A PROGRESS REPORT

to

The Atomic Energy Commission
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SPIN-LABELED MEMBRANES

submitted by

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PROGRESS REPORT

During the period of AEC support the following research and review articles have been advanced. The treatment of the progress report is aimed at being brief and accurate with no aspect overstated.

The general observation that Neurospora crassa could incorporate ^{12}NS into phospholipids and neutral lipids had some uncertainty about it since the analysis was based on spin content and Neurospora, as well as many other cells, can reduce spin labels to diamagnetic species¹. Sometimes organism-mediated spin destruction may be more severe than simple reduction. Yeast was used as a test organism together with tritiated ^{12}NS (^3H -9,10- ^{12}NS) to verify that ^{12}NS was incorporated into phospholipid and neutral lipid in ester form without having to rely on spin content². The observation was positive and conclusive. The percentages were about the same as observed for Neurospora previously.

We devoted considerable effort to the development of the use of unsaturated fatty acid auxotrophs both as subjects for spin label studies and as a direct measure of getting useful information about biological membranes. From these mutants it was determined that the double bond position has considerable flexibility as well as does chain length^{3,4}. In some cases trans double bonds can replace cis double bonds in molecular configuration. The essential fatty acids of mammals serve as adequate replacements for the unsaturated fatty acid of yeast; therefore they offer the possibility of serving as models for essential fatty acid studies. Later, we isolated saturated fatty acid requirers of yeast and used these both to determine saturated fatty acid requirements and as subjects for spin label studies^{5,6}. Odd chain lengths serve as well as even chain lengths and a certain amount of saturated fatty acid is an absolute requirement for growth.

We have undertaken several special projects about which I will give a sentence of two each in an attempt to label its general content and significance.

Sucrose effects ⁷. We determined that sucrose alters the physical properties of mitochondrial membranes and appears to disrupt the normally narrow interface boundary.

Monolayers ⁸. These studies were carried out to determine the degree of similarity or difference that spin labels have in common with their natural product analogs. We concluded that the spin label moiety confers extra properties to the parent molecule.

Red blood cells and essential fatty acids ^{9,10}. Spin labels were used as assay methods to determine alterations in rat liver mitochondrial membrane structure resulting from altered cholesterol or fatty acid composition.

¹⁵N spin labels ¹¹. We have synthesized spin labels from ¹⁵NH₃ and have carried out some experiments in an attempt to take advantage of the special ¹⁵N properties. ¹⁵N has a nuclear spin factor of one-half and results in a two-line signal having about 3/2 the An of ¹⁴N spin labels. The tensor elements, however, have 2/3 hν less to accomplish the same time-dependent hyperfine averaging process compared to ¹⁴N spin labels; consequently, the spectra can be accurately analyzed to a slower state of motion than ¹⁴N labels. ¹⁵N labels are also promising in experiments where a ¹⁴N and a ¹⁵N spin label can be simultaneously used.

Protoplasm viscosity ¹². We have carried out a preliminary study dealing with the internal viscosity of cells, primarily of the aqueous areas inside cells. This viscosity is several times that of water and has the following approximations in the cells listed: E. coli, 20 times water; Pseudomonas

BAL-31, 18 times water; yeast, 19 times water; Chlamydomonas, 100 times water; Kentucky Wonder bean root-tip, 120 times water; and human lung embryo cell, 150 times water. We will continue to pursue this line of work as outlined under Proposed Work.

Spin label motion in solids.¹³ As was proposed in the 1972-1973 proposal we carried out experiments in this area and demonstrated that some spin labels move quite freely in tristearin in the solid state. These studies demonstrated that the degree of impurity effect that a spin label exerts on its environment is very different depending on the structural similarity. Very small spin labels, in a matrix containing large molecules move very differently than the matrix molecules. If the structures are similar, however, the motion more nearly reflects that of the matrix. Perhaps the major point, however, was that structurally dissimilar spin labels can undergo translational diffusion in a solid matrix, at least 55°C below the observable melting point. We believe these results are quite relevant to permeability and translocation of small molecules.

We carried out an experiment defining the crystal parameters of Tempon.¹⁴

We have carried out various other research which is listed in the references or in my bibliography which relate to phase transitions in various membranes or to the membrane physical state as a function of some biological function (references 15-26).

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