Cytologia **45**: 453–466, 1980

The Cell as a Membranous Network under Microscope

Suad Artvinli

Department of Biochemistry, Hacettepe University,
School of Medicine, Ankara, Turkey

Received November 18, 1978

An eukaryotic cell has been visualized as a structure walled of from the outside by a surface membrane. Inside this membranous wall there are some membranous organelles separated by a medium called the cytoplasm. Until recent years, biochemical findings have also been agreed with this morphological finding as that they metabolically compartmentalized the cell with specific enzymes of substructures or organelles.

The compartmentalized cell, however, has raised an important question that how a hormone can control cell metabolism by binding to a receptor on the plasma membrane unless reaching substructures which contain related enzymes. To solve this problem, some messenger or mediator molecules operating between the plasma membrane and the other membranous substructures of the cell have been proposed.

On the other hand, the literature involves some data which emphasize the ability of the membranous systems of the cell to control cell metabolism. For example, molecules which react with phospholipids or glycosyl residues of membranes, such as phospholipases and lectins could modulate cell metabolism (Rodbell 1966, Czech and Lynn 1973).

An attempt has therefore been made to see whether the membrane systems of the cell or the cell itself has a different structure, which can explain the control of cell metabolism by the membrane alone from the outside of the cell.

It has been observed that the cell is not a compartmentalized structure, it shows a labyrinth structure formed by a united membrane or a biomolecular network.

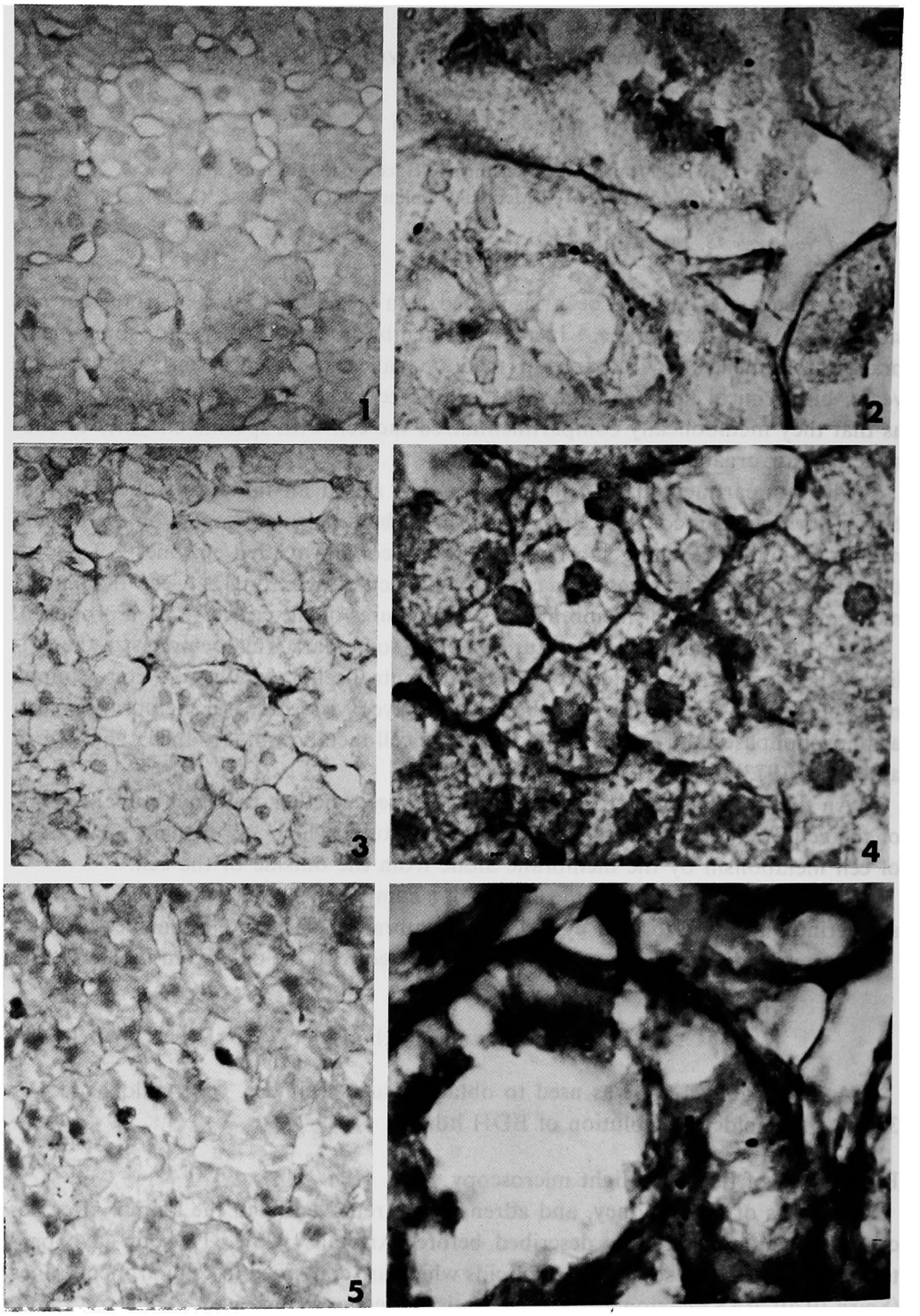
The existence of such a cell has been discussed with the data from the literature.

Materials and methods

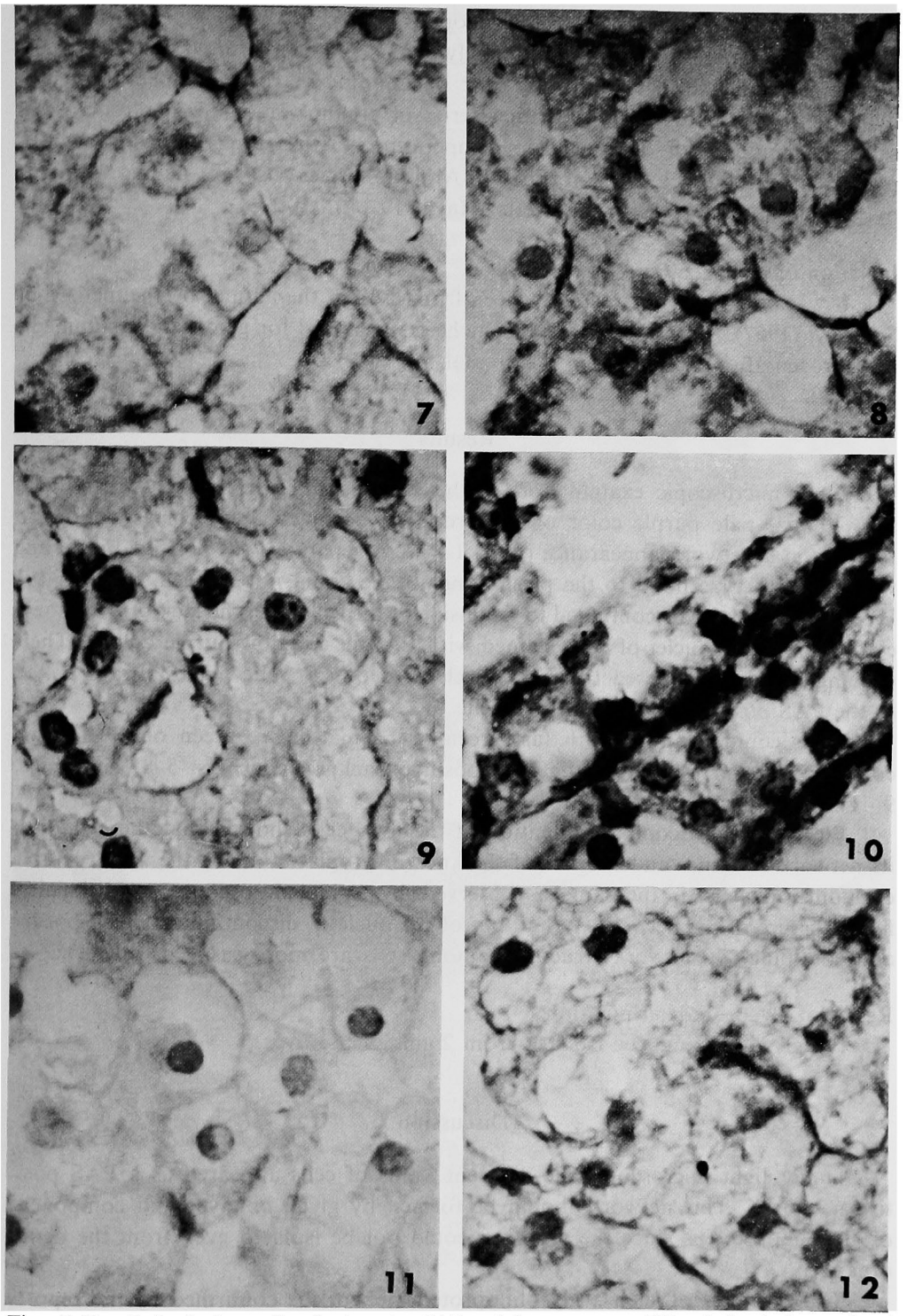
A fasted guinea pig was used to obtain tissue specimens. Formaldehyde was 37–40% formaldehyde solution of BDH ltd.

Preparation of tissues to light microscopy

Samples of liver, kidney, and adrenal were removed from the animal after decapitation and prepared as described before (Artvinli 1975). To stain glycoproteins, glycolipids and some phospholipids which are contained in cellular membranes (Spiro 1970), PAS reaction was applied to tissue sections (Barka and Anderson 1965). Fast green and haematoxylin were used to stain proteins and nucleoproteins.



Figs. 1–6. 1, liver cells stained with PAS. ×300. 2, tubuli cells of kidney stained with PAS. ×750. 3, fasciculata cells of adrenal stained with PAS. ×300. 4, fasculata cells of adrenal stained with PAS. ×750. 5, liver cells stained with PAS and fast green. ×300. 6, tubuli cells of kidney. PAS and fast green. ×750.



Figs. 7–12. 7, fasciculata cells of adrenal. PAS and fast green. ×750. 8, fasciculata cells of adrenal. PAS and fast green. ×750. 9, liver cells stained with PAS and haematoxylin. ×750. 10, tubuli cells of kidney. PAS and haematoxylin. ×750. 11, fasciculata cells of adrenal. PAS and haematoxylin. ×750. 12, glomerulosa cells of adrenal. PAS and haematoxylin. ×750.

Preparation of tissue to electron microscopy

A tissue block of liver, approximately $3 \times 3 \times 3$ mm in size, was fixed in bicarbonate formaldehyde (Artvinli 1975) for one minute at room temperature. Dehydration was performed in a graded series of ethanol prepared in physiological saline, beginning with 20%, at room temperature. After transitional solvent propylene oxide, the block was embedded in Araldite. Sections were stained with uranyl acetate and Reynold's lead citrate (Hayat 1970a).

Isolation of glycogen from liver

It was attempted to isolate glycogen from liver of the animal by the method of Somogyi (1957). The tissue sample which was analyzed for glycogen was immediately frozen after excising from the animal.

Results

Light microscopic examination of the sections of liver, kidney and adrenal showed that pale purple color of PAS reaction occupied areas corresponding to the cells in a network appearance (Figs. 1–4). A separate PAS positive borderline which would correspond to the plasma membrane could not be seen. Rather the PAS positive materials continued to the inside of the cells with the lack of the known cytoplasm. The nuclei of cells stained with PAS in a yellow-purple color. Their staining intensities differed from one nucleus to other. The nuclei always linked to the PAS positive network in each cell.

After counterstaining with fast green or haematoxylin, green or blue-purple colors were visualized upon the PAS positive background (Figs. 5–8) and (Figs. 9–12).

Electron microscopic examination of the liver cells showed that the cell, with its network structure and absence of the known cytoplasm and of the substructures excluding nucleus, is the same as observed by light microscope. Additionally, fine structure of the network, in and out of the nucleus, discontinuity of cell border and continuity of network structures among cells are more easily distinguishable.

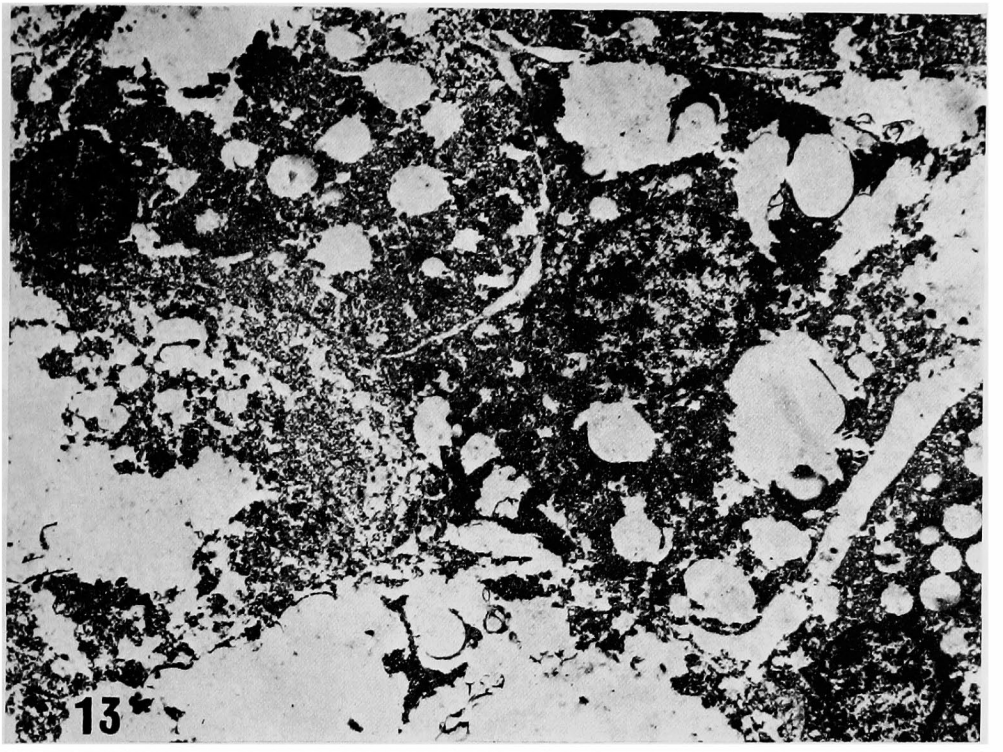
Result of glycogen determination

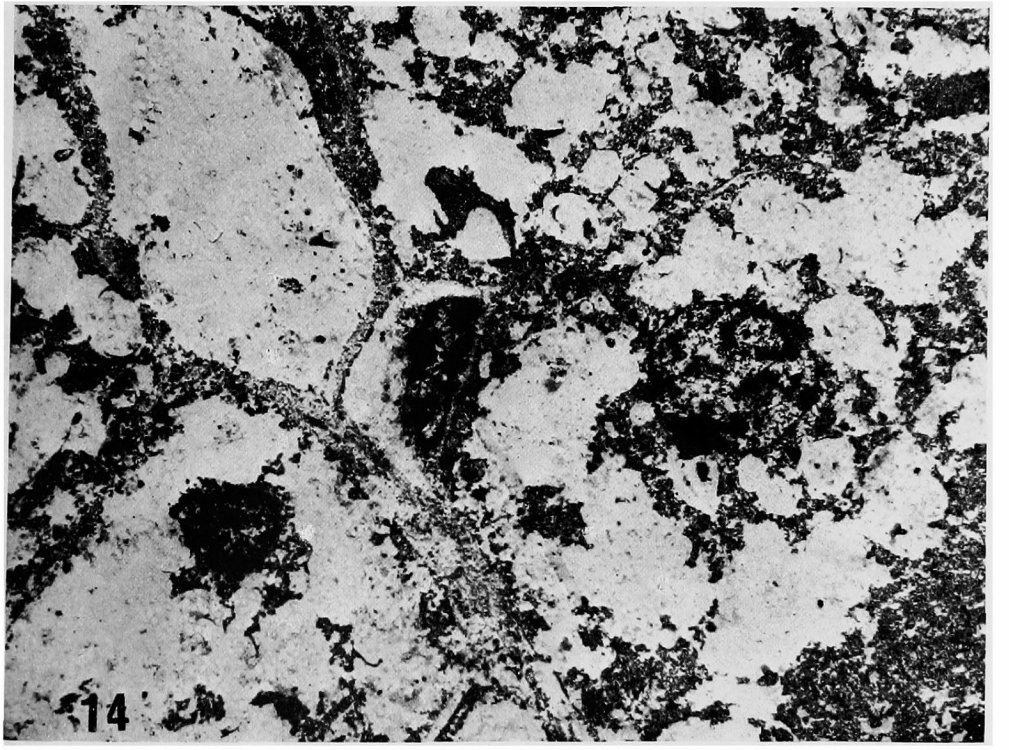
No glycogen could be isolated from 2 gms of the liver.

Discussion

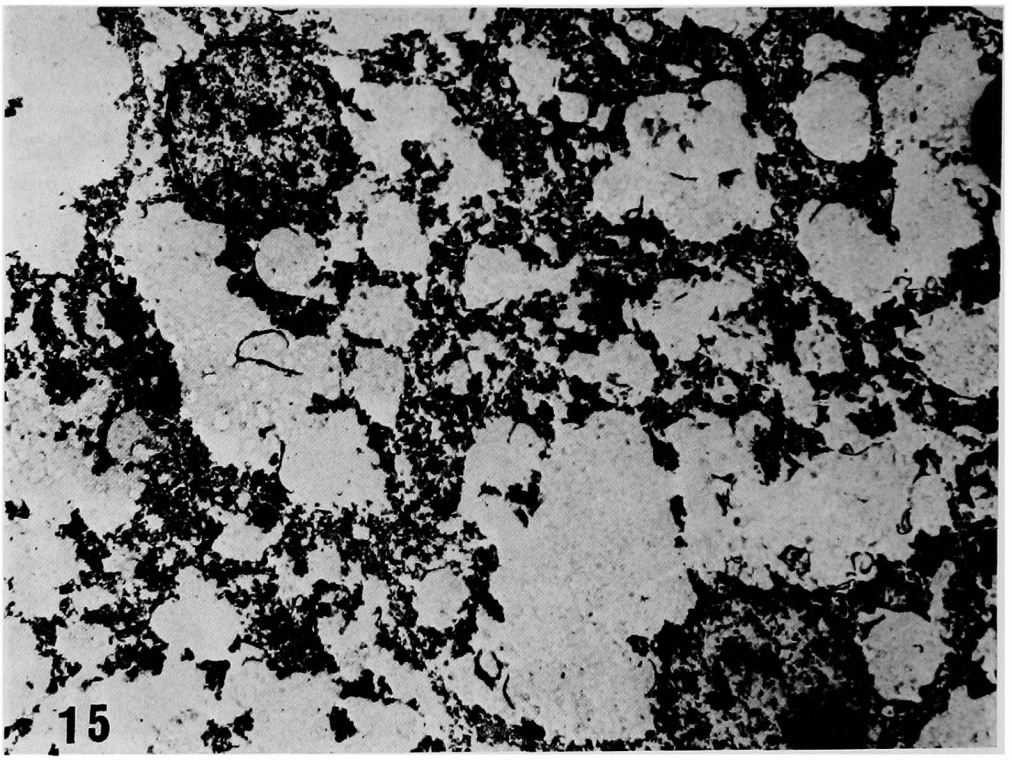
Under light microscope, the different kinds of cells appeared as a PAS positive network. This network should be formed by glyco or glycolipid component of glycolipoproteins since the glycogen could not be isolated even from the depot organ.

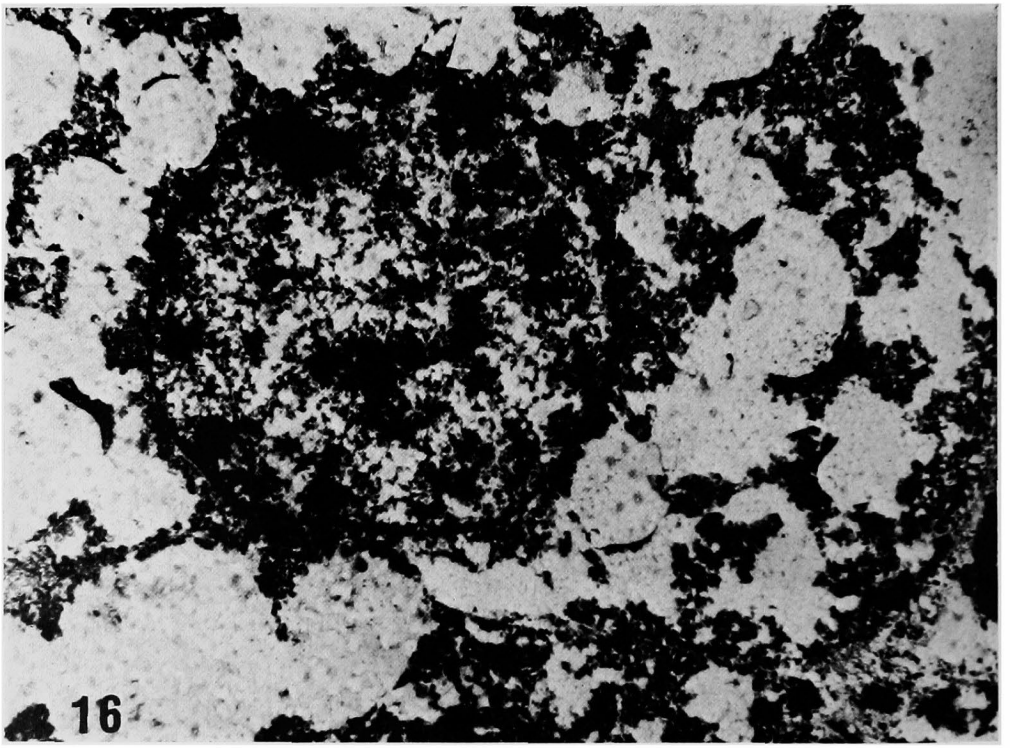
The existence of such a glycolipoprotein network is confirmed by the reports showing the transport of synthesized glycoproteins from the Golgi region to the surface membrane (Bennett *et al.* 1974), and of phospholipids from the same region to the all of membranous substructures (Dawson 1973). Because these transport





Figs. 13–14. 13, liver cells prepared as in the text and stained with lead citrate and uranyl acetate. 14, liver cells prepared as in the text and stained with lead citrate and uranyl acetate.





Figs. 15–16. 15, liver cells prepared as in the text and stained with lead citrate and uranyl acetate. 16, liver cells prepared as in the text and stained with lead citrate and uranyl acetate.

or exchange phenomena would require the existence of glycolipoprotein connections between subcellular structures or the absence of a cytoplasm separating subcellular structures, which correspond to the cell visualized here as a network.

Considering that the visualized cell has a glycolipoprotein network as the only structure, but does not have the known cytoplasm, cellular structural materials even those of which belong to the cytoplasm should be glyco or glycolipoproteins, but not unconjugated proteins. The report that shows a cytoplasmic structural material, cytoplasmic aspartate transaminase, is a glycoprotein (Denisova and Polyanovsky 1973) is therefore consistent with the visualized network.

In addition to the PAS positive network, the nuclei of cells contained PAS positive material. This also is not an artifact since there are some differences between staining intensities and since hexosamines, hexoses, and sialic acids could be isolated from the nuclear matrix (Berezney and Coffey 1975) and phospholipids from chromosomes (Tata *et al.* 1972).

Consequently, the existence of glycolipid or glycolipoprotein network which links the innermost part of the cell to its outermost part by occupying cytoplasmic and nuclear area is confirmed by the above mentioned data. Since a number of cellular membranes have been shown to be glycoproteins or glycolipoproteins it is possible to say that the cell is a membranous network.

The network appearance of the cell which visualized by PAS retained in the cells counterstained with fast green or haematoxylin. This proves that the network visualized by PAS reaction is in fact glyco or glycolipid component of glycolipoproteins, in other words, proteins or nucleoproteins of the cell are bound to a glycolipid network. Since the bulk of cellular proteins is enzymes one would expected that the enzymes of the cell are lipo, glyco or glycolipoproteins with respect to the above histochemical interpretation. In fact the cumulating data in the literature reveal the existence of such kind of enzymes. For example, mitochondrial hexokinase has been reported as a soluble lipoprotein (Craven and Basford 1972). Many other glycoprotein or glycolipoprotein enzymes have been defined (Wallace *et al.* 1973, Plapp and Cole 1966, Pazur *et al.* 1970, Bon and Rieger 1975, Forstner 1971, Evans *et al.* 1973, Starnes and Behal 1974, Norden and O'Brien 1974, Odds and Hierholzer 1973, Stepan and Ferwerda 1973, Kyte 1972).

The network structure of the cell retained under electron microscope. A discontinue border, almost undistinguishable and unseparated from neighbour cell, and a labyrinth structure reaching to the nucleus are formed by the biomolecular network. Although such appearance of the cell was not ordinary, it could be predicted and also confirmed by reviewing of the literature.

The literature emphasized the existence of such a cell that it is a changeable wound or network in which the plasma membrane is formed from the outermost parts of the network and the cytoplasm with all of the organelles is formed from the inner parts of the network.

Some data which emphasize such a wound or network membrane come from morphological field. For example, the cell can direct its own organelles to its periphery and eject them with or without their renewal. McKanna (1973) described this phenomenon for contractile vacuoles. Other similar organelle translocations

have been described such as mitochondrial elimination (Jurand and Yamada 1967, Simpson and Kling 1968), nuclear elimination (Skutelsky and Danon 1967, Campbell 1968), and release of secretory granules (Lagunoff 1973). Fadei (1963) revealed that nuclear transfer between cells takes place, involving nuclear-mitochondrial extrusions with successive transfer to another cell. The cell is also capable of inserting one or more of its organelles into another, whilst each of them is described as being a closed structure. For example mitochondria have been shown to exist in the nucleus (Klug 1966, Matsuyama and Suzuki 1972). Typical cytoplasmic organelles have been detected in the nucleus as nuclear inclusions (Nakayama and Nickerson 1972). The cell can lose some of its organelles with or without the formation of new organelles. Transformation of mitochondria into lipid granules (Agrell 1968) and into vacuoles (Beaulaton 1968) has been described. In nerve cells, rapid depletion of most nuclear and cytoplasmic organelles, with a marked increase in the number of neurofilaments has been observed (Torvik 1972). Decrease of endoplasmic reticulum and breakdown of nuclear membrane typically occur at the onset of cell mitosis (Robbins and Gonatas 1964). The cell can translocate its organelles through the cytoplasm. Such cytoplasmic organelle movements caused to the polarized appearance of lymphocytes (Biberfeld 1971), and oocytes (Beams and Sekhon 1966, Henzen 1966). Such movements have also been defined for endoplasmic reticulum (Dougherty and Lee 1967), and for nucleus (Rose 1966).

A biochemical finding agreed with this new concept of the cell. Soll and Sonneborn (1971) showed that the cell could become completely different with regard to the type of organelles it contained, without protein synthesis. Since the synthesis of a new organelle requires protein synthesis, the differentiation of these cells must involve a change in organization of the preformed wound or network membranous structure.

In accordance with the visualized network, some biochemical findings suggest that the mitochondrion is a membranous fragment but not a separate membranous organelle. It is in continuity with microsomal membranes in the intact cell. Protein contained in a mitochondrial pellet decreased after each centrifugation up to 26%, which indicates mitochondrial losses during centrifugation (Romani *et al.* 1974). Another relevant observation is that dumb-bell shaped mitochondria could be detected on the fourth day of isolation (Romani *et al.* 1974). Considering the known spheric structure of the mitochondrion, an explanation to these observations is difficult. A mitochondrial enzyme, glutamate dehydrogenate, has been shown to be synthesized in microsomes, and then translocated to mitochondria (Godinot and Lardy 1973). This report suggests that microsomal and mitochondrial membranes must be in structural integrity, which accounts for the observed functional integrity. Moldeus *et al.* (1973) also suggested the structural integrity between these membranes by interpreting their biochemical and morphological findings. A histological finding that is globular mitochondrial forms may be produced by excessive chromation (McManus and Mowry 1964) further suggests that the mitochondrion is a round membranous fragment produced by tissue fixation as a spherical separate organelle.

Since the organelles of the cell have been specified with respect to the type of

enzymes they contained, the existence of network or wound membranous structure of the cell must require organelles with unspecified enzymes. The presence of such a condition was recently shown by DePierre and Ernster (1977) in their review.

The enzymes belonging to the same metabolic pathway, namely the urea cycle, have been detected partly in cytosol and partly in mitochondria (Lehninger 1975). This biochemical finding is compatible with the visualized network integrating the cell metabolically.

The heterogeneity of the mitochondria of liver and brain cells (Berger 1973, Wilson and Cascarano 1972, Neidle *et al.* 1969), and the existence of usual organelles in unusual forms and of unusual organelles are also supporting data to the existence of the network cell (Ishihara *et al.* 1973, Knese 1971, Di-Augustine 1974, Krzyzowska Gruca *et al.* 1971, Anteunis *et al.* 1966, Armstrong *et al.* 1973, Stephens and Easterbrook 1968, Knyihar 1971, Jorke 1970, LeBeux 1972).

Membranous linkages between organelles could be detected, which is necessary to form the network structure of the cell. Some of these observed linkages were between endoplasmic reticulum and mitochondria (Bowman 1967, Ghidoni and Thomas 1969, Ruby *et al.* 1969), between outer nuclear and outer mitochondrial membrane (Franke *et al.* 1973), and between various other membrane systems including the plasma membrane (Agrell 1966, Carlson and Ollerich 1969, Arnold 1967). The detection of some membranous structures in the surroundings of extruded nuclei also suggests the presence of membranous continuities between subcellular structures (Wise and Prescott 1973). Moreover differences between the order of sedimentation of organelles in cell homogenates and in intact cell, as shown by Zalokar (1969), are an indirect evidence of membranous integrity within the cell.

The literature also includes several reports, which shows that the plasma membrane is not continuous structure, rather is in continuity with inner cytoplasmic structures. Such a concept is also essential for understanding of the cell as a membranous wound or network. There are some reports suggesting the surface membrane as being a folded structure. For example, during the cell cycle some surface antigenic determinants, known as H-2 antigens, are lost and restored (Pasternak *et al.* 1971). The surface area of a cell has been shown to widen two times in a spreading state without reduction in the charge density of the cell surface (Nordling and Mayhew 1969). Lymphocytes which are spheric in shape could develope long processes on their surface membrane (Clarke *et al.* 1971). About 85% of the surface membrane receptors for concanavalin A in transformed cells are in cryptic form in normal cells (Inbar and Sachs 1969). Nevertheless, the microscopy which is a more direct method in detection of folds on the surface membrane revealed that the surface membrane is discontinuous and the folded image found in the above experiments is presumably due to the existence of membranous continuities with cytoplasmic membranous structures. For example, during migration of fibroblasts, the cell membrane has been shown to be interrupted with granular cytoplasm extrusions (Franceschini and Santoro 1966). Direct continuity of intercellular space with intracellular structures, namely cytoplasmic lacunae, lysosomes, and endoplasmic reticulum has been visualized (Searle 1972, Oledzka 1972,

Hönigsmann and Wolff 1973). Dan and Hagiwara (1967) showed that the surface membrane of sperm cell could be disrupted with opening of lysosome to the outside. Transient holes in the erythrocyte membrane during hypotonic hemolysis have been detected (Seeman 1967). Additionally, biochemical findings reveal that enzymes belonging to the inner membranous structures can migrate to the surface membrane. Davidova *et al.* (1968) described this situation for glycolytic enzymes. Treatment with CCl_4 enabled microsomal and inner mitochondrial membrane enzymes to be detected on the plasma membrane (Masuda *et al.* 1973, Kamath and Rubin 1974). These biochemical findings, together with the morphological findings visualizing cytoplasmic extrusions suggest that the plasma membrane is in fact the continuation of inner membranous structures.

This morphological study has verified that the cell is a membranous network as predicted and confirmed by reviewing of the literature. Moreover it should be noted that this is not the first time for the visualization of this network. Similar images have already been observed in different kinds of cells. However, such images have been interpreted as intracellular ice crystals because of their visualization in frozen, unfixed, undehydrated cells (Hayat 1970b).

What is the meaning of such a structure of the cell? The literature also involves the data which gave a solution to this question. There seems no doubt that this biomolecular network alone is responsible for the control of metabolism which is accomplished by energetic changes in its shape.

Summary

Up until now, it has been visualized that the membrane systems of an eukaryotic cell include a limiting plasma membrane and separated organelles in the cytoplasm. They all constitute the cell.

The presence of a wide variety of experimental results which suggest that a united membrane constitutes the cell has therefore necessitated this morphological study.

To visualize the cell, the new tissue preparation technique introduced by Artvinli (1975), and the stains reacting or combining with chemical groups contained in biomolecules of the membranes as well as of the cytoplasm have been used. Light and electron microscopic appearance of the cell, under these conditions, is similar to a network. The cell was therefore defined as a membranous network. Its structure can also be visualized as a biomolecular network.

The existence of such a united membrane or a biomolecular network seems to be true since it could be predicted and also confirmed by reviewing of the literature.

Such a structure of the cell links any part of the cell to its other parts, and can control metabolism through changes which occur in its shape, in other words, interdependent changes in conformations of biomolecules.

Acknowledgements

My thanks to Dr. Afet Özoran, Dr. Deniz Balta, and Dr. Esin Yalçin for their valuable helps in electron microscopy.

References

Agrell, I. P. S. 1966. Continuity of the membrane systems in the cells of imaginal discs. Z. Zellforsch. **72**: 22–29.

— 1968. Differentiation of the membrane system in the cells of imaginal discs. Z. Zellforsch. **88**: 365–369.

Anteunis, A., Fautrez-Firlefyn, N. and Fautrez, J. 1966. A regular tubulo-mitochondrial complex in the young oocyte of *Artemia salina*. J. Ultrastruct. Res. **15**: 122–130.

Armstrong, E. M., More, I. A. R., McSeveney, D. and Carty, M. 1973. The giant mitochondrion-endoplasmic reticulum unit of the human endometrial glandular cell. J. Anat. **116**: 375–383.

Arnold, J. M. 1967. Organellogenesis of the cephalopod iridophore: Cytomembranes in development. J. Ultrastruct. Res. **29**: 410–421.

Artvinli, S. 1975. Biochemical aspects of aldehyde fixation and a new formaldehyde fixative. Histochem. J. **7**: 435–450.

Barka, T. and Anderson, P. J. 1965. Histochemistry. pp. 69–74. Harper and Row, New York, Evanston and London.

Beams, H. W. and Sekhon, S. S. 1966. Electron microscope studies on the oocyte of the freshwater mussel (Anadonta), with special reference to the stalk and mechanism of yolk deposition. J. Morphol. **119**: 477–501.

Beaulaton, J. A. 1968. Ultrastructural changes of the secretory cells of the prothoracic gland of the silkworm during the last two larval stages I. The chondriom and its relationship to the smooth reticulum. J. Cell Biol. **39**: 501–525.

Bennett, G., Leblond, C. P. and Haddad, A. 1974. Migration of glycoprotein from the Golgi apparatus to the surface of various cell types as shown by radioautographie. J. Cell Biol. **60**: 258–284.

Berezney, R. and Coffey, D. S. 1975. The nuclear protein matrix: Isolation, structure and functions. In Advances in Enzyme Regulation (ed. G. Weber), pp. 63–99. Pergamon Press.

Berger, E. R. 1973. Two morphologically different mitochondrial populations in the rat hepatocyte as determined by quantitative three-dimensional electron microscopy. J. Ultrastruct. Res. **45**: 303–327.

Biberfeld, P. 1971. Uropod formation in phytohaemagglutinin stimulated lymphocytes. Exp. Cell Res. **66**: 433–445.

Bon, S. and Rieger, F. 1975. Interactions between lectins and electric eel acetylcholinesterase. FEBS lett. **53**: 282–285.

Bowman, R. W. 1967. Mitochondrial connections in canine myocardium. Tex. Rep. Biol. Med. **25**: 517–524.

Campbell, F. R. 1968. Nuclear elimination from the normoblast of fetal guinea pig liver as studied with electron microscopy and serial sectioning techniques. Anat. Rec. **160**: 539–553.

Carlson, E. C. and Ollerich, D. A. 1969. Intranuclear tubules in trophoblast III of rat and mouse chorioallantoic placenta. J. Ultrastruct. Res. **28**: 150–160.

Clarke, J. A., Salsbury, A. J. and Willoughby, D. A. 1971. Some scanning electron microscope observations on stimulated lymphocytes. J. Path. **104**: 115–118.

Craven, P. A. and Basford, R. E. 1972. Properties of the glucose-6-phosphate solubilized brain hexokinase. Evidence for a lipo-protein complex. Biochim. Biophys. Acta **255**: 620–630.

Czech, M. P. and Lynn, W. S. 1973. Stimulation of glucose metabolism by lectins in isolated white fat cells. Biochim. Biophys. Acta **297**: 368–377.

Dan, J. C. and Hagiwara, Y. 1967. Studies on the acrosome IX. Course of acrosome reaction in the starfish. J. Ultrastruct. Res. **18**: 562–579.

Davidova, S. Y., Shapot, V. S. and Solowjeva, A. A. 1968. Hexokinase activity and glycolytic capacity of plasma membranes of hepatomas. Biochim. Biophys. Acta. **158**: 303–305.

Dawson, R. M. C. 1973. The exchange of phospholipids between cell membranes. Sub-Cell. Biochem. **2**: 69–89.

Denisova, G. F. and Polyanovsky, O. L. 1973. Carbohydrate components of aspartate transaminase: multiple forms of the enzyme from pig heart cytosol. FEBS lett. **35**: 129–132.

DePierre, J. W. and Ernster, L. 1977. Enzyme topology of intracellular membranes. Ann. Rev. Biochem. **46**: 201–262.

DiAugustine, R. P. 1974. Lung concentric laminar organelle. Hydrolase activity and compositional analysis. J. Biol. Chem. **249**: 584–593.

Dougherty, W. J. and Lee, M. M. 1967. Light and electron microscope studies of smooth endoplasmic reticulum in dividing rat hepatic cells. J. Ultrastruct. Res. **19**: 200–220.

Ewans, W. H., Hood, D. O. and Gurd, J. W. 1973. Purification and properties of a mouse liver plasma membrane glycoprotein hydrolyzing nucleotide pyrophosphate and phosphodiester bonds. Biochem. J. **135**: 819–826.

Fadei, L. 1963. Intercellular nuclear transfer in a culture of irradiated tumor. Arch. Anat. Micr. Morphol. Exp. **52**: 615–623.

Forstner, G. G. 1971. Release of intestinal surface membrane glycoproteins associated with enzyme activity. Biochem. J. **121**: 781–789.

Franceschini, M. P. and Santoro, A. 1966. Remarks on the ultrastructure of migrating fibroblasts in culture. Bull. Soc. Ital. Biol. Sper. **42**: 1268–1269.

Franke, W. W., Zentgraf, H., Scheer, U. and Kartenbeck, J. 1973. Membrane linkages at the nuclear envelope. Cytobiology **7**: 89–100.

Ghidoni, J. J. and Thomas, H. 1969. Connection between a mitochondrion and endoplasmic reticulum in liver. Experientia **25**: 632–633.

Godinot, C. and Lardy, H. A. 1973. Biosynthesis of glutamate dehydrogenase in rat liver. Demonstration of its microsomal localization and hypothetical mechanism of transfer to mitochondria. Biochemistry **12**: 2051–2060.

Hayat, M. A. 1970a. Principles and Techniques of Electron Microscopy. Biological applications. Vol. I. Van Nostrand Reinhold, New York, Cincinnati, Toronto, London, Melbourne.

— 1970b. Principles and Techniques of Electron Microscopy. Biological applications. Vol. **2** pp. 14, 17, 19 and 84. Van Nostrand Reinhold, New York, Cincinnati, Toronto, London, London, and Melbourne.

Henzen, M. 1966. Cytological and microcytological studies on the ooplasmic segregation during the meiosis of the tubifex egg. Z. Zellforsch. **71**: 415–440.

Hönigsmann, H. and Wolff, K. 1973. Continuity of intercellular space and endoplasmic reticulum of keratinocytes. Exp. Cell Res. **80**: 191–209.

Inbar, M. and Sachs, L. 1969. Structural difference in sites on the surface membrane of normal and transformed cells. Nature (London) **223**: 710–712.

Ishihara, T., Tsukayama, C., Uchino, F. and Matsumoto, N. 1973. Intramitochondrial filatentous structures in human reticulum cells in the bone marrow. J. Electron Microscopy **22**: 39–44.

Jorke, D. 1970. The submicroscopic structure of some new cytoplasmic organelles in lymphocytes and lymphoid cells. Folia Haemat. **94**: 1–10.

Jurand, A. and Yamada, T. 1967. Elimination of mitochondria during wolffian lens regeneration. Exp. Cell Res. **46**: 636–638.

Kamath, S. A. and Rubin, E. 1974. Effects of carbon tetrachloride and phenobarbital on plasma membranes. Lab. Invest. **30**: 494–499.

Klug, H. 1966. Occurrence of mitochondria in the cell nucleus. Naturwissenschaften **53**: 339.

Knese, K. H. 1971. Transformation of endoplasmic reticulum of glycoprotein synthetizing skeletal cells. Acta Anat. **79**: 504–525.

Knyihar, E. 1971. Glial spheroids. ATPase active double walled structures in Bergmann glial

cells. Acta Biol. Acad. Sci. Hung. **22**: 435–441.

Krzyzowska Gruca, S., Gruca, S. and Vorbrodt, A. 1971. Cytochemical studies on the appearance of lysosomal enzymes in unusual structures of rat liver cells. Acta Histochem. **40**: 98–107.

Kyte, J. 1972. Properties of the two polypeptides of sodium and potassium dependent adenosine triphosphatase. J. Biol. Chem. **247**: 7642–7649.

Lagunoff, D. 1973. Membrane fusion during mast cell secretion. J. Cell Biol. **57**: 252–259.

LeBeux, Y. J. 1972. An ultrastructural study of a cytoplasmic filamentous body, termed nematosome, in the neurons of the rat and cat substantia nigra. The association of nematosomes with the other cytoplasmic organelles in the neuron. Z. Zellforsch. **133**: 289–325.

Lehninger, A. L. 1975. Biochemistry pp. 580. Worth, New York.

Masuda, Y., Kuchii, M., Yamamoto, H. and Murano, T. 1973. Studies on the function of cell membrane 5th report: Characterization of NADH-cytochrome C reductase of liver cell membrane in normal and CCl_4-treated rats. Jap. J. Pharmacol. **23**: 653–663.

Matsuyama, M. and Suzuki, H. 1972. Seizing mechanism and fate of intranuclear mitochondria. Experientia **28**: 1347–1348.

McKanna, J. A. 1973. Membrane recycling: vesiculation of the amoeba contractile vacuole at systole. Science **179**: 88–90.

McManus, J. F. A. and Mowry, R. W. 1964. Staining methods pp. 83. Harper and Row, and John Weatherhill, New York, Evanston, London.

Moldeus, P. W., Cha, Y. N., Cinti, D. L. and Schenkman, J. B. 1973. Hepatic organelle interaction 3. Mitochondrial modification of microsomal drug metabolism. J. Biol. Chem. **248**: 8574–8584.

Nakayama, I. and Nickerson, P. A. 1972. Intranuclear inclusions in mammotrophs of the female Mongolian gebril. Amer. J. Anat. **135**: 93–103.

Neidle, A., Van Den Berg, C. J. and Grynbaum, A. 1969. The heterogeneity of rat brain mitochondria. J. Neurochem. **16**: 225–234.

Norden, A. G. W. and O'Brien, J. S. 1974. Binding of human liver beta-galactosidases to plant lectins insolubilized on agarose. Biochem. Biophys. Res. Commun. **56**: 193–198.

Nordling, S. and Mayhew, E. 1966. Surface area changes and the electrophoretic mobility of tissue culture cells. Exp. Cell Res. **43**: 77–83.

Odds, F. C. and Hierholzer, J. C. 1973. Purification and properties of a glycoprotein acid phosphatase from *Candida albicans*. J. Bacteriol. **114**: 257–266.

Oledzka Slotwinska, H. 1972. Unusual shape of lysosomes and their connections with intercellular spaces in cholestatic rat liver cells. Acta Histochem. **42**: 168–177.

Pasternak, C. A., Warmsley, A. M. H. and Thomas, D. B. 1971. Structural alterations in the surface membrane during the cell cycle. J. Cell Biol. **50**: 562–564.

Pazur, J. H., Knull, H. R. and Simpson, D. L. 1970. Glycoenzymes: A note on the role for the carbohydrate moieties. Biochem. Biophys. Res. Commun. **40**: 110–115.

Plapp, B. V. and Cole, R. D. 1966. Purification and characterization of bovine liver beta-glucuronidase. Arch. Biochem. Biophys. **116**: 193–206.

Robbins, E. and Gonatas, N. K. 1964. The ultrastructure of a mammalian cell during the mitotic cycle. J. Cell Biol. **21**: 429–463.

Rodbell, M. 1966. Metabolism of isolated fat cells II. The similar effects of phospholipase C and of insulin on glucose and aminoacid metabolism. J. Biol. Chem. **241**: 130–139.

Romani, R. J., Tuskes, S. E. and Özelkök, S. 1974. Survival of plant mitochondria in vitro. Form and function after 4 days at 25°C. Arch. Biochem. Biophys. **164**: 743–751.

Rose, G. G. 1966. Zeiosis I. Ejection of cell nuclei into zeiotic blebs. J. Roy. Microsc. Soc. **86**: 87–102.

Ruby, J. R., Dyer, R. F. and Skalko, R. G. 1969. Continuities between mitochondria and endoplasmic reticulum in the mammalian ovary. Z. Zellforsch. **97**: 30–37.

Searle, R. F. 1972. Observations on the unusual cytoplasmic lacunae of porcine hepatocyte. J. Cell Biol. **54**: 434–438.

Seeman, P. 1967. Transient holes in the erythrocyte membrane during hypotonic hemolysis and

stable holes in the membrane after lysis by saponin and lysolecithin. J. Cell Biol. **32**: 55–70.

Simpson, C. F. and Kling, J. M. 1968. The mechanism of mitochondrial extrusions from phenylhydrazine-induced reticulocytes in the circulating blood. J. Cell Biol. **36**: 103–109.

Skutelsky, E. and Danon, D. 1967. An electron microscopic study of nuclear elimination from the late erythroblasts. J. Cell Biol. **33**: 625–635.

Soll, D. R. and Sonneborn, D. R. 1971. Zoospore germination in *Blastocladiella emersonii*: Cell differentiation without protein synthesis. Proc. Nat. Acad. Sci. USA **68**: 459–463.

Somogyi, M. 1957. Preparation of glycogen, nitrogen- and phosphorus-free from liver. In Methods in Enzymology. (eds. Colowick, S. P. and Kaplan, N. O.) Vol. 3, pp. 3. Academic Press, New York.

Spiro, R. G. 1970. Glycoproteins. Ann. Rev. Biochem. **39**: 629–630.

Starnes, W. L. and Behal, F. J. 1974. A human liver amino peptidase. Biochemistry **13**: 3221–3227.

Stepan, J. and Ferwerda, W. 1973. Phosphatases IX. Differences in sialic acid content of rat liver alkaline phosphatase isoenzymes. Experientia **29**: 948–949.

Stephens, R. J. and Easterbrook, N. 1968. Development of the cytoplasmic membranous organelle in the endodermal cells of the yolk sac of the bat *Taderida brasilensis cynocephala*. J. Ultrastruct. Res. **24**: 239–248.

Tata, J. R., Hamilton, M. J. and Cole, R. D. 1972. Membrane phospholipids associated with nuclei and chromatin: Melting profile, template activity and stability of chromatin. J. Mol. Biol. **67**: 231–246.

Torvik, A. 1972. Phagocytosis of nerve cells during retrograde degeneration. An electron microscopic study. J. Neuropath. Exp. Neurol. **31**: 132–146.

Wallace, E. F., Krantz, M. J. and Lovenberg, W. 1973. Dopamine-beta-hydroxylase: A tetrameric glycoprotein. Proc. Nat. Acad. Sci. USA **70**: 2253–2255.

Wilson, M. A. and Cascarano, J. 1972. Biochemical heterogeneity of rat liver mitochondria separated by rate zonal centrifugation. Biochem. J. **129**: 209–218.

Wise, G. E. and Prescott, D. M. 1973. Ultrastructure of enucleated mammalian cells in culture. Exp. Cell Res. **81**: 63–72.

Zalokar, M. 1969. Intracellular centrifugal separation of organelles in Phycomyces. J. Cell Biol. **41**: 494–509.