



National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

**THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED**

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

**LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE**

A STUDY OF BIOLOGICAL MEMBRANES AND
RELATED SYSTEMS BY INFRARED SPECTROSCOPY.

by

Hector L. Casal

Thesis Submitted to the School of
Graduate Studies in Partial Fulfillment
of the Requirements for the Degree
of Doctor of Philosophy in
Chemistry.

Ottawa, 1980.

CONTENTS

ACKNOWLEDGMENTS.	v
PREFACE.	vi
ABSTRACT.	viii
LIST OF FIGURES.	1
LIST OF TABLES.	10
ABBREVIATIONS.	12
CHAPTER 1: INTRODUCTION.	
1.1. Biological Membranes.	14
1.1.1. Chemical Composition.	14
1.1.2. Membrane Structure.	18
1.2. Model Membranes.	20
1.3. Thermotropic Transitions in Bio-mem- branes.	21
1.3.1. The 1,2-Diacyl-phosphatidylcholines.	23
1.3.2. <i>Acholeplasma laidlawii</i> Membranes.	29
1.4. Scope of the Studies.	30.
CHAPTER 2: MATERIALS AND METHODS.	
2.1. Materials.	34
2.2. Sample Preparations.	36
2.3. Infrared Spectroscopy.	40
2.3.1. Instrumentation.	40
2.3.2. Spectral Acquisition.	42
2.3.3. Data Reduction.	43

CHAPTER 3: HYDROCARBONS.

3.1.	Solid Phase Behaviour of Normal Alkanes.	55
3.2.	The Infrared Spectra of Orthorhombic n-C ₁₇ H ₃₀ D ₆ ; n-C ₁₉ H ₄₀ and n-C ₂₁ H ₄₄ .	59
3.2.1.	C-H Stretching.	60
3.2.2.	Methylene Scissoring and Methyl Bending.	62
3.2.3.	Methylene Wagging.	66
3.2.4.	Methylene Rocking and Twisting.	69
3.2.5.	Carbon-Carbon Stretching.	73
3.3.	Effects of the "Rotator" Phase Transition on the Infrared Spectra.	73
3.3.1.	C-H Stretching.	73
3.3.2.	Methylene Scissoring and Methyl Bending.	81
3.3.3.	Methylene Wagging.	85
3.3.4.	Methylene Rocking and Twisting.	89
3.3.5.	Carbon-Carbon Stretching.	93

CHAPTER 4: 1,2-DIPALMITOYL-sn-GLYCERO-3-PHOSPHOCHOLINE.

4.1.	The Infrared Spectrum of DPPC.	95
4.1.1.	C-H Stretching.	97
4.1.2.	Carbonyl Stretching.	101
4.1.3.	Fingerprint Region.	101
4.2.	The Thermotropic Behaviour of DPPC Multilamellar Dispersions.	106
4.2.1.	Acyl Chain Modes.	106

4.2.1.1.	Scissoring and Rocking.	106
4.2.1.2.	Methylene Wagging Band Progression.	114
4.2.1.3.	C-H Stretching.	118
4.2.1.4.	Discussion.	123
4.2.2.	Head-Group Modes.	130
4.2.2.1.	Ester Linkage.	130
4.2.2.2.	Phosphate Modes.	135
4.2.2.3.	Choline Group.	137
4.2.3.	Specifically-Deuteriated Derivatives.	139
4.2.3.1.	DPPC-2'-d ₂ .	142
4.2.3.2.	DPPC-3'-d ₂ , DPPC-7',8'-d ₄ and DPPC-13'-d ₂ .	146
4.2.3.3.	DPPC-16'-d ₃ .	152
CHAPTER 5: <i>ACHOLEPLASMA LAIDLAWII</i> MEMBRANES.		
5.1.	General Characteristics.	159
5.2.	Membranes Enriched in C16:0-d ₃₁ .	160
5.2.1.	Absorbance Changes.	163
5.2.2.	Frequency Shifts.	166
5.2.3.	Halfbandwidth Variations.	169
5.2.4.	Effect of Temperature.	171
5.2.5.	Effect of Proteins.	174
5.3.	Membranes Enriched in C15:0-d ₂₉ .	175
5.3.1.	Absorbance Changes.	178
5.3.2.	Frequency Shifts.	182
5.3.3.	Halfbandwidth Variations.	184

5.3.4.	Discussion.	186
--------	-------------	-----

CHAPTER 6: CONCLUDING REMARKS.

6.1.	Fourier-Transform Infrared Spectroscopy.	190
------	--	-----

6.2.	Hydrocarbons.	192
------	---------------	-----

6.3.	Bilayers of DPPC.	193
------	-------------------	-----

6.4.	<i>Acholeplasma laidlawii</i> Membranes.	197
------	--	-----

REFERENCES.		200
-------------	--	-----

• ACKNOWLEDGMENTS

I would like to express my gratitude to my Supervisor Dr. Ian C.P. Smith for his hospitality, encouragement and help of all kinds during the course of my studies.

It is a privilege to thank Drs. David G. Cameron and Henry H. Mantsch; their scientific guidance, support and advice made this work possible.

I would also like to acknowledge the help and friendship of all the members of the Molecular Biophysics and Molecular Spectroscopy sections of the National Research Council of Canada and especially my fellow students for the atmosphere of comradeship that always prevailed.

• The financial support received from ICRO-UNESCO, the School of Graduate Studies and the Province of Ontario is gratefully acknowledged.

PREFACE

The study of biological membranes constitutes an important part of Molecular Biology since membranes not only maintain the physical integrity of cells and organelles but they also serve as the medium for cellular communication and through them all metabolites are transported.

In the last few years there has been a growing interest in the state of lipids in membranes, particularly in the nature of their packing. Spectroscopic methods are well suited for these studies and until recently only magnetic resonance techniques have been successful in the study of natural membranes.

It is the purpose of this thesis to develop appropriate techniques and to initiate the study of biological membranes using infrared spectroscopy.

Parts of the following publications originated from the work presented in this thesis:

Casal, H.L., Smith, I.C.P., Cameron, D.G. & Mantsch, H.H.
Biochim. Biophys. Acta. 550, 145 (1979).

Cameron, D.G., Casal, H.L. & Mantsch, H.H.
J. Biochem. Biophys. Methods. 1, 21 (1979).

Casal, H.L., Cameron, D.G., Smith, I.C.P. & Mantsch, H.H.
Biochemistry. 19, 444 (1980).

Cameron, D.G., Casal, H.L. & Mantsch, H.H.
Biochemistry. 19, 3665 (1980).

7
-vii-

Cameron, D.G., Casal, H.L., Mantsch, H.H., Boulanger, Y. &
Smith, I.C.P. Biophys. J. in the press.

ABSTRACT.

Fourier-transform infrared transmission spectroscopy has been used to investigate the thermal behaviour of lipids in bio-membranes. Artificial membranes as well as natural membranes were studied.

An appropriate methodology for sample preparation, spectral recording and data handling has been developed.

The orthorhombic to hexagonal solid-solid phase transition in the hydrocarbons 1,1,1,17,17,17-hexadeuterioheptadecane ($n\text{-C}_{17}\text{H}_{30}\text{D}_6$); nonadecane ($n\text{-C}_{19}\text{H}_{40}$) and heptacosane ($n\text{-C}_{21}\text{H}_{44}$) has been studied. The effects of this transition on the infrared spectra of the compounds have been characterized and used in the understanding of the thermal behaviour of lipid bilayers. The infrared data on the phase transition are interpreted as indicating that there is no conformational disorder introduced in the hexagonal phase. However, the data are also consistent with the existence of torsions or twists in the chains.

The infrared spectrum of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) has been assigned with the aid of the spectra of specifically-deuteriated derivatives.

The thermotropic mesomorphism of fully hydrated multibilayers of DPPC was studied. Spectral parameters were measured for the acyl chain vibrational modes. Correlation with the effects observed in the case of the orthorhombic-hexagonal phase transition in *n*-alkanes allowed the charac-

terization of the pretransition at 36 - 38 °C as a phase change in the acyl chain packing from an orthorhombic to a hexagonal subcell as the temperature is raised. The orthorhombically-packed chains just below 36°C are not rigidly held in position, but undergo torsions. The full rigidity of the lattice is attained at -60°C. It is also shown that in the gel phase at all temperatures below the main transition the acyl chains are in the all-trans conformation. The main transition in DPPC bilayers was confirmed to involve primarily the introduction of conformational disorder in the acyl chains. The spectral changes for the "head-group" of the molecule indicate minor perturbations occurring in the polar area of the bilayers. Evidence is presented supporting the concept of two slowly interchanging conformations of the "head-group" in the liquid-crystalline phase and demonstrating an even slower rate of interchange in the gel phase.

The thermotropic mesomorphism of bilayers formed with a series of derivatives of DPPC specifically-deuteriated in the *sn*-2 chain was investigated. It is shown that the pretransition results in greater changes in the intermolecular interactions in the central segments of the chains than in the center of the bilayer, suggesting the existence of a "plateau" in the strength of the interchain interactions in the gel phase. The rate of rotation of the terminal methyl group decreases steadily as the temperature is reduced.

The phase transition of the lipid components of the plasma membrane of *Acholeplasma laidlawii* B was studied. Membranes enriched biosynthetically to 60 % in perdeuteriated palmitic acid and to 94 % in perdeuteriated pentadecanoic acid were investigated. A comparison of the behaviour of intact and deproteinated membranes allowed the study of the influence of the endogenous proteins on the lipid organization and dynamics.

The thermal rearrangement in the *A. laidlawii* membranes enriched with palmitic acid- d_{31} is shown to cover a 20°C temperature range. It consists of two overlapping stages, in the lower temperature range the principal change is a reduction in the rigidity of the lipid matrix, in the upper temperature range the principal effect is a large change in the gauche/trans conformer ratio of the acyl chains.

The phase transition in *A. laidlawii* membranes enriched with pentadecanoic acid- d_{29} covers a narrower temperature range. It also involves two stages; one at lower temperatures whereby the rigidity of the lattice decreases, and a second at higher temperatures involving the introduction of conformational disorder in the acyl chains.

The effects of the intrinsic membrane proteins on the lipid phase transition are seen to be relatively minor. They produce a decrease in the general freedom of motion of the acyl chains in the gel phase. In the liquid-crystalline phase the presence of proteins increases the population of gauche conformers in the acyl chains. Membrane proteins also produce a residual broadening of the phase transition.

LIST OF FIGURES

- Fig. 1. Chemical structure of phospholipids.
A. General structure and B. different head-group structures. PC: phosphatidylcholine; PS: phosphatidylserine and PE: phosphatidylethanolamine.
- Fig. 2. Representation of the "fluid-mosaic" model of biological membranes.
- Fig. 3. Schematic representation of different lipid mesomorphs in water.
- Fig. 4. Differential Scanning Calorimetry (DSC) scan of fully hydrated bilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine. (Kindly supplied by Mrs. Anne Joyce).
- Fig. 5. Layout of a Harrick demountable cell for liquid samples consisting of: (A) stainless steel cup with Luer fittings (B), O-rings (C), windows (D), spacers (E), retaining ring (F), and knurled screw collar (G). The cell assembly is from left to right.
- Fig. 6. Lower curve: infrared spectrum of doubly distilled water at 30°C. The sample was contained in a 6 μm -thick cell with BaF_2 windows. Upper curve: difference spectrum obtained by subtracting the above spectrum from one recorded under identical conditions at 40°C. Inset: vertically expanded water and difference spectra in the region of the "association" band which interferes with the C-D stretching bands.
- Fig. 7. Infrared spectrum of fully hydrated DPPC- $13'\text{-d}_2$. The sample was a 50 μm -thick film between CaF_2 windows. A. Before subtraction of the "water association" band and B. after subtraction of the water

band.

Fig. 8. Bottom: Infrared spectrum of fully hydrated DPPC bilayers in the C-H stretching region at 39°C (solid line) and 43°C (broken line). The sample was a 6 μm -thick film between CaF_2 windows. Top: Corresponding difference spectrum showing the change in integrated intensity and the ΔA parameter.

Fig. 9. Temperature dependence of the CH_2 antisymmetric stretching band in the spectra of DPPC as $\Delta A/\Delta T$ and A versus temperature.

Fig. 10. Representation of a band contour as an example for the determination of band positions.

Fig. 11. Hydrocarbon chain crystal-packing patterns: A, orthorhombic or monoclinic; B, hexagonal and C, triclinic. In all cases, the long axes of the chains are projecting from the page. In the case of hexagonal packing, the circles represent that the orientations of chains relative to each other at a given moment are random.

Fig. 12. Carbon-hydrogen stretching region in the infrared spectra of orthorhombic $\text{n-C}_{17}\text{H}_{30}\text{D}_6$; $\text{n-C}_{19}\text{H}_{40}$ and $\text{n-C}_{21}\text{H}_{44}$.

Fig. 13. Carbon-deuterium stretching region in the infrared spectrum of orthorhombic $\text{n-C}_{17}\text{H}_{30}\text{D}_6$.

Fig. 14. Bending region ($1500 - 1350 \text{ cm}^{-1}$) in the infrared spectra of orthorhombic $\text{n-C}_{17}\text{H}_{30}\text{D}_6$; $\text{n-C}_{19}\text{H}_{40}$ and $\text{n-C}_{21}\text{H}_{44}$.

Fig. 15. Wagging region ($1360 - 1180 \text{ cm}^{-1}$) in the infrared spectra of orthorhombic $\text{n-C}_{17}\text{H}_{30}\text{D}_6$; $\text{n-C}_{19}\text{H}_{40}$ and $\text{n-C}_{21}\text{H}_{44}$.

Fig. 16. Rocking region ($1190 - 680 \text{ cm}^{-1}$) in the infrared spectra of orthorhombic $n\text{-C}_{17}\text{H}_{30}\text{D}_6$; $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{21}\text{H}_{44}$.

Fig. 17. Carbon-hydrogen stretching region in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$.
Bottom: A, orthorhombic; B, hexagonal and C, melt.
Top: Corresponding difference spectra.

Fig. 18. Temperature dependence of the CH_2 antisymmetric stretching mode in the spectrum of $n\text{-C}_{21}\text{H}_{44}$ as: A, frequency; B, halfbandwidth and C, integrated intensity.

Fig. 19. Temperature dependence of the positions of the two components of the CH_3 asymmetric stretching mode in the spectrum of $n\text{-C}_{21}\text{H}_{44}$.

Fig. 20. Bending region in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$.
Bottom: A, orthorhombic; B, hexagonal and C, melt.
Top: Corresponding difference spectra.

Fig. 21. Wagging region ($1360 - 1180 \text{ cm}^{-1}$) in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$; A, orthorhombic; B, hexagonal and C, melt.

Fig. 22. Temperature dependence of the $k = 2$ component of the methylene wagging band progression in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$ as: A, halfbandwidth and B, integrated intensity.

Fig. 23. Rocking region ($1190 - 680 \text{ cm}^{-1}$) of the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$; A, orthorhombic; B, hexagonal and C, melt.

Fig. 24. Temperature dependence of the intensity of the P_{11} component of the methylene rocking band progression in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$.

Fig. 25. Temperature dependence of the intensity of the methyl symmetric in-plane rocking band (δ) in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$.

Fig. 26. Temperature dependence of the intensity of the R_3 component of the C-C stretching band progression in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$.

Fig. 27. Chemical structure of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

Fig. 28. Infrared spectrum of solid DPPC.

Fig. 29. Carbon-hydrogen stretching region in the infrared spectra of DPPC and derivatives (solid samples).
A, DPPC; B, DPPC- d_9 ; C, DPPC-16',16"- d_6 and D, DPPC- d_{62} .

Fig. 30. Bending region ($1500 - 1300 \text{ cm}^{-1}$) in the spectra of DPPC and derivatives (solid samples).
A, DPPC; B, DPPC- d_9 ; C, DPPC-16'-16"- d_6 and D, DPPC- d_{62} .

Fig. 31. Infrared spectra of DPPC and derivatives (solid samples) in the $1300 - 900 \text{ cm}^{-1}$ region.
A, DPPC; B, DPPC- d_9 ; C, DPPC-16',16"- d_6 and D, DPPC- d_{62} .

Fig. 32. Infrared spectra of DPPC and derivatives (solid samples) in the $900 - 600 \text{ cm}^{-1}$ region.
A, DPPC; B, DPPC- d_9 ; C, DPPC-16'-16"- d_6 and D, DPPC- d_{62} .

Fig. 33. Methylene scissoring (A) and methylene rocking (B) regions of the infrared spectrum of fully hydrated DPPC at 0° , 30° , 38° , 43° and 55°C (bottom) and corresponding difference spectra (top). The spectra in A were recorded from a $6 \text{ }\mu\text{m}$ -thick sample in a cell

with BaF_2 windows. The spectra in B were recorded from a 6 μm -thick sample in a cell with ZnSe windows and were smoothed with an 11-point function. In addition, baselines in B were corrected by removing the slope from the underlying water band.

Fig. 34. Methylene scissoring (A) and methylene rocking (B) regions of the infrared spectrum of fully hydrated DPPC at -60 (solid line) and 0°C (broken line). Spectra were recorded under the same conditions as described in Fig. 33.

Fig. 35. Temperature dependence of the CH_2 scissoring and rocking modes in the spectrum of fully hydrated DPPC: A. CH_2 scissoring modes, as $\Delta A/\Delta T$ (solid line) and A ($\diamond$) vs. temperature; B. CH_2 rocking mode, as $\Delta A/\Delta T$ (solid line) and A (∇) vs. temperature; C. halfbandwidth of the CH_2 rocking mode vs. temperature; D. frequency positions of the minima in the difference spectra of the CH_2 scissoring mode vs. temperature.

Fig. 36. Infrared spectrum of fully hydrated DPPC in the $1360 - 1190 \text{ cm}^{-1}$ region at 0° , 30° , 38° , 43° and 55°C (bottom) and corresponding difference spectra (top). Spectra are from a 25 μm -thick sample between CaF_2 windows.

Fig. 37. Temperature dependence of the $k = 2$ component of the methylene wagging band progression in the spectrum of fully hydrated DPPC as $\Delta A/\Delta T$ (solid line) and A (Δ) vs. temperature.

Fig. 38. Carbon-hydrogen region in the infrared spectrum of fully hydrated DPPC at 0° , 30° , 38° , 43° and 55°C (bottom) and corresponding difference spectra (top). Spectra are from a 2.5 μm -thick sample between CaF_2 windows.

Fig. 39. Temperature dependence of the C-H stretching modes: A. CH_2 symmetric stretching as $\Delta A/\Delta T$ vs. temperature; B. CH_2 symmetric stretching as $\Delta B/\Delta T$ (change in integrated intensity) vs. temperature; C. frequencies: CH_3 asymmetric stretching ($\diamond$), CH_2 antisymmetric stretching (*) and CH_2 symmetric stretching (+); D. halfbandwidths: CH_2 antisymmetric stretching (o) and CH_2 symmetric stretching (Δ).

Fig. 40. Carbonyl stretching region in the spectrum of fully hydrated DPPC at 0° , 30° , 38° , 43° and 55°C (bottom) and corresponding difference spectra (top). Spectra are from a $2.5\text{ }\mu\text{m}$ -thick sample between CaF_2 windows dispersed in H_2O (A) and D_2O (B).

Fig. 41. Temperature dependence of the carbonyl stretching band in the spectra of fully hydrated DPPC as: A. halfwidth vs. temperature and, B. $\Delta B/\Delta T$ (solid line) and B (x) vs. temperature.

Fig. 42. Infrared spectrum of fully hydrated DPPC in the $1200 - 900\text{ cm}^{-1}$ region (bottom) and corresponding difference spectra (top). Spectra were recorded from a $25\text{ }\mu\text{m}$ -thick sample between BaF_2 windows.

Fig. 43. Temperature dependence of fully hydrated DPPC- $2'\text{-d}_2$. A. Infrared spectrum of fully hydrated DPPC- $2'\text{-d}_2$ recorded at 5° , 30° , 38° and 45°C . Spectra are from a $50\text{ }\mu\text{m}$ -thick sample between CaF_2 windows and are plotted at the same scale. The "water-association" band has been removed by subtraction; B. $\Delta A/\Delta T$ derived from the CD_2 antisymmetric stretching band vs. temperature; C. Frequency of the CD_2 antisymmetric (*) and symmetric (+) stretching modes vs. temperature; D. halfbandwidth of the CD_2 antisymmetric (Δ) and symmetric (∇) stretching modes vs. temperature.

Fig. 44. Temperature dependence of fully hydrated DPPC-3'-d₂; DPPC-7',8'-d₄ and DPPC-13'-d₂; A - C. C-D stretching regions of the spectra of DPPC-3'-d₂; DPPC-7',8'-d₄ and DPPC-13'-d₂ at 5°, 30°, 38° and 45°C. Spectra are from 25 μm-thick samples between CaF₂ windows and are plotted at the same scale. The "water-association" band has been removed by subtraction. D - F. ΔA/ΔT vs. temperature plots derived from the CD₂ antisymmetric stretching bands in the spectra of DPPC-3'-d₂; DPPC-7',8'-d₄ and DPPC-13'-d₂.

Fig. 45. A - C. Temperature dependence of the frequencies of the CD₂ antisymmetric stretching bands in the spectra of DPPC-3'-d₂; DPPC-7',8'-d₄ and DPPC-13'-d₂; D - F. Temperature dependence of the halfbandwidth of the CD₂ antisymmetric stretching bands in the spectra of DPPC-3'-d₂; DPPC-7',8'-d₄ and DPPC-13'-d₂.

Fig. 46. Carbon-deuterium stretching region of the infrared spectrum of fully hydrated DPPC-16'-d₃ at 5°, 30°, 38° and 45°C. Spectra are from a 25 μm-thick sample between CaF₂ windows and are plotted at the same scale. The "water-association" band has been removed by subtraction.

Fig. 47. Temperature dependence of the CD₃ asymmetric stretching mode in the spectra of DPPC-16'-d₃; A. frequency vs. temperature; B. halfbandwidth vs. temperature.

Fig. 48. Progressive motional collapse of the doubly degenerate CD₃ asymmetric mode in the spectra of DPPC-16'-d₃. Bottom: absorption spectra at 5°, 20°, 30° and 38°C. Top: Difference spectra obtained by subtraction of

adjacent pairs of absorption spectra. All difference spectra are plotted to the same size, hence absolute comparisons of spectral changes can not be made.

Fig. 49. Infrared spectra of solid unhydrated samples of:
A. intact *A. laidlawii* C16:0-d₃₁ plasma membranes;
B. deproteinated *A. laidlawii* C16:0-d₃₁ plasma membranes; and C. DPPC-d₆₂.

Fig. 50. Infrared spectra of fully hydrated samples of:
A. intact *A. laidlawii* C16:0-d₃₁ plasma membranes (50 μ m sample); B. deproteinated *A. laidlawii* C16:0-d₃₁ plasma membranes (25 μ m sample); C. DPPC-d₆₂ (12 μ m sample).

Fig. 51. $\Delta A/\Delta T$ vs. temperature plots derived from the CD₂ symmetric stretching bands in the spectra of fully hydrated: A. intact *A. laidlawii* C16:0-d₃₁ membranes; B. deproteinated *A. laidlawii* C16:0-d₃₁ membranes; C. DPPC-d₆₂.

Fig. 52. Temperature dependence of the frequency of the CD₂ symmetric stretching modes in the spectra of fully hydrated: A. intact *A. laidlawii* C16:0-d₃₁ membranes; B. deproteinated *A. laidlawii* C16:0-d₃₁ membranes; C. DPPC-d₆₂.

Fig. 53. Temperature dependence of the halfwidth of the CD₂ symmetric stretching bands in the spectra of fully hydrated: A. intact *A. laidlawii* C16:0-d₃₁ membranes; B. deproteinated *A. laidlawii* C16:0-d₃₁ membranes; C. DPPC-d₆₂.

Fig. 54. Infrared spectrum of *A. laidlawii* C15:0-d₂₉ plasma membranes. The sample was a pellet composed of 2 mg membranes and 200 mg KBr.

Fig. 55. Infrared spectrum of fully hydrated *A. laidlawii* C15:0-d₂₉ plasma membranes at 5° (solid line) and 45 °C (broken line). The sample was 50 µm-thick between CaF₂ windows. The "water-association" band has been removed by subtraction.

Fig. 56. $\Delta A/\Delta T$ vs. temperature plots derived from the CD₂ symmetric stretching bands in the spectra of fully hydrated: A. intact *A. laidlawii* C15:0-d₂₉ plasma membranes; B. deproteinated *A. laidlawii* C15:0-d₂₉ membranes.

Fig. 57. Temperature dependence of the frequency of the CD₂ symmetric stretching modes in the spectra of fully hydrated: A. intact *A. laidlawii* C15:0-d₂₉ plasma membranes; B. deproteinated *A. laidlawii* C15:0-d₂₉ membranes.

Fig. 58. Temperature dependence of the halfwidths of the CD₂ symmetric stretching bands in the spectra of fully hydrated: A. intact *A. laidlawii* C15:0-d₂₉ plasma membranes; B. deproteinated *A. laidlawii* C15:0-d₂₉ membranes.

LIST OF TABLES

- Table I: Chemical composition of cell membranes.
- Table II: Heat and temperature of transition for 1,2-diacyl-*sn*-glycero-3-phosphocholines.
- Table III: Relevant physical constants of normal alkanes.
- Table IV: Infrared frequencies (cm^{-1}) of the methylene scissoring and methyl bending modes of $n\text{-C}_{21}\text{H}_{44}$; $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{17}\text{H}_{30}\text{D}_6$.
- Table V: Infrared frequencies (cm^{-1}) of the methylene wagging band progression of $n\text{-C}_{21}\text{H}_{44}$; $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{17}\text{H}_{30}\text{D}_6$.
- Table VI: Infrared frequencies (cm^{-1}) of the methylene rocking band progression and methyl rocking of $n\text{-C}_{21}\text{H}_{44}$ and $n\text{-C}_{19}\text{H}_{40}$.
- Table VII: Infrared frequencies (cm^{-1}) of the methylene twisting bands of $n\text{-C}_{21}\text{H}_{44}$ and $n\text{-C}_{19}\text{H}_{40}$.
- Table VIII: Infrared frequencies (cm^{-1}) of the C-C stretching modes in $n\text{-C}_{21}\text{H}_{44}$; $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{17}\text{H}_{30}\text{D}_6$.
- Table IX: Temperature dependence of the two components of the CD_3 symmetric stretching mode in the spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$.
- Table X: Observed frequencies (cm^{-1}) in the infrared spectra of DPPC and deuteriated derivatives as polycrystalline solids and CDCl_3 solutions: C-H stretching region.
- Table XI: Observed frequencies (cm^{-1}) in the infrared spectra of DPPC and deuteriated derivatives as polycrystalline solids and CDCl_3 solutions: Fingerprint region.

Table XII: Temperature dependence of the peak position of the first three components of the CH_2 wagging band progression in the infrared spectrum of fully hydrated DPPC bilayers.

Table XIII: Temperature dependence of the asymmetry of the carbonyl stretching band in the spectra of multibilayers of DPPC dispersed in D_2O , measured as the variation in the high frequency component and the low frequency component relative to the peak maximum.

Table XIV: Infrared vibrational frequencies of the C-D stretching bands of specifically deuteriated derivatives of DPPC and palmitic acid.

ABBREVIATIONS

$n\text{-C}_{17}\text{H}_{30}\text{D}_6$:	1,1,1,17,17,17-Hexadeuterioheptadecane.
$n\text{-C}_{19}\text{H}_{40}$:	Nonadecane.
$n\text{-C}_{21}\text{H}_{44}$:	Heneicosane.
$n\text{-C}_{33}\text{H}_{68}$:	Tritriacontane.
DMPC:	1,2-Dimyristoyl- <i>sn</i> -glycero-3-phosphocholine.
DPPE:	1,2-Dipalmitoyl- <i>sn</i> -glycero-3-phosphoethanolamine.
DPPC:	1,2-Dipalmitoyl- <i>sn</i> -glycero-3-phosphocholine.
DPPC- d_9 :	1,2-Dipalmitoyl- <i>sn</i> -glycero-3-phospho-(N,N,N-trideuteriomethyl)-choline.
DPPC-2'- d_2 :	1-Palmitoyl-2-(2'-dideuterio)-palmitoyl- <i>sn</i> -glycero-3-phosphocholine.
DPPC-3'- d_2 :	1-Palmitoyl-2-(3'-dideuterio)-palmitoyl- <i>sn</i> -glycero-3-phosphocholine.
DPPC-7',8'- d_4 :	1-Palmitoyl-2-(7',8'-tetra deuterio) <u>-palmitoyl</u> - <i>sn</i> -glycero-3-phosphocholine.
DPPC-13'- d_2 :	1-Palmitoyl-2-(13'-dideuterio)-palmitoyl- <i>sn</i> -glycero-3-phosphocholine.
DPPC-16'- d_3 :	1-Palmitoyl-2-(16'-trideuterio)-palmitoyl- <i>sn</i> -glycero-3-phosphocholine.
DPPC-16',16''- d_6 :	1-(16''-Trideuterio)-palmitoyl-2-(16'-trideuterio)-palmitoyl- <i>sn</i> -glycero-3-phosphocholine.
DPPC- d_{62} :	1,2-Diperdeuteriopalmityl- <i>sn</i> -glycero-3-phosphocholine.
DSC:	Differential Scanning Calorimetry.
ESR:	Electron Spin Resonance.

FT-IR: Fourier transform infrared.
IR: Infrared.
NMR: Nuclear magnetic resonance.
 T_m : Temperature of the gel to liquid-crystal phase transition in phospholipid-water systems.
 T_{pre} : Temperature of the pretransition in phosphocholines-water dispersions.

CHAPTER 1. INTRODUCTION.

1.1. Biological Membranes.

Biological membranes have a number of important functions in a living organism. They act as structural barriers that maintain the integrity of a cell; they are selective permeability barriers for the passage of molecules into and out of a cell or organelle; they are the site at which a number of important enzymes act; they are also the site at which cellular communication occurs. In summary, they are responsible for maintaining the cell identity and life itself [1].

The study of membrane properties at the molecular level provides insight not only into how life occurs, but also into the mechanisms of drug action. Ultimately, the detailed knowledge of membrane physical and chemical properties will serve to characterize these mechanisms, to predict drug effects and to aid in drug design.

1.1.1. Chemical Composition.

Biological membranes are composed of two main classes of compounds, lipids and proteins, with lesser amounts of carbohydrates often combined in glycoproteins and glycolipids [2].

The relative proportions of membrane components vary

greatly from species to species, from tissue to tissue, and also from organelle to organelle in the same cell. The differences in composition may be correlated with the specific function of the membrane in each case. Thus, in the myelin membrane sheathing nerve fibers, the proportion of lipid to protein is 4 to 1, while in the mitochondrial membrane the ratio is about 1 to 1 [3]. The composition of different membranes is given in Table I.

The membrane proteins are responsible for most of the functional roles, cell recognition, active transport of metabolites and enzymatic activity.

The constituent lipids of membranes can be classified in two general classes: neutral lipids and polar lipids. Neutral lipids are generally triglycerides and sterols, they serve as storage or for immediate use for the metabolic needs of the cell. Polar lipids form the largest group found in membranes [4]. The major classes are phospholipids, sphingolipids, and glycolipids. Most are derivatives of glycerol. Phospholipids are the most commonly found polar lipids in membranes, their general structure is shown in Fig. 1. Positions 1 and 2 of the glycerol backbone are ester-linked to fatty acids. These fatty acids are heterogeneous in chain length (usually from C_{14} to C_{24}) with varying degrees of unsaturation. Usually saturated acids are found at position 1 while unsaturated acids are linked to position 2.

Position 3 of the glycerol molecule is ester-linked to phosphoric acid where different substituents (X) are

TABLE I. Chemical composition of cell membranes

Membrane	Protein (%)	Lipid (%)	Carbohydrate (%)	Weight fraction of protein	Ratio of protein to lipid
Myelin	18	79	3	0.18	0.23
Plasma membranes					
Acholeplasma laidlawii	57	32	(1-2)	0.57	1.80
Mouse liver cells	46	54	(2-4)	0.46	0.85
Human erythrocyte	49	43	8	0.49	1.1
Amoeba	54	42	4	0.54	1.3
Rat liver cells	58	42	(5-10)	0.58	1.4
L cells	60	40	(5-10)	0.6	1.5
HeLa cells	60	40	2	0.6	1.5
Retinal rods, bovine	51	49	4	0.51	1.0
Mitochondrial outer membranes.	52	48	(2-4)	0.52	1.1
Sarcoplasmic reticulum	67	33		0.67	2.0
Nuclear membrane, chicken liver.	73	21		0.73	3.5
Mitochondrial inner membranes.	76	24	(1-2)	0.76	3.2
Gram-positive bacteria	75	25	(10)	0.75	3.0
Halobacterium purple membrane.	75	25		0.75	3.0

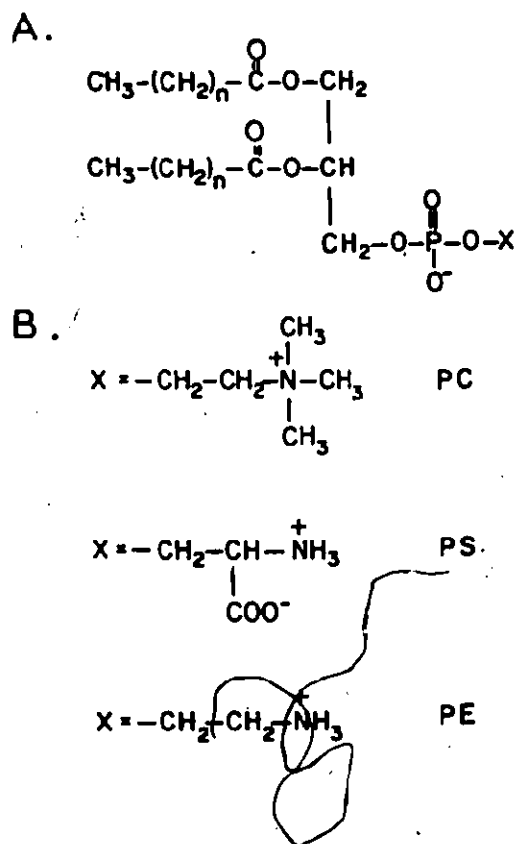


Fig. 1. Chemical structure of phospholipids.
 A. General structure and B. different head-group structures. PC: phosphatidylcholine; PS: phosphatidylserine and PE: phosphatidylethanolamine.

found. The generic names of the phospholipids are derived from the nature of the substituent linked to the phosphate moiety. The common substituents are shown in Fig. 1.

The molecular structure of phospholipids is unusual in that the polar and non-polar portions of the molecule are segregated to give a hydrophilic head and a hydrophobic tail connected by a belt region of intermediate polarity. Because of this segregation of polar and non-polar moieties of the molecule there is no suitable solvent for both head and tail. This gives rise to one important property in relation to membrane structure, the lyotropic mesomorphism of membrane lipids. In water, the solvent of biology, polar membrane lipids spontaneously form bilayer structures [5].

1.1.2. Membrane Structure.

The different membrane components must be arranged so as to ensure the viability of such an organization to carry out the functions it must perform. At the same time the arrangement must be compatible with the properties of the individual membrane components. Several models have been proposed for the architecture of biological membranes. The currently accepted view, the fluid-mosaic model [6] is represented in Fig. 2. The lipid components are arranged in a bimolecular array (bilayer). The polar moieties of these lipids are in contact with the aqueous media outside and inside the cell. The membrane proteins are imbedded in the lipid bilayer, some are in close association with the li-

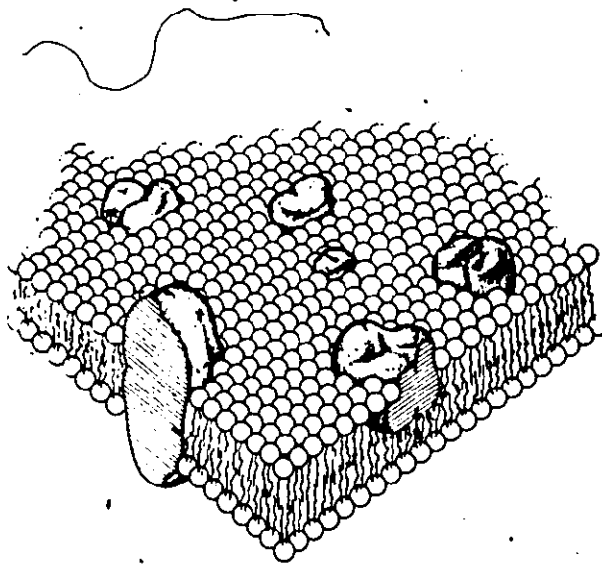


Fig. 2. Representation of the "fluid-mosaic" model of biological membranes [6].

pids, sometimes spanning the lipid bilayer (intrinsic proteins) while others are in a more loose association with the bilayer (extrinsic proteins).

Evidence of this model has come from studies of membrane disaggregation and the realization that certain membrane-bound enzymes require the association of specific classes of lipids in order to function. The similarity of physical properties of bio-membranes and bilayers of polar lipids, in particular as revealed by X-ray diffraction and electron microscopy, gives support to the bilayer hypothesis of bio-membranes [7].

The close association of protein and lipid molecules in membranes gives rise to lipid-protein interactions, the nature of which is not clearly understood [8].

1.2. Model Membranes.

Much of the present knowledge of the membrane organization has come from studies of artificial membranes composed of synthetic or naturally-occurring phospholipids. As stated above, the amphipatic nature of phospholipids determines the formation of particular aggregations when dispersed in water [5]. The hydrophobic moieties will tend to repel the water molecules and in doing so they aggregate with other lipid molecules in such a way that there is a maximum interaction between apolar acyl chains. The polar ends will find themselves interacting with each other and with water. The macroscopic arrangement depends on the particular way in

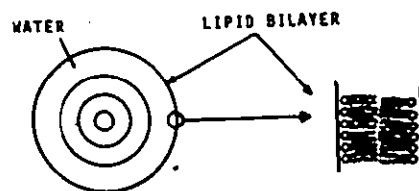
which lipid and water are brought together, the relative amounts of each and the temperature [9], examples of different lipid mesomorphs are shown in Fig. 3.

The microscopic arrangement of polar lipids in water is a bimolecular membrane, the hydrocarbon "tails" directed inwards and the hydrophilic "head-groups" outwards. The final packing is determined by the molecular shape. All major phospholipids found in membranes are roughly rectangular in shape and this makes a bilayer the best arrangement for lipids in water.

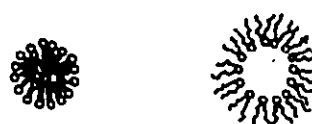
Different lipid aggregates are used for studies of membrane properties, the most commonly used are multilamellar lipid dispersions (Fig. 3A).

1.3. Thermotropic Transitions in Bio-membranes.

One of the characteristics of protein-free lipid bilayers and bio-membranes alike is their ability to undergo a reversible thermotropic transition from a fluid state at high temperature to a crystalline state at low temperature. Such transitions were first characterized in phospholipids before they were found in bio-membranes. They resemble some transitions found in related systems such as soaps in water and share some features in common with the melting of hydrocarbons. The surprising parallel between these order-disorder transitions in phospholipids and bio-membranes has emphasized the relevance of the study of lipids in water as model membranes [10].



A) MULTILAMELLAR VESICLES.



B) MICELLES: NORMAL AND INVERTED.



C) MONOLAYER.

Fig. 3. Schematic representation of different lipid mesomorphs in water.

Phase transitions have been most extensively studied in phospholipids, (and in particular, in the phosphatidylcholines) but the similarity in molecular structure between these and the other membrane polar lipids suggests that the characteristics of their transitions will be very similar [10,11].

Thermotropic transitions in a bio-membrane were first found in the plasma membrane of the microorganism *Acholeplasma laidlawii* [12]. The coincidence of the general thermodynamical characteristics of the transition in synthetic and natural membranes [13] gave much insight into the structure of the membrane matrix. Other organisms also show similar transitions and their physiological implications are a matter of current research [11].

1.3.1. The 1,2-Diacyl-phosphatidylcholines.

This class of phospholipids have been extensively studied with regard to both their lyotropic and thermotropic mesomorphism. Of particular importance in the context of this work are the studies of their thermotropic mesomorphism in the presence of excess water.

Phase transitions in bilayers of these compounds have been studied using a wide variety of physical techniques. In particular, calorimetry is specially suited to detect these transitions since they involve a definite enthalpic change [13].

Table II shows the heats and temperatures of transition for some representative 1,2-diacyl-phosphatidylcholines. The temperature of the transition decreases with decreasing chain length of the acyl substituents, and it also decreases with increasing degree of unsaturation in the acyl chains [14]. As shown in Fig. 4, differential scanning calorimetry (DSC) heating curves of these compounds indicate that at a few degrees below the main melting transition (T_m) there is another endothermic event which has been called the pretransition, the temperatures at which it occurs are listed in Table II. The nature of this pretransition event has been the subject of much study and controversy in the past.

X-ray diffraction techniques have also been applied to the study of the different phases of bilayers of the phosphatidylcholines [15,16]. In particular the hydrocarbon chain organization has been studied with X-rays. It has been determined that the acyl chain organization in the liquid-crystalline phase is like that of liquid hydrocarbons [9] while in the gel phase the reflection spacings are typical of hydrocarbon chains packed in a hexagonal lattice [17]. The hydrocarbon chains are fully extended in the gel phase and they are oriented such that the plane formed by the carbon atoms forms an angle with respect to the bilayer normal. The value of the tilt angle seems to depend on temperature, and there is still some controversy about it [18-21]. Recently, the crystal structure of 1,2-dimyristoyl-sn-glyce-

TABLE II. Heats and temperatures of transition for
1,2-diacyl-*sn*-glycero-3-phosphocholines [14].

 Compound	Transition temperature T_m (°C)	Heat absorbed at T_m (kcal/mole)	Entropy change at T_m (cal°mole⁻¹)	Pretransition temperature T_{pre} (°C)
Dibehenoyl	75	14.90	42.8	75
Distearoyl	58	10.70	32.8	56
Dipalmitoyl	41	8.65	27.6	35
Dimyristoyl	23	6.65	22.4	14
Dioleoyl	-22	7.60	30.3	--

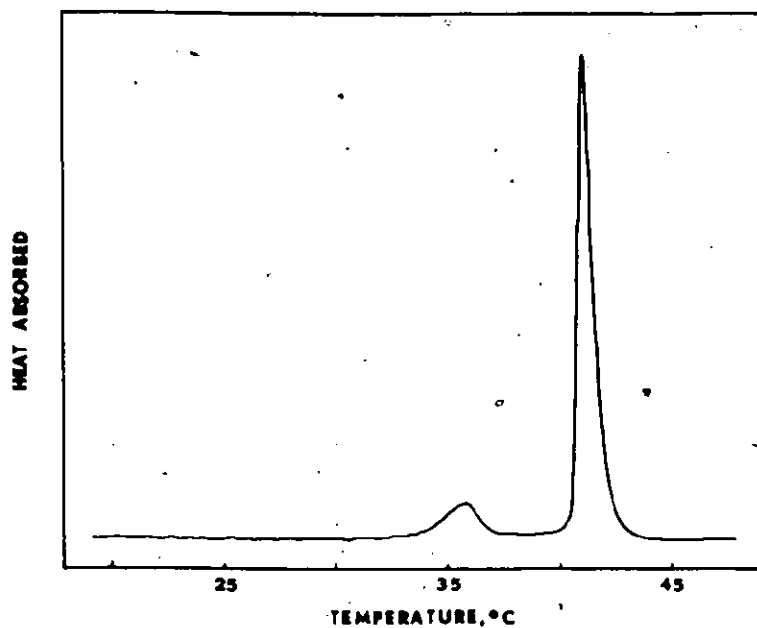


Fig. 4. Differential Scanning Calorimetry DSC scan of fully hydrated bilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine. (Kindly supplied by Mrs. Anne Joyce).

ro-3-phosphocholine (DMPC) dihydrate has been determined [22]. In the gel phase the chains were shown to pack in a monoclinic lattice with neighbouring chains perpendicular to each other at positions close to the ester carbonyl group. However, the chains at positions farther away from the "head-group" were found to pack parallel to each other, as in a triclinic lattice.

The nature of the pretransition has been reported to be a distortion of the hexagonal packing as the temperature is lowered [19-21] and also to involve a change of the polar "head-group" organization [10].

Magnetic resonance spectroscopic methods (ESR and NMR) have been extensively used to study the dynamical aspects of the hydrocarbon chain organization in lecithin bilayers [23,24]. In particular, the study of deuteriated derivatives of different phospholipids using ^2H NMR [25] has been successful in providing detailed pictures of the dynamical behaviour of the hydrocarbon chains in the liquid-crystalline phase [26]. An order parameter "plateau" has been found in bilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) [27] in the liquid-crystalline phase and has since been observed in other systems, including natural membranes [28]. Recently, ^2H NMR [29] and saturation transfer ESR [30] studies on DPPC have shown that in the gel phase there is considerable motion of the acyl chains. This motion has been proposed to be rotation of the chains about their long axes with the rate of rotation decreasing with

temperature.

Vibrational spectroscopy, both infrared and Raman, have also been applied to the study of phase transitions of the phosphatidylcholines [31]. In fact, it was with infrared spectroscopy that the molecular nature of the phase transition in anhydrous films of related systems was first described [32].

Raman spectroscopic studies of hydrated phospholipid bilayers have confirmed the phase transition as involving the melting of the hydrocarbon chains [31]. The melting implies the introduction of conformational disorder in the acyl chains [33]. Raman spectroscopic studies of the gel phase of DMPC and DPPC (and their deuteriated derivatives) have detected considerable changes in the spectra as the temperature is varied. These changes have been interpreted as arising from the introduction of conformational disorder (gauche conformers) in the acyl chains as the temperature is raised throughout the gel phase [33,34,35]. These conclusions contrast with those from the above-mentioned X-ray studies which indicated that the chains are fully extended in the all-trans conformation in the gel phase. The Raman spectral changes observed during the pretransition in DPPC have been interpreted as resulting also from changes in interchain interactions and the introduction of triclinic packing in the acyl chains as the temperature is lowered [34].

Infrared spectroscopy has not been so extensively used for the study of hydrated bilayers of phosphatidylcholines. Water is an intense infrared absorber and masks several

regions of the infrared spectrum. However, melting curves for phospholipids have been constructed using infrared spectroscopy [36,37].

1.3.2. *Acholeplasma laidlawii* Membranes.

As noted above the plasma membrane of the microorganism *Acholeplasma laidlawii* was the first natural membrane whose lipids were shown to undergo a thermal phase transition [12] similar to that found in artificial membranes.

The physical state of membrane lipids is believed to regulate not only the architecture and physical properties of biological membranes but also various physiological functions that ensure the viability of the organism [2]. Enzyme activities have been found to depend to a large extent on the "fluidity" of the lipid matrix which is a function of the temperature and of the lipid composition [38].

A. laidlawii is a microorganism capable of regulating the "fluidity" of its plasma membrane when the growth temperature or the external fatty acid source is varied [39]. The enzymatic activity in *A. laidlawii* has been found to depend critically on the phase behaviour of the membrane lipid pool, as determined with the membrane-bound ATPase [40].

The phase transition of *A. laidlawii* plasma membrane lipids has been studied with different physical techniques such as calorimetry [12], X-ray diffraction [41], ^2H NMR [42] and ESR [43]. In all cases, it was found that the onset

of molecular disorder in the lipid acyl chains occurs over a wide temperature range. This is in contrast to model membrane systems where the phase transition is very sharp.

The broad temperature range of this phase transition could be explained either by lipid-protein interactions which modulate the thermal behaviour of the lipid matrix or by the heterogeneity in the nature of the lipid acyl chains and head-groups. Studies with *A. laidlawii* membranes enriched to >90% in a particular fatty acid have revealed narrower phase-transition ranges, supporting the latter explanation [44].

1.4. Scope of the Studies.

The present work has been devoted to the study of phase transitions in model membranes (in particular, those constituted of bilayers of DPPC) and in natural membranes (the plasma membrane of *A. laidlawii*) using infrared spectroscopy.

Infrared spectroscopy has had limited success in the study of bio-membranes; the main reason for this lack of success has been the complexity of the systems involved coupled with the need to carry out the studies in the presence of a large excess of water. However, infrared spectroscopy is a technique extremely well suited to study both the structure and dynamics of long chain systems. At the same time, it is a complementary spectroscopic technique

of Raman scattering. It presents the advantage over Raman spectroscopy that it is not an inherently weak phenomenon. Minor quantities of fluorescent impurities can hamper Raman studies of complex biomolecules while these impurities have no particular effect on infrared spectra. The main disadvantage of infrared compared to Raman spectroscopy is the obscuring effect of water which is a very strong infrared absorber and not an intense Raman scatterer.

The study of the dynamics of a system requires that the time scale of the spectroscopic technique used be of the same order of magnitude as the molecular motions under investigation. The great disparity of time scales, between vibrational spectroscopy and magnetic resonance spectroscopy makes IR a complementary tool for dynamical studies. At the same time, with infrared spectroscopy the membrane itself can be studied without the need of introducing spectral probes such as nitroxide spin labels for ESR work.

In this work, a general methodology to study both natural and model membranes with infrared spectroscopy has been developed and it is presented in Chapter 2.

This methodology was then applied to the study of fully hydrated multibilayers of DPPC; the results and conclusions of that study are presented in Chapter 4. The IR spectra of DPPC and phospholipids in general arise predominantly from vibrations involving the fatty acyl chains; therefore, the spectra are comparable with those of hydrocarbons. Some normal alkanes present a series of solid-solid

phase transitions which involve changes in crystal packing and molecular motion [45]. The solid-solid phase transition of the n-alkanes, nonadecane ($n\text{-C}_{19}\text{H}_{40}$), heneicosane ($n\text{-C}_{21}\text{H}_{44}$) and 1,1,1,17,17,17-hexadeuterio-heptadecane ($n\text{-C}_{17}\text{H}_{30}\text{D}_6$) was studied by IR spectroscopy. The results of this study are presented in Chapter 3. The aim of the work there presented was principally to obtain information on how the infrared spectra of acyl chains are affected by changes in the crystal packing and molecular motion. With the aid of the results obtained from the hydrocarbon spectra the changes observed in the spectrum of DPPC in the gel phase have been characterized. In particular, the pretransition event at 35-37°C in DPPC has been characterized as a change in the crystal packing from a monoclinic or orthorhombic crystal lattice (orthorhombic subcell) to a hexagonal lattice.

Specifically-deuteriated derivatives of DPPC have been studied in order to aid in the assignment of its infrared spectrum. The derivatives in which the deuterium label is located at different positions in the acyl chains were also studied as fully hydrated multibilayers in order to gain insight into the dynamics of specific sites in the membrane (Chapter 4).

Finally, deuteriated fatty acids were incorporated biosynthetically into the plasma membrane of *A. laidlawii*. The C-D stretching vibrations arising from these fatty acyl chains in the membrane were used to study the organization and dynamics of this natural membrane. The lipids obtained

after removal of the membrane proteins, were also studied. In this manner, information on the influence of protein on lipid dynamics was obtained (Chapter 5). It is also shown that considerable insight may be gained into the various types of motion displayed by the fatty acyl chains by considering the different responses of several IR spectral parameters to motional effects.

CHAPTER 2. MATERIALS AND METHODS.

2.1. Materials.

The normal alkanes n-nonadecane ($n\text{-C}_{19}\text{H}_{40}$) and n-heneicosane ($n\text{-C}_{21}\text{H}_{44}$) were a generous gift of Dr. Robert G. Snyder (University of California at Berkeley, USA).

1,1,1,17,17,17-Hexadeuterio-heptadecane (abbreviated as $n\text{-C}_{17}\text{H}_{30}\text{D}_6$) was kindly supplied by Dr. L.C. Leitch (National Research Council of Canada). It was further purified by gas liquid chromatography on a 5% OV-101 column (5'x3/8") at 170°C in a Varian model 920 aerograph.

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Sigma Chemical Company (St. Louis, MO, USA). 1,2-Diperdeuteriopalmityl-*sn*-glycero-3-phosphocholine (DPPC- d_{62}) and 1-(16'-trideuterio)-palmitoyl-2-(16"-trideuterio)-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC-16',16"- d_6) were purchased from Serdary Research Laboratories (London, Ont., Canada).

1-Palmitoyl-2-(2'-dideuterio)-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC-2'- d_2); 1-palmitoyl-2-(3'-dideuterio)-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC-3'- d_2); 1-palmitoyl-2-(7',8'-tetra-deuterio)-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC-7',8'- d_4); 1-palmitoyl-2-(13'-dideuterio)-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC-13'- d_2); 1-palmitoyl-2-(16'-trideuterio)-palmitoyl-*sn*-glycero-3-phospho-

choline (DPPC-16'-d₃) and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(N,N,N-trideuteriomethyl)-choline (DPPC-d₉) were a generous gift of Dr. Yvan Boulanger (National Research Council of Canada).

Acholeplasma laidlawii B plasma membranes were kindly supplied by Drs. Keith W. Butler and Harold C. Jarrell (National Research Council of Canada). Two different membrane preparations were used: a.- the microorganism was grown at 37°C in a medium supplemented with palmitic acid-d₃₁; and b.- the growth temperature was also 37°C, but the medium was supplemented with pentadecanoic acid-d₂₉ in the presence of avidin. The details of the procedure for the growth of *Acholeplasma laidlawii* and the isolation of its plasma membrane are given in reference [46]. Avidin is a biotin-binding protein which inhibits the synthesis of fatty acids by the organism without affecting its growth [44]. In the presence of avidin, *A. laidlawii* incorporates the exogenous fatty acid in such a way that the membrane is practically homogeneous in that fatty acid.

The composition of the fatty acid acyl chains, determined by gas-liquid chromatography was the following: a.- membranes enriched in C16:0-d₃₁: C12:0, 7.0 mol %; C14:0, 26.6 mol %; C14:1, 2.0 mol %; C16:0, 64.5 mol %; and b.- membranes enriched in C15:0-d₂₉: C14:0, 1.0 mol %; C15:0, 94.2 mol %; C16:0, 2.0 mol %; C18:0, 1.0 mol %; C18:1, 1.4 mol %.

For lipid analysis, the membranes were hydrolyzed and

methyated in 0.7 N methanolic HCl and extracted with petroleum ether (bp 30-60°C). The methyl esters of the fatty acids were analyzed on a Hewlett-Packard HP5710A gas chromatograph with 15 % stabilized on Chromosorb W,A/W, 80-100 mesh, columns at 175°C. Heptadecanoic acid was used as the internal standard.

The extraction of membrane lipids was carried out following the method of Bligh and Dyer [47]. In the case of membranes enriched in Cl6:0-d₃₁, 25 mg of membrane were taken in 2 ml H₂O, and 5 ml CH₃OH with 2.5 ml CHCl₃ were added. The mixture was stirred for 4 hours at room temperature and 1 ml H₂O and 1 ml CHCl₃ were added. After centrifugation (10 min), the precipitate was reextracted with CH₃OH-CHCl₃ (2:1 v/v). The CHCl₃ was dried over Na₂SO₄, filtered and dried under nitrogen: yield; 3.4 mg of lipids. In the case of membranes enriched in Cl5:0-d₂₉ the same procedure was followed with 30 mg of membranes which yielded 4.5 mg of lipids.

2.2. Sample preparations.

Infrared spectra of solid samples were recorded as KBr pellets (13 mm diameter) which were prepared according to standard techniques. KBr pellets of n-C₁₇H₃₀D₆ (liquid at room temperature) were prepared as follows: the desired amount of KBr was combined with a toluene solution of n-C₁₇H₃₀D₆, the mixture was evaporated under a stream of dry N₂ for 12 hours; the residual solvent was eliminated under

vacuum for 12 hours. The resultant mixture of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ and KBr was ground and pressed to form a 13 mm diameter disc.

In general, 2 mg of sample with 200 mg KBr were used to record survey spectra. In order to record very weak bands in the spectra of the n-alkanes (see Chapter 3) pellets composed of 10 mg hydrocarbon and 100 mg KBr were used.

The spectra of CHCl_3 solutions of the different deuteriated derivatives of DPPC were recorded as 10 % solutions (byweight) in a 0.5 mm-thick cell with NaCl windows.

For the studies of hydrated systems, water-insoluble windows were used such as CaF_2 , BaF_2 or ZnSe. These are materials of high refractive index, and fringes, or channel spectra are frequently encountered, for that reason the windows were wedged (20 minutes). In spite of wedging the spectra of samples taken with ZnSe windows showed fringes!

In the cases of multilamellar dispersions of specifically-deuteriated derivatives of DPPC or *A. laidlawii* membranes, only the C-D stretching region ($2500 - 2000 \text{ cm}^{-1}$) of their infrared spectra was recorded. Since these bands are quite weak compared with the rest of the spectrum, fairly thick samples were used (50, 75 and 100 μm); these samples were prepared in conventional amalgamated cells with CaF_2 windows.

For studies involving the complete mid-IR spectrum

(i.e. all but the intense O-H stretching modes of water) very thin cells were used with pathlength of 2.5; 6; 12 and 25 μm . These thin films were prepared using demountable cells from Harrick Scientific Corporation (Ossining, NY, USA). Fig. 5 shows a layout of this cell, it presents a series of advantages over conventional amalgamated cells in that it can be assembled and disassembled in a few minutes, the spacers are made from mylar film and it is round which matches the circular optics used in Fourier transform instruments rather than the linear optics of grating spectrometers.

Fully hydrated multibilayers of DPPC and its deuteriated derivatives were prepared with a variation of the technique used for ESR studies [48]. The bottom half of the Harrick cell is pre-assembled by placing a cell window with an O-ring in the stainless steel cup. Between 50 and 100 μg of dry lipid were placed in the center of the cell window and a few drops of CHCl_3 were added. The CHCl_3 first dissolves the lipid, then evaporates rapidly leaving a thin film of the lipid. For thicker films the process was repeated several times until a film of the desired thickness was obtained. Alternatively, concentrated solutions of the lipid in CHCl_3 were deposited drop by drop until a film was obtained. The CHCl_3 was eliminated under vacuum for a period not shorter than 12 hours. The films were then hydrated with doubly distilled water by placing a few drops of water on the film and maintaining it at 50°C for 30 min. The cell was

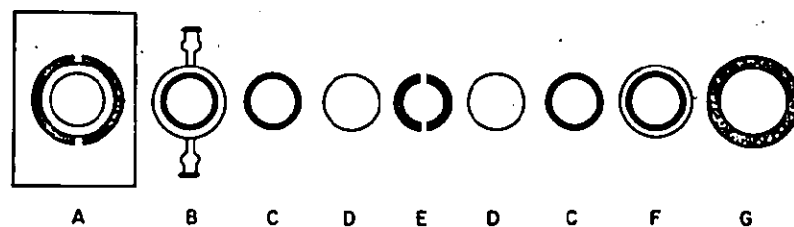


Fig. 5. Layout of a Harrick demountable cell for liquid samples consisting of: (A) stainless steel cup with Luer fittings (B), O-rings (C), windows (D), spacers (E), retaining ring (F), and knurled screw collar (G). The cell assembly is from left to right.

assembled after placing the two spacers, the second window, the O-ring and the rest of the assembly. The cells were then stored at 4°C for 12 hours before spectra were recorded.

Hydrated samples of lipids were also prepared by combining the lipid sample and water and centrifugated for 10-15 min. In this manner, a gel was obtained which was placed in the center of a window and the cell assembled.

The samples of *A. laidlawii* membranes were prepared by combining 3 to 5 mg. of lyophilized membranes with 0.5 ml of water, the mixture was heated at 45°C for 5 min. and then centrifuged for 15 min. The supernatant was removed and the resultant gel placed in the center of a window and the cell assembled.

These techniques always yielded fully hydrated systems; the presence of water in large excess was confirmed by recording a spectrum and checking the water bands.

2.3. Infrared Spectroscopy.

2.3.1. Instrumentation.

Two different Nicolet 7199 Fourier-transform infrared spectrometers were used. Permission to use one of them at the Standards Branch of Consumers and Corporate Affairs Canada is gratefully acknowledged as is the help of D.J. Moffat. They were equipped either with a deuteriated triglycine sulphate (TGS) detector or a mercury cadmium telluride (MCT)

detector. A Digilab FTS-11 (equipped with a MCT detector) and a Digilab FTS-15 (equipped with an indium antimonide, InSb, detector) Fourier-transform infrared spectrometers were used. The MCT and InSb detectors were purchased from Infrared Associates Inc. (New Brunswick, NJ, USA). All spectrometers were purged with dry nitrogen.

Low frequency band-pass optical filters were used in order to cut all the energy above a desired frequency. In this manner the intensity of the center burst in the interferogram is reduced and the aperture can be further opened resulting in increased intensity in the wings of the interferogram yielding an increased signal-to-noise ratio. The use of filters also allows a faster computation of spectra since undersampling is possible [49].

Temperature regulation in the range 0 to 60°C was achieved by circulating a thermostated ethanol/water mixture through a hollow cell mount, giving a temperature stability of better than $\pm 0.1^\circ\text{C}$ across the sample. A copper-constantan thermocouple was mounted directly on the cell window and temperatures were recorded by a Newport digital pyrometer equipped with a printer. In all cases during a sequence of measurements the sample and spectrometer were untouched. The complete sequence of recording a spectrum, printing and incrementing the temperature, waiting for equilibration and recording the next spectrum was under the control of the spectrometer computer. The computer was programmed to take the desired number of spectra at different

temperatures

For spectra at temperatures below 0°C the samples were placed in an evacuable low-temperature chamber located in the sample compartment of the spectrometers. Temperature was regulated by flowing cold nitrogen from a liquid nitrogen container. The rate of flow could be regulated and in that manner the temperature was controlled. Temperature was monitored with a copper-constantan thermocouple.

2.3.2. Spectral Acquisition.

Nicolet instruments: Interferograms were collected with a maximum optical retardation of 1 cm. The mirror velocity was $0.14 \text{ cm} \cdot \text{s}^{-1}$ when using the TGS detector and $0.64 \text{ cm} \cdot \text{s}^{-1}$ with the MCT detector. In all cases the interferograms were apodized with a Happ-Genzel function [50] whose form is:

$$0.54 + 0.46 \cos \frac{\pi}{2} \left| \frac{\delta}{\Delta} \right| \quad \begin{array}{l} |\delta| < |\Delta| \\ |\delta| > |\Delta| \end{array}$$

where δ is the optical path difference and Δ the maximum optical retardation. One level of zero filling was used and interferograms were Fourier-transformed to yield a resolution of 0.9 cm^{-1} . The number of scans was selected in each case according to the desired signal-to-noise ratio.

Digilab instruments: Interferograms were also collected with a maximum optical retardation of 1 cm at an optical velocity of $1.26 \text{ cm} \cdot \text{s}^{-1}$ for both spectrometers. Interferograms were apodized with a triangular function [49] of the

$$\text{form:} \quad 1 - \left| \frac{\delta}{\Delta} \right| \quad \begin{array}{l} |\delta| < |\Delta| \\ 0 \quad \quad \quad |\delta| > |\Delta| \end{array}$$

and were Fourier-transformed to yield a spectral resolution of 0.9 cm^{-1} . One level of zero filling was also used. The total collection time (number of interferograms to be co-added) was selected according to the desired signal-to-noise ratio.

2.3.3. Data Reduction.

The temperature-induced changes in the physical state of the different systems studied in this work produce changes in their IR spectra. In general, all experiments were carried out by increasing the temperature and collecting spectra at different temperatures.

In this section the methods used to measure all IR band parameters as a function of temperature will be described using some of the results presented in following sections as examples.

Water subtraction: In the studies of the specifically-deuteriated derivatives of DPPC (Chapter 4) and *A. laidlawii* membranes (Chapter 5) the bands of interest are those due to the C-D stretching modes. These bands are located in the $2300\text{--}2000 \text{ cm}^{-1}$ spectral region. The mid-IR spectrum of water presents a broad band at $\sim 2130 \text{ cm}^{-1}$ which extends from 2600 to 1900 cm^{-1} . This band has been called the "water association" band. In spectra of fully hydrated samples this

broad water band can mask the C-D stretching bands from a single CD_2 or CD_3 group in a molecule like DPPC (MW = 702). The study of the molecule in D_2O instead of H_2O would in principle overcome the problem of the H_2O absorption, but the strong O-D stretching band in the spectrum of D_2O can also mask spectral features up to 2000 cm^{-1} . However, the spectral region of the O-H bending bands around 1640 cm^{-1} can be readily studied with D_2O as the hydrating agent, this is particularly important for the C=O stretching band in DPPC at about 1740 cm^{-1} (Chapter 4).

For the studies of the C-D stretching bands, the obscuring effect of the "water-association" band was circumvented by subtracting the water band.

In order to carry the subtraction with a minimum of distortion effects the following procedure was used. Since anomalous dispersion is a function of the refractive indices of the cell windows and of the cell contents but is independent of the pathlength, while the absorption is a function of the pathlength [51], cells of the same pathlengths with identical window materials were used to record the spectra of the water and of the samples of interest.

The spectrum of water also changes with temperature. Fig. 6 shows the mid-IR spectrum of water collected at 30°C and 40°C but otherwise under identical conditions. The difference spectrum (top) shows that there are changes in all absorption bands. The insert to that figure shows the changes observed in the region of the "water-association" band. These changes in the water spectrum can produce distortions

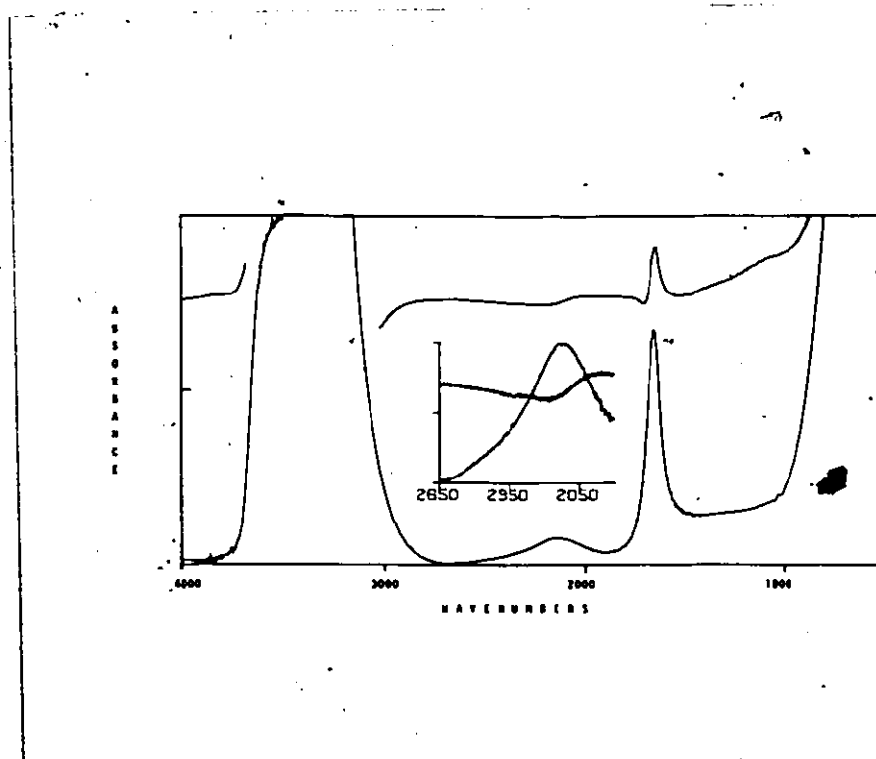


Fig. 6. Lower curve: infrared spectrum of doubly distilled water at 30°C. The sample was contained in a 6 μ m-thick cell with BaF₂ windows. Upper curve: difference spectrum obtained by subtracting the above spectrum from one recorded under identical conditions at 40°C. Inset: vertically expanded water and difference spectra in the region of the "association" band which interferes with the C-D stretching bands.

when the subtraction is carried out; therefore, all the water spectra used in the subtractions were recorded at the same temperature as the spectra of the samples.

As an example, Fig. 7 shows the C-D stretching region of the IR spectrum of a 50 μm thick sample of DPPC- $13'\text{-d}_2$. Fig. 7A shows the spectrum before water subtraction and Fig. 7B after the broad water band has been subtracted.

Difference spectra: The absorption spectra obtained at different temperatures permit the monitoring of such band parameters as peak position, halfwidth and intensity. However, small spectral variations induced by the temperature change or the presence of weak or overlapping bands can be emphasized using difference spectra.

By taking the absorbance of the ratio of a higher temperature single beam spectrum to that at a lower temperature, a spectrum is generated which comprises all the temperature-induced changes in the sample. Alternatively, the same result is obtained if an absorbance spectrum is subtracted from one recorded at a higher temperature. The subtraction of two absorbance spectra amounts to the operation: $\log(I_0/I_h) - \log(I_0/I_l)$, where I_0 is a background spectrum, and I_h and I_l are respectively, spectra at high and low temperature. This operation is equivalent to: $\log(I_l/I_h)$. These difference spectra reflect only the temperature-induced changes in the spectra because, as noted above, the sample and spectrometer remain untouched while a series of spectra at different temperatures are collected.

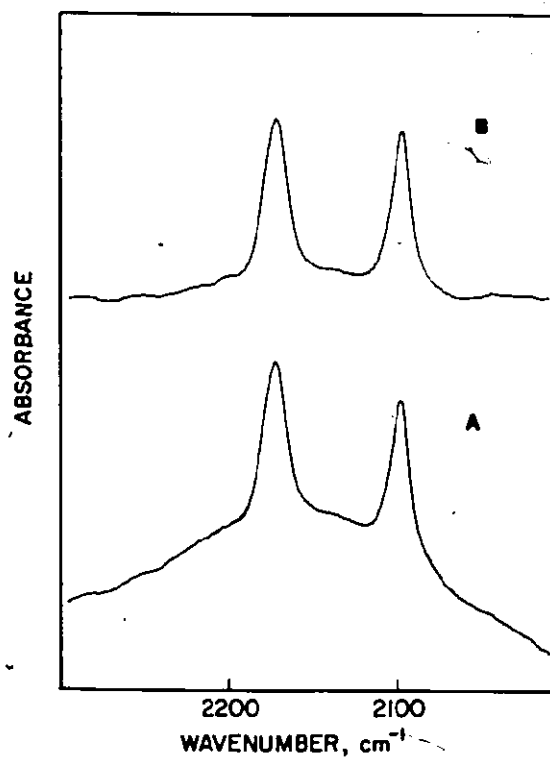


Fig. 7. Infrared spectrum of fully hydrated DPPC-13'-d₂, the sample was a 50 μ m-thick film between CaF₂ windows. A. Before subtraction of the "water-association" band and B. after subtraction of the water band.

All instrument settings are also constant throughout the experiment.

Difference spectra are centered around zero absorbance with deviations from zero reflecting absorbance changes at a given frequency. Positive deviations indicate an increase in absorbance while negative deviations indicate a decrease. However, concomitant changes in band position, shape and intensity make the interpretation of difference spectra complicated and in general, it is not possible to extract information from them in terms of a single band parameter.

Nevertheless, difference spectra have been used to detect small changes in absorbance and to determine the positions of very weak bands.

Fig. 8 shows the C-H stretching region of the spectrum of fully hydrated DPPC at two different temperatures. On the top part of the figure the difference spectrum obtained by subtracting the 42°C spectrum from that at 39°C is shown. An empirical parameter, ΔA , has been measured from difference spectra, such as that shown in Fig. 8. ΔA reflects the temperature-induced changes in all band parameters simultaneously. This parameter when divided by the temperature increment ΔT yields $\Delta A/\Delta T$, a gradient type parameter which was found very useful when detecting small variations in the spectra. From the computed values of $\Delta A/\Delta T$ a value A has been derived using the formula:



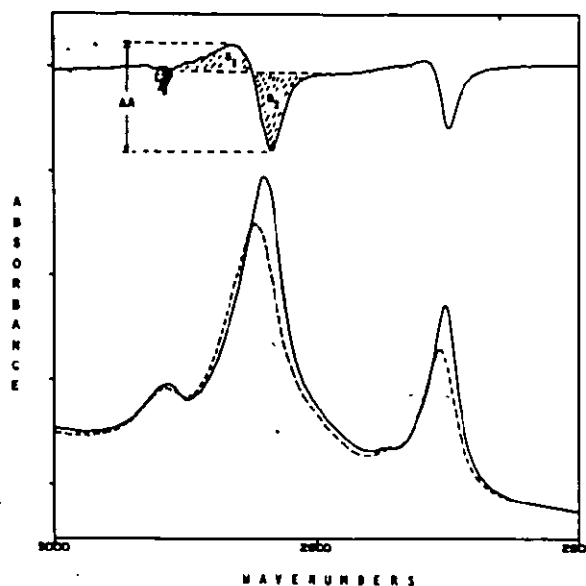


Fig. 8. Bottom: Infrared spectrum of fully hydrated DPPC bilayers in the C-H stretching region at 39°C (solid line) and 43°C (broken line). The sample was a 6 μm -thick film between CaF_2 windows. Top: Corresponding difference spectrum showing the change in integrated intensity and the ΔA parameter.

$$A_n = A_1 - \sum_{i=2}^n \Delta A_i$$

where A_1 is an arbitrary value assigned to the first spectrum in a series; and ΔA_i represents the ΔA value measured from the difference spectrum generated by subtracting the i^{th} spectrum from the $(i-1)^{\text{th}}$. Fig. 9 shows a plot of $\Delta A/\Delta T$ and A versus temperature derived from difference spectra of the CH_2 antisymmetric stretching mode in DPPC. In all $\Delta A/\Delta T$ and A versus temperature plots the ordinate axes have been normalized between zero and one; consequently, quantitative comparisons can not be made.

Changes in integrated intensity, ΔB were also measured from difference spectra as indicated in Fig. 8. Since B_2 in that figure represents a decrease in intensity and B_1 an increase; their difference represents the total change in intensity ΔB for a given band. When divided by the temperature increment $\Delta B/\Delta T$ is obtained which reflects the rate of change of intensity.

The $\Delta A/\Delta T$ parameter is a general monitor of spectral changes and is sensitive to any effect producing changes in the IR spectrum. More commonly employed spectral parameters are peak positions and bandwidths.

Frequencies were measured from absorbance spectra by determining the center of area of the topmost five data points of the peak using the formula:

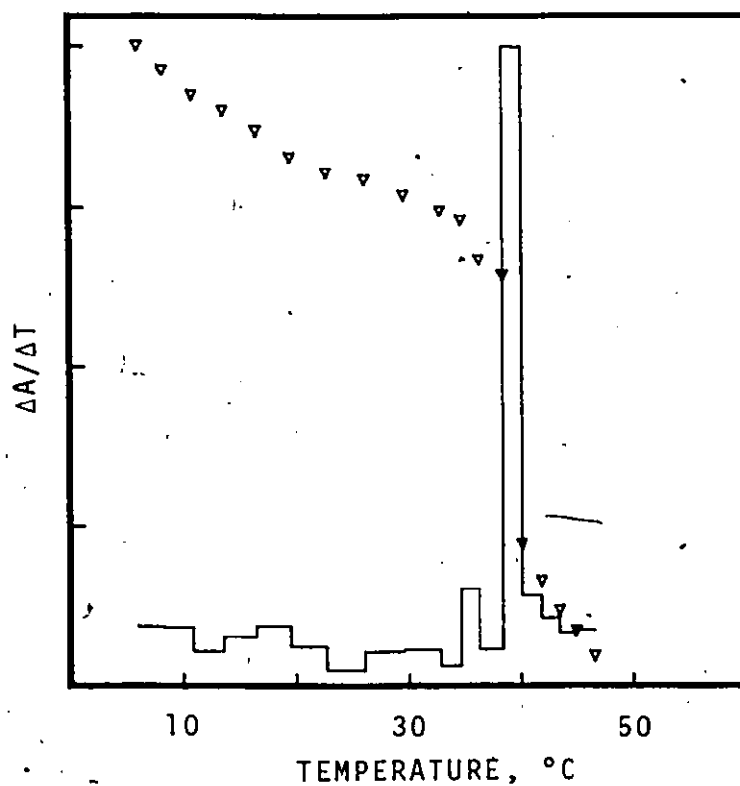


Fig. 9. Temperature dependence of the CH_2 antisymmetric stretching band in the spectra of DPPC as $\Delta A/\Delta T$ and A versus temperature.

$$\text{band position} = \nu_0 + \nu = \nu_0 + \Delta\nu \frac{\sum_i f_i}{\sum_i f_i}$$

where: ν_0 is the position of the maximum point in the band contour; ν is the center of area of the band; $\Delta\nu$ is the frequency separation between data points (at 1 cm^{-1} resolution and one level of zero filling $\Delta\nu = 0.5 \text{ cm}^{-1}$); f_i area under i^{th} point; see Fig. 10. Note that the value of ν can be negative since the formula has been derived by setting $\nu_0 = 0$ as indicated in Fig. 10.

Since $\Delta\nu = 0.5 \text{ cm}^{-1}$ and the topmost five data points were used, the values computed are centers of area for the topmost 2.5 cm^{-1} wide segment of the peaks.

This method for the measurement of frequencies provides a very precise determination of the position of a band [52]. The wavenumber scale of a FT-IR spectrometer, using fringes from a HeNe laser to determine the optical path difference, is reproducible to within 0.005 cm^{-1} . Using a spectral resolution substantially less than the halfwidth of the bands the only significant factor affecting the precision to which temperature-induced shifts can be measured is the signal-to-noise ratio since it determines the reproducibility of the absorbance scale. The signal-to-noise ratios usually employed in this study were such that temperature-induced shifts in frequency as small as 0.05 cm^{-1} could be measured.

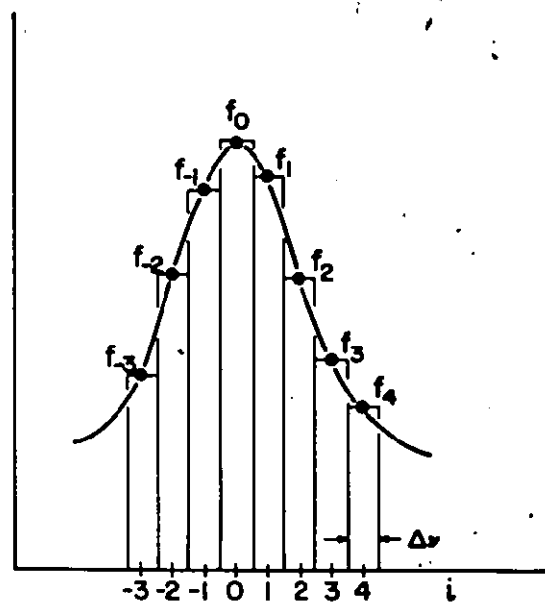


Fig. 10. Representation of a band contour as an example for the determination of band positions.

Peak positions derived from the topmost five data points were verified against those derived from the topmost three and seven data points. They were also checked against those obtained from midpoint values at 0.90 height and against the position of the peak maximum.

In the case of the specifically-deuteriated derivatives of DPPC where peak positions were measured from band contours obtained after subtracting the water background, incorrect water subtraction could affect the measured values. However, no effects was found when the scaling factor was varied by $\pm 50\%$. This lack of effect is due to the difference in bandwidths, the lipid bands have bandwidths in the range $10 - 30 \text{ cm}^{-1}$ while the water band is more than 300 cm^{-1} wide.

Bandwidths were measured at halfheight by visually drawing a baseline through the spectra and measuring the halfbandwidth, and by digitally subtracting a linearly interpolated baseline from the spectrum before computing the width at halfheight of the resultant band contour.

CHAPTER 3. HYDROCARBONS.

3.1. Solid Phase Behaviour of Normal Paraffins.

Normal paraffins can exist in different solid phases. Four solid forms are sufficient to characterize the structures of all odd numbered n-paraffins above $n\text{-C}_9\text{H}_{20}$ and all even numbered n-paraffins above $n\text{-C}_{10}\text{H}_{22}$. The different solid forms are: hexagonal, orthorhombic, triclinic and monoclinic [45]. The hexagonal phase, when present, is stable at temperatures close to the melting point, and the chains are normal to the plane formed by the methyl groups. The other three phases are stable at lower temperatures; in the orthorhombic phase the chains are also normal to the methyl groups plane while in the triclinic and monoclinic phases the chains are tilted with respect to that plane. Fig. 11 shows the subcells of the different crystalline forms.

In the orthorhombic and monoclinic forms the subcell is orthorhombic (Fig. 11A) and in the triclinic form it is triclinic (Fig. 11C).

The hexagonal phase is characterized by no long-range order. While in the orthorhombic and triclinic subcells the chains are rigidly oriented and coupled to each other, in the hexagonal phase they are essentially decoupled; the chains perform rotations about their long axes and the orientations of a given chain are random at any given moment with respect to the orientations of all the others [53].

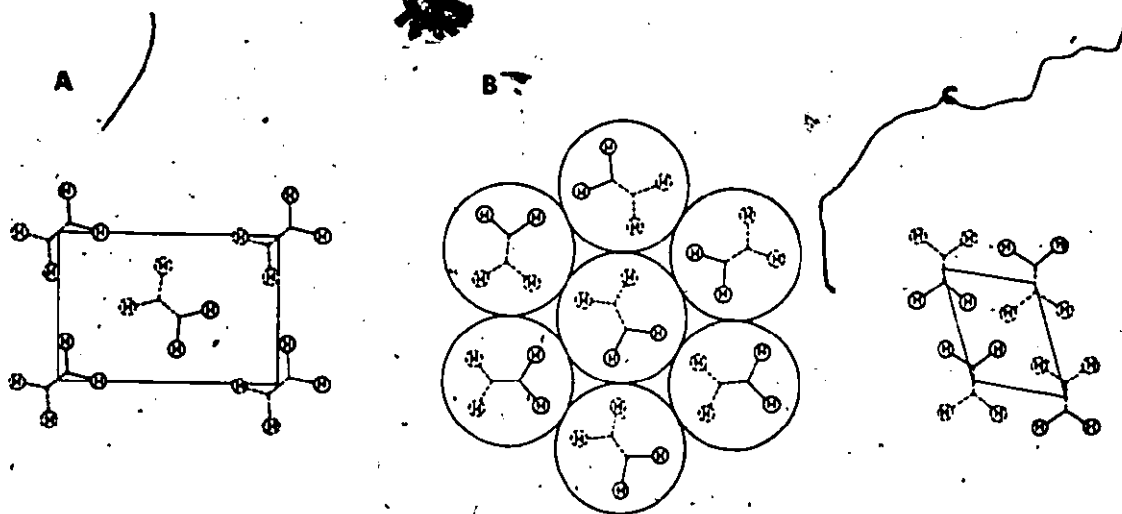


Fig. 11. Hydrocarbon chain crystal-packing patterns:
 A. orthorhombic or monoclinic; B. hexagonal
 and C. triclinic. In all cases, the long axes
 of the chains are projecting from the page.
 In the case of hexagonal packing, the circles
 represent random orientations of chains relative
 to each other at a given moment.

Different alkanes present different phases and transitions between them; some members of the alkane series show one solid-solid phase change while others, in general the long members, show a complex polymorphic behaviour. The compounds studied in this work ($n\text{-C}_{17}\text{H}_{30}\text{D}_6$, $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{21}\text{H}_{44}$) present only one solid-solid phase change some ten degrees below their melting point; this is a transition from the orthorhombic phase (orthorhombic subcell) to the hexagonal phase [45] which has been called the "rotator" phase transition. The temperature of transition as well as some relevant thermodynamic data are presented in Table III.

The detailed microscopic nature of the "rotator" phase transition is unclear; with an increase in temperature of the orthorhombic crystals, anisotropic expansion of the lattice occurs, at the transition temperature there is suddenly a discontinuous change in the lattice structure [54] together with an increase in the amplitudes of rotation of the molecules about their long axes.

Somewhat conflicting evidence for the presence of chain conformational disorder in the hexagonal phase has been reported. Strobl et al. [55,56] have concluded that the hexagonal phase of $n\text{-C}_{33}\text{H}_{68}$ is composed of chains with defects (0.72 gauche conformers per chain). On the other hand, Barnes and Fanconi [57] have determined that the frequency of the longitudinal acoustical mode in the Raman spectrum of $n\text{-C}_{19}\text{H}_{40}$ remains constant at 124 cm^{-1} during the "rotator" phase transition, indicating that there is no conforma-

TABLE III. Relevant physical constants of normal alkanes [45,54].

	Melting point, (°C)	Transition* point, (°C)	ΔH_f (fusion) kcal/mole	ΔH_t (transition) kcal/mole	% volume contraction at: solidification transition
n-C ₁₇ H ₃₆	21.9	10.5	9.6	2.6	10.8
n-C ₁₉ H ₄₀	32.0	22.1	10.9	3.3	10.5
n-C ₂₁ H ₄₄	40.2	32.5	11.4	3.7	10.0
					2.4
					2.5
					2.5

*Transition refers to the orthorhombic-hexagonal phase transition.

tional disorder (gauche conformers) being introduced in the hexagonal phase. However, the two systems ($n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{33}\text{H}_{68}$) are not strictly comparable; $n\text{-C}_{33}\text{H}_{68}$ shows a very complex polymorphism undergoing several solid-solid phase changes. At the same time, the "rotator" phase transition involves a larger thermal expansion for the longer member of the series [54].

In the following sections the results obtained from a study by infrared spectroscopy of the "rotator" phase transition in $n\text{-C}_{17}\text{H}_{30}\text{D}_6$, $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{21}\text{H}_{44}$ are presented. The aim has been to determine how the different intermolecular interactions and molecular motions present in the two crystalline forms affect the infrared spectra.

3.2. The infrared spectra of orthorhombic $n\text{-C}_{17}\text{H}_{30}\text{D}_6$; $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{21}\text{H}_{44}$.

The mid-infrared spectrum of normal alkanes is composed of bands arising from the following vibrational modes: carbon-hydrogen stretching; methyl bending and methylene scissoring; methylene wagging; methylene rocking and twisting and carbon-carbon stretching. The assignment of the observed bands in the spectra to particular vibrational modes has been thoroughly studied by Snyder et al [58,59,60] and in the following sections the assignment and labelling of bands given in reference [58] will be followed. The abbreviations to be used for the designation of bands are as follows:

δ , methylene scissoring; α , methyl asymmetric bending; U,

methyl symmetric bending; W, methylene wagging (W_1 is the 1th component of the band progression); T, methylene twisting (T_1 is the 1th component of the band progression); P, methylene rocking (P_1 is the 1th component of the band progression); β , methyl symmetric in-plane rocking; and R, carbon-carbon stretching (R_1 is the 1th component of the band progression).

In the orthorhombic crystals there are 4 molecules per unit cell while in the hexagonal phase there is only one. In the orthorhombic crystal there is a great deal of interchain coupling of vibrational modes due to the rigid characteristics of the packing. The infrared spectrum of hydrocarbons packed in the orthorhombic phase show vibrational correlation splitting [61,62] in the CH_2 rocking and bending modes. Factor group splitting of the C-H stretching modes has not been observed.

The comparison of the spectra of $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{21}\text{H}_{44}$ with that of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ permits confirmation of the assignment and at the same time some clarification in cases where methyl and methylene bands overlap.

3.2.1. C-H Stretching.

Fig. 12 shows the infrared spectra, in this region, of orthorhombic $n\text{-C}_{21}\text{H}_{44}$, $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{17}\text{H}_{30}\text{D}_6$. The spectra of $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{21}\text{H}_{44}$ are almost identical while that of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ differs only in that it lacks the bands arising from CH_3 vibrations. The strongest bands in the spectra in this region appear at 2918 and 2850 cm^{-1} and

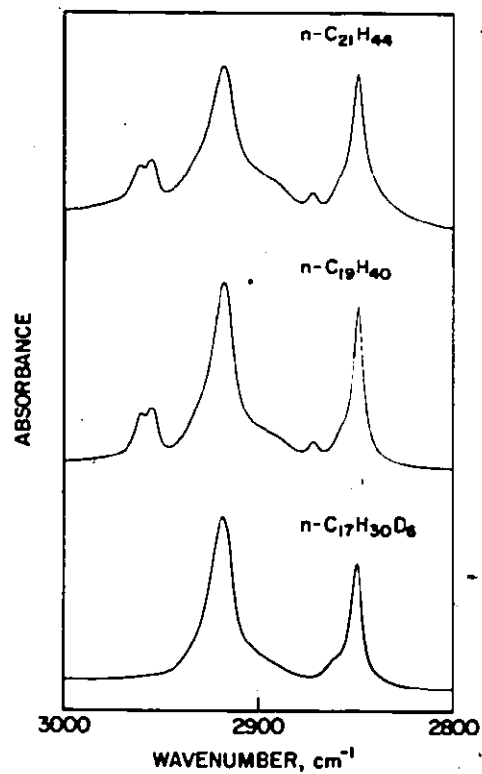


Fig. 12. Carbon-hydrogen stretching region in the infrared spectra of orthorhombic n-C₁₇H₃₀D₆; n-C₁₉H₄₀ and n-C₂₁H₄₄.

result from the antisymmetric and symmetric methylene stretching modes, respectively. The two bands at 2895 and 2856 cm^{-1} are also present in all three spectra and have been assigned to methylene stretching in resonance with binary combinations of methylene scissoring [60].

The bands at 2960 and 2955 cm^{-1} in the top two spectra in this figure result from the asymmetric methyl stretching (in-skeletal-plane and out-of-skeletal-plane, respectively). The band at 2935 cm^{-1} overlapping the antisymmetric CH_2 stretching band represents the symmetric CH_3 stretch in Fermi resonance with the first overtone of the methyl bending; the other component of the symmetric CH_3 stretching appear at 2872 cm^{-1} . The absence of these bands in the spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ confirms their assignment.

Correspondingly, the spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ in the C-D stretching region (Fig. 13) shows bands at 2223, 2212 cm^{-1} (in-plane and out-of-plane asymmetric CD_3 stretching); 2126 and 2075 cm^{-1} (the two components of the CD_3 stretch in Fermi resonance with CD_3 bending).

3.2.2. Methylene Scissoring and Methyl Bending.

All bands in the spectral region 1500 - 1350 cm^{-1} in n-alkanes arise from bending vibrations of either methylene or methyl groups. Fig. 14 shows the spectra of $n\text{-C}_{21}\text{H}_{44}$, $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ in this region; the methylene scissoring fundamentals appear between 1480 and 1460 cm^{-1} . Table IV lists the observed peaks in the spectra

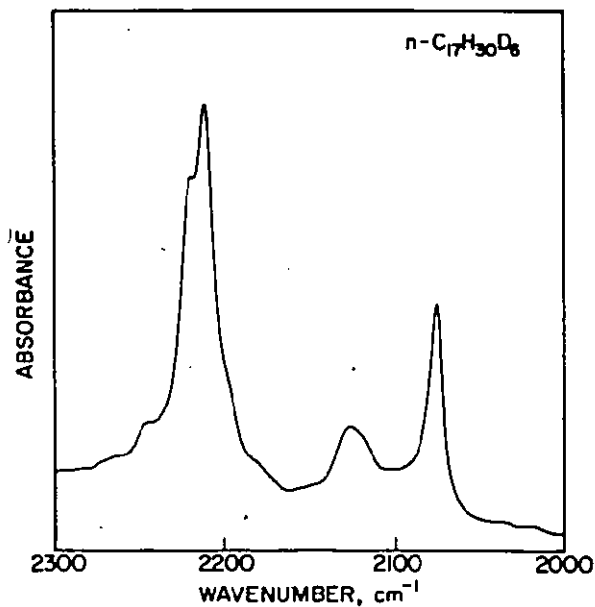


Fig. 13. Carbon-deuterium stretching region in the infrared spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$.

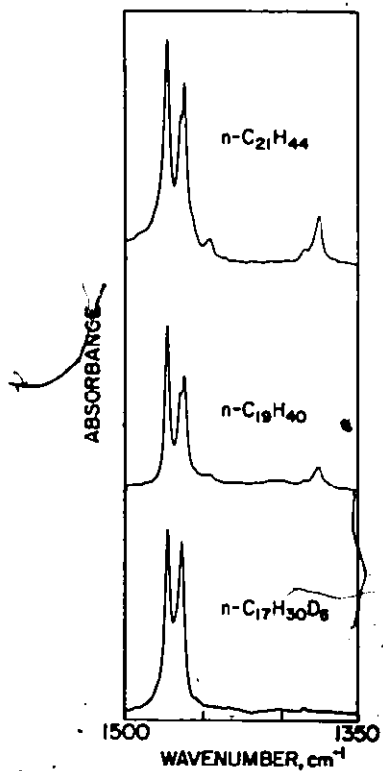


Fig. 14. Bending region (1500 - 1350 cm⁻¹) in the infrared spectra of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$; $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{21}\text{H}_{44}$.

TABLE IV. Infrared frequencies (cm^{-1}) of the methylene and methyl bending modes of $n\text{-C}_{21}\text{H}_{44}$; $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{17}\text{H}_{30}\text{D}_6$.†

	$n\text{-C}_{21}\text{H}_{44}$			$n\text{-C}_{19}\text{H}_{40}$			$n\text{-C}_{17}\text{H}_{30}\text{D}_6$	
	ORTHORHOMBIC	HEXAGONAL		ORTHORHOMBIC	HEXAGONAL		ORTHORHOMBIC	HEXAGONAL
δ	1473.6 s	1468.5 s	δ	1473.6 s	1468.5 s	δ	1471.3 s	1466.5 s
α	1465.3		α	1465.3				
δ	1462.6 s		δ	1461.8 s		δ	1463.4 s	
$\alpha+\delta$	1456.7 sh	1458.3 sh	$\alpha+\delta$	1459.8 sh	1459.8 sh	δ	1435.0 w,br	1437 w,br
	1446.0 m			1446.0 m				
	1384.2 m	1384.9 sh	U+W	1374.5	1377.2			1369.7 w,br*
U+W	1375.0 m	1379.4						
						α	1107.0 m	1105.0 m
						U	1054.0 m	1052.0 m

† The symbols s,m,w,sh represent: strong, medium, weak and shoulder respectively; br, re- presents broad.

The symbols δ , α , U and W represent methylene scissoring, methyl bending (asymmetric and symmetric) and methylene wagging respectively.

* Methylene wagging.

in the orthorhombic and hexagonal lattices. It is generally not possible to assign individual bands in this region because of severe overlap with methyl bending bands. However, comparison with the spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ permits clarification in the assignment.

The bands at 1474 and 1465 cm^{-1} in all three spectra in Fig. 14 arise from CH_2 scissoring while the band at 1462 cm^{-1} in the spectra of $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{21}\text{H}_{44}$ represents the asymmetric methyl bending which is absent in the spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$. The symmetric methyl bending in $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{21}\text{H}_{44}$ gives rise to the band at 1375 cm^{-1} ; this band is absent in the bottom spectrum of Fig. 14. In the spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$, the asymmetric and symmetric CD_3 bending bands appear at 1106 and 1054 cm^{-1} , respectively.

The assignment of the bands at 1457 and 1446 cm^{-1} ($n\text{-C}_{21}\text{H}_{44}$); 1460 and 1446 cm^{-1} ($n\text{-C}_{19}\text{H}_{40}$), and 1435 cm^{-1} ($n\text{-C}_{17}\text{H}_{30}\text{D}_6$) is not clear. The bands are thought to represent methyl bending coupled with methylene scissoring [58,59]; and the different pattern observed for $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ seems to support this argument. However, the temperature-dependent behaviour of the spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ in this region is quite similar to that of $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{21}\text{H}_{44}$ (Section 3.3.2.), suggesting that most of the intensity in the region 1460-1440 cm^{-1} must come from methylene scissoring. The assignment of the bands at about 1440 cm^{-1} to the first overtone of the methylene rocking fundamental at $\sim 720 \text{ cm}^{-1}$ is untenable due to the temperature-dependent behaviour.

Methylene wagging modes contribute to the intensity of bands in the region [58] and the band at 1384 cm^{-1} observed in all spectra in Fig. 14 can be attributed to methylene wagging.

3.2.3. Methylene Wagging.

The methylene wagging vibration gives rise to a band progression between 1360 and 1200 cm^{-1} in the spectra of n-alkanes and compounds with aliphatic chains such as fatty acids and alkyl halides.

The progression results from the wagging of n coupled oscillators in an all-trans conformation. The phase difference, Ψ , allowed between the motion of adjacent groups are given by:

$$\Psi = k / (n+1)$$

where k is an integer from 1 to n representing the number of loops in the stationary wave representing the normal mode [58,59]. The number of components in the progression as well as their frequency are specific to the length of the chain [63]. The intensity of the bands is dependent on the nature of the substituents attached to the chain. In the case of n-alkanes these bands are weak while their intensity is greatly enhanced when polar substituents are attached to the chain [63,64].

Fig. 15 shows the spectra of $n\text{-C}_{21}\text{H}_{44}$; $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ in the wagging region. Table V lists the frequencies of the CH_2 wagging bands in the spectra of the three

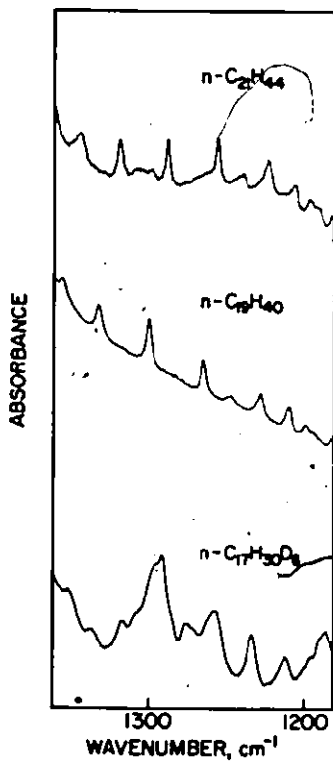


Fig. 15. Wagging region (1360 - 1180 cm⁻¹) in the infrared spectra of n-C₁₇H₃₀D₆; n-C₁₉H₄₀ and n-C₂₁H₄₄.

TABLE V. Infrared frequencies (cm^{-1}) of the methylene wagging band progression of $n\text{-C}_{21}\text{H}_{44}$; $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{17}\text{H}_{30}\text{D}_6$.*

	ORTHORHOMBIC	HEXAGONAL
$n\text{-C}_{21}\text{H}_{44}$	W_1 1196.0 w W_2 1204.6 w W_3 1222.0 w W_4 1238.0 vw W_5 1254.2 w W_6 ~1268 vw W_7 1286.3 w W_8 ~1305 vw W_9 1317.0 w W_{11} ~1342 vw W_{12} ~1362 vw	W_1 1199.2 vw W_2 1207.0 w W_3 1224.6 w W_4 - W_5 1256.4 w W_6 ~1270 vw W_7 1288.4 w W_8 - W_9 1319.6 w W_{11} 1343 vw W_{12} 1362 vw,sh
$n\text{-C}_{19}\text{H}_{40}$	W_1 1196.5 vw W_2 1207.5 w W_3 1228.7 w W_4 1245.5 w W_5 1262.8 vw W_6 ~1280.0 vw W_7 1298.5 W_8 ~1317 vw W_9 ~1330 vw	W_1 ~1198.6 vw W_2 1209.5 w W_3 1230.4 w W_4 1246.9 w W_5 1264.2 vw W_6 ~1281.0 vw W_7 1299.2 w W_8 - W_9 -
$n\text{-C}_{17}\text{H}_{30}\text{D}_6$	W_1 ~1195 vw W_2 1211.0 vw W_3 1233.3 w W_4 1256.1 W_5 1275.5 vw W_7 1316 vw	W_1 ~1195 vw,br,sh W_2 1212.3 vw W_3 1234.1 w W_4 1257.3 w W_5 1276.1 w W_7 ~1316 vw,sh

* The symbols w,v, br and sh. represent weak, very, broad and shoulder, respectively.

compounds in the orthorhombic and hexagonal phases, the progression bands are labelled $W_1, W_2 \dots$ following reference [58].

3.2.4. Methylene Rocking and Twisting.

In the frequency region $1100-700 \text{ cm}^{-1}$ the spectra of n-alkanes show a series of fundamentals; they are methylene rocking-twisting modes [58]. The band at 720 cm^{-1} is the head of the observed band progression, these bands show correlation splitting when the hydrocarbons are packed in an orthorhombic subcell [62]. While the head band is strong, higher frequency components show much weaker bands. The head band is almost purely CH_2 rocking in character while the contribution of CH_2 twisting to the band intensity increases for the high frequency components. The spectra of the three investigated hydrocarbons in the region $1190-680 \text{ cm}^{-1}$ are shown in Fig. 16 and the observed frequencies in the orthorhombic and hexagonal lattices are listed in Table VI.

Methylene twisting modes appear in the $1300-1170 \text{ cm}^{-1}$ region, Fig. 15; the observed frequencies are listed in Table VII. These bands are very weak and are obscured because of overlap with methylene wagging bands also appearing in this frequency region.

The methyl symmetric in-plane rocking mode (β) gives rise to two bands at 890 and 892 cm^{-1} in the spectrum of orthorhombic $\text{n-C}_{19}\text{H}_{40}$, and at 892 cm^{-1} in the spectrum of orthorhombic $\text{n-C}_{21}\text{H}_{44}$. The methyl rocking mode for $\text{n-C}_{17}\text{H}_{30}\text{D}_6$

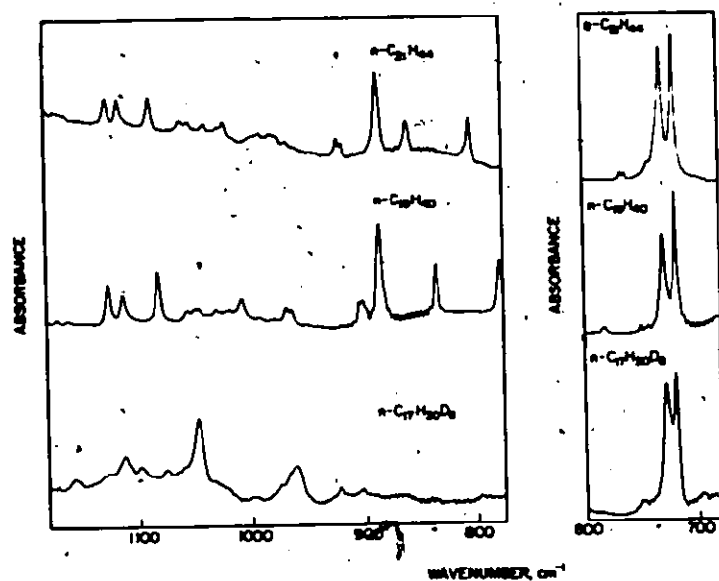


Fig. 16. Rocking region (1190 - 680 cm⁻¹) in the infrared spectra of n-C₁₇H₃₀D₆; n-C₁₉H₄₀ and n-C₂₁H₄₄.

TABLE VI. Infrared frequencies (cm^{-1}) of the methylene rocking band progression and methyl rocking mode of $n\text{-C}_{21}\text{H}_{44}$ and $n\text{-C}_{19}\text{H}_{40}$.*

		ORTHORHOMBIC	HEXAGONAL
$n\text{-C}_{21}\text{H}_{44}$	P_1	719.8 s 730.8 s	721.2 s
	P_7	743.0 m	~740.0 sh
	P_9	762.4 m 766.3 m	764.3 w
	P_{11}	808.5 m	808.3 m
	P_{13}	862. w, sh 865 m	864.3 m
	P_{15}	923.8 w 927.9 m	924.7 m
	P_{17}	980 w, ?† 986 w, ?†	- -
$n\text{-C}_{19}\text{H}_{40}$	P_1	719.2 s 729.6 s	719.5 s
	P_7	743.8 m 749.5 m 783.5 m	744.9 m
	P_9	781.9 m 779.0 w	782.2 w
	P_{11}	841.5 m	839.2 m
	P_{13}	905.6 w 908.0 w	904.9 m
	P_{15}	969.8 w 973.8 w	970 w
	P_{17}	~1029 vw	~1030 vw

TABLE VI (cont.)

		ORTHORHOMBIC	HEXAGONAL
n-C ₂₁ H ₄₄	β	892.4 m,br	890.2 m
n-C ₁₉ H ₄₀	β	891.8 m 889.	888.8 m

* The symbols s,m,v,w,br,sh represent strong, medium, very, weak, broad and shoulder respectively.

† Overlap with C-C stretching.

TABLE VII. Infrared frequencies (cm^{-1}) of the methylene twisting bands of n-C₂₁H₄₄ and n-C₁₉H₄₀.*

		ORTHORHOMBIC	HEXAGONAL
n-C ₂₁ H ₄₄	T ₁₉	1178 vw	-
	T ₂₀	1172 vw	1175 vw
n-C ₁₉ H ₄₀	T ₁₆	1286 vw	1286 vw
	T ₁₇	1180.8 w	1180.8 w
	T ₁₈	1171.6 w	1172 vw

* The symbols v and w represent very and weak respectively.

appears in a spectral region below 600 cm^{-1} which was not recorded.

The methyl rocking coordinate contributes to all of the bands in the methylene rocking and twisting series and it also contributes to the intensity of the terminal C-C stretch [58].

3.2.5. Carbon-carbon stretching.

The stretching of C-C bonds gives rise to several bands in the region $1150\text{--}950\text{ cm}^{-1}$ shown in Fig. 16. The first components of the series are the strongest due to coupling with methyl symmetric in-plane rocking. The rest of the bands are extremely weak, their frequencies are listed in Table VIII.

3.3. Effects of the "Rotator" Phase Transition on the Infra-red Spectra.

3.3.1. C-H Stretching.

The thermotropic behaviour of the C-H stretching bands has been investigated for all three hydrocarbons. The results obtained from the spectra of $n\text{-C}_{21}\text{H}_{44}$ will be presented in detail; those obtained from the spectra of $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ are almost identical.

Fig. 17 shows the C-H stretching region of the spectrum of $n\text{-C}_{21}\text{H}_{44}$ at 1, 36, and 48°C , representing the orthorhombic and hexagonal lattices and the melt, respectively.

TABLE VIII. Infrared frequencies of the C-C stretching modes in $n\text{-C}_{21}\text{H}_{44}$; $n\text{-C}_{19}\text{H}_{40}$ and $n\text{-C}_{17}\text{H}_{30}\text{D}_6$.*

	ORTHORHOMBIC	HEXAGONAL
$n\text{-C}_{21}\text{H}_{44}$	R_1 1134.8 m R_2 1124.1 m R_3 1094.5 m R_4 1067.0 w R_{13} 1046 w R_{12} 1034 sh R_5 1028 w R_{11} 1023 w,sh R_{10}, R_6 998 vw R_9 986 vw R_7 975 vw	R_1 1133.5 m R_2 1123.0 m R_3 1094.0 m R_4 1066.4 w R_{13} ~1045 vw R_{12} 1035 vw R_5 ~1028 w R_{11} - R_{10}, R_6 - R_9 - R_7 -
$n\text{-C}_{19}\text{H}_{40}$	R_1 1134.2 m R_2 1121 vw R_3 1088.6 m R_{14} 1057 vw R_{13} 1054 vw,sh R_{12} 1044 vw R_{11} 1037 vw R_{10} 1024 vw R_9 1000 vw R_9 997 vw R_6 988 vw R_8 984 vw	R_1 1132.6 m R_2 ~1119 vw R_3 1087.3 m R_{14} 1055 w,br R_{13} ~1044 vw R_{12} ~1038 vw R_{11} ~1026 vw R_{10} ~ 998 vw R_9 - R_6 - R_8 -
$n\text{-C}_{17}\text{H}_{30}\text{D}_6$	R_1 1136.0 w R_2 1120.5 m R_3 1083.5 m	R_1 1136.0 w R_2 1119.5 m R_3 1082.5 m

* The symbols m,w,v,sh, and br represent medium, weak, very, shoulder and broad respectively.

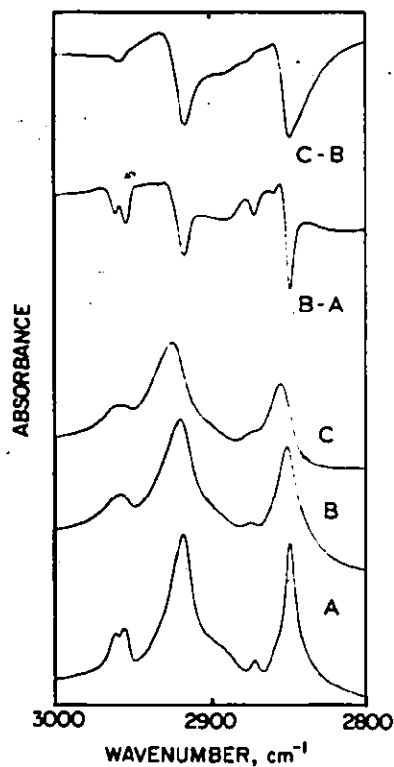


Fig. 17. Carbon-hydrogen stretching region in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$.
Bottom: A. orthorhombic; B. hexagonal and C. melt.
Top: Corresponding difference spectra.

The top traces in Fig.17 show the corresponding difference spectra obtained as described in section 2.3.3.; changes in all bands are evident.

The temperature dependence (in the 0 to 60°C range) of the antisymmetric methylene stretching band (position, halfwidth and integrated intensity) is shown in Fig. 18. The "rotator" phase transition in $n\text{-C}_{21}\text{H}_{44}$ occurs at 32.5°C.

The peak positions (Fig. 18A) are invariant at all temperatures when the hydrocarbon is solid. There is no detectable shift in frequency upon the change from orthorhombic to hexagonal packing. On melting (40.2°C), there is a 6 cm^{-1} shift to higher frequency. The integrated intensity (Fig. 18C) shows a steady decrease with increasing temperature throughout the temperatures representative of the orthorhombic crystal. On transition to the hexagonal form there is a large decrease in intensity, comparable with the decrease observed on melting. The halfwidth of this band (Fig. 18B) remains constant throughout the orthorhombic phase, but undergoes a steady increase at the temperature of the solid-solid phase transition with a further increase at the melting.

The symmetric methylene stretching mode in the spectra of the three compounds showed behaviour identical with that of the antisymmetric mode.

The constancy in frequency of the methylene stretching modes indicates that in both solid phases (orthorhombic and hexagonal) the chains are in the all-trans conformation. The C-H stretching modes in hydrocarbons are essentially de-

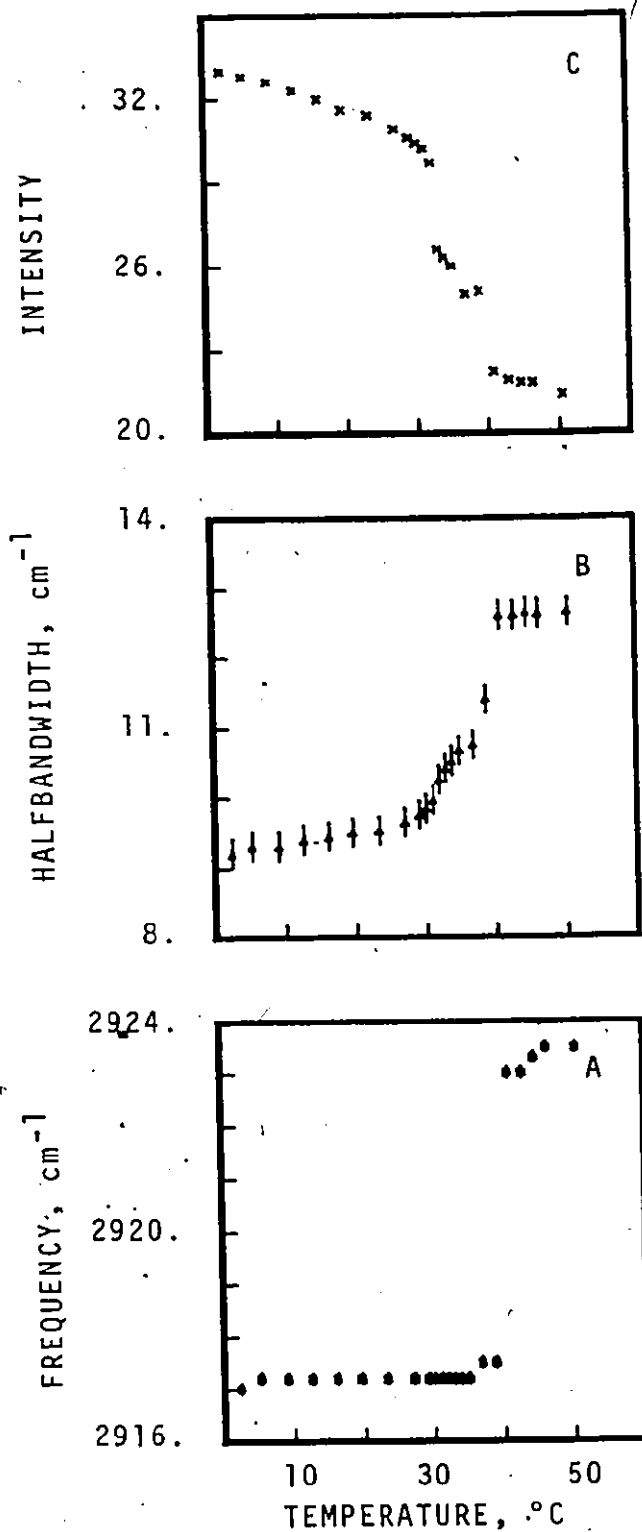


Fig. 18. Temperature dependence of the CH₂ antisymmetric stretching mode in the spectrum of n-C₂₁H₄₄ as: A. frequency; B. halfbandwidth and C. integrated intensity.

coupled from all other vibrations; they shift in frequency as a consequence of conformational changes, but are almost insensitive to other effects [65,66]. The shift to higher frequencies on melting is typical for these compounds [66] and reflects the introduction of conformational disorder in the chains.

The increase in motional freedom of the alkane molecules on transition from the highly rigid orthorhombic lattice to the less rigid hexagonal lattice is reflected by the increase in bandwidth. This is a general phenomenon in vibrational spectroscopy. The linear increase in bandwidth with increasing temperature throughout the hexagonal phase indicates an increase in the rate of longitudinal molecular rotations. The broadening of bands in the vibrational spectra of polyatomic molecules in condensed phases has been treated theoretically as arising from intramolecular anharmonic coupling of high frequency modes to low frequency modes undergoing energy exchange with the bath. The intermolecular energy exchange is temperature-dependent and the resulting frequency modulation will lead to a temperature-dependent band broadening [67]. Thus, the increase in bandwidth on transition from an orthorhombic to a hexagonal lattice could reflect a more efficient energy exchange in the latter case. However, band broadening arises from many contributions and no clear analysis is available [68].

The peak positions of the in-plane and out-of-plane components of the asymmetric CH_3 stretching are temperature

dependent as shown in Fig. 19 for the case of $n\text{-C}_{21}\text{H}_{44}$. At temperatures corresponding to the orthorhombic crystal the two components are observed at 2959 and 2954 cm^{-1} respectively. These two components collapse at the solid-solid phase transition and only one band is observed at 2956 cm^{-1} , corresponding to the center of the two bands at low temperatures. The band remains constant in frequency in the hexagonal phase and increases slightly ($\sim 1 \text{ cm}^{-1}$) upon melting. Similar effects are seen for the CD_3 asymmetric stretch in the spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$.

The collapse of the in-plane and out-of-plane components of the asymmetric methyl stretching has been observed previously for hexadecane in an urea clathrate and in a neat sample of eicosane [69]. The splitting of the methyl asymmetric mode is due to intramolecular effects; it is related to the rate of rotation of the methyl group relative to the adjacent methylene group. If the rate of rotation of the methyl group is sufficiently slow, two bands are observed. An increase in the rate of rotation results in the collapse of the band contour into a single peak, the rate of rotation at this point being of the order of 10^{12} s^{-1} .

The fact that the bands collapse abruptly on transition to the hexagonal structure, indirectly reflects the reduced intermolecular interactions in this phase as compared to the orthorhombic. Reduced intermolecular interactions will produce larger interchain distances allowing the methyl groups to rotate more freely.

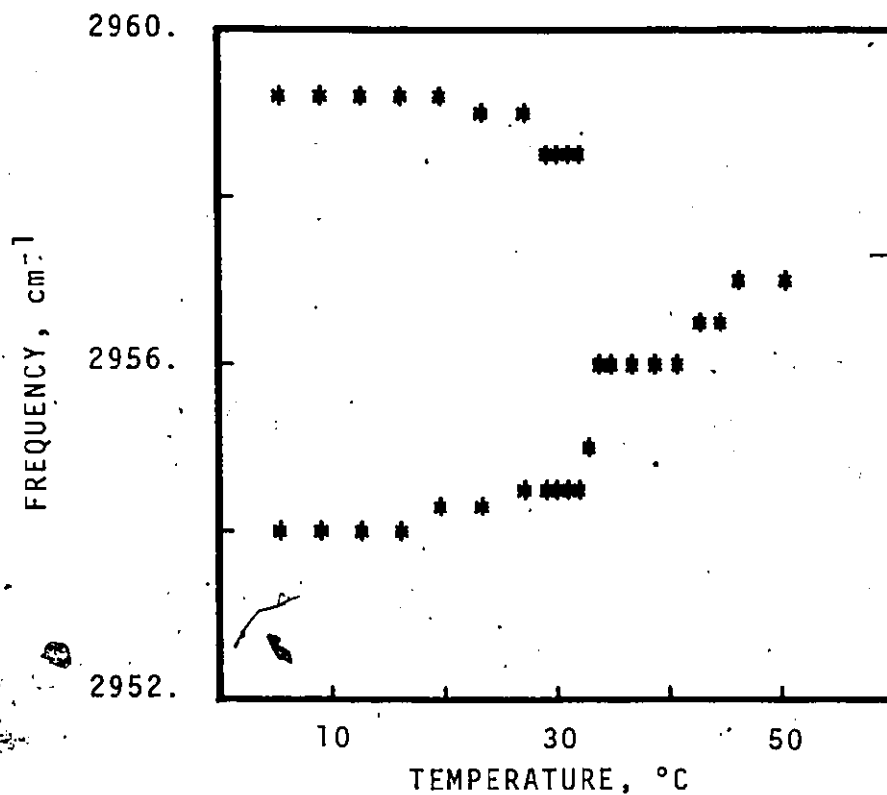


Fig. 19. Temperature dependence of the positions of the two components of the CH_3 asymmetric stretching mode in the spectrum of $n\text{-C}_{21}\text{H}_{44}$.

The components of the symmetric methyl stretching mode are heavily obscured by overlap with methylene stretching bands (Section 3.2.1.). They have been studied as a function of temperature in the spectra of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$. Their peak positions and halfbandwidth vary as shown in Table IX.

The symmetric methyl stretching band is split because of Fermi-resonance interaction with the first overtone of the methyl symmetric bending mode. The temperature-dependent effects observed reflect both changes in the crystal packing as well as in the Fermi-resonance interaction. It is not possible to assign these effects to any particular cause.

3.3.2. Methylene Scissoring and Methyl Bending.

The frequencies of the bending modes for the three compounds in the orthorhombic and hexagonal phases are listed in Table IV. Fig. 20 shows the bending region of the spectrum of $n\text{-C}_{21}\text{H}_{44}$ at three temperatures, 1, 36 and 48°C , which represent the orthorhombic and hexagonal crystal structures and the melt, respectively. The top of Fig. 20 shows the two corresponding difference spectra generated as described in Section 2.3.3.

The principal change on transition from orthorhombic to hexagonal is the removal of band splitting due to vibrational coupling. The two components of the methylene scissoring mode at 1473.6 and 1462.6 cm^{-1} collapse to one at 1468.5 cm^{-1}

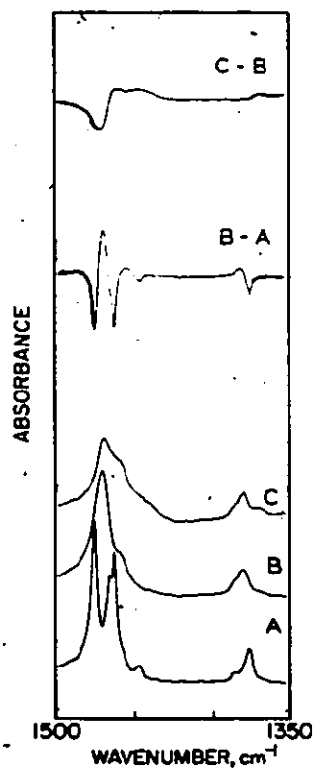


Fig. 20. Bending region in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$.
Bottom: A. orthorhombic; B. hexagonal and C. melt.
Top: Corresponding difference spectra.

TABLE IX. Temperature dependence of the two components of the methyl symmetric stretching mode in the spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$.

ORTHORHOMBIC		HEXAGONAL		MELT	
ν	$\Delta\nu_{\frac{1}{2}}$	ν	$\Delta\nu_{\frac{1}{2}}$	ν	$\Delta\nu_{\frac{1}{2}}$
2125.8	20.5	2124.2	15.1	2125.0	14.4
2075.0	10.3	2076.2	14.4	2076.5	16.0

* ν , band position in cm^{-1} ; $\Delta\nu_{\frac{1}{2}}$, halfbandwidth in cm^{-1} .

There is an apparent shift to higher frequency of the two bands at 1384.2 cm^{-1} and 1375.6 cm^{-1} (methyl symmetric bending and methylene wagging) readily seen in the difference spectrum B - A. Since there are two bands overlapping this shift cannot be analyzed unequivocally, most probably the derivative shaped difference spectrum is due to a shift to higher frequency of the wagging band along with a shift to lower frequency of the methyl bending band. The wagging bands shift to higher frequency (vide infra) and the CD_3 symmetric bending band in $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ shifts to lower frequency during this transition (Table IV).

The asymmetric methyl bending at 1465.3 cm^{-1} in the orthorhombic phase is overlapped in the spectrum of the hexagonal phase. The corresponding CD_3 asymmetric bending in the spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ shifts from 1107.0 to 1105.0 cm^{-1} (Table IV).

On transition from orthorhombic to hexagonal there is an increase in intensity in the region $1460 - 1440\text{ cm}^{-1}$. The bands at 1456.7 and 1446.0 cm^{-1} in the orthorhombic phase of $n\text{-C}_{21}\text{H}_{44}$ are not uniquely assigned; they probably represent methyl bending coupled with methylene bending. They are not totally absent in the spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ (Fig. 14). The gain in intensity in the region could arise from a shift to higher frequency of the 1446 cm^{-1} band, but the gain in intensity is also observed in the spectra of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ (not shown) where this band is not present. When the hydrocarbon melts there is a further increase in intensi-

ty in this region due to the bending of methylene groups in chains with gauche conformers [70].

3.3.3. Methylene Wagging.

As noted in Section 3.2.3. the methylene wagging gives rise to a band progression in the region $1360 - 1200 \text{ cm}^{-1}$. The frequencies of the observed band progression in the spectra of the three compounds in the orthorhombic and hexagonal phases are listed in Table V. Fig. 21 shows the $1360 - 1180 \text{ cm}^{-1}$ region of the spectrum of $n\text{-C}_{21}\text{H}_{44}$ at 1, 36, and 48°C (orthorhombic, hexagonal and melt, respectively).

The characteristics of the CH_2 wagging bands were measured in the spectra of the three compounds. The peak positions are listed in Table V. On transition to the hexagonal crystal the bands shift approximately 2 cm^{-1} to higher frequencies; the components of the progression with low k values are affected more than those with high k values.

Fig. 22A shows a plot of the halfbandwidth of W_3 (1222 cm^{-1}) in $n\text{-C}_{21}\text{H}_{44}$ versus temperature. The width remains constant throughout the temperature range of the orthorhombic crystal. On transition to hexagonal there is a slight broadening. This behaviour for W_3 was exhibited for all measured wagging bands in the spectra of the three compounds.

Fig. 22B shows a plot of the integrated intensity of the W_3 band versus temperature. The transition to the hexagonal results in a large decrease in intensity. The same

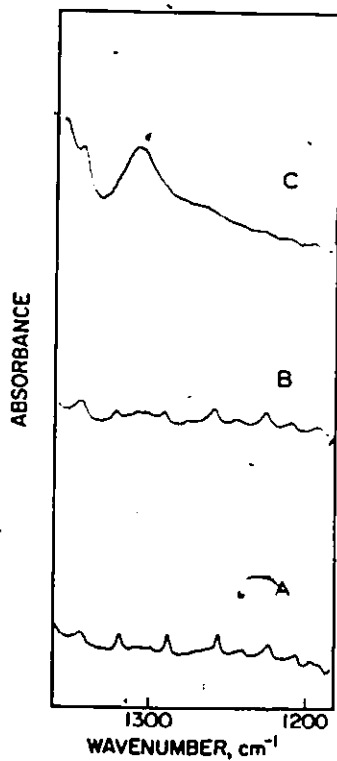


Fig. 21. Wagging region in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$; A. orthorhombic; B. hexagonal and C. melt.

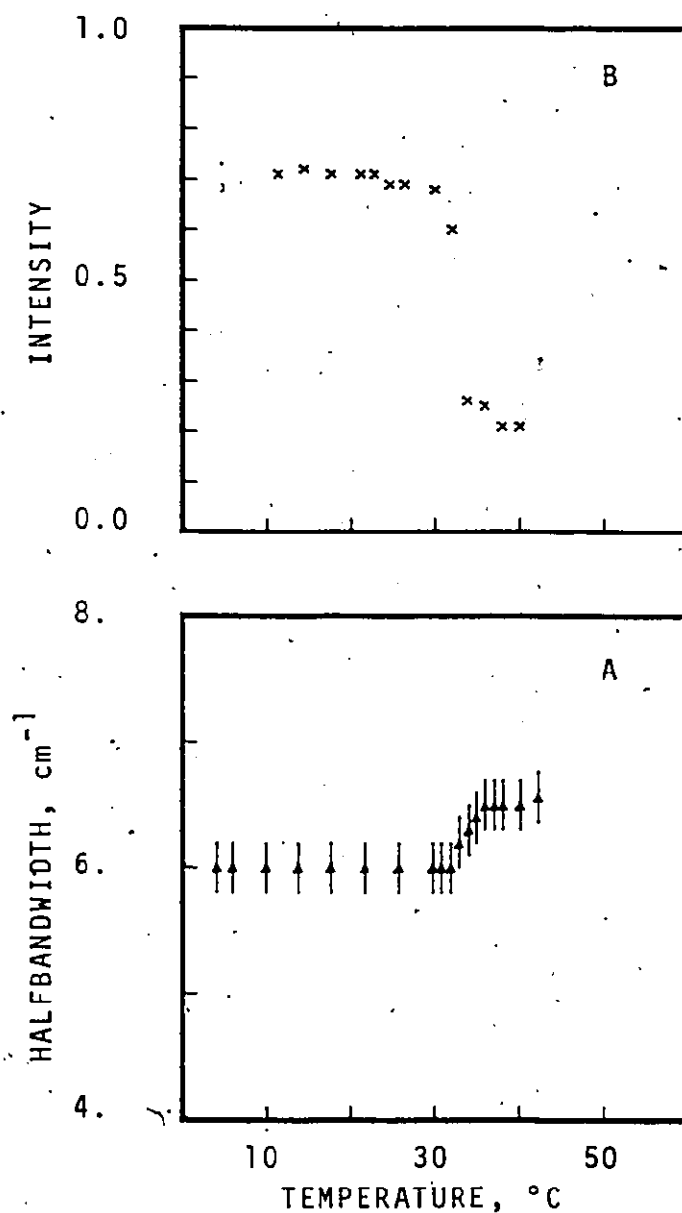


Fig. 22. Temperature dependence of the $k=2$ component of the methylene wagging band progression in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$ as: A. halfbandwidth and B. integrated intensity.

was observed for all wagging bands. At the melting temperature the wagging band progression disappears.

The spectrum of liquid $n\text{-C}_{21}\text{H}_{44}$ (Figs. 20 and 21) contains bands at 1378, 1300 and 1080 cm^{-1} and weaker bands at 1370, 1352 and 1340 cm^{-1} . These bands are due to the wagging vibrations of methylene groups adjoining gauche conformers [70].

In Fig. 21, there is an apparent increase in intensity at about 1350 and 1300 cm^{-1} with increasing temperature; intensity in this region arises from methylene wagging in non-planar chains.

The shift to higher frequencies of the wagging band during the "rotator" phase transition can be interpreted as arising from the differences in intermolecular interactions in the two solid phases. Wagging bands are not split because of interchain coupling in the orthorhombic phase, but, as shown here, their frequency is dependent on the type of crystal structure.

The introduction of gauche conformers into the hydrocarbon chains should produce shifts to lower frequency of the wagging bands [70]. The observation of shifts to higher frequency indicates that the molecular conformation is not changing upon transition. However, the increase in intensity at 1450, 1350 and 1300 cm^{-1} can be interpreted as arising from the introduction of non-planar conformations. In the case of $n\text{-C}_{33}\text{H}_{68}$ [56] this increase in intensity was correlated with small angle X-ray scattering measurements which showed a shortening of the chains in the hexagonal phase; a value of

0.72 defects per chain was determined. The buildup in infrared intensity was interpreted as confirming the introduction of defects. Since the behaviour of most bands in the infrared spectra (vide infra) and in the Raman spectra [57] indicates conservation of the all-trans conformation; the buildup of intensity in the infrared spectra can be interpreted as arising from chain imperfections originating from torsional motions about the long axes of the molecules. The thermal expansion during the "rotator" phase transition is larger for the longer member of the series than for the shorter ones [45,54]. The longer chains might be able to perform torsions of greater amplitude giving rise to the chain shortening effects observed in $n\text{-C}_{33}\text{H}_{68}$.

3.3.4. Methylene Rocking and Twisting.

The components of the methylene rocking band progression are split in the orthorhombic phase due to vibrational correlation. The principal effect of the "rotator" phase transition is to eliminate the splitting because there is no vibrational coupling the hexagonal phase. This is clearly seen in Fig. 23 which shows the spectrum of $n\text{-C}_{21}\text{H}_{44}$ at 1, 36 and 48°C. Table VI lists the observed frequencies for the rocking bands in the orthorhombic and hexagonal phases.

The head band in the spectrum of $n\text{-C}_{21}\text{H}_{44}$ appears at 719.8 and 730.8 cm^{-1} (P_1) in the orthorhombic phase; these values remain constant throughout the orthorhombic phase. In the hexagonal phase the band appear at 721.2 cm^{-1} and re-

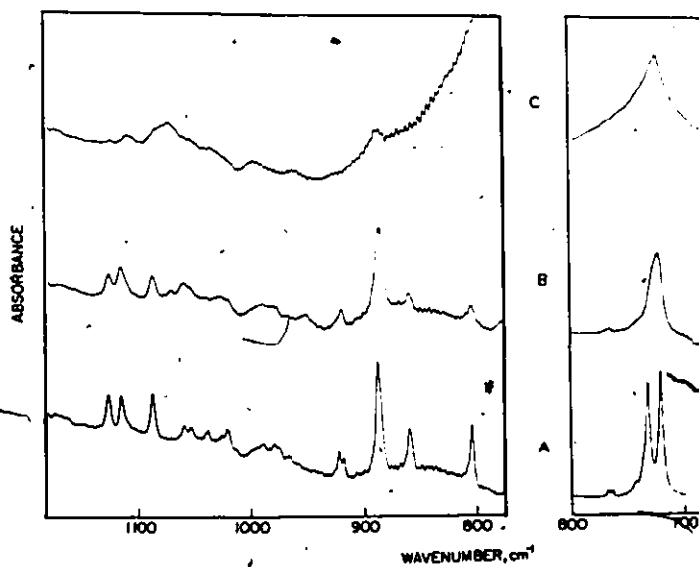


Fig. 23. Rocking region ($1190 - 680 \text{ cm}^{-1}$) of the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$; A. orthorhombic; B. hexagonal and C. melt.

mains constant in frequency up to the melting point.

In both compounds ($n\text{-C}_{21}\text{H}_{44}$ and $n\text{-C}_{19}\text{H}_{40}$) the P_{11} mode gives rise to only one band because it is at this point where the value of the correlation splitting is equal to zero [62]. The intensity of this band drops by ~50 % during the "rotator" phase transition; upon melting it becomes extremely weak and it is not possible to measure its intensity.

Fig. 24 shows the intensity of P_{11} in the spectrum of $n\text{-C}_{21}\text{H}_{44}$ versus temperature. All other bands in the rocking band progression show a similar behaviour during this transition.

The methyl symmetric in-plane rocking (β) shifts to lower frequency during the "rotator" transition (Table VI). There is a drop in its intensity as shown in Fig. 25, comparable to that observed for methylene rocking P_{11} (Fig. 24). At the melting point there is a further decrease in intensity of about 50 %.

The bands of the rocking band progression are sensitive to conformational changes that result in non-planar chains [70]. These changes in conformation should produce shifts in frequency. The frequency of the rocking bands remains constant throughout the hexagonal phase indicating that there are no conformational changes or that they are very small.

The methyl symmetric in-plane rocking mode has been found to be insensitive to conformational changes [70]. However, changes in intermolecular interactions such as those occurring during the "rotator" transition have marked effects

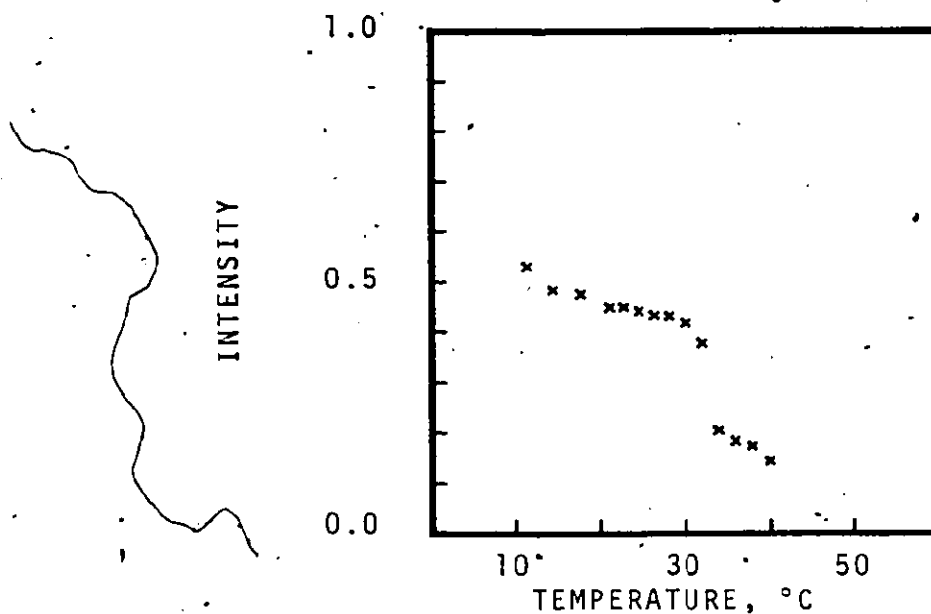


Fig. 24. Temperature dependence of the intensity of the P_{11} component of the methylene rocking band progression in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$.

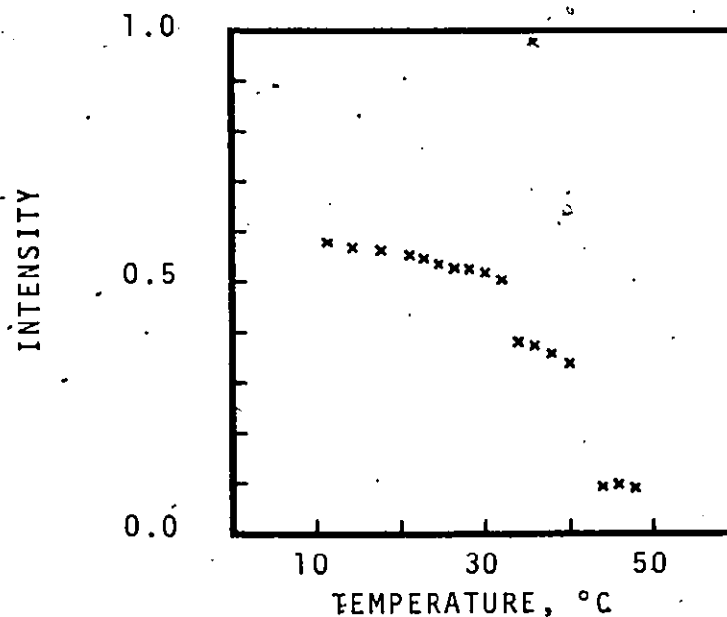


Fig. 25. Temperature dependence of the intensity of the methyl symmetric in-plane rocking band (β) in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$.

on its intensity and frequency.

Methylene twisting bands are extremely weak. They are not split due to intermolecular effects. Table VII lists the observed bands in the spectra of $n\text{-C}_{21}\text{H}_{44}$ and $n\text{-C}_{19}\text{H}_{40}$ in the orthorhombic and hexagonal phases. It can be seen that their frequency is insensitive to the "rotator" phase transition. The intensity of these bands, however, decreases sharply during the transition (Figs. 21 and 23). Quantitative measurements were not possible due to overlap with other bands and their behaviour is affected by the behaviour of the overlapping bands.

3.3.5. Carbon-carbon Stretching.

Fig. 23 shows the region where the C-C stretching bands appear. Table VIII lists the observed frequencies of the C-C stretching bands. In general, there is a shift to lower frequency of the strong components.

Fig. 26 shows a plot of the intensity of the R_3 component of the C-C stretching progression in the spectrum of $n\text{-C}_{21}\text{H}_{44}$ versus temperature. It shows a drop of 50 % of the band intensity during the "rotator" phase transition. As the intensity of this band is due not only to carbon-carbon stretching but also due to methyl symmetric rocking, the drop in intensity could reflect the similar drop observed for the methyl mode (Fig. 25). However, the R_3 band in the spectrum of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ (where this coupling is not possible) shows a comparable temperature-dependent behaviour. The coupling with methyl rocking does not affect the dependence of the band cha-

racteristics on the crystal structure.

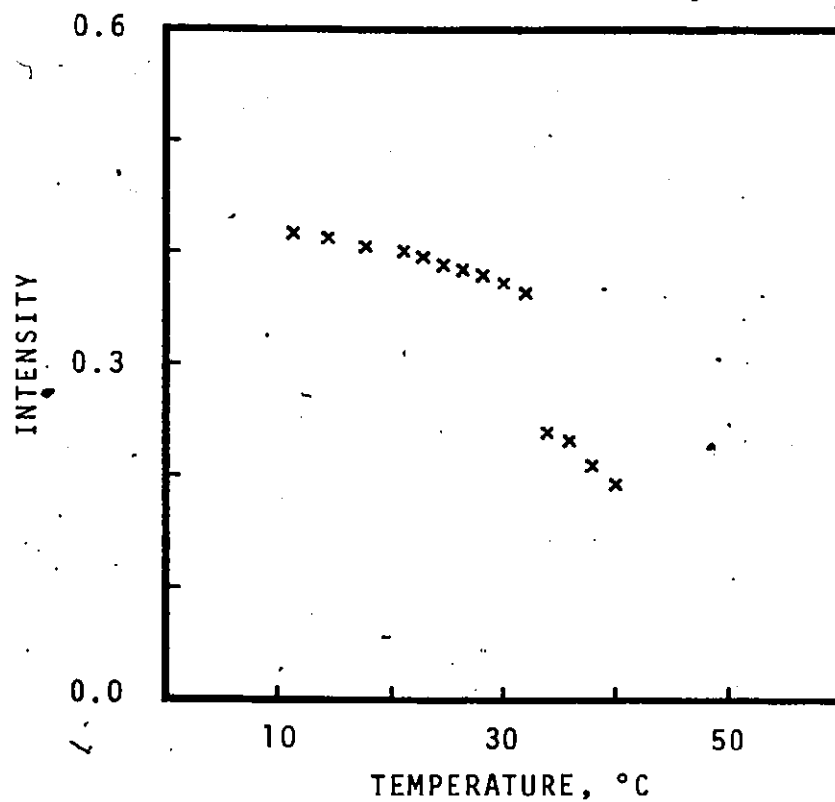


Fig. 26. Temperature dependence of the intensity of the R_3 component of the C-C stretching band progression in the infrared spectrum of $n\text{-C}_{21}\text{H}_{44}$.

CHAPTER 4. 1,2-DIPALMITOYL-*sn*-GLYCERO-3-PHOSPHOCHOLINE.

4.1. The Infrared Spectrum of DPPC.

As noted in Section 1.3.1., phosphatidylcholines have been extensively studied as models for bio-membranes. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) is probably the most widely studied compound in this class. The chemical structure is shown in Fig. 27. In order to use infrared spectroscopy to study the dynamical aspects of bilayers formed by this compound it is necessary to assign all or most of the bands in its spectrum. Fig. 28. shows the mid-IR spectrum taken as a polycrystalline solid.

The infrared spectrum of DPPC has been previously reported [31] and studies of its temperature dependence have been made [37]. However, few bands were considered in these reports. Comparison with the spectra of its deuteriated derivatives permits a clearer assignment. The use of state of the art infrared instrumentation, yielding spectra with high signal-to-noise ratios, permits the observation of very weak bands which were overlooked in the early reports.

The following deuteriated derivatives of DPPC were used in order to aid in the assignment: DPPC-d₆₂; DPPC-16',16"-d₆ and DPPC-d₉. The assignment is discussed in terms of the bands in the spectrum of DPPC and no assignment is made of the spectra of the deuteriated derivatives. However, the assignment for the deuteriated compounds can be readily es-

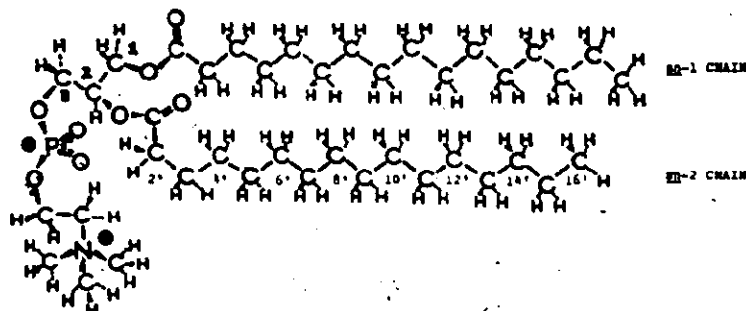


Fig 27. Chemical structure of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC).

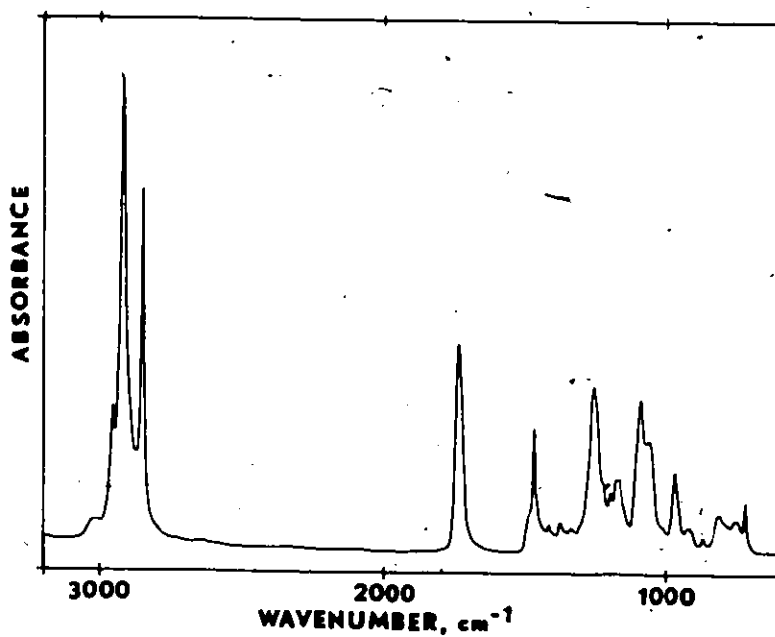


Fig. 28. Infrared spectrum of solid DPPC.

established by reference to the final assignments for the undeuteriated compound.

4.1.1. C-H Stretching.

Fig. 29 shows the infrared spectrum of DPPC and its deuteriated analogs in the C-H stretching region taken as polycrystalline solids. Table X lists the observed frequencies in this region for DPPC and its derivatives from spectra of polycrystalline solids and CDCl_3 solutions.

The spectrum of DPPC shows five resolved bands with a weak underlying band at 2895 cm^{-1} . The assignment of these bands is straightforward. The vibrations from the palmitoyl chains in the molecule dominate this spectral region and therefore the assignment follows from that of hydrocarbons given in Section 3.2.1. Furthermore, the assignment is confirmed by the disappearance of bands in the spectra of the deuteriated derivatives (Fig. 29). The two strong bands at 2917 and 2850 cm^{-1} are respectively the antisymmetric and symmetric methylene stretching modes of the palmitoyl chains. The asymmetric and symmetric modes of the terminal methyl groups appear at 2956 and 2872 cm^{-1} , respectively.

The band at 3030 cm^{-1} in the spectrum of solid DPPC corresponds to the asymmetric stretch of the choline methyl groups. It is not present in the spectrum of DPPC- d_9 (Fig. 29B).

As in the case of the C-H stretching region in the spectra of alkanes the underlying bands at 2895 and 2856 cm^{-1}

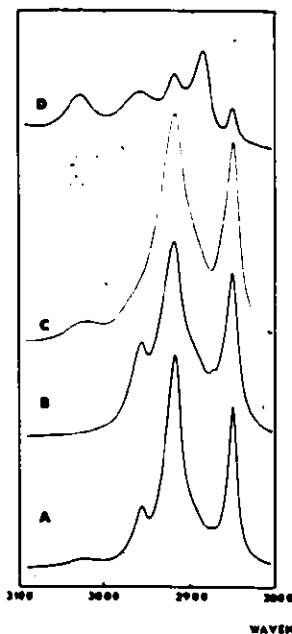


Fig. 29. Carbon-hydrogen stretching region in the infrared spectrum of DPPC and derivatives (solid samples). A. DPPC; B. DPPC-d₉; C. DPPC-16',16"-d₆ and D. DPPC-d₆₂.

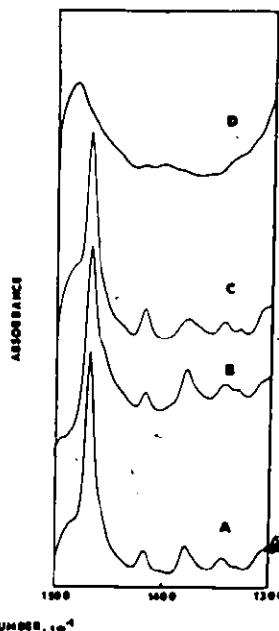


Fig. 30. Bending region (1500-1300 cm⁻¹) in the spectra of DPPC and derivatives (solid samples). A. DPPC; B. DPPC-d₉; C. DPPC-16',16"-d₆ and D. DPPC-d₆₂.

TABLE X. Observed frequencies (cm^{-1}) in the infrared spectra of DPPC and deuteriated derivatives as polycrystalline solids and CDCl_3 solutions: C-H stretching region.

DPPC		DPPC-d ₆₂		DPPC-16', 16"-d ₆		DPPC-d ₉		Assignment.
Solid	Solution	Solid	Solution	Solid	Solution	Solid	Solution	
3030		3030	3023	3023	3027			$\text{N}^+(\text{CH}_3)_3$ stretch. asym.
		2960	2960					$\text{N}^+(\text{CH}_3)_3$ stretch. sym.
2956	2956					2957	2955	CH_3 asym. stretch.
2917	2927	2918	2928	2917	2926	2918	2927	CH_2 antisym. stretch.
2900				2900		2900		CHD stretch.
2872	2872	2887	2890			2873	2871	CH_3 sym. stretch.
2850	2856	2850	2855	2850	2855	2850	2855	CH_2 sym. stretch.

represent methylene stretching in resonance with binary combinations of methylene scissoring [60].

There are some other bands to be expected in the C-H stretching region. These are, the symmetric stretch of the choline methyl groups; modes arising from the $(\text{PO}_4)\text{-CH}_2\text{-CH}_2\text{-N}^+$ moiety, and the glycerol C-H stretching modes. Some of these can be detected by comparison with the spectra of the deuteriated compounds.

In the spectrum of DPPC- d_{62} (Fig. 29D) the band at 2958 cm^{-1} can be assigned to the choline methyl symmetric stretch; it disappears on hydration, behaviour characteristic of the stretching modes of the choline group. The strong band in the spectrum of DPPC- d_{62} at 2887 cm^{-1} is assigned to stretching of palmitoyl CHD groups arising from incomplete deuteration. This assignment is supported by the fact that the band broadens and shifts to higher frequency when the compound is dissolved in CDCl_3 . Acyl chain methylene stretching bands broaden and shift to higher frequency when the compounds melt (Section 3.3.1.) or are in solution [66]. The bands at 2850 and 2918 cm^{-1} arise from the stretching of non-deuteriated methylene groups in the palmitoyl chains; they show the same expected behaviour on going into solution.

There is an underlying band at $\sim 2930\text{ cm}^{-1}$ which shows virtually the same frequency in the spectrum of the solid or the solution, and shows the same characteristic behaviour of "head-group" vibrations upon hydration. It is assigned

to the $-\text{CH}_2-\text{N}^+-$ symmetric stretch.

4.1.2. Carbonyl Stretching.

The carbonyl stretching mode is observed at 1737 cm^{-1} in solid samples and at 1736 cm^{-1} in CDCl_3 solution. In these samples the band was found to be symmetric (Fig. 28) and no evidence of multiple component bands could be observed in the solid sample in spite of there being two carbonyl groups in the molecule. However, the carbonyl band in the spectra of multilamellar dispersion of DPPC is quite asymmetric (vide infra) and two bands have been resolved in the spectrum of a sample of the racemic mixture D,L-DPPC [71] and two bands were observed in a dry sample of DPPE [72].

4.1.3. Fingerprint Region.

The $1500 - 1300\text{ cm}^{-1}$ spectral region is shown in Fig. 30 for DPPC and its deuteriated derivatives as polycrystalline solids. It contains the bending modes of methylene and methyl groups in several different functional groups. The peak positions are listed in Table XI. The region is dominated by the scissoring vibration of acyl chain methylenes at 1467 cm^{-1} which overlaps with a broad band structure spanning from 1500 to 1430 cm^{-1} , comprised of several components.

The band at 1485 cm^{-1} is absent in the spectrum of DPPC- d_9 (Fig. 30B) and remains in the spectra of DPPC- d_{62} (Fig. 30D) and DPPC- $16',16''\text{-d}_6$ (Fig. 30C) hence it is assigned

TABLE XI: Observed frequencies (cm^{-1}) in the infrared spectra of DPPC and deuteriated derivatives as polycrystalline solids and CDCl_3 solutions: fingerprint region.

DPPC		DPPC-d ₆₂		DPPC-16',16"-d ₆		DPPC-d ₉	
solid	solution	solid	solution	solid	solution	solid	solution
1485	1479	1484	1480	1484	1480		
1467	1468			1467	1467	1467	1467
		1469	1469				
1458			1458	1458	1458	1458	
						1455	1456
1440	1441	1441		1441	1442	1438	1445
1418	1417	1416	1416	1416	1417	1416	1417
1405							
	1398	1398					
1378	1379	1381	1380				1378
1368					1367	1362	
1340	1342			1343	1342	1342	
1331		1326					
1318		1316		1317		1319	
1284				1284		1284	
1266							
1258	1260	1256	1260	1258	1256	1256	1256
1246				1244		1246	
	1242	1244			1240		
1222				1223		1223	
		1215					
1198				1198		1198	
1180	1179	1175		1177	1175	1174	
1167				1170			
1153							
	1149	1148	1144	1141		1146	1148
1138							
1118							
1106		1098				1106	
1094	1092	1090	1090	1094	1090	1094	1092
						1084	1083
1075	1066	1070	1068		1066		1068
1062	1057	1058	1058	1056	1058		
						1036	1036
1014	1017	1018	1018	1019			
980						980	982
970	970		969	968	968		
955	954		956	956	954		
933		927		925			
915		912					
908						900	
890						888	
874		877		875			
845						846	
828							
820		819		817		815	
782						784	
760		763		760		766	
721				723		721	
		715					

to the asymmetric bending of the choline methyl groups. On the same basis the weak band at 1405 cm^{-1} is assigned to the corresponding symmetric CH_3 mode. Comparing the spectrum of DPPC with that of DPPC-16',16''-d_6 , and following the assignment of hydrocarbons (Section 3.2.2.), the bands at 1458 and 1378 cm^{-1} can be assigned to the antisymmetric and symmetric bending of the acyl chain terminal methyl groups.

The band at 1418 cm^{-1} has previously been assigned in the spectra of related compounds to the scissoring vibration of the methylene group next to the C=O group in the acyl chains [73]. The intensity of this band is noticeably decreased in the spectrum of a dry sample of DPPC-2'-d_2 supporting this argument.

As in the case of normal alkanes, the assignment of the band at 1440 cm^{-1} is unclear (Section 3.2.2.). In the case of hydrated multibilayers of DPPC this band has a thermotropic behaviour (vide infra) comparable to the one described in Section 3.3.2.

The spectrum of DPPC as a polycrystalline solid in the region $1300 - 900\text{ cm}^{-1}$, Fig. 31, consists of four strong bands overlapped by many weaker bands. The strong bands at 1258 and 1094 cm^{-1} represent the antisymmetric and symmetric phosphate stretching modes. The series of weak bands and shoulders at $1368, 1340, 1331, 1310, 1284, 1266, 1246, 1222$ and 1198 cm^{-1} are the components of the methylene wagging band progression. The frequencies are those characteristic of the palmitoyl chain [74].

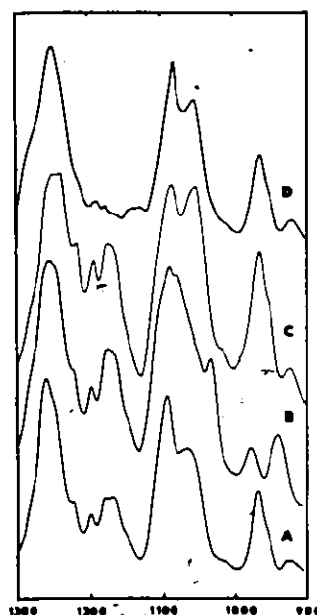


Fig. 31. Infrared spectra of DPPC and derivatives (solid samples) in the 1300- 900 cm^{-1} region.
A. DPPC; B. DPPC-d₉;
C. DPPC-16',16''-d₆ and
D. DPPC-d₆₂.

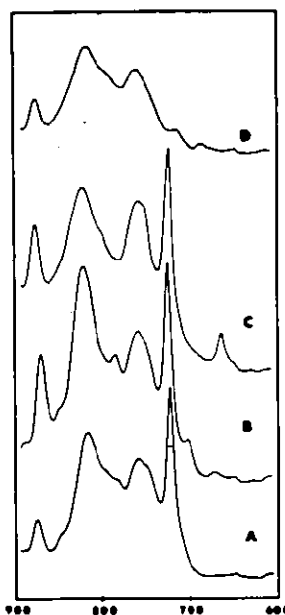


Fig. 32. Infrared spectra of DPPC and derivatives (solid samples) in the 900 - 600 cm^{-1} region.
A. DPPC; B. DPPC-d₉;
C. DPPC-16',16''-d₆ and
D. DPPC-d₆₂.

The C-O-C stretch gives rise to bands at 1180-1160 cm^{-1} (antisymmetric) and 1080-1060 cm^{-1} (symmetric) [65,72,73,75]. The bands at 1180 and 1167 cm^{-1} are assigned to asymmetric C-O-C stretching. These pairs of bands each collapse to one on hydration and studies of partially hydrated systems show gradual merging of the two doublets. The appearance of two bands instead of one for each C-O-C stretching mode can be rationalized in terms of two different conformations for the ester groups [72].

The bands at 1153 and 1138 cm^{-1} are also present in the spectrum of DPPC- d_{62} indicating that they are associated with vibrations of the "head-group".

The band at 1118 cm^{-1} can be associated with carbon-carbon stretching coupled with end groups [73].

From 1015 to 915 cm^{-1} a sequence of bands is observed which undergoes large changes on deuteration of the choline methyl groups. The weak band at 1014 cm^{-1} can be assigned to the $\text{N}^+\text{-CH}_2$ stretch, although largely masked by CD_3 bending the band is still evident as a weak broad feature in the spectrum of DPPC- d_9 .

The bands at 970, 933 and 915 cm^{-1} , and the weak shoulder at 955 cm^{-1} , all shift on deuteration of the choline methyl groups. The triplet has been assigned [72] to antisymmetric and symmetric $\text{-N}^+(\text{CH}_3)_3$ stretching modes (970 and 930 cm^{-1}).

The 900 - 600 cm^{-1} spectral region is shown in Fig. 32. The bands at 820 and 760 cm^{-1} represent the antisymmetric and symmetric P-O single bond stretching in P(OR)_2 [72].

The strong band at 721 cm^{-1} in the spectrum of DPPC represents the acyl chain methylene rocking mode [31].

4.2. The Thermotropic Behaviour of DPPC Multilamellar Dispersions.

In Section 1.3.1., the general characteristics of the thermotropic mesomorphism of phosphatidylcholine bilayers were given. In the following sections the results obtained from the study of this mesomorphism in DPPC bilayers by infrared spectroscopy are presented.

Fully hydrated multibilayers of DPPC were prepared for infrared spectroscopy as described in Section 2.2. These multibilayers show two endothermic events in the temperature range $0 - 60^\circ\text{C}$ (Fig. 4). One at 41.5°C , called the main transition, involves the melting of the acyl chains. The nature of the second, at $36-37^\circ\text{C}$ and called the pretransition will be characterized herein.

The liquid-crystalline phase, above 41.5°C , is composed of bilayers where the hydrocarbon chains contain large numbers of gauche conformers [27]. In the gel phase, below 41.5°C , the chains are essentially all-trans and packed in a regular lattice [16-21].

4.2.1. Acyl Chain Modes.

4.2.1.1. Scissoring and Rocking.

The infrared spectrum of fully hydrated multibilayers

in the CH_2 scissoring and rocking regions is shown in Fig. 33A and B respectively. Each part shows in the bottom section a set of five absorbance spectra taken at the indicated temperatures, and in the upper section the four corresponding difference spectra generated from the absorbance spectra. The temperatures are selected so as to bracket the various stages of the temperature-dependent behaviour of DPPC: the liquid-crystalline phase, the gel to liquid-crystal phase transition, the pretransition, and the gel phase below the pretransition.

The dominant features in Fig. 33 are the strong fundamentals at 1468 and 721 cm^{-1} which represent the scissoring and rocking modes of the methylene groups in the acyl chains (Section 4.1.).

In the liquid-crystalline phase these bands are relatively insensitive to temperature variations; however, they undergo abrupt changes in intensity during both transitions. Below the temperature of the pretransition, the principal changes in the absorbance spectra are broadening of the band contours as the temperature is decreased. In the case of the CH_2 scissoring mode this occurs primarily on the high-frequency side, and at 0°C (Fig. 33A, broken line) the band contour is comprised of two barely resolved components, at about 1472 and 1467 cm^{-1} . In the case of the CH_2 rocking mode, the maximum has shifted to lower frequency, but the band contour is now asymmetric as a result of the broadening occurring solely on the high frequency

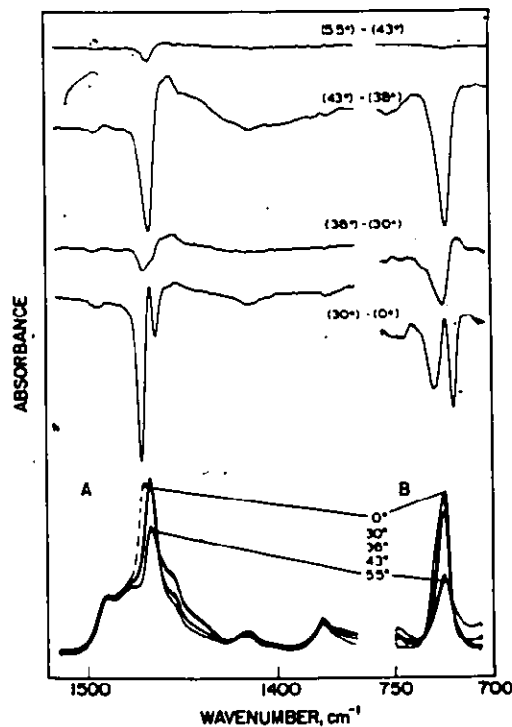


Fig. 33. Methylene scissoring (A) and methylene rocking (B) regions of the infrared spectrum of fully hydrated DPPC at 0°, 30°, 38°, 43° and 55°C (bottom) and corresponding difference spectra (top). The spectra in A were recorded from a 6 μm -thick sample in a cell with BaF_2 windows. The spectra in B were recorded from a 6 μm -thick sample in a cell with ZnSe windows and were smoothed with an 111-point function. In addition, baselines in B were corrected by removing the slope from the underlying water band.

side of the peak maximum (Fig. 33B).

In the difference spectra of Fig. 33 more subtle effects are evident. Throughout the liquid-crystalline phase (55 - 43 °C), only minor reductions in intensity occur as the temperature is raised, while the main transition (43 - 38 °C) produces large intensity decreases. In the case of the scissoring band, the intensity loss is accompanied by an increase in the intensity in the region 1450 - 1440 cm^{-1} . This behaviour is the same as that observed in the melting of hydrocarbons (Section 3.3.2.) and is typical of these modes on melting.

The changes observed in the temperature range covering the pretransition (38 - 30 °C) are smaller than those in the main transition and also different in nature. Although there are similar reductions in intensity of the fundamentals, and an intensity increase in the region 1450 - 1440 cm^{-1} , both difference spectra are shifted relative to those in the main transition. Furthermore, in the case of the scissoring mode, a low-frequency shoulder is evident in the negative peak.

The gel phase difference spectrum (30 - 0 °C) is completely different. In both the CH_2 scissoring and the CH_2 rocking regions there are two minima. As the difference spectra are an absolute measure of the absorbance differences resulting solely from the variation in temperature, the observation of two minima confirms the detection in the absorbance spectra of splitting of the CH_2 scissoring mode at 0°C

They also indicate that the changes in the CH_2 rocking mode result from splitting of the band, one component shifting to lower frequency and the second, weaker component appearing at higher frequency.

The splitting of the CH_2 scissoring and rocking modes continues progressively with decreasing temperature, and at about -60°C both modes are split by 10 cm^{-1} .

Fig. 34 shows the scissoring (Part A) and rocking (Part B) modes of DPPC at -60°C and 0°C .

As in the spectra of hydrocarbons (Sections 3.2.2. and 3.2.4.) the simultaneous observation of two bands in each of these regions is assigned to factor group (correlation field) coupling of these modes. This coupling is only observed when the acyl chains are packed in an orthorhombic subcell (monoclinic or orthorhombic crystal lattice) and are not observed when the subcell is hexagonal or triclinic.

The nature of the changes in the methylene scissoring and rocking modes was characterized by quantitatively evaluating several spectral parameters. The absorbance and $\Delta A/\Delta T$ (rate of change of absorbance, see section 2.3.3.) plots of the scissoring band (Fig. 35A) and of the rocking band (Fig. 35B) show similar behaviour. Large changes are observed at the main transition at 41.5°C , while at a slightly lower temperature, 37°C , smaller changes associated with the pretransition are observed. Both in the gel phase.

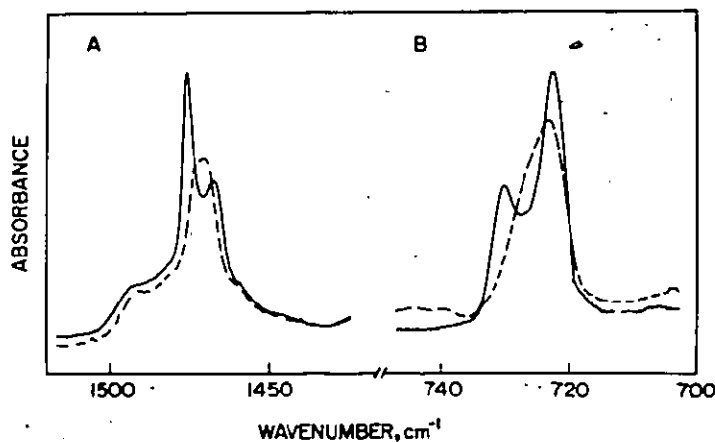


Fig. 34. Methylene scissoring (A) and methylene rocking (B) regions of the infrared spectrum of fully hydrated DPPC at -60° (solid line) and 0°C (broken line). Spectra were recorded under the same conditions as described in Fig. 33.

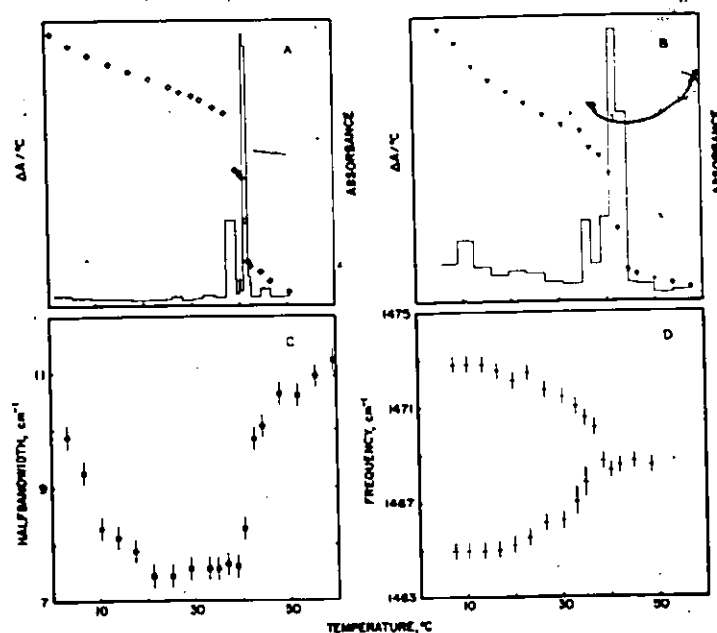


Fig. 35. Temperature dependence of the CH₂ scissoring and rocking modes in the spectrum of fully hydrated DPPC: A. CH₂ scissoring modes, as $\Delta A / \Delta T$ (solid line) and A ($\diamond$) vs. temperature; B. CH₂ rocking mode, as $\Delta A / \Delta T$ (solid line) and A (∇) vs. temperature; C. half-bandwidth of the CH₂ rocking mode vs. temperature; D. frequency positions of the minima in the difference spectra of the CH₂ scissoring mode vs. temperature.

and in the liquid-crystalline phase, the rates of change are much smaller and nearly constant.

The behaviour of the bandwidth of the CH_2 rocking mode (Fig. 35C) is in contrast to that shown by the absorbance plots. The bandwidth undergoes a large change at the main transition and is constant over the range 40 to 20°C . It then increases again when the temperature is further reduced, as a result of the factor group splitting. The same trend was observed for the CH_2 scissoring band in the gel phase. In addition, no evidence of a pretransition could be obtained from the width of either band.

The onset of factor group splitting is evident at temperatures below 10°C in the absorbance spectra. However, the halfwidths of the CH_2 rocking and scissoring bands indicate that splitting is present in the range 10 to 20°C , while the shoulder in the methylene scissoring band in the difference spectrum covering the pretransition ($38 - 30^\circ\text{C}$) suggests that splitting occurs at even higher temperatures. In order to observe the onset of the splitting, the positions of the minima in the difference spectra of the scissoring mode were monitored. These frequencies are plotted as a function of temperature in Fig. 35D. At all temperatures above 37°C a single minimum is observed at the same frequency as that of the peak in the absorbance spectrum, demonstrating that in this temperature range there is only one band, at constant frequency, the peak height of which decreases with increasing temperature. Below this temperature,

splitting of the CH_2 scissoring mode commences and increases in magnitude with decreasing temperature. This is evident from the appearance of a low-frequency shoulder in the difference spectrum, which is resolved into a second minimum below 30°C , and the simultaneous shift of the stronger minimum to higher frequency. The separation of the two components increases steadily with decreasing temperature until the splitting in the difference spectra can be clearly related to the splitting in the absorbance band contours.

The behaviour of the CH_2 scissoring and rocking bands demonstrate that as the temperature is reduced below 37°C there is a progressive factor group splitting of these modes. As noted above, factor group splitting of these modes is only compatible with acyl chains packed in an orthorhombic subcell.

4.2.1.2. Methylene Wagging Band Progression.

The methylene wagging band progression appears in the $1360 - 1190 \text{ cm}^{-1}$ region dominated by the antisymmetric phosphate stretching band (Section 4.1.). This region of the infrared spectrum of fully hydrated multibilayers of DPPC is shown in Fig. 36 where in the bottom part five absorbance spectra at selected temperatures are presented, and in the top part the four corresponding difference spectra are shown.

The components of the band progression are present at all temperatures below the main transition; these bands

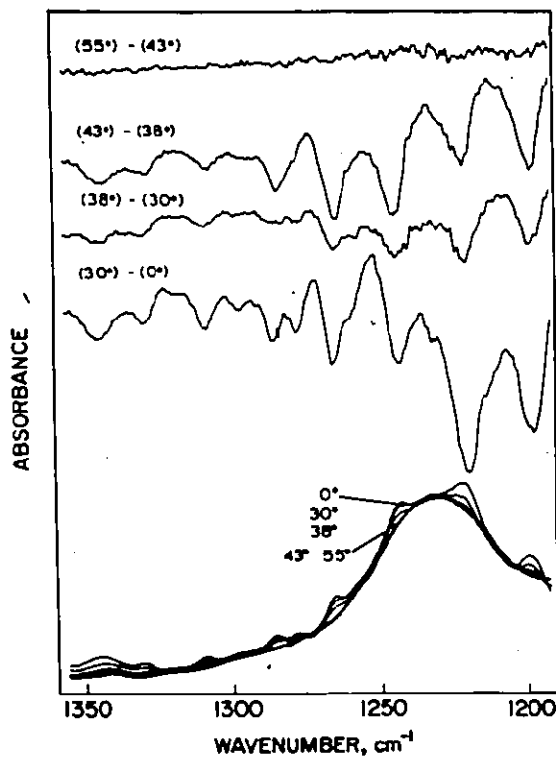


Fig. 36. Infrared spectrum of fully hydrated DPPC in the 1360 - 1190 cm^{-1} region at 0°, 30°, 38°, 43° and 55°C (bottom) and corresponding difference spectra (top). Spectra are from 25 μm -thick sample between CaF_2 windows.

are typical of all-trans chains [58] and therefore they are absent in the spectra of the liquid-crystalline phase. They increase considerably in intensity as the temperature is reduced in the gel phase. In fact, as is evident from the spectra in Fig. 36, the phosphate band is practically invariant with temperature, and the difference spectra result entirely from changes in the band progression.

The difference spectrum for the liquid-crystalline phase is featureless, confirming the absence of the band progression in the spectra of this phase, as a result of the high proportion of gauche conformers present in the acyl chains. When the temperature is reduced from 43 to 38 °C, large changes are evident in the band progression due to the transition to a predominantly all-trans conformation. Smaller intensity changes are observed in the pretransition range 38 - 30 °C, and, as the temperature is further reduced, the intensities of the band progression components continue to increase. Also evident in the 30 - 0 °C difference spectrum is a pair of weak bands at about 1300 and 1278 cm^{-1} . They could be components of the twisting-rocking band progression appearing in this spectral region, which are considerably weaker than the wagging bands (Section 3.3.4. and [74]).

The way in which the bands change was determined by monitoring $\Delta A/\Delta T$ for all components of the progression. Fig. 37 shows a plot of $\Delta A/\Delta T$ and A versus temperature for the $k = 2$ band at 1220 cm^{-1} . Plots derived from other components of the progression were practically identical. The

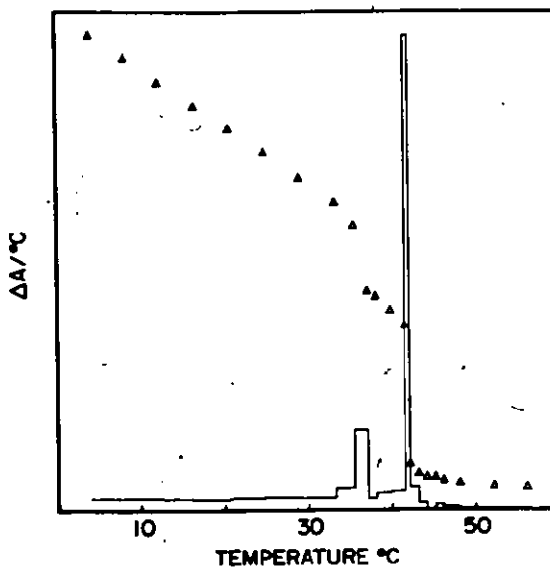


Fig. 37. Temperature dependence of the $k = 2$ component of the methylene wagging band progression in the spectrum of fully hydrated DPFC as $\Delta A/\Delta T$ (solid line) and A (Δ) vs. temperature.

plot in Fig. 37 shows the same behaviour as that derived from the CH_2 scissoring and rocking bands shown in Fig. 35.

The behaviour of A versus temperature in Fig. 37 can be compared to that of the intensity of the W_3 band in the spectra of $n\text{-C}_{21}\text{H}_{44}$ (Fig. 22B).

The frequencies of the band progression were derived from the positions of the minima in the difference spectra and are listed in Table XII. As the individual bands of this progression show no splitting, the values in Table XII correspond to their actual peak positions, except when a shift in frequency is occurring. It is evident that in the range 36 - 38 °C there is an abrupt shift to higher frequency as the temperature is raised. This shift is comparable to that of the wagging bands in the spectra of hydrocarbons during the "rotator" phase transition (Table V).

4.2.1.3. C-H Stretching.

The C-H stretching region in the spectrum of DPPC is shown in Fig. 38. The bottom part shows five spectra at selected temperatures and the top part the corresponding four difference spectra.

As is evident from Fig. 38, the C-H stretching bands change substantially in the pre- and main transitions, the frequencies of the methylene bands have previously been used to monitor the main phase transition in racemic D,L-DPPC [75]. The antisymmetric and symmetric methylene bands show identical behaviour. They are almost invariant in frequency

TABLE XII. Temperature dependence of the peak positions (cm^{-1}) of the first three components of the CH_2 wagging band progression in the infrared spectrum of fully hydrated DPPC bilayers; determined from the indicated difference spectra.

Temperature range in $^{\circ}\text{C}$	$k = 1$	$k = 2$	$k = 3$
42-40	1200	1222.5	1246
40-38	1200	1222.5	1246
38-36	1199	1221.5	1244.5
36-33	1198	1221	1244
33-30	1198	1221	1244
30-0	1198	1221	1244

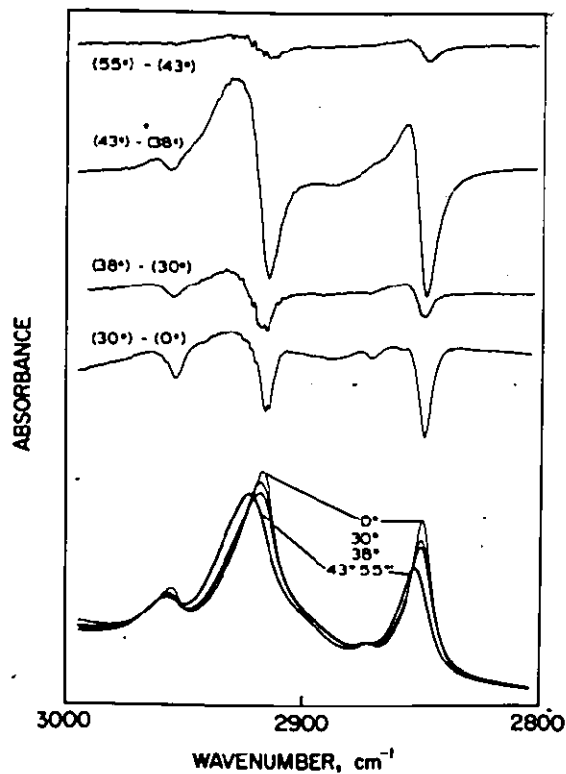


Fig. 38. Carbon-hydrogen region in the infrared spectrum of fully hydrated DPPC at 0°, 30°, 38°, 43° and 55°C (bottom) and corresponding difference spectra (top). Spectra are from a 2.5 μm -thick sample between CaF_2 windows.

throughout the gel phase, with an abrupt peak height change at the temperature of the pretransition. The main transition results in changes in all band parameters, while in the liquid-crystalline phase changes are minor and restricted to the peak height. In contrast, the methyl bands at 2956 and 2872 cm^{-1} undergo large changes in frequency, bandwidth, and peak height in the gel phase and in the pretransition. This is particularly evident in the corresponding difference spectra.

Detailed data regarding the temperature dependence of these bands are given in Fig. 39. The plots of $\Delta A/\Delta T$ (Fig. 39A) and $\Delta B/\Delta T$, rate of change in integrated intensity, (Fig. 39B) of the methylene symmetric stretching mode closely resemble the $\Delta A/\Delta T$ plots of the scissoring and rocking modes in Fig. 35.

The temperature dependence of the frequencies and bandwidths of the C-H stretching modes are shown in Fig. 39C and D respectively. The frequencies of the methylene modes are invariant (Fig. 39C) except at the main transition where they shift to higher frequency reflecting the acyl chain melting. This also produces the large bandwidth changes at 41.5°C (Fig. 39D). Below 41°C there are steady decreases in the methylene bandwidths with inflections at 20°C which can be correlated with the factor group splitting effect observed in the CH_2 rocking band.

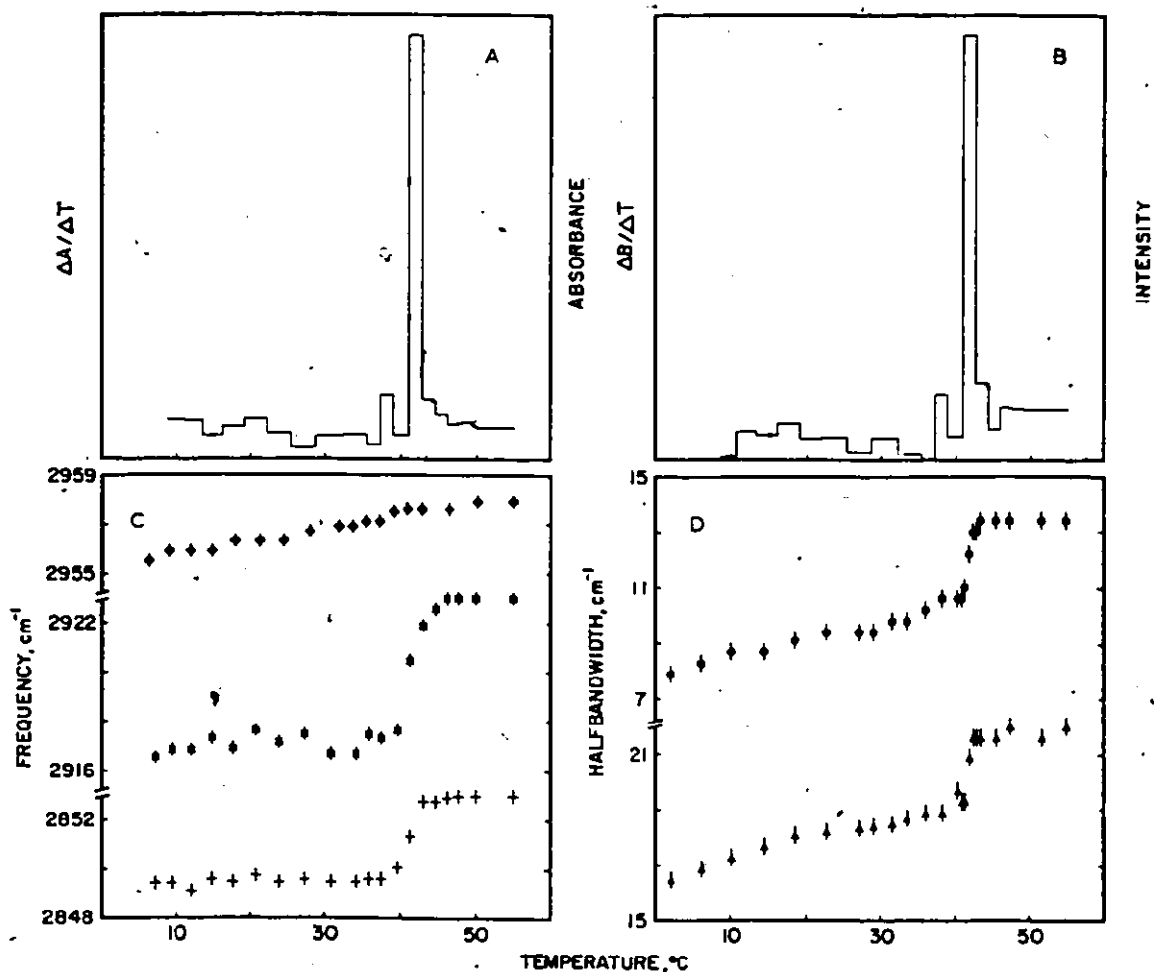


Fig. 39. Temperature dependence of the C-H stretching modes: A. symmetric stretching as $\Delta A/\Delta T$ vs. temperature B. CH_2 symmetric stretching as $\Delta B/\Delta T$ (change in integrated intensity) vs. temperature; C. frequencies: CH_3 asymmetric stretching ($\diamond$), CH_2 antisymmetric stretching (*) and CH_2 symmetric stretching (+); D. halfbandwidths: CH_2 antisymmetric stretching (o) and CH_2 symmetric stretching (Δ).

4.2.1.4. Discussion.

The main transition at 41.5°C in DPPC marks a change from a solid-like to a fluid-like phase. At this temperature, the infrared spectrum of the acyl chain modes exhibits large concomitant variations in peak height, frequency and bandwidth similar to the changes observed in the melting of n-alkanes (Section 3.3.)[66]. In the liquid-crystalline phase much smaller changes of the same type are observed as the temperature is raised. This correlates with results of ^2H NMR studies [27] which indicated an increase in the average number of gauche conformers per acyl chain as the temperature is raised in the liquid-crystalline phase.

The most specific data, from the infrared spectra relate to the gel phase and are obtained from the absorptions due to the methylene groups. Below the pretransition temperature, the dominant factor determining the band shapes and positions is the crystal packing. The resultant effects are first evident at 37°C in the difference spectra of the CH_2 scissoring band (Figs. 33A and 35D). At about 20°C the effects are also evident in the bandwidths of the CH_2 stretching, rocking and scissoring modes. Below 10°C there is clear evidence for two bands in the band contour of the CH_2 scissoring mode, and at lower temperatures both the scissoring and rocking modes are split (Fig. 34).

The pretransition event at 36 - 38 °C is characterized by abrupt decreases in peak height of all methylene bands. The only other band parameter observed to change in the temperature interval 36 - 38 °C is the frequencies of the wag-

ging band progression (Table XII). The key to the interpretation of the above results lies in the crystal packing of the acyl chains in DPPC. The observation of factor group splitting in the infrared spectrum at all temperatures below 36°C is compatible only with the assumption that the acyl chains are packed in an orthorhombic subcell in an orthorhombic or monoclinic crystal lattice, such splitting is absent in the spectra of acyl chains packed in triclinic or hexagonal lattices [62].

Factor group splitting (also called correlation field splitting and dynamic splitting) results from interchain coupling of vibrational modes and depends upon molecular motion [76]. The temperature dependence of splitting shown here can be rationalized in terms of variations in the interchain coupling resulting from distortions of the orthorhombic subcell. These distortions must result from motions of fully extended chains about their long axes. The progressive splitting on lowering the temperature of the scissoring and rocking modes shows that the chain torsional motions are decreasing in rate. At -60°C the rate of rotation seems to have reached a minimum since the splitting is constant at lower temperatures.

The relatively large changes observed in the methyl asymmetric stretching band throughout the gel phase supports the argument of continuously increasing rates of motion and therefore an increasingly mobile central area of the bilayer

in the gel phase as the temperature is raised.

This description of the bilayer structure below the pretransition is in accord with the observation of distorted hexagonal packing found from X-ray diffraction studies [21]. In those studies it was also found that the distortion increased as the temperature is reduced and it is accompanied by a decrease in the rate of motion about the long axes of the chains. A similar conclusion (with regard to the rates of motion was also reached from ^2H NMR [29] and ESR studies [30].

The determination of the crystal structure of DMPC dihydrate at 15°C [22] showed that the molecules are packed in a monoclinic lattice. The chains are packed perpendicular to each other at positions close to the "head-group" while towards the end they are twisted to a mutually parallel orientation resembling a triclinic unit cell. This illustrates the potential for torsional motions about the long axes, particularly in the fully hydrated system where the packing is less rigid.

On the basis of X-ray diffraction [22] and Raman spectroscopic studies [33,34], the general nature of the pretransition has been reported as a change from a regular close hexagonal lattice above the pretransition to one involving a greater degree of interchain interactions below the pretransition. The results from this infrared study at temperatures in the range $41 - 38^\circ\text{C}$ are compatible with close

hexagonal packing and below 36°C with orthorhombic packing; hence it is concluded that the pretransition is a crystal-lattice phase change from a hexagonal subcell (above 38°C) to an orthorhombic subcell (below 36°C).

The confirmation of this characterization of the pretransition comes from the studies of the "rotator" phase transition in n-alkanes presented in Section 3.3. The major changes observed from hexagonal to orthorhombic in the hydrocarbon spectra were: appearance of factor group splitting, slight decreases in bandwidth of the CH₂ stretching modes, relatively large increases in peak heights of the CH₂ stretching modes and of the wagging band progression, a decrease in intensity in the 1450-1440 cm⁻¹ region and shifts to lower frequency of the wagging band progression. The frequency shifts of the CH₂ wagging band progression and the abrupt increases in peak height of all CH₂ modes are exactly the changes observed at the pretransition in DPPC. The other effects, observed in the spectra of n-alkanes; i.e., slight frequency shifts and narrowing of bands, are observed in the spectra of DPPC as the temperature is reduced below 36°C, and can be correlated with increased splitting of the CH₂ scissoring and rocking bands. Further evidence for an orthorhombic subcell is found in the low-temperature Raman spectrum of D,L-DPPC [77] where a band is observed at 1420 cm⁻¹.

The exact nature of the crystal lattice, orthorhombic or monoclinic, cannot be determined directly from the infrared spectra. The two unit cells are nearly identical, the difference being in the angle of tilt of the chains

with respect to the *ab* plane, which in this case is the plane on which the terminal methyl groups lie. In the orthorhombic unit cell the chains are perpendicular to this plane, while in the monoclinic unit cell the chains are tilted with respect to the plane [45]. Generally, the crystal lattice of similar compounds such as fatty acids [78], fatty acid esters [79] and dipalmitoylphosphatidylethanolamine [80] is monoclinic with orthorhombic lattices restricted to odd-numbered *n*-alkanes and polyethylene [45]. X-ray diffraction studies of DPPC and DMPC [19,21,81] demonstrate substantial tilting of the acyl chains with respect to the plane of the bilayer in completely hydrated systems (water content greater than 40 wt%) and the crystal lattice in DMPC dihydrate was found to be monoclinic [22]. These data thus favor monoclinic over orthorhombic lattices. However, in a partially hydrated sample of DMPC orthorhombic packing has been reported [81].

This description of the pretransition is compatible with the observation that it disappears with the addition of perturbants such as cholesterol and anesthetics [35,75,82]. The orthorhombic subcell requires a highly specific packing of the acyl chains. The introduction of molecules in the bilayer that cannot pack into the orthorhombic cell will destroy the potential for such packing and hence alter the pretransition. Similar effects were found in the case of *n*-alkanes where the presence of small amounts of impurity changes the crystal lattice at a given temperature [54].

7

The Raman spectroscopic studies mentioned above [33,34] lead to the conclusion that the pretransition is marked by large increases in lateral chain interactions as the temperature is decreased. However, it was concluded that the interactions were typical of a triclinic lattice and that as the temperature is decreased to -14°C there is a gradual increase in the amount of triclinic phase. This infrared study points to an increase in the orthorhombic nature of the crystal lattice, which is in apparent conflict with the above conclusion. However, if on reduction of the temperature the chains pack as they do in the DMPC dihydrate, evidence for both orthorhombic and triclinic lattice may be accommodated. The "triclinic" lattice, if present, is relatively minor as the low-temperature infrared (Fig. 34) and Raman spectra [77] are both characteristic of orthorhombic packing, the main evidence for triclinic packing being a weak unresolved band at 2860 cm^{-1} in the Raman spectrum [34].

A further conclusion has been reached from Raman spectroscopic studies of DPPC multibilayers in the gel phase. It is that the presence of gauche conformers in the acyl chains can be monitored by following changes in the peak height of the C-C skeletal optical mode at 1130 cm^{-1} in the Raman. An "order" parameter S_{trans} has been derived [33] which relates the peak height of the band to the number of trans conformers in the chain, by assuming that the band intensity is the sum of intensities from all-trans segments.

From the changes in height of this band, the S_{trans} parameter predicts that as many gauche conformers are introduced when the temperature is raised from 15 to 37 °C as are introduced in the main transition, which is estimated to result in the introduction of three to four gauche conformers per chain [33,34,35]. The introduction of such a large number of gauche conformers should result in several types of change in the infrared spectrum. In particular, the CH_2 stretching bands should broaden and shift to higher frequency as observed at the main transition or when melting n-alkanes. In fact, both parameters from the CH_2 stretching bands (Fig. 39) are invariant throughout the pretransition range. At the same time, the introduction of gauche conformers should produce shifts to higher frequency of the $k = 1$ component of the wagging band progression [70] and the opposite is observed (Table XII).

This lack of confirmation by infrared spectroscopy of the conclusions reached from Raman spectroscopic studies may indicate that the assumptions used in the derivation of the S_{trans} parameter are not totally correct. Recently, it has been suggested that the integrated intensity rather than the peak height of the 1130 cm^{-1} Raman band more correctly reflects the number of gauche conformers per chain [83,84]. The number of gauche conformers estimated using the intensity are considerable lower than those estimated using peak heights.

✓ That the intensity of the skeletal optical mode is

not solely due to the number of trans segments has been demonstrated in a Raman study of the "rotator" phase transition in $n\text{-C}_{21}\text{H}_{44}$ [85]. The change in intermolecular interactions during the "rotator" phase transition produced a considerable loss of intensity and peak height of the 1130 cm^{-1} band, demonstrating that both peak height and intensity are sensitive not only to conformational disorder but also to intermolecular interactions.

It can then be concluded that the S_{trans} parameter is an overall measure of the total freedom of motion of the acyl chains and cannot be used unequivocally to monitor intrachain interactions without taking into consideration the effects of interchain interactions in its value.

4.2.2. Head-Group Modes.

4.2.2.1. Ester Linkage.

In the spectra of fully-hydrated DPPC multibilayers the ester carbonyl band is found at 1736 cm^{-1} . The band is overlapped to some extent by the H_2O bending mode at 1650 cm^{-1} but it could be monitored via difference spectra. Its temperature-dependent behaviour was also studied in the spectra of multilamellar dispersions using D_2O as the hydrating agent. Fig. 40 shows spectra of DPPC in the C=O stretching region using H_2O as the hydrating agent (Fig. 40A) and D_2O (Fig. 40B). As in the previous cases the bottom part shows absorbance spectra at five temperatures while the top part shows the

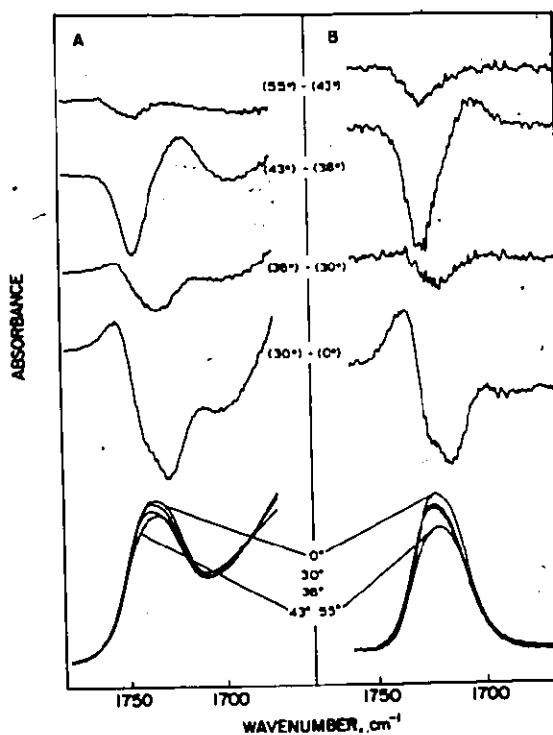


Fig. 40. Carbonyl stretching region in the spectrum of fully hydrated DPPC at 0°, 30°, 43° and 55°C (bottom) and corresponding difference spectra (top). Spectra are from 2.5 μm -thick samples between CaF_2 windows dispersed in H_2O (A) and D_2O (B).

four corresponding difference spectra.

The difference spectra in Fig. 40 show the following effects in the C=O stretching band; at temperatures below the pretransition there is a decrease in intensity, a broadening predominates in the pretransition range while during the main transition a shift to lower frequency is observed.

Fig. 41 shows the temperature dependence of the halfbandwidth and intensity. The halfwidths (Fig. 41A) show changes at 20, 32 and 41.5 °C. The largest effect is seen at the main transition. The $\Delta B/\Delta T$ plot (Fig. 41B) is different from that obtained from the acyl chain modes (Fig. 39B). In this case the changes at the pre- and main transitions are approximately equal in magnitude whereas in the case of the acyl modes the main transition dominates. The frequency of the band is almost invariant at the pretransition but decreases by $1-2 \text{ cm}^{-1}$ at the main transition.

The spectra at temperatures below the main transition show that the carbonyl band is quite asymmetric whereas the band in the liquid-crystalline phase is almost completely symmetric. This trend is further evident in Table XIII where the halfbandwidth has been divided in higher and lower frequency components. The general trend is to greater asymmetry at lower temperatures.

The observation of the asymmetric broadening can be explained in terms of two carbonyl bands, one at slightly higher frequency than the other. As the temperature increases the higher frequency band moves to lower frequency and in the

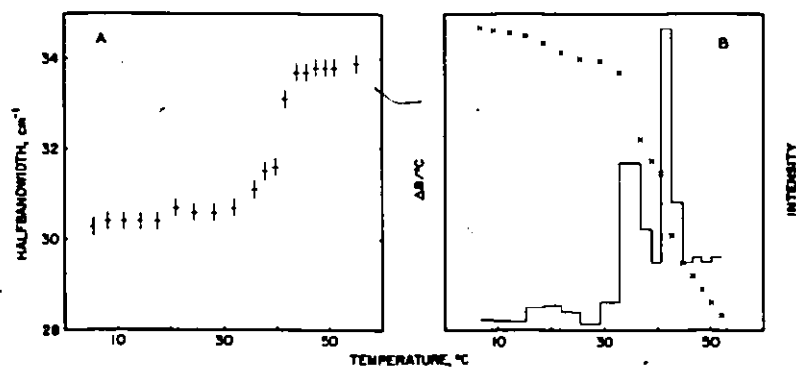


Fig. 41. Temperature dependence of the carbonyl stretching band in the spectra of fully hydrated DPPC as: A. halfwidth vs. temperature and, B. $\Delta B/\Delta T$ (solid line) and B (x) vs. temperature.

TABLE XIII. Temperature dependence of the asymmetry of the carbonyl stretching band in the spectra of multibilayers of DPPC dispersed in D_2O , measured as the variation in the high frequency component and the low frequency component relative to the peak maximum.

Temperature, °C	Bandwidth, cm^{-1}	
	High frequency	Low frequency
3	12.8	17.3
10	13.4	17.3
15	13.6	17.0
20	13.8	16.8
25	13.8	16.5
30	13.8	16.5
35	14.2	17.3
40	15.0	18.1
45	15.1	18.6
50	15.7	17.4

liquid-crystalline phase the bands are at essentially the same frequency.

The cause for the observation of two bands for the carbonyl stretching mode is the conformational inequivalence of the *sn*-1 and *sn*-2 chains as has been shown comparing the infrared spectrum of D,L-DPPC with that of lysolecithin [71]. Additional support comes from studies of the behaviour of the C-O-C stretching modes in DPPE and DPPC [72] where it was concluded that the fatty acid ester group in position 1 of the glycerol backbone exhibits a planar configuration while the ester group in position 2 has a non-planar configuration. These conclusions are in agreement with X-ray diffraction [80,86] and ^2H NMR [87] studies.

Fig. 42 shows the $1200 - 900 \text{ cm}^{-1}$ spectral region of fully hydrated multibilayers of DPPC. From this figure it is evident that the asymmetric C-O-C stretch at about $1180 - 1170 \text{ cm}^{-1}$ is composed of two bands (Section.4.1.) in the spectrum at 0°C and that at higher temperatures they have collapsed to one; the effect can also be seen in the $30 - 0^\circ\text{C}$ difference spectrum. The effect is not so clearly seen in the symmetric C-O-C stretching mode because of overlap with the phosphate band.

4.2.2.2. Phosphate Modes.

Both the asymmetric (1258 cm^{-1}) and symmetric

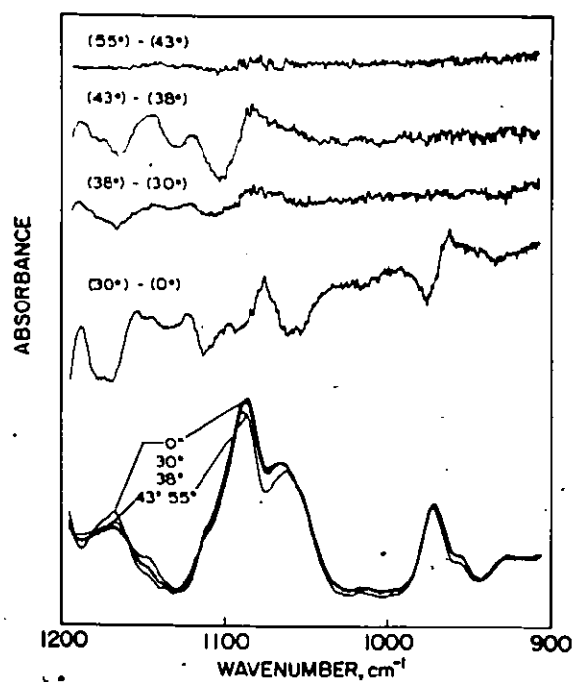


Fig: 42. Infrared spectrum of fully hydrated DPPC in the 1200 - 900 cm^{-1} region (bottom) and corresponding difference spectra (top). Spectra were recorded from a 25 μm -thick sample between BaF_2 windows.

(1094 cm^{-1}) phosphate stretching modes are overlapped and only a qualitative monitoring of their behaviour is possible. The symmetric mode shows a steady shift to lower frequency (Fig. 42) when the temperature is increased, the shift being about 2 cm^{-1} in the main transition. Changes in the pretransition are much smaller. Overall, the change in integrated intensity appears to be extremely small. The broader asymmetric stretching band (Fig. 36) is so heavily overlapped that the only possible observation is the fact that the integrated intensity is almost invariant in the 0 - 55 °C temperature range.

The other phosphate modes (Section 4.1.) give rise to bands below 900 cm^{-1} which should convey information regarding the conformation of the phosphate group. Unfortunately, they were found to be very weak and it was not possible to determine precisely their temperature-dependent behaviour.

4.2.2.3. Choline Group.

Absorptions from the choline group are observed in three spectral regions; methyl asymmetric stretching (3030 cm^{-1}), methyl asymmetric bending (1485 cm^{-1}), and N^+ -C stretching modes near 1000 cm^{-1} .

The methyl asymmetric stretching band is absent in the spectra of hydrated samples, it probably broadens to the extent of becoming indistinguishable from the baseline.

The changes observed in the methyl asymmetric bending band at about 1485 cm^{-1} are extremely small relative to the band intensity (Fig. 33); there is a slight shift to lower frequency ($<0.5\text{ cm}^{-1}$) in the gel phase and at the main transition.

The absorption from the $\text{CH}_2\text{-N}^+$ stretching mode at 1020 cm^{-1} (1014 cm^{-1} in the dry film, Section 4.1.) is so weak (Fig. 42) that if there are temperature-induced changes it is not possible to detect them.

The behaviour of the asymmetric $\text{N}^+(\text{CH}_3)_3$ stretching mode at 972 cm^{-1} can be seen in Fig. 42. There are large changes in intensity and frequency in the gel phase ($30 - 0^\circ\text{C}$) difference spectrum. The rest of the difference spectra are virtually featureless indicating that there are no more changes at higher temperatures.

The shift to lower frequency of the choline band is striking by virtue of its absence at higher temperatures. As the group is charged and relatively decoupled from adjacent functional groups it probably reflects a slight increase in hydration as the temperature is raised.

The pretransition is observed in the phosphatidylcholines and not in the other classes of phospholipids [10]. It is therefore surprising that the vibrations arising from the choline group are not affected by the pretransition. The presence of the choline group probably has an indirect effect on the chain packing. The size of the "head-group" could well determine the interchain distances in the gel phase and these will determine the chain packing.

4.2.3. Specifically-Deuteriated Derivatives.

Deuterium NMR studies of a series of specifically-deuteriated derivatives of DPPC have provided order parameter profiles of the liquid-crystalline phase [27]. It was found that the first ten methylene groups of the *sn*-2 palmitoyl chain present an almost constant order parameter, at positions further down the chain the order parameter drops off linearly. This order parameter "plateau" has been observed in a variety of model and natural membranes [26,28,88]. Studies of the gel phase have been carried out almost exclusively by monitoring simultaneously the properties of all methylene groups.

There is only one Raman study of specifically-deuteriated derivatives of DMPC [89] in the gel phase. However, due to the signal-to-noise ratio the study was restricted to changes in bandwidth of CD_2 stretching bands at the main transition, and no detailed data are available on the general gel phase changes and the specific changes at the pretransition.

In this section are presented the changes observed in the C-D stretching region of the infrared spectra of a series of specifically-deuteriated dipalmitoyl phosphatidylcholine. The compounds with CD_2 or CD_3 groups located at the 2', 3', 7', 8', 13' and 16' positions of the *sn*-2 palmitic chain were studied.

The carbon-deuterium stretching region of the vibrational spectra of compounds containing specifically-

deuteriated acyl chains generally consists of two bands near 2200 cm^{-1} and 2100 cm^{-1} [90]. In all cases the band near 2200 cm^{-1} is assigned to the asymmetric (CD_3) or antisymmetric (CD_2) stretching mode. When a single band is observed near 2100 cm^{-1} it is assigned to the symmetric stretching mode. An additional band is sometimes observed at about 2130 cm^{-1} and the two (2130 and 2100 cm^{-1}) are assigned to the symmetric stretching fundamental in Fermi resonance with the first overtone of the CD_2 or CD_3 bending mode. In any case, the thermotropic behaviour of symmetric stretching bands may be more complex than that of the antisymmetric band, as in the case of $n\text{-C}_{17}\text{H}_{30}\text{D}_6$ (Section 3.3.1.). Spectral changes may result from variations in the Fermi resonance interaction in addition to changes in acyl chain conformations or molecular motion. Therefore in the following results will be restricted to the bands near 2200 cm^{-1} . However, the changes observed in the two stretching bands at 2200 and 2100 cm^{-1} are similar in magnitude. In the case of the peak positions this is evident in Table XIV which summarizes the changes in frequency of the C-D stretching bands during the main transition. Also included are data taken from the spectra of anhydrous films of the specifically-deuteriated DPPC and data taken from the spectra of a series of specifically-deuteriated palmitic acids as polycrystalline solids.

The main transition results in changes in both the asymmetric and symmetric C-D stretching bands of most of the compounds. The magnitudes of the changes in the spectra

TABLE XIV. Infrared vibrational frequencies (cm^{-1}) of the C-D stretching modes of specifically-deuteriated derivatives of DPPC and palmitic acid.*

		C O M P O U N D				
		D P P C				
		Palmitic acid solid	anhydrous solid	hydrated 5°C	hydrated 38°C	hydrated 50°C
Position Labelled	2'-d ₂	ν_{as}	2219.0 2216.0	2212.6	2213.2	2213.4
		ν_{s}	2075.0	2075.1	2075.0	2075.0
	3'-d ₂	ν_{as}	2203.2	2196.8	2197.6	2198.0
		ν_{s}	2118.0	2112.2	2112.8	2113.4
	7;8'-d ₄	ν_{as}	2180.6 †	2180.0	2180.2	2180.8
		ν_{s}	2079.4 †	2078.4	2078.9	2079.1
	13'-d ₂	ν_{as}	2169.8	2169.6	2169.7	2169.7
		ν_{s}	2094.9	2095.6	2096.2	2096.5
	16'-d ₃	ν_{as}	2221.2 2210.2	2216.0 2212.0	2212.2	2212.8
		ν_{s}	2073.6	2074.0	2074.6	2074.8

* ν_{as} , frequency of the antisymmetric mode; ν_{s} frequency of the symmetric mode.

† Values are taken from the spectrum of palmitic acid 5,6-d₄. However, in the central region of the chain, frequencies of the CD₂ stretching bands are invariant with position [91].

of DPPC-16'-d₃ and DPPC-2'-d₂ are smaller than those in the spectra of the other compounds.

4.2.3.1. DPPC-2'-d₂.

The CD₂ group of DPPC-2'-d₂ exhibits spectral properties substantially different from those of DPPC-3'-d₂, DPPC-7',8'-d₄ and DPPC-13'-d₂. The CD₂ stretching bands are extremely weak, the antisymmetric stretching band contains two components separated by 3 cm⁻¹ (Table XIV), and the frequency of this band is higher than those of the other specifically-deuteriated phospholipids. In addition, hydration or solution in chloroform of an anhydrous sample results in a shift to lower frequency of the band, whereas in all other cases both the antisymmetric and symmetric bands shift to higher frequency (Table XIV). The characteristics of the spectra of hydrated DPPC-2'-d₂ are given in Fig. 43. Fig. 43A shows the C-D stretching region of the infrared spectrum of a fully hydrated multibilayer sample at 5, 30, 38 and 45 °C. Fig. 43B shows a plot of $\Delta A/\Delta T$ versus temperature for the antisymmetric CD₂ stretching band at about 2213 cm⁻¹. Figs. 43C and 43D show, respectively, plots of frequency and bandwidth versus temperature for the antisymmetric CD₂ stretching band and the symmetric CD₂ stretching component at 2075 cm⁻¹.

As shown in Fig. 43A, the main spectral changes resulting from an increase in temperature are decreases in peak height and the relative heights of the two peaks comprising

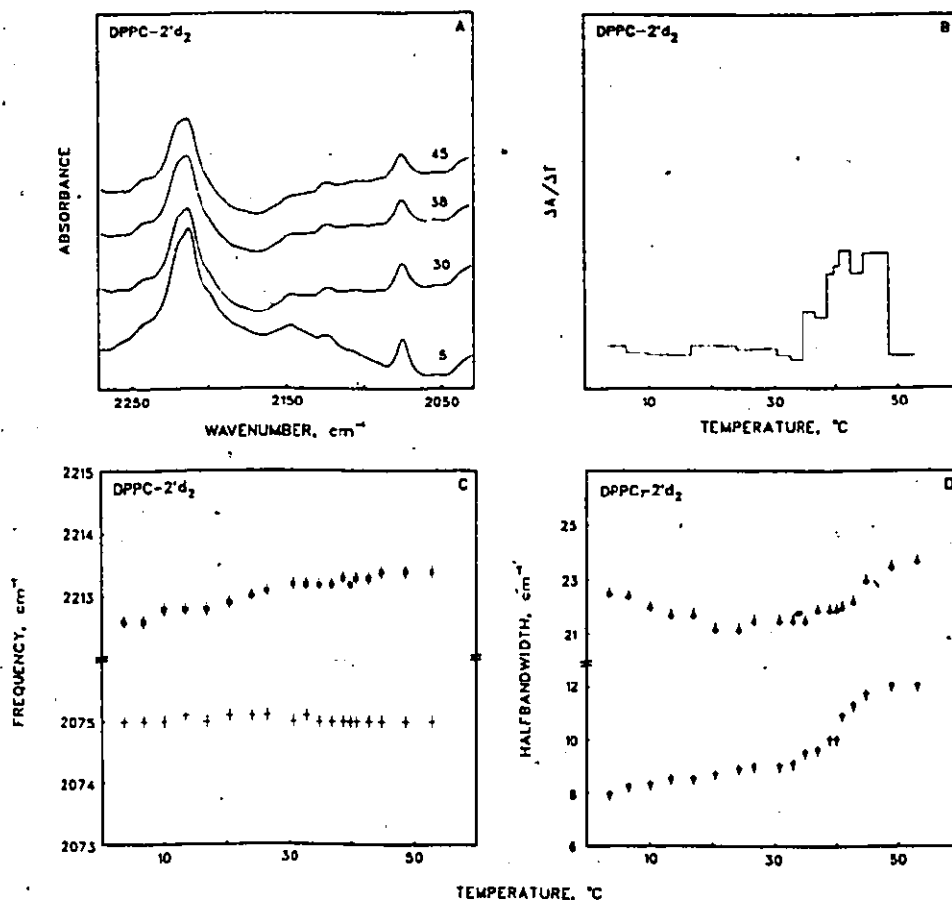


Fig. 43. Temperature dependence of fully hydrated DPPC-2'-d₂. A. Infrared spectrum of fully hydrated DPPC-2'-d₂ at 5°, 30°, 38° and 45°C. Spectra are from a 50 μm-thick sample between CaF₂ windows and are plotted at the same scale. The "water-association" band has been removed by subtraction; B. ΔA/ΔT derived from the CD₂ antisymmetric stretching band vs. temperature; C. Frequency of the CD₂ antisymmetric (*) and symmetric (+) stretching modes vs. temperature; D. halfbandwidth of the CD₂ antisymmetric (Δ) and symmetric stretching modes vs. temperature (∇).

the antisymmetric CD_2 stretching band.

The plot of $\Delta A/\Delta T$ versus temperature, Fig. 43B, shows a broad maximum in the range 35 - 45 °C. The $\Delta A/\Delta T$ plot derived from the CH_2 wagging band progression in the spectra of the same sample showed that the main transition occurred within a 1 - 2 °C interval. The breadth in Fig. 43B may then indicate that slight changes in this specific part of the bilayer occur over a wider temperature range than is the case in the more hydrophobic regions of the bilayer.

Temperature-induced variations in frequency and bandwidth are minimal. The frequency of the band at 2075 cm^{-1} is invariant while only a small, monotonic increase in the frequency of the antisymmetric stretching band is evident as the temperature is increased (Fig. 43C). The bandwidths (Fig. 43D) show a slightly higher temperature dependence, both bands broaden at the main transition. However, while the 2075 cm^{-1} band narrows as the temperature is reduced, there is a broadening of the antisymmetric stretching band at temperatures below 20°C.

There is a large inductive interaction between the polar carbonyl group and the methylenes in the acyl chains [89,91] affecting the frequencies, bandwidths and intensities of the methylene stretching vibrations. The chain positions closer to the C=O group are the most affected, the interaction becomes negligible at position 5. Therefore, in the case of the $2'\text{-CD}_2$ group the spectral parameters will

be dependent on the inductive interaction and on the conformations and mobility of the acyl chain.

However, the temperature-induced changes in the CD_2 stretching bands (in the spectra of DPPC-2'- d_2) are minor. Consequently, although the effects of conformational and inductive perturbations cannot be distinguished, the lack of change indicates that over the temperature range studied, there are no major changes in the inductive interaction or the conformational order at this location.

The small changes that are observed can be related to motional effects. 2H NMR studies [87] have demonstrated that, in the liquid-crystalline phase, there are two slowly interchanging conformations of the glycerol head-group which result in two conformations for the *sn*-2' chain methylene group adjacent to the carbonyl group. The anomalous shape observed for the antisymmetric CD_2 stretching band suggests that these two conformations are also present in the gel phase and that in both phases the CD_2 stretching modes of the two conformers occur at slightly different frequencies. The rate of interchange decreases with decreasing temperature in the liquid-crystalline phase [87]; if it also decreases in the gel phase it may result in the increased distinction of the two components of the antisymmetric CD_2 stretching band of DPPC-2'- d_2 at lower temperatures. Similarly, the slight changes in bandwidth at the main transition may result from a relatively abrupt decrease in the rate of interchange on transition from the liquid-crystalline to the gel phase.

The observation of two bands for the C-O-C stretching modes described in Section 4.2.2.1. supports the argument of two slowly interchanging conformations in the head-group and correlates with the observations just described for the CD_2 antisymmetric stretching mode in the spectra of DPPC-2'- d_2 .

4.2.3.2. DPPC-3'- d_2 , DPPC-7',8'- d_4 and DPPC-13'- d_2 .

The inductive interaction of the carbonyl group is greatly reduced at position 3' compared to position 2' and negligible at position 5' [91]. The inductive effects were found to remain constant with temperature at position 2', therefore, the temperature dependence of the CD_2 stretching bands of the groups located at positions 3', 7', 8' and 13' of the *sn*-2 chain of DPPC can be analyzed in terms of the fatty acid acyl chain conformational and motional behaviour.

Figs. 44A, 44B and 44C show, respectively, the C-D stretching region of the infrared spectra of fully hydrated samples of DPPC-3'- d_2 , DPPC-7',8'- d_4 and DPPC-13'- d_2 at 5, 30, 38 and 45°C while Figs. 44D, 44E and 44F show plots of $\Delta A/\Delta T$ versus temperature derived from the antisymmetric CD_2 stretching bands at $\sim 2200 \text{ cm}^{-1}$.

At temperatures below T_{pre} the main effect of an increase in temperature on the spectra is to decrease the peak heights. The rates of decrease are small compared with those at the main transition, as shown in the spectra and in the $\Delta A/\Delta T$ plots. The behaviour at T_{pre} is different for these three compounds. For DPPC-3'- d_2 and DPPC-13'- d_2 the

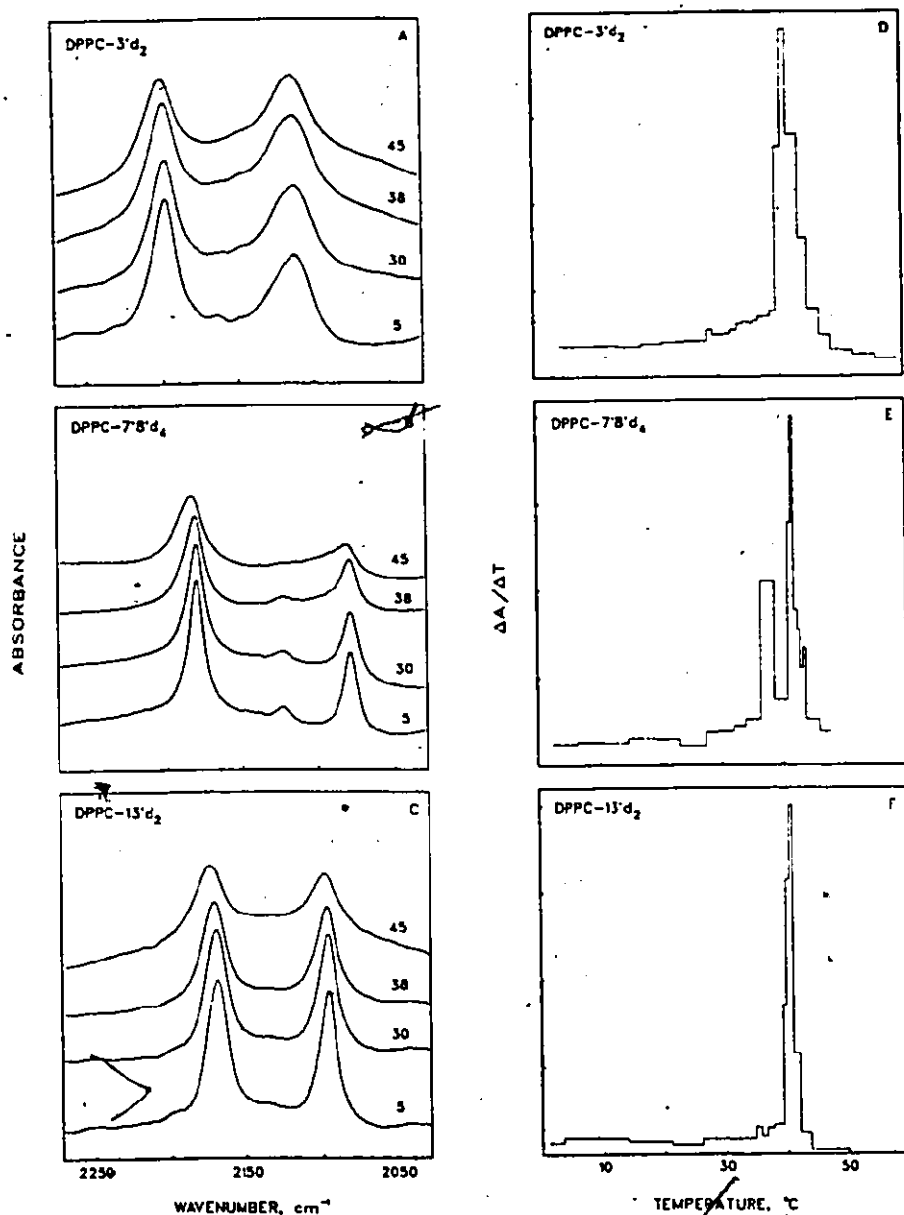


Fig. 44. Temperature dependence of fully hydrated DPPC-3'-d₂; DPPC-7',8'-d₄ and DPPC-13'-d₂; A - C. C-D stretching regions in the spectra of DPPC-3'-d₂; DPPC-7',8'-d₄ and DPPC-13'-d₂ at 5°, 30°, 38° and 45°C. Spectra are from 25 m-thick samples between CaF₂ windows and are plotted at the same scale. The "water-association" band has been removed by subtraction. D - F. ΔA/ΔT vs. temperature plots derived from the CD₂ antisymmetric stretching bands in the spectra of DPPC-3'-d₂; DPPC-7',8'-d₄ and DPPC-13'-d₂.

only effects evident in the temperature range 30 - 40 °C are slightly enhanced values of $\Delta A/\Delta T$, as compared to the values below 30°C, with slight evidence of a weak feature in the $\Delta A/\Delta T$ plot of DPPC-13'-d₂ in the range 34 - 38 °C.

However, in the spectrum of DPPC-7',8'-d₄, the peak heights of the antisymmetric and symmetric CD₂ stretching bands decrease abruptly at T_{pre} , resulting in the large peak in the $\Delta A/\Delta T$ plot in the range 35 - 37 °C.

At the main transition the spectra of all three compounds show large changes in all band parameters. These changes occur within a narrow temperature range and result in the strong peaks centered at 41.5°C in the $\Delta A/\Delta T$ plots. Above this temperature, in the liquid-crystalline phase, minimal temperature dependencies of the spectra are observed.

The variations in frequency and bandwidths as a function of temperature provide a detailed insight into the thermotropic behaviour of the different segments of the acyl chains. These data are presented in Fig. 45 which shows the temperature dependencies of the bandwidths and frequencies of the antisymmetric modes of DPPC-3'-d₂ (Figs. 45A and 45D respectively), DPPC-7',8'-d₄ (Figs. 45B and 45E respectively) and DPPC-13'-d₂ (Figs. 45C and 45F respectively).

As shown in Table XIV, in all cases the frequencies of the antisymmetric and symmetric CD₂ stretching modes of the dry and hydrated phosphatidylcholines agree to within

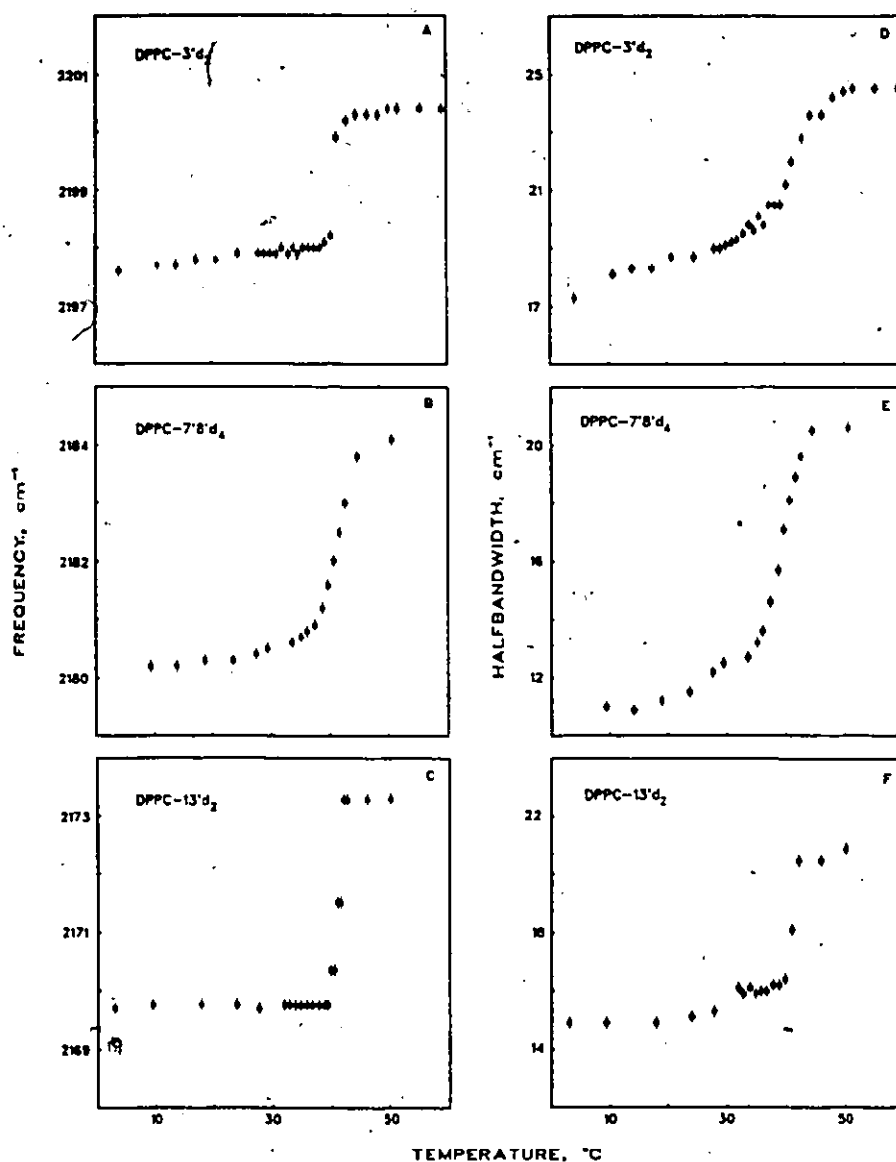


Fig. 45: A - C. Temperature dependence of the frequencies of the CD₂ antisymmetric stretching bands in the spectra of DPPC-3'-d₂; DPPC-7',8'-d₄ and DPPC-13'-d₂;

D - F. Temperature dependence of the half-bandwidths of the CD₂ antisymmetric stretching bands in the spectra of DPPC-3'-d₂; DPPC-7',8'-d₄ and DPPC-13'-d₂.

$\pm 0.3 \text{ cm}^{-1}$. The frequencies also agree to within $\pm 0.3 \text{ cm}^{-1}$ with those of bands in the spectra of the corresponding specifically-deuteriated palmitic acids, excepting the case of palmitic acid-3-d₂ where the large difference results from the stronger inductive effects of the acid group as compared to the ester linkage of DPPC. The bandwidths are also in agreement with those in the spectra of dry films.

Within the temperature range 0 - 38 °C the frequencies of the antisymmetric CD₂ stretching bands are almost invariant. Only slight, steady increases with increasing temperature are observed, the maximum shift being less than 0.6 cm^{-1} in the case of DPPC-7',8'-d₄. Similar results are evident in the frequency shifts of the symmetric CD₂ stretching bands (Table XIV). Within the same temperature range the bandwidths increase slightly on raising the temperature. There is no evidence of any change associated with the pre-transition in either the frequency or the bandwidth plots.

The above observation may be explained as follows. The match in the frequencies between the anhydrous films, the fatty acids and the hydrated samples in the gel phase indicates that the chains in the three systems are in the same trans conformation since the frequencies of these vibrational modes are sensitive to the introduction of gauche conformers (Sections 3.3.1.; 4.2.1. and reference [75]). X-ray diffraction of fatty acids [78] and DMPC [22] have shown that the chains are all-trans from position 3' to the end and therefore it can be concluded that the acyl chains in DPPC are in the all-trans conformation in the gel

phase.

The slight increases in bandwidth, without concomitant frequency shifts, are indicative of increased rates of motion of the acyl chains as the temperature is increased in the gel phase. These motions are ascribed to librations and torsions about the long axes of the acyl chains.

The spectral changes associated with the pretransition are evident only in the central portion of the acyl chains, and are restricted to an abrupt decrease in the peak height with no change in frequency or bandwidth. This is identical with the behaviour observed in non-deuteriated DPPC at the pretransition (Section 4.2.1.). In Section 3.3. it was shown that the CH_2 stretching modes of n-alkanes undergo an abrupt decrease in peak height in the orthorhombic-hexagonal phase transition without changes in frequency. The pretransition then, produces an abrupt change in the interchain interactions at positions 7' and 8' but not at positions 3' and 13'.

The difference between positions 7' and 8', and positions 13' indicates that at temperatures just below T_{pre} the orthorhombic packing is more rigid in the central area of the chains than in the center of the bilayer. This difference in the degree of interchain interactions is in accord with the concept of the chains undergoing torsional motions about their long axes, with the amplitudes and hence the degree of reduction of interchain interactions being greatest towards the end of the chains. The "twisted" chains found in DMPC dihydrate [22] give support to this argument.

The absence of pretransition effects in the spectrum of DPPC-3'-d₂ indicates that there is no change in interchain interactions in this location. The 2'-3' bond in the sn-2 chain is gauche [22] and this makes the packing of the 3' CD₂ group different from that of the other methylene groups and the lack of changes at T_{pre} may indicate a lack of sensitivity of the group to changes in interchain interactions.

At the main transition, all the C-D stretching bands of DPPC-3'-d₂, DPPC-7',8'-d₄ and DPPC-13'-d₂ show large changes in both frequency and bandwidth, together with decreases in peak height. These changes are the same as those observed at the main transition in the C-H stretching modes in the spectra of non-deuteriated DPPC (Section 4.2.1.3.) and in the spectra of n-alkanes (Section 3.3.1.); they reflect the introduction of conformational disorder in the acyl chains. As the temperature is further increased in the liquid-crystalline phase only slight increases in frequency are evident, similar to those observed in the spectra of DPPC in the liquid-crystalline phase.

4.2.3.3. DPPC-16'-d₃

Fig. 46 shows the C-D stretching region of the infrared spectrum of DPPC-16'-d₃ at 5, 30, 38 and 45 °C. The spectra show extremely small temperature dependencies compared to those shown by the spectra of DPPC-3'-d₂; DPPC-7',8'-d₄ and DPPC-13'-d₂. There is very little change at the main transition.

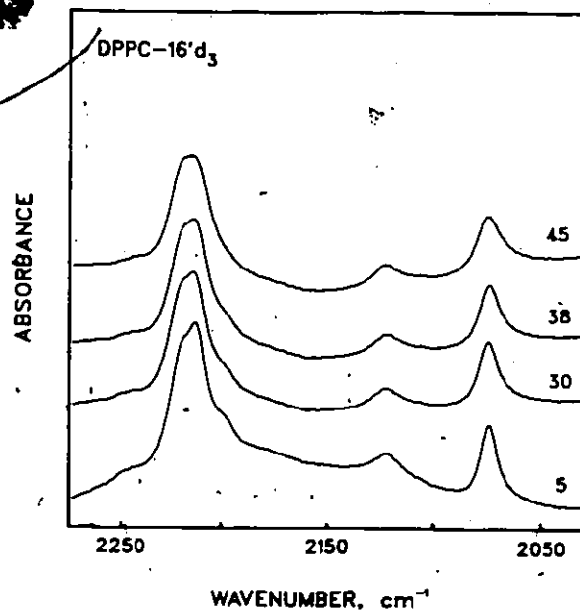


Fig. 46. Carbon-deuterium stretching region of the infrared spectrum of fully hydrated DPPC-16'-d₃ at 5°, 30°, 38° and 45°C. Spectra are from a 25 μm-thick sample between CaF₂ windows and are plotted at the same scale. The "water-association" band has been removed by subtraction.

Plots of the temperature dependence of the peak position and bandwidth of the asymmetric CD_3 stretching band of DPPC-16'- d_3 are shown in Figs. 47A and 47B, respectively. At 5°C the peak maximum is at 2212.2 cm^{-1} , close to the lower frequency maximum in the spectrum of anhydrous DPPC-16'- d_3 (Table XIV). As the temperature is increased the frequency of the band increases, accompanied by a slight broadening. The main transition produces a substantial band broadening and increase in frequency, relative to those observed in the gel phase. Constant values are observed after completion of the main transition.

In the liquid-crystalline phase, the terminal methyl group [75] and the environment in the center of the bilayer are highly disordered. The reduction in bandwidth and decrease in frequency at T_m on lowering the temperature indicates a decrease in the rate of rotation of the methyl group due to restrictions by the hexagonal packing in the gel phase. The same was observed for the methyl group in the spectra of $n\text{-C}_{21}\text{H}_{44}$ on going from the melt to the hexagonal phase (Fig. 19, Section 3.3.1.).

The factors affecting the methyl group as the temperature is reduced in the gel phase are different from those occurring at T_m . Fig. 48 shows the asymmetric CD_3 stretching band of DPPC-16'- d_3 at 5, 20, 30 and 38°C , together with the corresponding difference spectra generated by subtraction of adjacent spectra from each other.

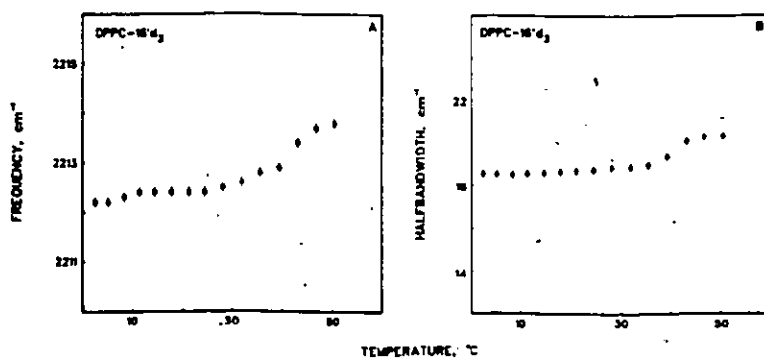


Fig. 47. Temperature dependence of the CD_3 asymmetric stretching mode in the spectra of DPPC-16'- d_3 ; A. frequency vs. temperature; B. halfbandwidth vs. temperature.

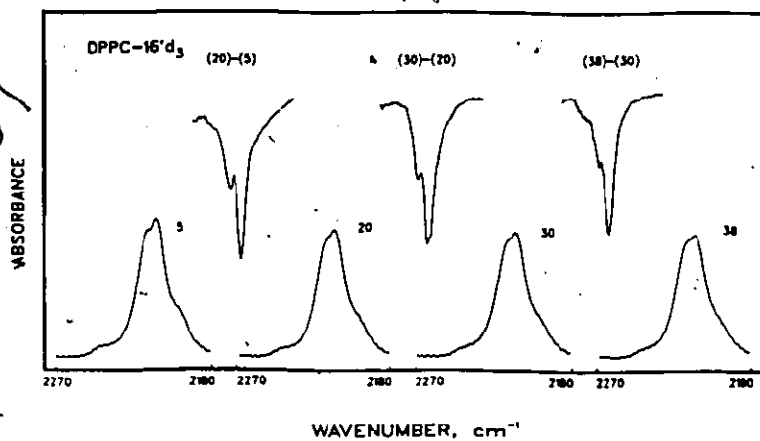


Fig. 48. Progressive motional collapse of the doubly degenerate CD_3 asymmetric mode in the spectra of DPPC-16'-d₃.

Bottom: absorption spectra at 5°, 20°, 30° and 38°C.

Top: Difference spectra obtained by subtraction of adjacent pairs of absorption spectra. All difference spectra are plotted to the same size, hence absolute comparisons of spectral changes can not be made.

At all temperatures in the gel phase the asymmetric CD_3 stretching band is comprised of two overlapped peaks. As the temperature is reduced, the frequency separation of the peaks increases. This results in the two minima in the difference spectra, even in that obtained by subtraction of the spectra recorded at 30°C from that at 38°C . Although the two peaks are not completely resolved at 5°C the splitting is about 4 cm^{-1} and reduction of the temperature to -50°C resulted in a splitting of about 6 cm^{-1} .

As noted in Section 3.2.1. splitting of the doubly degenerate methyl asymmetric stretching mode is related to the rate of rotation of the methyl group relative to the adjacent methylene group. The high frequency mode is the "in-skeletal-plane" stretching and the low frequency one is the "out-of-plane" stretching [60,92]. As mentioned earlier if the rate of rotation is sufficiently slow, two bands are observed and an increase in the rotation produces the collapse of the two bands into a single peak with the rate of rotation being of the order of 10^{12} s^{-1} [69]. The progressive splitting observed in Fig. 48 as the temperature is lowered indicates a decrease in the rate of rotation of the CD_3 group on a time scale of about one picosecond.

Since splitting of the CD_3 asymmetric stretching mode is an intrachain property it cannot be directly related to the packing of the acyl chains; however, as shown in Section 3.3.1., it gives an indirect indication of the rigidity of the crystal packing and in this case provides an

indication of a progressive reduction in the mobility of the acyl chains.

The reduction in the rate of rotation of the terminal methyl group can be correlated with the ^2H NMR studies of Davis [29] on DPPC- d_{62} . Although the ^2H NMR signals from the methylene groups of DPPC- d_{62} are superimposed in the spectra of the gel phase, the signal from the terminal methyl groups is readily distinguished. Quadrupole splittings of 12.2, 14.9 and 17.3 kHz were observed at 20, 2 and -7°C . These values may be compared with values of 15 and 36 kHz for the CD_3 signals in the ^2H NMR spectrum of $n\text{-C}_{19}\text{D}_{40}$ [93] in the hexagonal and orthorhombic phases respectively. It is evident that as the temperature is reduced the quadrupole splitting progresses steadily from a value near that of the hexagonal phase of $n\text{-C}_{19}\text{D}_{40}$ towards the value obtained in the orthorhombic phase. However, the value obtained for the quadrupole splitting at -7°C indicates that at this temperature there are motions affecting the CD_3 groups. The separation between the components of the methyl asymmetric stretching at this temperature is also indicative of a non-rigid methyl group; and the frequency separation between components of the CH_2 scissoring band in DPPC (Section 4.2.1.1.) is indicative of chains undergoing motions with respect to each other. Therefore, these ^2H NMR results give an indirect confirmation of the nature of the pretransition in DPPC bilayers.

CHAPTER 5. *ACHOLEPLASMA LAIDLAWII* MEMBRANES.

5.1. General Characteristics.

The microorganism *Acholeplasma laidlawii* is an appropriate system for membrane studies. The limiting plasma membrane is the only membranous structure and can be readily isolated from the rest of the organism through osmotic lysis [94]. The possibility of varying the fatty acyl group composition [39] has been used to study the role of lipids in the membrane structure and functions.

The ability of the organism to grow under several different conditions such as temperature, and presence of fatty acid synthetic inhibitors has been studied [39,95]. The lipid:protein ratio shows a marked dependence on the lipid composition [44].

The constituent lipids of these membranes belong to the following classes: glycolipids (monoglucosyldiglycerides and diglucosyldiglycerides), phospholipids (phosphatidylglycerol and diphosphatidylglycerol), phosphoglycolipids (glycerophosphoryl-diglucosyldiglycerides and glycerophosphoryl-monoglucosyldiglycerides) [96,97].

In the following sections the results from an infrared study of the phase transition in *A. laidlawii* membranes are given. Two membrane preparations were used: one in which the organism was grown in the presence of palmitic acid- d_{31} , the other in which the organism was grown in a me-

dium supplemented with pentadecanoic acid- d_{29} in the presence of avidin. In both cases the growth temperature was 37°C .

When the organism is grown in the presence of fatty acids it incorporates them into the glycerolipids of the plasma membrane without alteration; in this manner the membrane is enriched in a particular class of fatty acid. The C-D stretching vibrations of the deuteriated fatty acyl chains provide a direct probe of the membrane thermotropic behaviour. The C-D stretching region is free from other vibrations of the membrane components.

In both cases the results obtained from intact membranes are compared with those of the membrane lipids after separation of the proteins.

5.2. Membranes Enriched in $\text{C16:0-}d_{31}$.

Fig. 49 shows the spectra of *A. laidlawii* $\text{C16:0-}d_{31}$ membranes, the isolated lipids and the model compound DPPC- d_{62} (DPPC with fully deuteriated acyl chains).

In the spectrum of *A. laidlawii* $\text{C16:0-}d_{31}$ intact membranes (Fig. 49A) the carbon-deuterium stretching bands are evident in the $2400 - 2000 \text{ cm}^{-1}$ region. The spectrum is dominated by protein bands. Strong amide A and B bands appear at 3300 and 3100 cm^{-1} . Amide I and II bands appear at 1650 and 1530 cm^{-1} , respectively; their frequencies and relative intensities indicate that the dominant conformation adopted

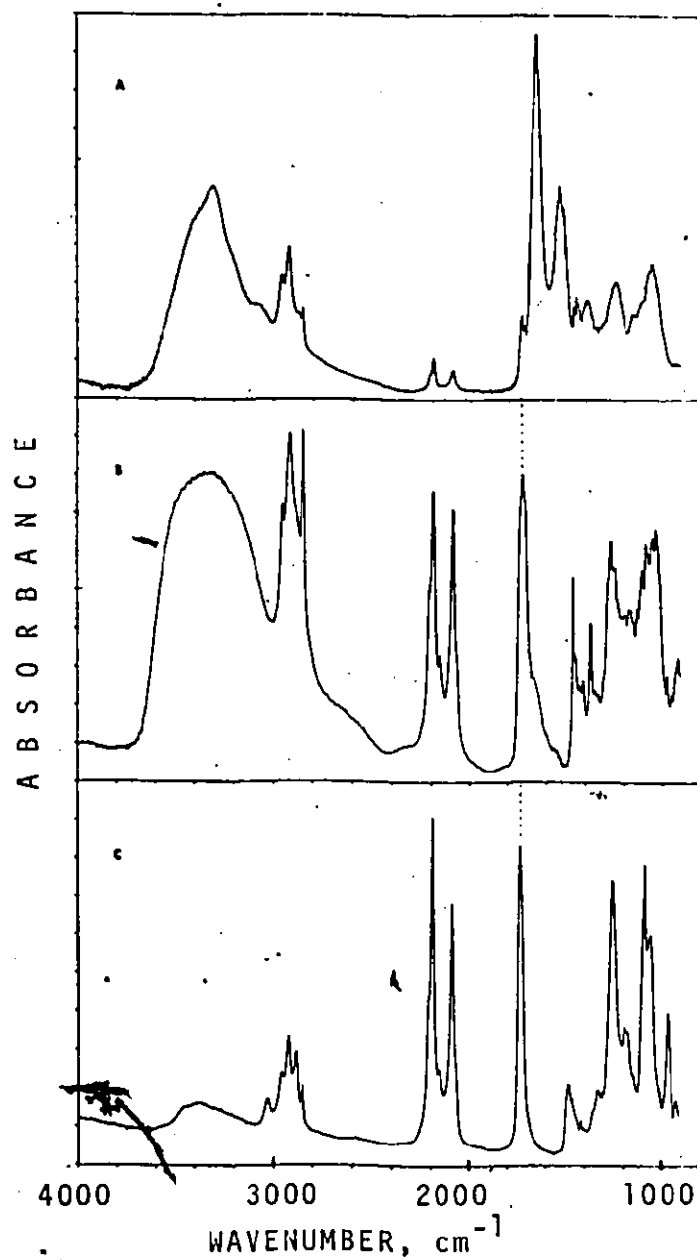


Fig. 49. Infrared spectra of solid unhydrated samples of: A. intact *A. laidlawii* C16:0-d₃₁ plasma membranes; B. deproteinated *A. laidlawii* C16:0-d₃₁ plasma membranes; and C. DPPC-d₆₂.

by the *A. laidlawii* membrane proteins is a helical (amide I, 1650 cm^{-1}) with some random coil structure (amide II, 1535 cm^{-1}) [98,99]. There is no evidence of β structure. A strong C-H stretching band characteristic of proteins can be observed at 2978 cm^{-1} [100].

The spectrum of the lipid component of the membranes is shown in Fig. 49B. The protein bands have disappeared and the C-D stretching bands are the strongest in the spectrum; the antisymmetric and symmetric CD_2 stretching modes are observed at 2194 and 2089 cm^{-1} , respectively, with the terminal CD_3 modes appearing at 2212 and 2169 cm^{-1} . The two CH_2 stretching modes at 2920 and 2850 cm^{-1} and the CH_2 scissoring mode at 1468 cm^{-1} resulting from the non-deuteriated lipid components are characteristic of highly-ordered acyl chains (Sections 3 and 4) [101,102]. The carbonyl band (1736 cm^{-1}) of the ester linkage in the head-groups is very strong, whereas in Fig. 49A it was only evident as a weak shoulder on the amide I band. In the fingerprint region there are many sharp bands between 1400 and 1000 cm^{-1} , typical of solid state carbohydrate spectra [103] from the mono- and diglucosyl lipids. The O-H stretching band at 3400 cm^{-1} can also be associated with the carbohydrate components, although it is partly due to residual water, as indicated by the weak H_2O bending band at 1640 cm^{-1} .

Fig. 49C shows the spectrum of DPPC- d_{62} already discussed in Section 4.1. The C-D stretching bands and the carbonyl band are almost identical with those in the spectrum

of the *A. laidlawii* lipids.

The spectra in Fig. 49 illustrate the general features of the spectra of intact and deproteinated *A. laidlawii* plasma membranes compared to those of a single phospholipid. However, in order to monitor their biologically-relevant properties, these membranes preparations were studied in the presence of a large excess of water. The spectra in the C-D stretching region of such hydrated samples are shown in Fig. 50. The "water-association" band appears in this region (Section 2.3.3., Fig. 6) and it is evident in Fig. 50A, the spectrum of fully hydrated *A. laidlawii* intact membranes. The C-D stretching bands are evident only as weak features on top of the broad water band, most other spectral regions are opaque to infrared radiation. As shown in Fig. 50B, the removal of proteins, and the consequent change in the proportion of lipids in the sample, produces a spectrum with considerable less interference from the water background. In the case of DPPC-d₆₂ multilamellar dispersions (Fig. 50C) the water band is even weaker and is evident only as a slight background curvature.

5.2.1. Absorbance Changes.

The temperature-induced absorbance changes in the CD₂ stretching bands were monitored by measuring the parameter $\Delta A/\Delta T$ versus temperature. The results of these measurements are shown in Fig. 51 for the three systems. The thermal response of DPPC-d₆₂ in Fig. 51C is typical of this model membrane and can be compared with that observed

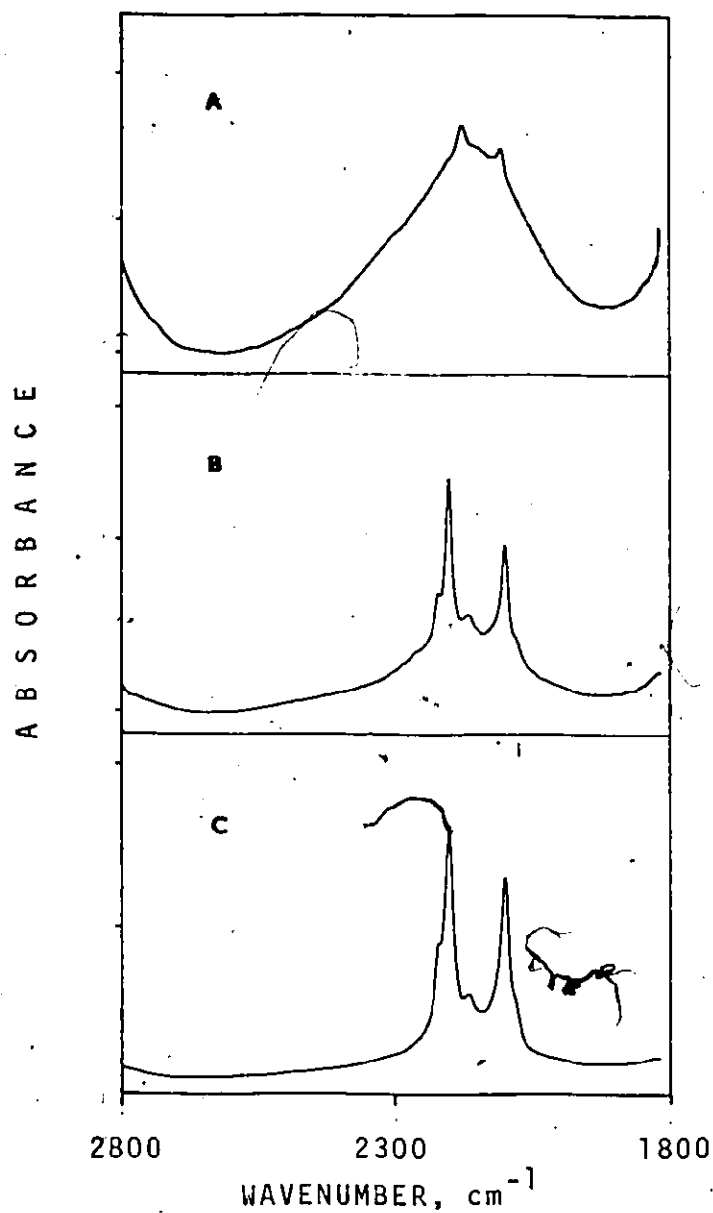


Fig. 50. Infrared spectra of fully hydrated samples of:
 A. intact *A. laidlawii* C16:0-d₃₁ plasma membranes
 (50 μ m sample); B. deproteinated *A. laidlawii* C16:0-d₃₁
 plasma membranes (25 μ m sample); C. DPPC-d₆₂ (12 μ m sample).

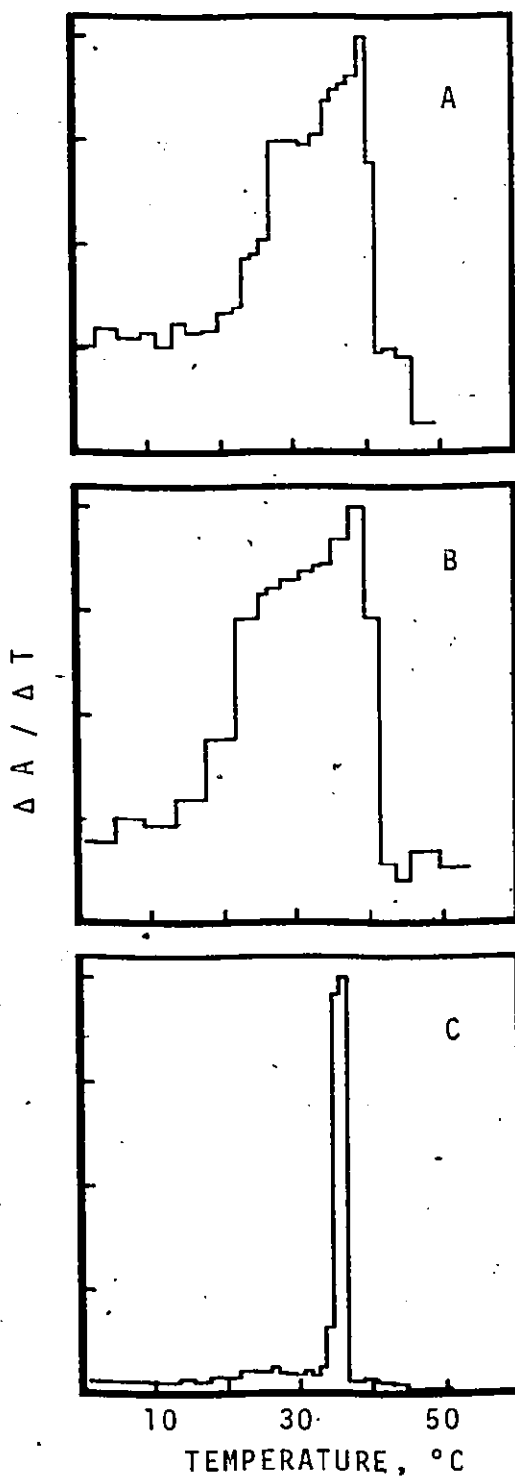


Fig. 51. $\Delta A / \Delta T$ vs. temperature plots derived from the CD_2 symmetric stretching bands in the spectra of fully hydrated: A. intact *A. laidlawii* C16:0- d_{31} membranes; B. deproteinated *A. laidlawii* C16:0- d_{31} membranes; C. DPPC- d_{62} .

for DPPC (Section 4.2.1.3., Fig 39). A maximum rate of change for DPPC- d_{62} is observed in a narrow temperature range centered around 37°C with minor perturbations observed before and after the gel-to-liquid-crystal phase transition.

In the cases of intact (Fig. 51A) and deproteinated (Fig. 51B) *A. laidlawii* membranes, changes are observed over a much wider temperature range. The $\Delta A/\Delta T$ plots show that the transition has the form of a ramp. Although intact and deproteinated membranes were previously studied by DSC [104] it was not evident from such studies that there are subtle differences between the two systems. This is apparent in the plots of Fig. 51, parts A and B, the $\Delta A/\Delta T$ values are identical above and in the region of the growth temperature. Below this temperature, in the case of the intact membranes, the rate of change decreases steadily and achieves a constant value at 22°C. The plot obtained from the deproteinated membrane is more plateau-like. The total width of the phase transition in deproteinated membranes is also slightly greater, the onset occurring at about 15 - 18 °C compared to 22°C in the case of the intact membranes.

5.2.2. Frequency Shifts.

Shifts in the frequency of C-H and C-D stretching vibrational modes can be used to detect the introduction of conformational disorder in ~~acyl~~ systems as it was shown in Sections 3.3.1. and 4.2.1.3. In a given physical state the C-D stretching bands are observed at the characteristic

frequencies of the state regardless of the terminal functional groups and they may shift only slightly as a result of interactions with molecules of a different class.

The temperature dependence of the frequency of the symmetric CD_2 stretching mode is shown in Fig. 52 for the three systems. Below 15°C the frequencies of both intact and deproteinated membranes are 0.5 cm^{-1} higher than those observed in the model membrane DPPC- d_{62} . Above 15°C changes are observed in all three systems. In the case of DPPC- d_{62} there is no change in frequency with increasing temperature until the main transition when there is a large shift to higher frequency. This behaviour is the same as the one found for DPPC in Section 4.2.1.3. (Fig. 39).

The total temperature range over which the frequencies are observed to change is $28 - 41^\circ\text{C}$ in the case of intact membranes and $22 - 41^\circ\text{C}$ for the deproteinated membranes. However, the rate of change is not constant within this temperature range; it increases with temperature, such that 80 % of the total change in the case of intact membranes and 70 % with deproteinated membranes occur within the range $35 - 40^\circ\text{C}$.

The frequencies of the CD_2 bands in the liquid-crystalline phase reflect the average number of gauche conformers introduced into the systems by the phase change. A large variation is observed among the three systems; intact membranes have the highest frequency, the deproteinated membranes are 1 cm^{-1} lower and the model membrane is 1 cm^{-1} lower.

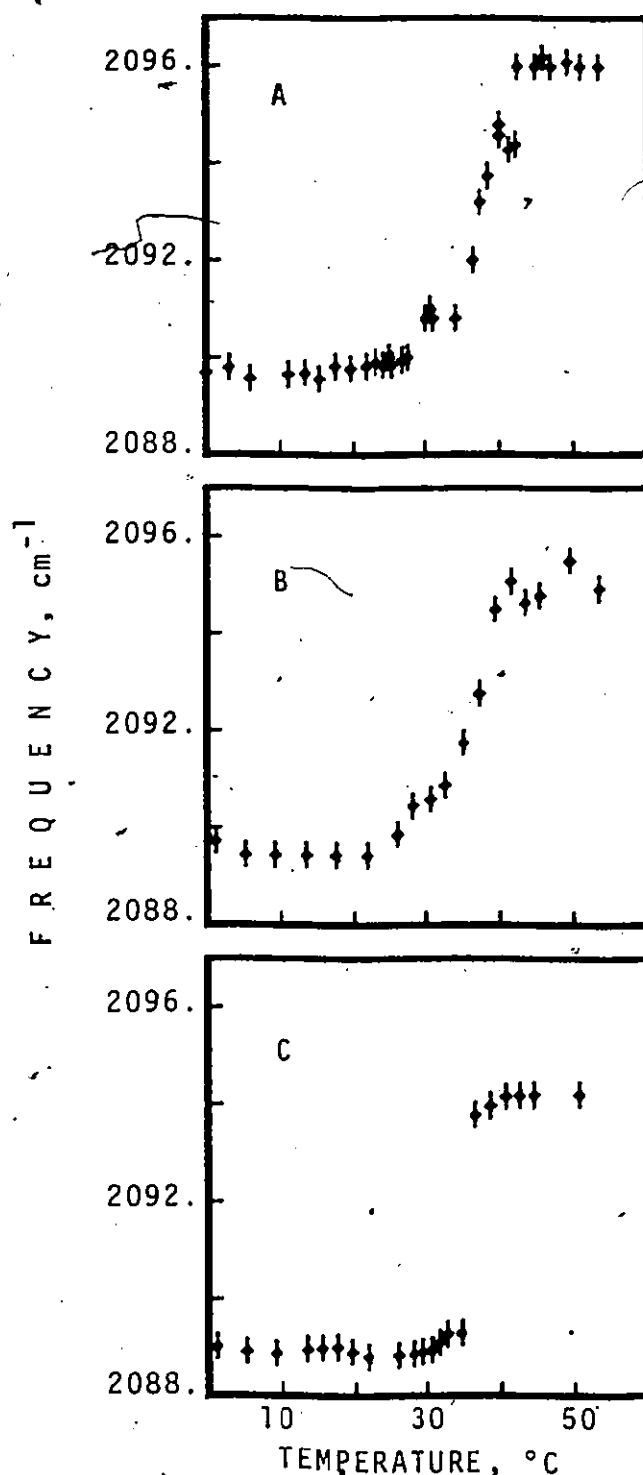


Fig. 52. Temperature dependence of the frequency of the CD_2 symmetric stretching modes in the spectra of fully hydrated: A, intact *A. laidlawii* C16:0- d_{31} membranes; B, deproteinated *A. laidlawii* C16:0- d_{31} membranes; C, DPPC- d_{62} .

wer again.

5.2.3. Halfbandwidth Variations.

The width of C-H and C-D stretching bands are sensitive to motional effects. In Section 3.3.1. it was shown that the halfwidths of the C-H stretching bands increase during the "rotator" phase transition as a result of the increased freedom of the chains in the hexagonal packing. This broadening occurred without concomitant shifts in frequency of the same modes. The bands also broadened considerably on melting of the acyl chains.

Thus, the bandwidth monitors the freedom of motion of the absorbing group, i.e. the amplitudes and rates of motion within its immediate environment.

In the case of *A. laidlawii* membranes the bandwidths will reflect the main thermal transition where a considerable change in the environment occurs. It is also sensitive to other changes which do not introduce gauche conformers, such as a decreased freedom of librational or torsional motion of the chains.

The plots showing the halfbandwidths of the symmetric CD₂ stretching modes as a function of temperature are given in Fig. 53 for the three cases. In the gel phase the halfbandwidths of the intact and deproteinated membranes are identical from 0 to 15 °C, increasing slowly with increasing temperature. In the model system a slightly lower value was found at 0°C, but between 10 and 15 °C the bandwidth is iden-

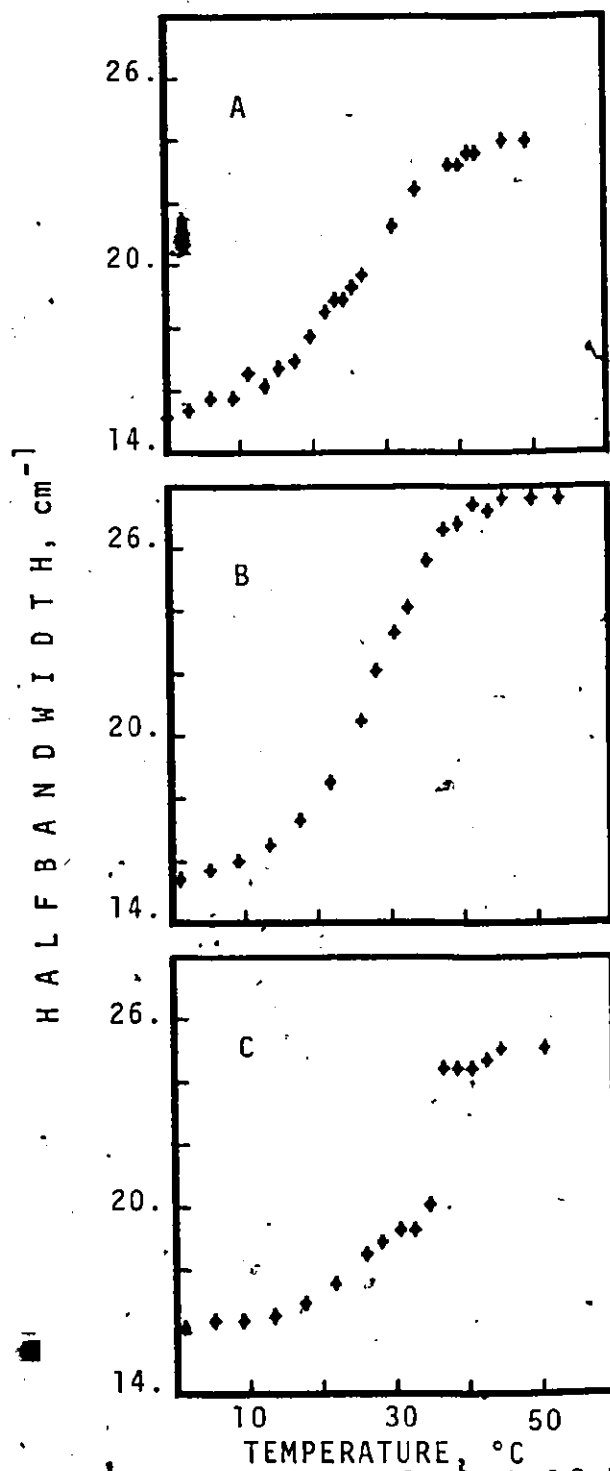


Fig. 53. Temperature dependence of the halfwidth of the CD₂ symmetric stretching bands in the spectra of fully hydrated A. intact *A. laidlawii* C16:0-d₃₁ membranes; B. deproteinated *A. laidlawii* C16:0-d₃₁ membranes; C. DPPC-d₆₂.

tical with the other two systems. Above 15°C the bandwidth in the model system shows a steady increase followed by a large change at the main transition. Above this temperature the width remains constant.

In the temperature range 20 - 40 °C the halfbandwidth of intact and deproteinated membranes change linearly, indicating a continuous variation in freedom of motion. However, in the case of deproteinated membranes the bandwidth increases more rapidly than in the intact membranes. This results in a difference in halfbandwidth of 2 cm^{-1} at 30°C and 4 cm^{-1} in the liquid-crystalline phase.

While the greater part of the frequency shift was observed to occur between 35 and 40 °C, the halfbandwidth did not show a sharp increase in that range. In fact, in both systems there is a greater change in bandwidth in the range 20 - 30 °C than in the range 30 - 40 °C. This indicates that the phenomenon occurring between 20 and 40 °C is not just a change in conformational equilibrium of the acyl chains.

5.2.4. Effect of Temperature.

From the close agreement of frequency and halfbandwidth values observed for the three systems below 15°C it can be concluded that the chain conformations, and the motions of the chains are very similar. The gel phase of DPPC (Section 4.2.1.) and DPPC-d₆₂ [29] were shown to be composed of all-trans chains undergoing considerable motion about the long

axes. This indicates that in both the intact and deproteinated membranes the lipids are also in the all-trans conformation in the gel phase. The reduction in halfbandwidth with temperatures approaching 0°C results from reduction of the amplitudes of torsional motions. The same conclusion was reached from ^2H NMR studies [42] where it was found that the profiles of intact *A. laidlawii* membranes at 0 and 22°C closely resembled those of the model membrane DPPC-d₆₂.

This similarity of the model and natural membranes in the gel state is not reflected in the nature either of the phase transition or of the liquid-crystalline phase. There is a clear distinction between the behaviour of DPPC-d₆₂ and the *A. laidlawii* systems in terms of the width of the transition and the values of the spectral parameters in the liquid-crystalline phase.

Although dissimilar to that of the model system, the transition of the intact and deproteinated *A. laidlawii* membranes are similar to one another. The $\Delta A/\Delta T$ plots resemble those obtained by DSC and ^2H NMR; they are ramplike in form, cover a 20 - 25°C temperature range, and terminate abruptly a few degrees above the growth temperature.

This general form of the phase transition has previously been suggested to result from the various proportions of different chain lengths in the lipids of natural membranes. The phase transition is proposed to be a cooperative melting of the acyl chains, with the shorter chains melting at lower temperatures and facilitating the melting.

of the longer chains [105]. If this were the case, the shifts in frequency should be observed simultaneously with an increase in bandwidth. However, almost all the frequency shifts observed for the *A. laidlawii* membranes occur in the range 35 - 40 °C, demonstrating that the melting phenomenon is concentrated in a small range centered at the growth temperature (37°C).

In contrast to the behaviour of the peak positions the halfbandwidths change throughout the entire range from 20 to 40 °C and the changes are maximal in a range where only minimal variations in peak positions are found. Thus, in the 20 - 35 °C range the principal phenomenon is an increase in the freedom and rate of motion of the chains without a concomitant large increase in the number of gauche conformers. In the range 35 - 40 °C, the change is primarily associated with the melting phenomenon (introduction of gauche conformers).

The increase in motional freedom in the range 20 - 35 °C must result from a more rapid and greater amplitude of reorientation about the acyl chain long axes and a decrease in the packing of the acyl chains, i.e., the restrictions imposed by forming a regular close-packed matrix. This is in accord with the behaviour observed for the C-H stretching bands in $n\text{-C}_{21}\text{H}_{44}$ (Section 3.3.1.).

The loosening of the lipid matrix leads to a condition where gauche conformers are introduced, as evidenced by the

frequency shifts and in the range 35 - 40 °C a change in the conformer population is the main phenomenon.

5.2.5. Effect of Proteins.

The presence of proteins produces no dramatic changes in the thermotropic behaviour of intact membranes on the general form of the phase transition; however, there are some subtle effects.

The presence of proteins delays the onset of the phase transition by 5 - 7 °C. This is particularly clear in the $\Delta A/\Delta T$ plots (Fig. 51) but it is also evident from the frequency and bandwidth data (Figs. 52 and 53). Although there is a delay in the onset of the transition, there is no effect on the temperature at which the transition terminates.

The frequency shift in the temperature range 35 - 40 °C is greater in the case of intact membranes than for the isolated lipids, demonstrating that the presence of proteins results in a greater disordering of the fatty acyl chains (as measured by the increase in the number of gauche conformers) in the liquid-crystalline phase of the intact membranes.

In the spectra of deproteinated membranes the half-bandwidths are greater at all temperatures above 15°C (Fig. 53). This indicates that the proteins limit the total freedom of motion of the lipid acyl chains in the gel phase, during the transition and in the liquid-crystalline phase. This occurs despite the fact that more gauche conformers are introduced in the intact membranes by the phase change.

The immobilization of membrane lipids in the presence of proteins has also been reported from ESR experiments on complexes of DMPC and cytochrome oxidase [106]. However, ^2H NMR studies on the same system indicate that the lipids are more disordered in the presence of protein [107]. The apparently contradictory statements from ESR and ^2H NMR studies have been explained in terms of the different time scales of the two spectroscopic techniques; the contradiction could also arise from the effects of the bulky nitroxide group; spin labels have been shown to give disparate results than ^2H NMR experiments on the same systems [108]. The duality of effects that proteins have on membrane lipids as found in the case of *A. laidlawii* (a restriction of acyl chain mobility accompanied by a greater number of gauche conformers) could also serve as an explanation for contradictory results obtained with other techniques.

5.3. Membranes Enriched in $\text{C}_{15:0}\text{-d}_{29}$

Avidin is a fatty acid synthetic inhibitor, when *A. laidlawii* is grown in its presence it becomes incapable of fatty acid synthesis [109]. If the medium is enriched in a suitable fatty acid, the cells incorporate the exogenous fatty acyl species into their membrane lipids to levels approaching homogeneity (in this case the membranes were 94 % homogeneous in $\text{C}_{15:0}\text{-d}_{29}$, Section 2.1.).

In these fatty acid homogeneous bio-membranes the lipid phase transition has been shown to occur within a narrow temperature range compared with those observed for

non-homogeneous membranes [44]. This demonstrates that lipid-protein interactions do not play a major role in the regulation of the lipid matrix thermal behaviour. In membranes, such as those enriched up to 60 % in a given fatty acid there are two factors operating simultaneously which affect the lipid thermal behaviour; the effects of proteins and the heterogeneity of the lipid pool. In the membranes enriched almost to homogeneity in a given fatty acid the only operating effects affecting the thermal response of the membrane lipids is the presence of proteins and the heterogeneity of the head-group classes [44]. The comparison between the thermal behaviour of non-homogeneous and homogeneous membranes can in principle clarify the ideas about lipid-protein interactions.

Fig. 54 shows the infrared spectrum of lyophilized *A. laidlawii* membranes enriched to 94 % in C15:0-d₂₉. Comparing it with the spectrum of a dry sample of *A. laidlawii* C16:0-d₃₁ membranes (Fig. 49A) shows that there are no dramatic differences between the two, demonstrating that the general membrane characteristics are maintained. Therefore, a comparison between the thermal response of the two membrane preparations is a valid exercise.

The C-D stretching bands are relatively stronger in the spectrum of *A. laidlawii* C15:0-d₂₉ than in the previous case. From the relative intensities and peak positions of the amide I and II bands at 1650 and 1535 cm⁻¹ it can be concluded that the proteins are in the same general conformation.

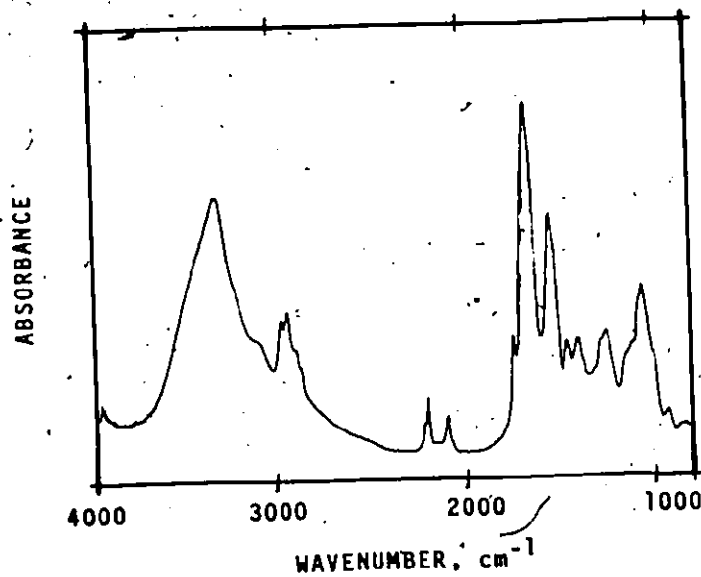


Fig. 54. Infrared spectrum of *A. laidlawii* C15:0-d₂₉ plasma membranes. The sample was a pellet composed of 2 mg membranes and 200 mg KBr.

as in the C16:0-d₃₁ membranes, i.e., α helical with some random coil structure.

The thermal response of intact and deproteinated *A. laidlawii* C15:0-d₂₉ membranes was studied in the presence of a large excess of water. Fig. 55 shows the C-D stretching region in the spectrum of fully hydrated *A. laidlawii* C15:0-d₂₉ membranes at 5 and 45 °C after water subtraction. The antisymmetric and symmetric CD₂ stretching bands appear at 2194 and 2090 cm⁻¹ respectively and the CD₃ bands at 2214 (asymmetric stretching), 2155 and 2070 cm⁻¹ (symmetric stretching) in the spectrum at 5°C. It is evident that all spectral characteristics change drastically in this temperature range because of the phase transition in the membrane lipids.

The deproteinated C15:0-d₂₉ membranes produced an infrared spectrum in the C-D stretching region which is practically identical with the one shown in Fig. 55 for the intact membranes.

5.3.1. Absorbance Changes.

The way in which the spectra change as a function of temperature was monitored via difference spectra and measuring from them the parameter $\Delta A/\Delta T$ versus temperature (Section 2.3.3.). Almost identical results were obtained when monitoring either the antisymmetric or the symmetric CD₂ stretching bands. Therefore, only results obtained for the symmetric CD₂ stretching band are presented.

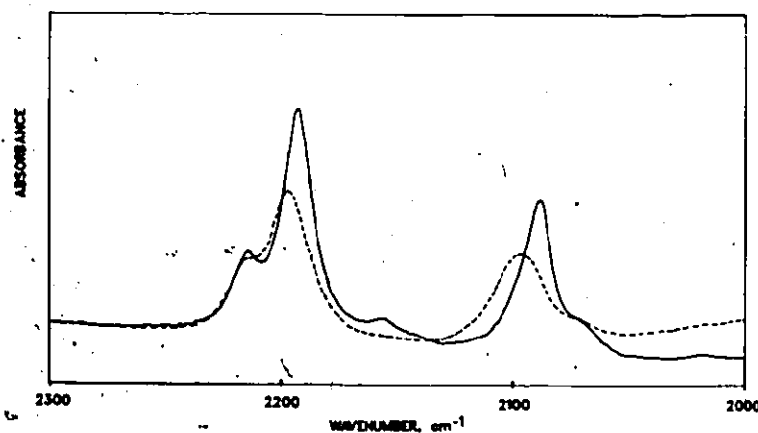


Fig. 55. Infrared spectrum of fully hydrated *A. laidlawii* C15:0-d₂₉ plasma membranes at 5° (solid line) and 45°C (broken line). The sample was 50 μ m-thick between CaF₂ windows. The "water-association" band has been removed by subtraction.

Fig. 56 shows the $\Delta A/\Delta T$ versus temperature plots. Fig. 56A shows the plot obtained from the spectra of the intact membranes and Fig. 56B the plot obtained from the deproteinated membranes.

In the case of intact membranes (Fig. 56A) the transition occurs within a 10°C temperature range (from 30 to 40°C) and it has the same general ramplike form as that observed in the *A. laidlawii* $\text{C}_{16}:0\text{-d}_{31}$ membranes (Fig. 51A). It also terminates abruptly a few degrees above the growth temperature (37°C). The changes observed at all temperatures below 30°C are small compared with those observed in the transition range. After the completion of the transition changes in the bands are minimal.

In the case of the deproteinated membranes (Fig. 56B) the phase transition is seen to occur within a narrow temperature range ($37 - 40^\circ\text{C}$) with very small absorbance changes above 40°C and almost no variation below 35°C as can be seen from a near zero value of $\Delta A/\Delta T$. This plot obtained from the deproteinated membranes is almost the same as that obtained from DPPC- d_{62} (Fig. 51C), reflecting the fact that in this case the membrane lipids are homogeneous in a single class of fatty acid.

A comparison of Figs. 56A and 56B demonstrate that the phase transition in the intact membranes is somewhat broader than in the deproteinated membranes. This contrasts with what was observed for *A. laidlawii* $\text{C}_{16}:0\text{-d}_{31}$ membranes

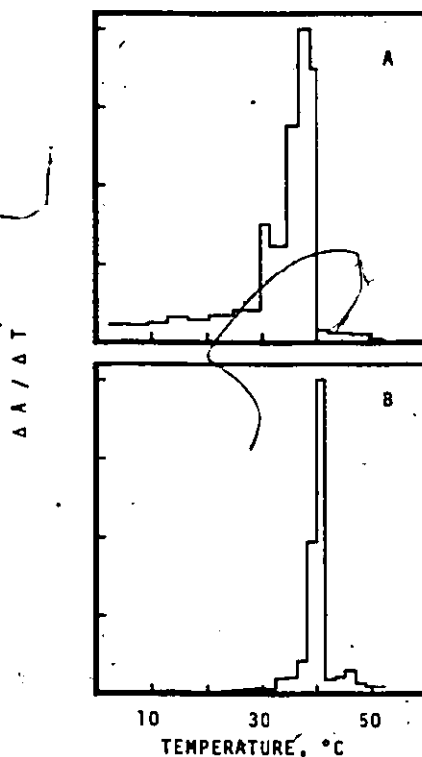


Fig. 56. $\Delta A / \Delta T$ vs. temperature plots derived from the CD_2 symmetric stretching bands in the spectra of fully hydrated: A. intact *A. laidlawii* $C15:0-d_{29}$ plasma membranes; B. deproteinated *A. laidlawii* $C15:0-d_{29}$ membranes.

(Figs. 51A and 51B). The changes observed in the gel phase (below 30°C) for the C15:0-d₂₉ intact membranes are larger than those for the deproteinated membranes indicating that in the intact membranes there are thermal effects perturbing the acyl chain packing. The transition ends at the same temperature in the two cases confirming that membrane proteins do not alter the transition temperature.

5.3.2. Frequency Shifts.

As in the case of *A. laidlawii* C16:0-d₃₁ membranes the frequency of the CD₂ stretching modes can be used to monitor the introduction of conformational disorder in the acyl chains. The frequency value in the liquid-crystalline phase is an indirect measure of the number of gauche conformers in the acyl chains.

Figs. 57A and 57B show the temperature dependence of the frequency of the symmetric CD₂ stretching modes in the spectra of intact and deproteinated *A. laidlawii* C15:0-d₂₉ membranes respectively.

In the case of intact membranes (Fig. 57A) the frequency of the band is invariant at all temperatures in the gel phase and shifts abruptly to higher frequencies at 35°C. The shift ends at 40°C and at higher temperatures the frequency remains constant. The range in which the frequency is observed to change is much smaller than in the $\Delta A/\Delta T$ plot (Fig. 56A). It is also smaller than the range observed in the case of C16:0-d₃₁ membranes (Fig. 52A).

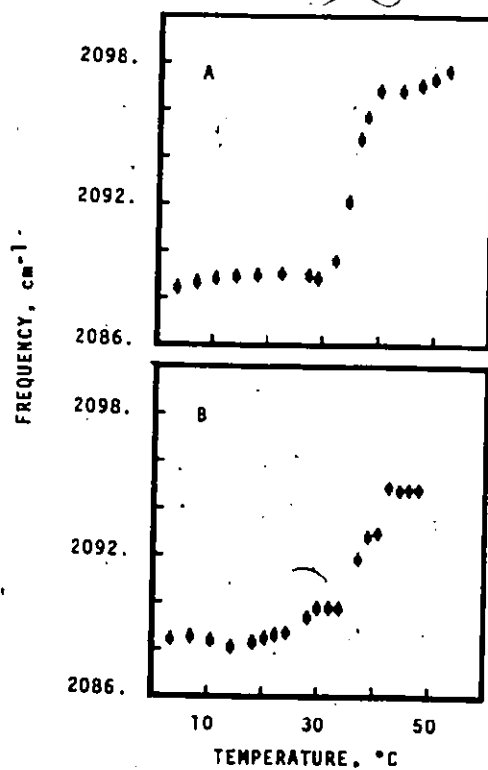


Fig. 57. Temperature dependence of the frequency of the CD₂ symmetric stretching modes in the spectra of fully hydrated: A. intact *A. laidlawii* C15:0-d₂₉ plasma membranes; B. deproteinated *A. laidlawii* C15:0-d₂₉ membranes.

The frequency of the symmetric CD_2 stretch in the spectra of deproteinated C15:0-d_{29} membranes (Fig. 57B) is also invariant at all temperatures in the gel phase and shifts to higher frequency at 37°C coinciding with the onset of the phase transition according to the $\Delta A/\Delta T$ plot (Fig. 56B). The frequency is constant at temperatures above 40°C .

The frequency value in the liquid-crystalline phase in the spectra of deproteinated membranes is lower than for intact membranes coinciding with the case of C16:0-d_{31} membranes (Figs. 52A and 52B). The difference is $\sim 2 \text{ cm}^{-1}$ in the C15:0-d_{29} membranes and $\sim 1 \text{ cm}^{-1}$ in C16:0-d_{31} membranes.

5.3.3. Halfbandwidth Variations.

The halfwidths of the CD_2 symmetric stretching bands were monitored as a function of temperature and the results of their temperature dependence are plotted in Fig. 58. Fig. 58A for the case of intact and Fig. 58B for the case of deproteinated C15:0-d_{29} membranes.

In Fig. 58A it can be seen that the band broadens continuously from 10 to 40°C . However, the rate of increase in halfbandwidth is not constant, there is an inflection point at approximately 32°C and the rate of change is higher in the $32 - 40^\circ\text{C}$ range than in the $10 - 30^\circ\text{C}$ range. After completion of the transition at 40°C the halfbandwidth remains constant. This behaviour is not identical with that

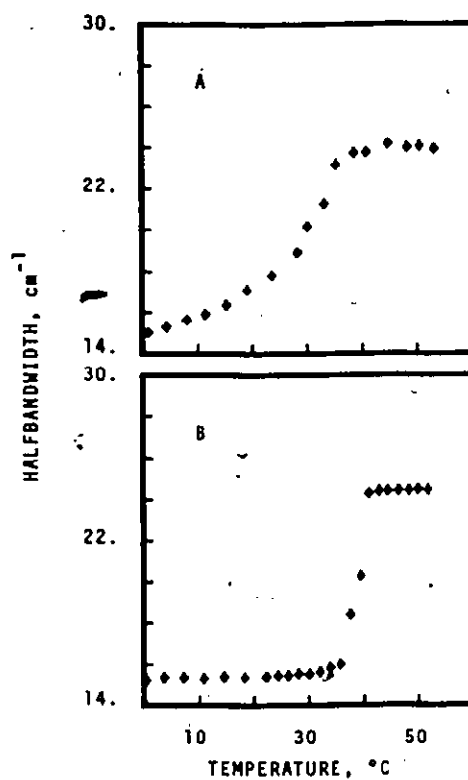


Fig. 58. Temperature dependence of the halfwidths of the CD_2 symmetric stretching bands in the spectra of fully hydrated: A. intact *A. laidlawii* $\text{C}_{15:0}\text{-d}_{29}$ plasma membranes; B. deproteinated *A. laidlawii* $\text{C}_{15:0}\text{-d}_{29}$ membranes.

observed for C16:0-d₃₁ where the halfbandwidth values were observed to change almost linearly in the 20 to 40 °C temperature range.

In the case of deproteinated C15:0-d₂₉ membranes (Fig. 58B) the halfbandwidths are almost invariant with temperature until 37°C where a large increase is observed. The values of halfbandwidth are constant after completion of the phase transition. In the liquid-crystalline phase they are slightly larger (0.5 cm^{-1}) than those observed for the intact membranes (Fig. 58A).

The behaviour of the bandwidths in the deproteinated C15:0-d₂₉ membranes contrasts with that of the deproteinated C16:0-d₃₁ membranes (Fig. 53B). In that case, the halfbandwidths were observed to change over the entire 20 - 40 °C temperature range and no sharp increase could be detected.

5.3.4. Discussion.

From the $\Delta A/\Delta T$ vs. temperature plots for these fatty acid-homogeneous membranes it is seen that the phase transition occurs within a narrow temperature range (10°C for intact membranes and 3°C for deproteinated membranes), which is in accord with DSC measurements [44].

This narrowing of the phase transition in lipid-homogeneous membranes demonstrates that most of the broadening observed in non-homogeneous membranes results from the lipid heterogeneity (acyl chain and head-group heterogeneity). The deproteinated C15:0-d₂₉ membranes are at least 90 % homogeneous and the residual broadening observed

in their transition (3°C , Fig. 56B) indicates the effects of the head-group heterogeneity.

In contrast to the Cl6:0-d_{31} enriched membranes the phase transition for the Cl5:0-d_{29} membranes was observed to be broader in the intact membranes as compared to the deproteinated ones. This indicates that there is a broadening effect originating from the presence of proteins. In the case of Cl6:0-d_{31} membranes it seems then that the fatty acyl chain heterogeneity (length and degree of unsaturation) predominates and produces a broader phase transition. This residual broadening of proteins on lipid phase transitions has been observed with preparations of glycophorin and pure DPPC and DPPC-d_{62} [110].

As in the case of Cl6:0-d_{31} enriched membranes, the transition is also centered at the growth temperature (37°C). This can indicate that the phase transition has specific physiological implications serving as a regulatory mechanism since enzymatic activity has been found to depend critically on the lipid phase behaviour [40]. However, it has been found with *A. laidlawii* membranes enriched in a variety of different fatty acids that the phase transition temperature bears no direct relation with the growth temperature [95]. In the case of outer membranes from *E. Coli* the phase transition temperature was found to depend on the growth temperature but the cytoplasmic membranes showed no effect in their transition temperature with growth temperature [111]. There is no consensus then as to whether the growth temperature

determines the phase transition temperature. In the present case of *A. laidlawii* membranes the same transition temperature was found for C16:0-d₃₁ and C15:0-d₂₉ membranes, this coincidence may result from the close similarity of the fatty acyl chains.

The behaviour of the frequencies for the C15:0-d₂₉ membranes is very similar to that observed for C16:0-d₃₁ membranes. The temperature range of the frequency shifts is very narrow in accord with a more homogeneous lipid pool. The effect of proteins seems to be the same, the intact membranes show a greater concentration of gauche conformers (higher frequency values in the liquid-crystalline phase). However, the difference in frequency is larger (2 cm^{-1}) in the case of the C15:0-d₂₉ membranes, indicating that the homogeneity of the lipid matrix has an "ordering" effect on the acyl chains compared to the C16:0-d₃₁ deproteinated membranes.

The close agreement between the frequencies in the gel phase points to a similar conformation for the acyl chains and indicates that the presence of proteins does not alter the frequency of the CD₂ stretching modes.

The behaviour shown by the halfbandwidths in the C15:0-d₂₉ membranes is in contrast to that observed for C16:0-d₃₁ membranes. In the case of the intact membranes the general freedom of motion of the acyl chains varies at all temperatures in the gel phase whereas in the deproteinated membranes it remains constant (Figs. 58A and 58B).

In the case of C16:0-d₃₁ membranes it was concluded that the freedom of motion was greater in the deproteinated membranes. This apparent contradiction may be explained by the homogeneity of the lipid pool.

In the deproteinated C15:0-d₂₉ membranes the acyl chains can pack in a well defined lattice with the inter-chain interactions being maximal. The small percentage of chains with different chain lengths or unsaturations can pack as impurities creating a small concentration of defects that will not destroy the interchain interactions. The deproteinated membranes will then behave as a pure lipid showing a rigid packing in the gel phase. The thermal behaviour is thus almost identical with that observed for the model membrane DPPC-d₆₂.

Membrane proteins are seen to have only minor effects on lipid thermal transitions. The presence of proteins seems to increase the number of gauche conformers in the liquid-crystalline phase, probably through stabilization of the lipid preferred conformations. In the gel phase they seem to have an immobilizing effect on chain motions but membranes enriched in C15:0-d₂₉ seem to indicate the opposite.

CHAPTER 6. CONCLUDING REMARKS.

6.1. Fourier-Transform Infrared Spectroscopy.

From the context of this work it is apparent that the nature of spectral studies on bio-membranes require spectra with good signal -to-noise ratios and reproducibility. Fourier-transform infrared spectroscopy has been presented as a technique with great potential in the study of bio-membrane systems including natural membranes.

Fourier-transform infrared spectroscopy presents a series of advantages over conventional dispersion infrared spectroscopy. These advantages arise from the use of the Michelson interferometer and the possibility to use state of the art cooled detectors and have been extensively discussed in the literature [112,113].

The principal advantages are: multiplexing or Fellgett's advantage, which is the time saving gained by taking all the resolution elements in the spectrum at once. Derives from the fact that every point in the interferogram contains information about every point in the spectrum.

The high energy throughput or Jacquinot's advantage relates to the amount of energy reaching the detector; this advantage is of particular importance in energy limited cases [114].

A third advantage is frequency scale accuracy or Connes' advantage [115]. The wavenumber scale of an FT-IR spectrometer is determined from the fringes produced from a HeNe laser

making it accurate to within 0.005 cm^{-1} . The reproducibility of the wavenumber scale is 0.01 cm^{-1} . This allows the monitoring of extremely small frequency shifts in spectra with high signal-to-noise ratios.

An added advantage is the dedicated computer required to drive the instrument, this allows for self re-calibration and feedback loops giving effective stabilities much higher than the built-in ones [116]. The computer also adds the advantage of extreme simplification of the data handling.

In the case of this study, difference spectra provided an excellent tool for monitoring phase changes, not only to subtract the solvent bands but also through the determination of changes in absorbance and intensity, as exemplified in Section 2.3.3. Difference spectra were also used to detect the presence of weak and unresolved bands like the components of the methylene wagging band progression.

The splitting in the CH_2 scissoring and rocking bands in the spectrum of DPPC were first found via difference spectra, as was the separation of the asymmetric methyl stretching mode in the spectra of $\text{DPPC-16}'\text{-d}_3$. This type of difference spectroscopy is not subject to the multitude of errors encountered in spectral stripping [117,118], whereby the spectrum of one component from a mixture is obtained by subtracting the spectra of the other components. The subtraction of the "water-association" band was seen not to produce considerable distortions in the C-D stretching bands. This results from the difference in bandwidth between the subtracted and subtracting bands (Section 2.3.3.).

6.2. Hydrocarbons.

The solid phase behaviour of n-alkanes was used to characterize spectral effects arising from changes in crystal packing and molecular motions. This characterization allowed a comparison with spectral effects observed in the thermotropic mesomorphism of lipid bilayers.

From the study of the n-alkane spectra it is concluded that the "rotator" phase transition results in changes of different nature in different spectral regions. The presence of conformational disorder in the hexagonal phase cannot be ruled out from the data. However, most of the observations point to conservation of the molecular conformation on going from the orthorhombic subcell to the hexagonal in accord with the results of Raman spectroscopic studies on $n\text{-C}_{19}\text{H}_{40}$ [57]. Some observations point to the introduction of non-planar conformations in the hexagonal phase in accord with small angle X-ray diffraction studies on $n\text{-C}_{33}\text{H}_{68}$ [56]. The explanation for this apparent contradictory information could be that in the hexagonal phase the chains perform torsions and not only rotations like rigid rods; thus, the conformations will have a slight non-planar nature while maintaining an overall trans character. In this respect, a considerable amount of information could be obtained studying specifically-deuteriated hydrocarbons and obtaining quantitative dynamical information from an analysis of the band shapes [119]. This is very difficult in the case of large molecules like hydrocarbons but the ever-increasing capability

of computers and spectroscopic methods will lessen this difficulty in the near future.

6.3. Bilayers of DPPC.

The infrared spectrum of DPPC was assigned with the aid of the spectra of some specifically-deuteriated derivatives.

The thermotropic behaviour of DPPC bilayers was studied in the temperature range -60 to $+60^{\circ}\text{C}$. In accord with previous studies it was concluded that these bilayers exist in distinctly different phases above and below the main transition at 41.5°C . The main transition was confirmed as a melting of the acyl chains, and evidence was obtained for further reduction of the conformational order as the temperature is raised in the liquid-crystalline phase.

The changes observed in the spectra of the gel phase reflect effects associated with interchain interactions and with the packing of fully extended all-trans acyl chains. Above 38°C the infrared spectra are in accord with the chains being packed in a hexagonal lattice with a high degree of motion about their long axes. Reduction of the temperature below 36°C leads to an interlocking of the chains reducing the rate of axial motions. This leads to a gradual introduction of orthorhombic packing and the gradual increase in the magnitude of factor group splitting.

The gradual introduction of orthorhombic packing is in contrast with the case of n-alkanes where the change in packing occurs within 1°C.

From the gradual increase in the magnitude of the splitting, information regarding molecular motion could be obtained through an appropriate intermolecular potential [120,121]. However, in the case of the spectra of these molecules where bands overlap heavily such a study seems not feasible at the present time.

The fact that the appearance of orthorhombic packing occurs progressively on lowering the temperature could be explained by assuming that the acyl chains perform torsions. The torsions can be expected to be of greater amplitude at positions close to the terminal methyl group; this is in accord with the determination of the crystal structure of DMPC [22]. The existence of torsions or twists in the chains will produce a gradient effect in the interchain interactions, with minimum interactions at the end of the chains and maximum interactions at positions in the middle of the chains.

The study performed on the thermal behaviour of the specifically-deuteriated derivatives of DPPC points to the existence of a "plateau" effect in the gel phase with the interchain interactions dropping off rapidly near the center of the bilayer. A more detailed series of specifically-deuteriated derivatives would be required to confirm this postulate.

The terminal methyl group was seen to rotate rapidly at temperatures just below the melting phenomenon but the rate of rotation decreases progressively in the gel phase pointing to a more rigid crystalline packing. The progressive reduction in the rate of rotation of the methyl group agrees with the observation of increasing values for the ^2H NMR quadrupole splitting [29] as the temperature is reduced.

Unfortunately, the Raman study on specifically-deuteriated derivatives of DMPC [89] does not provide enough information on the gel phase for a detailed comparison. A general conclusion reached from that study was that the "disorder" was greater towards the center of the bilayer which can be interpreted as reduced inter-chain interactions and hence increased freedom of motion.

Previous studies on the gel phase of phospholipid bilayers were not very successful in providing detailed information. Magnetic resonance spectroscopic experiments which have provided very detailed information on the liquid-crystalline phase of model and natural membranes [23,26-28, 88] have not been so successful in characterizing the gel phase. Infrared spectroscopy involves a very different time scale than magnetic resonance spectroscopy. In addition, there is an opposite temperature effect on IR and NMR; decreasing temperatures sharpen IR features, but broaden NMR signals. Any reduction in the rate of motion favours

observation by IR over observation by NMR, and vice-versa. The broadening of NMR signals does not imply that the dynamical information is lost; on the contrary, ^2H NMR spectra of bilayers in the gel phase might well be more informative than those of the liquid-crystalline phase. However, the analysis of the gel phase spectra is more difficult, recent developments in ^2H NMR spectral analysis will facilitate the interpretation of gel phase spectra [29].

As mentioned above, Raman spectroscopy has been applied to the study of lipid bilayers [31,33-35]. The conclusions reached from this IR study on DPPC bilayers do not agree with those from Raman spectra in relation to the existence of chain conformational disorder in the gel phase of DPPC bilayers. The appearance of gauche conformers in the gel phase of DPPC was inferred from the thermotropic behaviour of the carbon-carbon skeletal optical mode at 1130 cm^{-1} in the Raman spectra [33,34]. The thermotropic behaviour was analyzed solely in terms of intrachain effects. However, interchain interactions have a strong effect on the intensity of this mode [85]. Since, as it is shown in this study, the crystal packing and hence the interchain interactions are the major factor affecting the gel phase, it is concluded that the analysis of the Raman spectra is incomplete and should include these interactions.

6.4. Acholeplasma laidlawii Membranes.

The work on the thermal behaviour of *A. laidlawii* membranes represents the first such study by vibrational spectroscopy and provides detailed information on the temperature-dependent behaviour and the effects of proteins on lipid conformation and dynamics.

The data demonstrate that the temperature-dependent behaviour has the same general form in intact and deproteinated membranes. The phase transition in *A. laidlawii* membrane lipids occurs in two stages, one centered in a narrow temperature range, involving the melting of the acyl chains and the other encompassing a broader temperature range involves the gradual breakdown of the rigid crystal lattice present in the gel phase.

The breadth in the phase transition in membrane lipids was determined to originate from the heterogeneity in the lipid classes in the membrane. Proteins were also found to produce a broadening of the phase transition.

A comparison of the behaviour of *A. laidlawii* C16:0-d₃₁ (non-homogeneous) and C15:0-d₂₉ (homogeneous) membranes allows the study of the effects of membrane proteins.

In the case of the non-homogeneous C16:0-d₃₁ membranes in the gel phase, the chain mobility was greater in deproteinated membranes, pointing to an immobilizing effect of the membrane proteins. In the gel phase of the homogeneous C15:0-d₂₉ membranes the opposite was observed, the chain

mobility is greater when proteins are present while the behaviour of the extracted lipids is the same as that of model membranes composed of a single lipid. Thus, the proteins in the gel phase of the homogeneous membranes have the effect of increasing the mobility of the lipid acyl chains; this can be correlated with the concept of a mechanical match between protein and lipid motions [122].

In the liquid-crystalline phase and during the phase transition similar effects are seen in homogeneous and non-homogeneous *A. laidlawii* membranes. More gauche conformers are introduced in the presence of proteins than in their absence; however, the acyl chain mobility is restricted in their presence. That more gauche conformers are introduced in intact than in deproteinated membranes has also been observed by ^2H NMR in the same membranes [123].

A general conclusion to be reached from this IR study is that in the liquid-crystalline phase, membrane proteins have a dual effect on the lipid dynamics, they restrict the acyl chain mobility while at the same time the intrachain conformational disorder is greater. These findings emphasize the inadequacy of the popular description of membrane lipid properties in terms of "fluidity". Despite a disordering (greater number of gauche conformers) of the acyl chains, their rates of motion are apparently slower in the presence of proteins.

The study of bio-membranes by Fourier-transform infrared spectroscopy provides detailed information concerning the physical state of lipids under conditions of varying

composition and temperature. It is hoped that further studies on biological systems will facilitate the understanding of the functions of the various membrane components and their interactions with one another.

REFERENCES

1. Weissmann, G. & Claiborne, R., eds. "Cell Membranes - Biochemistry, Cell Biology and Pathology" HP Publishing Co., New York, 1975.
2. Andreoli, T.E.; Hoffman, J.F. & Fanestil, D.D., eds. "Membrane Physiology" Plenum Press, New York, 1978.
3. Guidotti, G. Ann. Rev. Biochem. 41, 731 (1972).
4. Ansell, G.B., Hawthorne, J.N. & Dawson, R.M.C. "Form and Function of Phospholipids" BBA Library, vol. 3. Elsevier Scientific Pub., Amsterdam, 1973.
5. Tanford, C. "The Hydrophobic Effect: Formation of Micelles and Biological Membranes" J. Wiley & Sons., New York, 1980.
6. Singer, S.J. & Nicolson, G.L. Science 175, 720 (1972).
7. Bangham, A.D. in Chapter 3 of reference 1.
8. Chapman, D., Gómez-Fernández, J.C. & Goñi, F.M. FEBS Letts. 98, 211 (1979).
9. Luzzati, V. in "Biological Membranes", D. Chapman, ed. p. 71. Academic Press, London, 1968.
10. Chapman, D. Quart. Revs. Biophys. 8, 185 (1975).
11. Melchior, D.L. & Steim, J.M. Ann. Rev. Biophys. Bioeng. 5, 205 (1976).
12. Steim, J.M., Tourtellotte, M.E., Reinert, J.C., McElhaney, R.N. & Rader, R.L. Proc. Natl. Acad. Sci. USA, 63, 104 (1969).

13. Ladbroke, B.D. & Chapman, D. Chem. Phys. Lipids. 3, 304 (1969).
14. Chapman, D., Williams, R.M. & Ladbroke, B.D. Chem. Phys. Lipids, 1, 445 (1967).
15. Andersen, H.C. Ann. Rev. Biochem. 47, 359 (1978).
16. Luzzati, V. & Tardieu, A. Ann. Rev. Phys. Chem. 25, 79 (1974).
17. Tardieu, A., Luzzati, V. & Reman, F.C. J. Mol. Biol. 75, 711 (1973).
18. Rand, R.P., Chapman, D. & Larsson, K. Biophys. J. 15, 1117 (1975).
19. Janiak, M.J., Small, D.M. & Shipley, G.G. Biochemistry, 15, 4575 (1976).
20. Brady, G.W. & Fein, D.B. Biochim. Biophys. Acta, 464, 249 (1977).
21. Janiak, M.J., Small, D.M. & Shipley, G.G. J. Biol. Chem. 254, 6068 (1979).
22. Pearson, R.H. & Pascher, I. Nature 281, 499 (1979).
23. Smith, I.C.P. Can. J. Biochem. 57, 1 (1979).
24. Bocian, D.F. & Chan, S.I. Ann. Rev. Phys. Chem. 29, 307 (1978).
25. Mantsch, H.H., Saito, H. & Smith, I.C.P. Progress NMR Spectroscopy. 11, 211 (1977).
26. Seelig, J. Quart. Revs. Biophys. 10, 353 (1977).
27. Seelig, A. & Seelig, J. Biochemistry. 13, 4839 (1974).
28. Stockton, G.W., Johnson, K.G., Butler, K.W., Tulloch, A.P., Boulanger, Y., Smith, I.C.P., Davis, J.H. & Bloom, M. Nature, 269, 267 (1977).

29. Davis, J.H. Biophys. J. 27, 339 (1979).
30. Delmelle, M., Butler, K.W. & Smith, I.C.P. Biochemistry. 19, 698 (1980).
31. Wallach, D.F.H., Verma, S.P. & Fookson, J. Biochim. Biophys. Acta. 559, 153 (1979).
32. Chapman, D. J. Chem. Soc. 784 (1958).
33. Gaber, B.P. & Peticolas, W.L. Biochim. Biophys. Acta. 465, 260 (1977).
34. Gaber, B.P., Yager, P. & Peticolas, W.L. Biophys. J. 21, 161 (1978).
35. Mendelsohn, R. & Taraschi, T. Biochemistry. 17, 3944 (1978).
36. Bulkin, B.J. & Krishnamachari, N. Biochim. Biophys. Acta. 211, 592 (1970).
37. Bulkin, B.J. & Krishnamachari, N. J. Am. Chem. Soc. 94, 1109 (1972).
38. Baldassare, J.J., Brenckle, G.M., Hoffman, M. & Silbert, D.F. J. Biol. Chem. 252, 8797 (1977).
39. McElhaney, R.N. J. Mol. Biol. 84, 145 (1974).
40. Silvius, J.R. & McElhaney, R.N. Proc. Natl. Acad. Sci. USA. 77, 1255 (1980).
41. Engelman, D.M. J. Mol. Biol. 47, 115 (1970).
42. Smith, I.C.P., Butler, K.W., Tulloch, A.P., Davis, J.H. & Bloom, M. FEBS Letts. 100, 57 (1979).
43. Huang, L., Lorch, S.K., Smith, G.G. & Haug, A. FEBS Letts. 43, 1 (1974).

44. Silvius, J.R., Mak, N. & McElhaney, R.N.
Biochim. Biophys. Acta. 597, 199 (1980).
45. Broadhurst, M.G. J. Res. Nat. Bur. Standards., Sect. A.
66, 241 (1962).
46. Stockton, G.W., Johnson, K.G., Butler, K.W., Pol-
naszek, C.F., Cyr, R. & Smith, I.C.P. Biochim. Biophys.
Acta. 401, 535 (1975).
47. Bligh, E.G. & Dyer, W.J. Can. J. Biochem. Physiol.
37, 911 (1959).
48. Smith, I.C.P., & Butler, K.W. in "Spin Labeling -
Theory and Applications" L.J. Berliner, ed. p 411.
Academic Press, New York, 1976.
49. Griffiths, P.R. "Chemical Infrared Fourier Transform
Spectroscopy" Wiley-Interscience, New York, 1975.
50. Happ, H. & Genzel, L. Infrared Phys. 1, 39 (1961).
51. Young, R.P. & Jones, R.N. Chem. Revs. 71, 219 (1971).
52. Kauppinen, J., Kärkkäinen, T. & Kyrö, E. J. Mol. Spectrosc.
71, 15 (1978).
53. Muller, A. Proc. Roy. Soc. (London). 127A, 417 (1930);
138A, 514 (1932).
54. Schaerer, A.A., Busso, C.J., Smith, A.E. & Skinner, L.B.
J. Am. Chem. Soc. 77, 2017 (1955).
55. Ewen, B., Fischer, E.W., Piesczek, W. & Strobl, G.
J. Chem. Phys. 61, 5265 (1974).
56. Strobl, G.R. J. Polym. Sci. (Polym. Symp.) 59, 121 (1977).

57. Barnes, J.D. & Fanconi, B.M. J.Chem.Phys. 56, 5190 (1972).
58. Snyder, R.G. & Schachtschneider, J.H. Spectrochim. Acta. 19, 85 (1963).
59. Schachtschneider, J.H. & Snyder, R.G. Spectrochim. Acta. 19, 117 (1963).
60. Snyder, R.G., Hsu, S.L. & Krimm, S. Spectrochim. Acta. 34A, 395 (1978).
61. Snyder, R.G. J.Mol.Spectrosc. 4, 411 (1960); 7, 116 (1961).
62. Snyder, R.G. J.Chem.Phys. 71, 3229 (1979).
63. Fischmeister, I. in "Progress in the Chemistry of Fats and Other Lipids", R.T.Holman, ed., vol. XIV (part 3), pp. 91-162. Pergamon Press, Oxford, 1975.
64. Primas, H. & Günthard, Hs.H. Helv.Chim.Acta. 39, 1182 (1956).
65. Bellamy, L.J. "The Infra-red Spectra of Complex Molecules" Chapman and Hall, London, 1975.
66. Sheppard, N. in "Advances in Spectroscopy", vol. 1, p. 288, H.W.Thompson, ed. Interscience Publishers, New York, 1959.
67. Shelby, R.M., Harris, C.B. & Cornelius, P.A. J.Chem.Phys. 70, 34 (1979).
68. Bailey, R.T. in "Molecular Spectroscopy", vol. 2, p. 173. A Specialist Periodical Report, The Chemical Society, London, 1974.
69. MacPhail, R.A., Snyder, R.G. & Strauss, H.L. J.Am.Chem.Soc. 102, 3976 (1980).
70. Snyder, R.G. J.Chem.Phys. 47, 1316 (1967).

71. Bush, S.F., Levin, H. & Levin, I.W. Chem. Phys. Lipids. 27, 101 (1980).
72. Fringelli, U.P. Z. Naturforsch. 32c, 20 (1977).
73. Jones, R.N. Can. J. Chem. 40, 301 (1962).
74. Susi, H. & Pazner, S. Spectrochim. Acta. 18, 499 (1962).
75. Asher, I.M. & Levin, I.W. Biochim. Biophys. Acta. 468, 63 (1977).
76. Decius, J.C. & Hexter, R.M. "Molecular Vibrations in Crystals" McGraw-Hill Book Co. New York, 1977.
77. Yellin, N. & Levin, I.W. Biochim. Biophys. Acta. 489, 177 (1977).
78. von Sydow, E. Ark. Kemi. 9, 231 (1956).
79. Aleby, S. Acta Crystallogr. 15, 1248 (1962).
80. Hitchcock, P.B., Mason, R., Thomas, K.M. & Shipley, G.G. Proc. Natl. Acad. Sci. USA. 71, 3036 (1974).
81. Stamatoff, J.B., Graddick, W.F., Powers, L. & Moncton, D.E. Biophys. J. 25, 253 (1979).
82. Pringle, M.J. & Miller, K.W. Biochemistry, 18, 3314 (1979).
83. Pink, D.A., Green, T.J. & Chapman, D. Biochemistry. 19, 349 (1980).
84. Caille, A., Pink, D., de Verteuil, F. & Zuckermann, M.J. Can. J. Phys. 58, 581 (1980).
85. Shyder, R.G., Cameron, D.G., Casal, H.L., Compton, D.A.C. & Mantsch, H.H. in "Proceedings of the VIIth International Conference on Raman Spectroscopy" W.F. Murphy, ed., p. 622, North-Holland, Amsterdam. 1980.

86. Hitchcock, P.B., Mason, R. & Shipley, G.G.
J. Mol. Biol. 94, 297 (1975).
87. Seelig, A. & Seelig, J. Biochim. Biophys. Acta.
406, 1 (1975).
88. Stockton, G.W., Polnaszek, C.F., Tulloch, A.P., Hasan, F.
& Smith, I.C.P. Biochemistry. 15, 954 (1976).
89. Bansil, R., Day, J., Meadows, M., Rice, D. & Oldfield, E.
Biochemistry. 19, 1938 (1980).
90. Hill, I.R. & Levin, I.W. J. Chem. Phys. 70, 842 (1979).
91. Goto, R. & Takenaka, T. Nippon Kagaku Zasshi. 81, 1504 (1960),
Chem. Abstr. 55:3136f; Proc. Intern. Symp. Mol. Struct.
Spectry. (A212) Tokyo, 1962. Chem. Abstr. 61:1395f.
92. Holland, R.F. & Nielsen, J.R. J. Mol. Spectrosc. 8, 383 (1962).
93. Taylor, M.G. Personal communication.
94. Razin, S. Microbiol. Revs. 42, 414 (1978).
95. McElhahey, R.N. J. Supramol. Struct. 2, 617 (1974).
96. Wieslander, A. & Rilfors, L. Biochim. Biophys. Acta.
466, 336 (1977).
97. Rottem, S. Biochim. Biophys. Acta. 604, 65 (1980).
98. Thomas, G.J. & Kyogoku, Y. in "Infrared and Raman Spec-
troscopy", E.G. Brame & J.G. Grasselli, eds., vol. 1,
part C, p. 717. M. Dekker, New York, 1977.
99. Oldfield, E., Chapman, D. & Derbyshire, W.
Chem. Phys. Lipids. 9, 69 (1972).
100. Parker, F.S. "Applications of Infrared Spectroscopy
in Biochemistry, Biology and Medicine" Plenum Press,
New York, 1971.

101. Krimm, S., Liang, C.Y. & Sutherland, G.B.B.M.
J.Chem.Phys. 25, 549 (1956).
102. Koyama, Y., Yanagishita, M., Toda, S. & Matsuo, T.
J.Colloid Interface Sci. 61, 438 (1977).
103. Vasko, P.D., Blackwell, J. & Koenig, J.L.
Carbohydr.Res. 23, 407 (1972).
104. Chapman, D. & Urbina, J. FEBS Letts. 12, 169 (1971).
105. Phillips, M.C., Ladbroke, B.D. & Chapman, D.
Biochim.Biophys.Acta. 196, 35 (1970).
106. Marsh, D., Watts, A., Maschke, W. & Knowles, P.F.
Biochem.Biophys.Res.Commun. 81, 397 (1978).
107. Oldfield, E., Gilmore, R., Glaser, M., Gutowsky, H.S.,
Hshung, J.C., Kang, S.Y., King, T.E., Meadows, M. &
Rice, D. Proc.Natl.Acad.Sci.USA. 75, 4657 (1978).
108. Taylor, M.G. & Smith, I.C.P. Biochim.Biophys.Acta.
599, 140 (1980).
109. Silvius, J.R. & McElhaney, R.N. Can.J.Biochem.
56, 462 (1978).
110. Taraschi, T. & Mendelsohn, R. Proc.Natl.Acad.Sci.USA.
77, 2362 (1980).
111. Janoff, A.S., Gupte, S. & McGroarty, E.J.
Biochim.Biophys.Acta. 598, 641 (1980).
112. Bell, R.J. "Introductory Fourier-Transform Spectroscopy"
Academic Press, New York, 1972.
113. Geick, R. in "Topics in Current Chemistry" vol. 58,
p. 73. Springer-Verlag, Berlin, 1975.

114. Bertie, J.E. in "Analytical Applications of FT-IR to Molecular and Biological Systems" J.R. Durig, ed., p. 51. D. Reidel Publishing Co., Dordrecht, 1980.
115. Pitts, J.M., Finlayson-Pitts, B.J. & Winer, A.M. Environ. Sci. Technol. 11, 568 (1977).
116. Hirschfeld, T. in "Fourier-Transform Infrared Spectroscopy. Applications to Chemical Systems. J.R. Ferraro & L.J. Basile, eds., vol. 2, p. 193. Academic Press, New York, 1979.
117. Hirschfeld, T. Appl. Opt. 17, 1400 (1978).
118. Anderson, R.J. & Griffiths, P.R. Anal. Chem. 50, 1804 (1978).
119. Gordon, R.G. J. Chem. Phys. 43, 1307 (1965).
120. Dows, D.A. J. Chem. Phys. 32, 1342 (1960).
121. Fox, D. & Hexter, R.M. J. Chem. Phys. 41, 1125 (1964).
122. Bloom, M. Can. J. Phys. 57, 2227 (1979).
123. Deslauriers, R., Jarrell, H.C., Butler, K.W. & Smith, I.C.P. Personal communication.