Lipid Molecular Motion and Enzyme Activity in Sarcoplasmic Reticulum Membrane†

Donald G. Davis,‡ Giuseppe Inesi,* and Tadeusz Gulik-Krzywicki

ABSTRACT: In biochemically active sarcoplasmic reticulum vesicles (SR) the physical state of the membrane lipids was studied by high angle x-ray diffraction and proton nuclear magnetic resonance (NMR) at 220 MHz, and related to thermal effects observed in SR functional parameters. It is shown by high angle x-ray diffraction that even at temperatures as low as 1 °C nearly all the SR lipid hydrocarbon chains are in a disordered conformation and only a very small part (less than 3%) are in rigid crystalline order. Consistent with this observation, the NMR data indicate that the majority of SR phospholipid molecules are in a state of restricted anisotropic motion having no apparent crystalline order at temperatures as low as 5 °C. At this temperature most of the resonance signal is contained in a broad featureless line of 700-Hz half-width. On the other hand, as the temperature is raised, high-resolution NMR signals, representing groups with highly isotropic motion, begin to grow in intensity. It is estimated that by 35 °C 90–100% of the phosphatidylcholine *N*-methyl protons and 35% of the hydrocarbon-chain protons give high-resolution signals. Concurrent studies on functional parameters reveal thermal effects giving rise to nonlinear Arrhenius plots for the rates of calcium transport and calcium activated ATPase. The thermal effects observed on functional parameters and on the character of phospholipid molecular motion exhibit a parallel behavior, suggesting a relationship between enzyme activity and the physical state of the membrane lipids.

Almost interesting question regarding membrane-bound enzymes is how their function is influenced by the physical state of the membrane lipids. In this respect temperature is a convenient means by which perturbations may be induced in both the physical state of the lipids and the biochemical properties of the membrane so as to reveal any possible relationships between these two parameters.

For example, a well-characterized change that can be induced by temperature is the order–disorder transition in which the lipid hydrocarbon chains undergo a transformation from a rigid, crystalline conformation to a disordered, fluid-like state. Although evident by a variety of techniques, this transition is best described by x-ray diffraction analysis (Luzzati, 1968; Ranck et al., 1974). For homogeneous lipid systems, the transition occurs within a narrow temperature range like that of a melting process; for heterogeneous systems, the transition is broader, involving phase separations which can be detected via the analysis of spin-label spectra (Shimshick and McConnell, 1973). These transitions can also be observed in certain biological membranes and are accompanied by an aggregation of membrane proteins which are excluded from the rigidly ordered lipid regions (Kleeman and McConnell, 1974; Shechter et al., 1974). Furthermore, these order–disorder transitions can be correlated with the temperature dependence of membrane functional parameters such as those observed in *Escherichia coli* fatty acid autotrophs (Shechter et al., 1974).

In sarcoplasmic reticulum (SR)[1] membranes, thermally induced effects give rise to nonlinear Arrhenius plots for the rates of Ca^{2+} transport and the Ca^{2+}-activated hydrolysis of ATP (Deamer, 1973; Inesi et al., 1973; Madeira et al., 1974). As judged by parallel spin-label studies both on native SR (Inesi et al., 1973) as well as on SR ATPase prepared with a controlled lipid environment (Lee et al., 1974) these functional changes are accompanied by changes in the ordering of the membrane lipids. Previous studies have amply demonstrated the dependence of SR Ca^{2+}-coupled ATPase activity on the fluidity of phospholipids (Kielly and Meyerhof, 1950; Martonosi, 1964; Meissner and Fleisher, 1972; Seelig and Hasselbach, 1971). The spin-label studies (Inesi et al., 1973) suggested, however, that the observed changes in the physical state of the lipids which accompany the functional changes were not transformations involving crystalline order since no spin-label spectral components were observed which could be attributed to the presence of separate lipid phases.

In order to characterize the physical state of the SR lipids more completely and to gain a better understanding of how thermally induced structural alterations are related to the thermal dependence of SR membrane functional parameters, we have undertaken variable-temperature studies on native SR using high-angle x-ray diffraction and proton nuclear magnetic resonance (NMR) techniques. We have also extended previous functional studies to include numerous determinations of "total", Ca^{2+}-independent and Ca^{2+}-dependent ATPase as well as Ca^{2+}-transport at different temperatures. As discussed below both x-ray diffraction and NMR data show clearly that by 5 °C most of the lipid hydrocarbon chains are "melted". Thus, the changes in ac-

† From the Laboratory of Physiology and Biophysics, University of the Pacific, San Francisco, California 94115 (D.G.D., G.I.), and the Centre de Genetique Moleculaire du Centre National de la Recherche Scientifique, Gif-sur-Yvette, France (T.G.-K.). *Received June 26, 1975.* The authors are deeply indebted to Professor Melvin Klein of the University of California in Berkeley for permission to use his NMR facility and for very helpful discussions, and to Professor Vittorio Luzzati for suggesting some of the reported experiments. This work was partially supported by the National Institutes of Health (HL 16607), the American Heart Association, and the Muscular Dystrophy Association.

‡ Present address: Department of Chemistry, Adelphi University, Garden City, N.Y. 11530.

[1] Abbreviation used is: SR, sarcoplasmic reticulum.

tivation energies occurring between 15 and 25 °C for the functional parameters are not related to a crystalline to liquid-crystalline transformation (e.g., involving crystalline order of the lipid hydrocarbon chains). Over this same temperature range, however, the character of the motion for some lipid proton groups becomes more isotropic. This feature can be correlated with the effect of temperature on functional parameters.

Methods

Vesicular fragments of sarcoplasmic reticulum membrane (SR) were prepared from white muscle of rabbit hind legs by homogenization and differential centrifugation as previously described (Eletr and Inesi, 1972). Further purification was then carried out on a discontinuous gradient (Meissner and Fleisher, 1972) collecting the fraction sedimenting between 26 and 29% sucrose.

Protein concentration was determined with the biuret reagent standardized with micro-Kjeldal nitrogen determinations.

Membrane lipids were prepared by a procedure (Folch et al., 1957) involving extraction with chloroform–methanol solutions (2:1, v/v). This step was then followed by repeated washing in 0.1 M KCl solutions.

ATPase activity was measured in the presence of 20 mM morpholinopropanesulfonate (Mops), 80 mM KCl, 2 mM potassium ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetate (EGTA), 5 mM potassium oxalate, and 0.18 mg of SR protein/ml. $CaCl_2$ (2 mM) was added when "total" ATPase, as opposed to "basic" ATPase, was measured. The reaction was started by the addition of 5 mM ATP·Mg. The pH of the reaction mixture was adjusted to 6.8 for each temperature. Samples were taken at different times and quenched in equal volumes of 10% trichloroacetic acid. Inorganic phosphate was measured by the method of Fiske and Subbarow (1925).

Calcium transport was measured in the presence of 20 mM Mops, 80 mM KCl, 0.1 mM EGTA, 0.1 mM $^{45}CaCl_2$, and 22–25 μg of SR protein/ml. The reaction was started by the addition of 2.5 mM ATP·Mg. At appropriate times samples were filtered through HA 0.45-μm Millipore filters for separation of the SR vesicles from the medium (Martonosi and Feretos, 1964). The residual calcium in the medium was then measured in a scintillation radioactivity counter. Control samples were made in which SR, but not ATP, was present. The pH of the reaction mixture was adjusted to 6.8 for each temperature.

High-angle x-ray diffraction experiments were carried out on membrane fragments concentrated to 30% dry weight by a 90-min high-speed centrifugation. The pellet was transferred into a thermostated x-ray sample holder between two beryllium plates separated by an aluminum spacer 1 mm thick.

Lipid–water samples were prepared by mixing fixed amounts of dried lipid and water, generally 40% lipid and 60% water, w/w. The mixture was allowed to equilibrate overnight.

An anode rotating Elliot GX 6 generator was used as the x-ray source. The beam was linearly focused by a gold mirror and filtered by a 0.02-mm thick Ni foil. The diffracted x-ray beam was detected with a linear, position-sensitive, proportional counter (Dupont et al., 1972). The high-angle x-ray spectra were recorded directly on a multichannel analyzer.

X-ray diffraction spectra of each sample at a given temperature were recorded within 6 min. The samples were equilibrated at each temperature for 10 min before recording. A complete set of determinations on a given sample at various temperatures was carried within 2 to 3 h.

The amount of ordered paraffin chains in the various samples was determined quantitatively by comparison of the integrated intensity of their 4.2-Å reflection to that of a standard consisting of egg lecithin mixed with 5% water. At 0 °C, practically 100% of the paraffin chains of this lipid are in an ordered state (Tardieu et al., 1973). In some cases, high-angle x-ray diffraction was recorded by conventional film methods after overnight exposure.

Proton NMR spectra of SR vesicles (38–50 mg of membrane protein/ml) in D_2O (buffered at pD 6.8 with 10^{-2} M maleate) were recorded at 220 MHz (Varian Associates 220-NMR spectrometer). NMR experiments at different temperatures were performed using the variable temperature accessory provided with the instrument. Before recording spectra, the samples were allowed to equilibrate for at least 30 min at each temperature setting. The temperature of the probe was determined before and after SR sample runs by comparing the chemical shifts of the hydroxyl protons in methanol and ethylene glycol standards with precalibrated values. It is estimated that the temperatures are known with an accuracy of ±1 °C.

Results

High-Angle X-ray Diffraction. High-angle x-ray diffraction spectra of SR membranes and their total lipid extract display a broad band centered at around 4.5 Å. This band is characteristic of disordered lipid paraffin chains (Luzzati, 1968).

As the temperature is decreased, a sharp reflection centered at around 4.2 Å (as judged from the x-ray films) appears superimposed on the broad band, indicating that some of the paraffin chains have become ordered (Luzzati, 1968). The sharp reflection is hardly visible at 18 °C, but becomes more definite at 1 °C (Figure 1). However, the percentage of SR lipids involved in this transition is very small. Its exact value is difficult to calculate especially in the case of SR membranes, due to significant water and protein contributions.

Comparing the integrated intensity of the 4.2-Å reflection for the membranes and that of standard samples (see Methods) we estimate this percentage to be: 3% at +1 °C, 2% at +5 °C and 1% at 9 °C. At 18 °C no sharp reflection was obtained with the counter, but a weak 4.2-Å reflection was still produced on films after overnight exposure. At 22 °C only a 4.5-Å broad band was observed even by this method.

Similar values are obtained for hydrated (40% dry weight) SR total lipid extract.

Proton NMR. Proton NMR spectra of SR membranes are shown in Figures 2 and 3. At 5 °C (Figure 2) nearly all of the resonance intensity is contained in a broad, featureless line having a half-width of ~700 Hz. As estimated by comparison with a known amount of an added standard (10^{-2} M maleate), the intensity of the broad line exceeds that expected for the total quantity of SR lipid protons by a factor of ~1.5. The excess intensity is attributed to protons of the membrane proteins.

Superimposed upon the broad line are sharper (≤100-Hz half-width) "high-resolution" lines. These signals can be assigned unambiguously to the proton resonances of phosphatidylcholine *N*-methyl groups (−3.3 ppm) groups. As the

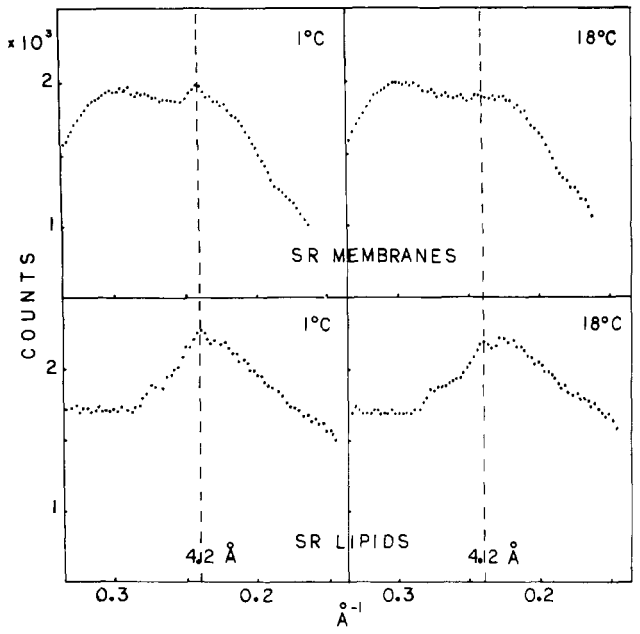

FIGURE 1: High angle x-ray diffraction spectra of SR membrane (30% dry weight) and SR lipid extract (40% dry weight). The 4.2-Å reflection appears much sharper in x-ray films after overnight exposure.

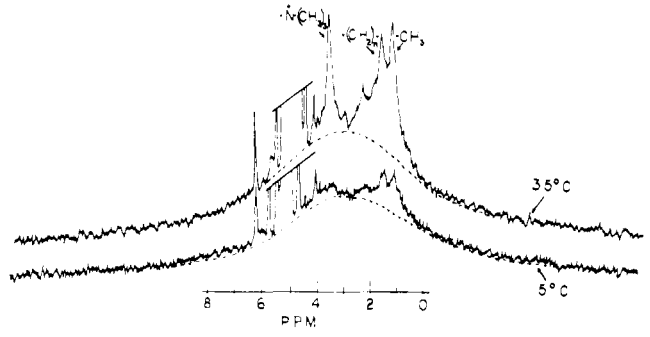

FIGURE 2: ^{1}H HMR spectra (220 MHz) of SR membrane vesicles (37 mg of protein/ml of D_2O) at 5 and 35 °C. The membrane suspensions were buffered at pD 6.8 by 10 mM sodium maleate (signal at 6.2 ppm). The scale is the parts per million from sodium 2,2-dimethyl-2-silapentane-5-sulfonate.

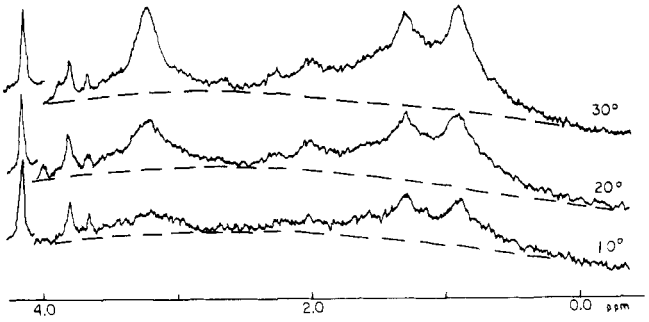

FIGURE 3: ^{1}H NMR spectra (220 MHz) of SR showing the "high-resolution" components on an expanded frequency scale. Note the increasing intensities as the temperature in raised. The sharp lines at the left are of the maleic acid buffer (10 mM) which was used as an intensity standard.

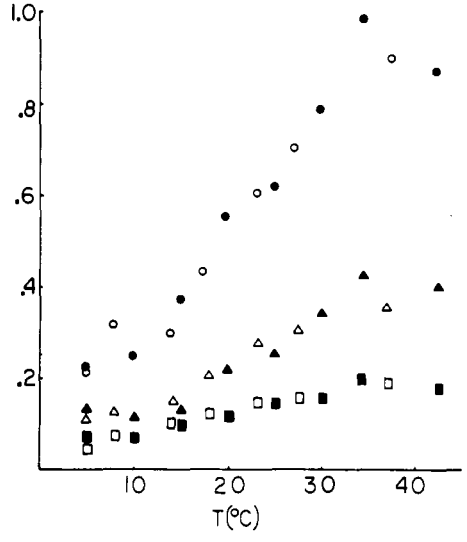

FIGURE 4: Intensity of lipid hydrocarbon methylene (△,▲), terminal methyl (□,■), and choline *N*-methyl (○,●) protons high-resolution lines in NMR spectra of SR, as a function of temperature. The figures on the vertical axis express fractional values relative to total expected intensities. Solid and open symbols correspond to different SR preparations.

temperature is raised the intensities of the high-resolution lines increase markedly (Figure 3). A somewhat smaller increase (~20%) is also observed in the intensity of the broad-line fraction as well.

In order to measure these intensity changes as accurately as possible the following procedure was used. A smooth baseline corresponding to the line shape of the underlying broad component was interpolated as indicated by the dashed lines in Figures 2 and 3. Copies of the spectra were then cut out and weighed. Absolute values for the intensities were estimated by referring to the intensity of the 10^{-2} M maleate–buffer protons at −6.2 ppm. As a consequence of the signal-to-noise ratio the accuracy of this method is limited by an error of ±10%.

The measured intensities of the high-resolution signals are shown in Figure 4. It can be seen that within experimental accuracy these intensities increase smoothly from 5 to 35 °C. Based on: (1) a lipid to protein weight ratio of 60:100 for rabbit SR (Meissner and Fleisher, 1972); (2) a phosphatidylcholine content of 60% for the total lipids (Meissner and Fleisher, 1972); and (3) an average chain length of 18 carbons (Morai and Kuksis, 1973), it is estimated that at 5 °C, 20% of the total phosphatidylcholine *N*-methyl protons and ~10% of the lipid hydrocarbon methyl and methylene protons contribute intensity to the high-resolution signals. At 35 °C, these intensities correspond to ~95% of the *N*-methyl protons, 40% of the hydrocarbon methyl, and 20% of the hydrocarbon methylene protons (Figure 4).

As the temperature is raised, the half-width of the phosphatidylcholine *N*-methyl protons line is continuously reduced from 55 ± 5 Hz at 5 °C to 20 ± 2 Hz at 38 °C, with an activation energy of 5 kcal mol^{-1}. On the other hand, with regard to the half-width of the hydrocarbon chain lines (115 ± 5 Hz) and the broad line (~700 Hz), no significant changes can be measured between 5 and 38 °C.

Functional Studies. Ca^{2+} transport and ATP hydrolysis catalyzed by SR occur at rates close to maximal velocity in the presence of oxalate and saturating substrate concentration (Hasselbach, 1964). Within the temperature range of our studies (4–45 °C), it is possible to measure constant rates of activity over appropriate time intervals (Figure 5).

In the presence of suitable Ca^{2+} concentration, a "total"

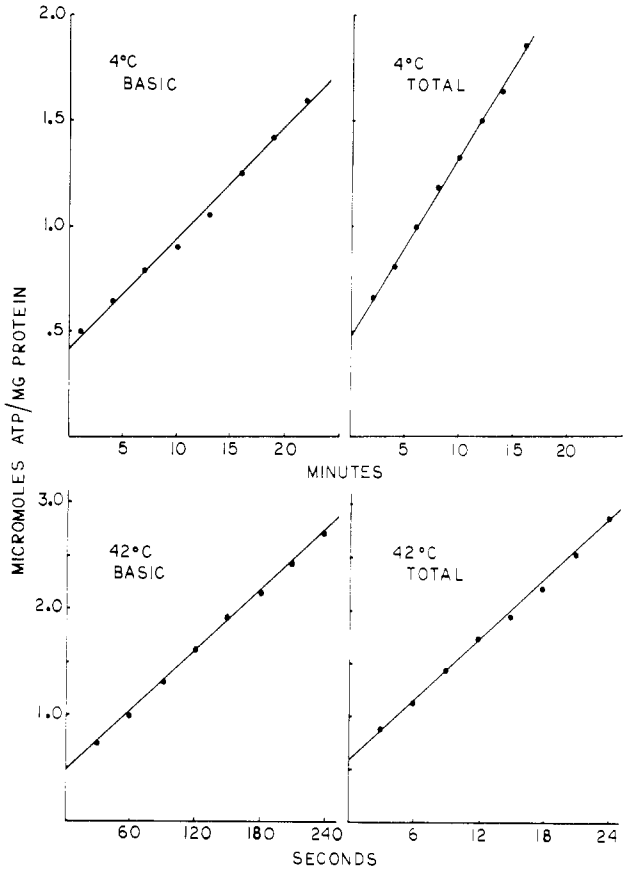

FIGURE 5: Amount of ATP hydrolyzed vs. time, in the absence ("basic") and in the presence ("total") of Ca^{2+}. Note the different temperatures and time intervals. See section on Methods for experimental details.

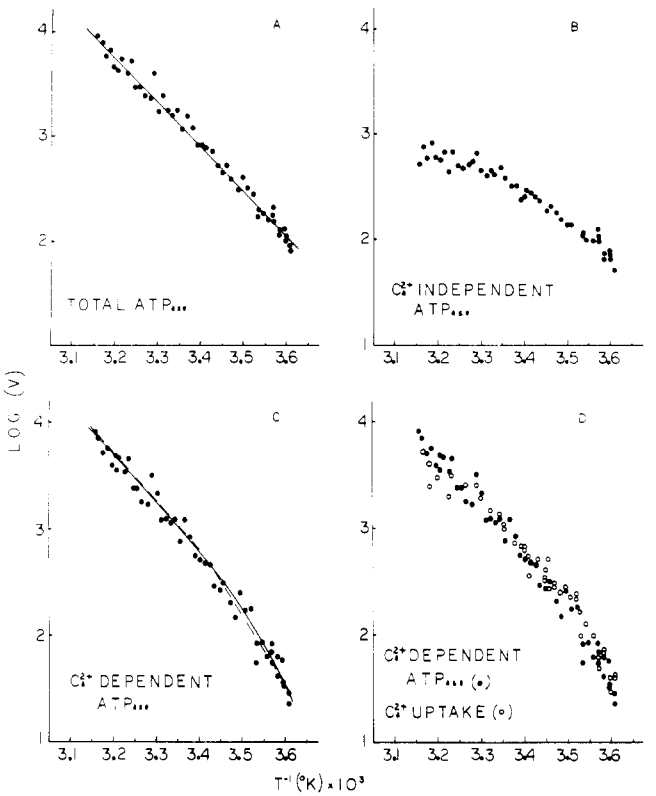

FIGURE 6: Semilogarithmic plots vs. $1/T$ for SR ATPase-catalyzed hydrolysis, measured in nanomoles/minute per milligram of SR protein: (A) "total" ATPase velocity; dots are experimental data; solid line is best linear regression fit; (B) Ca^{2+}-independent ATPase velocity; (C) Ca^{2+}-dependent ATPase velocity; dots are data points calculated from "total" minus "basic" velocities; solid line is fitted by second degree polynomial, dashed lines are fits by linear regression on the upper and lower temperature ranges; (D) "extra" ATPase velocity and rate of Ca^{2+} uptake; solid dots are "extra" ATPase velocity from part C; open dots are experimental data for Ca^{2+} uptake rate/2, measured in nanomoles/minute per milligram of SR protein.

ATPase activity is obtained which generates a semilogarithmic plot of V vs. $1/T$ having a constant slope over the entire temperature range (4–45 °C) used in these studies. The plot (Figure 6A) is fitted extremely well by a straight line.

On the other hand, in the absence of Ca^{2+} (2 mM EGTA and no added calcium) a "basic" or "Ca^{2+}-independent" ATPase activity is obtained. Contrary to the "total" ATPase, a semilogarithmic plot of "basic" ATPase V vs. $1/T$ displays a varying slope (Figure 6B).

The difference between "total" and "basic" ATPase is the "extra" or "Ca^{2+}-independent" enzyme activity. In this case, as for the "basic" ATPase, a semilogarithmic plot of V vs. $1/T$ is *not* fitted satisfactorily by linear regression, as its slope undergoes a change between 15 and 25 °C. The experimental points are better fitted with a second degree polynomial or two straight lines intersecting at approximately 20 °C (Figure 6C).

It was previously shown that the "extra" portion of the "total" ATPase is coupled to Ca^{2+} transport (Hasselbach, 1964). We have found that such coupling persists tightly over a temperature range between 4 and 40 °C, with an approximately 2.0 calcium:ATP molar ratio. Above 40 °C the two activities become uncoupled and Ca^{2+} transport is reduced while ATPase activity continues to increase (Inesi et al., 1973). The close relation between "extra" (Ca^{2+}-dependent) ATPase activity and Ca^{2+} transport is evident in Figure 6D, in which the rates of enzyme activity and rates of transport (reduced by 2.0 coupling ratio) closely coincide over the temperature range between 4 and 40 °C. It is important to notice that this relation is maintained even in the low-temperature range, where the plots of transport and "extra" enzyme vs. $1/T$ undergo identical deviations from linear behavior (Figure 6D).

Discussion

Measurements of SR functional parameters as a function of temperature show that semilogarithmic plots of V vs. $1/T$(K) for Ca^{2+}-ATPase and Ca^{2+}-transport are not linear, but display instead a change in their activation energies between 15 and 25 °C (Figure 6). Inasmuch as this transition in the SR kinetic parameters is also reflected by parallel changes in the spectral parameters for both lipid-soluble and protein-bound spin-labels (Inesi et al., 1973), it suggests that the two phenomena are related to a thermally induced change in the physical state of the lipids. This transformation does not, however, involve a crystalline to liquid–crystalline transition.

The virtual absence of any 4.2-Å (crystalline) reflections in the x-ray diffraction studies at temperatures as low as 1 °C indicates clearly and directly that most of the lipid chains are in a disordered conformation. This conclusion is confirmed independently by the NMR data which provide information concerning both the disorder as well as the mobility of the lipid chains.

As judged by the NMR spectra taken at 5 °C, most of the proton groups in SR give a resonance line of 700-Hz

half-width, corresponding to a 10^2-fold reduction from the rigid-lattice limit (Davis and Inesi, 1971).[2] Since the SR vesicles of the kind prepared for these studies have diameters of $\sim 10^3$ Å, their tumbling rates (~1 s) are too slow to account for the reduction in line width (Davis and Inesi, 1971). Instead, segmental motion of the lipid hydrocarbon chains is a more likely and realistic mechanism for the line-narrowing.

In fact, as described and demonstrated by Chan and coworkers (Lichtenberg et al., 1975; Seiter and Chan, 1973), proton NMR line widths of lipids in bilayers are reduced to this magnitude ($\sim 10^3$ Hz) only when the vectors connecting proton pairs can move or oscillate without restrictions at angles, $\Delta\beta$, up to 65° from the axis of rapid rotational motion. For the bulk of the lipid protons the latter is parallel to the long axis of the hydrocarbon chains. Moreover, for vesicles of this size (10^3 Å diameter), the amplitude rather than the rate of the motion is the critical and significant parameter that governs this degree of line narrowing (Lichtenberg et al., 1975). Such amplitudes ($\Delta\beta \simeq 65°$) are possible only above the melting transition where the chains are no longer packed in hexagonal, crystalline arrays. Since the intensity of the 700-Hz line obtained from SR is sufficient to account for all lipid protons, it must be concluded that even at 5 °C essentially all the SR lipid hydrocarbon chains are "melted" as is consistent with the x-ray diffraction data.

It should be pointed out, however, that after the completion of the melting transition, the character of the lipid thermal motion continues to change and an increasing number of lipid protons acquire isotropic, fluid-like motion with increasing temperature. This is indicated most clearly by the intensities of the high-resolution lines in the NMR spectra (Figure 3). According to the Seiter–Chan model (Seiter and Chan, 1973; Lichtenberg et al., 1975) such extreme narrowing ($<10^2$ Hz) occurs in bilayers when the angular deviations of the proton pairs approach an angle of 90° to the axis of fast rotation (i.e., the chain axis) and simultaneously, the rates of this flexing motion $\tau_\perp^{-1}$ approach the rate $\tau_\parallel^{-1}$ for reorientation about the chain axis. On a localized, molecular scale, motion of this nature is clearly characteristic of an isotropid fluid.

Not all of the SR lipid proton groups take on this fluid-like motion, however. Only 40% of the possible intensity for the chain terminal methyl protons and 30% of that for the chain methylene protons are observed as high-resolution signals at 35 °C. As judged by a variety of proton and deuterium NMR studies this appears to be a general feature for linear hydrocarbons that are restricted suitably in a lamellar array (Chan et al., 1971; Horowitz and Klein, 1973; Seelig and Seelig, 1974). Although the rates and amplitudes for the motion of the terminal methyl and neighboring methylene groups are large, they are constrained by their packing in the bilayer from bending back upon the chain axis. Consequently, the angularly dependent dipolar splitting of some of the spin transitions is not averaged out completely. For a 3-spin, isolated and rotating methyl group, only the $\frac{1}{2} \rightarrow -\frac{1}{2}$ transitions, which are orientation independent and make up 50% of the allowed spin transitions, appear as a high-resolution signal (Seiter and Chan, 1973). The remaining 50% of the methyl group signal, namely from $|\frac{3}{2}| \rightarrow |\frac{1}{2}|$ transitions, is broadly distributed in the wings of the main signal and cannot be observed easily[3] (Chan et al., 1971). For the methylene protons, the interpretation is more complicated since the separations of both of the allowed spin transitions, $\pm \leftrightarrow 0$, are orientation dependent. For a spherical vesicle it is estimated that ~4% of the lipid chains[4] reorient rapidly within ±5° of the "magic angle", thus making a contribution to the high-resolution signal (Lau and Chan, 1975). If the remaining 16% of the observed signal is assigned heuristically to the methylene groups nearest the chain terminus, it is estimated that these highly mobile groups belong to the first three carbons (i.e., C_{15} through C_{12}, assuming an average chain length of 16 carbons). This agrees well with independent estimates of the chain mobility derived from proton and deuterium NMR studies on lipid bilayers (Chan et al., 1971; Seelig and Seelig, 1974).

In contrast to the hydrocarbon chains, the *N*-methyl groups of the phosphatidylcholine component of the SR lipids become totally unrestricted in their motion, for their full intensity is observed as a high-resolution signal at 35 °C. It is interesting to note that the maximum rate of development of this signal occurs between 10 and 30 °C, i.e., over the same temperature range where the activation energy for the SR-functional parameters is not constant. In fact, the slopes of the upper and lower temperature ranges in the Arrheinus plots shown in Figures 6C and 6D intersect at approximately 20 °C, at which temperature the *N*-methyl protons high-resolution signal has grown to its half-maximal intensity (Figure 4).

Whether these thermally induced changes in the physical state of the lipids and in the SR membrane functional properties can be associated with mechanisms such as the breakdown of lipid "clusters" (Lee et al., 1974) or fluid–fluid phase separations (Wu and McConnell, 1975) cannot be determined from the data reported here. Based upon spin-label observations (Wu and McConnell, 1975), cluster-breakdown and fluid-state phase separations have been reported to occur in pure or in binary mixtures of pure lipids. These phenomena have rather subtle influences on the spin-label parameters and they may be masked entirely, if they occur at all, in such a heterogeneous mixture as are the lipids of the SR membrane.

In summary, a variety of physical studies indicate that in this mammalian, excitable membrane of heterogeneous lipid composition, melting of the lipid hydrocarbon chains is virtually complete at least 30 °C below the physiologic temperature. Nevertheless, a relationship between SR functional parameters and the character of phospholipid molecular motion is still obtained even at temperatures above the melting transition, very close to the physiologic temperature

[2] Comparable reductions in line widths are observed in dipalmitoyllecithin suspensions only at temperatures above their melting transition (41 °C) or for dipalmitoyllecithin vesicles that have been reduced to ~300 Å diameter by sonication (Darke et al., 1972; Sheetz and Chan, 1972).

[3] If these satellite lines could be observed, measurement of their separation would give information about the average orientation of the methyl group relative to the chain axis. In fact, magnetic resonance spectra of any spin system for which either the "quadrupolar" terms (i.e., terms $\propto m_I^2$) or the anisotropic Zeeman terms (e.g., via chemical shift or hyperfine anisotropy) of its spin-Hamiltonian are not averaged out by isotropic motion can provide, in principal, "order parameter" information.

[4] The fraction of lipid molecules with chains rapidly reorienting within ±5° of the "magic angle" (54° 44′) to the applied field is given by: $(2 \times d\Omega)/4 = \sin(54° 44') \times (10°/180°) \simeq 0.04$.

range. In such a relationship dipole orientation on the surface of the bilayer, in addition to hydrocarbon chain flexibility, may play a role.

References

Bartlett, G. (1959), *J. Biol. Chem. 234,* 466.

Chan, S. I., Feigenson, G. W., and Seiter, C. H. A. (1971), *Nature (London) 231,* 110.

Darke, A., Finer, E. G., Flook, A. G., and Phillips, M. C. (1972), *J. Mol. Biol. 63,* 265.

Davis, D., and Inesi, G. (1971), *Biochim. Biophys. Acta 241,* 1.

Deamer, D. W. (1973), *J. Biol. Chem. 248,* 5477.

Eletr, S., and Inesi, G. (1972), *Biochim. Biophys. Acta 282,* 174.

Dupont, Y., Gabriel, A., Chabre, M., Gulik-Krzywicki, T., and Shechter, E. (1972), *Nature (London) 235,* 331.

Fiske, H., and Subbarow, Y. (1925), *J. Biol. Chem. 66,* 375.

Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957), *J. Biol. Chem. 226,* 497.

Hasselbach, W. (1964), *Prog. Biophys. Mol. Biol. 14,* 167.

Horowitz, A. F., and Klein, M. D. (1973), *Biochim. Biophys. Acta 298,* 1.

Inesi, G., Millman, M., and Eletr, S. (1973), *J. Mol. Biol. 81,* 483.

Kielly, W. W., and Meyerhof, O. (1950), *J. Biol. Chem. 183,* 391.

Kleeman, W., and McConnell, H. M. (1974), *Biochim. Biophys. Acta 345,* 220.

Lau, A. L. Y., and Chan, S. I. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 2170.

Lee, A. A., Birdsell, N. J. M., Metcalfe, J. C., Tson, P. A., and Warren, G. B. (1974), *Biochemistry 13,* 3699.

Lichtenberg, D., Petersen, N. O., Girardet, J., Kainosho, M., Kroon, P. A., Seiter, C. H. A., Feigenson, G. W., and Chan, S. I. (1975), *Biochim. Biophys. Acta 382,* 10.

Luzzati, V. (1968), in Biological Membranes, Chapman, D., Ed., New York, N.Y., Academic Press, pp 71–123.

Madeira, V. M. C., Antunes-Madeira, M. C., and Carvalho, A. P. (1974), *Biochem. Biophys. Res. Commun. 58,* 897.

Martonosi, A. (1964), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 23,* 913.

Martonosi, A., and Feretos, R. (1964), *J. Biol. Chem. 239,* 648.

Meissner, G., and Fleisher, S. (1972), *Biochim. Biophys. Acta 255,* 19.

Morai, L., and Kuksis, A. (1973), *Can. J. Biochem. 51,* 1248.

Ranck, J. L., Mateu, L., Sadler, D. M., Tordieu, A., Gulik-Krzywicki, T., and Luzzati, V. (1974), *J. Mol. Biol. 85,* 249–277.

Seelig, A., and Seelig, J. (1974), *Biochemistry 13,* 4839.

Seelig, J., and Hasselbach, W. (1971), *Eur. J. Biochem. 21,* 17.

Seiter, C. H. A., and Chan, S. I. (1973), *J. Am. Chem. Soc. 95,* 7591.

Shechter, E., Letellier, L., and Gulik-Krzywicki, T. (1974), *Eur. J. Biochem. 49,* 61.

Sheetz, M. P., and Chan, S. I. (1972), *Biochemistry 11,* 4573.

Shimshick, E. J., and McConnell, H. M. (1973), *Biochemistry 12,* 2351.

Tardieu, A., Luzzati, V., and Reeman, F. C. (1973), *J. Mol. Biol. 75,* 711.

Wu, S. H., and McConnell, H. M. (1975), *Biochemistry 14,* 847.