Proc. Natl. Acad. Sci. USA
Vol. 75, No. 10, pp. 5076–5080, October 1978
Cell Biology

Translocation of a hydrocarbon fluorescent probe between Epstein–Barr virus and lymphoid cells: An assay for early events in viral infection

(liposomes/lymphocyte membranes/diphenylhexatriene/fluorescence polarization)

KEN S. ROSENTHAL, SAUL YANOVICH, MICHAEL INBAR*, AND JACK L. STROMINGER

Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115

Contributed by Jack L. Strominger, August 7, 1978

ABSTRACT **Translocation of the hydrocarbon fluorescent probe diphenylhexatriene (DPH) between membranes was studied by fluorescence polarization (*P*) analysis. First, using a model system, the high *P* value (0.324) of DPH-labeled cholesterol/phosphatidylcholine liposomes and the low *P* value (0.157) of DPH-labeled phosphatidylcholine liposomes allowed detection of DPH translocation between interacting liposomes. This was monitored by the change in *P* in either direction. Early events during cell–virus interactions were similarly studied by monitoring DPH translocation. The *P* value of DPH-labeled Epstein–Barr Virus (EBV) was significantly higher (0.350–0.392) than the *P* value of DPH-labeled lymphoid cells (0.238–0.289). Hence, DPH translocation could be detected by changes in *P* following incubation of DPH-labeled EBV and nonlabeled cells. A marked decrease in *P* was observed after incubation of DPH-labeled EBV with either nonlabeled lymphoblastoid Raji cells or fresh human B lymphocytes. However, only a slight decrease in *P* was obtained when DPH-labeled EBV was incubated with either nonlabeled fresh human T lymphocytes or fresh T or B rabbit lymphocytes. Moreover, incubation of fresh human B lymphocytes with the purified C3 component of complement (a putative inhibitor for the EBV receptor) prior to the addition of DPH-labeled EBV abolished the observed decrease in the *P* value. Most of these experiments were carried out with both the P3HR-1 and the B95-8 strains of EBV. DPH translocation, as determined by fluorescence polarization analysis, is, therefore, measuring some early event during interaction of this enveloped virus and mammalian cells. The potential applicability of this technique to other viruses is illustrated by an experiment with Semliki Forest virus.**

Epstein–Barr Virus (EBV) is an enveloped, DNA-containing human herpesvirus, which is capable of interaction with human B lymphocytes *in vivo* and *in vitro* (1). Although interactions of EBV with some T lymphoblasts have been reported (2), the infectivity and transformation spectra of EBV are limited to B lymphocytes of humans and New World monkeys (3). Such a limitation is presumably due to the presence of specific EBV receptors on B but not on T human lymphocytes. This receptor is closely associated with the receptor for the C3 component of complement (4, 5). However, further studies on binding, fusion, and infectivity of EBV have been limited by the lack of an assay to determine early biochemical events during cell–virus interactions.

In this report such an assay is described, as well as its application to measure events following interaction of EBV with different types of lymphoid cells. The method is based on translocation of the hydrocarbon fluorescent probe diphenylhexatriene (DPH) between viral envelopes and cellular membranes as monitored by fluorescence polarization analysis. Fluorescence polarization (*P*) analysis (6) is, in principle, an indirect measurement of the thermal mobility of the DPH molecules incorporated into a lipid bilayer. This is determined to a large extent by the lipid composition of the membrane (7). The degree of fluidity/rigidity of a membrane could be envisaged as a mechanical barrier imposed by the lipid bilayer on the degree of rotational mobility of DPH molecules (8). Therefore a slow rotation of DPH in a "rigid" membrane domain is recorded by a high *P* value, whereas a fast rotation of DPH in a "fluid" membrane region is measured by a low *P* value (6–8). Based on this assumption, fluorescence polarization analysis of DPH has been extensively used, in the last few years, to analyze the dynamic structural organization of lipids in cellular membranes (9–11) and viral envelopes (12, 13). These studies have independently shown that the *P* values of DPH-labeled lymphoid cells are markedly lower (14, 15) than those of DPH-labeled enveloped viruses (12, 13). Moreover, it has also been shown that hydrophobic molecules such as cholesterol (16–19) and DPH (20, 21) can be translocated between interacting membranes upon collision of exposed lipid regions. We have therefore measured translocation of DPH between viral envelopes and cellular membranes by monitoring the appropriate changes in the *P* values observed after incubation of DPH-labeled enveloped viruses and nonlabeled lymphoid cells.

It appears that early events during cell–virus interactions such as binding to specific receptors or intermixing of hydrophobic molecules (fusion) can be quantitatively analyzed by DPH fluorescence polarization in a cell–virus mixture.

MATERIALS AND METHODS

Chemicals and Reagents. DPH was purchased from Koch and Light; tetrahydrofuran from Aldrich; cholesterol from Sigma; egg lecithin from Lipid Products; dextran T-10 and Ficoll/Hypaque from Pharmacia; nylon wool from Fenwal; Dulbecco's phosphate-buffered saline (P_i/NaCl) and RPMI 1640 medium from GIBCO; and fetal calf serum from Microbiological Associates. Purified C3 component of complement was kindly provided by R. Frade.

Viruses. EBV producer cell lines P3HR-1 and B95-8 (22) were grown in RPMI 1640 medium supplemented with 5% fetal calf serum. Viruses were purified according to methods previously described (23), modified by the use of discontinuous dextran T-10 density gradient centrifugation from 10% to 30% in 5% steps. Purified Semliki Forest virus was kindly provided by A. Helenius and K. Simons (24). All viruses were kept frozen at −70°C.

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

Abbreviations: *P*, fluorescence polarization; P_{25}, *P* at 25°C; DPH, diphenylhexatriene; EBV, Epstein–Barr virus; SFV, Semliki Forest virus; PtdCho, phosphatidylcholine; Chol, cholesterol; P_i/NaCl, phosphate-buffered saline.

* On leave of absence from the Weizmann Institute of Science, Rehovot, Israel.

Cells. Raji, a Burkitt lymphoma cell line, and JY, a lymphoblastoid cell line established with EBV, were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells were used in the experiments 24 hr after the growth medium had been changed. Fresh mixed populations of T and B lymphocytes were obtained from human and rabbit peripheral blood, by Ficoll/Hypaque gradient centrifugation (25). T and B subpopulations were purified according to methods previously described (26).

Liposomes. Phosphatidylcholine (PtdCho) and cholesterol (Chol) were kept as stock solutions in chloroform/methanol (2:1 vol/vol.) at −20°C at concentrations of 100 and 50 mg/ml, respectively. For preparation of PtdCho liposomes, 0.1 ml of stock solution was evaporated with nitrogen and resuspended in 10 ml of P_i/NaCl; for preparation of Chol/PtdCho liposomes, 0.1 ml of PtdCho and 0.1 ml of Chol stock solutions were mixed, evaporated, and resuspended in 10 ml of P_i/NaCl. Lipid dispersions were than sonicated at 4°C with a Branson sonicator. PtdCho and Chol/PtdCho dispersions were sonicated for 6 and 12 min, respectively, at maximum energy output (8), and only fresh liposomes were used in the experiments.

Fluorescence Labeling with DPH. DPH (2 mM) in tetrahydrofuran was kept at 25°C as a stock solution. For labeling of samples, a dispersion of DPH in P_i/NaCl (2 μM) was prepared as previously described (7). Liposomes, viruses, and cells were labeled with DPH by incubating the samples in a 10-ml DPH/P_i/NaCl dispersion for 30 min at 25°C. Free DPH was removed by washing the DPH-labeled samples with 3 vol of 10 ml P_i/NaCl. No DPH was measurable in the final wash, in all cases.

Fluorescence Polarization Analysis. The degree of fluorescence polarization (P) of the DPH-labeled liposomes, viruses, and cells was determined with the aid of the Elscint MV-1 microviscosimeter as described (15, 20), and the P value was recorded directly by the instrument according to the equation $P = [(I_\parallel - I_\perp)/(I_\parallel + I_\perp)]$, in which $I_\parallel$ and $I_\perp$ are the fluorescence intensities polarized parallel and perpendicular to the direction of polarization of the excitation beam, respectively (6). All fluorescence measurements were carried out at 25°C, and the accuracy of the obtained P value was ±0.005.

RESULTS

Translocation of DPH upon Mixing of Liposomes. To analyze the possibility that DPH can be translocated between membranes, and that this translocation can be monitored by fluorescence polarization analysis, translocation of DPH was studied in an artificial membrane model system. Previous studies have shown that incorporation of cholesterol molecules into a phospholipid bilayer is associated with an increase in the rigidity of the membrane (27). These changes in the structural organization of the membrane can be monitored by the difference in the degree of fluorescence polarization of DPH embedded in liposomes of pure phospholipids and in mixed liposomes of cholesterol and phospholipids (28). A marked increase in the P value results from incorporation of cholesterol in lecithin liposomes. The P values obtained with DPH-labeled PtdCho liposomes and DPH-labeled Chol/PtdCho liposomes at 25°C were 0.157 and 0.324, respectively. On the basis of this large difference in the P values, translocation of DPH between the two liposomes was measured by fluorescence polarization analysis. Experiments summarized in Fig. 1 show a marked decrease in the P value after incubation of DPH-labeled Chol/PtdCho liposomes with nonlabeled PtdCho liposomes and a significant increase in the P value after incubation of DPH-labeled PtdCho liposomes with nonlabeled Chol/PtdCho liposomes. The results have also shown (Fig. 1) that partitioning

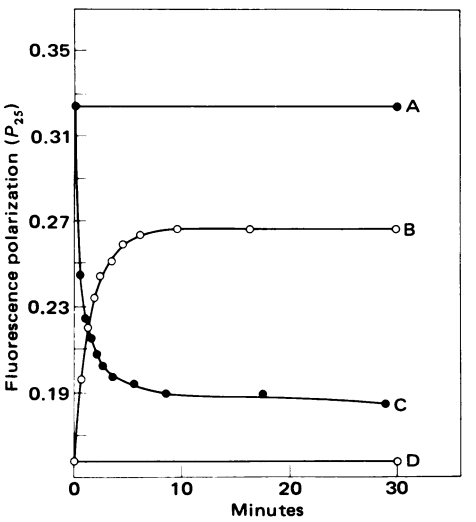

FIG. 1. Translocation of DPH between liposomes. P_{25} was determined for Chol/PtdCho (A) and PtdCho (D) liposomes by using the Elscint microviscosimeter. Liposomes were prepared, labeled, and washed three times with 10 ml of P_i/NaCl to eliminate free DPH. The absence of free DPH was determined by incubation of the wash supernatants with unlabeled liposomes and determination of the total fluorescence intensity after 30 min of incubation at 25°C. For DPH translocation experiments, 1.5 ml of DPH-labeled PtdCho liposomes was mixed with 1.5 ml of unlabeled Chol/PtdCho liposomes (B) or 1.5 ml of DPH-labeled Chol/PtdCho liposomes was mixed with 1.5 ml of unlabeled PtdCho liposomes (C). The degree of fluorescence polarization of the mixtures was determined at different time intervals up to 30 min after mixing.

of DPH is a rapid process and that the reaction reaches an equilibrium after less than 10 min of incubation at 25°C. Therefore, lipid–lipid interactions during membrane intermixing can be studied by fluorescence polarization analysis of DPH provided that (*i*) the two interacting membranes show a significant difference in their P values and (*ii*) no free DPH is associated with the labeled membranes.

Translocation of DPH from EBV to Raji Cells. Based on the results obtained with the membrane model system, an attempt was made to study cell–virus interaction. A large difference in the P values of EBV and lymphoblastoid cells, including both EBV-transformed cell lines and nontransformed lymphoid target cells, provided the basis for DPH translocation studies. The envelopes of the B95-8 and P3HR-1 strains of EBV had P values of 0.350 ± 0.004 and 0.380 ± 0.004, respectively, while the membranes of the Raji, B95-8, and P3HR-1 cell lines had P values between 0.171 and 0.214, depending on the growth phase and serum concentration of the culture (29). The difference in membrane fluidity of the envelopes of the P3HR-1 and B95-8 strains of EBV may be due to growth conditions of the host cells or inherent differences in characteristics of the host cells or virus strain.

Incubation of DPH-labeled EBV and labeled Raji cells brought about a rapid decrease in the P value (Fig. 2). The P value of DPH changed from that of the virus to a value representative of Raji cells upon mixing of DPH-labeled EBV with unlabeled Raji. The half-time of the change was 8–10 minutes at 25°C. This change in P was due to translocation of DPH from the viral envelope to the cellular plasma membrane. This assumption is supported by the P value of the Raji cell-associated DPH after incubation and centrifugation and of the residual DPH-labeled EBV (Table 1). Similar results were obtained with the P3HR-1 and B95-8 strains of EBV, although only the former can superinfect Raji cells (Fig. 2).

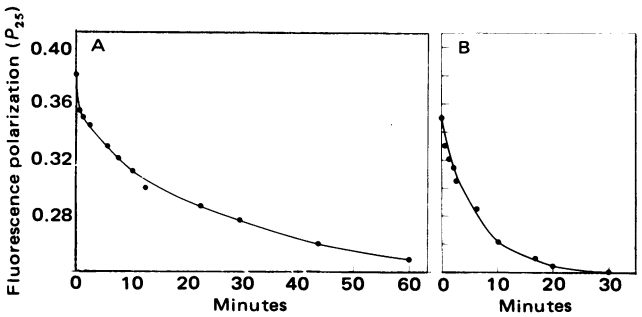

FIG. 2. Translocation of DPH from EBV to Raji. EBV was labeled with DPH by incubation of the purified virus with a P_i/NaCl/DPH dispersion for 30 min at 25°C. The labeled virus was washed three times with P_i/NaCl; under these conditions all the fluorescence signal was associated with the virus envelope and no free DPH could be detected in the wash supernatants. A sample of Raji cells (5×10^6) was washed twice with P_i/NaCl, resuspended in 3 ml of P_i/NaCl, and allowed to equilibrate to 25°C in the Elscint microviscosimeter. An aliquot (0.1 ml) of the DPH-labeled P3HR-1 (*A*) or B95-8 (*B*) strain of EBV, corresponding to 0.5 to 1.0 liters of concentrated supernatant, was then added to the nonlabeled cells and the P_{25} values of the mixtures were recorded.

Translocation of DPH from EBV to Lymphoid Cells. To test the possibility that translocation of DPH from EBV to lymphoid cells can be related to the infectivity spectrum of EBV, DPH-labeled EBV was incubated with nonlabeled T and B subpopulations of fresh human lymphocytes. These experiments were possible due to the following observations: (*i*) the *P* value of DPH-labeled EBV was found to be much higher that that of fresh human lymphocytes and (*ii*) the *P* values of DPH-labeled T and B subpopulations of fresh human lymphocytes were very similar (Table 2). Results presented in Fig. 3 show a marked decrease in the *P* value of DPH-labeled EBV after incubation with unlabeled human B lymphocytes for 30 min at 25°C. These results were observed for both the B95-8 and the P3HR-1 strains of EBV even though the P3HR-1 strain of EBV is not capable of transforming B lymphocytes *in vitro* (22). In contrast, only a slight decrease in the *P* value was observed when DPH-labeled EBV was incubated with unlabeled human T lymphocytes (Fig. 3 *A* and *B*). Results similar to those obtained with human T lymphocytes were also obtained when DPH-labeled EBV was incubated with unlabeled T and B subpopulations of rabbit lymphocytes (Fig. 3*C*). On the basis of these observations it can be concluded that DPH translocation occurs at a much higher efficiency during specific interactions of EBV with either Raji cells or fresh human B lymphocytes than during nonspecific interactions of EBV with human T lymphocytes or rabbit T and B lymphocytes.

Translocation of DPH upon mixing of dye-labeled EBV with human T lymphocytes or rabbit B and T lymphocytes could be the result of (*i*) nonspecific interactions of the virus and cell leading to transient lipid bilayer contact; (*ii*) the presence of some DPH in a form that is readily exchanged nonspecifically with a variety of cells—e.g., present in defective virus particles; (*iii*) the presence of DPH in contaminating cellular membranes that non-specifically interact with cells; or (*iv*) in the case of human lymphocytes, contamination of the T cell subpopulation by a small fraction of Ig-negative B lymphocytes that have EBV receptors. It is therefore suggested that in order to calculate the specific DPH translocation, the amount of DPH translocation between DPH-labeled EBV and human T lymphocytes can be subtracted from the amount of DPH translocation between DPH-labeled EBV and human B lymphocytes.

Table 1. Superinfection of Raji cells with EBV*

EBV strain	Virus	Raji cells	Raji + EBV†	Cell pellet‡	Supernatant
P3HR-1	0.380	0.171	0.249	0.233	0.330
P3HR-1	0.378	0.214	0.259	ND	ND
B95-8	0.349	0.189	0.238	0.199	0.317

* All data are expressed as P_{25}. ND, not determined.
† Values are shown after 60-min incubations for P3HR-1 and 30-min incubation for B95-8 virus.
‡ After the incubation period, cells were pelleted at $500 \times g$ for 10 min and resuspended to the original volume in P_i/NaCl.

Table 2. EBV interaction with lymphocytes*

Exp.	Virus	Cells	EBV + cells	Cell pellet†	Supernatant
	Human B cells with P3HR-1 or B95-8 virus‡				
1	0.392	0.277	0.337	0.297	0.367
2	0.380	0.281	0.327	0.285	0.382
3	0.354	0.282	0.291	0.280	0.345
	Human T cells with P3HR-1 or B95-8 virus‡				
1	0.392	0.274	0.370	0.334	0.382
2	0.380	0.267	0.359	0.312	0.392
3	0.354	0.282	0.320	0.283	0.356
	Rabbit B cells with B95-8 virus§				
1	0.350	0.289	0.315	0.299	0.351
	Rabbit T cells with B95-8 virus§				
1	0.350	0.284	0.318	0.283	0.345

* All data are expressed as P_{25}.
† After 30 min, cells were pelleted at $500 \times g$ for 5 min and resuspended to the original volume in P_i/NaCl.
‡ Human lymphocytes were separated on a B cell immunoabsorbent column (26). T cells for exp. 2 were further purified by passage over a nylon wool column. Exps. 1 and 2 were carried out with P3HR-1 virus and exp. 3 with B95-8 virus.
§ Rabbit lymphocytes were separated on a nylon wool column.

Inhibition of DPH Translocation from EBV to B Cells by the C3 Component of Complement. The specificity of DPH translocation upon fusion of EBV and human B lymphocytes was further analyzed by competition experiments with the C3 component of complement. Recent reports (4, 5) have suggested that the complement receptors on the cell surface of human B lymphocytes are associated with specific binding of EBV particles, suggesting that the complement receptor is part of the EBV receptor. Therefore, the effect of the purified C3 component of complement on DPH translocation was studied. Experiments summarized in Fig. 3*D* show, as expected, a marked decrease in the *P* value following incubation of DPH-labeled EBV and human B lymphocytes. However, the significant decrease in the *P* value was markedly inhibited by a prior incubation of the B lymphocytes with purified C3 (Fig. 3*D*). This is indicative of a competition between EBV and C3 for the same receptors on the cell surface of the B-lymphocytes. Note that the decrease in the *P* value obtained after incubation

Table 3. SFV interaction with JY cells*

Exp.	SFV virus†	JY cells‡	SFV + JY§
1	0.350	0.257	0.280
2	0.355	0.235	0.244

* All data are expressed as P_{25}.
† SFV labeled with DPH and washed free of DPH.
‡ JY cells labeled with DPH for 30 min at 25°C.
§ SFV + JY complex after 30 min of incubation of DPH-labeled SFV and nonlabeled JY cells.

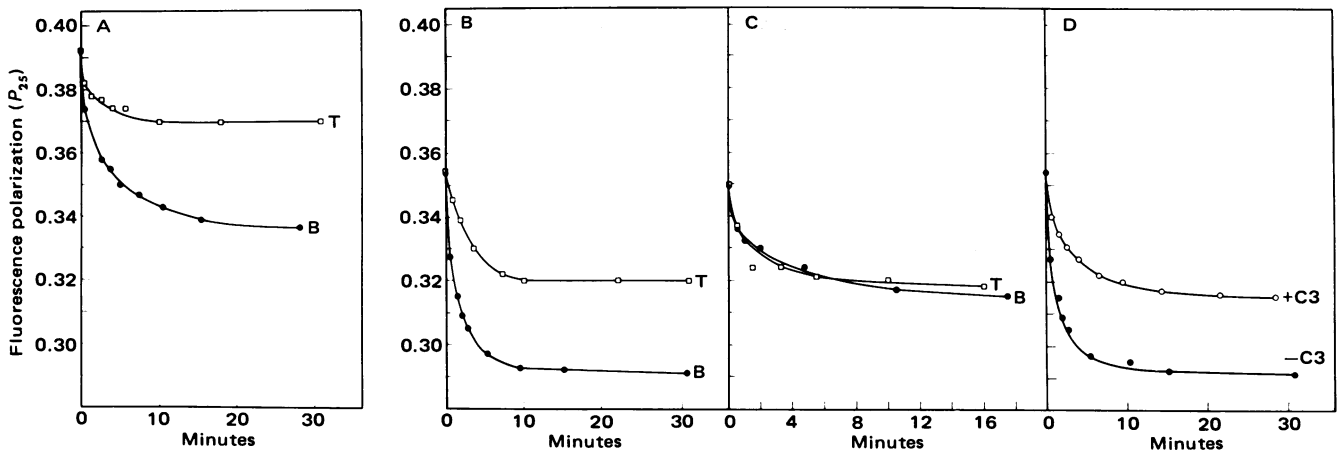

FIG. 3. Translocation of DPH from EBV to human and rabbit lymphocytes. EBV was labeled with DPH as described in the legend to Fig. 2. Subpopulations of normal human B and T lymphocytes were isolated from the peripheral blood of normal donors by Ficoll/Hypaque density gradient centrifugation followed by a B cell immunoabsorbent column. Rabbit B and T lymphocyte subpopulations were separated on a nylon wool column. The lymphocytes were washed twice in P_i/NaCl, and 4×10^6 cells in 3 ml of Pi/NaCl were allowed to equilibrate to 25°C in the microviscosimeter. A 0.1-ml aliquot of DPH-labeled EBV, corresponding to 0.5 to 1.0 liter of concentrated supernatant, was added to the nonlabeled cells and the P_{25} value was recorded at different time intervals. (*A*) Addition of DPH-labeled P3HR-1 strain of EBV to human B (●) and T (□) lymphocytes. (*B*) Addition of DPH-labeled B95-8 strain of EBV to human B (●) and T (□) lymphocytes. (*C*) Addition of DPH-labeled B95-8 strain of EBV to rabbit B (●) and T (□) lymphocytes. (*D*) Inhibition of EBV interaction with human B lymphocytes by the C3 component of complement. Two aliquots (4×10^6) of human B lymphocytes were suspended in 3 ml each of P_i/NaCl. Purified C3 (30 μg) was added to one sample and the two aliquots were incubated for 30 min at 37°C. An aliquot (0.1 ml) of DPH-labeled B95-8 was added to each sample and the changes in the P_{25} value were monitored with the microviscosimeter. (○) B cells with C3; (●) B cells without C3. Translocation of DPH from B95-8 to T cells of the same donor is shown in *B*.

of DPH-labeled EBV and human B lymphocytes preincubated with C3 (Fig. 3*D*) is similar to the decrease in the *P* value obtained when DPH-labeled EBV was incubated with human T lymphocytes (Fig. 3 *A* and *B*). If indeed the DPH translocation obtained with human T lymphocytes represents a nonspecific interaction, then C3 completely abolished the specific DPH translocation between EBV and human B lymphocytes.

DPH Translocation from Semliki Forest Virus to JY Cells. To see if DPH translocation can also be used to study interactions of other enveloped viruses and target cells, an experiment was carried out with Semliki Forest virus (SFV) and the JY human lymphoblastoid cell line to which SFV is known to bind (30). The results summarized in Table 3 clearly indicate that the *P* values of DPH-labeled SFV are significantly higher (0.350–0.355) than those of the DPH-labeled JY cells (0.235–0.257). Incubation of DPH-labeled SFV with nonlabeled JY cells resulted in a marked decrease in the *P* value, indicative of DPH translocation from SFV envelopes to JY cellular membranes.

DISCUSSION

Interaction of enveloped viruses and their target cells requires a specific virus–receptor interaction, presumably a protein–protein interaction. Such an interaction brings the viral envelope and the target cell plasma membrane into close proximity, perhaps allowing lipid–lipid interactions followed by either fusion of the viral and cellular membranes or viropexis (31). In the case of EBV, fusion of the viral envelope and the plasma membrane has been observed by electron microscopy (32). Incorporation of DPH in the viral envelope provides an assay for virus–cell interaction upon translocation of the dye during collision and intermixing of the cellular and viral membrane lipids. Aqueous transfer of the dye is unlikely due to the hydrophobicity of the compound. Measurement of the DPH transfer is made possible by differences in the fluorescence polarization of DPH incorporated into viral and cellular membranes. Translocation of DPH between membranes can be analyzed by changes in the *P* value after co-incubation of virus and cells provided that (*i*) the initial *P* values of the membranes of the two systems are significantly different and (*ii*) that only one membrane is labeled with DPH. The envelopes of all viruses tested, including vesicular stomatitis virus (12, 13), Sindbis virus, (12) SFV (12), and both the P3HR-1 and B95-8 strains of EBV (this paper) have high *P* values indicative of rigid lipid domains. This is in contrast to the much more fluid membranes of their host and target cells. The feasibility of measuring interactions of lipid domains by DPH translocation was illustrated by using liposomes of different *P* values. Upon mixing of DPH-labeled Chol/PtdCho liposomes (high *P*) with PtdCho liposomes (lower *P*), a rapid decrease in polarization for the system was observed. Such a change could only be due to the translocation of the fluorescent probe from a rigid to a more fluid membrane environment.

In the liposomal model system, interaction and possible fusion of liposomes takes place readily upon mixing, allowing translocation of hydrophobic molecules such as DPH. However, the transfer of hydrophobic molecules from the viral envelope to the plasma membrane of the cell, upon interaction of lipid regions, can be prevented by cell surface glycoproteins (17). The transfer of DPH from the lipid environment of an enveloped virus to its target cell most likely requires a specific virus–receptor interaction either to bring the lipid phases into juxtaposition or to fuse them. The large decrease in *P* upon interaction of DPH-labeled EBV or SFV with its natural target cells indicated that a significant DPH exchange occurred. Such an exchange was not observed upon incubation of EBV with cells that are not infectable by EBV, such as human T cells or rabbit B and T cells. Nonspecific interactions of EBV, as with T cells or rabbit cells, does not allow a significant DPH transfer to occur. The requirement for a specific virus–cell receptor interaction to precede DPH translocation was supported by the inhibition of DPH transfer from EBV to human B cells by the purified C3 component of complement. Competition by C3 for binding of EBV to its receptor prevented the close contact of lipid domains necessary for DPH transfer from the viral envelope to the target cell membrane. The complement receptor is either identical with or closely related to the EBV receptor (2, 4, 5).

The specific interaction of EBV with its receptor was not strain specific. Although the P3HR-1 strain of EBV is capable of superinfection of Raji but does not transform peripheral B lymphocytes and the B95-8 strain of EBV transforms B lymphocytes but does not superinfect Raji, interaction of both the B95-8 and P3HR-1 strains of EBV with both Raji and peripheral human B cells could be observed by DPH translocation. Hence, the biological distinction between the B95-8 and P3HR-1 strain of EBV does not seem to be the result of some phenomenon during interaction of the virus and the cell receptor. However, as can be seen in Figs. 2 and 3, the rate of translocation of DPH from the B95-8 strain of EBV to the target cell may be faster than for the P3HR-1 strain of EBV.

Although the mechanism of DPH translocation is not clearly understood, an experiment carried out with SFV corroborated the findings with EBV. Fluorescence polarization measurements of DPH translocation can serve as a method to study the early events of interaction of cells with enveloped viruses. At this time, it cannot be determined whether interaction of viral and cellular membranes at a virus receptor site is sufficient for DPH translocation or whether DPH transfer requires subsequent fusion of the cellular plasma membrane and viral envelope. The problem might be approached by studying viral mutants that bind to cells but are not capable of infection. This technique can also be used as an assay to study the properties of viral receptors, as is illustrated in the case of EBV by inhibition of DPH translocation with C3. Fluorescence polarization measurement of DPH translocation can also be used to study the lipid interactions or fusion of other membrane systems differing in lipid fluidity, such as interactions of liposomes with cells, lipoproteins with cells, or cell to cell fusion.

This study was supported in part by research Grants CA 21082 and A1-09576 from the National Institutes of Health and by a fellowship to K.S.R. from the American Cancer Society (PF 1369).

1. Epstein, M. A. & Achong, B. G. (1977) *Annu. Rev. Microbiol.* **31,** 421–445.
2. Menezes, J., Seigneurin, J. M., Patel, P., Bourkos, A. & Lenoir, G. (1977) *J. Virol.* **22,** 816–821.
3. Jondal, M. & Klein, G. (1973) *J. Exp. Med.* **138,** 1365–1378.
4. Yefenof, E. & Klein, G. (1977) *Int. J. Cancer* **20,** 347–352.
5. Yefenof, E., Klein, G., Jondal, M. & Oldstone, M. B. A. (1976) *Int. J. Cancer* **17,** 693–700.
6. Weber, G. (1971) *J. Chem. Phys.* **55,** 2399–2407.
7. Shinitsky, M. & Inbar, M. (1974) *J. Mol. Biol.* **85,** 603–615.
8. Shinitsky, M. & Inbar, M. (1976) *Biochim. Biophys. Acta* **422,** 133–149.
9. Fuchs, P., Parola, A., Robbins, P. W. & Blout, E. R. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3351–3355.
10. Inbar, M., Yuli, I. & Raz, A. (1977) *Exp. Cell Res.* **105,** 325–335.
11. de Laat, S. W., van der Saag, P. T. & Shinitzky, M. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4458–4461.
12. Moore, N. F., Barenholz, Y. & Wagner, R. R. (1976) *J. Virol.* **19,** 126–135.
13. Barenholz, Y., Moore, N. F. & Wagner, R. R. (1976) *Biochemistry* **15,** 2766–2772.
14. Inbar, M. & Ben Basset, H. (1976) *Int. J. Cancer* **18,** 293–297.
15. Petitotou, M., Tuy, R., Rosenfeld, C., Mishal, Z., Paintrand, M., Jasnin, C., Mathe, G. & Inbar, M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2306–2310.
16. Poznansky, M. & Lange, Y. (1978) *Biochim. Biophys. Acta* **506,** 256–264.
17. Lenard, J. & Rothman, J. E. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 391–395.
18. Inbar, M. & Shinitsky, M. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4229–4231.
19. Hagerman, J. S. & Gould, R. G. (1951) *Proc. Soc. Exp. Biol. Med.* **78,** 329–332.
20. Berke, G., Tzur, R. & Inbar, M. (1978) *J. Immunol.* **120,** 1378–1384.
21. Collard, J. G., de Wildt, A. & Inbar, M. (1978) *FEBS Lett.* **90,** 24–28.
22. Miller, G., Robinson, J., Heston, L. & Lipman, M. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4006–4010.
23. Dolyniuk, M., Pritchett, R. & Kieff, E. (1976) *J. Virol.* **17,** 935–949.
24. Helenius, A. & Söderlund, H. (1973) *Biochim. Biophys. Acta* **307,** 287–300.
25. Bøyum, A. (1968) *Scand. J. Clin. Lab. Invest.* **21,** Suppl. 97.
26. Chess, L. & Schlossman, S. F. (1976) in *In Vitro Methods in Cell Mediated and Tumor Immunity,* eds. Bloom, G. & David, J. (Academic, New York), Vol. 2.
27. Yeagle, P. L., Martin, R. B., Lala, A. K., Lin, H. K. & Bloch, K. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4924–4926.
28. Lala, A. K., Lin, H. K. & Bloch, K. (1978) *Bioorg. Chem.,* in press.
29. Pessin, J. E., Salter, D. W. & Glaser, M. (1978) *Biochemistry* **17,** 1997–2004.
30. Helenius, A., Morein, B., Fries, E., Simons, K., Robinson, P., Schirrmacher, V., Terhorst, C. & Strominger, J. L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3846–3850.
31. Dales, S. (1973) *Bacteriol. Rev.* **37,** 103–135.
32. Seigneurin, J. M., Vuillaume, M., Lenoir, G. & de The, G. (1977) *J. Virol.* **24,** 836–845.