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An X-Ray Diffraction Study of the Physical State of the Lipid Phase in Biological Membranes

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Abstract—High angle X-ray diffraction patterns have been recorded from a number of isolated cell membrane preparations and total lipid extracts from such preparations, maintained at various levels of hydration. Consistent differences in the positions of the peak at 4.5 Å to 5 Å have been observed as between surface membranes and cytoplasmic membranes and between their lipid extracts. Such differences might be explained in terms of the higher cholesterol content of cell surface membranes. Small differences were also observed as between each membrane preparation and its corresponding lipid extract but the significance of this is not yet established.

The physical state of the lipid phase in biological membranes is probably an important factor in the determination of permeability characteristics and also in the control of enzyme activities associated with many membrane properties.

Analyses of lipid extracts from a variety of membrane preparations have revealed some striking variations[1,2] which may have structural and functional significance. In many tissues, for instance, the cell surface membrane (plasma membrane) has an appreciably higher content of cholesterol than do cytoplasmic membranes and the lipid hydrocarbon chains have a significantly lower overall level of unsaturation. It was of interest, therefore to see if such differences in lipid characteristics have any measurable effect on the high angle X-ray diffraction in the region of 4.5 to 5 Å. A comparison was made between readily available samples

of surface membranes and cytoplasmic membranes and between the lipids extracted from such membranes, all samples being examined at several levels of hydration. Only the data on erythrocyte membranes and on muscle microsomes are available for this preliminary communication.

Materials and Methods

Haemoglobin-free human erythrocyte membranes were prepared in phosphate buffers according to the method of Dodge, Mitchell and Hanahan.[3] Microsomes from rat skeletal muscle were prepared according to the method of Martonosi and Feretos.[4] Both preparations were washed with distilled water prior to the final ultracentrifugation.

Lipid was extracted from each membrane preparation by the method of Rand and Luzzati[5] and reduced to the dried state and stored in an atmosphere of nitrogen.

The membrane preparations, sedimented as pellets by centrifugation for 30 mins. at 100,000 $\times$ g (average), initially contained more than 90% water. This water level was gradually reduced by passing a stream of nitrogen at controlled humidity over a sample of the preparation suspended in a closed cell on the X-ray diffraction camera.

Small samples of the lipid extracts were dispersed in small amounts of distilled water and sealed in glass capillary tubes for two days to equilibrate. The sample was then extruded onto the sample holder in the controlled humidity chamber and examined at a variety of levels of hydration by the same procedures as for the membrane samples.

In some experiments the sample holder was weighed before use and then withdrawn from the controlled humidity chamber immediately following an X-ray exposure and weighed again in order that an estimation of the amount of water remaining in the sample at this point could be made when the final dried weight was eventually determined.

The beam of X-rays from a Philips fine focus X-ray source was

defined by a lead glass capillary, six centimeters long and with a bore of 0.5 mm. Lead pinhole guard apertures were located on either side of the Melinex window at the entrance to the controlled humidity chamber (Fig. 1). The specimen-to-film distance was

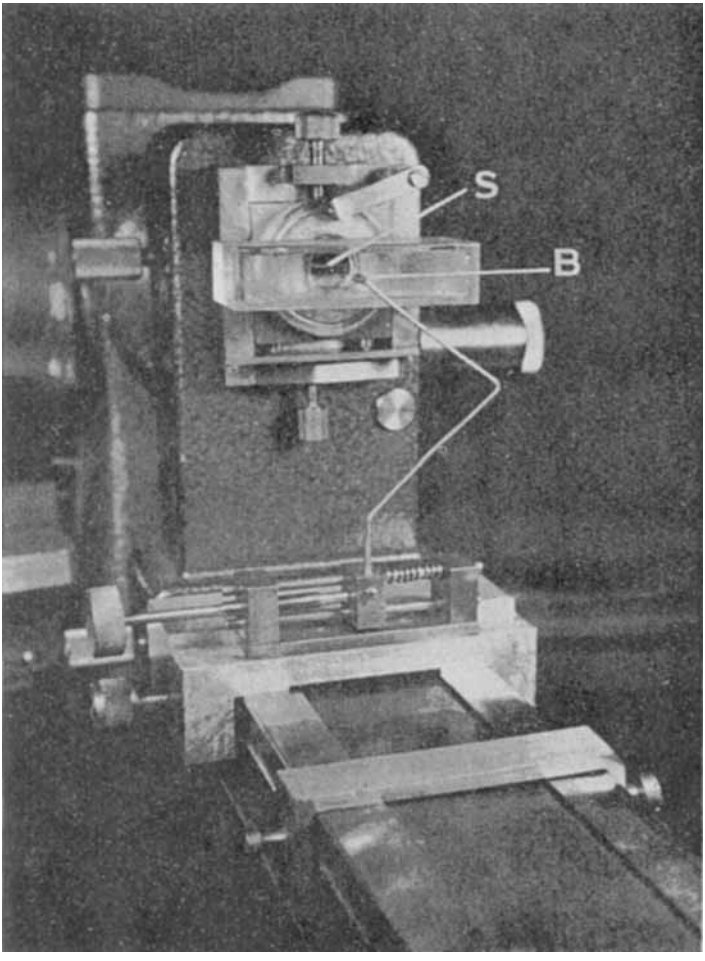

Figure 1. Photograph of controlled humidity cell mounted on X-ray collimator
S—sample holder B—lead beam stop in contact with window of cell.

rigorously controlled by locating the specimen chamber and the film holder against fixed stops and calibrated with reference to a sample of quartz powder. The lead beam stop was placed in contact with the forward window of the sample chamber in order that diffraction from this window should not reach the film.

X-ray diffraction patterns were recorded on Ilford Industrial G X-ray film and diffraction profiles and intensities were obtained from microdensitometer traces recorded using a Joyce-Loebl Model III CS automatic recording microdensitometer. Exposures with Nickel-filtered Cu Kα radiation were for a minimum of one hour.

Results

Examples of microdensitometer traces obtained from X-ray diffraction patterns recorded from samples of membrane preparations and from lipid extracts at two different levels of hydration are shown in Fig. 2. The peak at 3.4 Å could be reproduced from water alone and it became progressively less intense as the samples were dehydrated. With both membrane samples and lipid samples this peak could no longer be detected when the water levels reached about 20% with respect to final dried weight. This was established both by weighing and by reference to the appearance of low angle diffractions which have been studied more extensively from this point of view.

The peak in the region of 4.5 to 5 Å strengthened as water was gradually removed from the sample. It retained its smooth profile down to very low water levels and its peak position did not change appreciably. Sharp lines eventually became superimposed on these broad profiles but in most cases these did not become apparent until the sample had been left in the final dried state for several days. Cooling to $-20\,^{\circ}C$ did not have any substantial effect on this change.

The central (~ 10 Å) peak initially appeared to be an artefact arising from absorption by the highly hydrated and bulky sample, but in the case of the membrane preparations significant diffrac-

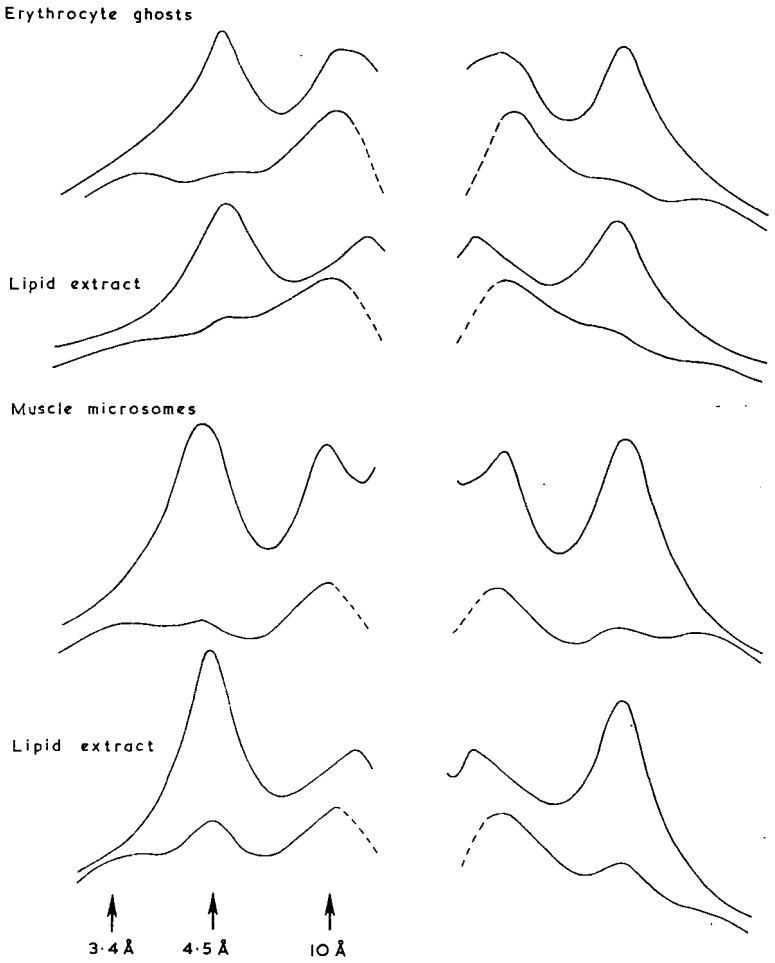

Figure 2. Microdensitometer traces across a selection of high angle diffraction patterns of membrane preparations and of lipid extracts maintained at various levels of hydration. Upper curve—low water content (~20%) Lower curve—high water content (50–90%).

tions soon appeared in this region and at low levels of hydration a multiplicity of the peaks became evident. Lipid samples gave much less intense diffraction in this region but subtraction of the lipid diffraction at low water levels from that of the corresponding

membrane sample produced a single peak in the region of 10.3 to 10.4 Å for the membrane.

The peaks of primary interest in this study are those in the region of 4.5 to 5 Å. Their profiles are remarkably similar in shape and in half width but the peak positions show appreciable variation. The exact positions of these peaks expressed as Bragg reflections are given in Table 1. The number of patterns measured is not yet sufficient to quote statistical variations but it is estimated that the maximum possible error in estimating peak values arising from variations in position of sample and from inaccuracies in measurement of peak positions on microdensitometer traces is of the order of 0.03 to 0.05 Å.

TABLE 1 Comparison of Peak Positions for Membrane Preparations and Their Lipid Extracts

Comparison is made at point where the water diffraction at 3.2 to 3.4 Å can no longer be detected. Peak positions are expressed as Bragg reflections

	Membranes	Lipid extract
Erythrocyte ghosts	4.65 Å	4.74 Å
Muscle microsomes	4.47 Å	4.52 Å

Discussion

The figures so far available show consistent differences in peak positions between one membrane and another and between their lipid extracts and also between each membrane and its corresponding lipid extract compared at an appropriate level of hydration. The differences between erythrocyte membranes and muscle microsomes and between their lipid extracts are certainly significant and may arise from the demonstrated differences in chemical composition, particularly the higher cholesterol content of the surface membrane. The addition of cholesterol to oleic acid has been reported by Segerman[6] to increase the high angle spacing appreciably. Lecithin-cholesterol mixtures have also

been studied by X-ray diffraction methods[7] but the effects of increasing proportions of cholesterol on the high angle reflections have not yet been reported.

The difference between each membrane preparation and its lipid extract examined at a comparable level of hydration is relatively small and in the case of muscle microsomal material cannot yet be considered of established significance. However, the magnitude of the difference in peak positions between erythrocyte membranes and the lipid extract is undoubtedly significant and several possible explanations can be suggested for consideration.

(1) The proportion of cholesterol in the final lipid extract may be higher than it is in the membrane. This could be conceived of as arising from a preferential loss of phospholipid or other lipids during extraction and purification but the possibility has not yet been checked experimentally.

(2) The position of the predominantly lipid peak in the intact membrane may be affected by an overlap with diffraction from non-lipid components. This possibility has not yet been investigated directly because of difficulties in the isolation of non-lipid components in an unmodified form. A peak shift in the observed direction could conceivably arise from overlap of diffraction from a β-type polypeptide component but spectroscopic studies of erythrocyte membranes[8,9,10] have indicated that there is very little β-polypeptide chain configuration in the membrane protein. This protein is mainly in coiled configuration which would be expected to have an effect in the reverse direction to that observed.

It should also be noted that the presence of β-type polypeptide in muscle microsome membranes could not account for the different peak position as compared with the lipid extract.

(3) Interaction with protein in the membrane may make the lipids pack more tightly than they do in the isolated lipid system. This is a very interesting possibility but it would not be profitable to speculate further until possibilities 1 and 2 have been further investigated.

The significance of the profiles of the peaks attributed to lipid

in terms of detail of packing of lipid molecules in the lipid phase of the membrane is difficult to assess. The profile is clearly indicative of a high degree of disorder but to what extent this is a molecular disorder and to what extent a disorder of small regions of partial order is not directly indicated by this technique.

REFERENCES

1. Finean, J. B., Coleman, R., and Green, W. A., *Annals N.Y. Acad. Sci.* **137**, 414 (1966).
2. Coleman, R., *Chem. Phys. Lipids*, **2**, 144 (1968).
3. Dodge, J. T., Mitchell, C., and Hanahan, D. J., *Arch. Biochem. Biophys.* **100**, 119 (1963).
4. Martonosi, A. and Feretos, R., *J. Biol. Chem.* **239**, 648 (1964).
5. Rand, R. P. and Luzzati, V., *Biophysic J.* **8**, 125 (1968).
6. Segerman, E. in International Conference on Biological Membranes, Frascati, Italy, 1967 (North-Holland Publishing Co. 1968).
7. Ladbrooke, B. D., Williams, R. M., and Chapman, D., *Biochim. Biophys. Acta*, **150**, 333 (1968).
8. Maddy, A. H. and Malcolm, B. R., *Science*, **153**, 213 (1966).
9. Wallach, D. F. H. and Zahler, P. H., *Proc. Natn. Acad. Sci. U.S.A.* **56**, 1552 (1966).
10. Lenard, J. and Singer, S. J., *Proc. Natn. Acad. Sci. U.S.A.* **56**, 1828 (1966).