Thermodynamic Statistical Evaluation of \( S \) and \( F \) from ESR Experimental Order Parameters.

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**Summary.** — A statistical analytical procedure for calculating entropy \( S \) and Helmholtz free energy \( F \) from the order parameters \( \bar{P}_q \) obtained from ESR spectra of spin-labelled membranes is described. The method is here applied to some literature data. A brief discussion on the results is also reported.

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1. — Introduction.

The spin-labelling technique has proved to be a continual source of information on the structure, dynamics and function of a variety of biological systems. In the past few years the number of papers dealing with spin labelling technique and its applications is greatly increased. Many reviews have also appeared and chapters have been devoted to this subject in the more important specialist periodical reports (1). The local technique of spin labelling reveals the existence

(1) See, for example, Specialist Periodical Reports, *Electron Spin Resonance*, nearly annually edited by The Royal Society of Chemistry.
of particular features of the sample, sometimes also in disagreement with other techniques which provide mean information on the system under consideration (see, for example, ref. (2)).

The only lack of this technique is that spin label molecules are never identical with the natural components of biological systems. However, this difficulty can be obviated by assaying of biological functions in the presence of the label or by other well-stated stratagems (5).

On the other hand, other local probing techniques such as deuterium magnetic resonance of selectively deuterated lipids, though resorting to not perturbing probes, sometimes reveal other severe limitations such as a lack of sensitivity at temperatures lower than the gel-to-liquid crystalline-phase transition temperature (see, for example, ref. (4)).

In the light of these considerations, ESR spin-labelling technique must be considered as a valid help to deepen our knowledge of biological-membrane dynamics and structure. The latter, in particular, can be studied, as well known, through the order parameter $P = \langle 3 \cos^2 \beta - 1 \rangle / 2$ which is a measure of the distribution of molecular orientations $\beta$ relative to a reference axis, generally the normal to the membrane surface. An approximate evaluation of such an order parameter can be generally provided by ESR spectra when the lifetime of the preferential distribution of orientations must be longer than the inverse of the orientational-dependent part of the Hamiltonian, that is by assuming that motions within the restricted space are fast on the ESR time scale. In this case the following approximate relation holds:

$$P = \frac{(T_1 - T_\perp)(T_{yy} - T_{xx})a_{y'y'}}{T_{11}},$$

where $T_1$, $T_\perp$ and $a_{y'y'}$ are parameters directly obtainable from the experimental spectra and $T_{yy}$, $T_{xx}$ and $a_y$ are rigid lattice parameters obtainable from single-crystal spectra. $T_1$, $T_\perp$, $T_{yy}$, $T_{xx}$, $a_y$, $a_{y'y'}$ have here their usual meaning reported elsewhere (see ref. (5-7)).

Comparison between experimental values of the order parameters and