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Closing Remarks

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Closing remarks

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In these closing remarks I would like to take a broad look at some aspects of the structure and function of biological membranes and envelopes. First, however, I think the organizers should be congratulated on a very stimulating and successful meeting. In my view they have been right to restrict the range of the Discussion to a few specific aspects of what is now a very large field; in this way it has been possible to consider the chosen subjects in depth. If in my remarks I step beyond the range of papers presented in the last two days, it will be to remind us all of the range of phenomena that still remain to be explained; and that thorough understanding will only come from the strenuous application of physical, chemical and biochemical principles in the broadest sense to biological material and to various types of model system.

The plasma membrane

First, the plasma membrane and its ubiquity; it is present in all cells and is a component – possibly in modified form – of many organelles. We are increasingly aware that a unit membrane – a definition that removes it from the restrictive specificity of the cell surface – is more than it seems from an electron micrograph. Mitochondrial membranes have long been known to be the seat of enzymic action and some of these proteins may be periplasmic and distinct from the membrane itself. There is evidence – presented at this meeting by Northcote – that internal membranes of the cells of higher plants are involved in synthesis and transport of wall material to the cell surface. There is therefore a degree of coordination and control between the internal and surface membranes over a wide range of cell types and organisms. The subject of cell fusion is both fashionable and important and it serves to emphasize the dynamic character of plasma membranes.

The picture of the plasma membrane put forward in 1943 by Davson & Danielli as a bi-lipid leaflet bounded by protein has received further confirmation quite recently from rigorous X-ray diffraction analysis of frog photoreceptor membranes. It is also generally agreed that the lipid phase of the plasma membrane is somewhat disorganized; moreover, we cannot be sure of detail because the X-ray method inevitably averages the structure. For this reason and others there is uncertainty about the uniformity or otherwise of membrane structure and uncertainty about the details of protein conformation and location. Information from studies of fractured membranes as discussed at this meeting by Bullivant may become increasingly important, but problems of interpretation as we have seen are substantial. Other than in a very broad sense, structural studies have so far given only limited guidance to possible transport mechanisms.

Differentiation of membranes

I pointed out more than ten years ago that the topography of the surface of flagellar and ciliate organisms and tissues and the very existence of cilia or flagella was suggestive of a differentiated surface or cortex. That a pattern in the nature and structure of such surfaces exists receives further support from the work of Roberts discussed at this meeting. The 'collar' at the external base of the flagellum in Chlamydomonas represents this differentiation in a structural context more clearly than one had any hope to expect. The plasma membrane is under as strict a genetic control as any other cellular organelle. Several reports have shown that in Chlamydomonas there are flagellar mutants in which the most striking phenotypic character is a swollen membrane. I have observed, that such effects may be simulated by the presence of lipids such as lysolecithin in the medium of the wild-type organism. The highly developed surface organization so frequently present in the protozoa should not however lead us to any sweeping general conclusions.

Cooperative properties

It seems likely that the plasma membrane is a dynamic – and possibly dissipative – system with properties which suggest cooperative phenomena over significant areas that are not necessarily constant in nature, in size, or in time and this feature lends a stochastic element to the behaviour of the membrane. Any molecular species approaching the membrane surface from either side and ultimately appearing on the other has first to be attracted not to a random point on that surface, but to one that is energetically, spatially and chemically significant for forward movement. The pores formed by head-to-head hydrogen-bonded dimers of gramicidin are of interest in this connexion. These considerations become more restrictive as the complexity of the molecule entering or leaving the cell increases.

The physico-chemical approach to membrane model systems are outside the scope of this meeting, but with the aid of model systems the complexity of the biological membrane, especially in relation to its multifarious functions, remains; and no one model system gives the answers; nor has any such approach revealed outstanding new principles.

Complex envelopes

Turning now to complex envelopes, these are striking for a number of reasons. First there is great diversity in their chemical nature. It has taken a generation to work out the essentials of the chemistry of bacterial cell walls. While many arguments and statements about function are teleological, it seems that in micro-organisms cell walls ensure strength and higher survival in face of lytic and osmotic adversities without preventing the ingress of nutrients and the egress of a variety of molecular species.

There are several ways in which an external envelope might be formed and appropriate criteria satisfied. The following considerations are by no means comprehensive.

(I) If the macromolecular species of which the envelope are composed are synthesized within the cytoplasm or at the inner surface of the plasma membrane, these molecules – inevitably of very substantial size – must somehow be carried through the plasma membrane to its outer surface. (See also Meldolesi in this volume).

(II) Alternatively, smaller chemical units of the ultimate envelope structure may be transported through the plasma membrane and synthesized externally into more complex structures.

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There could clearly be variations on the themes of (I) and (II).

(III) If the envelope consists of a number of biochemically distinctive parts, these must be laid down in a definitive order – a pointed reminder that the whole process is under genetic control.

(IV) By whatever means and in whatever form the components of the envelope system reach their locus, it is necessary by definition for them to remain there. Processes of local condensation and attachment must be presumed to exist to prevent dispersal. Even if envelope-turnover exists the same general principle must apply, otherwise the envelope would lose its overall integrity. (See Hicks *et al.* in this volume.)

(V) The envelope has to meet different criteria in different types of cell and tissue. Bacteria, for example, derive their whole nutrition from the medium via the envelope and the plasma membrane. On the other hand, in ciliates, food is imbibed by the oral apparatus and the pellicle may have no nutritional significance.

During the Discussion we have had an interesting example of category (I) in the paper by Thornley & Glauert. The outer layer of the *Acinetobacter* strain consists of subunits which may be detached from the organism and reassembled independently. Evidently the subunits possess at least two classes of binding site, one of which relates to forces between neighbouring subunits and the other to the binding of subunit to substrate. It is not clear of course whether these subunits are synthesized at the surface or are moved whole by carrier mechanism from the interior of the cell to the exterior.

To discuss the transport of large or even small molecules satisfactorily would require at least one whole Discussion. I would point out here only that the presence of exogenous macromolecules, including enzymes in cultures of micro-organisms and in tissue generally is so well known that it excites little comment as to how they get there. But it has been elegantly put to very good use by several workers in the study of the regulation of bacterial spore formation. In plants fungal pathogens secrete enzymes in a strict order, a situation now being exploited with particular reference to chemical studies of the primary cell wall. Again a definite time sequence of biochemical and genetic control is discernible. It also follows from these general considerations that if exogenous macromolecules – whether free or bound – are not synthesized in the cytoplasm, the appropriate mechanisms and enzymes must exist at, within or without the plasma membrane. In this context 'at or within' is virtually a semantic distinction and we arrive at a complex and dynamic picture of the membrane a world away from the structurally static picture of the electron micrograph which to a large extent denies the biochemical complexity it conceals.

If the alternative of (II) is preferred ((I) and (II) are not mutually exclusive) it follows that final stages of synthesis must take place either at or in the membrane–envelope complex or alternatively in the medium. If the latter, then it follows that completely exogenous synthesis is a major mechanism for envelope development. This in turn implies the additional and subsequent process of deposition of the products on the cell surface in the correct sequence. It would be hazardous at present to assume that self-assembly is the sole mechanism for deposition.

With regard to (III) the work of Roberts and his colleagues reported in this publication has shown that the envelope of *Chlamydomonas reinhardii* is a useful organelle for genetic investigation.

There are many known examples of the existence of free extracellular macromolecules in biological systems of varying complexity as I have already implied. I will refer to two only: the extracellular formation of cellulose by *Acetobacter xylinium* and the formation of collagen fibres

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in animal connective tissues. I do not wish to enter into the complex biochemistry of formation of either of these macromolecules although in both instances it appears that exogenous enzymes are involved in the later stages. These examples and many others show that the mere appearance of macromolecules exterior to the cell surface does not in itself automatically provide the conditions for the formation of an envelope. Genetically controlled chemical and physical processes must ensure that the inner moiety of the envelope is firmly attached to the plasma membrane. That the outer layer may be less firmly attached as shown from the work of Thornley & Glauert in this Discussion and the genetic control of cell wall attachment of Chlamydomonas is another interesting system. (See Roberts in this Discussion.)

It has long been known that cells of higher plants are enclosed in a rigid wall in which cellulose, hemicellulose, pectic polysaccharides, protein and lignin are the major structural components. Early work on such algae as *Valonia ventriculosa* implicated cellulose as a major component of the walls of this group. In others, xylans and mannans have been shown to be major single components. In *Chlamydomonas reinhardii* it has been shown that glycoproteins are a major structural component susceptible to structural analysis by optical diffraction of electron micrographs. (See Roberts in this volume.) In my laboratory we have been interested in producing heterokaryons – between wild-type and various flagellar mutants to which normal mating procedures are inapplicable. We have found that pronase almost completely digests bulk preparations of isolated Chlamydomonas walls. The same complex of proteases may be applied to the living cells. After exposure to the enzymes of 37 °C for 24 h, there is a substantial survival of living cells.

Thin sections of these cells viewed in the electron microscope subsequent to Franke fixation and the normal embedding and sectioning procedures, show that the laminate structure of layers W 2–W 7 disappears.

The chemical nature of the cell wall of *Chlamydomonas reinhardii* has been recently studied by several workers who have already demonstrated the presence of hydroxyproline, arabinose, galactose, mannose and xylose as major components (see Roberts in this volume and his references). In addition these investigations have shown that crude wall preparations contain a number of hydroxyproline hetero-oligosaccharides, whereas in higher plants the general rule is for the hydroxyproline to be associated with homopolysaccharides. Among the five new hydroxyproline glycosides observed is hydroxyproline-*o*-galactose. Moreover, the bands separated by gel electrophoresis stained with dyes appropriate for glycoprotein. Although each band probably corresponds to a single glycoprotein, it would be difficult to show whether each layer of the wall as seen in the electron micrographs consists of a single glycoprotein or of a selective mixture.

In collaboration with R. Hynes of the M.R.C. Muscle Biophysics Unit and J. Cooper formerly of the University of Durham Glycoprotein Research Unit we have been able to extend the chemical studies of the wall. (Cooper, Hyams, Hynes, Macdougall & Randall, in preparation.) The chemical analyses made by Roberts and his colleagues together with our own observations have shown beyond all reasonable doubt that the overall content of the wall of *Chlamydomonas reinhardii* is glycoprotein in nature. The wall material has now been examined for phosphorus or lipid, but it seems unlikely from current work that there can be more than traces of either. The wall material – which from electrophoretic evidence contains about six protein components – has a high carbohydrate moiety. The protein is also notable for its amino acid content; the molar ratios of proline and hydroxyproline (in terms of histidine = 1) are 7

and 14 respectively. There are also substantial amounts of the hydroxy amino acids serine and threonine which are known to be sugar acceptors. The sugars present in decreasing order are arabinose, galactose, mannose, xylose, glucose and a trace of glucosamine.

The sugar analysis of the wild-type wall shows that arabinose and galactose are the most abundant, followed by mannose, glucose and xylose in that order. Only a trace of glucosamine was found.

It will not have passed unnoticed that Chlamydomonas is a valuable eukaryotic organism with well charted genetics. Already its flagella, its cell wall, chloroplasts and mitochondria have been extensively investigated and offers a considerable scope for further study.

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