SUMMARY

The present thesis consists of three sections.

Section I

This gives a comprehensive account of the up-to-date "consensus" view of structure and properties of biological membranes.

Section II

In this section, studies on an extremely sensitive plant, *Mimosa pudica*, have been described.

Cellular and chloroplast lipids of the leaves of this plant have been analysed. Qualitatively the over-all lipid composition of this plant is not much different from that reported in the case of other photosynthetic tissues. Phosphatidylethanolamine (predominant in leaves), phosphatidylglycerol, phosphatidylcholine (predominant in chloroplast), phosphatidyl inositol and lysophosphatidylcholine are the phospholipid components present. Glycolipid fraction contains mainly galactolipids and these are identified as monogalactosyldiglyceride, digalactosyldiglyceride (predominant in both leaves and chloroplast), cerebrosides and sulfoquinovosyldiglyceride. Sterol has been found to be a mixture of β-sitosterol and dihydro β-sitosterol. Chloroplast lipid profile shows some resemblance with that of algae. Amongst fatty acids palmitic acid and α-linolenic acid have been found to be predominant in both
leaves and chloroplast. Cerebroside fraction has been found to contain a polyunsaturated fatty acid (20:4,3) and a long chain sphingosine base whose Rf-value (0.8) in thin-layer chromatography coincides with that (0.8) from ox brain cerebroside and not with that (0.37) of phytosphingosine from spinach.

Chloroplast consists of two membrane systems, the outer one is called envelope membrane and the inner one thylakoid membrane. These two membranes have been separated by sucrose density gradient centrifugation. Further studies have been carried out with the envelope membrane because the thylakoid membrane contains lot of chlorophyll which may interfere with our experiments. The following parameters have been studied with envelope membrane.

(i) Lectin (RCA1) - mediated agglutination of envelope membrane and liposome prepared from total polar lipid of chloroplast -

RCA1 agglutinates both envelope membrane and liposome, however, agglutination is about 58% more in the case of liposome.

(ii) Effect of divalent cations on lectin-induced agglutination -

the divalent cations Ca++ and Mg++ do not have any effect on agglutination.

(iii) Thermotropic phase transition by fluorescence method incorporating an excimer forming, lipophilic fluorescent probe, pyrene -
the $I_E/I_M$ ratio (where $I_E$ = intensity maximum for excimer band and $I_M$ = intensity maximum for monomer band in the fluorescence spectrum of pyrene) was plotted against temperature. The curve shows a major phase transition below $15^\circ C$ and another weak transition around $19^\circ -21^\circ C$. This is in agreement with the reported values for the chloroplast membranes of other plants.

(iv) Effect of RCA$_1$ on phase transition of envelope membrane -

RCA$_1$ has considerably decreased the $I_E/I_M$ ratio indicating decrease in over-all fluidity due to agglutination.

Section III

In this section, studies on erythrocyte membranes of four different vertebrates viz. goat, chicken, turtle and fish (katla) have been carried out. Followings parameters have been investigated.

(i) Lipid composition of turtle and fish erythrocytes -

out of these four systems, lipid profiles of turtle and fish erythrocytes have not been reported before. Therefore, lipid composition of these two systems have been analysed. Phospholipid and cholesterol contents have been found to be more in fish erythrocyte than in turtle. However, phospholipid:cholesterol molar ratio is almost same ($\approx 1.0$) in the two systems. Glycolipid content (water insoluble fraction) of turtle erythrocyte is
remarkably high compared to that of fish. Glycolipid fraction has not been analysed in detail. These appear to be asialoglycosphingolipids since these are resistant to alkali hydrolysis and do not contain gangliosides. Choline containing phospholipids have been found to be predominant (>70% of total phospholipid) in both the systems. Phosphatidylcholine: sphingomyelin ratio is widely different in these two systems. Sphingomyelin is the predominant phospholipid (47%) in turtle whereas in fish phosphatidylcholine is the major constituent (60%). Amongst the fatty acids, palmitic acid is present in considerable amount in both the systems. Long chain fatty acids above C₁₈ are not detected in turtle. Over-all unsaturation has been found to be much more in fish.

(ii) Asymmetric distribution of phospholipids in turtle and fish erythrocyte membranes -

asymmetry in phospholipid distribution in turtle and fish erythrocytes has been studied with the help of phospholipase A₂ from Naja naja (Indian cobra). The result shows that, in turtle 45% of total phosphatidylcholine and 25% of total phosphatidylethanolamine are present in the outer half of the membrane. In fish a major portion of total phosphatidylcholine appears to be present on the outer surface since sphingomyelin content is very low here. During phospholipase A₂ reaction considerable hemolysis has been observed in the case of fish erythrocyte and not in turtle. Sphingomyelin is not degraded by phospholipase A₂ and has been shown previously to be exclusively located in the outer
(v)

surface of erythrocyte membrane. As this is the major phospholipid in turtle erythrocyte, this perhaps imparts stability to the system.

(iii) RCA₁₃ mediated agglutination of erythrocyte ghosts of the above four vertebrates and respective liposomes -

as in Mimosa pudica, in this case also, agglutination has been found to be more in liposomes compared to that in ghosts (except chicken). This may be due to presence of 'self-neutralised, closed pairs' formed between receptors and complementary proteins (endogenous lectins?).

(iv) Effect of divalent cations on RCA₁₃ mediated agglutination -

Mg⁺⁺ has been found to specifically stimulate agglutination in both erythrocyte ghosts and liposomes. Stimulation is more in liposomes. Ca⁺⁺ has been found to be stimulatory to some extent in chicken only.

(v) Thermotropic phase transition of erythrocyte ghosts and respective liposomes by fluorescence method using fluorescent probe, pyrene -

in all the cases broad phase transition within the temperature range 23°-35°C has been observed. This is probably due to high content of cholesterol in erythrocyte membranes. Besides this in chicken and fish, there appears to be another major phase transition below 20°C.
(vi) Effect of RCA on phase transition of the above systems -
with goat and fish erythrocyte ghosts, Ie/Im ratio at a particular temperature has been found to increase in presence of RCA. This indicates that, in these systems, redistribution of lectin receptors probably induce a phase separation so that pyrene has been squeezed out in a more fluid region. In turtle and chicken erythrocyte ghosts and also in liposomes of all the four systems, the Ie/Im ratio is decreased in presence of RCA. Probably increased agglutination (either due to the presence of more receptors or due to the proximity of receptors to form cross-bridge) has imposed on over-all rigidity to the system.

(vii) Effect of RCA concentration on phase transition of goat erythrocyte ghost -
with increase in the concentration of RCA (10 to 50 µg/ml), the Ie/Im value at a particular temperature decreases, though the ratio is always higher than the corresponding value in the normal phase transition curve of the system.

(viii) Effect of cholesterol on phase transition of liposome prepared from total polar lipid of goat erythrocyte ghost -
in the absence of cholesterol, a distinct phase transition between 32.5°C-36°C is observed. With increase in cholesterol concentration, fluidity decreases and the sharp phase transition is abolished. Fluidity is minimum when phospholipid:cholesterol molar ratio is 1:0.9 (normal composition of goat erythrocyte
membrane). With further increase in cholesterol concentration fluidity increases.

The following important deductions may be made from our study:

(a) glycolipids play a larger role in lectin-mediated agglutination of cells,

(b) redistribution of lectin receptors affects fluidity of biological membranes,

(c) membrane proteins can act as endogenous lectins and form 'closed' complex with the complementary endogenous glycosylated receptors.