Origin and Continuity of Cell Organelles

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Assembly, Continuity, and Exchanges in Certain Cytoplasmic Membrane Systems

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Basic to the definition of a cell is a membrane separating the activities within it from the surrounding environment. In even the simplest organisms, the plasma membrane, or plasmalemma, bounding the cellular mass is characterized by definable structure and distinctive physiological properties. The structure, composition, and properties of membranes have all been subjects of extensive, interrelated studies. This work will deal with questions about their assembly, continuity, and exchange. Only brief consideration will be given to structure, composition, and properties to provide a basic understanding that will lend coherence to the other questions.

The eukaryotic cell is characterized by a substantial amount of intracellular compartmentalization by membranes. The membranes function both as selective barriers and reaction surfaces. That some sort of membrane transfer or flow exists in such cells has been apparent for some time (Schnepf, 1969). The focus here will be on exchanges among cellular membranes and the types of modifications that must occur concurrently with such exchanges. Partly because of lack of sufficient knowledge about the fundamental structure of membranes and its relation to specific functioning and about the precise molecular components of membranes, many of the basic questions cannot be answered with certainty. However, some observations can be made with assurance and the feasibility of some suggestions concerning interrelationships can be evaluated.

I. The Nature of the Membrane

Much knowledge about the physiological properties of biological membranes was accumulated in early studies of permeability, which also sparked a series of interpretations of the structure of membranes (Gortner and Grendel, 1925, and others; see Danielli, 1967). Ultrastructural studies of recent years have accepted as one of their major challenges the testing of these interpretations and contributing to an understanding of the structure of the membrane. Although subject to certain limitations in meeting these challenges, these studies have strengthened the view that there are common elements of organization among all biological membranes. Combined with cytochemical and radioautographic investigations, they have served to broaden the concept of a membrane from that of essentially a permeability barrier to one which additionally includes its being a surface on which many reactions are carried out (see Dalton and Haguenau, 1968). Further, they have demonstrated that many membranes, or at least many membrane-associated materials, are characterized by specificity factors (see Davis and Warren, 1967; Sjöstrand, 1968). This broadened concept of a biological membrane holds that many of the functional

characteristics of the cell are associated with one or another of its membranes. Still more recent direct chemical analyses of purified membrane fractions (MADDY, 1966, 1967; BENEDETTI and EMMELOT, 1968; COOK, 1968a, b; MALHOTRA and VAN HARREVELD, 1968; ROTHFIELD and FINKELSTEIN, 1968; ROUSER et al., 1968; KORN, 1969) confirm postulates from the earlier work that the membranes are composed largely of lipids and proteins. They also make it evident that carbohydrates are frequent constituents. Information about the specific constituents of membranes associated with particular functions may well lead to much more satisfactory understanding of the differentiation of membranes and the transfers and transformations they undergo.

The advancement of techniques to the point where fractions of different cellular membranes can be separated and analysed has indicated that, despite the more or less common images, the makeup of membranes differs from species to species, from tissue to tissue, and from one cellular organelle to another in terms of the proportions of the various components and of the particular molecules in each class (Table 1, from KORN, 1969). As will be seen there are also clear indications of area differences in given membranes. Functional attributes of membranes relate to their specific molecular composition and architecture. It follows that if membrane transfer occurs, since it involves functional alteration, it must also be accompanied by changes in composition, structure, and specificity factors.

Table 1. *Protein and lipid content of membranes. From* KORN (1969). *The abbreviations are:* Cer, *cerebrosides;* DPG, *diphosphatidylglycerol;* GalDG, *galactosyldiglyceride;* PA, *phosphatidic acid;* PC, *phosphatidylcholine;* PE, *phosphatidylethanolamine;* PGaa, *amino acyl esters of phosphatidylglycerol;* Plas, *plasmalogen;* SL, *sulfolipid;* Sph, *sphingomyelin. For further explanation of sources, see* KORN (1969)

Membrane	Protein/Lipid	Cholesterol/Polar Lipid	Major Polar Lipids
	wt/wt	mole/mole	
Myelin	0.25	0.7—1.2	Cer, PE, PC
Plasma membranes			
Liver cell	1.0—1.4	0.3—0.5	PC, PE, PS, Sph
Ehrlich ascites	2.2		
Intestinal villi	4.6	0.5—1.2	
Erythrocyte ghost	1.5—4.0	0.9—1.0	Sph, PE, PC, PS
Endoplasmic reticulum	0.7—1.2	0.03—0.08	PC, PE, Sph
Mitochondrion			DPG, PC, PE, Plas
Outer membrane	1.2	0.03—0.09	
Inner membrane	3.6	0.02—0.04	
Retinal rods	1.5	0.13	PC, PE, PS
Chloroplast lamellae	0.8	0	GalDG, SL, PS
Bacteria			
Gram-positive	2.0—4.0	0	DPG, PG, PE, PGaa
Gram-negative		0	PE, PG, DPG, PA
PPLO	2.3	0	
Halophilic	1.8	0	Ether analogue PGP

The membrane images revealed by early electron microscopy studies (FINEAN, 1953; SJÖSTRAND and RHODIN, 1953; SJÖSTRAND and HANZON, 1954a; ROBERTSON, 1955) led ROBERTSON (1959) to postulate a fundamental structure which he termed

the "unit membrane". The unit membrane concept relates, in part, to the paucimolecular theory of membrane structure set forth by DAVSON and DANIELLI (see DAVSON and DANIELLI, 1943; see also DANIELLI, 1967). This theory was based largely on permeability studies and the properties of membranes revealed by them. The concept also relates, in part, to X-ray diffraction and other studies of the myelin sheath, many of them by SCHMITT and his co-workers (see ROBERTSON, 1959). Both the theoretical base of the unit membrane concept and the applicability of the results of the myelin studies to other cellular membranes have been questioned. Many investigators have called attention to the fact that uniform lipid bilayer structure does not adequately explain certain membrane phenomena, including the degree of differentiation that must exist between various cellular membranes or even spatially and/or ontogenetically within a given cellular membrane (see LUCY, 1968a). SJÖSTRAND (1968) has presented a summary of serious qualifications about the use of the myelin sheath as a model membrane. These questions have led several investigators to seek alternative interpretations of cellular membrane structure. Before considering these alternatives, a further description of the unit membrane concept is in order. This concept holds that cellular membranes are composed of double lipid layers and associated nonlipid material. (The concept of the unit membrane initially had reference to cell surface membrane. SJÖSTRAND had pointed out [SJÖSTRAND, 1953a, b] that there might be common principles of organization in both surface and intracytoplasmic membranes, although he also observed differences [SJÖSTRAND, 1956].)

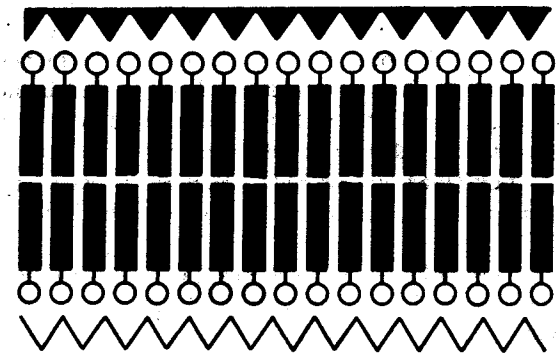


Fig. 1. Highly schematic diagram of the unit membrane pattern. The lipid polar groups are indicated by circles; the nonpolar carbon chains by bars and nonlipid monolayers by zig-zag lines. The upper zig-zag lines are partially blocked in to indicate that there is a chemical asymmetry in the membrane surface, the outside being chemically different from the inside in some important way. This may be due to the presence of a high concentration of mucopolysaccharide or mucoprotein on the outside surface with a dominance of protein on the inside. (This diagram is based upon ROBERTSON's interpretation of the myelin sheath which is an outer bounding membrane.) Diagram and legend from ROBERTSON (1965)

A recent (1965) version of ROBERTSON's diagram of the unit membrane is shown in Fig. 1. This version differs from the original diagram in that it takes into account the frequently emphasized asymmetry of cellular membranes and proposes that the nonlipid layer on one side may be protein and that on the opposite side partly polysaccharide.

Most of the alternatives to the unit membrane concept hold that the molecular components of membranes are arranged in subunits which are, in turn, associated to make up the membrane. The molecules of phospholipids in experimental phospholipid-water systems have been shown to be grouped in specific ways (Luzzati and Husson, 1962; Stoeckenius, 1962). Membrane breakdown studies have suggested that certain molecular components, perhaps including both phospholipids and proteins, are not degraded to the molecular level (Hokin, 1968). These observations

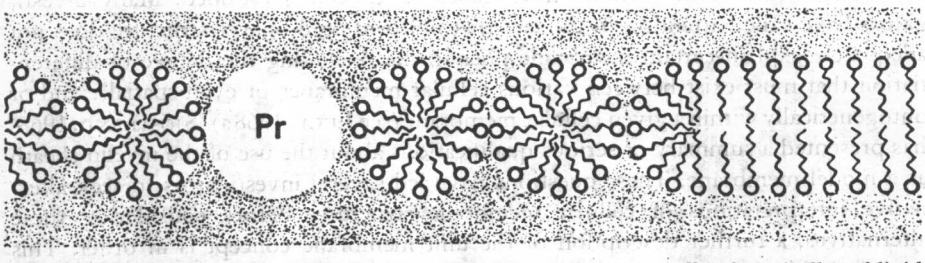


Fig. 2. Diagram of a cross-sectional view of a membrane in which globular micelles of lipid are in dynamic equilibrium with a bimolecular leaflet of lipid. A layer of protein and/or glycoprotein is shown on each side of the lipid layer. The structure of each lipid molecule is illutrated in a diagrammatic fashion: only a polar group (o) and a nonpolar moiety (—) are shown, and the lipid may be phospholipid or nonphospholipid. One globular micelle of lipid has been replaced by a globular protein molecule (pr) which may be a functional enzyme. Figure and legend from Lucy (1968b)

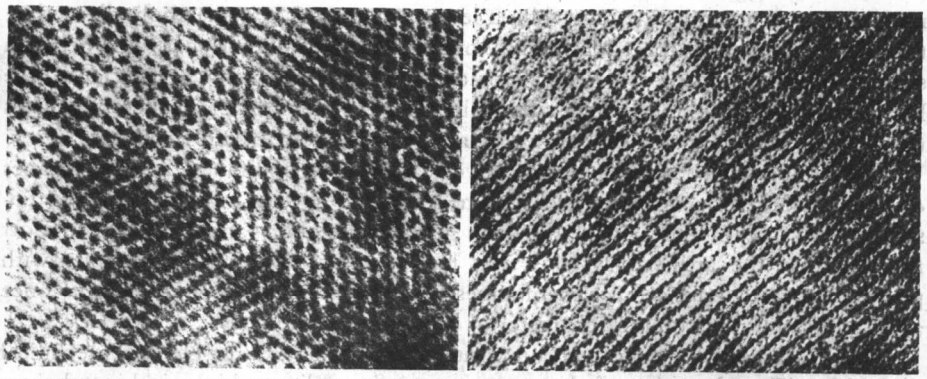
lend credence to the possibility of subunit structures in membranes. What appear to be subunits are detectable along the fracture planes of membranes prepared for electron microscopy studies by freeze etching (Branton, 1966). The subunit hypothesis is attractive for the possibilities it offers for explaining functional variation within membranes. Lucy (1964, 1968a, b) has suggested that membranes may be, in part, organized into subunits, and, in part, into molecular leaflets of the Danielli type (Fig. 2).

Some studies of phospholipid-water systems have shown different patterns of association of the phospholipid molecules and changes from one type of association to another as a result of modified conditions (Stoeckenius, 1962; see Fig. 3). Presumably these different patterns of association and perhaps others could exist within a cellular membrane (see Stoeckenius and Engelman, 1969). The obvious functional differentiation in membranes may well relate to differences in structure in different parts of the membrane.

For further considerations of alternatives to the unit membrane concept, the reader is referred to Korn (1966), Lucy and Glauert (1967), Glauert and Lucy (1968), Chapman (1968), Sjöstrand (1969) and Stoeckenius and Engelman (1969). Various models representing hypothetical interpretations of subunits of different character have now been adduced by a number of investigators.

Membrane dynamics is a complex subject which must deal with synthesis of membrane components, assembly, changes in area organization, continuous turnover of molecular constituents, and transfer. Some of the considerations have been summarized by Siekevitz et al. (1967). No attempt will be made here to deal with

questions of synthesis, but brief mention must be made of the turnover of membrane constituents because this represents transfer at one level. OMURA, SIEKEVITZ, and PALADE (1968) have shown continuing turnover of membrane proteins and lipids and demonstrated differences in the rates of this turnover between the proteins and the lipids and between different categories of lipids. HOKIN (1968) has reviewed evidence from a series of secretion stimulation studies which suggests that in membrane breakdown certain components are broken down to their building blocks



a b

Fig. 3a and b. Electronmicrographs of phospholipids in water: (a) hexagonal phase; (b) lamellar phase. From STOECKENIUS (1962). Magnification (a) 532,000 ×; (b) 430,000 ×

whereas others are not. His results have led him to suggest that in the recirculation of membrane components, some may participate at the molecular level whereas others participate as part of subunits containing several phospholipids and proteins (see Fig. 19). This turnover, whether partly at the subunit level or not, must be considered in reference to the changing composition and specific functional state of the membrane. LUZZATI (1968) and LUZZATI et al. (1969) have postulated that any given portion of a membrane may be in a transient state. Such transient states relate to changes in functional characteristics of membranes, including those attendant upon transfer of membrane segments.

II. The Assembly of Membranes

A discussion of membrane assembly at this time must revolve around an unanswered question: are all membranes developed from already existing membrane or may at least some of them be formed by the assembling of components under the influence of conditions existing at a particular site in the cell?

There are no critical data to guide a choice of alternatives nor grounds for supposing that one or the other always pertains. Whatever the method of membrane assembly, there must be pools in the cell with which the molecular components of the membranes may be exchanged.

LUZZATI and HUSSON (1962) and LUZZATI et al. (1969) have developed a series of interpretations of the molecular arrangements in several different phases of dispersed phospholipids of the sort commonly found in membrane systems. STOECKENIUS (1962) was able to take electron micrographs that showed good agreement

with the X-ray diffraction data concerning both hexagonal and lamellar phases of phospholipids and by altering conditions was able to bring about formation of the lamellar phase. MADDY (1967) has been able to fractionate membranes, separate their lipid and protein components, and reconstitute them structurally. Such *in vitro* experiments as these demonstrate that membrane-like arrangements of phospholipids and proteins can be formed in the absence of pre-existing membranes, but they do not prove that this is how membranes are assembled in the cell. MADDY's work (1967, 1969) suggests that much of the protein associated with the membrane lipids may have a structural function while a lesser amount of it is metabolically active, though he emphasizes the undesirability of attempting to draw a sharp line between structural and other protein. He concludes, in agreement with earlier ideas (see DANIELLI, 1967), that the protein adds stability to the membrane and then postulates that the mutability of membranes may relate primarily to the lipid fraction.

The comparability and differences between artificial "membranes" and membranes of living cells has been considered at some length by several investigators (see TOSTESON, 1969; TRIA and SCANU, 1969). In general, the observations tend to support the idea that the artificial systems may have some structural comparability with portions of functional cellular membranes. But the differences are numerous, once again indicating the complex character of biological membranes.

Direct studies of membrane composition and structure are beset by technical difficulties. The adaptation of electron microscopic studies to biological membranes brought the hope that some of the questions could be resolved by visualization of membrane structure. However, none of the techniques available for preparing cells for study is without the possibility of greatly modifying structure. Nonetheless it is possible to add to the earlier knowledge of the composition and general properties of membranes some observations concerning the nature of the differentiations involved and the changes associated with changes in cellular activity including various transfers and transformations.

III. The Growth and Transfer of Membranes

The movement of membrane components in the cell is readily detectable microscopically only in terms of organized membranes. There are detectable transfers of organized membranes that link together the nuclear envelope, the endoplasmic reticulum, the Golgi apparatus, the plasma membrane, the lysosomal system, and perhaps some other vesicles and vacuoles. This provides the rationale for considering these membranes part of an exchange system. There is little evidence that membranes of the plastids and the mitochondria are linked to this system at this level. Hence, they, and some other membranes about which evidence is not adequate, will be omitted from consideration here. It must be recognized, however, that visible transfers of membrane must constitute only a part of the total movement of membrane components and that exchanges between all of them and cellular pools supported by synthesis must occur.

A. The Nuclear Envelope

In all eukaryotic cells the nucleus is separated from the cytoplasm by a double-membrane envelope. This double-membrane structure encloses the so-called perinuclear space in which certain enzymes have been demonstrated cytochemically and

a number of reactions presumably are carried out. This envelope is characterized by pores (Fig. 4). Some materials pass between nucleus and cytoplasm through the pores but materials pass through the membranes of the envelope as well. The relative

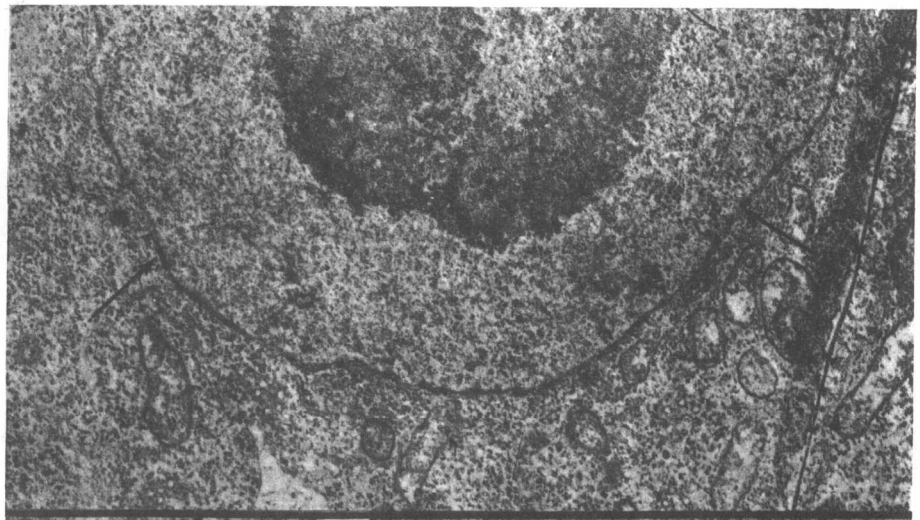


Fig. 4

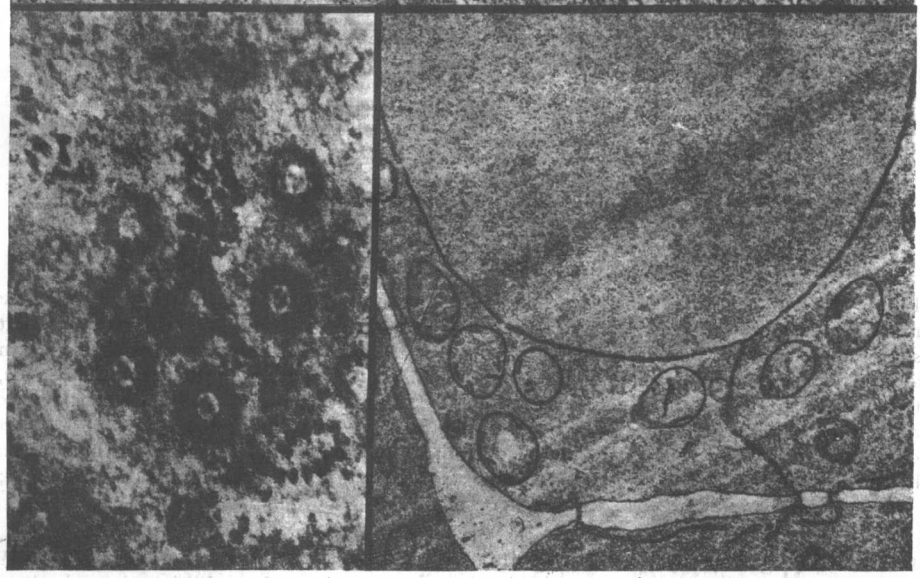


Fig. 5

a b

Fig. 4. Pores (see arrows) in a nuclear envelope demonstrated by treatment with $Zn(MnO_4)_2$. *Zea mays*. By H. H. MOLLENHAUER. Magnification 11,500 ×

Fig. 5a. Surface view of nuclear envelope showing pores and attached ribosomes. *Zea mays*. By MARIANNE DAUWALDER. Magnification 80,000 ×

Fig. 5b. Micrograph showing continuity of nuclear envelope with endoplasmic reticulum. Permanganate fixation fails to show ribosomes. *Zea mays*. By H. H. MOLLENHAUER. Magnification 9,500 ×

amounts of the nuclear surface occupied by pores and apparently uninterrupted membrane differ from one species to another (Franke, 1966, 1967) and during development (Franke and Scheer, 1970).

The outer membrane of the nuclear envelope is frequently demonstrated to be ribosome-studded and in this characteristic at least comparable to the rough endoplasmic

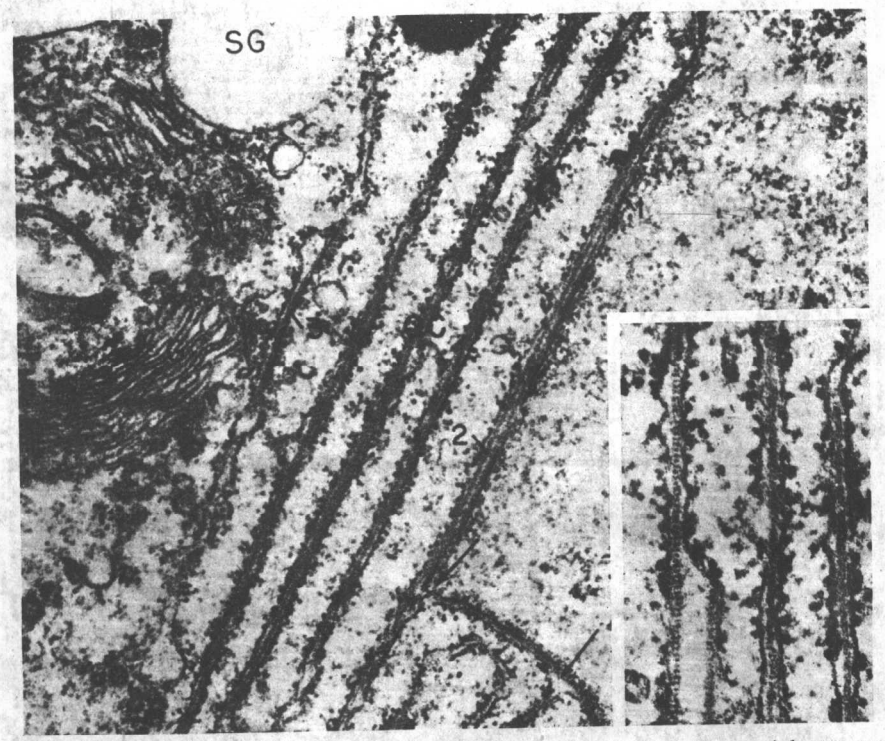


Fig. 6. Part of a young Paneth cell, showing two communications (arrows) between the nuclear envelope and oriented cisternae (OC) containing material. The cisterna to the left shows a fine line (arrow 3) in its cavity. The cavities in the remaining cisternae are filled with a rather dense material, which in places shows transverse striations. In the perinuclear space, two bands of material are seen (arrows 1 and 2) which continue into a cisterna of the reticulum. Golgi region (G). Secretory granule (SG). Insert: Higher magnification of a part of three oriented cisternae, showing the periodicity of the material contained in the cisterna to the left. Figure and legend from Behnke and Moe (1964). Magnification 37,500 ×; Insert: 66,300 ×

reticulum (Fig. 5a). Because of this and the fact that there is often direct continuity between the perinuclear space and the lumen of the rough endoplasmic reticulum (Fig. 5b), it seems in order to look upon these as different specializations of one membrane system (see Watson, 1955). Not only have membrane continuities been demonstrated, but identifiable contents of the lumens have sometimes been seen to have a common pattern (Behnke and Moe, 1964; Fig. 6). Observations of this sort and evidence that under certain conditions the nuclear envelope and the endoplasmic reticulum are sequentially interconnected and then separate have led to the postulate that the endoplasmic reticulum is a derivative of the nuclear envelope (see Porter,

1961; PARKS, 1962; BEHNKE and MOE, 1964). While interconnections often appear to be transitory they are consistently seen in some organisms. Nuclear envelope extensions sometimes envelop other organelles, as in the case of the chloroplasts (GIBBS, 1962; BOUCK, 1962, 1965).

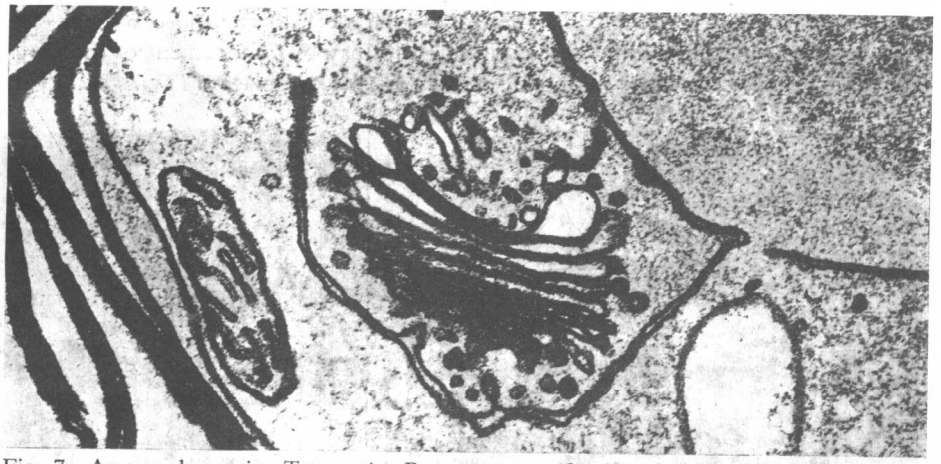


Fig. 7. An amplexus in *Tetracystis*. Permanganate fixation fails to show ribosomes. By H. J. ARNOTT. Magnification 40,000 ×

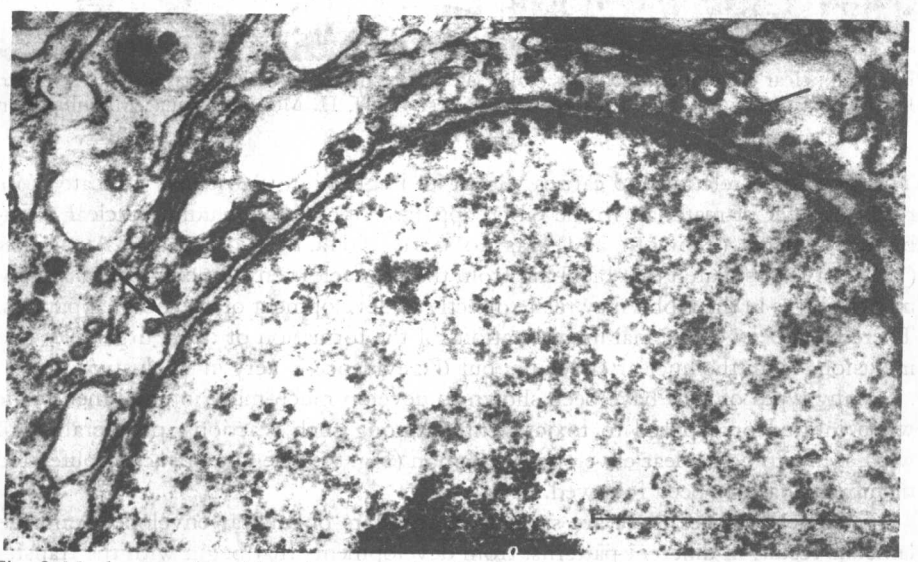


Fig. 8. Nuclear envelope blebs (see arrows) in the vicinity of the Golgi apparatus. *Botrydium*. From FALK, personal communication. Magnification 41,000 ×

What LANG (1963) has termed "amplexi" are extensions from the nuclear envelope to the immediate vicinity of the Golgi apparatus. These extensions frequently show blebs on the profile adjacent to the proximal face of the apparatus (see below; Fig. 7). In many instances there is actual separation of membrane from the nuclear envelope itself; small vesicles formed by blebbing appear to transfer perinuclear