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Membrane phenomenons in relation to vitamin A

By J. T. DINGLE and J. A. LUCY, *Strangeways Research Laboratory, Cambridge*

One way of studying the organization and properties of the lipid components of membranes is to investigate the effect on membranes of various agents known to interact with lipids. Vitamin A has recently been found to have this property; studies made at the Strangeways Laboratory on the interactions of the vitamin with membranes have yielded information that may be relevant both to the structure and function of biological membranes and to the mechanisms of action of vitamin A.

Excess of the vitamin affects the membranes of a number of cells and intracellular organelles (reviewed by Fell, 1965*a*, and Lucy & Dingle, 1964*a*). Examples of such changes have been seen in electron micrographs of fibroblasts grown in the presence of retinol (Daniel, Dingle, Glauert & Lucy, unpublished observations). A disappearance of the granular endoplasmic reticulum of these cells was observed, which was accompanied by a marked increase in the number of free ribosomes. The appearance of small invaginations in the plasma membrane and the formation of many myelinated bodies were noted. Mitochondrial swelling was observed after 6 h growth in the presence of the vitamin, at which time a depression of respiratory activity occurred; these findings may be related to the swelling seen in isolated mitochondria after treatment with retinol (Lucy, Luscombe & Dingle, 1963).

Lysosomes were present in both the control and experimental cells, but there was an increase in the number of cytolysomes in retinol-treated fibroblasts. Cytolysomes are believed to be lysosomes that are particularly concerned in the degradation of damaged cellular organelles (Novikoff & Essner, 1962). This change in lysosomal type may be correlated with the decreased stability of lysosomes in retinol-treated tissues in culture (Fell & Dingle, 1963) and in hypervitaminotic animals (Dingle, Sharman & Moore, 1963). As stated in Dame Honor Fell's (1965*b*) paper, the release of lysosomal enzymes by vitamin A causes many of the changes observed in cartilage and probably those produced in bone also (Fell & Mellanby, 1952).

Information on the mode of action of the vitamin on lipoprotein membranes has come from studies on both biological and artificial membranes. Studies made with the electron microscope on rabbit erythrocytes treated with excess of vitamin A in vitro have shown that retinol causes indentations of the membrane and the production of vacuoles by a process resembling micropinocytosis (Glauert, Daniel, Lucy

& Dingle, 1963). The penetration of membranes observed in these experiments is thought to be analogous to the penetration of a lecithin–cholesterol monolayer by the vitamin, which appears to depend on an interaction between vitamin A and the phospholipid (Bangham, Dingle & Lucy, 1964). The molecular structural requirements for the action of the vitamin on lipid monolayers, and for many of its actions on membranes, are highly specific and are similar to the requirements for reversing hypovitaminosis in animals (*cf.* Dingle & Lucy, 1962).

Further information about the action of vitamin A on membranes has come from the work of Kinsky (1963) on the lysis of bacterial protoplasts, and from that of Blough (1963) who observed that infection of eggs with influenza virus followed by treatment with vitamin A resulted in the formation of very pleomorphic forms of virus. Blough suggested that the changes in viral morphology reflected an alteration by the vitamin of the structure of the cell membrane.

The sequence of events that follow the penetration of a membrane by retinol is thought to depend upon the chemical composition of the membrane. With the erythrocyte, rapid lysis ensues at 37°. Since this rapid haemolysis is inhibited by vitamin E, oxidation of vitamin A and lipids may be involved in the lytic process (Lucy & Dingle, 1964*b*). Investigation of the molecular specificity for the inhibition of haemolysis led us to conclude that inhibition by the tocopherols may not be due to neutralization of free radicals and the breaking of auto-oxidative chain reactions, but may be associated in some way with the long isoprenoid chain of the inhibitory compounds.

The nature of the changes produced in the lysosomal membrane after penetration by the vitamin is obscure since the release of lysosomal enzymes in vitro (Dingle, 1961) is not prevented by vitamin E (Dingle, unpublished observations). It is of interest therefore to consider at this point some of the possible mechanisms by which retinol may modify the structure and function of lipoprotein membranes.

An hypothesis for the structure of biological membranes has been advanced in which it is suggested that, under certain circumstances, the lipids may be arranged in small globular micelles, each having a lipophilic core of about 40 Å in diameter (Lucy, 1964*a*). Membranes might normally be composed of both bimolecular leaflets and micelles in dynamic equilibrium, and this equilibrium could be altered by the presence of small quantities of physiologically active molecules, such as vitamin A or hydrocortisone. In the theoretical micellar model for the lipids of membranes (Lucy, 1964*a*), areas of membrane that are composed of micelles contain small water-filled pores, about 8 Å in diameter, which would easily allow water and small ions to pass through the membrane. This model provides a dynamic and reversible mechanism that may be of physiological importance in the control of membrane permeability. A membrane containing a large quantity of retinol might be expected to have an abnormally high proportion of its structure in the micellar form. It has been suggested therefore that the leakage of potassium ions, followed by slow haemolysis, that is observed when erythrocytes are treated with retinol and α-tocopheryl acetate simultaneously may reflect the presence of a greatly increased proportion of micellar-membrane in these cells (Lucy, 1964*a*). This change in structure would result in an

increased permeability to water and eventual osmotic rupture of the membrane. We have recently proposed that the release of hydrolytic enzymes from lysosomes by retinol may occur by a similar mechanism (Lucy & Dingle, 1964*b*).

An alternative way by which the membranes of cells and intracellular particles, including lysosomes, may be irreversibly damaged by retinol and other surface-active agents is indicated by the observations of Glauert, Dingle & Lucy (1962) and Lucy & Glauert (1964) on the interactions of saponin with membranes and with membrane lipids. Micelles formed by the interactions of lytic molecules with the lipids of membranes are sometimes able to arrange themselves into a variety of artificial structures. The geometry of these arrangements depends intimately on the properties and concentrations of the lytic molecules. For example, bizarre helical structures that resemble certain viruses in appearance, and hexagonal structures containing water-filled areas that are 80 Å in diameter, are observed in preparations of erythrocytes lysed with saponin in vitro. These structures are incapable of maintaining the osmotic integrity of the red cell. The utilization of membrane lipids in the formation of artificial structures of this kind may be generally applicable to the processes by which membranes are irreversibly damaged. It is possible that irreversible damage to membranes produced in this way by retinol in vivo may be associated with the observed formation of cytolysomes, since these organelles are believed to be responsible for the digestion of damaged cellular structures.

How far studies on the mode of action of excess of the vitamin shed light on its normal function, is uncertain. Since, however, the molecular structural requirements for penetration of membranes by excess of vitamin A are essentially similar to those for the reversal of hypovitaminosis in vivo and, since even small quantities of the vitamin have been shown to react with the erythrocyte membrane (Lucy & Dingle, 1964*b*), it seems that the 'membrane-activity' (cf. Lucy, 1964*b*) of retinol may be a necessary and perhaps initial stage in its normal physiological action. Thus vitamin A or a metabolite is envisaged as playing a functional role in membrane physiology (Dingle & Lucy, 1965), but at present this hypothesis must be regarded merely as a guide for future work.

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