

compounds the reduction followed the relationship  $K = AP^B$  where  $K$  is the equilibrium constant  $C_{II}/C_{III}$ . For the acetate and acetyl acetonate,  $B$  decreased with increasing temperature, whereas both oxalates showed an increase in  $B$  with increasing temperature. These observations are discussed in terms of thermodynamic implications. The unusual behavior of the isomer shift and quadrupole splitting of the ferric ion in the acetyl acetonate is briefly discussed.

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### SPIN-LABELED MITOCHONDRIAL LIPIDS IN NEUROSPORA CRASSA\*

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The current interest in membrane models and membranes has increased the search for new probing techniques. Recently, spin labeling has been explored as a technique for investigating micelles.<sup>1,2</sup> Although micelles are a rather primitive type of membrane model, the system is simple and forms a logical starting point for more ambitious studies. The main result of the micelle work was the demonstration that a water-insoluble molecule, in this case a nitroxide free radical, remains highly mobile when solubilized by a micelle. It also became clear during the course of this work that nitroxide spin labels would be useful as probes of more complex membranelike systems.

The chemistry of nitroxide free radicals is now well understood and a wide choice of probes may be synthesized by known procedures. Electron spin resonance (ESR) is the main spectroscopic tool for observing the probes. The analysis of the ESR spectra when the nitroxide probes are tumbling at intermediate rates or aggregating in solution is still an active field of investigation. Even though the analysis is not yet complete, it is obvious from the work of many laboratories that the ESR spectrum contains a large amount of information. The number of probes, the rotational mobility of the probes, the polarity of the environment, and the state of aggregation of the probes are types of information that can, in principle, be obtained from the ESR data. A number of investigations of proteins have already been published<sup>3</sup> and a good review of the chemistry of nitroxides and the spin-labeling technique is available.<sup>4</sup>

At least three important questions remain unanswered from the earlier work. (1) How can one incorporate a nitroxide into the lipid portion of a membrane in a meaningful way? (2) Is the nitroxide moiety sufficiently stable to remain paramagnetic in a living system? (3) Will the system survive in the presence of nitroxide free radicals? The present paper is an attempt to answer these questions for one specific case. The approach in this work was to allow an organism to take up a lipid spin label. The fate of the spin label in the membrane-rich mitochondria was then followed by chemical and ESR methods. The organism chosen for this study was *Neurospora crassa*, the common bread mold.

**Experimental.**—*Synthesis of the 12-nitroxide methyl stearate spin label:* The 12-nitroxide methyl stearate (I) was prepared from 12-keto methyl stearate by the procedure of Keana, Keana, and Beetham<sup>5</sup> for converting ketones into stable nitroxide free radicals. The details of this synthesis are described elsewhere.<sup>5</sup>

