COMPREHENSIVE REPORT OF THE OVERALL ACTIVITIES OF THE CONTRACT
DURING THE PAST THREE YEARS

Pennsylvania State University

The research accomplishments carried out during the last three years of support by ERDA are outlined in the Progress Report. These entail various experiments that were carried out using spin labels to uncover information about the physical properties of biological membranes. A second general approach that was developed by this laboratory during that period was the use of paramagnetic broadening agents as an approach to explore certain restricted areas of cell and cellular systems through the use of spin labels. By utilization of this approach it is possible to remove the spin label signal from heterogeneous environments from all zones except a particular one. The third general area of accomplishment during this interval was the initiation of a class of spin labels which we call surface labels. These labels are designed to label the aqueous hydrocarbon interface of one side of a membrane enclosure. This approach is still new to us and its full potential is not yet developed, but we believe it is a profitable approach; it overcomes a number of the criticisms that have been leveled at spin labels over the years.

The plans for the continuation of the present objectives are outlined in the new proposal for the period of November 1, 1976 through October 31, 1977. These include the continued development of surface labels to obtain useful information about membrane surfaces, the continued development of spin label line broadening agents and particular stress on studies dealing with the measurement of diffusional processes in the cellular cytoplasm and at the membrane interfaces.

I came from the University of California at Berkeley to the Pennsylvania State University in the spring of 1972. Two students have obtained their Ph.D. degrees in that increment

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of time. One of these, Jim Lepock, has taken a postdoctoral fellowship at Tufts University Medical School in the laboratory of Prof. Wallach. The other student, Davor Juretić, who was completely supported on the ERDA research grant throughout his Ph.D. training, has taken a postdoctoral fellowship in the laboratory of Prof. Tien in the Biophysics Department at Michigan State University. In addition to these, one student, Linda Adams, achieved a M.S. degree and is presently engaged in pursuing a career in industry. Dr. Gerald Cohn was a postdoctoral fellow in this laboratory and is presently employed by the Illinois Institute of Technology, Physics Department, as Assistant Professor. Another postdoctoral fellow, Dr. Philip Morse II, is presently Assistant Professor in the Biology Department at Wayne State University in Detroit, Michigan. Other students are in the process of being trained.

All publications resulting from our work during this interval of time are given in the bibliography, and those that have special reference to ERDA support are commented upon by number in the Progress Report.

My opinion as to the present state of knowledge in this area of research is that it is significant to biology and medicine. Needed future investigations are somewhat difficult to project. The general field of membrane research has developed rapidly, and since the entrance of molecular biologists into the field (in the neighborhood of 1967-1968) progress has been substantially accelerated. At the present time there are investigators exploring the biological and physical properties of membranes from many directions so that it is difficult now to devise experiments which are purely phenomenological without some unique approach on the part of the experimenters. I believe it is vital to continue work on biological membranes, but I do not feel that a tremendous amount of work, all essentially the same, should be carried out. I believe that my approach to

the study of membranes is sufficiently unique that it does have a high probability of acquiring information useful to the field and that will be helpful to others over a fairly long period of time.

While membranes have been exploited and explored to the limit of present capability by a variety of researchers, the interactions between the properties of biological membranes and the cytoplasm have not been suitably explored. While there are many investigations that have been carried out on water itself, most of these have not been done in a very sophisticated way with respect to the aqueous component of cytoplasm. I believe our approach in studying rotational and diffusional properties of spin labeled solutes has an extremely good chance of being important to many aspects of biophysics. I think that these findings may well answer or help to answer a number of questions about cell function which cannot possibly be answered by studies on biological membranes or by present approaches being utilized by most investigators.

The present division of federal support for my entire research program is presented in the appropriate location in the Research Proposal. All of the various sources of support are documented at that point and the subjects covered by the various ones are well defined by the accompanying titles.

PROGRESS REPORT

The research efforts described here were carried out between the fall of 1973 and the fall of 1976. Most of this research has been published. These publications are briefly described and are placed into appropriate categories. Current research or research efforts that have not yet been published are placed in the same classifications.

The three categories shown below comprise the general approach that has been used by this laboratory in the past three years:

Spin labeled membranes as a general approach

Spin exchange and broadening agents

Surface labels

Spin labeled membranes

References 1, 7, 14 and 16 all directly relate to measuring the physical properties of biological membranes with respect to the phenomenological phase transition event. Reference 1 deals with chloroplast membranes and attempts to measure a different type of induced conformational change in the membrane than we had previously dealt with. In this case the chloroplast membranes were photoexcited and the spin label

spectral properties were assayed before and after the photoexcitation. After a careful analysis of this work it was determined that there is a slight measurable modification in the spin label detectable physical properties of the chloroplast membrane after photoexcitation. This type of study is complicated since the reducing environment of the chloroplast membrane, which under some conditions will reduce paramagnetic spin labels to diamagnetic species, is substantially modified by different conditions of illumination and because the entire metabolic state of the chloroplast is changed by illumination conditions. It is easy to measure apparent large changes in the chloroplast membrane under conditions of illumination; however, most of these large changes in spin label measurements are due to loss of spin label signal caused by modification in the reduction environment. This study distinguishes between spectral changes caused by modifications in the reduction environment and spectral changes caused by conformational modifications of the membrane.

References 7 and 14 deal directly with the inferred detection of phase transitions in a plant and animal membrane system respectively. The detectable phase transition events correlate with changes in the activity of biological events since both occur at the same temperature. Reference 16 is a somewhat more sophisticated treatment of the detection of phase transitions in an animal system and deals with a

hibernating animal. Hibernating animals seem to have different mitochondrial membrane properties while in hibernation and while in phase between hibernations. It was determined in this study that animals in the hibernation state lose a detectable phase transition in mitochondria and quickly regain the phase transition upon returning the animal to nonhibernating conditions. The assignment of the cause of the loss of a phase transition at the hibernating temperature was made. The assignment was attributed to a much higher proportion of lysophospholipids which were found to exist in the lipids of the hibernating animals' mitochondria. Adding approximately the same percentage of lysophosphatides to normal mitochondria or mitochondrial extracted lipids also took away the previously detectable phase transition.

I have included three other studies that were primarily carried out on spin labels, under this category, because the findings have relevance to membrane research. Reference 6 is a straightforward study which deals with a measurement of crystal parameters of the spin label TEMPONE. Reference 9 deals with the characterization of an ^{15}N TEMPONE molecule. Since ^{15}N spin labels have two hyperfine lines rather than three, the spectra can be dealt with by more simple analyses. The general motional properties of ^{15}N TEMPONE are described in this paper. Reference 20 is a sophisticated treatment of rapid passage ESR carried out on the ^{15}N TEMPONE molecule.

All of the theoretical and experimental work in this study was carried out by Larry Dalton and associates at Vanderbilt University. My part was to supply the ^{15}N TEMPONE and to contribute a few telephone conversations to the study.

Spin exchange and broadening agents

Our first experiments on this subject are described in reference 2. In this study hydrocarbon spin labels having various chain lengths were dissolved in highly purified tristearin. The tristearin containing spin label was taken to the liquid phase and vigorously mixed. Samples of this liquid phase were rapidly cooled in microcapillary tubes by immersing the microcapillary tubes in liquid freon at -160°C . The samples cooled in this manner had a characteristic signal of spin labels spaced far apart and rigidly immobilized. Heating the microcapillary tubes for very brief periods of time to temperatures still considerably below the melting point of the tristearin resulted in the spectra changing from that of immobilized spectra to spin exchange spectra. All signals were taken at liquid nitrogen temperatures. These data indicated that spin labels were capable of migrating through space at temperatures as far as 55° below the melting point of the tristearin. Simultaneous NMR measurements taken on these samples indicated that the tristearin molecules were in fact rigidly immobilized. The main

point of this study was to point out that a solute molecule dissolved in a solvent whose molecular structure is somewhat different allows the solute to still undergo extensive rotational diffusion and to some extent translational diffusion even when the solvent is in the solid state. The spin exchange spectra were characteristic of typical exchange narrowed spectra indicating that the spin label molecules were grouped together so that they had overlap of the wave functions of the unpaired electrons.

In order to carry out more extensive and more sophisticated studies of this type it became necessary to characterize fundamental aspects of spin exchange so that otherwise well-known principles could be better applied to biophysical experiments. The following is a brief account of the experiments we have carried out in an attempt to characterize aspects of the spin exchange that are relevant to biophysical experiments.

The data shown in Fig. 1 is a considerable condensation of the work presented in reference 31. Figure 1A shows the concentration dependence of line broadening of ^2H -TEMPONE signal. ^2H -TEMPONE was used in this experiment because it has a more narrow line width than TEMPONE. ^2H -TEMPONE at 10^{-4} M in degassed water has a minimum line width of approximately 0.18 gauss. In Fig. 1A for the line labeled TEMPONE, the TEMPONE concentration was varied. For all other lines the species shown at the end of the line had its concentration

varied while that of TEMPONE was maintained constant. This figure illustrates that line broadening is directly proportionate to molarity and that different species have different efficiencies of line broadening. Figure 1B illustrates that as viscosity of the solvent is increased the effectiveness of line broadening decreases. The four lines shown from left to right represent varying the concentration of TEMPONE in water, in 65% glycerol, in 75% glycerol, and in 85% glycerol. As can be seen, the concentration dependence for line broadening increases as rotational correlation time (τ_c) increases. Figure 1C shows the effect of negative charges on the TEMPOL spin label interacting with a negatively charged broadening agent, potassium ferricyanide. The TEMPOL molecule interacts with the potassium ferricyanide molecule to result in about the expected degree of line broadening based on the concentration dependence for collisional events. A TEMPOSULFATE molecule shows a much lower collision frequency and the TEMPOPHOSPHATE molecule is still lower. These two negatively charged spin labels illustrate the effect of charge repulsion. Figure 1D shows similar data, only this time a positively charged broadening agent, nickel chloride, is used, and the effects of repulsion from positively charged spin labels carrying a +1 charge, TEMPAMINE and TRIMETHYLTEMPAMINE, show a smaller magnitude effect with respect to charge repulsion.

Figure 1E shows data that is extremely relevant to bio-

physical experiments. For this experiment we used polyacrylamide beads containing different pore sizes. The P notation means that all molecules of that size and larger are excluded from the bead pore volume. P100 means 100,000 molecular weight, P30 means 30,000 molecular weight, and so on. As TEMPONE enters the beads having smaller pore sizes the line broadening that results from spin label-spin label interaction is drastically reduced. These data are perhaps presented more clearly in Fig. 1F where line broadening is plotted against the reciprocal of rotational correlation time. The open and closed circles are for TEMPONE dissolved in various mixtures of water and glycerol. Media of this composition is approximately isotropic. The line characterized by squares is taken from the data shown in Fig. 1E. These data indicate that rotational correlation time is reduced only slightly while collisional dependent line broadening is reduced drastically. These data mean that heterogeneous environments such as the trapped volume in polyacrylamide beads may well have barriers to translational diffusion which do not effectively operate as barriers to rotational diffusion. This is the type of environment expected to be encountered in the cell interior. The aqueous cytoplasm of cells may be expected to have a multitude of diffusion barriers of various types and shapes.

A general approach of using ionic or chelate broadening

agents to remove spin label signal from certain selected environments has been put to use in a number of experiments carried out in this laboratory. Reference 3 is a preliminary attempt to put this principle to use. Several cell types were analyzed with TEMPONE and nickel chloride in an attempt to measure the restriction to rotational diffusion of the TEMPONE molecule while located in the aqueous cytoplasm. Results of this experiment showed that the restriction to rotational diffusion of the TEMPONE molecule was greater to varying degrees in different cells than what would be predicted from NMR experiments (32) which have been carried out on water in cells. At present there is no absolute way of drawing a one-to-one correlation between the two types of experiments. The NMR experiments carried out on water molecules inside cells indicate that water is about a factor of 2 reduced in translational diffusion based on self-diffusion measurements using the spin echo technique (33). Spin label experiments indicate that the rotational diffusion of TEMPONE or other similar size spin labels dissolved in mammalian cells is restricted considerably more. The two experiments may be perfectly consistent with each other since it is not always clear that the same forces that restrict the diffusion of molecules considerably larger than water will always have the same degree of restriction to the motion of water molecules themselves.

Reference 15 was a somewhat more sophisticated approach

aimed at characterizing the viscosity profile of sarcoplasmic reticular vesicles. For this study, a variety of spin labels were used. Some of the spin labels were hydrocarbon soluble and some were water soluble. Information was accumulated relating both to the membrane of the sarcoplasmic reticular vesicles and to the trapped aqueous interior. The variety of hydrocarbon soluble spin labels used revealed that the hydrocarbon phase of the membrane is essentially the same with respect to the outer bilayer half or the inner bilayer half. Alkylating and water soluble spin labels, however, revealed that the interface zone localized on the inside of the vesicles was several times the viscosity of the equivalent locality on the surface of the sarcoplasmic reticular vesicles.

Reference 18 shows that Mycobacterium tuberculosis undergoes a change in its cytoplasm such that water soluble spin labels undergo an increase in restriction to rotational diffusion several hours after the bacterium has been exposed to a specific antibiotic specific for the tuberculosis bacterium.

Extensive studies were carried out on bull sperm measuring the properties of the aqueous cytoplasm. In this study a slightly different approach was also used. In this study the number of sperm were carefully quantified, nickel chloride was used in connection with a water soluble spin label and the integrated intensity of the resulting ESR spectra constitute an effective measurement of the protected aqueous volume inside

the sperm cell. In the course of this study it was shown that bull sperm briefly treated at 0° lose about 90% of the trapped aqueous volume. It was further revealed that if the bull sperm are treated with BHT prior to the cold exposure then essentially none of the trapped aqueous volume is lost. This study illustrates that it is the exact state of the cytoplasmic membrane that determines if cold shock or environmental modifications will render the cytoplasmic membrane permeable.

Studies have also been carried out on yeast lipid auxotrophs to determine if the quality of the cytoplasm undergoes changes during starvation for specific lipid requirements. It was shown that yeast auxotrophs requiring saturated fatty acids, *fas*⁻, die when starved for the saturated fatty acid for several hours. Simultaneous measurements carried out on the restriction to rotational diffusion of a water soluble spin label localized inside the yeast cells revealed that there was a simultaneous increase in restriction to rotational diffusion. More extensive studies carried out on this strain and on an inositol requiring mutant of yeast which also underwent rapid loss of viability during the starvation process revealed that there was a concomitant increase in cytoplasmic viscosity with loss of cell viability. Subsequent studies on these two auxotrophs using measurements based on

collision frequency reveal that the major modification in the yeast cytoplasm relates mainly to translational diffusion even though there is also a sizeable modification in rotational diffusion.

Surface labels

In recent months we have concentrated on the synthesis of amphiphilic spin labels which bear a hydrocarbon tail, a charge or other polar group, and a spin label moiety terminal to these other components. These spin labels, when properly constructed, localize almost entirely on the cell surface of most cells and only undergo transition to the cellular interior at a slow rate. We feel that these spin labels are promising and offer the opportunity of examining properties of the cell surface or the surface of any other membrane enclosure without having interfering signals emanating from other parts of the system. In an attempt to determine some of the important properties that may be relevant to the membrane surface we have carried out preliminary experiments using these spin labels, surface labels, on a variety of cells to see if they have good potential. For example, spin label measurements carried out on cells taken from human mammary tumors reveal phenomenological differences between the motional properties of these spin labels localized on the surface of the tumor cells compared to the normal breast cells. We have

carried out another fairly extensive study on the surface properties of bull sperm and rabbit sperm and have found that slight modification of the surface charge on the sperm cells drastically modifies the resulting ESR spectra. Studies using these spin labels are only in their preliminary stages. We have published one preliminary publication, reference 21, dealing with these labels.

Bibliography

1. Torres-Peterson, J., R. Mehlhorn, A. D. Keith and L. Packer. Changes in membrane lipid structure of illuminated chloroplasts - Studies with spin-labeled and freeze-fractured membranes. *Arch. Biochem. Biophys.* 160: 90-99 (1974).
2. Cohn, G., A. D. Keith and W. Snipes. Spin-label translational diffusion in solid tristearin. *Biophys. J.* 14:178-188 (1974).
3. Keith, A. D. and W. Snipes. Viscosity of cellular protoplasm. *Science* 183:666-667 (1974). Technical comment *re* 186:158-159 (1974).
4. Snipes, W., J. Cupp, J. Sands, A. Keith and A. Davis. Calcium requirement for assembly of the lipid-containing bacteriophage PM2. *Biochim. Biophys. Acta (Biomembranes)* 339:311-322 (1974).
5. Eletr, S., M. A. Williams, T. Watkins and A. D. Keith. Perturbations of the dynamics of lipid alkyl chains in membrane systems: Effect on the activity of membrane-bound enzymes. *Biochim. Biophys. Acta* 339:190-201 (1974).
6. Snipes, W., J. Cupp, G. Cohn and A. Keith. ESR analysis of the nitroxide spin label TEMPONE in single crystals of the reduced TEMPONE matrix. *Biophys. J.* 14:20-32 (1974).
7. Wade, N., R. W. Breidenbach, A. D. Keith and J. M. Lyons. Phase changes in castorbean mitochondria. *J. Plant Physiol.* 54:320-323 (1974).
8. Keith, A. D. and M. A. Williams. Yeast lipid mutants: Isolation, maintenance and spin label studies, in *Methods of Enzymology*, vol. XXXII, Part B (ed. S. Fleisher and L. Packer) Academic Press, 1974. pp. 819-837.
9. Keith, A. D., D. Horvat and W. Snipes. Spectral characterization of ^{15}N spin labels. *Chem. Phys. Lipids* 13:49-62 (1974).
10. Snipes, W., A. Keith and P. Wanda. Active transport of choline by a marine pseudomonad. *J. Bacteriol.* 120:197-202 (1974).
11. Snipes, W., J. Douthwright, J. Sands and A. D. Keith. Control of phospholipid synthesis and viral assembly by bacteriophage PM2. *Biochim. Biophys. Acta* 363:340-350 (1974).
12. Sands, J. A., J. Cupp, A. Keith and W. Snipes. Temperature sensitivity of the assembly process of the enveloped bacteriophage $\phi 6$. *Biochim. Biophys. Acta* 373:277-285 (1974).
13. Keith, A. D., D. Deamer and J. K. Reason. Biological membrane structure and function. *Science* 188:282 (1975).
14. Lyons, J. M., A. D. Keith and I. J. Thomason. Temperature induced phase transitions in nematode lipids and their influence on respiration. *J. Nematol.* 7:98-104 (1975).

15. Morse, P. D. II, M. Ruhlrig, W. Snipes and A. D. Keith. Viscosity profile of sarcoplasmic reticular vesicles. *Arch. Biochem. Biophys.* 168:40-56 (1975).
16. Keith, A. D., R. C. Aloia, J. Lyons, W. Snipes and E. T. Fengelley. Spin label evidence for the role of lysoglycerophosphatides in cellular membranes of hibernating mammals. *Biochim. Biophys. Acta* 394:204-210 (1975).
17. Cupp, J., M. Klynkowski, J. Sands, A. Keith and W. Snipes. Effect of lipid alkyl chain perturbations on the assembly of bacteriophage PM2. *Biochim. Biophys. Acta* 389:345-357 (1975).
18. Takayama, K., A. D. Keith and W. Snipes. Effect of isoniazid on the protoplasmic viscosity in Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy* 7:22-24 (1975).
19. Snipes, W., S. Person, A. Keith and J. Cupp. Butylated hydroxytoluene inactivates lipid-containing viruses. *Science* 188:64-66 (1975).
20. Dalton, L. R., P. Coffey, L. A. Dalton, B. H. Robinson and A. D. Keith. Theory of nonlinear spin response: Rapid passage for very slow molecular reorientation. *Phys. Rev. A* 11:488-498 (1975).
21. Lepock, J. R., P. D. Morse II, R. J. Mehlhorn, R. H. Hammerstedt, W. Snipes and A. D. Keith. Spin labels for cell surfaces. *FEBS Lett.* 60:185-189 (1975).
22. Cupp, J., P. Wanda, A. Keith and W. Snipes. Inactivation of lipid-containing bacteriophage PM2 by butylated hydroxytoluene. *Antimicrobial Agents and Chemotherapy* 8:608-706 (1975).
23. Keith, A. D. Spin labeling the hydrocarbon phase of biological membranes, in Mitochondria: Bioenergetics, Biogenesis, and Membrane Structure (ed. L. Packer). Academic Press, New York, 1976. pp. 291-302.
24. Hammerstedt, R., R. Amann, T. Rucinsky, P. Morse, J. Lepock, W. Snipes and A. Keith. Use of spin labels and electron spin resonance spectroscopy to characterize membranes of bovine sperm: Effect of butylated hydroxytoluene and cold shock. *Biol. of Reprod.* 14:381-397 (1976).
25. Henry, S. A., A. D. Keith and W. Snipes. Changes in the restriction of molecular rotational diffusion of water-soluble spin labels during fatty acid starvation of yeast. *Biophys. J.* 16:641-654 (1976).
26. Keith, A. D. and W. Snipes. Biological applications of spin exchange (Symposium on Magnetic Resonance in Colloid and Interface Science, ed. H. A. Resing) *Am. Chem. Soc. Publications* (in press).
27. Keith, A. D. and W. Snipes. The structure of biological membranes, in Horizons in Biochemistry and Biophysics (ed. E. Quagliariello). Addison-Wesley Pub. Co., Inc., Reading Mass. (in press).
28. Snipes, W., S. Person, G. Keller, W. Taylor and A. Keith. Inactivation of lipid-containing viruses by long-chain alcohols (in manuscript).

29. Pollard, E. C., W. Snipes, A. D. Keith, S. A. Henry and M. R. Culbertson. Inositolless death in yeast results in a simultaneous increase in intracellular viscosity (in manuscript).
30. Keith, A. D., W. Snipes and D. Chapman. Spin label studies on the aqueous regions of phospholipid multilayers (in manuscript).
31. Keith, A. D., W. Snipes, R. J. Mehlhorn and T. Gunter. Factors restricting diffusion of water soluble spin labels (in manuscript).
32. Abefsedarskaya, L. A., G. G. Miftakhutdinova and F. G. Fedotov. Biofizika 13:630 (1968).
33. Cope, F. W. Biophys. J. 9:303 (1969).

Legend to Figure 1

A. The concentration dependence for broadening the TEMPONE (VI) hyperfine lines is shown as a function of concentration of several broadening agents.

B. The viscosity dependence of broadening the TEMPONE (VI) hyperfine lines; relevant rotational correlation times (τ_c) are shown.

C., D. Charge dependence of broadening the TEMPONE hyperfine lines with a negatively charged broadening agent, $K_3Fe(CN)_6$, and a positively charged broadening agent, $NiCl_2$, are shown.

E. Pore size of BioGel beads is shown and its dependence on TEMPONE line broadening.

F. The relation between rotational correlation time (τ_c) and line broadening is shown for a homogeneous diffusion environment (O,●) and for a heterogeneous diffusion environment (■).

Legend to Figure 2

Structures I-IV are charged surface labels and have been synthesized using 18-carbon alkyl chains as well as 12-16-carbon chain lengths.

Structure V has not been synthesized. Structure VI has been synthesized, fully deuterated, and a ^{15}N -containing label of this type has also been synthesized. Structures VII and IX have been synthesized in fully deuterated form. Structure VIII is planned to be synthesized in deuterated form. Structures X through XIII are all in stock.

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A. D. Keith, R. C. Aloia, J. Lyons, W. Snipes and E. T. Pengelley.

Reprint: A Spin-Label Study of the Viscosity Profile of Sarcoplasmic
Reticular Vesicles. P. D. Morse II, M. Ruhlrig, W. Snipes
and A. D. Keith.

Reprint: Spin Labeling the Hydrocarbon Phase of Biological Membranes.
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Reprint: Spin Labels for Cell Surfaces. J. R. Lepock, P. D. Morse II,
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Reprint: Changes in the Restriction of Molecular Rotational Diffusion
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Preprint: Spin Label Studies on the Aqueous Regions of Phospholipid
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