A preparation of polysomes and free ribosomes isolated from rat liver by the method of Bloemendal, Bont, de Vries & Benedetti (1967) was mixed at 2° together with reticulosomes and micellar phospholipid, and left at that temperature for 20min. After that time the mixture was centrifuged for 60min. at 130000$g_{av.}$ and the pellet resuspended in 0·35M-sucrose in Medium B, and layered over a double gradient as described by Bloemendal *et al.* (1967). On such a gradient ribosomes form a pellet, while rough (ribosome-studded) membranes come to an equilibrium at the lower interface, and smooth membranes find their equilibrium at the upper interface. When all three components (ribosomes, reticulosomes and phospholipids) were mixed, only a small pellet was formed, while the bulk of the material was situated at the lower interface, and only small amounts of smooth membranes were found at the upper interface. On analysis the lower interface material was shown to consist of RNA, protein and phospholipid in proportions similar to those found in rough microsomal fractions. When viewed under the electron microscope, the lower interface material resembled rough microsomal vesicles.

The assembly of a membranous structure from non-membranous components *in vitro* does not have biological significance, unless the resulting structure also exhibits enzymic or other biological activities.

Smooth membranes formed by the interaction of reticulosomes and phospholipid show a significant change in glucose 6-phosphatase activity. The reconstituted rough vesicles were examined for their ability to incorporate amino acids into proteins. Their rate of incorporation of amino acids was considerably lower than that of free ribosomes and polysomes, but approximately of the same order as that of rough microsomes isolated by the method of Bloemendal *et al.* (1967).

The incorporation of amino acids by the reconstituted rough membranes was energy-dependent, stimulated by polyuridylic acid and inhibited by puromycin.

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The Isolation and Analysis of the Plasma Membrane Lining the Lumen of the Rat Bladder

By R. MARIAN HICKS, B. KETTERER and D. BEALE (*The Middlesex Hospital Medical School, London, W. 1, and A.R.C. Institute of Animal Physiology, Babraham, Cambridge*)

Rat transitional epithelium acts as a barrier to the flow of water between underlying tissue fluids and the hyperosmotic urine in the bladder. The maintenance of this permeability barrier depends on the asymmetrically thickened membrane that limits the luminal face of the superficial epithelial cells (Hicks, 1966). This membrane has now been isolated and subjected to preliminary chemical analysis.

The method of isolation is based on that of Warren, Glick & Nass (1966) and involves stabilization of the membrane *in situ* by treatment with fluorescein mercuric acetate. The superficial layer of tissue lining the bladder is scraped away, homogenized and sedimented through sucrose density gradients. In the final gradient the luminal membrane is recovered at a density of 1·13. Above this in the gradient is a fraction consisting apparently of unthickened plasma membrane originating from cell surfaces other than those lining the bladder lumen.

The determination of the protein content of fractions obtained direct from the final sucrose density gradient shows that the yield of luminal membrane protein is of the order of 200μg. However, the removal of sucrose is necessary for most analyses and the repelleting and washing required to do this result in a substantial decrease in yield.

The principal lipid components of the membrane are cholesterol, phosphatidylcholine, phosphatidylethanolamine and cerebroside. The molar ratio of cholesterol to phospholipid is 0·8.

The asymmetric thickening of the luminal membrane appears to be due to protein, since the ratio of phospholipid to protein of this membrane fraction is 0·5 whereas that of the unthickened membrane above it in the gradient is 0·8. Amino acid analysis of the luminal membrane shows that it has the unusually high proline content of 8 residues/100 residues.

What at this stage can be deduced about the chemical basis for the impermeability of the luminal membrane to water? It cannot depend on the lipid components of the membrane, since they resemble in type and content those of plasma membranes, which are water-permeable. It seems likely therefore that it is the large protein component that is of key importance.

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