with the materials and instructions supplied with the NIAMD assay kit distributed by NIH. Serum testosterone concentrations were determined by the unpublished method of R. L. Goodman, J. Hotchkiss, F. J. Karsch, and E. Knobil, with an antiserum supplied by Dr. I. H. Thorneycroft.
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* Career development awardee of the National Institute of Child Health and Human Development.

23 November 1973

Electron Diffraction of Wet Biological Membranes

Abstract. *Electron diffraction patterns were obtained for the first time from single wet phospholipid bilayers and from wet human erythrocyte membranes by using a temperature-controlled electron microscope hydration stage. Selective area diffraction showed the existence of semicrystalline domains. A structural transition was observed at the transition temperature of the wet dipalmitoyl lecithin bilayer.*

Electron diffraction has not been as popular as x-ray diffraction in the study of the structure of biological membranes. The main problem is that the membrane specimen to be studied by electron diffraction has to be dried. This problem has been overcome by the development of hydration stages for electron microscopes (*1*). A membrane specimen can now be studied in an electron microscope under more physiological conditions by both imaging and diffraction. Artifacts introduced during drying, fixation, and staining are thus eliminated, and it is only necessary to limit radiation damage from the electron beam by use of sensitive emulsions (*2*).

The advantages of the electron diffraction technique over other diffraction methods are the following: (i) Specimens of only one or several membrane thicknesses (25 to several hundred angstroms) can be examined because of the strong interaction between the specimen and the incident electron beam. (ii) The data collection time is very short (of the order of seconds). (iii) Very small areas can be observed by use of the selective area diffraction technique. The signal-to-noise ratio can also be improved by this method. (iv) A very small amount of specimen is required and impurities can be screened. (v) The shape and morphology of the specimen may be observed by imaging.

We have used the electron diffraction technique to study both phospholipid bilayers and human erythrocyte membranes. Dipalmitoylphophatidylcholine was spread on a Langmuir trough at a surface pressure of 40 dyne/cm, and a bilayer film was formed over micromesh squares by slowly dipping 1000-mesh grids through the surface. The grids were immediately inserted into the hydration stage in the electron microscope. The entire operation was performed under a water-saturated atmosphere. Human erythrocyte ghosts were prepared by a modified Dodge method (*3*). The sample was placed on a grid coated with carbon and Formvar. Excess solution on the grids was removed in a water-saturated atmosphere before they were inserted into the hydration stage.

A hydration stage has been constructed for a Siemens Elmiskop IA electron microscope. The stage is so designed that the temperature of the specimen can be controlled in the range −10° to 50°C under 100 percent humidity. The degree of hydration of the specimen can also be varied if an external water reservoir is used. The structure and function of this stage are described elsewhere (*4, 5*).

To minimize the radiation damage to the specimen, the electron beam dose was kept below 10^{-5} coulomb/cm^2 (measured by a Faraday cage) for each diffraction pattern recording. This was achieved by using a 10-μm second-condenser aperture, and by recording the patterns with Kodak No-Screen x-ray films, which were 17 times more sensitive than electron image plates (*2*). The patterns deteriorated if the radiation dose was increased 50-fold. All patterns were taken at 100 kv, the specimen being under saturated water vapor pressure for the corresponding temperatures.

Typical diffraction patterns of a single wet unsupported dipalmitoylphosphatidylcholine bilayer taken below the transition temperature are shown in Fig. 1, A and B. A streaked hexagonal pattern (Fig. 1A), or in some cases several similar patterns misoriented from one another (Fig. 1B), were usually seen when the observed area was about 5 μm in diameter. The streaking does not diminish if the observed area is further reduced. When the observed area is widened the pattern becomes a typical continuous ring powder pattern, although the ring always remains sharp. Outer rings of $1/3^{1/2}$ and $1/2$ times the spacing of the inner pattern can also be seen. These spacings are characteristics of the α-phase of lipid hydrocarbon chain packing (*6*). The streaking suggests imperfections in the hexagonal packing.

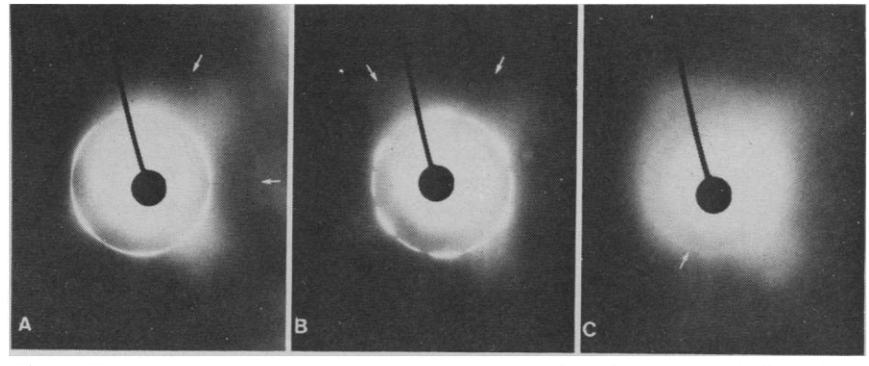

Fig. 1. Electron diffraction patterns of a wet, unstained, and unsupported dipalmitoyl lecithin bilayer. The observation area is 5 μm in diameter. (A) Pattern taken at 30°C showing a streaked hexagonal pattern at spacings of 4.25 and 2.45 Å (arrows). (B) Pattern taken at 39°C showing two misoriented hexagonal lattices. Both show spacings of 4.30 and 2.48 Å (arrows). (C) Pattern taken at 44.0°C showing a diffused ring (arrow) at 44 Å.

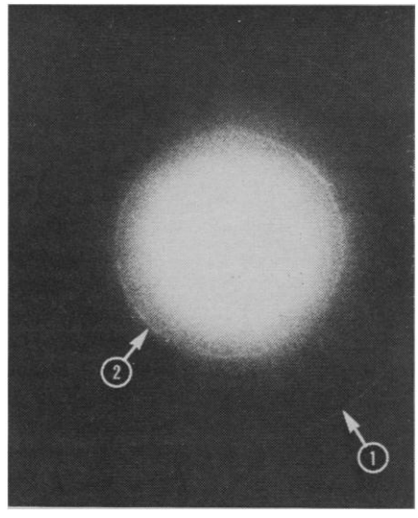

Fig. 2. Electron diffraction pattern of wet, unfixed, and unstained human erythrocyte membrane. The outer ring ① is from the aluminum-coated substrate; the inner ring ②, at a spacing of 4.18 Å, is from the membrane.

The two misoriented patterns in Fig. 1B show either the observation area straddling two neighboring domains, each with imperfect α-phase packing, or the packing of the two monolayers with different orientations. Yet the frequent simultaneous observation of three or more patterns indicates that domain boundaries must exist. The bilayer is made up of a mosaic of these domains, each with a dimension of several micrometers. This structure persists under any degree of hydration, as long as the temperature is below the transition temperature. When the temperature of the hydration stage was raised, an increase in unit cell dimensions was observed for various degrees of hydration, and a sharp transition to a less ordered state was indicated by the loss of sharp diffraction rings. Above the transition temperature, a diffuse ring with a spacing of 4.4 Å is observed, as shown in Fig. 1C. The transition temperature observed for small areas (< 1 μm) of single bilayers was identical with that obtained by other methods for larger volumes of multilayers (*7, 8*). The variation of the transition temperature with the degree of hydration agrees with the temperature-hydration phase diagram obtained from x-ray diffraction measurements of a bulk lipid-water system (*8*). A complete analysis of this work will be reported elsewhere (*9*).

The diffraction pattern from a wet human erythrocyte ghost membrane is shown in Fig. 2. The spacing for the discontinuous ring in Fig. 2 is 4.18 Å. The sharp ring on the outside is from aluminum deposited on the grids for calibration. The sharpness and unevenness of the inner ring in Fig. 2 suggest that the membrane may also consist of semicrystalline domains, as in the case of phospholipid bilayers. The diffused ring at 4.7 Å observed by x-ray diffraction was not seen here (*10*).

The advantage of electron diffraction for studying the structure of biological membranes can be readily seen from this report. By restricting the observed area to a size comparable to that of the domain, the mosaic structure of the membrane was directly observed for the first time. The ability to obtain data from a single sheet of membrane is important since, in general, biological membranes do not exist in nature in the multilayer forms required for most x-ray diffraction work. To meet the challenge of observing the structure of biological membranes in their natural states, electron diffraction seems to be a very useful technique among direct methods.

It is possible to use this technique to observe small angle diffraction from biological membranes, with an electron energy filter to discriminate the inelastically scattered electrons in the small angle region (*11*). It should be useful for studying large protein structures, such as intramembrane particles (*12, 13*), in biological membranes, and the structure of gap junctions (*13*).

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15 November 1973; revised 18 January 1974 ■

Spermidine in Hormone-Dependent Differentiation of Mammary Gland in vitro

Abstract. *Stimulation of milk-protein synthesis in mouse mammary epithelium in vitro requires insulin, hydrocortisone, and prolactin. The requirement for hydrocortisone can be replaced by spermidine. The possibility that spermidine mediates the effect of glucocorticoid is also supported by the observation that the cellular spermidine concentration increases before the accelerated synthesis of casein and α-lactalbumin.*

Spermidine is ubiquitous in living tissues, and relatively high concentrations of this polyamine are often found in mammalian tissues which exhibit relatively high rates of RNA and protein synthesis (*1, 2*). Furthermore, the spermidine concentration frequently undergoes marked changes during growth and differentiation (*3, 4*). In mammary gland, the spermidine concentration has been reported to increase dramatically during lactation (*2*). This prompted us to investigate the possible role of spermidine in lactogenesis. The results reported here demonstrate the involvement of spermidine in the synthesis of milk proteins during the hormone-dependent differentiation of mouse mammary epithelial cells in vitro and represent the first demonstration that spermidine can mediate the effect of glucocorticoid.

To determine the effects of spermidine on milk protein synthesis, mammary explants prepared from pregnant mice were cultured for 2 days on media containing several combinations of the polyamine, insulin, hydrocortisone, and prolactin. Maximal increases in casein synthesis (*5*) and α-lactalbumin