STRUCTURE OF THE LIPID PHASE OF RAUSCHER MURINE LEUKEMIA VIRUS

[a]Frank R. Landsberger, [b]Richard W. Compans, [c]John Paxton and [a*]John Lenard

[a]Sloan-Kettering Institute for Cancer Research, New York, N. Y. 10021

[b]Rockefeller University, New York, N. Y. 10021

[c]Varian Associates, Springfield, N. J. 07081

Summary: The lipid-containing membrane of Rauscher murine leukemia virus was studied using stearic acid spin labels with the nitroxide ring on the C_5 and C_{16} positions. The environment of the C_5 spin label was found to be much more rigid than that of the C_{16} spin label. This result, which parallels similar observations in red cell membranes and influenza virus, suggests that the lipid phase of Rauscher murine leukemia virus is arranged in a bilayer.

Electron spin resonance spectroscopy using spin labels has proved to be a useful tool in studying the geometric organization of lipids in both natural and synthetic membranes (1-9). Especially valuable for this purpose are spin labels which probe the lipid phase at specific distances from the membrane surface. The spectra from membranes into which a spin label has been intercalated may be qualitatively interpreted in terms of the rigidity or fluidity of the local environment. By comparing the rigidity at several different depths of the membrane interior, conclusions can be drawn regarding the overall structural organization of the membrane lipid phase (3,4,9).

We have previously shown that spectra of spin-labeled influenza virus and of human red blood cell membranes have similar characteristics which suggested that the lipid in the virus was arranged in a bilayer (9). The surface membranes of transformed cells have properties which may be reflected in the envelopes of oncogenic viruses which form by a process of budding at the plasma membrane. To investigate the arrangement of lipids in such a virus, we

* Established Investigator of the American Heart Association

compared the spectra of spin-labeled Rauscher murine leukemia virus (RLV) with the spectra of influenza virus and human red cell membranes which we have previously described (9).

The growth, purification and spin-labelling procedure for influenza virus has been described (9). For growth of RLV, the SLS-V9 line of mouse bone marrow cells transformed by this virus was used (10). Cells were grown in reinforced Eagle's medium (11) with 20% calf serum in 75 cm^2 Falcon plastic flasks. After monolayers were confluent, the growth medium was replaced with reinforced Eagle's medium with 2% calf serum. Virus was harvested after incubation at 37° for two or more days and was purified by precipitation with polyethylene glycol and equilibrium zonal centrifugation in a 5-40% potassium tartrate gradient as described previously for influenza virus (9). Samples containing 200-400 μg of virus were spin-labeled by incubating with 2 ml. of a bovine serum albumin-spin label complex (1 mg. of spin label and 50 mg. of bovine serum albumin per ml), and repurified in a potassium tartrate gradient. The number of spins in the sample was estimated relative to a pitch standard measured under the same conditions, and it was concluded that less than 0.1% of the lipid molecules were spin labels. Human red blood cells were obtained from freshly drawn heparinized blood by repeated washings with cold phosphate buffered saline and repeated removal of the buffy coat after centrifugation. They were spin-labeled as described previously (9). Spectra were obtained on a Varian E-12 electron spin resonance spectrometer.

In Fig. 1 the spectra of Rauscher virus are compared with those obtained from influenza virus and red blood cells. Each tracing represents superposition of two types of spectra, a "broad line" and a "liquid line" component. The "liquid line" spectrum consists of three sharp lines arising from isotropically tumbling spin labels. The amount of "liquid line" present can be estimated from the intensity of the narrow line denoted "LL" in Fig. 1. There is a negligible amount of "liquid line" in the red cell spectra, while the influenza virus spectra have a larger, but still quite small, amount. The RLV spectra,

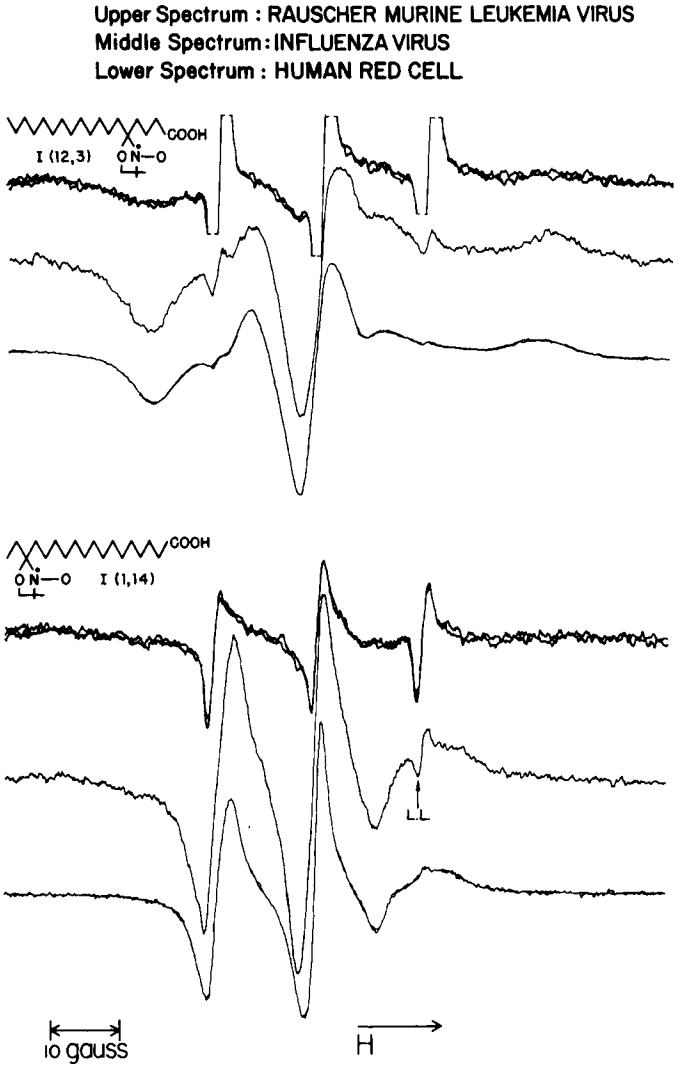

Fig. 1. Comparison of spectra of spin labels I (1,14) and I (12,3) incorporated into Rauscher murine leukemia virus, influenza virus, and human red blood cells. LL indicates the position of the high magnetic field peak of the "liquid line" superimposed on the "broad line" spectrum (see text). The three "liquid line" peaks of I (12,3) in RLV have been clipped in order to facilitate comparison between the "broad line" spectra. H denotes the magnetic field.

however, are characterized by an extremely intense "liquid line" component. This is thought to arise either from spin label which is adsorbed to the outer surface of the virus or to label associated with lipid droplets which may be present as contaminants in the virus preparation. Consequently, this component of the spectrum has not been interpreted in terms of membrane structure.

Apart from the intensities of the "liquid line", there is a striking similarity between the "broad line" spectra of the various membrane systems for each of the two spin labels. This component arises from spin label which is intercalated into the lipid phase of the virus. It may be interpreted in terms of the fact that the splitting between the low and high magnetic field peaks increases as the environment limits the motional freedom of the spin label. Thus, in terms of mechanical rigidity at different levels of the lipid phase, all these membranes are quite similar. In the vicinity of the polar head groups the lipid phase is quite rigid, as shown by spin label I (12,3) (Fig. 1, top). Deeper in the hydrocarbon region, as shown by spin label I (1, 14) (Fig. 1, bottom), there is greater fluidity and consequent narrowing of the "broad line" spectrum. A more careful inspection of the spectra obtained from I (12,3) shows that the "broad line" splittings for RLV and influenza virus are approximately the same, but are somewhat larger than the splitting observed for the erythrocytes. This indicates that the spin labels in RLV and influenza virus are in a somewhat more rigid environment than is the case in the red cell membrane. A similar comparison cannot be made with the spectra from I (1,14) because of the strong interference by the "liquid line" in the RLV spectrum.

It has recently been shown by several physical techniques that the lipids of a number of biological membranes, including red cell ghosts, are arranged in a bilayer (12-14). Spin label studies of these membranes and of synthetic multilayers indicates that these bilayer structures are characterized by a flexibility gradient, i.e. an increasingly rigid environment as the nitroxide ring is placed closer to the polar head group of a stearic acid molecule (4,15). The striking similarity of the spectra of spin-labeled influenza virus and of

red blood cell membranes led to the conclusion that the lipids of influenza virus are also arranged in a bilayer (9). The present data provide similar evidence for the presence of a lipid bilayer in the envelope of Rauscher virus. Thus, at the present level of resolution, the lipid component of an oncogenic virus has an arrangement which cannot be distinguished from that of a non-oncogenic enveloped virus.

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