inhibited by ouabain (10^{-4} mole/liter). It was temperature-dependent, occurring much more rapidly at 37°C than at room temperature.

The protein exhibited adenosine triphosphatase activity. The released inorganic phosphate was determined by the Marsh procedure (*10*) adapted for the determination of 0.1 μg of P_i. The *p*H optimum of the activity of the protein isolated from the rat was 6.8; that from the cat was 7.6. This was assayed in a medium containing in final concentration 0.2*M* imidazole-HCl, 0.1*M* KCl, $1 \times 10^{-3}M$ Mg^{2+}, and $5 \times 10^{-4}M$ ATP. The protein hydrolyzed approximately 10^{-2} μmole of ATP per minute per milligram of protein. The enzymatic activity was linear for the first 30 minutes and then gradually diminished over the next 30 minutes. This activity is approximately one-thirtieth that of actomyosin isolated from rabbit striated muscle, half that of smooth muscle (uterus) actomyosin, and twice that of contractile protein from sarcoma cells and blood platelets (*11*).

Table 1 shows the effect of Mg^{2+} and Ca^{2+} on the adenosine triphosphatase activity of the protein in media of different ionic strengths. In the absence of Mg^{2+} or Ca^{2+} the enzymatic activity of the protein was 10 percent of that achieved when $10^{-3}M$ Mg^{2+} was present. In contrast to $(Na^+ + K^+)$-activated adenosine triphosphatase (*1*), the hydrolysis of ATP was almost as good when Ca^{2+} (10^{-3} mole/liter) replaced the Mg^{2+} as the activating cation. Since the addition of both Mg^{2+} and Ca^{2+} were not additive, these ions are very probably stimulating the same enzyme and not two separate enzymes. The enzyme activity of the preparations was dependent upon the ionic strength and the cations of the medium. At low KCl concentration (0.03 mole/liter) Mg^{2+} activation was greatest, and at high KCl concentration (0.6 mole/liter) Ca^{2+} activation was greatest; in the latter case Mg^{2+} had an inhibitory effect. Actomyosin and myosin both have adenosine triphosphatase activity, and both are activated by Ca^{2+}. An important difference is that myosin is inhibited by Mg^{2+} (*12*). At low ionic strength actomyosin predominates, whereas at high ionic strength the addition of ATP causes dissociation of actomyosin and the appearance of myosin adenosine triphosphatase characteristics (*13*). Our data are in general accord with these observations. It is very likely that the preparations contain "actin," "myosin," and "actomyosin." The data suggest that the rat preparation more closely resembles the actomyosin of muscle.

The adenosine triphosphatase activity of the proteins was inhibited in a fashion similar to that observed with superprecipitation. Mersalyl (2.5×10^{-4} mole/liter) reduced the enzyme activity of the protein approximately 80 percent; *p*-chloromercuribenzoate (2.5×10^{-2} mole/liter) reduced the enzyme activity to less than 5 percent. Ouabain in a concentration (10^{-4} mole/liter) effective against the $(Na^+ + K^+)$-activated enzyme (*1*) had only a slight inhibitory effect (approximately 5 percent). Mersalyl has been described as a specific inhibitor of contractile protein adenosine triphosphatase activity (*14*).

The evidence indicates that at least part of the Mg^{2+}- or Ca^{2+}-activated adenosine triphosphatase activity in brain is due to a contractile protein similar to actomyosin.

Contractile proteins in cells may serve a universal function as in cell reproduction (*15*) or a specific function as in striated and smooth muscle contraction (*16*), clot retraction (*11*), or cell movement (*15*). In liver mitochondria it has been described as functioning in the regulation of glycolysis and energy metabolism (*17*). It has been suggested that in nervous tissue it is associated with changes in permeability during excitation (*2, 3*) and in control of acetylcholine storage and release (*4*). The basis for all its functions is very probably conformational changes transmitted to membranes.

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References

1. J. C. Skou, *Physiol. Rev.* **45**, 597 (1965).
2. B. Libet, *Fed. Proc.* **7**, 72 (1948).
3. K. Bowler and C. J. Duncan, *Nature* **211**, 642 (1966); *J. Cell Physiol.* **70**, 121 (1967).
4. M. Germain and P. Proulx, *Biochem. Pharmacol.* **14**, 1815 (1965); K. Kadota, S. Mori, R. Imaizumi, *J. Biochem.* **61**, 424 (1967).
5. A. Szent-Györgyi, *Chemistry of Muscle Contraction* (Academic Press, New York, 1951), p. 151.
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
7. E. Layne, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1957), vol. 3, p. 447.
8. A. Szent-Györgyi, *Chemistry of Muscle Contraction* (Academic Press, New York, 1951), p. 34.
9. Mersalyl (Salyrgan) sodium salt of *o*-[(3-hydroxymercuri-2-methoxypropyl)carbamyl]phenoxyacetic acid.
10. B. B. Marsh, *Biochim. Biophys. Acta* **32**, 357 (1959).
11. M. Bettex-Galland and E. F. Lüscher, in *Advances in Protein Chemistry* (Academic Press, New York, 1960), vol. 20, p. 1.
12. D. M. Needham, in *Structure and Function of Muscle*, G. H. Bourne, Ed. (Academic Press, New York, 1960), vol. 2, p. 72.
13. W. Hasselbach, *Z. Naturforsch.* **76**, 163 (1952).
14. H. Hoffman-Berling, *Biochim. Biophys. Acta* **19**, 453 (1956).
15. ———, in *Comparative Biochemistry*, M. Florkin and H. S. Mason, Eds. (Academic Press, New York, 1960), vol. 2, p. 341.
16. S. V. Perry, *ibid.*, p. 245.
17. S. A. Neifakh, J. A. Avramov. V. S. Gaitskhoki, T. B. Kazakova, N. K. Monakhov, V. S. Repin, V. V. Turovski, I. M. Vassiletz, *Biochim. Biophys. Acta* **100**, 329 (1965).
18. Supported in part by PHS grant NB-04064; PHS career development award K3-NB-5117 (S.B.); Cerebral Palsy Education and Research Foundation grant R-206-66; and by the Clinical Research Center for Parkinson's and Allied Diseases (PHS grant NB-05184) and the Parkinson Information Center under PHS contract PH 436454.

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Cytomembrane Differentiation in the Endoplasmic Reticulum-Golgi Apparatus-Vesicle Complex

Abstract. *Diversity of cytomembrane types is confirmed in hyphae of the fungus* Pythium ultimum *by electron microscopy. A transition in membrane morphology across stacks of dictyosome cisternae (from endoplasmic reticulum-like at one pole to plasma membrane-like at the opposite pole) suggests that dictyosomes of the Golgi apparatus are sites of membrane interconversion.*

Structural and functional diversity among cellular membranes is well established (*1–3*), but still remaining are the questions of how structurally different membrane types are related and whether they are interconvertible. If intermembrane conversion occurs, what is the nature and locus of the transformation? Our electron-microscopic evidence demonstrates a progressive transition in membrane morphology (Figs. 1–7) (*4*) across stacks of dictysome cisternae in hyphae of the plant pathogenic fungus *Pythium ultimum* Trow. These findings are consistent with the interpretation that membrane interconversion occurs in the endoplasmic reticulum-Golgi apparatus-vesicle complex.

Mycelia of *P. ultimum* were cultured at 25°C on potato-dextrose agar (Difco) overlaid with permeable cellophane. Hyphal mats were fixed for

1 hour at room temperature (about 24°C) with 4 percent glutaraldehyde prepared in 0.1*M* potassium phosphate (*p*H 7.0). This was followed by treatment with 1 percent OsO_4 in 0.1*M* potassium phosphate (*p*H 7.0) for 8 hours at room temperature (*5*). Hyphae were subsequently dehydrated in a graded series of increasing ethanol concentrations and finally in anhydrous acetone, and embedded in Araldite 6005 (CIBA). Thin sections of embedded hyphae were then stained for 10 minutes with 1 percent aqueous barium permanganate and examined in a Philips EM 200. A 54,864-line diffraction grating replica (Ladd Research Industries) was used as the magnification standard.

The Golgi apparatus of *P. ultimum* is comprised of dictyosomes which are polarized stacks of membrane-bound cisternae (Fig. 6) (*5*, *6*). The cisterna at one pole of each dictyosome is adjacent to endoplasmic reticulum or nuclear envelope. This is the proximal pole (Fig. 6). Profiles of endoplasmic reticulum or nuclear envelope adjacent to the proximal pole are frequently characterized by smooth-surfaced blebs, and small vesicular profiles of similar appearance are aligned in this region parallel to the proximal dictyosome cisterna (*5*). These configurations are important as possible stages in the formation of new cisternae at the proximal pole (*6*). Numerous secretory vesicles are associated with the periphery of the dictyosome, especially near the distal pole (Fig. 6).

Three characteristics enable us to differentiate morphological classes of cytomembranes in electron micrographs: (i) overall membrane thickness, (ii) staining intensity, and (iii) substructural patterns of stain deposition. At one extreme, plasma membrane and the membranes of vesicles (both free in the cytoplasm away from dictyosomes, and those associated with dictyosomes near the distal pole) stain most intensely, are thickest (up to 75 Å), and clearly exhibit the dark-light-dark pattern that characterizes unit membranes (Figs. 3–5) (*7*). Nuclear envelope and endoplasmic reticulum, however, stain faintly, appear thinnest (approximately 25 to 40 Å), and rarely reveal the dark-light-dark pattern (Figs. 1 and 2). Membranes of dictyosome cisternae do not conform uniformly to either of these extreme types.

Dictyosome membranes are differentiated across the stack of cisternae so that those at the proximal pole appear similar to endoplasmic reticulum and

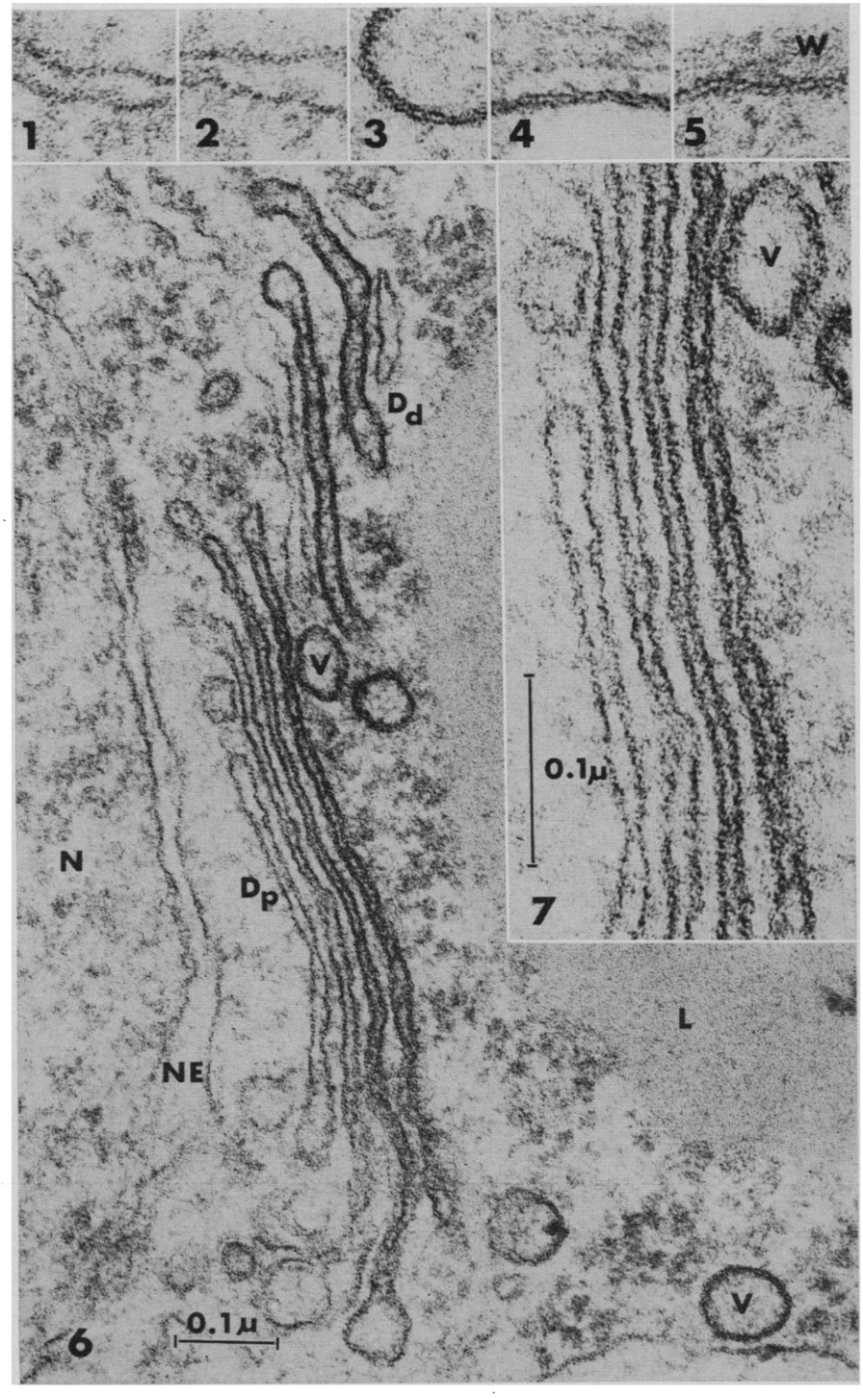

Fig. 1 (right). Membranes of nuclear envelope (× 280,000). Fig. 2. Endoplasmic reticulum membranes (× 280,000). Fig. 3. Membrane of a vesicle that is associated with the distal pole of a dictyosome (× 280,000). Fig. 4. Membrane of an enlarged secretory vesicle, free in the cytoplasm (× 280,000). Fig. 5. Plasma membrane adjacent to the hyphal wall (*W*) (× 280,000). Fig. 6. A dictyosome and associated secretory vesicles (*V*) adjacent to a nucleus (*N*). The membrane of the cisterna at the proximal pole of the dictyosome (D_p), is similar to nuclear envelope (*NE*). The membranes of each successive cisterna stain more intensely and appear thicker toward the distal pole (D_d). Lipid droplets (*L*) (× 137,000). Fig. 7. Enlargement of part of the dictyosome in Fig. 6 (× 280,000).

nuclear envelope, whereas those at the distal pole (including vesicle membranes) are similar to plasma membrane (Figs. 6 and 7). The intercalary cisternae (midregion) are morphologically intermediate; each successive cisterna, progressing toward the distal pole, is more like plasma membrane (that is, denser, thicker, and showing the dark-light-dark pattern more clearly). These observations have been confirmed by microdensitometric tracings across images of dictyosomes. Certain cytochemical stains also reveal progressive changes in membrane images across dictyosomes (*8*). Insofar as these differences are seen in juxtaposed membranes within single electron micrographs, they reflect inherent differences in the membranes. However, the type of image depends on the manner in which the specimen is fixed and stained. If, for example, uranyl acetate is used as a stain immediately after fixation, all dictyosome membranes appear similar.

The phenomenon illustrated here probably is not unique to *P. ultimum* and may be of general occurrence. Under appropriate conditions of dictyosome functioning, and with proper staining, similar membrane differentiation should be demonstrable in other biological systems [see, for example, suggestions of this phenomenon in figures in (*9*) and discussion in (*6*)].

The occurrence of dissimilar membranes in dictyosomes is significant for the concept of Golgi apparatus functioning. A major function of the Golgi apparatus is to elaborate secretory vesicles whose limiting membranes can fuse with plasma membrane (*6*). This property of vesicle membranes facilitates the discharge of secretory products from the protoplast and provides a potential source of new plasma membrane. Functional polarity within the Golgi apparatus is evidenced by progressive changes in cisternal and vesicle contents across dictyosomes (*6*, *10*). These observations led to the concept of a forming (proximal) face and a secreting (distal) face, in which the dictyosome was visualized as being in a dynamic steady state (*6*). Accordingly, as proximal cisternae were formed, presumably from membrane material derived from endoplasmic reticulum (the Golgi apparatus is not known to engage in protein synthesis), vesicles were considered to be discharged from the cisternae at the distal pole with the concomitant loss of the distal cisterna—each cisterna being progressively displaced from the forming face through continued production of new cisternae. Membrane differentiation of the type reported here (from endoplasmic reticulum-like to plasma membrane-like) is implicit in this concept of Golgi apparatus functioning.

Morphological dissimilarity of various cytomembranes has led to the view that common origin of membranes is unlikely and that membranes of one morphological type will not likely be derived from those of another type (*2*). Membrane systems are dynamic, however, and certain membranes in the living cell may be interconvertible or subject to a variety of transformations (*1*, *11*). Our results not only confirm that morphologically distinct membrane types occur, but demonstrate that in one cell component intermediate forms also exist. If these morphological differences arise from an interconversion of membrane types, then the dictyosomes function as sites of membrane transformation.

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References and Notes

1. E. Benedetti and P. Emmelot, in *The Membranes*, A. Dalton and F. Haguenau, Eds. (Academic Press. New York, in press).
2. P. Grun, *J. Ultrastruct. Res.* **9**, 198 (1963); M. Ledbetter, in *Proceedings of Fifth International Congress for Electron Microscopy* (Academic Press, New York, 1962), vol. 2, W-10.
3. K. Porter, K. Kenyon, S. Badenhausen, *Protoplasma* **63**, 262 (1967); F. Sjostrand, *J. Ultrastruct. Res.* **9**, 561 (1963); M. Girbardt, *Biol. Rundsch.* **4**, 1 (1965).
4. In this report, we use the expression membrane morphology to denote the features of membranes seen in images formed by electron microscopy. Thus, the observed differences in membrane morphology represent differences in sites of stain deposition and do not necessarily reflect the morphological forms of the living membranes.
5. S. Grove, C. Bracker, D. Morré, in preparation; S. Grove, D. Morré, C. Bracker, *Proc. Indiana Acad. Sci. 1966* **76**, 210 (1967).
6. H. Mollenhauer and D. Morré, *Ann. Rev. Plant Physiol.* **17**, 27 (1966); W. Whaley, in *Probleme der Biologischen Reduplikation*, (Springer-Verlag, New York, 1966), p. 340.
7. J. Robertson, in *Cellular Membranes in Development*, M. Locke, Ed. (Academic Press, New York, 1964), p. 1.
8. S. Grove, D. Morré, C. Bracker, *Amer. J. Bot.* **54**, 638 (1967).
9. A. Sakai and M. Shigenaka, *Cytologia* **32**, 72 (1967).
10. J. Berlin, *J. Cell Biol.* **32**, 760 (1967); I. Manton, *J. Cell Sci.* **1**, 429 (1966); H. Mollenhauer and W. Whaley, *J. Cell Biol.* **17**, 222 (1963).
11. G. Dallner, P. Siekevitz, G. Palade, *J. Cell Biol.* **30**, 73 (1966); J. Kavanau, *Structure and Function in Biological Membranes* (Holden-Day, San Francisco, 1965), vols. 1 and 2; A. Novikoff, E. Essner, S. Goldfischer, M. Heus, in *The Interpretation of Ultrastructure*, R. Harris, Ed. (Academic Press, New York, 1962), p. 149.
12. This work was supported by NSF grant GB-3044. Purdue University AES journal Paper No. 3328.

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Infectious Mononucleosis: Complement-Fixing Antibodies to Herpes-Like Virus Associated with Burkitt Lymphoma

Abstract. *Complement-fixing antibodies to a herpes-like virus derived from a Burkitt tumor-cell line developed in each of 21 patients with infectious mononucleosis. These antibodies were absent in all serums before the patients became ill, appeared during the early phases of illness, and persisted for long periods of time. These antibodies are distinct from heterophile antibodies. None of the patients developed immune responses to herpes simplex, cytomegalo-, or reoviruses in the course of their illness. The data suggest that the development of complement-fixing antibodies to this herpes-like virus in these patients may be linked to infectious mononucleosis.*

Henle *et al.* (*1*) and Neiderman *et al.* (*2*) have reported that herpes-like virus (EBV) associated with a cell line derived from a Burkitt lymphoma (EB) may be the etiologic agent of infectious mononucleosis. By indirect immunofluorescence tests, these investigators demonstrated the appearance of antibodies to EBV in serums of patients with infectious mononucleosis.

Complement-fixing antibodies to a herpes-like virus (HLV) derived from another Burkitt tumor-cell line (P_3J) are widespread among apparently healthy children and adults and among subhuman primates (*3*). The relation of these antibodies to disease remained to be determined. We now report evidence on the appearance and persistence of complement-fixing antibodies to HLV in 21 patients with confirmed diagnosis of infectious mononucleosis.

Serial serum specimens were available from cases of prospective studies of infectious mononucleosis conducted among students at Yale University