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Sendai virus-induced hemolysis: Reduction in heterogeneity of erythrocyte lipid bilayer fluidity

(spin label/protein–lipid interaction/divalent cations/hemolysis activation/hemagglutination)

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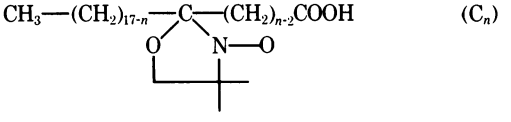
ABSTRACT **Hemolysis of human or chicken erythrocytes by Sendai virus causes a change in the structure of the erythrocyte membrane lipid bilayer that can be detected by spin label electron spin resonance. In the intact erythrocyte, the phosphatidylcholine derivative spin label exists in a more rigid environment than the corresponding phosphatidylethanolamine label. Virus-induced hemolysis tends to abolish this difference in fluidity, i.e., the region of the phosphatidylcholine spin label becomes more fluid and that of the phosphatidylethanolamine spin label becomes more rigid. Fatty acid derivative spin labels, which may detect some "average" environment, show no change in fluidity. The fluidity change is detected at several different positions in the fatty acyl chain of the phosphatidylcholine spin label. Sendai virions grown in Madin–Darby bovine kidney (MDBK) cells or grown in eggs and harvested early, which lack hemolytic activity, cause no significant change in bilayer structure. Hemolytic activity and the ability to alter erythrocyte bilayer fluidity can be activated in MDBK-grown Sendai virions by trypsin treatment *in vitro* and in early-harvest egg-grown Sendai virions by freezing and thawing. Erythrocyte ghosts prepared by osmotic hemolysis and resealed by treatment with Mg^{2+} or elevated ionic strength exhibit a difference in fluidity between phosphatidylcholine and phosphatidylethanolamine spin labels, although less than that observed in whole cells. Incubation of resealed ghosts with Sendai virus abolishes the difference in fluidity. Unsealed ghosts that have been extensively washed show no heterogeneity in membrane bilayer fluidity, and incubation with Sendai virus causes no further fluidity change. Virus-induced hemolysis as measured by hemoglobin release is more sensitive to inhibition by Ca^{2+} than is the associated fluidity change in the bilayer.**

In parainfluenza viruses such as Sendai virus, several biological activities are associated with the glycoprotein "spikes" present on the outer surface of the membrane-like envelope. Hemagglutination and neuraminidase activities are associated with one glycoprotein, HN, while hemolytic and cell-fusing activities are associated with the other glycoprotein, F (1–4). Interaction of enveloped viruses with cell membranes can cause changes in membrane structure that can be detected using the techniques of spin label electron spin resonance (ESR). Agglutination by influenza and Sendai viruses causes the membrane lipid bilayer of chicken but not human erythrocytes to become more fluid (5). This change in bilayer fluidity reflects changes in the interaction of the virus receptor with the lipid bilayer that may be mediated by microtubule-like systems, since the hemagglutination-induced fluidity change is sensitive to drugs that interfere with the function of microtubules (5).

We present here results indicating that changes in bilayer fluidity occur in both human and chicken erythrocytes following hemolysis by Sendai virus. Most of the experiments were performed on human erythrocytes, because the fluidity changes following hemolysis are not superimposed on hemagglutination-induced changes. The nature of the fluidity change depends upon the region of the lipid bilayer examined. The regions probed by the phosphatidylcholine spin label derivatives become more fluid, while those of the phosphatidylethanolamine label become more rigid. The effects on erythrocyte membrane structure of virus-induced hemolysis are similar in many respects to the effects of osmotic hemolysis (6). The viral factors involved in the fluidity change of the erythrocyte membrane were examined by altering the biological activities of the virus using appropriate conditions of virus cultivation. These experiments show that the viral glycoprotein F is required to alter membrane fluidity, but that the action of this protein does not lead to a fluidity change under conditions where lysis does not occur. It is suggested that the observed structural changes are probably due to alterations in the interaction between the lipids and proteins on the interior surface of the membrane.

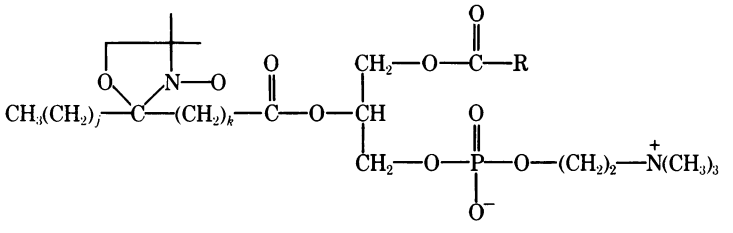
MATERIALS AND METHODS

Chemicals. The stearic acid derivative spin labels, C_n, have the structure



(C_n)

The spin labels C_5 and C_{16} were obtained from Syva, Palo Alto, CA. The C_7 label was synthesized according to Jost *et al.* (7) and C_{12} according to Waggoner *et al.* (8). The phosphatidylcholine spin labels (PC*n*)



PC5: $j = 10$, $k = 3$; PC7: $j = 10$, $k = 5$; PC12: $j = 5$, $k = 10$; PC16: $j = 1$, $k = 14$

were synthesized using carbonyl diimidazole (9). PC5 was synthesized using the nitroxide derivative of palmitic acid (10).

Abbreviations: ESR, electron spin resonance; MDBK cells, Madin–Darby bovine kidney cells; HAU, hemagglutinating units.

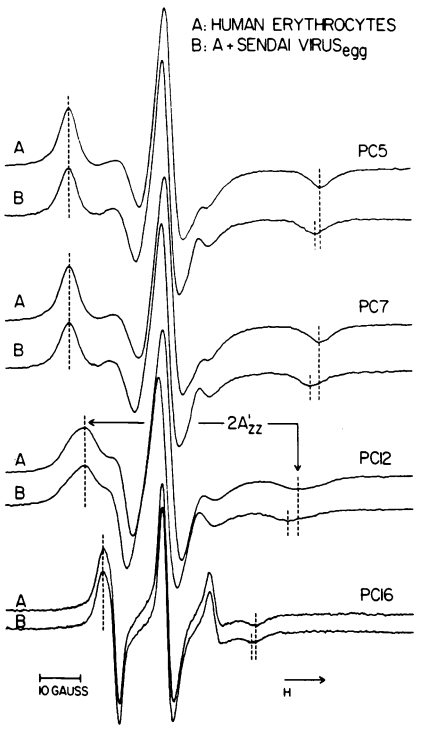


FIG. 1. Effect of Sendai virus-induced hemolysis on the structure of the lipid bilayer of the plasma membrane of human erythrocytes as detected by phosphatidylcholine derivative spin labels. Spectra were recorded at 21°. Vertical lines are drawn through the outermost peaks of the spectra. The splitting of the outermost peaks ($2A'_{zz}$) is defined for the control PC12 spectrum. Each spectrum is a time average of nine repetitive scans. One gauss = 10^{-4} tesla.

The phosphatidylethanolamine spin label (PE12) was synthesized from PC12 by the transphosphatidylation reaction of phospholipase D (Sigma Chemical Co.) (11), and purified on a silicic acid column (9). The phosphatidic acid spin label (PA12) was synthesized from PC12 by the action of phospholipase D (11) and purified by preparative thin-layer chromatography on silica gel in chloroform/methanol/acetic acid/water (80:13:8:0.3, vol/vol).

Virus. Sendai virus was grown in embryonated hen eggs or in Madin–Darby bovine kidney (MDBK) cells (4). Unless otherwise stated, the virus used in experiments was grown in eggs and harvested 48 hr after infection (late harvest). The WSN strain of influenza A_0 virus was grown in MDBK cells (12). The viruses were purified as described (13). Hemagglutination titrations were performed as described (4). Some variability in the ratio of hemolytic activity to hemagglutinating activity of different virus preparations was noted.

Spin Labeling of Erythrocytes. Erythrocytes were obtained from freshly drawn, heparinized human and chicken blood (5). The cells were washed four times with cold 150 mM NaCl/10 mM Tris·HCl (pH 7.4). A suspension of phospholipid derivative spin labels (1 mg/ml) in the same buffer was sonicated under N_2 at 4°, three times for 5-min intervals, with a Heat Systems Sonifier followed by centrifugation at 18,000 × g for 15 min. The supernatant was added to an equal volume of packed erythrocytes, and bovine serum albumin (14) (essentially free of fatty acids, 10 mg/ml, Sigma Chemical Co.) was added to a final concentration of 1 mg/ml. The cells labeled for 4 hr at 37° (6 hr for PE12) were washed three times with Tris-buffered saline (pH 7.4). Because PC5 and PE12 are not well suspended in the pure state, they were premixed with an equal amount of egg yolk phosphatidylcholine (15) in chloroform before the lipid suspension was formed (2 mg/ml of total phospholipid). Inclusion of an equal amount of egg lecithin in the PC12 vesicles does not alter the ESR spectrum. Erythrocytes were labeled with C_5 and C_{12} by exchange from unfiltered spin label–bovine serum albumin complex (13, 16).

Hemolysis of Spin-Labeled Erythrocytes. Spin-labeled erythrocytes (0.1 ml of packed cells) were incubated with virus [200–8000 hemagglutinating units (HAU)] in a final volume of 3.0 ml of Tris-buffered saline (pH 7.4) on ice for 10 min, followed by 1 hr at 37° with shaking (at which time the end point of hemoglobin release had been reached). The samples were cooled in an ice bath for 5 min and centrifuged at 18,000 × g for 15 min. The absorbance of the supernatant was determined at 590 nm. The absorbance is linear with hemoglobin concentration to an absorbance of 2.0. The experimental control without virus corresponds to no hemolysis, and the sample with cells incubated in buffer without NaCl or virus corresponds to 100% hemolysis. The ESR spectrum of the pellet in an aqueous cell with a capillary tip (James F. Scanlon Co.) was recorded at room temperature with a Varian E-12 spectrometer, interfaced to a Texas Instruments 980A computer (17). The pellet was not oriented in the sample cell. Data in each of the tables and figures are from representative experiments, each repeated at least two more times.

RESULTS

Hemolysis of human erythrocytes by Sendai virus results in a structural change in the lipid bilayer of the erythrocyte membrane as detected by spin-labeled derivatives of phospholipids incorporated into the erythrocyte membrane. Fig. 1 shows the ESR spectrum of phosphatidylcholine derivatives (PCn) containing a nitroxide free radical at various positions along the fatty acyl chain in the erythrocyte membrane before and after virus-induced hemolysis. Hemolysis by Sendai virus increases the fluidity of the erythrocyte membrane bilayer detected by phosphatidylcholine derivative spin labels, as indicated by a decrease in the splitting between the outermost peaks of the spectrum ($2A'_{zz}$, defined in Fig. 1) for each position in the fatty acyl chain examined (7, 10). As seen in Fig. 1, the motion of phospholipid fatty acyl chains in a lipid bilayer progressively increases at greater distances from the glycerol backbone, resulting in smaller spectral splitting (7, 10). The region probed by PC12 exhibits the largest change in $2A'_{zz}$, while the PC5 and PC16 labels reflect less of a change. The shapes of ESR spectra of PC16-labeled intact and hemolyzed erythrocytes indicate that the spin labels are incorporated into the lipid bilayer and that no significant fraction of the spin label is tightly bound to protein (16).

Heterogeneity in the distribution of membrane lipids may result in regions within the lipid bilayer that are characterized by different fluidity. Heterogeneity in the fluidity of the erythrocyte membrane has been observed (6). Table 1 illustrates the structural change in the membrane bilayer following virus-induced hemolysis detected by different types of spin labels. The heterogeneity in fluidity is readily seen by comparing $2A'_{zz}$ of the different labels in intact cells, which show that the phosphatidylcholine derivative spin label experiences a more rigid environment than does the phosphatidylethanolamine derivative. Following virus-induced hemolysis, this heterogeneity disappears with increasing virus concentration: the environment of the phosphatidylcholine spin label becomes more fluid while that of the phosphatidylethanolamine spin label becomes more rigid. The phosphatidic acid (PA12) derivative spin label gives results similar to those of the phosphatidylethanolamine label. The fatty acid derivative spin labels, C_5 and C_{12}, which may detect some "average" environment, show no change after hemolysis by Sendai virus. Direct

Table 1. ESR spectral splitting of spin labels in the human erythrocyte membrane after hemolysis by Sendai virus

Label	$2A'_{zz}$ (gauss)	
	Control	+ Sendai virus
PC12	51.5	49.1
PE12	45.4	48.5
C_5	58.3	58.5
C_{12}	52.9	52.6

Spin-labeled human erythrocytes (0.1 ml of packed cells) were incubated with late harvest Sendai virus grown in eggs (8000 HAU) for 1 hr at 37° as described in *Materials and Methods*. Spectra were recorded at a room temperature of 21°. The ESR spectral splitting ($2A'_{zz}$) is defined in Fig. 1. In general, differences in $2A'_{zz}$ greater than ±0.3 gauss can be detected by superposition of spectra.

comparison of the splitting of the C_{12} label with that of the phospholipid derivatives is difficult, because fatty acid derivative spin labels appear to probe regions slightly closer to the bilayer surface than do the corresponding phospholipid derivatives (18). The complex effect on membrane bilayer fluidity by virus-induced hemolysis is similar to the effect of osmotic lysis, which also increases the fluidity of phosphatidylcholine spin labels, decreases that of phosphatidylethanolamine spin labels, and has no effect on fatty acid derivative spin labels (6, 16).

Table 2 shows the effect of hemolysis on spin-labeled erythrocytes by Sendai virus preparations, the biological activities of which have been modified by the appropriate choice of the conditions of virus cultivation. Sendai virions grown in MDBK cells lack the F glycoprotein, which is responsible for the hemolytic and cell-fusing activities of the virus, but contain F_0, an inactive precursor to F which can be activated by trypsin cleavage *in vitro* (4). The structural change in the lipid bilayer of the erythrocyte following hemolysis is dependent upon an active F protein, since MDBK-grown Sendai virions have little effect on the spectral splitting of phosphatidylcholine derivative

Table 2. Effect of Sendai virus preparations lacking hemolytic activity on ESR spectrum of PC12 spin label in the human erythrocyte membrane

Virus	% hemoglobin release	$2A'_{zz}$ (gauss)
None	0	52.2
$Sendai_{egg}$—late harvest*	52.2	50.4
$Sendai_{MDBK}$†	11.9	51.5
$Sendai_{MDBK}$—trypsin activated†	30.8	50.1
$Sendai_{egg}$—early harvest*	2.5	51.9
$Sendai_{egg}$—early harvest, frozen and thawed*	46.2	50.5
None—human erythrocyte ghosts‡	100	49.3

PC12-labeled human erythrocytes (0.1 ml of packed cells) were incubated with Sendai virus (500 HAU) for 1 hr at 37° as described in *Materials and Methods*.

* Sendai virus grown in eggs was harvested at either 24 hr (early harvest) or the usual 48 hr after infection (late harvest). Hemolysis activity of early harvest virus was induced by freezing and thawing 10 times.

† Sendai virus was grown in MDBK cells and hemolysis activity was activated by trypsin treatment as described by Scheid and Choppin (4).

‡ Hemoglobin-free erythrocyte ghosts were prepared according to Steck (21).

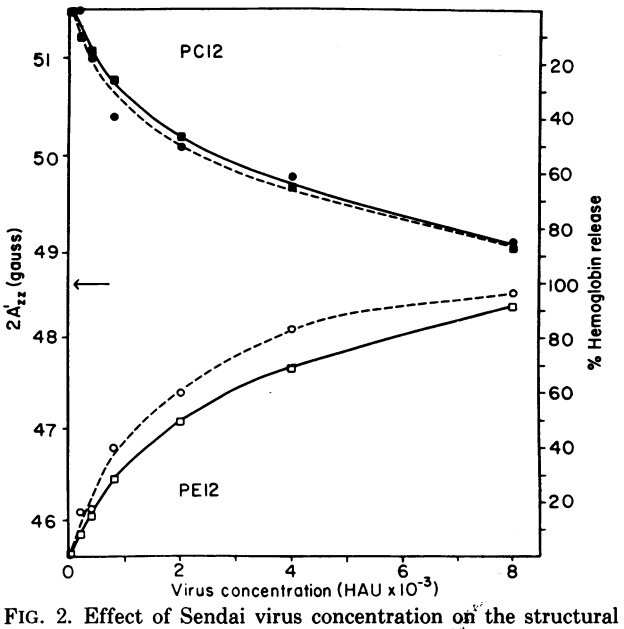


FIG. 2. Effect of Sendai virus concentration on the structural change in human erythrocyte plasma membrane as a result of hemolysis. PC12-labeled erythrocytes: ■—■, % hemoglobin release; ●- - -●, $2A'_{zz}$. PE12-labeled erythrocytes: □—□, % hemoglobin release; O- - -O, $2A'_{zz}$. Spectra were recorded at 21°. To demonstrate the correspondence between the observed hemoglobin release and the ESR spectral change for both labels, the direction of the % hemoglobin release axis is inverted, and the scale of the $2A'_{zz}$ axis is expanded slightly above the position indicated by the arrow for the PC12 data. The $2A'_{zz}$ value corresponding to 100% hemolysis was measured from spectra of spin-labeled hemoglobin-free ghosts prepared by osmotic lysis using the procedure described by Steck (21), whose spectra exhibit no difference in $2A'_{zz}$ between PC12 and PE12 (compare Table 3).

spin label PC12, while trypsin-activated, MDBK-grown Sendai virions cause a decrease in $2A'_{zz}$ of the spectrum consistent with their greater hemolytic activity (Table 2). When Sendai virus grown in embryonated eggs is harvested 24 hr after infection instead of the usual 48 hr, it lacks hemolytic activity even though it has an active F glycoprotein and is able to cause cell fusion. Hemolytic activity can be activated in early harvest virions by a number of techniques, including freezing and thawing, possibly by inducing a change in the virus envelope necessary for hemolysis (19). Early harvest Sendai virions cause no structural change in the lipid bilayer of human erythrocytes, while virions activated by freezing and thawing do (i.e., a decrease in $2A'_{zz}$ as seen in Table 2). Thus, only those Sendai virions possessing hemolytic activity are able to alter the bilayer fluidity as detected by phospholipid derivative spin labels.

The dependence on virus concentration of the fluidity changes detected by phosphatidylcholine and phosphatidylethanolamine derivative spin labels (PC12 and PE12) after hemolysis by egg-grown Sendai virus is shown in Fig. 2. The extent of hemolysis as measured by hemoglobin release is also shown as a function of virus concentration, and is superimposed on the plots of the fluidity changes by letting the spectral splitting of spin-labeled erythrocyte ghosts correspond to 100% hemolysis. The structural change in the erythrocyte lipid bilayer detected by phosphatidylcholine and phosphatidylethanolamine spin labels directly reflects the extent of hemolysis as measured by hemoglobin release. The close correspondence between hemoglobin release and the structural change in the erythrocyte lipid bilayer can be altered by including divalent cations in the hemolysis reaction. As shown in Fig. 3, Ca^{2+}, and to a lesser extent Mg^{2+}, inhibits hemolysis induced by Sendai virus as measured by hemoglobin release (20), but the fluidity

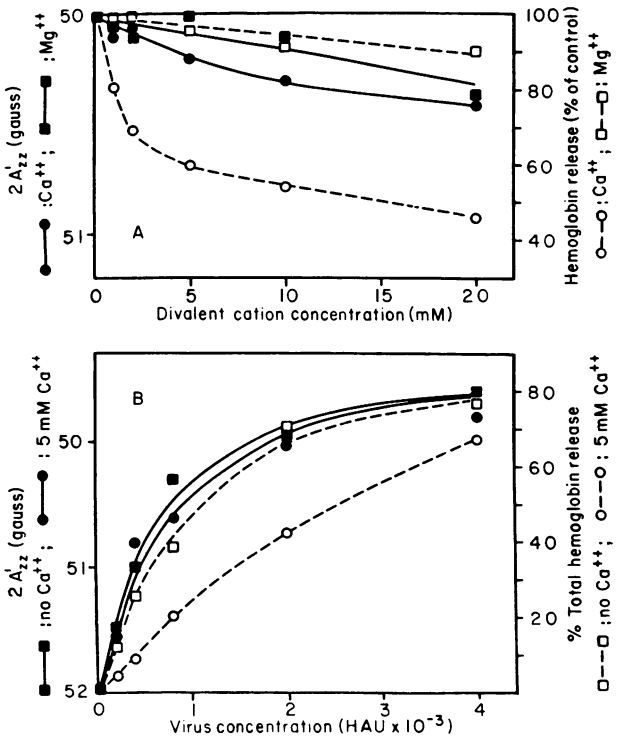


FIG. 3. Inhibition by divalent cations of Sendai virus-induced hemolysis of human erythrocytes labeled with PC12. (*A*) Effect of divalent cation concentration; $MgCl_2$ or $CaCl_2$ was added at the indicated concentrations; virus concentration is 1000 HAU. Hemoglobin release is plotted as percent of control (i.e., virus without divalent cations). (*B*) Effect of Sendai virus concentration; $CaCl_2$ concentration is 5 mM. Spectra were recorded at 20°. The $2A'_{zz}$ scales in both *A* and *B* were chosen to correspond to the appropriate percent hemoglobin release.

change in the erythrocyte bilayer is inhibited to a lesser extent.

The effect of Sendai virus on spin-labeled erythrocyte ghosts also illustrates the similarity of the effects of osmotic and virus-induced hemolysis. Erythrocyte ghosts, prepared by the usual procedure of lysis in hypotonic buffer followed by extensive washing to remove hemoglobin, do not show the heterogeneity in membrane fluidity between phosphatidylcholine and phosphatidylethanolamine spin labels seen in intact cells (ref. 6 and Table 3). Incubation with Sendai virus causes no further change in the spin label spectrum in addition to that resulting from osmotic hemolysis (Table 3). Ghosts can be resealed by including 1 mM $MgSO_4$ in the hypotonic buffer at 4° or by incubating ghosts, which have not been extensively washed, in isotonic salt solutions at 37° (21). Mg^{2+} ions inhibit the fluidity changes in the erythrocyte bilayer following osmotic lysis when hemolysis is carried out at 0° (although no inhibition is observed at 37°, the temperature at which virus-induced hemolysis is assayed) (ref. 6 and Table 3). Ghosts resealed in isotonic NaCl also exhibit some heterogeneity in bilayer fluidity, although much less than intact cells or Mg^{2+}-sealed ghosts (Table 3). This remaining heterogeneity in the ghosts is present before the addition of NaCl. Incubation of spin-labeled ghosts, resealed by either method, with Sendai virus for 1 hr at 37° abolishes the remaining heterogeneity in bilayer fluidity (Table 3).

Chicken erythrocyte plasma membranes undergo a structural change detectable by the PC12 spin label upon hemagglutination by influenza virus or Sendai virus grown in MDBK cells (Table 4). Upon hemolysis of chicken erythrocytes by egg-grown Sendai virus, a much larger decrease in spectral splitting occurs, similar to that seen upon hemolysis of human erythrocytes.

Table 3. Effect of Sendai virus on spectrum of PC12 and PE12 spin labels in human erythrocyte ghosts

Type of erythrocyte membrane	$2A'_{zz}$ (gauss)		
	PC12	PE12	PC12 − PE12*
Intact cell	50.8	45.5	5.3
+ Sendai virus	49.0	48.9	0.1
Mg^{2+}-sealed ghosts	49.6	46.2	3.4
+ Sendai virus	48.8	47.9	0.9
NaCl-sealed ghosts	48.8	47.4	1.4
+ Sendai virus	49.0	48.7	0.3
Unsealed ghosts (washed three ×)	48.7	48.6	0.1
+ Sendai virus	48.9	48.6	0.3

Spin-labeled human erythrocyte ghosts (0.1 ml of packed cells) prepared according to Steck (21) were incubated with late harvest egg-grown Sendai virus (8000 HAU) for 1 hr at 37° in the buffer in which they were prepared. Spectra in this table were recorded at a room temperature of 22°.

* The difference between the spectral splitting of PC12 and PE12 can be used as a measure of bilayer heterogeneity.

DISCUSSION

Spin label ESR methods have been useful in studying the various aspects of the interaction of enveloped viruses with cell membranes. A previous study revealed changes in membrane lipid bilayer fluidity upon agglutination of avian erythrocytes by viruses and lectins (5). It has been shown that cell fusion induced by Sendai virus results in the intermixing of lipids in the membranes being fused (22). As shown here, changes in erythrocyte membrane structure occur following hemolysis induced by Sendai virus.

The nature of the membrane structural change that follows virus-induced hemolysis is rather complex. In the erythrocyte membrane, the phosphatidylcholine derivative spin label exists in a more rigid environment than does the phosphatidylethanolamine derivative (ref. 6 and Table 1). This heterogeneity in membrane fluidity disappears upon hemolysis, i.e., the environment of the phosphatidylcholine spin label becomes more fluid and that of the phosphatidylethanolamine spin label becomes more rigid. Interestingly, the fatty acid derivative spin labels, which may reflect an "average" fluidity of the membrane bilayer, are unaffected by hemolysis. The same pattern of fluidity changes is seen following osmotic hemolysis (6, 16). Similar results have been obtained by complement-induced hemolysis (23).

Table 4. ESR spectral splitting of PC12 spin label in chicken erythrocyte membranes after viral agglutination and hemolysis

Virus	% hemoglobin release	$2A'_{zz}$ (gauss)
None	0	55.7
Influenza	1.8	55.1
$Sendai_{MDBK}$	8.3	54.6
$Sendai_{egg}$—late harvest	30.8	51.0

Spin-labeled chicken erythrocytes (0.1 ml of packed cells) were incubated with virus (1000 HAU) for 1 hr at 37° as described in *Materials and Methods*. Spectra in this table were recorded at a room temperature of 20°.

Tanaka and Ohnishi (6) have implicated proteins on the interior surface of the erythrocyte membrane in the maintenance of heterogeneity in lipid fluidity, and have suggested that alterations in these proteins occur following hemolysis which result in the disappearance of this heterogeneity. This hypothesis is supported by the present observation that erythrocyte ghosts that have not been extensively washed retain a limited amount of bilayer heterogeneity that disappears upon subsequent washing. Incubation of ghosts in low ionic strength buffers causes release of detectable amounts of membrane protein even at 4° (24). The similarity between the effects of virus-induced hemolysis and osmotic hemolysis suggests that virus-induced hemolysis also alters lipid–protein interactions on the interior surface of the erythrocyte membrane, and this alteration of protein on the interior surface of the membrane leads, in turn, to a change in structure of the lipid bilayer.

Sendai virions lacking full hemolytic activity at 37° can be obtained by growing Sendai virus in MDBK cells or by harvesting egg-grown Sendai virus 24 hr after infection instead of the usual 48 hr. MDBK-grown virions have an inactive precursor to the F glycoprotein responsible for hemolysis and cell fusion, which can be activated by trypsin treatment *in vitro* (4). Early harvest egg-grown virions, although containing an F protein that can cause cell fusion, are unable to lyse erythrocytes, possibly because of a structural difference in the viral envelope involving ion permeability (19). Neither MDBK-grown Sendai virus nor egg-grown virus harvested early causes a significant change in the fluidity of phospholipids in the human erythrocyte membrane, while trypsin-activated, MDBK-grown Sendai virus or frozen and thawed early harvest virus causes a change similar to that of fully active, egg-grown Sendai virus. Thus, it appears that not only for hemolysis itself, but also for the viral hemolysis-induced fluidity change in the erythrocyte membrane, the action of the F glycoprotein, although required, is not sufficient.

Ca^{2+} and to a limited extent Mg^{2+} inhibit virus-induced hemolysis as measured by hemoglobin release (ref. 20 and Fig. 3), but have less of an effect on the bilayer fluidity changes (Fig. 3). This discrepancy may arise from the ability of divalent cations to induce resealing of leaks in the erythrocyte membrane (21), thus preventing complete hemoglobin release from cells in which the structural change in the bilayer has already occurred. The effect of divalent cations on bilayer fluidity changes following osmotic hemolysis is complex. Mg^{2+} but not Ca^{2+} tends to preserve the heterogeneity in membrane fluidity if present in the hemolysis buffer at low temperature, but has no effect at 37°, the temperature at which virus-induced hemolysis is assayed (6). Thus, Mg^{2+}-resealed ghosts prepared according to Steck (21) show a considerable difference in fluidity between phosphatidylcholine and phosphatidylethanolamine spin labels (Table 3). Ghosts that have been prepared without Mg^{2+} and washed once can be resealed by incubation at 37° at isotonic salt concentrations. These ghosts retain some heterogeneity in bilayer fluidity (Table 3). Further washing of unsealed ghosts abolishes the remaining heterogeneity. Incubation of ghosts resealed by either Mg^{2+} or NaCl with Sendai virus abolishes the heterogeneity in membrane bilayer fluidity. Incubation of extensively washed, unsealed ghosts with Sendai virus causes no further changes in bilayer fluidity, as would be expected if osmotic and virus-induced hemolysis affect the bilayer in a similar manner.

The interaction of enveloped viruses with cell membranes may result in changes in membrane bilayer fluidity that probably involve altering lipid–protein interactions within the membrane. An earlier study showed that agglutination of avian (but not human) erythrocytes by viruses caused the lipid bilayer to become more fluid, possibly by inducing rearrangements of receptor molecules mediated by microtubule-like systems (5). The changes in erythrocyte membrane bilayer fluidity following virus-induced hemolysis described here are probably also due to an alteration in the interaction between membrane lipid and membrane protein that is associated with the interior surface of the erythrocyte plasma membrane. This change in membrane lipid–protein interaction resulting in fluidity changes in the bilayer is reminiscent of, but different from, the effects of hemagglutination.

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