quantitative dinitrophenylation of amino acids and peptides. 1953. *J. Am. Chem. Soc.* 75:4612–4615.
- Schwartz, B. A., and G. R. Gray. 1977. Proteins containing reductively aminated disaccharides. *Arch. Biochem. Biophys.* 181:542–549.
- Singer, S. J. 1976. The fluid-mosaic model of membrane structure: Some applications to ligand-receptor and cell-cell interactions. In *Surface Membrane Receptors*. R. A. Bradshaw, W. A. Frazier, R. C. Merrell, D. I. Gottlieb, and R. A. Hogue-Angeletti, editors. Plenum Press, NY. 1–24.
- Tank, D. W., E.-s. Wu, and W. W. Webb. 1982. Enhanced molecular diffusibility in muscle membrane blebs: release of lateral constraints. *J. Cell Biol.* 92:207–212.
- Van Lenten, L., and G. Ashwell. 1971. Studies on the chemical and enzymatic modification of glycoproteins. A general method for the tritiation of sialic acid-containing glycoproteins. *J. Biol. Chem.* 246:1889–1894.
- Weigel, P. H. 1980. Rat hepatocytes bind to synthetic galactoside surfaces via a patch of asialoglycoprotein receptors. *J. Cell Biol.* 87:855–861.
- Weis, R. M., K. Balakrishnan, B. A. Smith, and H. M. McConnell. 1982. Stimulation of fluorescence in a small contact region between rat basophilic leukemia cells and planar lipid membrane targets by coherent evanescent radiation. *J. Biol. Chem.* 257:6440–6445.