The fusion of Sendai virus at pH 4–7 with artificial lipid vesicles composed of phosphatidylserine or phosphatidylcholine was quantified by measuring fluorescence energy transfer from N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phosphatidylethanolamine to N-(lissamine-rhodamine-B-sulfonyl)-phosphatidylethanolamine in the target membranes. About 60% of the phosphatidylserine vesicles and virus appeared to fuse at pH 4 and about 100% at pH 5. Fusion was much less under all other conditions. The apparent fusion at pH 4, however, was due to a decrease in absorption of the acceptor probe, instead of dilution of acceptor as a result of fusion of labeled vesicles with unlabeled virus.

After correction for this fusion-independent effect of Sendai virus, the extent of fusion was only 4–20% at pH 4, but still 80–100% at pH 5. These findings parallel the loss of hemagglutinating and hemolytic activities of the virus induced by incubation at pH 4 but not at pH 5. Vesicle-virus hybrids were observed with the electron microscope after incubation at pH 5 but not at pH 7. The assay of membrane fusion by fluorescence energy transfer can be misleading unless correction is made for changes in energy transfer due to fusion-independent effects.

The measurement of changes in the fluorescence of amphiphilic probes on fusion of the probe-containing membranes is now one of the standard ways of quantifying membrane fusion (Blumenthal, 1987; Dzugunse and Bentz (1987)), including the fusion of membranes in the presence of proteins (Amselem et al., 1986; Chejanovsky and Loyer, 1985; Chejanovsky et al., 1986; Driessen et al., 1985; Eidelman et al., 1984; Hoeftstra et al., 1984; Stegmann et al., 1985; van Meer et al., 1985; Wharton et al., 1986). This widespread use of fluorescence assays attests to their reliability, although a few irregularities have been observed with methods relying on energy transfer (Driessen et al., 1985; Morris et al., 1985; Uster and Deamer, 1981; Wharton et al., 1986). Of relevance to the present work was the recent finding that membrane-depleted influenza hemagglutinin reduced energy transfer between N-NBD-PE' and N-Rh-PE in target vesicles, so that spurious fusion was observed in the absence of a virus membrane (Wharton et al., 1986).

As reported here, a similar fusion-independent change in energy transfer was suspected initially when the assay of PS vesicles indicated their fusion with Sendai virus at pH 4 and at 5, since the virus lost its hemagglutinating and hemolytic activities when incubated at pH 4 but not at pH 5. Decreased absorption of acceptor N-Rh-PE in vesicles incubated at pH 4 with Sendai virus showed subsequently that the decrease in energy transfer, by which fusion was quantified, was not entirely due to fusion-dependent dilution of the acceptor probe originally in the target vesicle membrane. After appropriate corrections were made for fusion-independent changes in probe fluorescence, it became clear that Sendai virus does fuse with PS vesicles at pH 5 but not at pH 4. Hence, fusion between Sendai virus and PS vesicles can be assayed by the degree of fluorescence energy transfer between N-NBD-PE and N-Rh-PE despite fusion-independent effects on probe fluorescence, provided energy transfer data are corrected for the latter effects.

**MATERIALS AND METHODS**

**Chemicals**—Egg PC, bovine brain PS, egg phosphatidylethanolamine, egg sphingomyelin, N-NBD-PE and N-Rh-PE were from Avanti Polar Lipids, Inc. Py-PC was from Molecular Probes, Inc. These lipids were analyzed for purity by thin layer chromatography and assayed by a modification of the Bartlett procedure (Bartlett, 1959). Only lots of N-Rh-PE free of isomers with pH-dependent quantum yields were used (van Meer et al., 1985). Cholesterol was from Sigma.

**Virus**—Sendai virus was grown in chicken eggs as described (MacDonald, 1986) and stored at ~70°C. Virus protein was measured (Markwell et al., 1978) and total viral lipid calculated to be 400 nmol/mg of viral protein (Hoekstra et al., 1984).

**Vesicle Preparation and Incubation with Virus**—Mixtures of lipids in chloroform-methanol were dried under high vacuum for 1 h, hydrated in 100 mM NaCl + 10 mM MOPS, pH 7.2, +1 mM EDTA + 3 mM Na+, at 37°C for 30 min and sonicated in a bath-type apparatus under nitrogen. Vesicles were oligomannolamellar and ranged in diameter from 40–400 nm. 10-μl aliquots of Sendai virus containing 11 nmol of lipid were incubated for 5 min at 37°C in 20 μl of 100 mM sodium acetate, pH 4.2 to 6.4, or 100 mM NaCl + 10 mM MOPS, pH 7.2, as well as 1 mM EDTA + 3 mM Na+, prior to the addition of 2 μl of vesicles containing 12 nmol of lipid. The pH given under "Results" refers to the pH of the virus-buffer-vesicle mixture determined by measuring the pH of a large volume of such a mixture. After 10 min at 37°C, 370 μl of 100 mM NaCl + 10 mM Tris-Cl, pH 8, or 10 mM MOPS, pH 7.2, as well as 1 mM EDTA + 3 mM Na+, were added to samples at pH 4.2 or at pH 5.1–7.2, respectively, to raise the pH to 7.0–7.2 prior to measurement of fluorescence. To minimize dilution of the samples for electron microscopy, the pH was adjusted with acetic acid and NaOH.

**Fluorescence Measurement**—Vesicles containing 0.5 mol % Py-PC, 1.0 mol % N-NBD-PE and/or 1.0 mol % N-Rh-PE were incubated with and without Sendai virus at pH 4–7. Fluorescence was proportional to concentration at these probe levels. Samples were excited at 350 nm and scanned from 360 to 500 nm to measure Py-PC fluorescence (peak emission = 380 nm), excited at 475 nm and scanned from 480 to 550 nm.
500 to 550 nm to measure N-NBD-PE fluorescence (peak emission = 530 nm), excited at 570 nm and read at 590 nm to measure N-Rh-PE fluorescence in a Farrand spectrophotometer with excitation and emission band widths of 5 nm. Light scattering due to Sendai virus was subtracted from all samples. Each sample was read in the absence and presence of 1% Triton X-100 which gave a 100% value of unquenched N-NBD-PE fluorescence after allowing for quenching by Triton X-100.

**Fluorescence Energy Transfer Assay**—This assay is based on the quenching of a donor, e.g. N-NBD-PE, by an acceptor, e.g. N-Rh-PE, in the same membrane through energy transfer, since their emission and excitation spectra, respectively, overlap. Because donor quenching or energy transfer depends on the acceptor concentration (Fung and Stryer, 1978), donor fluorescence can reflect the degree of acceptor dilution resulting from mixing of acceptor-containing membranes with acceptor-free membranes (Struck et al., 1981). Energy transfer is related to donor fluorescence according to the equation, 

\[ E = 1 - \frac{F}{F_0} \]

where \( F \) is the donor fluorescence in the presence of acceptor and \( F_0 \) being the donor fluorescence in the absence of acceptor (Fung and Stryer, 1978). According to a standard procedure (MacDonald and MacDonald, 1983; Struck et al., 1981, Uster and Deamer, 1981), a calibration curve was constructed to assign a percent lipid-mixing value to the \( F/F_0 \) value of an unknown sample by plotting the percent lipid mixing versus \( F/F_0 \) values of lipid vesicles “mock-fused” to varying proportions (i.e., PS or PC vesicles labeled with both N-NBD-PE and N-Rh-PE (for \( F \)) or with N-NBD-PE alone (for \( F_0 \)) and unequal amounts of unlabeled vesicles of a lipid composition similar to Sendai virus membranes, 18 mol % phosphatidylethanolamine, 8 mol % PS, 16 mol % PC, 14 mol % sphingomyelin, and 44 mol % cholesterol (Quigley et al., 1972). The \( F/F_0 \) versus percent lipid-mixing standard curves were not affected by pH but differed slightly if PS or PC were the labeled lipid.

**Difference Spectra of Absorbance**—Samples identical, except for their increased volume, to those prepared for fluorescence measurements were scanned from 700 to 400 nm in a Shimadzu double beam spectrophotometer with opal filters to minimize the effect of light scattering. The absorbance of N-Rh-PE in PS vesicles was obtained by scanning vesicles labeled with N-NBD-PE and N-Rh-PE in the presence or absence of Sendai virus in the sample cuvette and vesicles labeled with N-NBD-PE alone in the presence or absence of Sendai virus in the reference cuvette.

**Electron Microscopy**—Samples were applied to collodion-coated copper grids, stained with 2% sodium phosphotungstate, pH 7, and viewed in a JEOL electron microscope at 48,000 × magnification.

**Hemolytic Activity of Sendai Virus**—The hemolytic activity of Sendai virus, which is a measure of its fusion activity, was assayed as described (Kundrot et al., 1983) after preincubation of the virus from pH 4 to 7 in the absence of red cells.

**RESULTS**

**Apparent Fusion of Sendai Virus and Lipid Vesicles**—Fig. 1A, open bars, shows that lipid mixing between Sendai virus and N-NBD-PE + N-Rh-PE labeled PS vesicles after 10 min of incubation at 37 °C was a maximal 100% at pH 5.2 and 60% at pH 4.1. Lipid mixing did not occur at these pH values when unlabeled vesicles were substituted for Sendai virus (not shown). At pH 6.4 and at pH 7.2, lipid mixing was 25% or less, a relatively low level also found with Sendai virus and PC vesicles from pH 4 to 7 (Fig. 1A, open bars). The marked susceptibility of PS vesicles to fusion with Sendai virus at low pH was surprising since Sendai virus fuses over a broad pH range (i.e., pH 5–8) with cell membranes (Lenard and Miller, 1981; White et al., 1983), of which the acidic lipid content is only 10 mol % (van Deenen and de Gier, 1974). Particularly suspect was the apparent fusion with PS vesicles at pH 4, since incubation of Sendai virus at pH 4 for as brief a period as 1 min reduced its hemolytic activity to 30.8% of the original. The hemolytic activities of Sendai virus incubated for 20 min at 37 °C at pH 4, 5, and 7 were 0, 75.8, and 100%, respectively, of the original activity. Hemagglutinating activity was also destroyed at the original pH 4, as virus incubated for 5 min at pH 5 and at pH 7 contained 30,800 hemagglutination units/ml, whereas virus incubated for 5 min at pH 4 contained 0 hemagglutination units/ml.

**Fusion-independent Relief by Sendai Virus of N-NBD-PE Quenching**—To determine whether Sendai virus could affect the fluorescence of either the donor and/or acceptor probes without inducing virus-target fusion, the fluorescence of each probe alone in PS or PC vesicles was measured after incubation with or without Sendai virus. The effect of Sendai virus on the fluorescence of Py-PC-containing vesicles was also measured to determine whether the virus could affect the fluorescence of a probe bearing the fluorescent group in its hydrophilic region as well as a probe bearing the fluorescent group in its hydrophobic region. Table I shows that Sendai virus reduces the fluorescence of N-Rh-PE in PS vesicles, significantly at pH 4 (to 68%) and somewhat at pH 5 (to 80%), and of N-NBD-PE and Py-PC in PS vesicles slightly at pH 4 (to 90 and to 88%, respectively), and enhances the fluorescence of N-NBD-PE in PS vesicles slightly at pH 6 and 7 (to 113 and to 110%, respectively). In contrast with PS vesicles, PC vesicles incubated with Sendai virus displayed a slightly enhanced fluorescence of all three probes as the pH was raised from 4 to 7. Thus, some or all of the increased N-NBD-PE fluorescence attributed to virus PS vesicle fusion in Fig. 1B, open bars could reflect Sendai virus enhancement of the fluorescence of N-NBD-PE by effects on N-Rh-PE absorption and not by reducing the membrane concentration of N-Rh-PE through fusion.
Absorption of N-Rh-PE under Fusion Conditions—The fusion-independent reduction of N-Rh-PE fluorescence shown in Table I could be due either to a diminished excitability of some acceptor molecules and/or to a failure of those acceptor molecules to emit light on excitation. If the fluorescence reduction were due to a reduced excitability, this fusion-independent effect should be correctable by extrapolating the degree of lipid mixing from a percent lipid mixing versus F/Fo curve, based on the availability of N-Rh-PE for energy transfer and not on the N-Rh-PE physically present in the membrane. To ascertain diminished excitability of N-Rh-PE, absorption spectra of N-Rh-PE in PS vesicles were obtained as a function of pH and incubation with Sendai virus. Samples were identical to those prepared for the assay of fusion except for their larger volumes. The sample cuvette contained PS vesicles labeled with N-NBD-PE + N-Rh-PE and incubated with or without Sendai virus at the appropriate pH, and the reference cuvette contained PS vesicles labeled with N-NBD-PE alone and treated in the same way as those in the sample cuvette.

Fig. 2 shows the absorption spectra of N-Rh-PE in PS or PC vesicles incubated with or without Sendai virus at pH 4, 5, 6, or 7 and the percent change in absorbance due to the presence of Sendai virus. The absorbance of N-Rh-PE in PS vesicles incubated with Sendai virus is clearly lower than that without Sendai virus at pH 4 (average 101.6 ± 10%), but not at pH 5 (average 91.8 ± 84%), at pH 7 (average 90.1 ± 8.7%) and in PC vesicles at pH 4 (107.3%). Because Sendai virus incubated at pH 4 looked unusually turbid, N-NBD-PE + N-Rh-PE-labeled vesicles incubated alone at pH 4 were mixed after neutralization and dilution of all samples either with buffer or with Sendai virus incubated alone at pH 4. The N-Rh-PE absorbance of these two mixtures were the same (not shown). Hence, the reduced absorbance of N-Rh-PE in PS vesicles incubated with Sendai virus at pH 4 was not due to light scattering by the pH 4-treated Sendai virus.

True Fusion of Sendai Virus and Lipid Vesicles—Since Fig. 2 shows that the absorption of N-Rh-PE is reduced in PS vesicles incubated with Sendai virus at pH 4, it was necessary to re-evaluate fusion which had been determined under the apparently incorrect assumption that the absorption efficiency of 1 mol % N-NBD-PE was the same under all experimental conditions (Fig. 1, A and B, open bars). To correct for reduced absorption of N-Rh-PE, a curve was constructed in which the N-Rh-PE fluorescence of vesicles containing various concentrations of N-Rh-PE and 1 mol % N-NBD-PE was plotted against the corresponding F/Fo of N-NBD-PE. The resulting curve (Fig. 1C) resembles that reported by Struck et al. (1981). Because energy transfer resulting from lipid mixing is a function of the concentration of N-Rh-PE in the membrane (Fung and Stryer, 1978), the curve in Fig. 1C could be used to assign a corrected percent lipid-mixing value to experimentally obtained F/Fo values. The F/Fo value equal to true 0% lipid mixing on the curve corresponds to the concentration of N-Rh-PE commensurate with its absorption or fluorescence, not its actual concentration of 1 mol %. The effective N-Rh-PE concentration was calculated either as (1 mol % N-Rh-PE × (N-Rh-PE fluorescence in the presence of virus/N-Rh-PE fluorescence in the absence of virus)) or as (1 mol % N-Rh-PE × (N-Rh-PE absorbance in the presence of virus/N-Rh-PE absorbance in the absence of virus)). The N-Rh-PE fluorescence values were from Table I and the N-Rh-PE absorbance values were from Fig. 2. The F/Fo value at the true 100% lipid mixing corresponds to an effective N-Rh-PE concentration that calculated for true 0% lipid mixing, since complete mixing of the vesicles with the amount of virus added would result in a 1-fold dilution of the probes. Thus, the F/Fo values at true 0 and 100% lipid mixing demarcate a portion of the curve in Fig. 1C, the limits of which correspond to 0 and 100% lipid mixing on the abscissa. Accordingly, true fusion values were obtained from that portion of the curve relevant to the unquenched, i.e. fluorescent and/or light absorbing, N-Rh-PE in each case. Instead of 60% lipid mixing at pH 4 (Fig. 1B, open bar), the corrected lipid mixing between virus and PS vesicles at pH 4 is negligible, i.e. 20% (Fig. 1B, stippled bar based on N-Rh-PE fluorescence) or 4% (Fig. 1B, solid bar based on N-Rh-PE absorbance). After correction of values obtained at pH 5, in contrast, percent lipid mixing remains high, i.e. 80% (Fig. 1B, stippled bar based on N-Rh-PE fluorescence) or 100% (Fig. 1B, solid bar based on N-Rh-PE absorbance). The corrected values of percent lipid mixing between PS vesicles and virus at pH 6 and 7 in Fig. 1B (stippled and solid bars) and between PC vesicles and virus in Fig. 1A (stippled and solid bars) scarcely differ from the uncorrected lipid mixing (open bars). The reasonably good agreement between corrections based on fluorescence (stippled bars) and on absorbance (solid bars) indicates that decreases in N-Rh-PE fluorescence are largely due to decreases in N-Rh-PE absorbance.

Electron Microscopy Corroborates Preceding Results—Corroboration of fusion between PS vesicles and Sendai virus at pH 5 but not at 7 was obtained by electron microscopy. Fig. 3A is a representative electron micrograph of Sendai virus and PS vesicles incubated for 10 min at 37 °C at pH 7 at a magnification of x48,000. The arrow points to a nucleoprotein-containing virus particle which is adhering to but not fusing with a liposome. In contrast, Fig. 3B shows virus fused with PS vesicles after incubation at pH 5. The arrow points to a very large product of virus-liposome fusion which contains loosely arranged nucleoprotein and a fringe of virus spikes on its surface. Virus and PS vesicles incubated at pH 4 appeared as large aggregates of obscure structure (not shown), whereas fusion hybrids were not observed following incubation of virus and PC vesicles at pH 5 (not shown).

DISCUSSION

Since vesicles of biological origin, such as Sendai virus, can be leaky and/or are not easily loaded with aqueous space markers, the assay of membrane mixing, rather than compartment mixing, may be the method of choice for the rapid and accurate measurement of the fusion of such membranes. Alternative methods for quantifying membrane fusion present various disadvantages. The assay of virus-vesicle hybrids by sucrose gradient centrifugation (Haywood and Boyer, 1984) and the assay of trapped marker release from target liposomes (Kundrot et al., 1983; Ouku et al., 1982; Tsao and Huang, 1985)
| Sample | Reference | Scan from 400 to 700 nm | $A_{572}$ | $A_{572}$ w/ Virus | $A_{572}$ w/out Virus |
|--------|-----------|------------------------|----------|-------------------|----------------------|
| 1) NRPS + SV, pH 4 | NPS + SV, pH 4 | ![Graph](image1) | 0.024 | 63.5 ± 10.7 |
| 2) NRPS, pH 4 pH | NPS, pH 4 | ![Graph](image2) | 0.046 |
| 3) NRPS + SV, pH 5 | NPS + SV, pH 5 | ![Graph](image3) | 0.04 | 91.8 ± 8.4 |
| 4) NRPS, pH 5 | NPS, pH 5 | ![Graph](image4) | 0.048 |
| 5) NRPS + SV, pH 7 | NPS + SV, pH 7 | ![Graph](image5) | 0.036 | 90.1 ± 8.7 |
| 6) NRPS, pH 7 | NPS, pH 7 | ![Graph](image6) | 0.045 |
| 7) NRPC + SV, pH 4 | NPC + SV, pH 4 | ![Graph](image7) | 0.022 | 107.3 |
| 8) NRPC, pH 4 | NPC, pH 4 | ![Graph](image8) | 0.0205 |

Fig. 2. Absorption spectra of N-Rh-PE in PS or PC vesicles incubated at different pH values with or without Sendai virus. Sample mixtures containing vesicles labeled with N-NBD-PE and N-Rh-PE were identical to those prepared for energy transfer assays (Fig. 1) except for their increased volume, i.e., 1 ml instead of 0.4 ml with the reactants being increased in proportion. Reference mixtures were identical to the sample mixtures except that the vesicles were labeled with N-NBD-PE only. The spectra and $A_{572}$ given were obtained in the third experiment performed, except for mixtures 7 and 8, data for which were obtained in the fourth experiment. The last column gives the average ± SD of the percent change in $A_{572}$ calculated as ($A_{572}$ in the presence of Sendai virus) divided by ($A_{572}$ in the absence of Sendai virus) × 100.

Fig. 3. Electron microscopy of Sendai virus and PS vesicles. A, Sendai virus and PS vesicles were incubated for 10 min at 37 °C in the same proportions as in Fig. 1 at pH 7.2 prior to negative staining and electron microscopy at ×48,000 magnification, as described under “Experimental Procedures.” The arrow points to a virus particle adhering to but not fusing with a PS vesicle. B, Sendai virus and PS vesicles were incubated as in Fig. 3A but at pH 5.2 prior to negative staining and electron microscopy at ×48,000 magnification, as described under “Experimental Procedures.” The arrow points to a particularly large product of virus-vesicle fusion.
in analogy with Sendai virus-induced hemolysis do not specifically signify the merger of fusogenic membranes, whereas the countering of virus-vesicle fusion hybrids in electron micrographs (Tsao and Huang, 1986) is quite time consuming. Also, relatively complicated is a form of compartment mixing assay in which nucleases or proteases in the target vesicle degrade viral RNA or protein on virus-vesicle fusion (Hsu et al., 1983; White and Helenius, 1980).

On the other hand, the energy transfer assay data in Fig. 1, reflecting fusion-independent effects of Sendai virus, indicate the need for caution in regarding changes in donor fluorescence as due to fusion alone. Similarly, the fluorescence of N-NBD-PE in N-NBD-PE + N-Rh-PE-labeled target liposomes increased in the presence of membrane-free influenza virus hemagglutinin (Wharton et al., 1986). At least part of this increase in N-NBD-PE fluorescence of target liposomes in the presence of hemagglutinin or intact virus was attributed to “quenching” of N-Rh-PE by hemagglutinin and not to N-Rh-PE dilution by the fusion of labeled with unlabeled membranes. Wharton et al. (1986) alternatively labeled their target vesicles with a different donor-acceptor pair, i.e. cholesteryl anthracene-9-carboxylate and N-NBD-PE, which gave no spurious fusion signal. Since cholesteryl anthracene-9-carboxylate behaves anomalously in vesicles composed entirely of PS (Uster and Deamer, 1981) and PS appeared necessary in the present study, however, it was decided to continue using N-NBD-PE + N-Rh-PE-labeled target vesicles but to attempt to correct for fusion-independent changes in probe fluorescence if necessary.

The need to correct for fusion-independent changes in N-NBD-PE fluorescence was apparent from absorption spectra of N-Rh-PE in PS vesicles incubated with Sendai virus at pH 4, 5, or 7. According to the spectra, the absorbance of N-Rh-PE in PS, but not PC, vesicles was reduced on incubation with Sendai virus at pH 4 but not at pH 5 or 7. Because of this decreased absorbance of N-Rh-PE in PS vesicles incubated with Sendai virus at pH 4, the quenching by N-Rh-PE of a certain portion of N-NBD-PE in those vesicles was relieved. This decrease in absorption of N-Rh-PE in PS vesicles incubated at pH 4 with Sendai virus may be similar to the well known hypochromicity of nucleic acids (Freidel, 1982). As nucleic acids become more ordered, the extinction coefficient of the constituent nucleotide bases decreases without a change in the wavelength of maximum absorption. Evidence for an “orientation effect” on N-Rh-PE like that causing hypochromicity of nucleic acids is the sizable fluorescence anisotropy of N-Rh-PE in vesicles incubated with influenza virus at low pH (Wharton et al., 1986).

The corrections of percent lipid-mixing values obtained in the presence of Sendai virus (cf. open bars versus stippled and solid bars, Fig. 1, A and B) are valid for two reasons: 1) corrected (Fig. 1B, stippled bars and solid bars), as opposed to uncorrected (Fig. 1B, open bars), values for percent lipid mixing indicate negligible PS-virus fusion at pH 4 at which pH Sendai virus hemolysis and hemagglutination were completely inactivated. Although it is not clear why Sendai virus fuses poorly with PS vesicles at pH 6 and 7 but lyses red cells well (Lenard and Miller, 1981) and fuses BHK-21 cells moderately well (White et al., 1983) at those pH values, the correction nevertheless accurately reflects little fusion by a pH 4-inactivated virus. 2) Virus-vesicle fusion products were seen in electron micrographs of Sendai virus and probe-labeled PS vesicles incubated at pH 5 (Fig. 3B) but not at pH 7 (Fig. 3A). The occurrence of fusion at pH 4 seemed unlikely, since large aggregates of obscure structure formed on incubation of virus and PS vesicles at pH 4. Nevertheless, it should be noted that earlier reports involving the use of different assays and/or different fluorescent probes and/or under different conditions have indicated that Sendai virus (Anslem et al., 1986; Klappe et al., 1986) and reconstituted Sendai virus (Chejanovsky et al., 1986) fuse with acidic lipid vesicles at pH values lower than 5.

How the fusion of Sendai virus with PS vesicles at low pH, like the fusion of reconstituted vesicular stomatitis virus G protein (Eidelman et al., 1984) and of influenza virus (Stegmann et al., 1985) with acidic lipid vesicles at low pH, is related to virus fusion with cell membranes remains unanswered and is referred to at greater length in another report. What is clear is the importance of testing for fusion-independent conditions which may compromise the accuracy of energy transfer measurements. To ensure that accuracy, the influence of fusion-independent effects on energy transfer should be assessed by measuring the fluorescence anisotropy of the probes (Wharton et al., 1986) and/or the absorption of the acceptor probe as done here. It may then be possible, as in this instance, to correct the data for artifacts revealed in these and other ways. Thus, donor-acceptor pairs such as N-NBD-PE and N-Rh-PE should continue to be extremely useful in assaying membrane fusion, provided measures are taken to detect and correct for fusion-independent effects.

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