MONTE CARLO STUDY OF LIPID CHOLESTEROL INTERACTIONS IN BIOMEMBRANES

BY

SHASHIKANT D. KALASKAR

Bachelor of Science
Poona University
Poona, India
1971

Master of Science
Western Carolina University
North Carolina, USA
1982

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1988

MONTE CARLO STUDY OF LIPID

CHOLESTEROL INTERACTIONS

IN BIOMEMBRANES

Thesis approved:

Thesis Advisor

Dean of the Graduate College

ACKNOWLEDGEMENT

The author wishes to express his appreciation to his major advisor, Dr. H. L. Scott for his patience and help during the course of this study. Also I wish to thank Dr. Paul Westhaus and Dr. H. J. Harmon for their guidance. I wish to thank my friend Mr. Lokman Merican and Dr. Aslam Chaudhari for their help in computer and programming techniques. Thanks to my wife Shaila and daughters Anupama, Menaka for their love and encouragement.

TABLE OF CONTENTS

CHAPTER Page

I. INTRODUCTION....1

The Membrane Components....1
Membrane Models....17

II. THEORY....23

III. RESULTS and DISCUSSIONS....38

BIBLIOGRAPHY....46

APPENDIX A - COMPUTER PROGRAM FOR THE CALCULATION OF THE AVERAGE VALUES OF THE ORDER PARAMETERS....48

APPENDIX B - COMPUTER PROGRAM FOR THE CALCULATION OF THE WEIGHTED AVERAGES AND STANDARD DEVIATION....73

LIST OF TABLES

Table Page

I. Standard deviation for the bonds on the cholesterol neighboring chains..... 41

II Standard deviation for the bonds on all acyl chains..... 42

LIST OF FIGURES

Figure Page

1. Some Typical Phospholipid Molecules.....3

   Some Typical Sphingolipid Molecules.....4

2. Monolayers, Bilayers, Micelles and Vesicles.....5

3. Trans and Gauche Configurations.....7

4. Possible Hydrocarbon Chain Configurations

   in a Membrane.....8

5. Lipid Phase Transition.....9

6. Sterols: Cholesterol Molecule.....12

7. Orientation of Cholesterol in the Membrane Hydrogen Bonding...14

8. Effect of Cholesterol on lipid phase transition.....16

9. Proteins in Membranes.....18

10. Model Membranes.....19

11. Modes of Mobility of Membrane Components.....21

12. Flow Chart Displaying Monte Carlo Averaging Procedure.....28

13. A Model of a Unit Cell.....30

14. Gauche Rotation.....34

15. Plot of S_n vs. Bond Number for boundary lipids.....39

16. Plot of S_n vs. Bond Number for lipid chains.....40

CHAPTER I

INTRODUCTION

The purpose of this study is to explore the nature of lipid cholesterol interactions in biomembranes, using the Monte Carlo method.

Our understanding of the membrane and its functions has evolved considerably. It is widely accepted now that a membrane is a two dimensional bimolecular array of molecules of lipids, proteins and in some cases cholesterol. It is about 50 A^{o} thick. Biomembranes are usually curved continuously and form closed sacs. The major components of biomembranes, lipids and proteins, are present in the varying amounts, depending on the type of the membrane (1). The lipids and proteins have certain physical properties that determine the structural organization of the membrane.

The Membrane Components

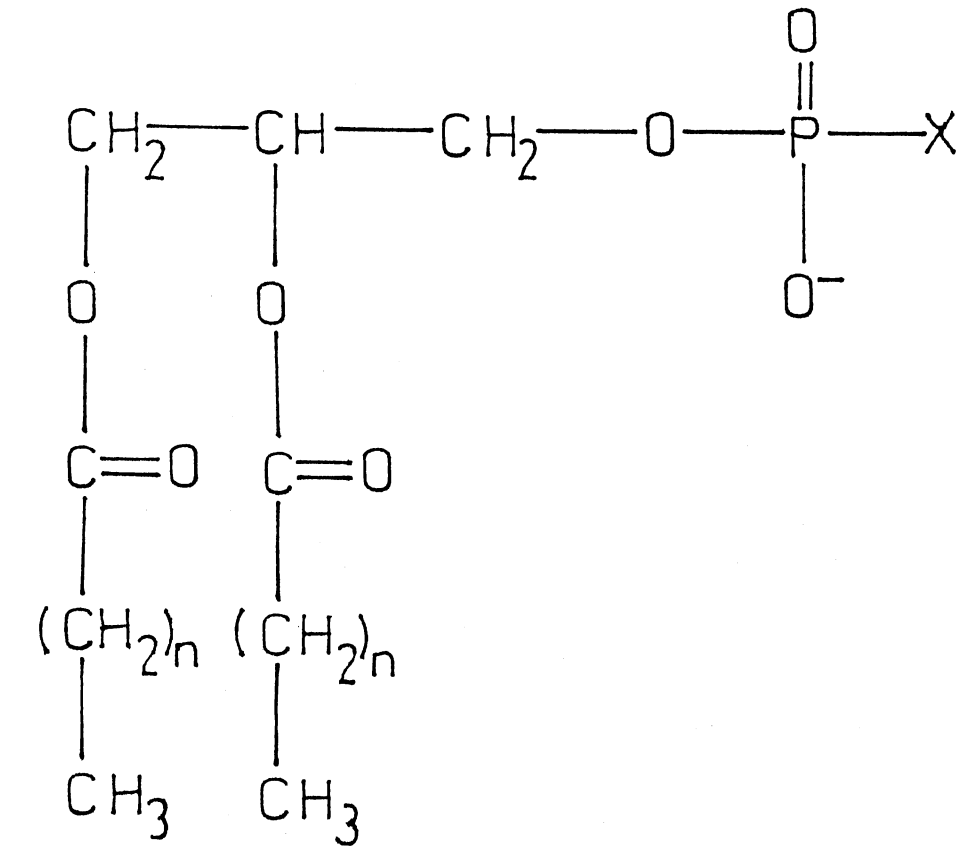
Lipids : These are organic molecules that can be extracted from wet membranes by use of non polar solvents such as chloroform, benzene or ether. Membrane lipids are amphipathic i.e. a lipid molecule contains both hydrophobic (non-polar) and hydrophilic (polar) regions. These

regions are usually bridged by glycerol moiety or a sphinganine derivative. The most common lipids found in the eukaryotic membranes are phospholipids, glycolipids, sphingolipids and sterols. The last two classes are usually absent in the prokaryotic membranes. Figure 1 shows some typical lipid molecules.

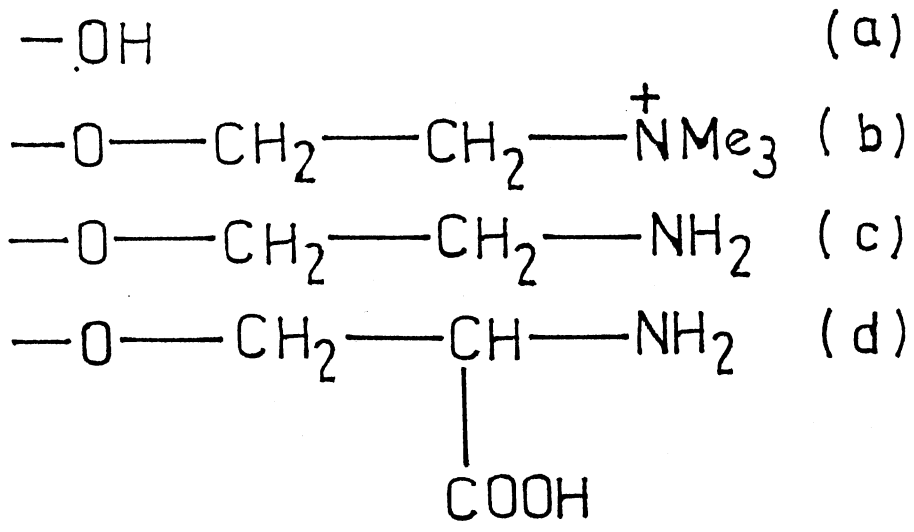
The polar headgroups determine the affinity of the phospholipids for the water, whereas the acyl chains determine the solubility of these molecules in the solvents - polar or non polar. In an aqueous phase the amphipathic molecules reorient themselves so as to minimize unfavorable interactions between hydrocarbon chains and water. This leads to various arrangements that the lipid molecules assume in an aqueous phase (Figure 2). As seen in this figure the hydrophilic region always faces the aqueous environment and the hydrophobic region moves away from the aqueous zone.

The hydrophobic effect (2) has its origin in the strong attractive forces between water molecules. If the solute is to dissolve in the water, it must disrupt these strong attractive forces in favour of attractive forces between water and solute. This is why the ionic solutes readily dissolve in the water.

Apolar groups on the other hand, cause an extensive rearrangement of the neighboring water molecules. This ordering of molecules apparently leads to a decrease in entropy which is thermodynamically unfavorable. For this reason the solution of the apolar solvents in the



X equal to



(a)

(b)

(c)

(d)

Figure 1. Some Typical Phospholipid Molecules. (a) Phosphatidic Acid. (b) Phosphatidyl Choline. (c) Phophatidyl Ethanolamine. (d) Phosphatidyl Serine.

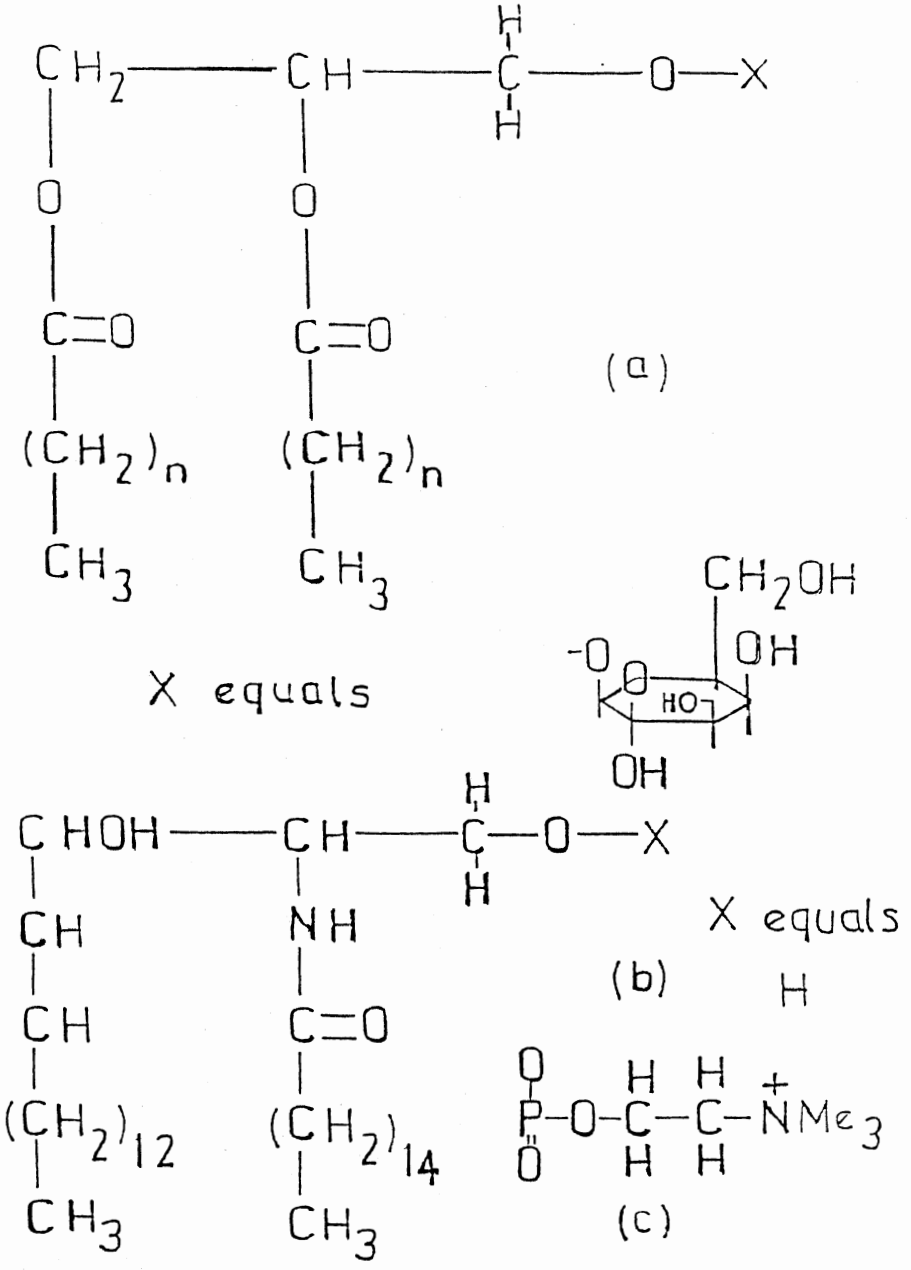


Figure 1. (continued) Sphingolipids and Glycolipids.
(a) Galactosyldiacylglycerol.
(b) Ceramide. (c) Sphingomylein.

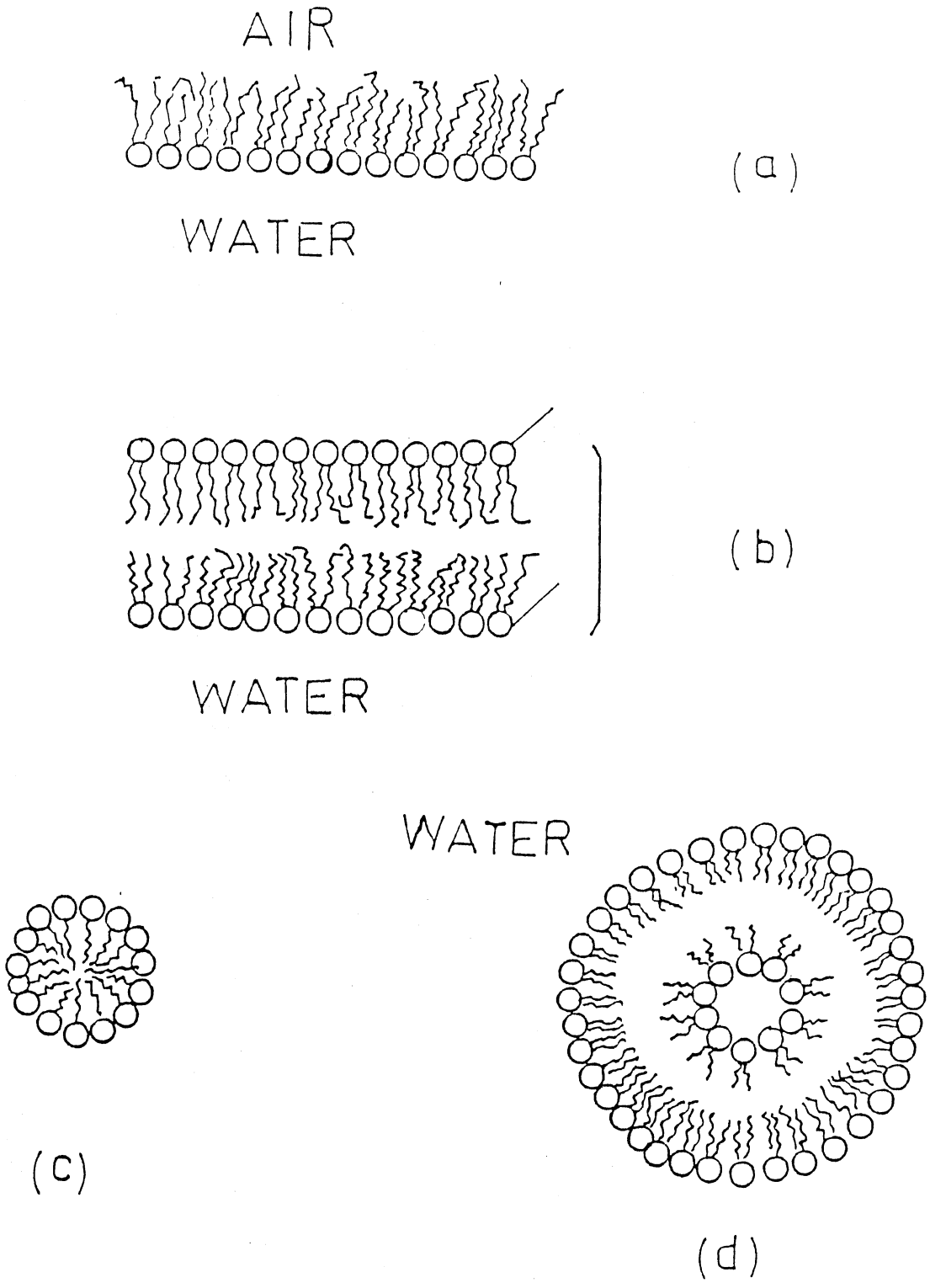


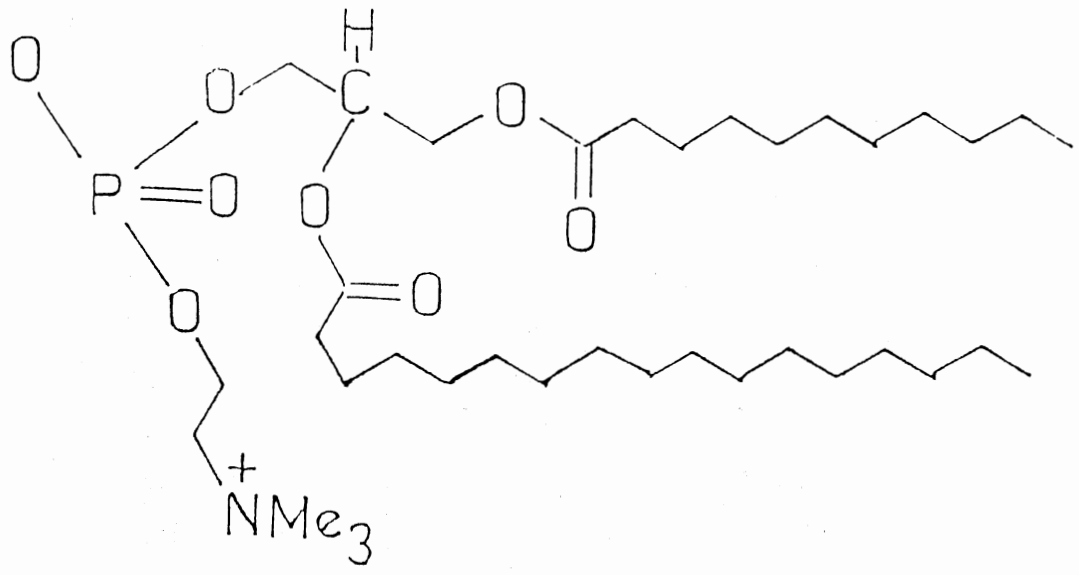
Figure 2. (a) Monolayer. (b) Bilayer. (c) Micelles. (d) Vesicle.

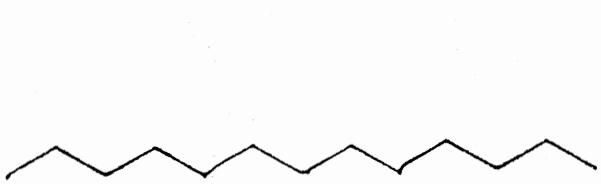
water is resisted. The apolar groups - hydrocarbon chains of the lipid molecules cluster together, excluding water so as to minimize the free energy of the system. The hydrophobic nature of the lipid chains is thus an entropic consideration rather than any other specific interaction.

The geometry of hydrocarbon chains in their stretched form, called all trans configuration, forms a hydrophobic cylinder (Figure 3).

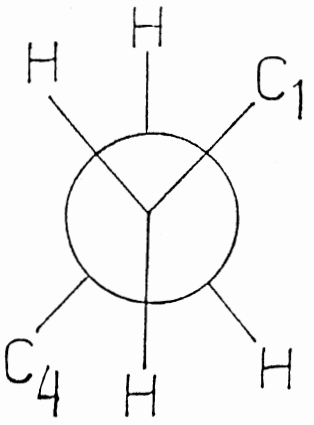
There is a rotation about C-C bonds. In trans configuration the dihedral angle between C_1 to C_2 and C_3 to C_4 bonds is 180 degrees. For gauche rotation the most stable and thus lowest in energy, dihedral angles are 120 degrees and 240 degrees (i.e. -120 degrees).

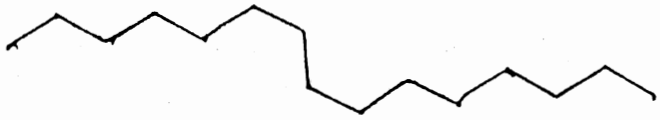
Because of the steric strain the gauche confirmation is less stable by about 0.5 kcal/mole or more, than trans configuration. It is also possible to have two gauche rotations which produce a common chain configuration by rotating about one C-C bond by 120 degrees then rotating either of the two next nearest neighbors by -120 degrees. This produces what is called a 'kink' in the chain. It is obvious that such kinks shorten the length of hydrocarbon chains and thereby increase the volume of the hydrophobic cylinder. The increase in temperature will allow more kinks in the acyl chains. Figure 4 shows some of the chain disorders that may take place in the membrane. At the lower temperatures however, when the phospholipids are in the solid





TRANS





GAUCHE

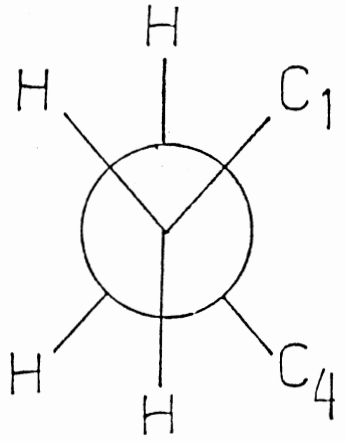


Figure 3: Trans and Gauche Configurations.

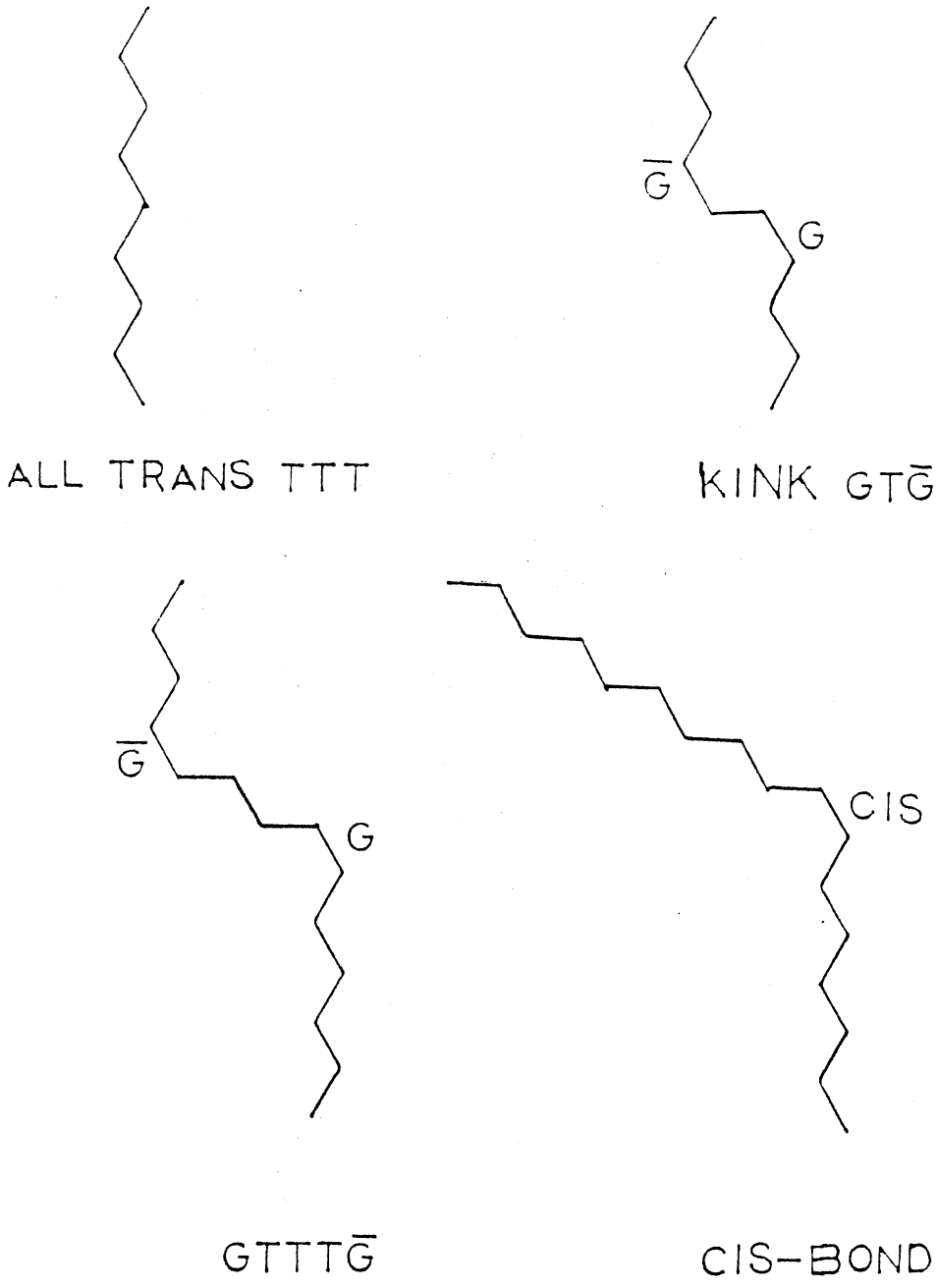


Figure 4. Possible Hydrocarbon Chain Configurations in a Membrane.

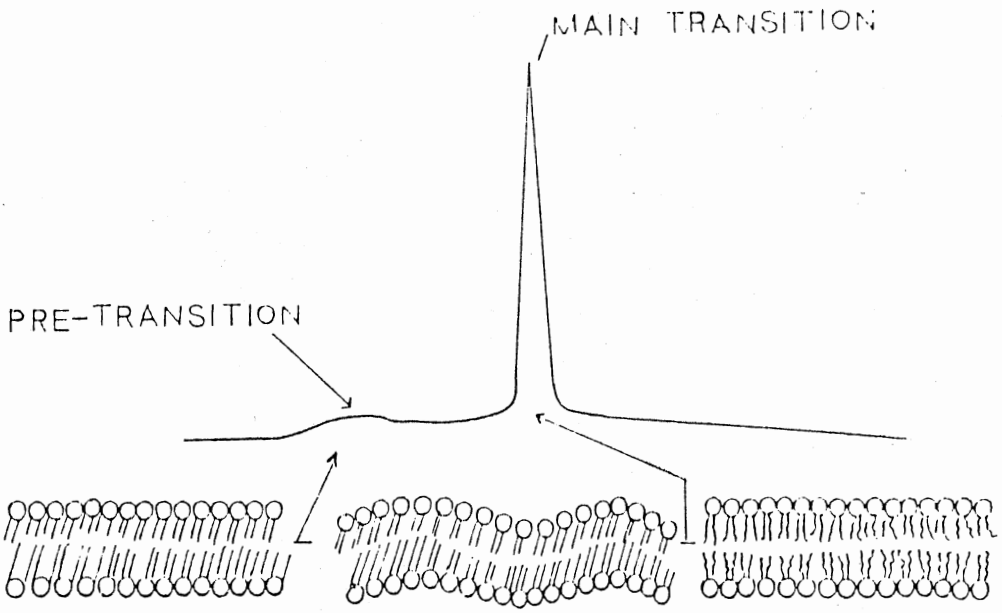


Figure 5. Lipid Phase Transition.

crystalline state the acyl chains are in fully stretched all trans state.

In the crystalline state the lipid molecules are packed together in an hexagonal array, with the acyl chains parallel to each other. If the temperature is increased the bilayers undergo distinct organizational changes, although the general structure of the membrane remains the same (Figure 5). At transition temperature the lipids change from crystalline solid state to the liquid gel state. The transition temperature depends on such factors as, type of phospholipid headgroup, acyl chain length (3), number of double bonds, their positions in the chains (4), and composition of the mixture of phospholipids. In general transition temperatures are higher for longer acyl chains and are lower for highly unsaturated acyl chains.

As seen in the Figure 5, below the transition temperature the acyl chains are predominantly in all trans state, parallel to each other but not perpendicular to the bilayer plane. The main transition is preceded by a pretransition which involves reorientation of chains from tilted to the perpendicular positions along with distortion of the bilayer by periodic ripple. Above the transition temperatures the kinks are introduced in the chains which decreases thickness of the bilayer and increases the area of the membrane. The hexagonal array is disrupted as well. The phase transition involves a small volume change (5). The change of pressure over temperature has been found (6) to be 00.44 atm per 1 degree Celsius, using Clausius-Claperon equation.

$$\Delta P / \Delta T = \Delta H / (T_c (V_l - V_g)) \qquad (1)$$

where ΔH = Enthalpy of Transition; T_c = Transition Temperature and ($V_l - V_g$) = Change in volume of two phases. The volume change of 00.037 ml/mg is observed (7) at the main transition temperature 41.4 degree Celsius for Diapalmitoyl phosphatidylcholine water system. The phase transition in the lipid bilayers are studied by several experimental techniques such as calorimetry (8), X-ray diffraction (9) and spin label resonance (10).

Sterols: are a third major class of lipids, first two being phospholipids and glycolipids. In animal membranes cholesterol is a predominant sterol but in plant membrane it is rare.

A cholesterol molecule consists of a rigid sterol ring about 1.1 nm in length with a hydroxyl group attached at one end (Figure 6). At the other end of the sterol ring structure there is a flexible hydrocarbon chain about 0.8 nm long. Most phospholipid molecules whose acyl chains are at least 9 $-CH_2$ units long can accommodate cholesterol molecules in bilayers. The molecule is inserted into the bilayer with its hydroxyl - OH, group towards polar headgroup of lipids and its apolar hydrocarbon tail towards hydrophobic acyl chains of the bilayer. The plane of the sterol ring is thus perpendicular to the plane of the bilayer. The hydrocarbon tail of cholesterol - with two methyl groups at the end - is rather mobile and interacts with neighboring acyl chains. The molecule is inserted deeply into the bilayer to allow hydrogen bonding between

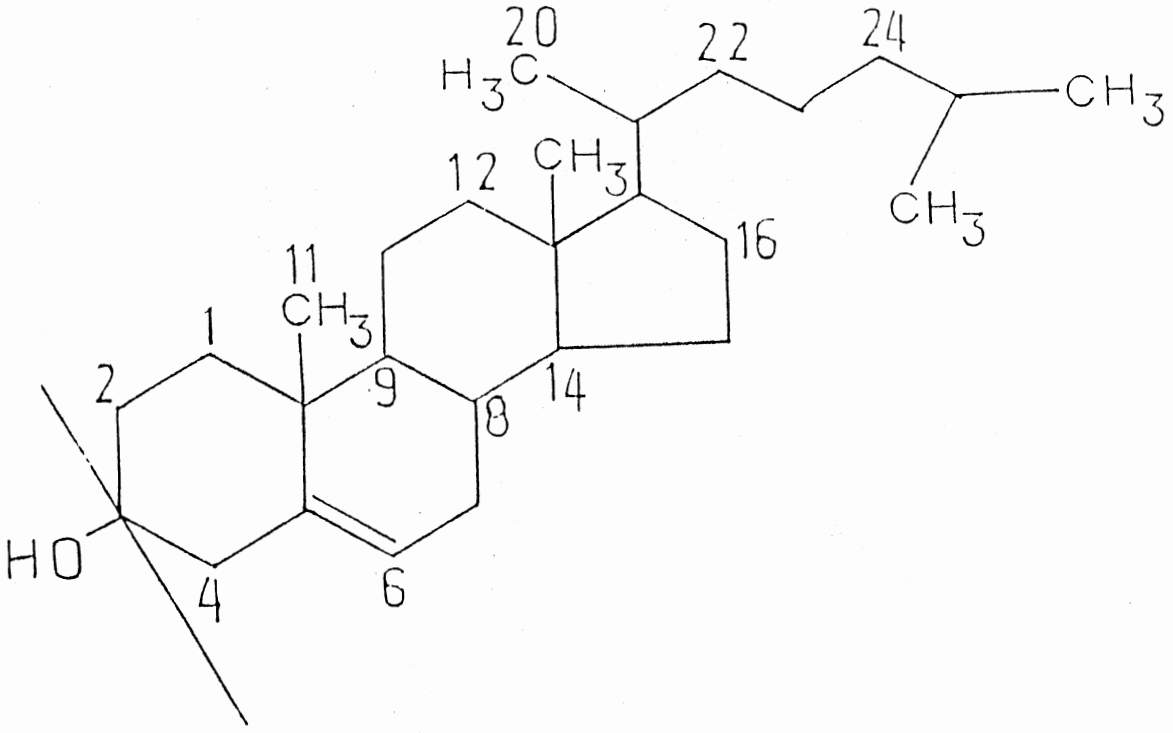


Figure 6. Sterols: Cholesterol Molecule.

oxygen of -OH group and oxygen of C=O group of the lipid molecule (Figure 7). The rigid ring structure of cholesterol prevents kinks from forming in neighboring acyl chains. This decreases the molecular area of the phospholipids. Thus the area of cholesterol-phospholipid (1:2) mixture is 25 % lower than the direct sum of their areas (11). This is so called 'Condensing effect'. On the other hand, the hydrogen bonding causes the separation between the polar headgroups of the lipids, thus minimizing electrostatic interaction among them allowing greater freedom of mobility. Incorporation of cholesterol has long been controversial to the extent that some of the early models do not propose hydrogen bonding at all, a claim later refuted (13). The structure of cholesterol is rather significant to the membrane properties but less so for its incorporation into the bilayer.

The amount of cholesterol that can be incorporated in the bilayer without disrupting the membrane function has a upper limit. At > 50 mole % some sterol molecules will have no phospholipid neighbors and there are too many sterol-sterol interactions destabilizing the membrane. At about 10 mole % cholesterol there is an increased rate of lateral diffusion of phospholipids. At about 22 mole % cholesterol the regions of pure phospholipids and cholesterol disappear and the bilayer consists of closely packed clusters of cholesterol and lipids. Between 33 to 50 mole % of cholesterol both species are distributed randomly with some sterols separated by only one lipid molecule. The increase in the amount of cholesterol slowly abolishes the phase transition. (14,15)

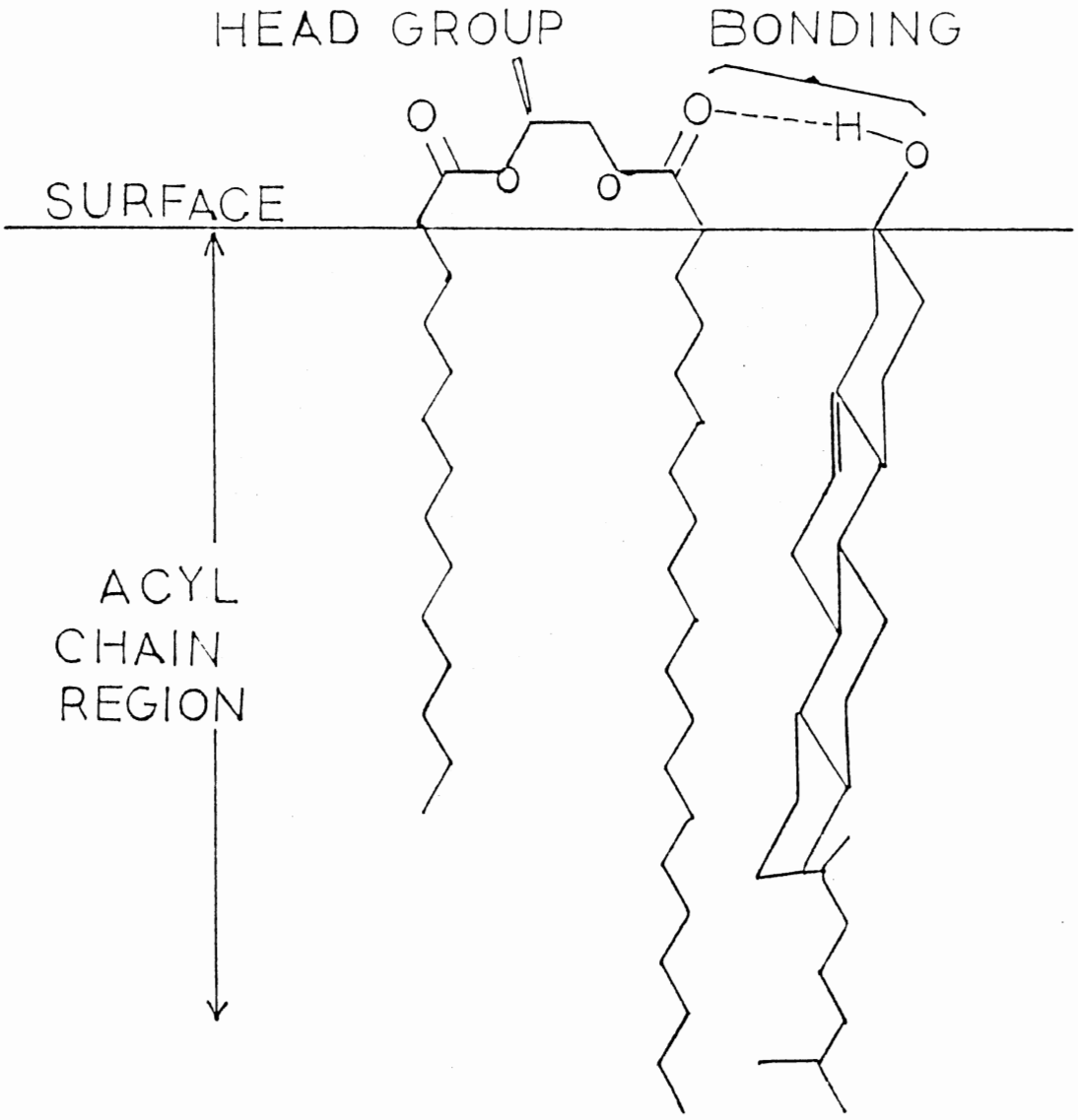


Figure 7. Orientation of Cholesterol in the Membrane, Hydrogen Bonding.

The effect of increase in cholesterol on lipid phase transition is shown in Figure 8.

Temperature plays a key role in the lipid-cholesterol interactions. At the temperatures higher than the transition temperature cholesterol increases bilayer rigidity by ordering the fluid state lipids whereas below this temperature it increases fluidity of the solid state lipid by disordering them.

Proteins: The proteins associated with the membranes can be classified in a number of ways - The ease of separation from membrane, geometric structure or simply by their chemical contents.

Weakly bound proteins (peripheral, extrinsic proteins)- The proteins that can be isolated easily using strong salt solutions or EDTA solution. Such an isolation does not significantly disrupt the membrane structure. Cytochrome C found in the inner membrane of mitochondria is an example of peripheral protein.

Tightly bound proteins (Integral, Intrinsic proteins) - Proteins can be isolated by the use of detergents such as Sodium dodecyl sulfate, SDS. The isolation of integral proteins from the membrane causes a serious damage to the membrane structure. The integral proteins are firmly built into the membranes. Globular proteins are quite bulky. They consist of several loops of polypeptide chains. Fibrous proteins consist of a single strand of a polypeptide chain. Both, the globular

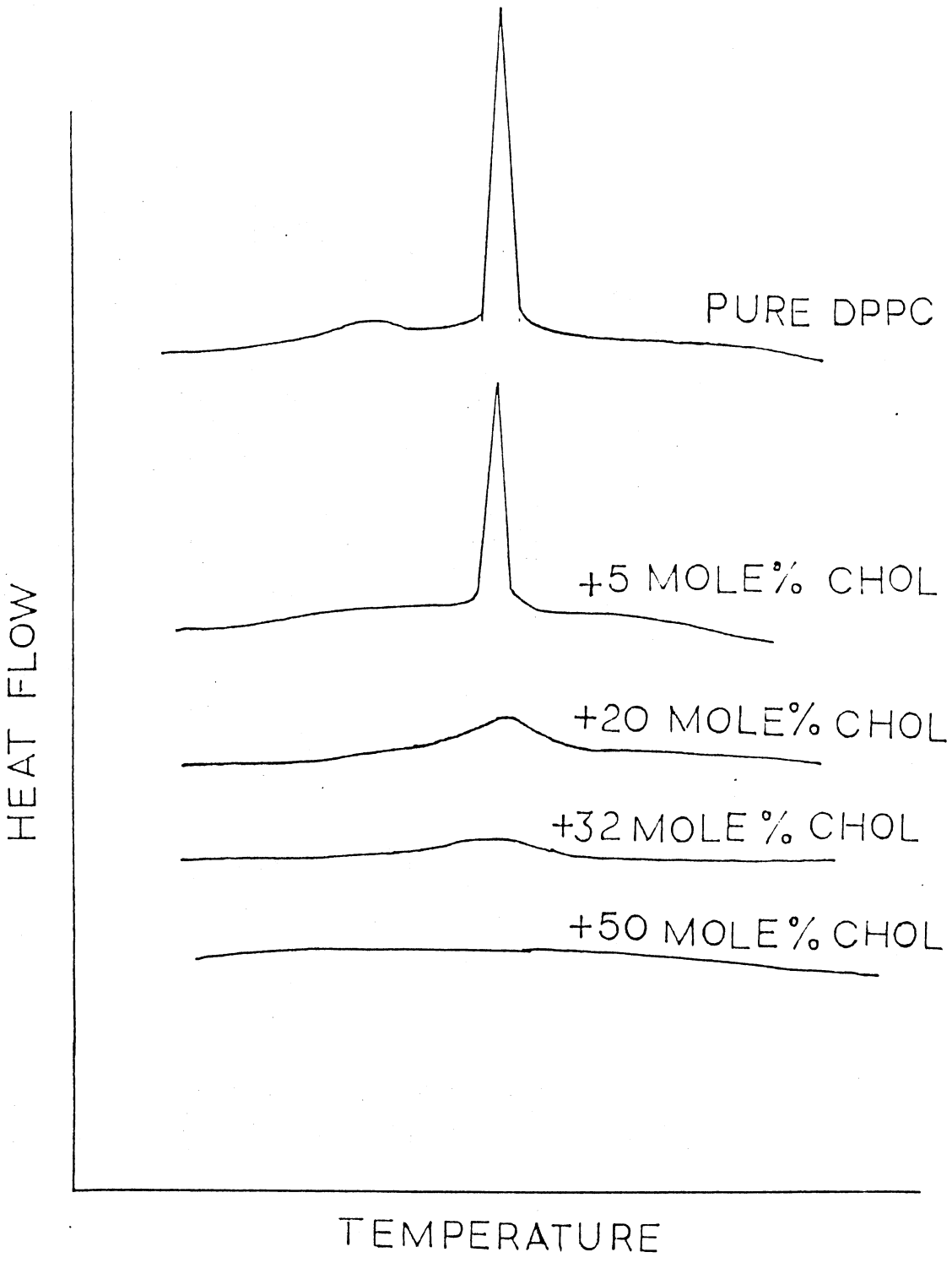


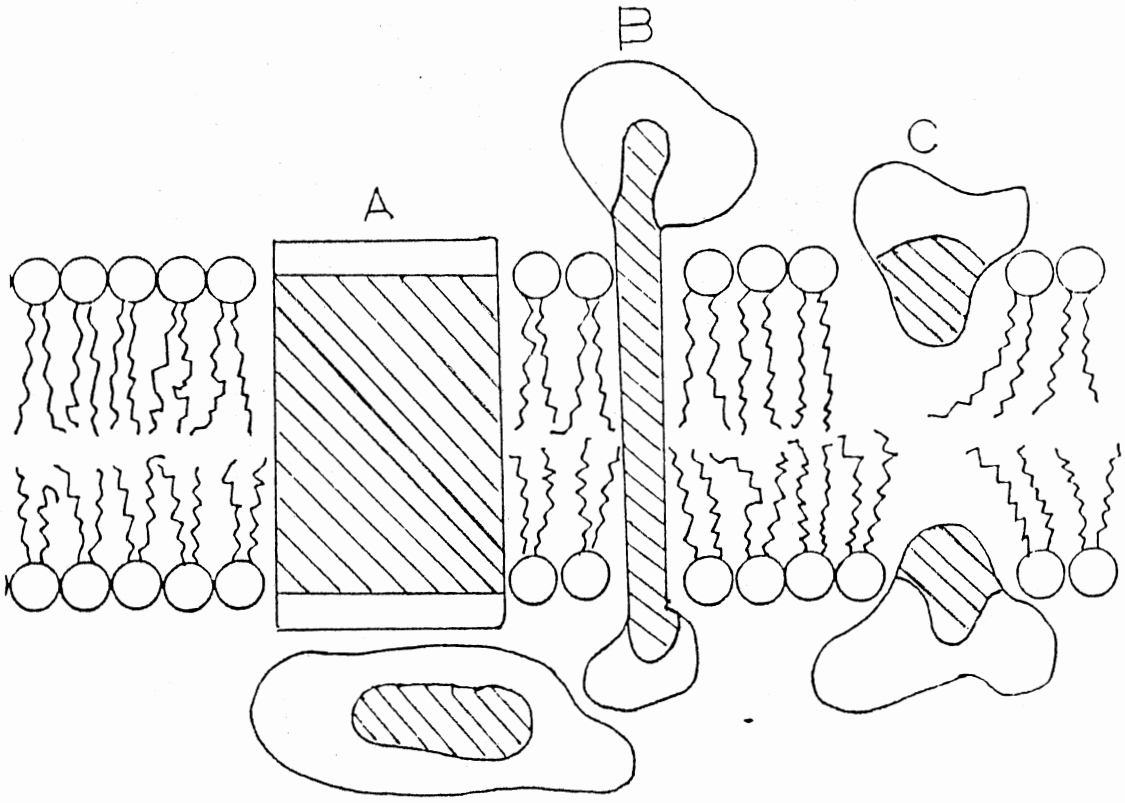
Figure 8. Effect of Cholesterol on lipid Phase Transition.

and the fibrous proteins may be transmembrane and therefore, integral proteins (Figure 9).

Proteins play key role in allowing passage of selective chemicals across the membrane. Specific proteins allow passage to a particular chemical species. Thus proteins too, like lipids, are found in the variety of kinds and amounts. The type and the amount of protein present depends on the function of the membrane in question. For example, inner mitochondrial membrane is highly active in its functions and therefore contains 25 different kinds of proteins which constitute 75% of its weight. On the other hand, mylein which acts as an insulator contains only 20% protein by weight.

Membrane Models

The membrane components described in the previous section namely lipids, cholesterol and proteins are organized in a special manner so as to form a functional membrane. Several models have been proposed by various researchers. Of these, the 'fluid mosaic model' is now widely accepted (15). This model stresses the dynamic aspects of the membrane structure such as membrane fluidity. The extrinsic proteins are external but close to the membrane and may interact electrostatically with the polar headgroups of the lipids. The globular proteins in this model float surrounded by lipids (Figure 10).



A. GLOBULAR PROTEIN

B. FIBROUS PROTEIN

C. EXTRINSIC PROTEIN

Figure 9. Proteins in the Membranes.

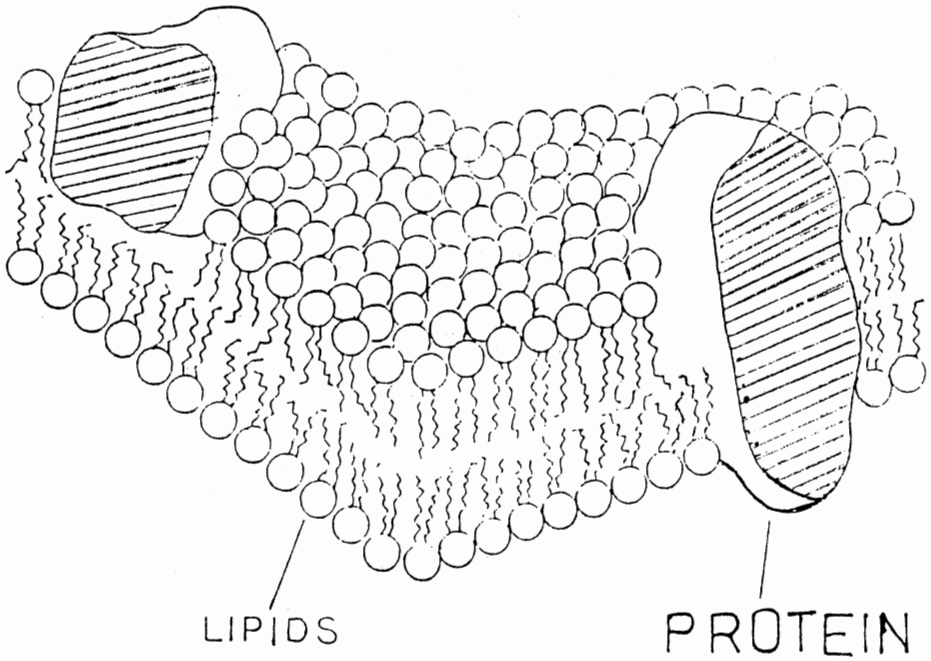


Figure 10. Model Membranes.

The lipids surrounding the proteins are further classified as the 'annular lipids' which are nearest neighbors of the protein and form a 'ring' around it. The lipids that are further away from the proteins are called 'bulk lipids'. Interaction of lipids and proteins has been under intense investigation both by experimentalists and theorists. Among experimental techniques x-ray diffraction (16), freeze etch fracture (17), thermal differential calorimetry (18, 19) and spin label spectroscopy (20, 21) have been used. On theoretical front, Monte Carlo simulation technique has been used by Scott (22), to study lipid-protein interaction.

The 'annular lipids' due to closeness of the proteins are more ordered and less mobile than the lipids in the bulk. Results of the ESR studies (20,21) indicated the existence of a single layer of lipids surrounding protein, cytochrome C oxidase. This result was further confirmed by theoretical models (23,24). The 'annular lipids' though relatively less mobile do frequently exchange themselves with the bulk lipids. This exchange is called 'hopping frequency'. In the bulk lipids this exchange takes place rather rapidly as compare to the exchange between bulk lipids and annular lipids. This reduction in exchange is attributed to the existence of interaction between protein and boundary lipids.

Although general structure of the membrane is preserved, the membrane components themselves are in continuous motion. (Figure 11)

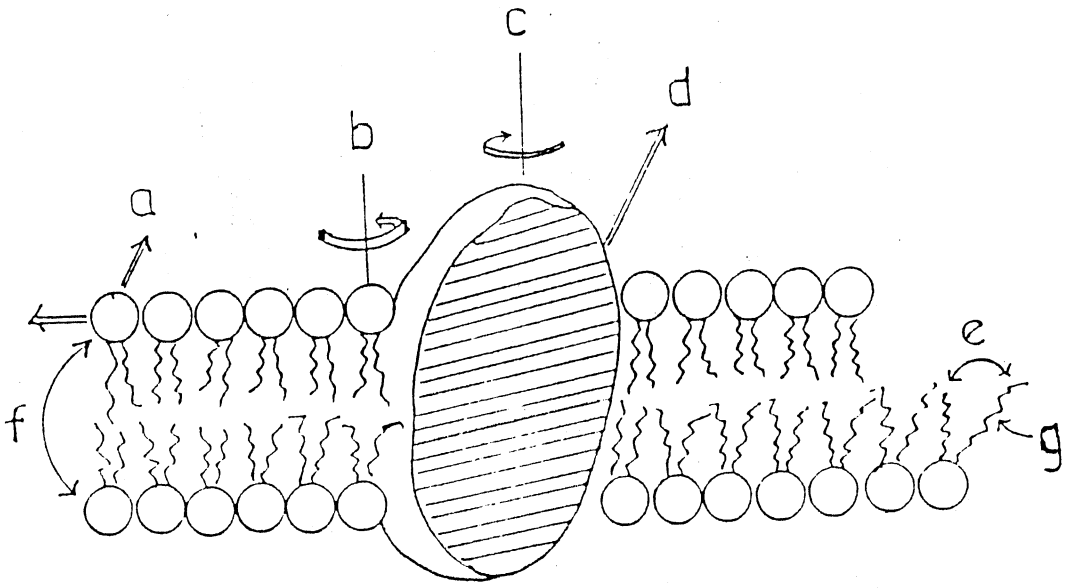


Figure 11. Modes of Mobility of Membrane Components. (a) Lateral Diffusion of Lipid. (b) Rotational Diffusion of Lipid. (c) Rotational Diffusion of Protein. (d) Lateral Diffusion of Protein. (e) Side to Side Chain Motion. (f) Lipid Exchange. (g) Rotation about C-C Bond.

A). Lateral Diffusion: Phospholipid and protein molecules diffuse laterally in the plane of the bilayer.

B). Rotational Diffusion: Phospholipid and protein molecules rotate about their long axis.

C). Lipid flip-flop motion: The phospholipid molecules mutually exchange themselves within the bilayers, going from one half of the bilayer to the other. But this motion is extremely slow on the order of days, compared to lateral diffusion which takes place in matter of seconds.

D). Chain motion: Above transition temperatures T_c, several disorders are introduced in the chain due to rotation about C-C bond. The various modes of motions of the membrane constituents along with their mutual interactions indeed form quite an intricate organization. This study attempts to investigate one important aspect, that of lipid cholesterol interaction by using Scott's method (22) of Monte Carlo simulation technique.

The following chapter describes the essentials of this technique. Following this, results and discussion are presented.

CHAPTER II

THEORY

The lipid cholesterol interaction study presented here is based on Monte Carlo simulation technique similar to one used by Scott (22) in his investigation of lipid protein interactions.

The Monte Carlo method is an efficient technique used to compute average quantities. In the present case we wish to calculate the average of the quantities called order parameters. This quantity, denoted by 'S' presents a measure of the interactions between the lipids and cholesterol by allowing us to find the extent to which the acyl chains are 'distorted' due the presence of a cholesterol molecule. The average order parameter $\langle S_n \rangle$, is defined as:

$$\langle S_n \rangle = \langle 3/2 \cos^2 \theta_n - 1/2 \rangle \qquad (2)$$

Where θ_n represents the angular deviation of nth C-C bond in an acyl chain, from its position while the chain is in all trans state. Thus, for example if the average value of the order parameters were 1.000 it would mean this particular bond did not deviate at all from its original all trans position. A smaller average value of the order parameters

would indicate greater degree of orientational disorder. The average values of the order parameters are thus expected to be in the range of 1 to 0. The values of the order parameters for each bond averaged over all the chains is then plotted against bond numbers as an pictorial indication of the lipid-cholesterol interaction.

In statistical mechanics the average of observable quantities Ai within canonical ensemble is given by (25,26).

$$\langle A \rangle = \left(\sum_i A_i \exp\left(- E_i/kT \right) \right) / \left(\sum_i \exp\left(- E_i/kT \right) \right) \qquad (3)$$

or

$$\langle A \rangle = \left(\int A \exp\left(- E / kT \right) dr \right) / \int \left(\exp\left(- E /kT \right) dr \right) \qquad (4)$$

where $r = (r_1, r_2, r_3, r_4, \ldots\ldots\ldots\ldots r_n)$

r_i = position vector of i th molecule, E = total energy of the system, T = Absolute temperature of the system and k = Boltzmann constant, 1.987 cal/mole-Kelvin. But evaluation of such an integral is rather complex and thus calculation of $\langle A \rangle$ demands alternative, simpler method. The Metropolis Monte Carlo method (27) developed in 1953, provides an efficient procedure of calculating average quantities. This method can be briefly described as follows:

1) Construct a model of the system under consideration. The initial configuration of the particles in the system - positions of carbon atoms of lipids and cholesterol in this case - should be known and saved.

2) Calculate energy E_o of this initial configuration and save it. Note that this energy consists of potential energy due to gauche rotations about C-C bonds plus sum of potential energies due to pairwise interactions between all atoms in all molecules.

3) Pick any particle--lipid molecule in this case- and translate it within the plane of the bilayer by a small distance dr.

$$r_i \longrightarrow r_i + edr_i$$

where r_i = co-ordinates of particle in question, dr_i = maximum allowed displacement, e = random number.

4) Pick a bond at random on this translated lipid molecule and perform gauch rotation about C-C bond according to rotational probabilities.

5) Apply periodic boundary condition to find if any of the carbon atoms on this chain have been displaced outside the unit cell. Also check for the chain overlaps.

6) Calculate the energy E_1 of this new configuration.

7) Calculate the energy change, $E = E_1 - E_o$, due to translation and rotation of the chain. (In our model system the perturbent

cholesterol molecule is always held fixed in its position w.r.t. the origin, except its mobile hydrocarbon tail.)

8) If $E < 0$ the transition is accepted and the lipid molecule is allowed to retain its new position. If $E > 0$,then a quantity $\exp(-E/kT)$ is calculated.

9) The quantity $\exp(-E/kT)$ is compared to a random number RANF (28). $0 <$ RANF < 1. If $\exp(-E/kT) <$ RANF then the move is rejected and the chain is moved back to its previous position. If $\exp(-E/kT) >$ RANF then move is accepted.

10) Above procedure is carried out for a large number of steps.

In Metropolis Monte Carlo method the configurations are not chosen randomly but are selected such that the transition probability P_{ij}, between states i and j is proportional to a Boltzman factor, thus

$$P_{ij} = (1/N)*(U_i/U_j) \qquad i \neq j;\ U_i > U_j \tag{5}$$

$$P_{ij} = (1/N) \qquad i = j;\ U_i < U_j \tag{6}$$

$$P_{ii} = 1 - P_{ij} \tag{7}$$

where $U_i \propto \exp(-E_i / kT)$

$$U_j \propto \exp(-E_j / kT) \tag{8}$$

and N = number of states

Under these conditions

$$\langle A \rangle = \left(\sum_i A_i \exp(- E_i/kT) \right) / \left(\sum_i \exp(- E_i/kT) \right) \qquad (9)$$

simply reduces to arithmetic average. The average value of the quantity A at some step s is given by

$$\langle A \rangle = (1/n) \sum_{n=1}^{s} A_s$$

where, n is the number of iterations carried out in the averaging process. In practice n is a large but finite number. Steps (8) and (9) above lead to selection of configurations that are lower in energy and thus eventually would take the system to its equilibrium state.

The average order parameter $\langle S_n \rangle$ for each bond number in all the chains is calculated by the procedure described above. For example, S_3 is an average order parameter for bond number 3 on all 35 chains. The flow chart (Figure 12) summarizes the procedure laid above for calculation of the average order parameters. It should be noted that the small displacement dr mentioned in the flow chart is chosen withsome consideration. If dr is too small then it will take long time for the system to reach equilibrium (wasting valuable computer time) and if dr is too large then too many moves will be rejected.

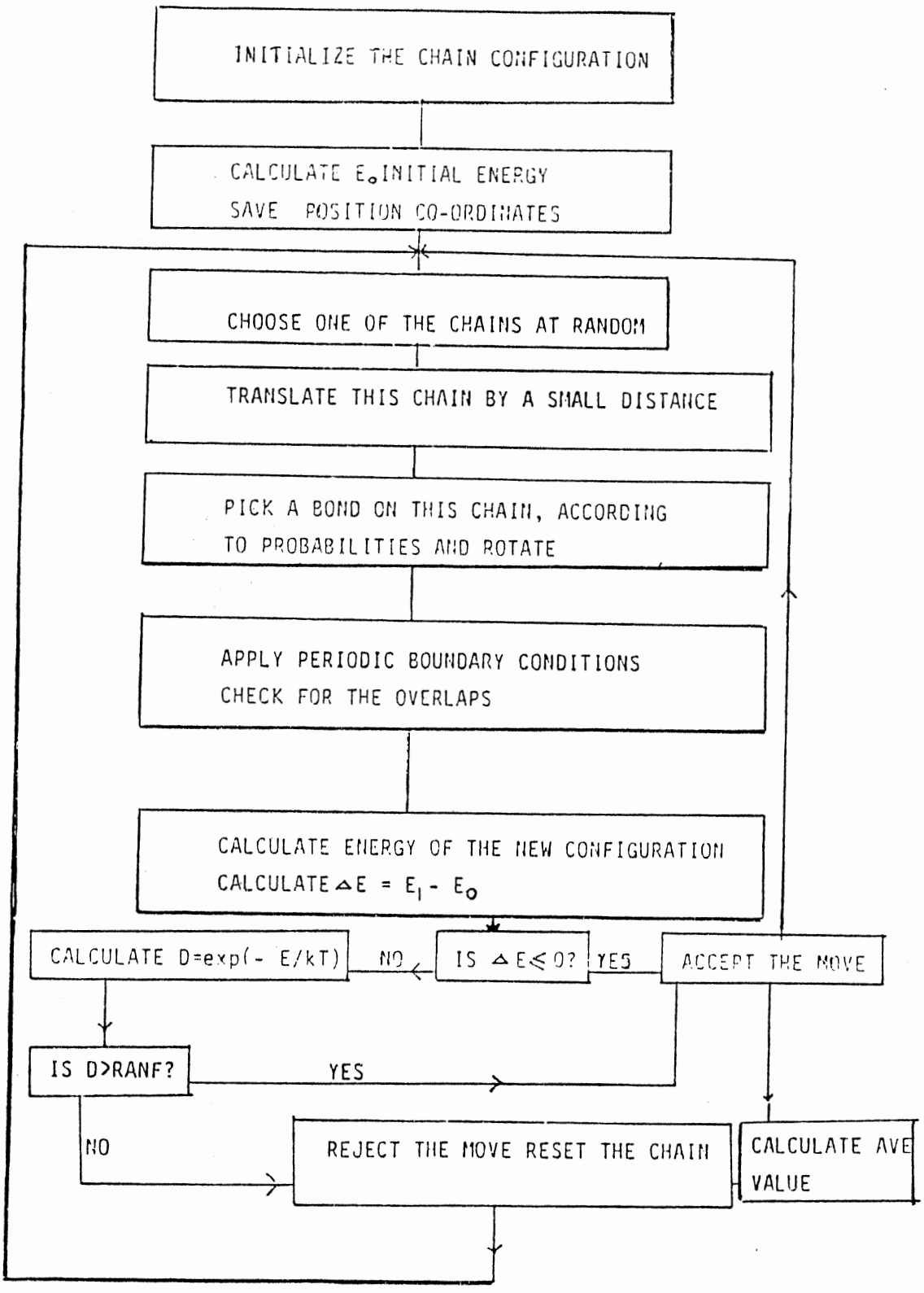


Figure 12. Flow Chart Displaying Monte Carlo Averaging Procedure.

hydrocarbon chains of the lipid molecules in this unit cell but one of the molecules namely molecule at the 22nd site is replaced bycholesterol molecule. The cell dimension are chosen so as to have approximately 29 Angstrom square area per acyl chain, (not including area of cholesterol). This area corresponds to lipid bilayer area per chain in its fluid state (29). The choice of site number 22 is arbitrary and was chosen to create an asymmetric unit cell. The cholesterol molecule with its surrounding lipids forms an hexagonal array (29). (figure 13). The first and the next nearest neighbors of cholesterol are clearly defined at the beginning of the program. Co-ordinates of the carbon atoms in the sterol ring structure were calculated assuming rings to be planar. The molecule itself is anchored to the membrane at the third carbon (at which B-hydroxy group is located, Figure 6).

Thirty six hydrocarbon chains were then simulated - including 'tail' on the cholesterol ring - by use of rotation matrix operator. C-C bond length was taken to be 1.53 A., and bond angle 109.5 degrees. The C-C bond position vector RVEC has its components as the projections along x,y,z axes. Initially components of RVECs for all chains are set to be (1.53,0,0). Next a rotation matrix operator ROT is defined,

$$ROT = \begin{bmatrix} \text{Cos } X & \text{Sin } X \text{ Cox } Y_i & \text{Sin } X \text{ Sin } Y_i \\ \text{Sin } X & -\text{Cos } X \text{ Cos } Y_i & -\text{Cos } X \text{ Sin } Y_i \\ 0 & -\text{ Sin } Y_i & \text{Cos } Y_i \end{bmatrix} \qquad (10)$$

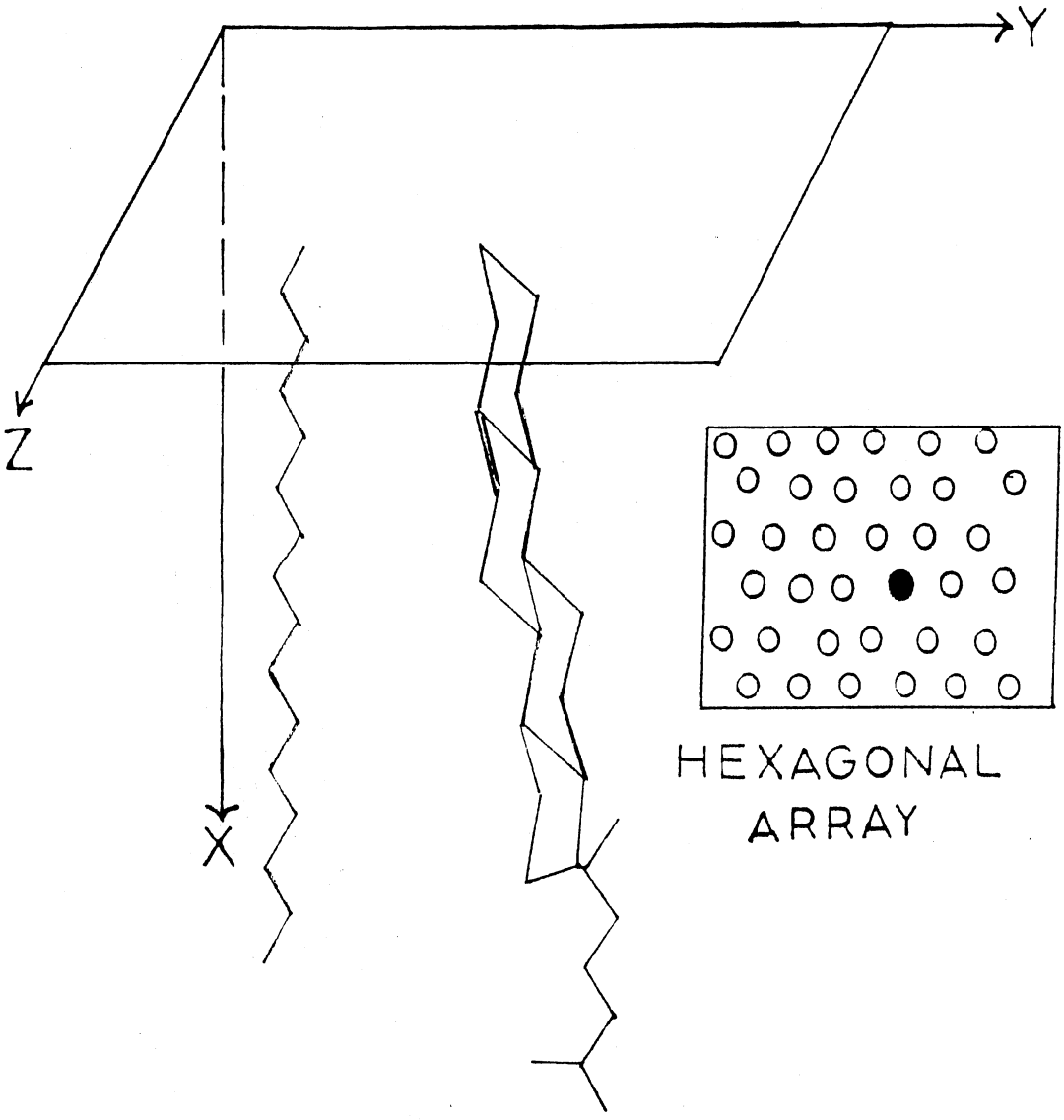


Figure 13. A Model of a Unit Cell.

where X = 70.5 degrees (complementary angle of 109.5 degrees) and Yi = gauche rotation angle = +120 or -120 degrees for bond i. But initially all the chains are desired to be in all trans state and so Yi is 0 degrees for all bonds. Operator ROT then becomes,

$$ROT = \begin{bmatrix} \text{Cos X} & \text{Sin X} & 0 \\ \text{Sin X} & -\text{Cos X} & 0 \\ 0 & 0 & 1 \end{bmatrix} \qquad (11)$$

The remaining chain i.e. x,y,z components of RVECs (giving positions of carbons on the chain) are obtained by successive application of the matrix operator.

$$\begin{bmatrix} \text{Cos X} & \text{Sin X} & 0 \\ \text{Sin X} & -\text{Cos X} & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x \\ y \\ z \end{bmatrix} = \begin{bmatrix} x' \\ y' \\ z' \end{bmatrix} \qquad (12)$$

The axes of these chains are not perpendicular to the y-z plane. In order to achieve this, these chains should be tilted. Therefore, another rotation matrix operator RT is defined.

$$RT = \begin{bmatrix} \text{Cos}\ (\ X/2) & \text{Sin}\ (\ X/2) & 0 \\ \text{Sin}\ (\ X/2) & -\ \text{Cos}\ (\ X/2) & 0 \\ 0 & 0 & 1 \end{bmatrix} \qquad (13)$$

This operator operates on each component of RVECs to produce chains that are perpendicular to the yz plane of the membrane.

$$\begin{bmatrix} \text{Cos}\ (X/2) & \text{Sin}\ (X/2) & 0 \\ \text{Sin}\ (X/2) & -\text{Cos}\ (X/2) & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x' \\ y' \\ z' \end{bmatrix} = \begin{bmatrix} x'' \\ y'' \\ z'' \end{bmatrix} \qquad (14)$$

The chains simulated in this manner are in all trans state, their planes being parallel to x-y plane. Each chain in our model is 16 CH_2 units long. The cholesterol tail is shorter, being only 6 CH_2 units long. This short chain is located on the 21st carbon atom of cholesterol. The chain is thus 'placed' on 21st atom simply by adding co-ordinates of 21st carbon to that of first carbon atom of the 'tail'.

Thus far a computer simulated model of the unit cell has been created, with 35 chains in all trans state and cholesterol molecule at the 22nd site. This defines initial configuration of our system. The position co-ordinates of all carbon atoms are known and saved.

The total energy of each chain in this configuration can be computed by summing its internal energy due to any gauche rotations

about C-C bonds and interaction energy between all carbon atoms of this chain and all other carbon atoms within interaction range, including those of cholesterol molecule.

$$E = E(i) + \sum_{CH_2's} \sum_{i \neq j} \epsilon \left(\left(\sigma / r_{ij} \right)^{12} - \left(\sigma / r_{ij} \right)^{6} \right) \qquad (15)$$

where E(i) = internal energy due to gauche rotation (+120 or -120 deg.) of bond number i. E(i) = 500 cal/mole for single gauche rotation and 2500 cal/mole for successive gauche rotations.

The pairwise interaction is obtained by summing Van der Waal interaction energies over all pairs i.e. all CH_2's of that chain and all other CH_2's within interaction range. Here ϵ and σ are Van der Waal parameters, with their values (30) ϵ = 118 cal/mole and σ = 3.905 A., r_{ij} = distance between CH_2's on i th and j th chain - including those on chain number 22 - within the interaction range. The condition $i \neq j$ is imposed so as to avoid 'self energy' being calculated. The total energy of initial configuration is thus known.

At this stage we are ready to put the system in a new configuration. A chain is picked and it is translated through a small distance dr. Its long axis still being perpendicular to y-z plane. On the same chain a bond is picked randomly according to the transition probability P_{ij} between states i and j and gauche rotation is attempted. The positions of the carbon atoms of a lipid chain, before and after a

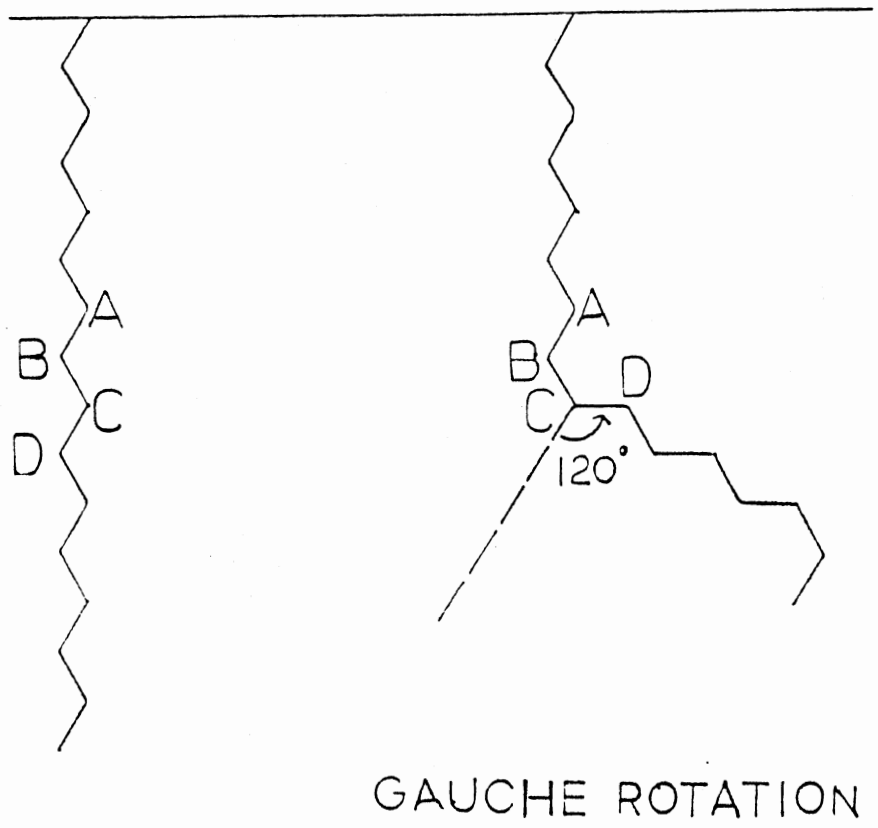


Figure 14. Gauche Rotation.

gauche rotation can be described in terms of a dihedral angle. Consider for example the atoms labelled as A, B, C, D in Figure 14. For the all trans configuration of this chain all four atoms A, B, C, D lie in one plane. More specifically the dihedral angle between the plane containing atoms A, B, C and the plane containing atoms B, C, D is 180 degrees. If a gauche rotation is performed about the bond joining atoms B-C, then the positions of atoms A, B, C remain unchanged. Atom D (and the subsequent atoms of this chain) will occupy new position as a result of gauche rotation. The dihedral angle between the plane containing atoms A, B, C and the plane containing atoms B, C, D is now 120^{o}. Note that the first bond between C_1-C_2 remains fixed at all times i.e. it is never picked for attempting gauche rotation. The transition probability P_{ij} can be calculated as follows.

(a) $P_{ij} = P(t \rightarrow g+) = P(t \rightarrow g-) = (1/N) \exp(-E / kT)$

$= 1/3 \exp((-500 \text{ cal/mole})) / ((1.987 \text{ cal/mole-K})(300 \text{ K})$

$= 0.144$

(b) $P_{g+ \rightarrow t} = 1/3$

(c) $P_{tt} = 1 - 2/3 \text{ epx}(- 500 / 1.987 T)$

(d) $P_{gg-} = 1/3 \exp(- 2200 / 1.98 T)$

(e) $P_{g+g+} = 1 - 1/3 - 1/3 \exp(- 2200 / 1.987 T)$

Energy of this new configuration is calculated as described previously (Equation 15). The energy difference between the previous (old) configuration and new configuration is calculated at this stage. Also, at this stage periodic boundary condition is applied. Following

which a subroutine CHECK, is devised in order to check overlap of two chains. If carbon atoms belonging to two chains come within a fixed hard-core diameter then they are said to overlap. If such an overlap occurs then the move is rejected and the chain is restored to its previous position. This makes saving the position co-ordinates very important in the program.

The energy difference ($DEL(E_{ij})$), between the previous configuration and the newly accepted one is then examined as follows.

If $DEL(E_{ij}) < 0$, then the new state is accepted. If $DEL(E_{ij}) > 0$ then a quantity $\exp(-DEL(E_{ij})/ kT)$, let it be denoted by D, is calculated and compared to a random number RANF between 0 and 1.

If $D > RANF$ then new state is accepted and If $D < RANF$ the new state is rejected. Such a test ensures that each new configuration is chosen so that system as a whole will reach the equilibrium in a large but finite number of steps.

Following this an average order parameter is calculated for all 15 bonds of all chains.

All computations were performed at the Oklahoma State University Computer Center facilities using IBM mainframe machine. The random number generated by the computer program RANF was developed by J. P.

Chandler of the Computer Science Department. The results of our study is presented in the next chapter followed by discussion.

CHAPTER III

RESULTS AND DISCUSSION

The results obtained by computer simulations are shown in Figures 15 and 16. The Tables in I and II show average values for the bond order parameter and the standard deviations of the bonds on cholesterol neighboring chains and all acyl chains respectively. Figure 15 shows graph of order parameter averaged over each bond in acyl chains neighboring cholesterol molecule versus bond number. Figure 16 shows values of order parameters for all chains plotted versus bond number. Each chain in our simulation had been 16 CH_2 units long.

Cholesterol ring structure was assumed to be planar although in reality the rings are 'puckered'. Yet our model is very close approximation of the actual molecule.

As mentioned earlier in Chapter II, the transition probabilities are proportional to Boltzman factor $\exp(- E_{ij} / kT)$. The absolute temperature of the simulated model was chosen to be 300 K at all times. The rotational probabilities were thus proportional to $\exp(- E_{ij} / kT)$, with appropriate values of E_{ij}. Since the temperature enters in the calculation of the probabilities, one might suspect that it plays a

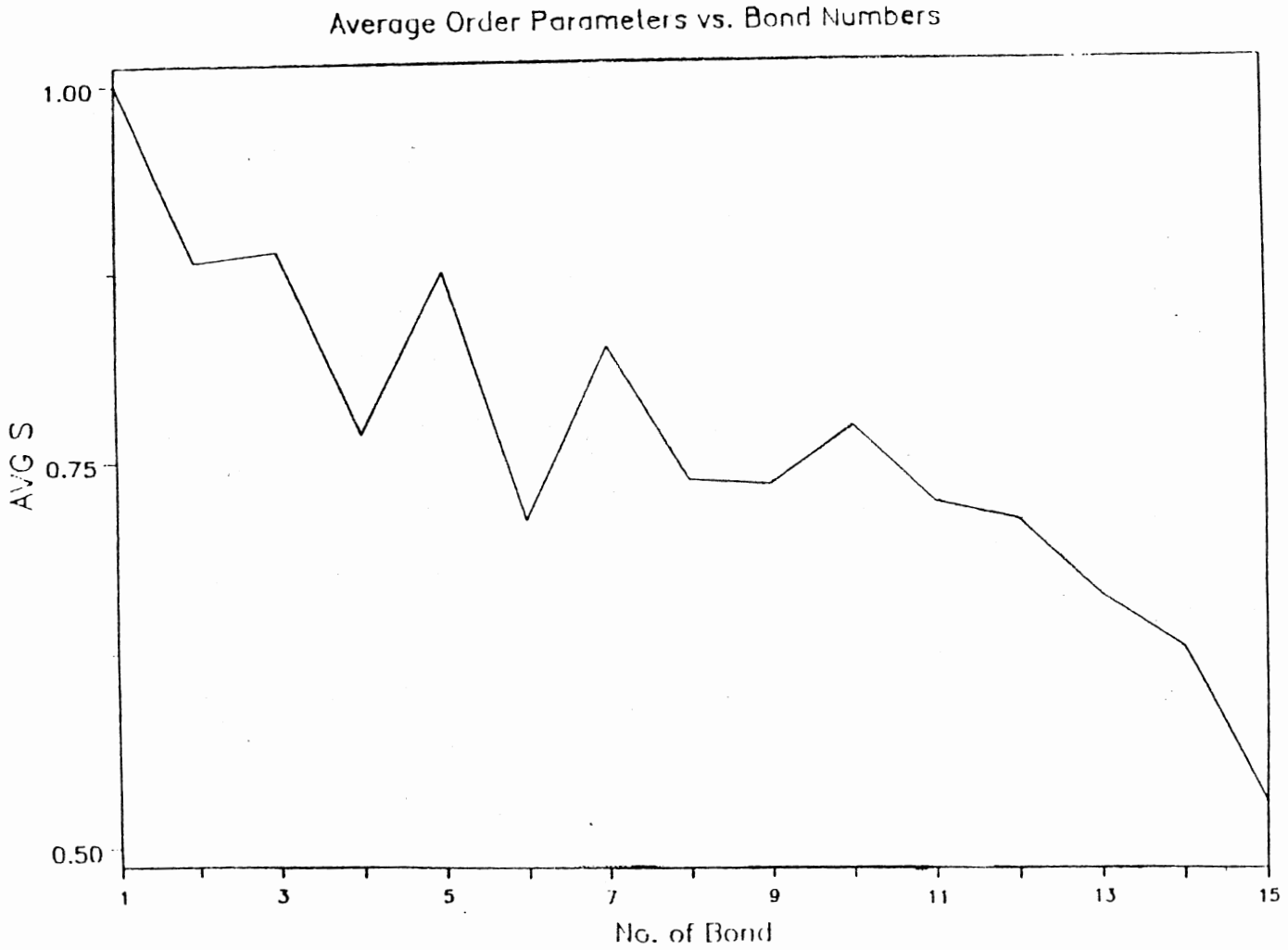


Figure 15. Plot of Sn vs. Bond Number for Boundary Lipids.

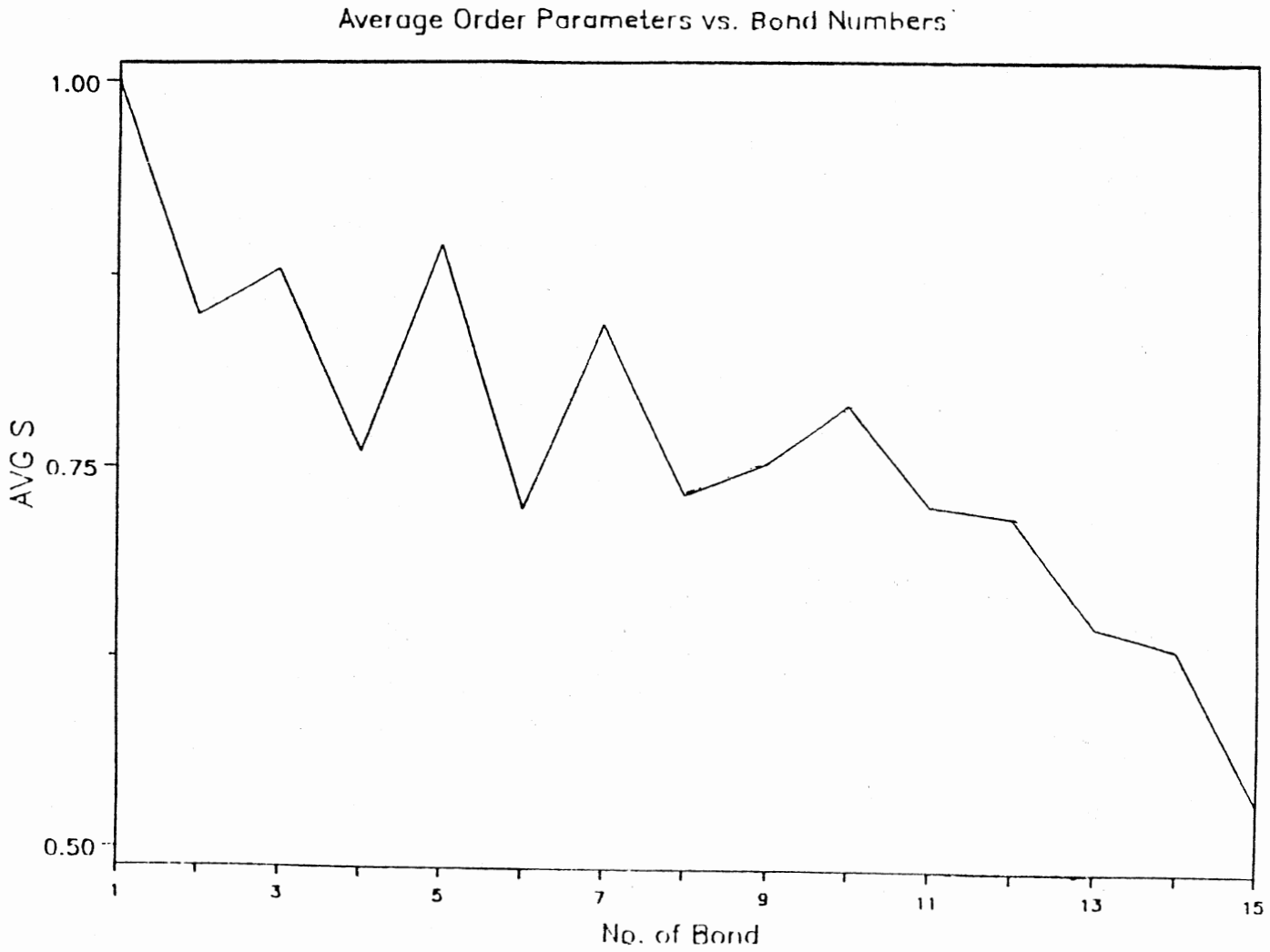


Figure 16. Plot of Sn vs. Bond Number for Lipid Chains.

TABLE I

AVERAGE VALUES OF THE ORDER PARAMETERS AND THE STANDARD DEVIATIONS FOR THE BONDS ON THE CHOLESTEROL NEIGHBORING CHAINS.

Bond Number	$\langle S_n \rangle$	Standard Deviation
1	1.00000	0.00000E+00
2	0.88200	0.98259E-02
3	0.88867	0.14815E-01
4	0.76817	0.18964E-01
5	0.87461	0.18454E-01
6	0.71137	0.51546E-01
7	0.82461	0.38985E-01
8	0.73685	0.42919E-01
9	0.73435	0.93022E-02
10	0.77325	0.74824E-01
11	0.72262	0.99968E-01
12	0.71149	0.33530E-01
13	0.66078	0.78854E-01
14	0.62665	0.33124E-01
15	0.52882	0.47242E-01

TABLE II

AVERAGE VALUES OF THE ORDER PARAMETERS AND THE STANDARD DEVIATIONS FOR THE BONDS ON THE ALL ACYL CHAINS.

Bond Number	$\langle S_n \rangle$	Standard Deviation
1	1.00000	0.00000E+00
2	0.84918	0.10306E-01
3	0.87891	0.93375E-02
4	0.76051	0.19716E-01
5	0.89466	0.14034E-01
6	0.72296	0.435117E-01
7	0.84420	0.35723E-01
8	0.73252	0.32989E001
9	0.75443	0.25289E-01
10	0.79258	0.85399E-01
11	0.72546	0.65777E-01
12	0.71829	0.29268E-01
13	0.64602	0.50648E-01
14	0.63105	0.43373E-01
15	0.53266	0.39056E-01

significant role in the outcome of the results. In fact, as found by Scott (22), the values of order parameters are not affected appreciably for two identical systems at different temperatures.

The Monte Carlo averages of the order parameters were found after 20,000 steps, where each step involves more of a single molecule. Although a large number of steps is desirable for more reliable results, it is believed that these averages are adequate, i.e. these values of S_n converge.

Superimposing plots shown in Figures 15 and 16 it is seen that there is very little difference in the order parameter profiles for the bulk and boundary lipids. This implies that the presence of a single cholesterol molecule in the unit cell simulated in our study did not affect the equilibrium of lipid chain states to a significant degree.

The length of a cholesterol molecule is about 19 angstroms. This includes the rigid ring structure (about 11 angstroms) and its tail (about 8 angstroms). The length of an acyl chain, in its all trans state, is also about the same. This was found by taking the product of the projection of the C-C bond vector on the x axis (1.27 angstroms) and the number of bonds. The rigid ring structure should restrict the motion of first 8 to 9 bonds. This is implied by higher values of the order parameters. Remaining 6 to 7 bonds are less restricted and highly interact with the tail of the cholesterol. The values of the order parameters for these bonds declined rapidly (Figure 16).

As described in the flow chart (Figure 12) the acyl chains were translated in the plane of the bilayer before attempting gauche rotations. The principal axis of an acyl chain was still perpendicular to the plane of the bilayer. The side-to-side motion of the chains (Figure 11) was not included in our simulation, nor did we include rotational diffusion of acyl chains.

The effect of tilting of the chains on the order parameters has been studied (31). It is reasonable to expect that such motion will further enhance the interactions between CH_2 groups on the neighboring chains. This will reduce the values of the order parameters. The values of the order parameters reflect the extent of disorder in the chains. Since an acyl chain is attached to the membrane surface at the carbon C_1, it is immobile. A first few top bonds show less disorder and tend to remain in their gauche states a longer time. The lower bonds being more free to rotate about C-C bonds alter their rotational states frequently. Therefore, there is a consistent decline in the values of the order parameters. But because of the comparable lengths of the acyl chains and the cholesterol molecule, the chains are unable to fold themselves.

The principal conclusion derived from this study is that a single molecule of cholesterol situated among 6x6 array of lipid chains cannot affect the equilibrium of lipid states. This is why the order parameter profiles for the boundary lipids and the bulk lipids follow the same

pattern. This conclusion is consistent with the lipid-protein interaction study by Scott (22).

Increasing lipid-cholesterol ratio will increase computation time to some extent and complexity of the problem. It will be interesting to include at least one more cholesterol molecule in our simulation and observe some changes due to increased interactions.

SELECTED BIBLIOGRAPHY

(1) Baker, D. A. and Hall, H. L., Membranes and Ion Transport Longman, p. 6, Longman Press, New York (1977).

(2) Tanford, C., Hydrophobic Effect., John Wiley, New York (1973)

(3) Davis, P. J., Keough K. M., Biochemistry., 18, 1453-1459 (1979)

(4) Barton, P. C. and Gunstone F. D., J. Biol. Chem., 250, 4470-4476, 6, (1975)

(5) Liu, N. I., Kay, R. I., Biochemistry., 16, 3484-3486 (1977)

(6) Trudell, J. R., Chin, J. H., Cohen, E. N., Payan, D. G., Biochim Biophys. Acta., 373, 436-443 (1974).

(7) Nagle, J. F., Wilkinson, D. A., Biophys. J., 23, 159-175, (1978).

(8) Chapman, D., Williams, R. M., Ladbrook, B. D., Chem Phys Lipid., 1, 455, (1967)

(9) Eagleman, D. M., J. Mol, Biol., 58, 153 (1971).

(10) Hubell, W. L., McConnell, H. M., J. Amer Chem. Soc., 93, 314 (1971).

(11) Jain, M. K., Current Topics in Membrane Transport., p. 1-57, J. Wiley, New York (1975).

(12) Lucy, J. A., Biological Membranes., Academic Press., 238-240.

(13) Chapman, D., Quart. Rev. Biophys., 8, 185-235 (1975).

(14) Chapman, D., William, R. M., Biochim Biophys Acta., 150, 333-340 (1968)

(15) Singer, S. J. and Nicholson, G. L., Science., 175, 720-731 (1972)

(16) Eagleman, D. M., Rothman, J. E., J. Biol. Chem., 247,3694 (1972)

(17) Vail, W. J., Papahadjopoulous, D., Biochim. Biophys. Acta., 163, 301 (1968)

(18) Chapman, D., Urbina, J., Keough K. M., J. Biol. Chem. 249, 2512 (1974)

(19) Papahadjopolous, D., Moscarello, M., Eylar, E. H., Isac, T., Biochim. Biophys. Acta., 401, 317 (1975)

(20) Jost, A., Griffith, O. H., Capaldi, R. A., Vanderkooi, Biochim. Biophys. Acta., 311, 141, (1973)

(21) Jost, A., Griffith, O. H., Capaldi, R. A., Vanderkooi, G., Proc. Natl. Acad. Sci. U.S.A., 70, 480 (1973)

(22) Scott, H. L., Biochim. Biophys. Acta., 469, 264 (1977)

(23) Kleeman, W., McConnell, H. M., Biochim. Biophys. Acta., 419, 206 (1976)

(24) Marcelja S., Biophys. Acta., 455, 1 (1979)

(25) Binder, K., Monte Carlo methods in statistical physics., Verlag.

(26) Curro. J. G., J. Chem. Phys., 61, 3, 1203-1207, (1974)

(27) Metropolis, N., Rosenbluth, M. N., Teller, A. H., Teller, E., J. Chem. Phys., 21, 1087 (1953)

(28) Chandler, J. P., RANF, Oklahoma State University., Computer Science Department

(29) Scott, H. L., Biochemistry, 25, 6122 (1986)

(30) Jorgensen, W. L., Madura, J. D., Swenson, C. J., J. Am. Chem. Soc., 106, 6638-6696 (1984)

(31) Peterson, N. O., Chan, S. I., Biochemistry., 16, 2657-2667 (1977)

APPENDIX A

COMPUTER PROGRAM FOR THE CALCULATION OF THE AVERAGE VALUES OF THE ORDER PARAMETERS

```
C
C
C     MONTE-CARLO SIMULATION PROGRAM FOR LIPID CHOLESTEROL INTERACTION
C     CHAIN NUMBER 22 IS CHOLESTEROL
C
C     THIS PROGRAM COMPUTES AN ORDER PARAMETER WHICH IS A MEASURE
C     OF INTERACTION BETWEEN LIPID CHAINS AND CHOLESTEROL MOLECULE
C     IN A MEMBRANE.
C
C     *********PARAMETERS USED IN THIS PROGRAM ****************
C     ANG=CHAIN LONG AXIS ROTATION
C     BKT = RECIPROCAL OF (BOLTZMAN CONST*TEMP)
C     CROT = RANDOM NUMBER FOR AXIAL ROTATION
C     CPS = SAVED CARBON CO-ORDINATES
C     CHK = SUBROUTINE :CHECKS OVERLAPS,CALCULATES ENERGY
C     CP = CARBON POSITION
C     DEL = ENERGY DIFFERENCE IN GAUCH TRANS CONFIGURATIONS
C     EPK = LIPID CHOLESTEROL INTERACTION ENERGY
C     EOLD = OLD LIPID CHOLESTEROL INTERACTION ENERGY
C     ENEW = NEW TOTAL ENERGY
C     EOLD = OLD TOTAL ENERGY
C     EVWD = VANDER WAALS ENERGY
C     ESV = SAVED ENERGY
C     EPIL = 6 - 12 ENERGY PARAMETER
C     GASIN = SIN 120 DEGREES
C     GACOS = COS 120 DEGREES
C     GR = +1(+120 DEGREES ROTATION OF BOND)
C     GS = SAVED CO-ORDINATES
C     IRAN = CALLS TO RAN BEFORE PROG STARTS
C     IPRT = PRINT INTERVAL
C     IC,ICL = USED TO PICK BONDS FOR GAUCH ROTATIONS
C
ISN    1      COMMON CP(3,27,36),RVEC(3,27,36),NCHAIN,NLINK,SIZEY,SIZEZ,RC
ISN    2      COMMON/LST/RANG,ILST,JLST,IDST
ISN    3      COMMON/VDW/SIG,EPIL
ISN    4      DIMENSION ROT(3,3),AVEC(3,27),D(3),ROTN(3,3),ROTP(3,3)
ISN    5      DIMENSION RVECH(3,4),CHP(3,4),TVEC(3,16,36)
ISN    6      COMMON/CHK/KTR,JR,SY,SZ,SAVG(27),SSVG(27),NPP(36),NNP(36)
ISN    7      DIMENSION GS(27,36),ESV(36),VRND(36,4,80),DPP(36)
ISN    8      DIMENSION XPR(15),YPR(15),ZPR(15),ZNN(15),NREJ(36),ANG(36)
ISN    9      DIMENSION GR(27,36)
ISN   10      DIMENSION NN(36,36),EVDW(36,36),RSV(3,27,36),CPS(3,27,36)
ISN   11      DIMENSION IC(36),ICL(36),E(36),LRJ(27),GASIN(3),GACOS(3)
ISN   12      DIMENSION DCH(2,27),CHOL(3,27),DST(16),DOV(3)
ISN   13         DIMENSION ENEW(36),EOLD(36)
ISN   14      DIMENSION DEL(36),EPK(36),EPOLD(36),CMP(36),RT(3,3),CROT(36)
ISN   15      PARAMETER(RAD=4.,RREP=7.05,T=300.,NMAX=50,NMIN=0)
ISN   16      PARAMETER(IPRT=25,IRAN=17,RRR=9.,RPEP=40.)
C     ******SOME MORE PARAMETERS IN THIS PROGRAM ********
C     KTR = CHAIN BEING TRANSLATED AND ROTATED
```

```
C      NMAX = # OF STEPS FOR AVERAGING
C      NMIN = # OF STEPS TO EQUILIBRATE
C      NN = NEIGHBOR MATRIX
C      NPP = CHOL NEICHBOR MATRIX
C      NCHAIN = # OF CHAINS
C      NLINK = # OF BONDS
C      RPEP = RADIUS OF PEPTIDE(ORIGINAL PROG PARAMETER)
C      A PARAMETER 'DPX' IS COMPARED TO RPEP AND RNNN AND SUBJECT
C      TO CONDITIONS NPP AND NNP ARE SET EQUAL TO 1(LNES 6150-6250)
C      RRR,RPEP,RAD = CUTOFF RADII
C      RT = SECOND ROT OPERATOP(LONG AXIS)
C      ROT = FIRST ROT OPERATOR (BOND-BOND)
C      RVEC = C TO C BOND VECTORS
C      SAVG = AVERAGE ORDER PARAMETER
C      SSVG = INITIAL AVG ORDER PARAMETER
C      SY,SZ = ARRAY SIZE
C      SIG = VANDER WAAL RADIUS
C      SIZEY,SIZEZ = CELL DIMENSION
C      X = COMPLEMENTARY ANGLE OF 109.5 DEGREES =70.5 DEGREES
C
C      INITIALIZE
C
C      **SIZEY,SIZEZ REFER TO THE DIMENSION OF THE CELL
C      SIG=3.905 ANGSTROM IS VAN DER WALL RADIUS:
C      EPIL=118 CAL/MOLE IS VANDER WALL ENERGY:
C      CELL DIMENSION IS ADJUSTED TO GIVE 29 ANGSROM SQUARE
C      AREA PER LIPID HEADGROUP.
C      THERE ARE 36 CHAINS IN A CELL ;ONE OF WHICH (CHAIN #22)
C      IS CHOLESTEROL(PERTUBANT).REMAINING LIPID CHAINS ARE
C      10 CH2 UNITS IN LENGTH,AND ARE PERPANDICULAR TO CELL SURFACE
C
C ***************************************************************
C                        SUBPROGRAMS
C  SUBPROGRAM :CHECK ,CHECKS FOR OVERLAPS AND CALCULATES 6-12
C  ENERGIES BETWEEN CHAIN NO. KTR AND ALL OTHER CHAINS.
C  SUBPROGRAM :CH2(XPR,YPR) ,CALCULATES ORDER PARAMETERS 'S'
C  SUBPROGRAM :CLIST ,CALCULATES THE NN ARRAY
C  SUBPROGRAM :CHOL1 , GENERATES THE 'TAIL'CHAIN OF CHOLESTEROL.
C
C
C***************************************************************
ISN       17          ILI=1
ISN       18          RNNN=4.*RPEP
C                     IRAN=PRIME NUMBER
ISN       19          RANG=(RRR)**2
ISN       20          NCHAIN=36
ISN       21          NLINK=16
ISN       22          BND=RAD/2.
ISN       23          DO 99 LL=1,NCHAIN
ISN       24    99    ESV(LL)=0.
ISN       25          JSKIP=3*NLINK
ISN       26          IREJ=0
ISN       27          LLINK=NLINK-1
ISN       28          LSKIP=3*LLINK
ISN       29          SIZEY=34.2
ISN       30          SIZEZ=29.61807
```

```
ISN        31          SIG=3.905
ISN        32             SIG=SIG**2
ISN        33          SIGLP=SIG
ISN        34          EPIL=118.
ISN        35          SZ=SIZEZ-RRR
ISN        36          SY=SIZEY-RRR
                  C
                  C     INITIALIZE LIPID-CHOLESTEROL NEIGHBOR ARRAYS;
                  C     CHOL MOLECULE IN THE CENTRE OF HEXAGONAL ARRAY OF LIPIDS
                  C     NPP REFERS TO THE NEAREST NEIGHBORS OF CHOL (SIX OF THEM)
                  C     NNP REFERS TO THE NEXT NEAREST NEIGHBORS (TWELVE OF THEM)
                  C     NUMBER IN BRACKET E.G. NPP(28) IS CHAIN NUMBER
                  C
ISN        37          DO 3 II=1,NCHAIN
ISN        38          NPP(II)=0
ISN        39             NNP(II)=0
ISN        40          DO 3 JJ=1,NCHAIN
ISN        41          IF(II.EQ.JJ)GO TO 2
ISN        42          NN(II,JJ)=0
ISN        43          GO TO 3
ISN        44     2    NN(II,JJ)=1
ISN        45     3    CONTINUE
ISN        46          DO 4 LL=1,LLINK
ISN        47             YPR(LL)=0.
ISN        48     4    XPR(LL)=0.
ISN        49          NPP(16)=1
ISN        50          NPP(17)=1
ISN        51          NPP(21)=1
ISN        52          NPP(22)=0
ISN        53          NPP(23)=1
ISN        54          NPP(28)=1
ISN        55          NPP(29)=1
ISN        56             NNP(9)=1
ISN        57             NNP(10)=1
ISN        58             NNP(11)=1
ISN        59             NNP(15)=1
ISN        60             NNP(18)=1
ISN        61             NNP(20)=1
ISN        62             NNP(24)=1
ISN        63             NNP(27)=1
ISN        64             NNP(30)=1
ISN        65             NNP(33)=1
ISN        66             NNP(34)=1
ISN        67             NNP(35)=1
                  C
                  C     *****CO-ORDINATES OF CARBON ATOMS OF CHOLESTEROL******
                  C    FIRST NUMBER IN THE BRACKET IS CO-ORD,SECOND IS CARBON NUMBER
                  C    UPPER LEFT CORNER OF THE CELL IS ORIGIN,CARTESIAN SYSTEM.
                  C    CARBON-CARBON BOND IS 1.53 A;BOND ANGLE IS 109.5 DEGREES.
                  C
ISN        68                      CHOL(1,1)=2.413
ISN        69                      CHOL(2,1)=18.524979
ISN        70                      CHOL(3,1)=16.404443
ISN        71                      CHOL(1,2)=0.883
ISN        72                      CHOL(2,2)=18.524979
ISN        73                      CHOL(3,2)=16.404443
```

```
ISN      74                    CHOL(1,3)=0.0
ISN      75                    CHOL(2,3)=18.524979
ISN      76                    CHOL(3,3)=15.155443
ISN      77                    CHOL(1,4)=0.883
ISN      78                    CHOL(2,4)=18.524979
ISN      79                    CHOL(3,4)=13.906443
ISN      80                    CHOL(1,5)=2.413
ISN      81                    CHOL(2,5)=18.524979
ISN      82                     CHOL(3,5)=13.906443
ISN      83                    CHOL(1,6)=3.300
ISN      84                    CHOL(2,6)=18.524979
ISN      85                    CHOL(3,6)=12.657443
ISN      86                    CHOL(1,7)=4.830
ISN      87                    CHOL(2,7)=18.524979
ISN      88                    CHOL(3,7)=12.657443
ISN      89                    CHOL(1,8)=5.713
ISN      90                    CHOL(2,8)=18.524979
ISN      91                    CHOL(3,8)=13.906443
ISN      92                    CHOL(1,9)=4.830
ISN      93                    CHOL(2,9)=18.524979
ISN      94                    CHOL(3,9)=15.155443
ISN      95                    CHOL(1,10)=3.300
ISN      96                    CHOL(2,10)=18.524979
ISN      97                    CHOL(3,10)=15.155443
ISN      98                    CHOL(1,11)=3.300
ISN      99                    CHOL(2,11)=20.0544979
ISN     100                    CHOL(3,11)=15.155443
ISN     101                    CHOL(1,12)=7.243
ISN     102                    CHOL(2,12)=18.524979
ISN     103                    CHOL(3,12)=16.404443
ISN     104                    CHOL(1,13)=5.713
ISN     105                    CHOL(2,13)=18.524979
ISN     106                    CHOL(3,13)=16.404443
ISN     107                    CHOL(1,14)=7.243
ISN     108                    CHOL(2,14)=18.524979
ISN     109                    CHOL(3,14)=13.906443
ISN     110                    CHOL(1,15)=8.132
ISN     111                    CHOL(2,15)=18.524979
ISN     112                    CHOL(3,15)=12.664443
ISN     113                    CHOL(1,16)=11.132
ISN     114                    CHOL(2,16)=18.524979
ISN     115                    CHOL(3,16)=13.191443
ISN     116                    CHOL(1,17)=9.593
ISN     117                    CHOL(2,17)=18.524979
ISN     118                    CHOL(3,17)=14.720943
ISN     119                    CHOL(1,18)=8.126
ISN     120                    CHOL(2,18)=18.524979
ISN     121                    CHOL(3,18)=15.155443
ISN     122                    CHOL(1,19)=8.126
ISN     123                    CHOL(2,19)=20.054979
ISN     124                    CHOL(3,19)=15.155443
ISN     125                    CHOL(1,21)=9.593
ISN     126                    CHOL(2,21)=20.054979
ISN     127                    CHOL(3,21)=15.155443
                C
                C****** GENERATING CHOLESTEROL CHAIN CO-ORDINATES **********
```

```
C  THE POSITIONS OF CARBONS(CP) ON THE CHAIN OF THIS MOLECULE
C  ARE FOUND BY USING THE SUBPROG CHOL1. THE CHAIN IS GENERATED
C  EXACTLY IN THE SAME MANNER AS THE OTHER CHAINS IN THE MAIN
C  PROGRAM EXCEPT THAT THIS CHAIN IS LOCATED ON THE 21ST CARBON
C  WHICH IS AT THE LOWER END OF THE RIGID RING SSTRUCTURE.
C  THUS FOR THIS CHAIN CP(1,1,K) IS NOT EQUAL TO 0.
C  BUT CP(1,1,K)=9.5930 WHICH IS THE X CO-ORD OF 21ST CARBON.
C**********************************************************************
ISN     128                                  CHOL(1,20)=8.34354
ISN     129                                    CHOL(2,20)=19.17194
ISN     130                                   CHOL(3,20)=15.15544
ISN     131                                  CHOL(1,22)=10.84246
ISN     132                                  CHOL(2,22)=19.17194
ISN     133                                   CHOL(3,22)=15.15544
ISN     134                                   CHOL(1,23)=12.09192
ISN     135                                   CHOL(2,23)=20.05498
ISN     136                                   CHOL(3,23)=15.15544
ISN     137                                    CHOL(1,24)=13.34138
ISN     138                                    CHOL(2,24)=19.17194
ISN     139                                    CHOL(3,24)=15.15544
ISN     140                                    CHOL(1,25)=14.59084
ISN     141                                    CHOL(2,25)=20.05498
ISN     142                                    CHOL(3,25)=15.15544
ISN     143                                    CHOL(1,26)=15.8403
ISN     144                                    CHOL(2,26)=19.17194
ISN     145                                    CHOL(3,26)=15.15544
ISN     146                                    CHOL(1,27)=14.59084
ISN     147                                    CHOL(2,27)=21.30444
ISN     148                                    CHOL(3,27)=16.03847
C *** THERE ARE 20 CARBON ATOMS IN THE RIGID RING STRUCTURE
C     AND THERE ARE 7 CARBON ATOMS IN THE "TAIL" CHAIN
C   KSTART=1 MEANS START FROM LATTICE; ELSE SET IT = 0
C
ISN     149          DO 6666 I=1,3
ISN     150          DO 6669 J=1,27
ISN     151  6669    CP(I,J,22)=CHOL(I,J)
ISN     152  6666    CONTINUE
ISN     153             KSTART=1
ISN     154          IF (KSTART.NE.0)GO TO 10
ISN     155          GO TO 15
ISN     156  10      CONTINUE
ISN     157          DO 13 I=1,NCHAIN
ISN     158          DO 13 J=1,NLINK
ISN     159  13      GR(J,I)=0.
ISN     160          DLL=5.7
ISN     161          RI=2.85
ISN     162           DLZ=5.0*(.8660254)
ISN     163           RLZ=DLZ/2.
ISN     164          DO 21 J=1,6,2
ISN     165          DO 21 L=1,6
ISN     166          XL=FLOAT(L-1)
ISN     167          XJ=FLOAT(J-1)
ISN     168          JL=L+6*(J-1)
ISN     169          IF (JL .EQ. 22) GO TO 21
ISN     170          CP(2,1,JL)=RI+1.425+DLL*XL
ISN     171              CP(3,1,JL)=RLZ+DLZ*XJ
```

```
ISN      172   21     CONTINUE
ISN      173          DO 22 J=2,6,2
ISN      174          DO 22 L=1,6
ISN      175          JL=L+6*(J-1)
ISN      176          XL=FLOAT(L-1)
ISN      177          XJ=FLOAT(J-1)
ISN      178          IF (JL .EQ. 22) GO TO 22
ISN      179          CP(2,1,JL)=DLL*XL+1.425
ISN      180              CP(3,1,JL)=RLZ+DLZ*XJ
ISN      181   22     CONTINUE
ISN      182          GO TO 48
ISN      183   15     CONTINUE
                C
                C     USING ROTATION MATRICES SUCCESSIVELY INITIAL CHAIN POSITIONS
                C     ARE CALCULATED .
                C
ISN      184          REWIND 9
ISN      185          DO 16 L=1,NCHAIN
ISN      186          READ(9,46)ANG(L)
ISN      187          IF (L .EQ. 22) THEN
ISN      188          IZ1=21
ISN      189          IZ2=27
ISN      190          ELSE
ISN      191          IZ1=1
ISN      192          IZ2=16
ISN      193          ENDIF
ISN      194          DO 16 J=1,IZ2
ISN      195          READ(9,45)(CP(K,J,L),RVEC(K,J,L),K=1,3),GR(J,L)
ISN      196   16     CONTINUE
ISN      197   45     FORMAT(7F12.6)
ISN      198   46     FORMAT(F12.6)
ISN      199   48     CONTINUE
ISN      200          XDN=NMAX-NMIN
ISN      201          BKT=1./(1.987*T)
ISN      202          PRINT 1233,(D(J),J=1,3),RREP,T
ISN      203   1233   FORMAT(4X,'CHOL RUN',3A4,'RREP= ',F10.3,5X,'TEMP= ',F10.3//)
ISN      204          PRINT 1230
ISN      205   1230   FORMAT(5X,'NMAX',6X,'NMIN',6X,'ILST',6X,'KSTART'//)
ISN      206          PRINT 1234,NMAX,NMIN,ILST,KSTART
ISN      207   1234   FORMAT(' ',4(I6,5X)//)
ISN      208          PI=+3.14159265
ISN      209          Y1=2.*PI/3.
ISN      210          Y2=0.
ISN      211          Y3=5.*Y1
ISN      212          GASIN(1)=SIN(Y1)
ISN      213          GACOS(1)=COS(Y1)
ISN      214          GASIN(2)=SIN(Y2)
ISN      215          GACOS(2)=COS(Y2)
ISN      216          GASIN(3)=SIN(Y3)
ISN      217          GACOS(3)=COS(Y3)
ISN      218          X=70.5*PI/180.
ISN      219          ESO=SIN(X)
ISN      220          ECO=COS(X)
ISN      221          ESON=SIN(-X)
ISN      222          ECON=COS(-X)
ISN      223             ES=SIN(X/2.)
```

```
ISN      224            EC=COS(X/2.)
ISN      225          ROTN(1,1)=ECON
ISN      226          ROTN(2,1)=ESON
ISN      227          ROTN(3,1)=0.0
ISN      228          ROTN(1,2)=-ESON
ISN      229          ROTN(2,2)=ECON
ISN      230          ROTN(3,2)=0.0
ISN      231          ROTN(1,3)=0.0
ISN      232          ROTN(2,3)=0.0
ISN      233          ROTN(3,3)=1.0
ISN      234          ROTP(1,1)=ECO
ISN      235          ROTP(2,1)=ESO
ISN      236          ROTP(3,1)=0.0
ISN      237          ROTP(1,2)=-ESO
ISN      238          ROTP(2,2)=ECO
ISN      239          ROTP(3,2)=0.0
ISN      240          ROTP(1,3)=0.0
ISN      241          ROTP(2,3)=0.0
ISN      242          ROTP(3,3)=1.0
                  C     ELEMENTS OF ROTATION MATRICES ROT AND RT ARE DEFINED
                  C     ANGLE X=70.5 DEGREES
ISN      243          ROT(1,1)=ECO
ISN      244          ROT(2,1)=ESO
ISN      245          ROT(3,1)=0.
ISN      246          ROT(1,2)=ESO
ISN      247          ROT(2,2)=-ECO
ISN      248          ROT(3,2)=0.
ISN      249          ROT(1,3)=0.
ISN      250          ROT(2,3)=0.
ISN      251          ROT(3,3)=1.
ISN      252          DO 116 I=1,NLINK
ISN      253          SAVG(I)=0.
ISN      254     116  CONTINUE
ISN      255          DO 6000 I=1,NCHAIN
ISN      256          IF(I.EQ.22) GO TO 6000
ISN      257          CP(1,1,I)=0.0
ISN      258     6000 ANG(I)=0.
ISN      259          RT(1,1)=EC
ISN      260          RT(2,1)=ES
ISN      261          RT(3,1)=0.
ISN      262          RT(1,2)=-ES
ISN      263          RT(2,2)=EC
ISN      264          RT(3,2)=0.
ISN      265          RT(1,3)=0.
ISN      266          RT(2,3)=0.
ISN      267          RT(3,3)=1.
ISN      268          IF(KSTART.EQ.0)GO TO 155
ISN      269          DO 140 K=1,NCHAIN
ISN      270          IF(K.NE.22)GOTO 6999
ISN      271          CALL CHOL1(ROT,RT,AVEC)
ISN      272          IY=22
ISN      273          DO 7048 IZ=21,27
ISN      274          GS(IZ,IY)=GR(IZ,IY)
ISN      275          DO 7049 IW=1,3
ISN      276          RSV(IW,IZ,IY)=RVEC(IW,IZ,IY)
ISN      277     7049 CONTINUE
```

```
ISN      278   7048  CONTINUE
ISN      279         DO 7019 IZ=1,27
ISN      280         DO 7029 IW=1,3
ISN      281         CPS(IW,IZ,IY)=CP(IW,IZ,IY)
ISN      282   7029  CONTINUE
ISN      283   7019  CONTINUE
               CL**************
ISN      284         GO TO 150
ISN      285   6999  CONTINUE
ISN      286         RVEC(1,1,K)=1.53
ISN      287         DO 6001 I=2,3
ISN      288   6001  RVEC(I,1,K)=0.
ISN      289         DO 145 J=2,NLINK
ISN      290         L=J-1
ISN      291         DO 147 I=1,3
ISN      292         RVEC(I,J,K)=0.
ISN      293         DO 6002 IY=1,3
ISN      294   6002  RVEC(I,J,K)=RVEC(I,J,K)+RVEC(IY,L,K)*ROT(IY,I)
ISN      295   147   CONTINUE
ISN      296   145   CONTINUE
ISN      297         DO 6003 I=1,3
ISN      298         DO 6004 J=1,NLINK
ISN      299   6004  AVEC(I,J)=RVEC(I,J,K)
ISN      300   6003  CONTINUE
ISN      301         DO 148 I=1,3
ISN      302         DO 148 J=1,NLINK
ISN      303         RVEC(I,J,K)=0.
ISN      304         DO 6005 IY=1,3
ISN      305   6005  RVEC(I,J,K)=RVEC(I,J,K)+AVEC(IY,J)*RT(IY,I)
ISN      306   148   CONTINUE
ISN      307         DO 149 J=2,NLINK
ISN      308         JJ=J-1
ISN      309         DO 6006 I=1,3
ISN      310   6006  CP(I,J,K)=CP(I,JJ,K)+RVEC(I,JJ,K)
ISN      311   149   CONTINUE
ISN      312   150   CONTINUE
ISN      313   140   CONTINUE
ISN      314   155   PRINT 1109
ISN      315   1109  FORMAT(18X,'INITIAL VALUES OF CP & IG' ///)
ISN      316         DO 165 L=1,NCHAIN
ISN      317         J1=1
ISN      318         J2=NLINK
ISN      319         IF(L.EQ.22)THEN
ISN      320         J1=21
ISN      321         J2=27
ISN      322         END IF
ISN      323         DO 165 J=J1,J2
ISN      324         PRINT 1110,(CP(K,J,L),K=1,3),GR(J,L)
ISN      325   165   CONTINUE
               C
               C     ***** INITIAL CONFIGURATION IS PRINTED AT THIS STEP *****
               C     MEANS CARBON POSITIONS CP ARE PRINTED,XYZ CO-ORDINATES.
               C
ISN      326   1110  FORMAT(5X,4F12.5)
ISN      327   1114  FORMAT(5X,'1114',I4,I4)
ISN      328   1115  FORMAT(5X,'1115',I4,I4)
```

```
ISN      329          II=0
ISN      330          KK=0
ISN      331          XINIT=RANF(0)
ISN      332          PRINT 1100
ISN      333    1100  FORMAT(12X,'XYZ=RANF(0)'//)
ISN      334          DO 120 I=1,IRAN
ISN      335          XYZ=RANF(0)
ISN      336          PRINT 1101,XYZ
ISN      337    120   CONTINUE
ISN      338    1101  FORMAT(10X,F12.6/)
ISN      339          DO 1210 ILST=1,NCHAIN-1
ISN      340          DO 1210 JLST=ILST+1,NCHAIN
ISN      341          CALL CLIST
ISN      342          IF(IDST.EQ.1)GO TO 1095
ISN      343          NN(ILST,JLST)=0
ISN      344          NN(JLST,ILST)=0
ISN      345          GO TO 1210
ISN      346    1095  NN(ILST,JLST)=1
ISN      347          NN(JLST,ILST)=1
ISN      348    1210  CONTINUE
ISN      349    3299  FORMAT(10X,36I2)
ISN      350          DO 130 K=1,NCHAIN
ISN      351    130   PRINT 3299,(NN(K,I),I=1,NCHAIN)
                      C
                      C  INITIAL ENERGIES OF THE CHAINS ARE CALCULATED AND PRINTED
                      C  FOR ALL 36 CHAINS.INTERNAL ENERGY OF A CHAIN DUE TO ITS BONDS'
                      C  CONFIGURATION AND INTERACTION WITH ALL OTHER CHAINS IS SUMMED.
                      C
ISN      352          DO 135 K=1,NCHAIN
ISN      353          KTR=K
ISN      354          IF(KTR.EQ.22)THEN
ISN      355          NLINK=27
ISN      356          ELSE
ISN      357          NLINK=16
ISN      358          END IF
ISN      359          CALL CHECK(IFLAG,NN,ESV)
ISN      360          DO 134 J=1,NCHAIN
ISN      361          EVDW(K,J)=ESV(J)
ISN      362    134      EVDW(J,K)=ESV(J)
ISN      363    135      CONTINUE
ISN      364             DO 1349 IRT=1,NCHAIN
ISN      365             IF(IRT.EQ.22)GOTO1349
ISN      366             EPOLD(IRT)=0.
ISN      367             EPK(IRT)=0.
ISN      368             IF(NPP(IRT).EQ.0)GO TO 1349
ISN      369             DO 1349 KP=1,NLINK
ISN      370             DO 1349 KH=1,27
ISN      371             DPK=0.
ISN      372             DO 1348 KD=1,3
ISN      373             DPK=DPK+(CP(KD,KP,IRT)-CHOL(KD,KH))**2
ISN      374    1348     CONTINUE
ISN      375             EPK(IRT)=EPK(IRT)+EPIL*((SIGLP/DPK)**6-(SIGLP/DPK)**3)
ISN      376    1349     CONTINUE
ISN      377          DO 161 J=1,NCHAIN
ISN      378          EE=0.
ISN      379          DO 163 K=1,NCHAIN
```

```
                  ...
ISN       380    163    EE=EE+EVDW(J,K)
ISN       381              EE=EE+EPK(J)
ISN       382              EPOLD(J)=EPK(J)
ISN       383           PRINT 3298,EE,J
ISN       384    161    CONTINUE
ISN       385           KMOV=20
ISN       386    3298   FORMAT('1OX,'INITIAL ENERGY',E18.7,5X,I6)
ISN       387           JPRT=IPRT
ISN       388           JLI=ILI
                 C
                 C      ***** MAIN MONTE CARLO LOOP BEGINS HERE*******
                 C
ISN       389           XDP=0.
ISN       390           YDP=0.
ISN       391           DO 1002 LMC=1,NMAX
ISN       392           IREJ=0
ISN       393           DO 1000 KMC=1,10
ISN       394           DO 6007 IY=1,36
ISN       395           DO 6008 IZ=1,16
ISN       396           GS(IZ,IY)=GR(IZ,IY)
                 C
                 C      ***** SAVE OLD CONFIGURATIONS HERE **************
                 C      CARBON POSITIONS CP SAVED AS CPS AND RVEC AS RSV,GR AS GS.

ISN       397           DO 6009 IW=1,3
ISN       398           RSV(IW,IZ,IY)=RVEC(IW,IZ,IY)
ISN       399    6009   CPS(IW,IZ,IY)=CP(IW,IZ,IY)
ISN       400    6008   CONTINUE
ISN       401    6007   CONTINUE
                 CL********* SAVES K=22 RVECS*********
ISN       402           IY=22
ISN       403           DO 6048 IZ=21,27
ISN       404           GS(IZ,IY)=GR(IZ,IY)
ISN       405           DO 6049 IW=1,3
ISN       406           RSV(IW,IZ,IY)=RVEC(IW,IZ,IY)
ISN       407    6049   CONTINUE
ISN       408    6048   CONTINUE
ISN       409           DO 6019 IZ=1,27
ISN       410           DO 6029 IW=1,3
ISN       411           CPS(IW,IZ,IY)=CP(IW,IZ,IY)
ISN       412    6029   CONTINUE
ISN       413    6019   CONTINUE
                 CL***************
                 C
                 C      GENERATE NEW CONFORMATIONS ON ALL CHAINS WITH PROB .5....
                 C
ISN       414    190      CONTINUE
                 C      DO 1010 L=1,NCHAIN
                 C      IF(L.EQ.22)GOTO 1009
                 C      NREJ(L)=0
                 C      XV=(NLINK-2)*RANF(0)
                 C      XVL=14*RANF(0)
                 C      IC(L)=INT(XV)+2
                 C      ICL(L)=IC(L)+1+INT(XVL)
                 C 1009 IF(L.EQ.22)CALL CHOL2(IC,ICL)
                 C 1010 CONTINUE
```

```
                C
                C      **** PICK A BOND ON CHAIN NO. LABELLED LROT AND
                C           "FLIP  A COIN " TO MAKE A ROTATION OR NOT *****
                C
ISN       415           DO 1030 LROT=1,NCHAIN
ISN       416              IF(LROT.EQ.22)THEN
ISN       417              IZ1=21
ISN       418              IZ2=27
ISN       419              ELSE
ISN       420              IZ1=1
ISN       421              IZ2=16
ISN       422              NLINK=16
ISN       423              ENDIF
ISN       424              DEL(LROT)=0.
ISN       425              ITR=LROT
ISN       426              KTR=LROT
ISN       427              FLIP=RANF(0)
ISN       428              IF(FLIP.LT.0.5)GO TO 6099
ISN       429              XV=(IZ2-2)*RANF(0)
ISN       430              XVL=(IZ2-3)*RANF(0)
ISN       431              IC(LROT)=INT(XV)+2
ISN       432              ICL(LROT)=IC(LROT)+1+INT(XVL)
ISN       433           IF(ICL(LROT).GE.IZ2)GO TO 1025
ISN       434           LU=IC(LROT)
ISN       435           LL=ICL(LROT)
ISN       436           GR(LU,LROT)=GR(LU,LROT)+1
ISN       437           IF(GR(LU,LROT).GT.1.1)GR(LU,LROT)=-1.
ISN       439           GR(LL,LROT)=GR(LL,LROT)-1
ISN       440           IF(GR(LL,LROT).LT.-1.1)GR(LL,LROT)=1.
ISN       442           AR=(GR(LL,LROT))*GR(LL,LROT)
ISN       443           AS=GS(LL,LROT)*GS(LL,LROT)
ISN       444           BR=GR(LU,LROT)*GR(LU,LROT)
ISN       445           BS=GS(LU,LROT)*GS(LU,LROT)
ISN       446           DL=AR-AS
ISN       447           DU=BR-BS
ISN       448           RRL=GR(LL-1,LROT)*GR(LL-1,LROT)+GR(LL+1,LROT)*GR(LL+1,LROT)
ISN       449           RRU=GR(LU-1,LROT)*GR(LU-1,LROT)+GR(LU+1,LROT)*GR(LU+1,LROT)
ISN       450           DEL(LROT)=-500.*(DL+DU)-2500.*(DL*RRL+DU*RRU)
ISN       451           GO TO 6099
ISN       452     1025  LU=IC(LROT)
ISN       453           GR(LU,LROT)=GR(LU,LROT)-1.
ISN       454           IF(GR(LU,LROT).LT.-1.01)GR(LU,LROT)=1.
ISN       456           BR=GR(LU,LROT)*GR(LU,LROT)
ISN       457           BS=GS(LU,LROT)*GS(LU,LROT)
ISN       458           RRU=GR(LU-1,LROT)*GR(LU-1,LROT)+GR(LU+1,LROT)*GR(LU+1,LROT)
ISN       459           DEL(LROT)=-500.*(BR-BS)-2500.*RRU*(BR-BS)
ISN       460     6099  CROT(LROT)=RANF(0)
ISN       461           IF(LROT.EQ.22)GOTO 169
ISN       462           CP(2,1,KTR)=CP(2,1,KTR)+.03*(1.-2.*RANF(0))
ISN       463           CP(3,1,KTR)=CP(3,1,KTR)+0.03*(1.-2.*RANF(0))
                C
                C      **** NEW CHAIN POSITIONS ARE CALCULATED AFTER ROTATION
                C           AND TRANSLATION OF CHAIN NO.LABELLED LROT. ****
                C
ISN       464     169   CONTINUE
ISN       465     170   ANGSV=ANG(ITR)
```

```
ISN      466          RVEC(2,1,ITR)=0.
ISN      467          RVEC(3,1,ITR)=0.
ISN      468          ANG(ITR)=ANG(ITR)+0.03*PI*(1.-2.*CROT(ITR))
ISN      469          EPS=SIN(ANG(ITR))
ISN      470          EPC=COS(ANG(ITR))
ISN      471          RT(1,1)=EC
ISN      472          RT(2,1)=ES
ISN      473          RT(3,1)=0.
ISN      474          RT(1,2)=-ES*EPC
ISN      475          RT(2,2)=EC*EPC
ISN      476          RT(3,2)=EPS
ISN      477          RT(1,3)=ES*EPS
ISN      478          RT(2,3)=-EC*EPS
ISN      479          RT(3,3)=EPC
ISN      480          RVEC(1,1,ITR)=1.53
ISN      481          DO 210 J=IZ1+1,IZ2
ISN      482          DO 200 K=1,3
ISN      483          LR=J-1
ISN      484          RVEC(K,J,ITR)=0.
ISN      485          DO 6011 IY=1,3
ISN      486  6011    RVEC(K,J,ITR)=RVEC(IY,LR,ITR)*ROT(IY,K)+RVEC(K,J,ITR)
ISN      487  200     CONTINUE
ISN      488  210     CONTINUE
ISN      489          DO 206 J=IZ1+1,IZ2-1
ISN      490          J1=IZ2-J
ISN      491          J2=IZ2+1-J
ISN      492          IF(GR(J2,ITR).EQ.0.)GOTO 206
ISN      493          XX1=.5*FLOAT(J1)
ISN      494          IXX1=2*INT(XX1)
ISN      495          IF(GR(J2,ITR).EQ. +1.0) N=1
ISN      497          IF(GR(J2,ITR).EQ. -1.0) N=3
ISN      499          IF(IXX1.NE.J1)THEN
ISN      500          DO 806 L=J1+1,IZ2-1
ISN      501          TVEC(2,L,ITR)=RVEC(2,L,ITR)
ISN      502          TVEC(3,L,ITR)=RVEC(3,L,ITR)
ISN      503          RVEC(2,L,ITR)=TVEC(2,L,ITR)*GACOS(N)+TVEC(3,L,ITR)*GASIN(N)
ISN      504          RVEC(3,L,ITR)=TVEC(3,L,ITR)*GACOS(N)-TVEC(2,L,ITR)*GASIN(N)
ISN      505  806     CONTINUE
ISN      506          ELSE
ISN      507          DO 807 L=J1,IZ2-1
ISN      508          DO 808 K=1,3
ISN      509          TVEC(K,L,ITR)=RVEC(K,L,ITR)
ISN      510  808     CONTINUE
ISN      511          DO 809 K=1,3
ISN      512          RVEC(K,L,ITR)=0.0
ISN      513          DO 810 IY=1,3
ISN      514  810     RVEC(K,L,ITR)=RVEC(K,L,ITR)+TVEC(IY,L,ITR)*ROTP(IY,K)
ISN      515  809     CONTINUE
ISN      516  807     CONTINUE
ISN      517          DO 811 L=J1+1,IZ2-1
ISN      518          TVEC(2,L,ITR)=RVEC(2,L,ITR)
ISN      519          TVEC(3,L,ITR)=RVEC(3,L,ITR)
ISN      520          RVEC(2,L,ITR)=TVEC(2,L,ITR)*GACOS(N)+TVEC(3,L,ITR)*GASIN(N)
ISN      521          RVEC(3,L,ITR)=TVEC(3,L,ITR)*GACOS(N)-TVEC(2,L,ITR)*GASIN(N)
ISN      522  811     CONTINUE
ISN      523          DO 812 L=J1,IZ2-1
```

```
ISN      524          DO 813 K=1,3
ISN      525          TVEC(K,L,ITR)=RVEC(K,L,ITR)
ISN      526   813    CONTINUE
ISN      527          DO 814 K=1,3
ISN      528          RVEC(K,L,ITR)=0.0
ISN      529          DO 815 IY=1,3
ISN      530   815    RVEC(K,L,ITR)=RVEC(K,L,ITR)+TVEC(IY,L,ITR)*ROTN(IY,K)
ISN      531   814    CONTINUE
ISN      532   812    CONTINUE
ISN      533          ENDIF
ISN      534   206     CONTINUE
ISN      535   160    DO 6015 IY=1,3
ISN      536          DO 6014 IZ=IZ1,IZ2
ISN      537   6014   AVEC(IY,IZ)=RVEC(IY,IZ,ITR)
ISN      538   6015   CONTINUE
ISN      539          DO 260 K=IZ1,IZ2
ISN      540          DO 240 M=1,3
ISN      541          RVEC(M,K,ITR)=0
ISN      542          DO 6016 IY=1,3
ISN      543   6016   RVEC(M,K,ITR)=RVEC(M,K,ITR)+AVEC(IY,K)*RT(IY,M)
ISN      544   240    CONTINUE
ISN      545   260    CONTINUE
ISN      546          DO 280 J=IZ1+1,IZ2
ISN      547          JJ=J-1
ISN      548          DO 6017 IY=1,3
ISN      549   6017   CP(IY,J,ITR)=CP(IY,JJ,ITR)+RVEC(IY,JJ,ITR)
ISN      550   280    CONTINUE
                      C
                      C      CHECK FOR LIPID-CHOLESTEROL OVERLAPS.....................
                      C
ISN      551          DO 320 J=1,NLINK
ISN      552           IF(CP(2,J,KTR).LT.0.)CP(2,J,KTR)=CP(2,J,KTR)+SIZEY
ISN      554           IF(CP(2,J,KTR).GT.SIZEY)CP(2,J,KTR)=CP(2,J,KTR)-SIZEY
ISN      556           IF(CP(3,J,KTR).LT.0.)CP(3,J,KTR)=CP(3,J,KTR)+SIZEZ
ISN      558           IF(CP(3,J,KTR).GT.SIZEZ)CP(3,J,KTR)=CP(3,J,KTR)-SIZEZ
ISN      560   320     CONTINUE
ISN      561          DO 7100 K=IZ1+1,IZ2
ISN      562   7100   IF(CP(1,K,ITR).LT.0.)GO TO 500
ISN      563             EPK(ITR)=0.
ISN      564          IF(NPP(ITR).EQ.1)GO TO 2140
ISN      565          GO TO 7800
ISN      566   2140     EPK(ITR)=0.
ISN      567           IF(ITR .EQ. 22) GO TO 7800
ISN      568   2150     DO 2180 KP=1,NLINK
ISN      569          DO 2179 KC=1,27
ISN      570             DPK=0.
ISN      571             DO 2170 KD=1,3
ISN      572             DPK=DPK+(CP(KD,KP,ITR)-CP(KD,KC,22))**2
ISN      573   2170     CONTINUE
ISN      574          IF(DPK.LT.2.35)GO TO 500
ISN      575             EPK(ITR)=EPK(ITR)+EPIL*((SIGLP/DPK)**6-(SIGLP/DPK)**3)
ISN      576   2179    CONTINUE
ISN      577   2180   CONTINUE
ISN      578   7800    CONTINUE
                      C
                      C      IF CHAIN IS MOVED CHECK PERIODIC B.C. & RECALC VAN DER W. ENERGY
```

```
                C
                C
                C       IF(KMC.EQ.KMOV)GO TO 2300
                C       GO TO 2500
ISN       579           CALL CHECK(IFLAG,NN,ESV)
ISN       580           ENEW(ITR)=0.
ISN       581           EOLD(ITR)=0.
ISN       582           DO 350 K=1,NCHAIN
ISN       583           EOLD(ITR)=EOLD(ITR)+EVDW(KTR,K)
ISN       584           ENEW(ITR)=ENEW(ITR)+ESV(K)
ISN       585     350   CONTINUE
                C
                C       **** CHANGE IN ENERGY EXP(-DELTA E/KT) IS COMPARED
                C           WITH RANDOM NO. TO ACCEPT OR REJECT THE CHANGE****
                C
ISN       586           DEL1=ENEW(ITR)-EOLD(ITR)+EPK(ITR)-EPOLD(ITR)
ISN       587           DELT=(DEL1-DEL(LROT))*BKT
ISN       588           IF(DELT.LE.0.)GO TO 400
ISN       589           IF(DELT.GT.10.)GO TO 500
ISN       590           DDD=EXP(-DELT)
ISN       591           TST1=RANF(0)
ISN       592           IF(DDD.LE.TST1)GO TO 500
ISN       593     400   DO 420 LE=1,NCHAIN
ISN       594           EVDW(KTR,LE)=ESV(LE)
ISN       595     420   EVDW(LE,KTR)=EVDW(KTR,LE)
ISN       596              EPOLD(ITR)=EPK(ITR)
ISN       597           GO TO 2000
                C
                C       RESET AFTER REJECTED MOVE.....................
ISN       598      500  CONTINUE
ISN       599           DO 6098 IY=1,3
ISN       600           DO 6097 IZ=IZ1,IZ2
ISN       601           CP(IY,IZ,KTR)=CPS(IY,IZ,KTR)
ISN       602     6097  RVEC(IY,IZ,KTR)=RSV(IY,IZ,KTR)
ISN       603     6098  CONTINUE
                C
ISN       604           ANG(KTR)=ANGSV
ISN       605     600   IREJ=IREJ+1
ISN       606           DO 601 IR=IZ1,IZ2
ISN       607     601   GR(IR,KTR)=GS(IR,KTR)
ISN       608     2000  CONTINUE
ISN       609     1030   CONTINUE
ISN       610           DO 3150 KC=1,NCHAIN
ISN       611           IF(NNP(KC).EQ.1)YDP=YDP+1
ISN       613           IF(NPP(KC).EQ.1)XDP=XDP+1.
ISN       615     3150  CONTINUE
ISN       616     2500  IF(LMC.LE.NMIN)GO TO 990
ISN       617     800   CALL CH2(XPR,YPR)
ISN       618           IF(LMC.EQ.JPRT)GO TO 900
ISN       619           GO TO 990
ISN       620     900   PRINT 2900,KMC,LMC,IREJ
ISN       621     2900    FORMAT(' AFTER',I7,' X 10 X',I4,'    MC STEPS...IREJ=',I8/)
ISN       622           PRINT 3110
ISN       623     3110  FORMAT('   VALUES OF CP & IG: WRITE ON DISK'/)
ISN       624           REWIND 9
ISN       625           DO 950 L=1,NCHAIN
```

```
ISN      626          IF (L .EQ. 22) THEN
ISN      627          IZ2=27
ISN      628          ELSE
ISN      629          IZ2=16
ISN      630          ENDIF
ISN      631          WRITE(9,46)ANG(L)
ISN      632          DO 950 J=1,IZ2
ISN      633          WRITE(9,45)(CP(K,J,L),RVEC(K,J,L),K=1,3),GR(J,L)
ISN      634   950    CONTINUE
ISN      635          PRINT 3100
ISN      636   3100   FORMAT('   ORDER PARAMETER PROFILE'/)
ISN      637          FLMC=FLOAT(LMC)
ISN      638          FNMIN=FLOAT(NMIN)
ISN      639          XDN=10.*(FLMC-FNMIN)-9.
ISN      640          YDN=1./((NCHAIN-1)*XDN)
ISN      641          DO 6096 IY=1,LLINK
ISN      642   6096   SSVG(IY)=SAVG(IY)*YDN
ISN      643          PRINT 3200,(K,SSVG(K),K=1,LLINK)
ISN      644   3200   FORMAT(5X,I4,5X,F12.6)
                      C
                      C      WRITE(18,3412),(K,SSVG(K),K=1,LLINK)
                      C3412  FORMAT(5X,I4,5X,F12.6)
                      C
ISN      645          DO 3140 JP=1,15
ISN      646             ZNN(JP)=YPR(JP)/YDP
ISN      647             ZPR(JP)=XPR(JP)/XDP
ISN      648   3140   CONTINUE
ISN      649          PRINT 3197
ISN      650          PRINT 3198,(ZPR(II),II=1,15)
ISN      651          PRINT 3198,(ZNN(II),II=1,15)
ISN      652   3198   FORMAT(5X,9F10.5 )
ISN      653   3197    FORMAT(25X,'ORDER PARAMETERS FOR CHOLESTEROL NEIGHBORS'//)
ISN      654          JPRT=JPRT+IPRT
                      C
                      C       ***** LIPID-CHOL :VARIOUS INTERACTION ENERGIES
                      C             EOLD,ENEW,EPK,EPOLD AND DEL(ENERGY DIFF
                      C             BETWEEN GAUCH-TRANS CONFIG.)PRINTED HERE ****
                      C
ISN      655          PRINT 3199
ISN      656   3199   FORMAT(12X,'ENEW',12X,'EOLD',12X,'EPK',12X,'EPOLD',12X,'DEL'/)
ISN      657             DO 5000 L=1,NCHAIN
ISN      658   5000    PRINT 4199,ENEW(L),EOLD(L),EPK(L),EPOLD(L),DEL(L)
ISN      659    4199      FORMAT(5X,5E16.6)
ISN      660             IF(LMC.EQ.NMAX)GO TO 1002
ISN      661   990    IF(LMC.EQ.JLI)GO TO 992
ISN      662          GO TO 1000
ISN      663   992    DO 999 ILST=1,NCHAIN-1
ISN      664          DO 999 JLST=ILST+1,NCHAIN
ISN      665   995    CALL CLIST
ISN      666          IF(IDST.EQ.1)GO TO 996
ISN      667          NN(ILST,JLST)=0
ISN      668          NN(JLST,ILST)=0
ISN      669          GO TO 999
ISN      670   996    NN(ILST,JLST)=1
ISN      671          NN(JLST,ILST)=1
ISN      672   999    CONTINUE
```

```
                C      SKIP THIS LOOP IN PURE LIPID PROG
ISN      673           DO 9992 L=1,NCHAIN
ISN      674           IF(L.EQ.22)GO TO 9992
ISN      675           NPP(L)=0
ISN      676              NNP(L)=0
ISN      677           DO 9990 M=1,NLINK
ISN      678           DO 9991 LL=1,27
ISN      679           DPX=(CP(2,M,L)-CP(2,LL,22))**2+(CP(3,M,L)-CP(3,LL,22))**2
ISN      680           IF(DPX.GE.RNNN)GO TO 9991
ISN      681              IF(DPX.LE.RPEP) NPP(L)=1
ISN      683              IF(DPX.LE.RNNN.AND.DPX.GT.RPEP) NNP(L)=1
ISN      685           GO TO 9992
ISN      686    9991   CONTINUE
ISN      687    9990   CONTINUE
ISN      688    9992   CONTINUE
ISN      689           JLI=JLI+ILI
ISN      690    1000   CONTINUE
ISN      691    1002   CONTINUE
ISN      692           DO 8888 L=1,NCHAIN
ISN      693           IF(L.EQ.22)GO TO 2001
ISN      694           PRINT 46,ANG(L)
ISN      695           DO 8444 J=1,NLINK
ISN      696           PRINT 45,(CP(K,J,L),RVEC(K,J,L),K=1,3),GR(J,L)
ISN      697    8444   CONTINUE
ISN      698           GOTO 8888
ISN      699    2001   DO 2444 J=1,27
ISN      700           PRINT 45,(CP(K,J,22),RVEC(K,J,22),K=1,3),GR(J,22)
ISN      701    2444   CONTINUE
ISN      702    8888   CONTINUE
ISN      703           STOP 111
ISN      704           END
```

```
C
C
C
C
C
ISN      1          SUBROUTINE CHECK(IFLAG,NN,ESV)
ISN      2          COMMON CP(3,27,36),RVEC(3,27,36),NCHAIN,NLINK,SIZEY,SIZEZ,RC
ISN      3          COMMON/VDW/SIG,EPIL
ISN      4          COMMON/CHK/KTR,JR,SY,SZ,SAVG(27),SSVG(27),NPP(36),NNP(36)
ISN      5          DIMENSION NN(36,36),ESV(36)
ISN      6          DIMENSION DIF(3,27),DIST(27),DEL(3,27),RECIP(27),EVV(27)
ISN      7          IF(KTR.EQ.22)THEN
ISN      8          IZ3=27
ISN      9          ELSE
ISN     10          IZ3=NLINK
ISN     11          ENDIF
ISN     12          DO 51 I=1,NCHAIN
ISN     13          IF(I.EQ.22)THEN
ISN     14          IZ4=27
ISN     15          ELSE
ISN     16          IZ4=NLINK
ISN     17          ENDIF
ISN     18          ESV(I)=0.
ISN     19          IF(NN(KTR,I).EQ.0)GO TO 51
ISN     20          ENEW=0.
ISN     21          DO 50 JC=1,IZ3
ISN     22          JCC=IZ3-JC+1
ISN     23          DO 8000 IZ=1,3
ISN     24          DO 8100 IY=1,IZ4
ISN     25          DIF(IZ,IY)=CP(IZ,JCC,KTR)-CP(IZ,IY,I)
ISN     26  8100     DIF(IZ,IY)=ABS(DIF(IZ,IY))
ISN     27  8000    CONTINUE
ISN     28          IF(I.EQ.KTR)GO TO 45
ISN     29  35      DO 38 J=1,IZ4
ISN     30          IF(DIF(2,J).GT.SY)DIF(2,J)=DIF(2,J)-SIZEY
ISN     32          IF(DIF(3,J).GT.SZ)DIF(3,J)=DIF(3,J)-SIZEZ
ISN     34          DIST(J)=0
ISN     35          DO 8200 IY=1,3
ISN     36  8200     DIST(J)=DIF(IY,J)*DIF(IY,J)+DIST(J)
ISN     37  38      CONTINUE
ISN     38          DO 41 IJ=1,IZ4
ISN     39  41      IF(DIST(IJ).LE.2.33)GO TO 150
ISN     40          GO TO 49
ISN     41  45      DO 48 JJ=1,IZ4
ISN     42          J=IZ4-JJ+1
ISN     43          JP=JCC+1
ISN     44             JPP=JCC+2
ISN     45          JM=JCC-1
ISN     46             JMM=JCC-2
ISN     47          IF(J.EQ.JP)GO TO 48
ISN     48             IF(J.EQ.JPP)GO TO 48
ISN     49          IF(J.EQ.JM)GO TO 48
```

```
ISN        50              IF(J.EQ.JMM)GO TO 48
ISN        51            IF(J.EQ.JCC)GO TO 48
ISN        52            IF(DIF(2,J).GT.SY)DIF(2,J)=DIF(2,J)-SIZEY
ISN        54            IF(DIF(3,J).GT.SZ)DIF(3,J)=DIF(3,J)-SIZEZ
ISN        56            DIST(J)=0
ISN        57            DO 8900 IY=1,3
ISN        58     8900    DIST(J)=DIF(IY,J)*DIF(IY,J)+DIST(J)
ISN        59            IF(DIST(J).LE.2.33)GO TO 150
ISN        60     48     CONTINUE
ISN        61            GO TO 50
ISN        62     49     EATT=0
ISN        63            DO 8300 IY=1,IZ4
ISN        64            RECIP(IY)=1./DIST(IY)
ISN        65            EVV(IY)=(RECIP(IY)*SIG)**6-(RECIP(IY)*SIG)**3
ISN        66            EVV(IY)=EPIL*EVV(IY)
ISN        67     8300    EATT=EATT+EVV(IY)
ISN        68            ENEW=ENEW+EATT
ISN        69     50     CONTINUE
ISN        70            ESV(I)=ENEW
ISN        71               GO TO 51
ISN        72     150     ESV(I)=1.E20
ISN        73               GO TO 300
ISN        74     51     CONTINUE
ISN        75     200    IFLAG=0
ISN        76     300    RETURN
ISN        77            END
```

```
                C
                C
                C
                C
                C
ISN         1           SUBROUTINE CLIST
ISN         2           COMMON CP(3,27,36),RVEC(3,27,36),NCHAIN,NLINK,SIZEY,SIZEZ,RC
ISN         3           COMMON/LST/RANG,ILST,JLST,IDST
ISN         4           COMMON/CHK/KTR,JR,SY,SZ,SAVG(27),SSVG(27)
ISN         5           DIMENSION DDF(3,27)
ISN         6           I=ILST
ISN         7           IDST=0
ISN         8           J=JLST
ISN         9           DO 7000 IY=1,3
ISN        10           DO 7010 IZ=1,16
ISN        11           DDF(IY,IZ)=CP(IY,IZ,J)-CP(IY,IZ,I)
ISN        12    7010   DDF(IY,IZ)=ABS(DDF(IY,IZ))
ISN        13    7000   CONTINUE
ISN        14           DO 40 L=1,NLINK
ISN        15           IF(DDF(2,L).GT.SY)DDF(2,L)=DDF(2,L)-SIZEY
ISN        17           IF(DDF(3,L).GT.SZ)DDF(3,L)=DDF(3,L)-SIZEZ
ISN        19           DST=0.
ISN        20           DO 7020 IY=1,3
ISN        21    7020   DST=DST+DDF(IY,L)*DDF(IY,L)
ISN        22           IF(DST.LE.RANG)GO TO 45
ISN        23    40     CONTINUE
ISN        24           GO TO 50
ISN        25    45     IDST=1
ISN        26    50     CONTINUE
ISN        27           RETURN
ISN        28           END
```

```
                C
                C
                C
                C
                C
ISN         1           SUBROUTINE CH2(XPR,YPR)
ISN         2           DIMENSION XPR(15),YPR(15)
ISN         3           COMMON CP(3,27,36),RVEC(3,27,36),NCHAIN,NLINK,SIZEY,SIZEZ,RC
ISN         4           COMMON/CHK/KTR,JR,SY,SZ,SAVG(27),SSVG(27),NPP(36),NNP(36)
ISN         5           DIMENSION DLX(27,36),SO(27,36)
ISN         6           LLINK=NLINK-1
ISN         7           DO 70 J=1,NCHAIN
ISN         8           LLINK=15
ISN         9           K1=1
ISN        10           K2=LLINK
ISN        11           IF(J.EQ.22)THEN
ISN        12           K1=21
ISN        13           K2=25
ISN        14           END IF
ISN        15           DO 70 K=K1,K2
ISN        16           DLX(K,J)=RVEC(1,K,J)
ISN        17           SO(K,J)=RVEC(2,K,J)*RVEC(2,K,J)+RVEC(3,K,J)*RVEC(3,K,J)
ISN        18    70     CONTINUE
ISN        19           DO 9000 IZ=1,36
ISN        20           I1=1
ISN        21           I2=15
ISN        22           IF(IZ.EQ.22)THEN
ISN        23           I1=21
ISN        24           I2=25
ISN        25           END IF
ISN        26           DO 9100  IY=I1,I2
ISN        27           SO(IY,IZ)=ABS(SO(IY,IZ))
ISN        28           SO(IY,IZ)=SQRT(SO(IY,IZ))
ISN        29           SO(IY,IZ)=DLX(IY,IZ)/SO(IY,IZ)
ISN        30           IF(SO(IY,IZ).EQ.0.000)GOTO 9000
ISN        31           SO(IY,IZ)=ATAN(SO(IY,IZ))
ISN        32           SO(IY,IZ)=SO(IY,IZ)-.955566
ISN        33           SO(IY,IZ)=COS(SO(IY,IZ))
ISN        34           SO(IY,IZ)=SO(IY,IZ)*SO(IY,IZ)
ISN        35           SO(IY,IZ)=1.5*SO(IY,IZ)
ISN        36    9100   SO(IY,IZ)=SO(IY,IZ)-0.5
ISN        37    9000   CONTINUE
ISN        38           DO 100 J=1,NCHAIN
ISN        39           L1=1
ISN        40           L2=15
ISN        41           IF(J.EQ.22)THEN
ISN        42           L1=21
ISN        43           L2=25
ISN        44           END IF
ISN        45           DO 100 L=L1,L2
ISN        46           SAVG(L)=SAVG(L)+SO(L,J)
ISN        47           IF(NPP(J).EQ.1.)XPR(L)=XPR(L)+SO(L,J)
ISN        49              IF(NNP(J).EQ.1)YPR(L)=YPR(L)+SO(L,J)
ISN        51    100    CONTINUE
ISN        52           RETURN
ISN        53           END
```

```
                C
                C
                C
                C
                C
                C     ************SUBPROG CHOL1 ********************************
ISN         1           SUBROUTINE CHOL1(ROT,RT,AVEC)
ISN         2           COMMON CP(3,27,36),RVEC(3,27,36),NCHAIN,NLINK,SIZEY,SIZEZ,RC
ISN         3           DIMENSION ROT(3,3),RT(3,3),AVEC(3,27)
ISN         4           K=22
ISN         5           RVEC(1,21,K)=1.53
ISN         6           DO 194 I=2,3
ISN         7    194    RVEC(I,21,K)=0.
ISN         8           NLINCH=26
ISN         9           DO 196 J=22,NLINCH
ISN        10           L=J-1
ISN        11           DO 198 I=1,3
ISN        12           RVEC(I,J,K)=0.
ISN        13           DO 200 IY=1,3
ISN        14           RVEC(I,J,K)=RVEC(I,J,K)+RVEC(IY,L,K)*ROT(IY,I)
ISN        15     200   CONTINUE
ISN        16    198    CONTINUE
ISN        17    196    CONTINUE
ISN        18           DO 202  I=1,3
ISN        19           DO 204  J=21,NLINCH
ISN        20    204    AVEC(I,J)=RVEC(I,J,K)
ISN        21    202    CONTINUE
ISN        22           DO 206 I=1,3
ISN        23           DO 206 J=21,NLINCH
ISN        24           RVEC(I,J,K)=0.
ISN        25           DO 208 IY=1,3
ISN        26           RVEC(I,J,K)=RVEC(I,J,K)+AVEC(IY,J)*RT(IY,I)
ISN        27    208    CONTINUE
ISN        28    206    CONTINUE
ISN        29           CP(1,21,K)=9.59300
ISN        30           CP(2,21,K)=20.054979
ISN        31           CP(3,21,K)=15.155443
ISN        32           DO 210 J=22,NLINCH
ISN        33           JJ=J-1
ISN        34           DO 212 I=1,3
ISN        35    212    CP(I,J,K)=CP(I,JJ,K)+RVEC(I,JJ,K)
ISN        36    210    CONTINUE
ISN        37           RETURN
ISN        38           END
```

```
                C     ************SUBPROG CHOL2 *******************************
ISN         1          SUBROUTINE CHOL2(IC,ICL)
ISN         2          DIMENSION NREJ(36),IC(36),ICL(36)
ISN         3           L=22
ISN         4           NLINCH=6
ISN         5          NREJ(L)=0
ISN         6          XV=(NLINCH-2)*RANF(0)
ISN         7          XVL=4*RANF(0)
ISN         8          IC(L)=INT(XV)+2
ISN         9          ICL(L)=IC(L)+1+INT(XVL)
ISN        10          RETURN
ISN        11          END
```

The original Computer program 'Monte Carlo Simulation program for lipid-protein interaction' written by Dr. H. L. Scott was modified in the present study of lipid-cholesterol interaction

The following changes were made:

1) Comments were inserted to define all parameters.

2) DIMENSION statements were modified to suit the cholesterol molecule structure. (ISN 1 - 16)

3) DIMENSION statements were modified so as to accommodate longer acyl chains containing 15 C-C bonds each. (ISN - 16)

4) Comments were introduced at appropriate places to clarify the main steps of the program.

5) Neighboring matrix of the cholesterol molecule was defined differently than in the earlier program. (ISN 49 - 67)

6) Coordinates of cholesterol molecules were included. (ISN 68 - 148)

7) ISN 187 - 194 were added.

8) ISN 225 - 242 define two new rotation operations ROTN and ROTP.

9) ISN 316 - 325 were re-written to suit the cholesterol molecule.

10) ISN 352 - 373 were modified for cholesterol.

11) ISN 402 - 413 were re-written.

12) Necessary changes were made in the main loop so as to make it suitable for the present study concerning cholesterol rather than the protein model used earlier. (ISN 415 - 550)

13) ISN 699 - 702.

14) Sub-programs, CHECK and SUBROUTINE, were adjusted to accommodate longer acyl chains and the peculiar structure of the cholesterol molecule.

15) Sub-program, CHOL1, generates cholesterol 'tail'.

The structure of the main program is essentially the same, except the changes made so as to make it suitable for the present study of lipid-cholesterol interaction.

APPENDIX B

COMPUTER PROGRAM FOR THE CALCULATION OF THE WEIGHTED AVERAGES AND THE STANDARD DEVIATIONS

```
                C *****A  PROGRAM TO CALCULATE WEIGHTED AVERAGES,STD DEVIATION.
ISN         1             IMPLICIT REAL(A-H,O-Z)
ISN         2             DIMENSION SUM1(15),SUM2(15),SUM3(15)
ISN         3             DIMENSION  TOTAL(15),AVE(15),STDEV(20)
ISN         4             DIMENSION  X(15,6),Y(15,6),Z(15,6),SQSUM(20)
ISN         5             DIMENSION SQSUM1(20),SQSUM2(20),SQSUM3(20)
ISN         6             N=17
ISN         7             DO 10 IBOND=1,15
ISN         8             TSUM1=0.00
ISN         9             READ(2,15)(X(IBOND,L),L=1,6)
ISN        10             WRITE(6,16)(X(IBOND,L),L=1,6)
ISN        11       15    FORMAT(6(F7.5,5X))
ISN        12       16    FORMAT(1H ,6(F7.5,5X))
ISN        13             DO 401 L=1,6
ISN        14             TSUM1=TSUM1+X(IBOND,L)*50.
ISN        15      401    CONTINUE
ISN        16             SUM1(IBOND)=TSUM1
ISN        17       10    CONTINUE
ISN        18       18    FORMAT(5(F7.5,5X))
ISN        19             DO 20 IBOND=1,15
ISN        20             TSUM2=0.00
ISN        21             READ(2,18)(Y(IBOND,M),M=1,5)
ISN        22             WRITE(6,16)(Y(IBOND,M),M=1,5)
ISN        23             DO 402 M=1,5
ISN        24             TSUM2=TSUM2+Y(IBOND,M)*100.
ISN        25      402    CONTINUE
ISN        26             SUM2(IBOND)=TSUM2
ISN        27       20    CONTINUE
ISN        28             DO 30 IBOND=1,15
ISN        29             TSUM3=0.00
ISN        30             READ(2,15)(Z(IBOND,N),N=1,6)
ISN        31             WRITE(6,16)(Z(IBOND,N),N=1,6)
ISN        32             DO 403 N=1,6
ISN        33             TSUM3=TSUM3+Z(IBOND,N)*200.
ISN        34      403    CONTINUE
ISN        35             SUM3(IBOND)=TSUM3
ISN        36       30    CONTINUE
ISN        37             DO 40 IBOND=1,15
ISN        38             TOTAL(IBOND)=SUM1(IBOND)+SUM2(IBOND)+SUM3(IBOND)
ISN        39             AVE(IBOND)=TOTAL(IBOND)/2000.
ISN        40       40    CONTINUE
ISN        41              DO 50 IBOND=1,15
ISN        42              DO 60 J=1,6
ISN        43              PRINT 11,(X(IBOND,J)-AVE(IBOND))**2
ISN        44       11     FORMAT(' ',F14.6)
ISN        45       60     CONTINUE
ISN        46       50     CONTINUE
ISN        47              DO 70 IBOND=1,15
ISN        48              DO 80 K=1,5
ISN        49              PRINT 22,(Y(IBOND,K)-AVE(IBOND))**2
ISN        50       22     FORMAT(' ',F14.6)
ISN        51       80     CONTINUE
ISN        52       70     CONTINUE
ISN        53              DO 71 IBOND=1,15
ISN        54              DO 81 I=1,6
ISN        55              PRINT 33,(Z(IBOND,I)-AVE(IBOND))**2
ISN        56       33     FORMAT(' ',F14.6)
ISN        57       81     CONTINUE
ISN        58       71     CONTINUE
ISN        59              DO 12 IBOND=1,15
ISN        60              DO 13 J=1,6
ISN        61              SQSUM1(IBOND)=(X(IBOND,J)-AVE(IBOND))**2
ISN        62       13     CONTINUE
ISN        63       12     CONTINUE
```

```
ISN     64              DO 34 IBOND=1,15
ISN     65              DO 35 K=1,5
ISN     66              SQSUM2(IBOND)=(Y(IBOND,K)-AVE(IBOND))**2
ISN     67      35      CONTINUE
ISN     68      34      CONTINUE
ISN     69              DO 36 IBOND=1,15
ISN     70              DO 37 I=1,6
ISN     71              SQSUM3(IBOND)=(Z(IBOND,I)-AVE(IBOND))**2
ISN     72      37      CONTINUE
ISN     73      36      CONTINUE
ISN     74              DO 99 IBOND=1,15
ISN     75              SQSUM(IBOND)=SQSUM1(IBOND)+SQSUM2(IBOND)+SQSUM3(IBOND)
ISN     76              PRINT 44,SQSUM(IBOND)
ISN     77      44      FORMAT(30X,F12.6)
ISN     78      99      CONTINUE
ISN     79              PRINT 55,(SQSUM(IBOND),IBOND=1,15)
ISN     80      55      FORMAT(20X,F8.5)
ISN     81              DO 95 IBOND=1,15
ISN     82              STDEV(IBOND)=(SQSUM(IBOND)/(N-1))**0.5
ISN     83              PRINT 98,STDEV(IBOND)
ISN     84      98      FORMAT(5X,F8.5/)
ISN     85      95      CONTINUE
ISN     86              PRINT 75
ISN     87      75      FORMAT(5X,'TOTAL',7X,'AVERG',7X,'SQSUM',7X,'STDEV'/)
ISN     88              DO 400 IB=1,15
ISN     89              PRINT 85,TOTAL(IB),AVE(IB),SQSUM(IB),STDEV(IB)
ISN     90      85      FORMAT(1H ,4(E12.5))
ISN     91      400     CONTINUE
ISN     92              STOP
ISN     93              END
```

VITA

Shashikant D. Kalaskar

Candidate for the Degree of

Master of Science

Thesis: MONTE CARLO STUDY OF LIPID CHOLESTEROL INTERACTIONS IN BIOMEMBRANES

Major Field: Physics

Biographical:

Personal Data: Born in Poona, India, September 28, 1948 the son of Mr. and Mrs. D. M. Kalaskar.

Education: High School diploma from Camp Education Society's high school in June, 1965; received Bachelor of Science degree in Chemistry (major), physics, mathematics (minor) from Poona University in June, 1971; received Bachelor of Education degree from Tilak College of Education, Poona University in June, 1975; received Master of Science degree in Chemistry (physical) from Western Carolina University, North Carolina, August, 1982.

Professional Experience: Science, Mathematics teacher, R.D. High School, Poona, India, 1971-1975; Science, Mathematics teacher, Trenchtown Comprehensive High School, Kingston, Jamaica (W.I.), 1975-1976; Physics, Mathematics Instructor, Holy Childhood High School, Kingston, Jamaica (W.I.), 1976-1979; Physics, Mathematics Instructor, Priory High School, Kingston, Jamaica, (W.I.), 1979-1980; Demonstrator of Physics, University of the West Indies, Department of Physics, 1978-1980; Mathematics, Physics Instructor Priory Adult Centre of Education (P.A.C.E.), 1976-1980; Graduate Teaching Assistant, Western Carolina University, Department of Chemistry, 1980-1982; Graduate Teaching Assistant, Oklahoma State University, Department of Chemistry, 1982-1983; Graduate Teaching Assistant, Oklahoma State University, Department of Physics, 1983-1987.