LIPID VESICLE-CELL INTERACTIONS

II. Induction of Cell Fusion

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ABSTRACT

The ability of lipid vesicles of simple composition (lecithin, lysolecithin, and stearylamine) to induce cells of various types to fuse has been investigated. One in every three or four cells in monolayer cultures can be induced to fuse with a vesicle dose of about 100 per cell. At such dosages and for exposures of 15 min to 1 h, vesicles have essentially no effect on cell viability. Under anaerobic conditions, these cells lyse rather than fuse. Avian erythrocytes are readily fused with lipid vesicles in the presence of dextran. Fusion indices increase linearly with the zeta potential of the vesicles (increasing stearylamine content), indicating that contact between vesicle and cell membrane is required. Fusion indices increase sublinearly with increasing lysolecithin content. Divalent cations increase fusion indices at high vesicle doses. The data presented are consistent with the hypothesis that cell fusion occurs via simultaneous fusion of a vesicle with two adhering cell membranes.

Cell fusion occurs in normal animal development during fertilization and bone and muscle differentiation, and in a wide variety of pathological situations, including infection by viruses and bacteria and in both benign and malignant tumors (21). The paramyxovirus Sendai is one of the agents that has been most frequently utilized to induce cell fusion in vitro both for purposes of investigating fusion processes per se (4) and of producing heterokaryons for studying animal cell genetics (6). We have sought even greater simplicity than that afforded by Sendai and have developed simple membrane vesicles which do not contain components of unknown structure but nevertheless possess fusion-promoting capabilities. In the preceding paper, we described the ability of lipid vesicles containing lecithin, lysolecithin, and stearylamine (SA) to mimic two of the membrane-associated properties, hemagglutination (HA) and hemolysis, of Sendai virus. Here, we describe the ability of these vesicles to mimic the induction of cell fusion by Sendai virus.

This study represents somewhat of an extension of the investigations of Ahkong et al. (1, 2), Poole et al. (20), and others (5, 8) on the use of lysolecithin to induce cell fusion. It was evident from those investigations that, although lysolecithin has deleterious effects on cells, it nevertheless induced the appropriate changes in their membranes that permitted them to fuse. We reasoned that if the effect of lysolecithin could be localized by incorporating this molecule in a lipid vesicle which had the ability to adhere to two cells simultaneously, cell fusion could occur without severe generalized membrane effects. Papahadjopoulos et al. (18) have reported similar results with phospholipid

vesicles containing acidic lipids such as serine or glycerol phosphatides.

MATERIALS AND METHODS

Phosphatides used in this study were diisostearoylphosphatidylcholine (L) and isostearoyllysophosphatidylcholine (LL). Synthetic methods, commercial sources of lipids, and zeta potential measurements were as described elsewhere (11). Liposomes were prepared essentially as described before (11), except that the dispersions were sonicated for a longer period (45 min at 20–25°C) to yield a population of mainly unilamellar vesicles (15). To check for the possibility of chemical degradation of one or more of the lipid components during sonication, a sonicated dispersion was extracted with chloroform-methanol and, after evaporation of the organic solvent, dispersed again into aqueous solution by hand shaking. The zeta potentials of these liposomes were found to be identical to those before sonication. It is thus assumed that zeta potentials of the lipid vesicles used here are independent of sonication time even though potentials of highly sonicated dispersions cannot be determined directly by microelectrophoresis. In addition, thin-layer chromatography revealed no changes with sonication (although this method is far less sensitive to the production of fatty acid than is electrophoresis). None of the lipids used in this study are subject to autoxidation. Dilutions of liposomes were made in Balanced Salt Solution (BSS: 0.12 M NaCl, 6 mM KCl, 6 mM glucose, 5 mM $MgSO_4$, 10 mM $CaCl_2$, 20 mM Tricine, pH 7.8) or Sucrose Fusion Medium (SFM: 0.28 M sucrose, 5 mM $MgSO_4$, 10 mM $CaCl_2$, 20 mM Tricine, pH 7.8) unless otherwise stated. Lipid composition of vesicles is given in weight percent.

Cells

Rooster blood was withdrawn into heparin; the erythrocytes were washed three times in BSS and suspended in BSS at a concentration of 10% (vol/vol). Ehrlich ascites tumor cells (EATC) were grown in adult Swiss mice. Weekly passages of 0.2 ml of freshly harvested ascitic fluid were made intraperitoneally. For fusion experiments, cells were harvested on the 7th–10th day after inoculation. The cells were freed of contaminating erythrocytes and leukocytes by washing three times in BSS. The cells were suspended at a final concentration of 10% (vol/vol) in BSS.

Rabbit kidney (RK), human epithelioid carcinoma (KB), and African green monkey kidney (VERO) cells were grown as monolayer cultures in 25 cm^2 plastic flasks. Cultures were grown to confluence and maintained in Eagle's minimal essential medium (MEM) supplemented with 10% heat-inactivated fetal calf serum.

Erythrocyte Ghosts

Freshly drawn human erythrocytes were washed in phosphate-buffered saline (PBS) as described previously (11), and resuspended at a concentration of 50 vol % in isotonic Tris buffer, pH 7.4.

Fusion Conditions

The ability of liposomes to induce fusion was monitored in both suspension and monolayer cultures. For fusion of cells in suspension, a modification of the technique of Okada et al. (17) for Sendai virus-induced fusion was used. 1 ml of the 10% suspension of EATC in BSS, containing about 5×10^6 cells, was added to a number of glass culture tubes containing 5 ml of MEM. The cells were preincubated at 37°C with constant shaking for 30 min. Next, the cells were pelleted and resuspended in 1 ml of cold SFM. 1 ml of the liposome dispersion to be tested (1.2 mg lipid/ml SFM) was added to each tube, and the cells were allowed to agglutinate in the cold for 30 min. The tubes were then transferred to a 37°C water bath and incubated for an additional 60 min with constant shaking.

For fusion of cells in monolayer cultures, the methods of Kohn were used (9) except that liposomes were substituted for virus. Confluent monolayer cell cultures were washed several times with prewarmed BSS. 1 ml of the liposome dispersion was added to each flask along with 5 ml of BSS. After an incubation period of between 15 and 60 min (37°C), the monolayers were washed with warm BSS, and fresh maintenance medium was added. The cells were incubated for an additional 24 h before fixation and staining.

Fusion of avian erythrocytes was carried out using a method similar to that reported by Ahkong et al. (2). Fresh, washed rooster cells were suspended at a concentration of 10% in BSS (which had been adjusted to pH 5.5 with potassium acetate) containing 80 mg/ml dextran (low fraction, mol wt 75,000). To 1 ml of the cell suspension, 0.25 ml of the liposome dispersion was added. The mixture was incubated at 37°C for several hours. At intervals, samples were removed and examined by phase-contrast microscopy.

Assay for Extent of Cell Fusion

The extent of liposome-induced fusion of EATC was determined as described by Okada et al. (17). At times, it was necessary to break up cell clumps by gently pipetting the cultures in the presence of high salt (0.3 M NaCl). A modification of the method of Kohn (9) was used to assay fusion in monolayer cultures. Monolayers were treated with a solution of 10^{-4} M EDTA in 0.14 M NaCl for 2 min at room temperature. The cells were then fixed in absolute methanol for 25 min and stained with May Grünwald-Giemsa stain. 1,000–2,000 cells and their nuclei were counted in each flask. Fusion indices (FI) for EATC and monolayer cells were calculated as described by Okada et al. (17) and Kohn (9), respectively.

Cell Viability

Viability of cultured cells was determined by the common Trypan blue exclusion technique (19).

HA

HA titers of lipid dispersions were determined as described previously (11).

Microscopy

Techniques used for the examination of thin sections under phase and electron microscopes have been described elsewhere (11, 12, 13). For the examination of treated erythrocyte suspensions, samples were introduced into a hemocytometer and photographed under phase and Normarski optics.

Virus

An egg-adapted strain of Sendai virus was used. The procedures for propagation and purification of the virus were as described by Okada et al. (17).

RESULTS

Liposome-Induced Fusion of Cells in Suspension

Lecithin liposomes (no net charge) did not fuse EATC. Liposomes composed of L and varying amounts of SA also showed little fusion activity although such positively charged vesicles caused extensive cell agglutination. Uncharged and negatively charged (5% dicetylphosphate, DCP) lipid vesicles containing 20% LL were similarly devoid of fusion activity. In contrast, liposomes containing both LL and SA promoted cell fusion. The relationship between liposome-induced cell fusion (FI_{susp}) and LL content of positively charged liposomes is shown in Fig. 1. The dependence of FI_{susp} on the zeta potential of liposomes (varied by increasing the proportion of SA) containing 20% LL is plotted in Fig. 2. The potentials plotted were obtained by microelectrophoresis of hand-shaken lipid dispersions before sonication as described in Materials and Methods. From these data, it is clear that the fusion activity of liposomes increases with both LL content and positive surface potential.

Viability data for EATC are plotted in Figs. 1 and 2. As may be seen, the percent of the cells that are viable after liposome treatment drops as the proportions of both SA and LL in the liposomes are increased. Since the fusion index overestimates the fraction of fused cells in proportion to the fraction of lysed cells, it is helpful to correct

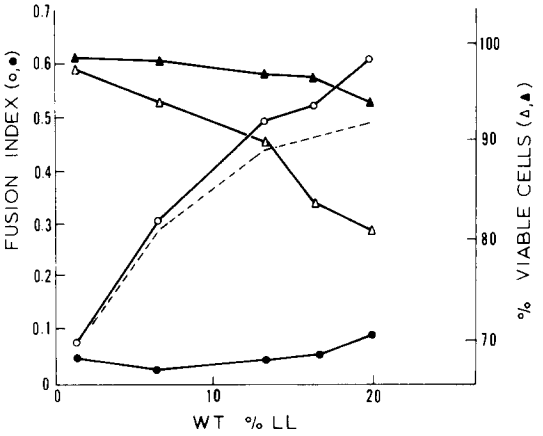

FIGURE 1 Dependence of fusion activity and cell viability upon lysolecithin (LL) content of lipid vesicles. FI_{susp} (open circles) and percent viable cells (open triangles) are plotted against % lysolecithin for vesicles containing 5% SA (zeta potential ≅ 50 mV). Filled circles and triangles are corresponding plots for control vesicles which contained no SA (zeta potential ≅ 0). Cells (EATC) were incubated with vesicles for 60 min at 37°C in SFM as described in Materials and Methods. Lipid vesicle concentration was 0.6 mg/ml. Corrected fusion index (FI × fractional viability) is given for SA-containing vesicles as the dotted line.

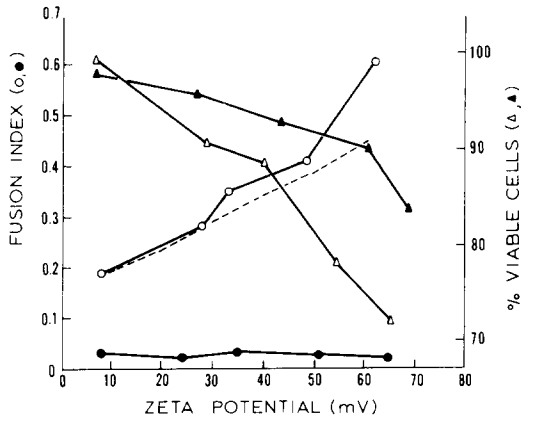

FIGURE 2 Fusion activity (circles) and cell viability (triangles) as a function of zeta potential of lipid vesicles measured before sonication containing 20% lysolecithin (open circles and triangles) and no lysolecithin (filled circles and triangles). Incubation conditions are the same as those described in the legend to Fig. 1. Corrected fusion index is given for LL-containing vesicles as the dotted line.

FI to reflect the number of fused cells that are actually viable. This can be done approximately by simply multiplying the fusion index by the fraction of viable cells. When this is done for the data

representing vesicles of ternary composition of Figs. 1 and 2, the dotted lines shown in those figures are obtained. Throughout the ranges of the figures, the corrected index increases approximately linearly with zeta potential. The relationship between corrected FI and LL content, on the other hand, is approximately hyperbolic.

Table I reveals that for EATC the corrected fusion index is maximal at a liposome concentration of about 0.6 mg/ml. This maximum is due to a sharp dropoff in viability above 1 mg/ml. Although fusion as a function of lipid concentration was not determined outside the concentrations given in Table I, this was done for monolayer cultures (see below), and it was found that concentrations could be reduced by factors of 100 or 1,000 without reducing the degree of fusion by more than about 20%. The corrected fusion index also drops when divalent ions are omitted from the medium, again because of increased lysis, although by reducing the liposome concentration, significant degrees of fusion may be obtained without too severe a loss of cells.

As would be predicted from the hemolytic effects described in the previous paper (11), liposomes causing significant degrees of fusion with minimal lysis became highly lytic to cultured cells under anaerobic conditions.

For purposes of comparison with lipid vesicles, a few fusion experiments were done with Sendai virus. At virus doses of 128 and 2,000 HA U/ml, we obtained FI of 1.2 and 2.4, respectively. For the LL- and SA-containing vesicles of Fig. 1, the corresponding HA titer was 10 U/ml at the very most (see Discussion).

EATC polykaryons formed by liposome treatment were also examined to determine whether they remain viable after treatment or whether such cells are more likely to die subsequently. The polykaryons were found to remain viable for 24 h in MEM at 37°C; after 24 h, both control and experimental cultures began to die. It thus appears that the period of contact with liposomes in the fusion medium is the most crucial and that cells that survive treatment are subsequently as viable as untreated cells.

Fig. 3 is an electron micrograph of a thin section of a liposome-induced EATC polykaryon. Like virus-induced polykaryons, liposome-induced polykaryons occasionally appear pleomorphic rather than spherical and often exhibit areas of the surface rich in microvilli. In our experience, Sendai virus- and liposome-induced EATC polykaryons are indistinguishable.

Liposome-Induced Fusion of Cells Grown as Monolayer Cultures

RK, VERO, and KB cells were grown to confluence and treated with liposomes composed of various proportions of SA, LL, and L. The results of such experiments are shown in Table II. These data again reveal that only those lipid vesicles which are positively charged and contain LL are active to any significant extent in cell fusion. Cell viability data are also given in Table II. FI of about 0.4 are easily attained with negligible cell death (a few percent or less). As was the case with EATC, extensive cell lysis occurred with vesicles containing more than 15% SA and 30% LL, however, we have not investigated the effects of such liposomes at concentrations lower than those given in the table. It is possible that such potent vesicles could be efficient at fusion at very low concentrations.

TABLE I

Fusion of EATC by Lipid Vesicles

Vesicle composition*	Vesicle concentration mg/ml	Medium‡	Fusion index	Viability	Corrected fusion index§
wt %				%	
100:0:0	0.6	SFM	0.00	93	0.00
95:0:5	0.6	SFM	0.04	94	0.38
70:20:5	0.6	SFM	0.54	84	0.45
70:20:5	1.2	SFM	0.58	73	0.42
70:20:5	2.4	SFM	–	<10	–
70:20:5	1.2	SFM – M^{++}	–	<10	–
70:20:5	0.3	SFM – M^{++}	0.48	75	0.36

* Composition given as the ratio, lecithin:lysolecithin:stearylamine.

‡ $-M^{++}$ indicates that calcium and magnesium were omitted from the medium.

§ Corrected fusion index = (Fusion index) (% viability)/100.

Corrected FI (again taken as the product of the fusion index and the fraction of viable cells) are also included in Table II. These are about 0.4 under the best circumstances for the three cell types tested.

The relationship between liposome concentration and the degree of fusion of KB cell monolayers treated with SA-LL-L (5:20:75) vesicles is shown in Fig. 4, where concentrations are given in mg lipid/flask and the volume of medium was 6 ml. The extent of fusion rises rather rapidly as the lipid concentration is increased from 10 ng to 1 μg

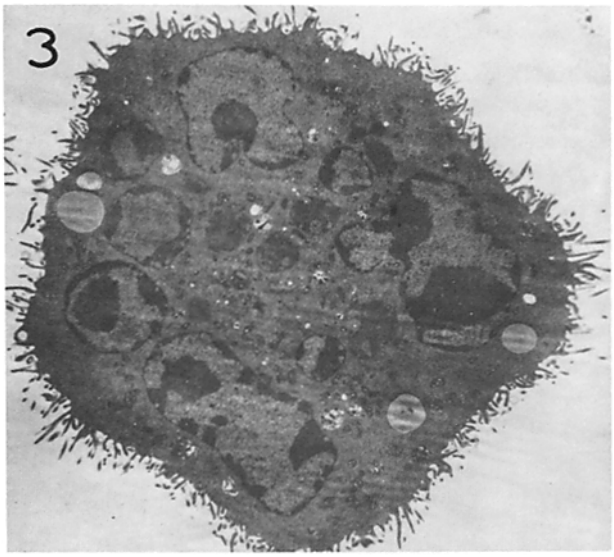

FIGURE 3 Electron micrograph of a lipid vesicle-induced EATC homopolykaryon. EATC were treated with vesicles composed of 20% LL, 5% SA, and 75% L as described in Materials and Methods. Gold-colored sections were stained with lead citrate and uranyl acetate. Cell contains at least four nuclei. × 2100.

TABLE II

Fusion of Cells in Monolayer Culture by Lipid Vesicles

Cell type	Vesicle composition*	Vesicle concentration	Medium‡	Fusion index	Viability	Corrected fusion index§
	wt %	*mg/ml*			%	
KB	100:0:0	0.1	BSS	0.04	100	0.04
KB	95:0:5	0.1	BSS	0.03	98	0.03
KB	75:25:0	0.1	BSS	0.08	90	0.07
KB	70:20:5	0.1	BSS	0.47	85	0.40
RK	100:0:0	0.1	BSS	0.09	100	0.09
RK	70:20:0	0.1	BSS	0.43	87	0.37
VERO	100:0:0	0.1	BSS	0.09	100	0.09
VERO	70:20:5	0.1	BSS	0.40	94	0.38
VERO	70:20:5	0.3	BSS	0.42	82	0.34
VERO	70:20:5	0.1	BSS − M^{++}	0.43	73	0.31
VERO	70:20:5	0.3	BSS − M^{++}	−	<10	−
VERO	70:20:5	0.3	SFM	−	<10	−

* See Table I.
‡ See Table I.
§ See Table I.

per flask and then more slowly as the concentration is further increased to 1 mg per flask. It is noteworthy that about 30% of the cells can be fused with as little as 1 μg per flask (167 ng/ml medium). Cell death is negligible at lipid concentrations less than 0.01 mg/ml, yet the fusion index is nearly maximal at this concentration. Under the same condition as used for the experiments of that figure, Sendai virus at doses of 128 and 2,000 HA U/ml gave FI of 0.67 and 1.21, respectively. Lipid vesicles at a concentration of 0.01 mg/flask correspond to less than 1 HA U/ml (see Discussion).

Polykaryons formed by treating KB cell monolayers with liposomes were found to remain viable for at least 5 days (after which time all cultures were fixed and stained) under normal culture conditions. The majority of polynuclear cells in the treated cultures appeared normal under the light microscope, but a few exhibited an unusually high degree of intracellular vacuolization. After treatment with concentrated liposome dispersions for prolonged periods, some cells exhibited large cytoplasmic lipid droplets. Fig. 5 is a light micrograph of several typical liposome-induced KB polykaryons fixed and stained as described in Materials and Methods.

The effect of divalent cations was tested in the case of VERO cells. As was true for EATC, cells are more prone to lysis in the absence of divalent cations (cf. lines 9 and 11, Table II), although significant fusion can be obtained with lowered vesicle concentration (cf. lines 8 and 10, Table II).

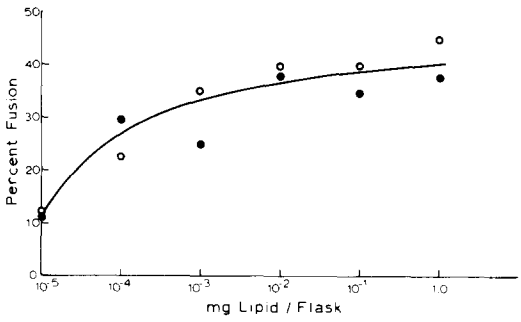

FIGURE 4 Dependence of fusion activity on lipid vesicle dose. Percent fusion ($FI_{mono} \times 100$) of KB cells was determined after treatment of the monolayers with varying concentrations of liposomes composed of 20% LL, 5% SA, and 75% L. Volume of medium was 6 ml. Fusion conditions were the same as described in the legend to Fig. 1. Open and closed circles represent duplicate samples.

Liposome-Induced Fusion of Avian Erythrocytes

Rooster erythrocytes are rapidly lysed when treated with SA-LL-L liposomes in the SFM. Lysis also occurs in BSS but at a much slower rate. After a procedure of Ahkong et al. (1), we have used low molecular weight dextrans to prevent lysis during fusion of erythrocytes by lipid vesicles.

Rooster erythrocytes were suspended in BSS containing 80 mg/ml dextran and incubated in the presence of vesicles composed of 12% SA, 25% LL, and 63% L for several hours at 37°C. Under these conditions, the cells immediately clump but do not lyse. After several minutes of incubation, the cells round up, and many begin to fuse, forming rather amorphous polynuclear cells which retain hemoglobin. After a 60-min incubation, many polynuclear cells are visible (Fig. 6). Less than 10% of the cells lysed.

DISCUSSION

The experiments described above are a logical extension of those of the preceding paper (11) in which evidence was presented that liposomes composed of diisostearoyllecithin, isostearoyllysolecithin, and SA have properties that closely mimic the HA and hemolysis properties of a paramyxovirus such as Sendai virus. Since Sendai virus causes fusion of actively metabolizing cells in culture, it was anticipated that lipid vesicles might mimic Sendai virus in this respect as well. This expectation was realized, particularly in the case of cells in monolayer culture, where about half of the cells can be induced to fuse with practically no lysis.

Under the conditions we have used to investigate fusion by vesicles, the degree of fusion depends very much on the cationic charge of the vesicle. The omission of SA leads to a very significantly reduced degree of cell fusion. This argues very strongly for the proposition that vesicles must adhere to the cell membrane for induction of cell fusion. It is equally obvious that mere adhesion of vesicle and cell membrane is insufficient for fusion, for vesicles containing only SA and L, which can be prepared to produce various degrees of cell aggregation, are devoid of fusion activity.

These results and those presented in the previous paper (11) suggest that the SA content of lipid vesicles is responsible for their ability to cause cells to adhere to one another and that the LL content is responsible for the subsequent fusion of adhering membranes. Although these ex-

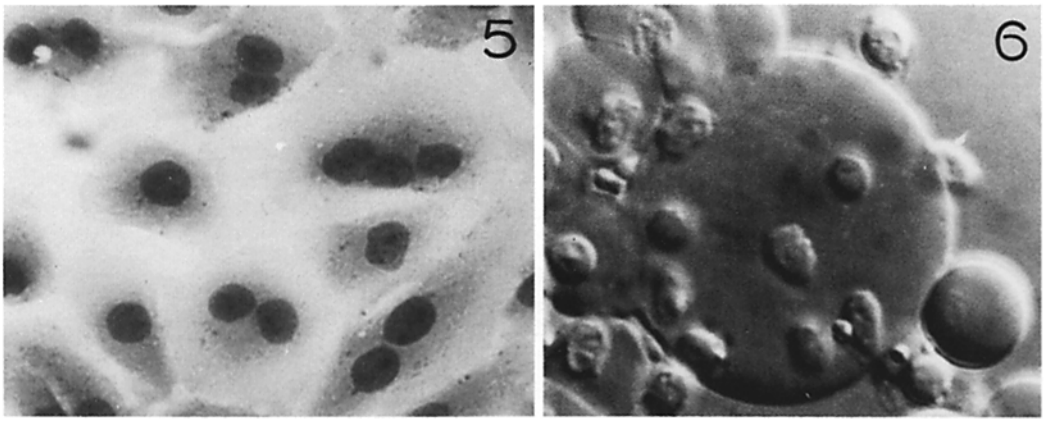

FIGURE 5 Photomicrograph of a KB cell monolayer treated with 1.0 mg/ml lipid vesicle dispersion for 30 min in BSS. The liposomes were composed of 20% LL, 5% SA, and 75% L. After liposome treatment, the cells were washed, fixed in methanol, and stained with May Grünwald-Giemsa stain. Polynuclear cells are visible in the culture. × 900.

FIGURE 6 Nomarski differential-interference micrograph of lipid vesicle-treated rooster erythrocytes. The cells were incubated for 60 min at 37°C in the presence of 1.0 mg/ml lipid vesicles composed of 12% SA, 25% LL, and 63% L in BSS containing 80 mg/ml dextran. × 1100.

periments do not distinguish among the several possible modes of cell fusion, it is plausible to assume that it occurs via simultaneous or near-simultaneous fusion of two cell membranes with a lipid vesicle. Evidence that L-LL-SA vesicles can fuse with cell membranes is presented in the following paper (12).

The mechanism by which micellar solutions of LL may induce cell fusion has been discussed by Lucy (10). Although we have used bilayer lipid vesicles with LL incorporated in them rather than free LL, it is possible that some of the same considerations apply to both systems. We would agree with Lucy (10) to the extent that membranes firmly committed to a compact bilayer structure are not likely to fuse readily, but we see no reason to invoke perturbation as extreme as the micellar structure he proposes in his mechanism of fusion. Until more information is available on membranes that fuse, we prefer to discuss fusion in terms of the general concepts of energetics rather than in terms of detailed hypothetical mechanisms.

Any perturbation by lysolecithin, for example, of even one membrane of a fusing pair that diminishes its commitment to a well-ordered structure, would be expected to raise the free energy of that membrane. Given that after fusion this perturbation is relaxed by, say, diffusion of the perturbing species away from the site of fusion, the effect of the perturbation would be to raise the free energy of the prefusion state relative to the less perturbed postfusion state. This would not only provide a driving force, but also lower the activation energy for the process in the direction of fusion. Thus, in thermodynamic if not in mechanistic terms, the fusion-promoting effect of substances such as lysolecithin is readily understood. On this basis, it is not surprising that substances like gangliosides (14) apparently promote fusion of lipid vesicles with each other. One might also reasonably expect that the small glycoprotein that is thought to be crucial in fusion-related activities of Sendai virus (7, 22) will be found to be accommodated into lipid bilayers only at the cost of some disruption of the latter.

When compared on the basis of number of particles at low doses, lipid vesicles and Sendai virus appear to have similar cell fusion-inducing efficiencies. Although comparison of vesicles and Sendai virus on the basis of particle numbers is somewhat uncertain because a determination of the size distribution of vesicles used in this study was not made, an approximate comparison is nevertheless possible. The minimum diam of bilayer vesicles is about 20 nm (15). Although our sonication conditions were somewhat milder than those necessary to produce a uniform population of such small vesicles, they were sufficient to reduce the turbidity well into the plateau region that follows

initial elimination of most of the large multilayer liposomes. A conservative estimate would be 10 times fewer vesicles than the maximum possible. This would correspond to about 3×10^{13} vesicles per mg lipid. Fusion of every fourth cell in monolayer culture requires 10^{-4} mg or at least 10^9 vesicles. At 10^7 cells per flask (25 cm^2), at least 100 vesicles per cell are required. At a dose of 100 particles per cell, Sendai virus induces fusion of, on the average, every other cell. At these low doses, then, virus and vesicle have similar cell fusion efficiencies. At higher doses, however, the virus is significantly more efficient. This is because, even with very large doses of vesicles, FI greater than about 0.6 could not be obtained, whereas with only moderate doses of virus, FI greater than 1.0 were found.

The differences between virus and vesicle at high doses cannot be that the degree of fusion is limited by vesicle-induced cytolysis; in the case of monolayer cells, the extent of fusion is close to maximal at concentrations at least 10 times less than those which lead to death of more than a few percent of the cells. A more plausible reason for the difference is that, at high doses, cells are so heavily coated with vesicles that their net surface charge becomes positive and adjacent cells repel one another. Reversal of cell surface charge at high vesicle concentrations is described in the previous paper (11).

It is possible that the difference between virus and vesicles could be diminished by proper choice of the length of time that lipid dispersions are sonicated. At the outset of this study, it appeared that a high proportion of small, unilamellar vesicles would give maximal interaction of a given amount of lipid with a cell surface and hence be more effective than coarser dispersions which would contain substantial proportions of multilayer liposomes. We therefore sonicated lipid dispersions for rather long times. In retrospect, our reasoning may have been erroneous. Subsequently, we found that the HA titer of a lipid dispersion goes through a maximum with increasing sonication time, the maximum HA titer being about 10 times that of unsonicated dispersions and about 50 times that of highly sonicated dispersions. HA titer is a measure of cell-vesicle-cell interaction, and since vesicle-cell adhesion is clearly required for fusion, vesicles may be considerably more efficient in inducing cell fusion when sonicated to the maximum HA titer. With regard to fusion per se, there is apparently nothing unusual about small vesicles that result from long or intensive sonication, for even unsonicated dispersions induce cell fusion. In addition to optimizing sonication conditions, it is likely that the efficiency of vesicle-induced cell fusion could be increased substantially by changes in the fusion medium. The medium we used for monolayer fusion contained a relatively high concentration of electrolyte. A lower concentration would increase vesicle-cell adhesion (11) and would be expected to likewise increase fusion efficiency.

Liposomes may offer an advantage over Sendai virus in the preparation of heterokaryons. Since cells coated with positively charged liposomes themselves become positively charged (11), the addition of liposome-coated cells of one type to untreated cells of a second type should greatly favor heterokaryon formation over homokaryon formation. The latter process should be diminished under such conditions because cells of the same type would have the same charge and therefore not adhere to one another.

Both Sendai virus (3) and lipid vesicles cause hemolysis (11) or fusion of osmotically unprotected or protected (dextran) erythrocytes, respectively. Both agents thus constitute osmotic challenges to cells, and it is therefore not surprising that cultured cells whose volume regulation capacity is handicapped by an anaerobic atmosphere are lysed by lipid vesicles.

Divalent cations increase the extent of fusion induced by both virus (16) and vesicles. In the latter case at least, the effect seems to result from general stabilization of cell membranes; divalent cations diminish lysis of cells that have been treated with vesicles.

Papahadjopoulos et al. (18) have investigated the ability of vesicles composed of a variety of natural and synthetic lipids to induce the fusion of BHK hamster cells and 3T3 and L929 mouse cells in both suspension and monolayer cultures. None of the vesicles they studied were positively charged but several, particularly those that were most effective, would be negatively charged in monovalent electrolyte solutions. Their FI for monolayer cultures are very similar to ours when comparisons are made on the basis of the same ratio of lipid to cells. It is somewhat surprising that positive and negative vesicles are so similar in effecting cell fusion, but it is possible, as they suggest, that calcium ions form bridges between anionic sites on the cell membrane and those on the vesicles. There may also be some differences in propensity

for fusion between the cell lines employed by Papahadjopoulos et al. (18) and by us, for, under the conditions described by them, we were unable to obtain significant fusion using vesicles consisting of L and phosphatidylserine, a mixture which they found to be one of the most effective.

There is a difference between our results and those of Papahadjopoulos et al. (18) with respect to the cytotoxicity of LL-containing vesicles. They found that vesicles containing LL, phosphatidylserine, and L were very toxic. In contrast, we find that SA-L-LL vesicles at about the same dose as they reported produce substantial fusion with practically no cell death. If, indeed, the difference in vesicle composition is the important factor, we can suggest no reason why substitution of SA for phosphatidylserine should so dramatically reduce toxicity.

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