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

(cases 1 to 9) and at the University of Chicago (cases A to L) in Tables 1 and 2. The clinical diagnosis was confirmed by the presence of atypical lymphocytes in the blood and by positive heterophile-antibody tests. In the case of the patients at Yale, heterophile-antibody tests (after the serums were absorbed with guinea pig kidney) and fluorescent-antibody titers to EBV were performed on specimens of serums obtained before the onset and during the acute and convalescent phases of illness (*4*). All serums were inactivated at 56°C for 30 minutes before use in a quantitative microcomplement-fixation test (*3*). We used a partially purified HLV antigen derived from the P_3J Burkitt tumor-cell line, and similarly prepared material derived from a virus-free Burkitt tumor (Raji) served as control.

None of the serums reacted with the control antigen. Results of complement fixation are expressed as the reciprocal of the serum dilution at which 50 percent of complement was fixed. A titer of <30 is considered negative for the purpose of this study.

We also attempted to use the microtiter technique for complement fixation (*5*), but with the available antigen the antibody titers were approximately 10 to 15 times lower compared with the more sensitive, quantitative method.

The results obtained with serums of 12 patients at the University of Chicago are summarized in Table 1. None of the serums collected between 2 and 38 days before the onset of illness contained complement-fixing antibodies to HLV. These antibodies appeared generally within 2 to 3 weeks after onset of disease; their titer decreased during convalescence in some cases, remained almost constant in others, and showed a definite increase in three cases (B, D, I). The immune response in these last three patients may be a reflection of continuous antigenic stimulation due to current infection.

The relationship among heterophile, fluorescent, and complement-fixing antibodies in infectious mononucleosis was studied in the nine patients at Yale University (Table 2). No antibodies were detected in serums obtained before the onset of illness by any of the three tests. In general, all three types of antibodies appeared early after onset and reached a peak within 2 to 6 weeks.

The complement-fixing antibodies persisted in all patients throughout the entire period of observation. That these

Table 1. Complement-fixing (CF) antibodies to herpes-like virus in the serums of patients with infectious mononucleosis.

Patient	CF antibody responses							
	Before onset		During acute and convalescent stages					
			Sample 1		Sample 2		Sample 3	
	Titer	Day	Titer	Day	Titer	Day	Titer	Day
A	< 30	5					405	145
B	< 30	11	370	29			> 810	155
C	< 30	36	450	25			270	74
D	< 30	2			224	52	392	136
E	< 30	30	187	11			104	265
F	< 30	33			405	44	380	85
G	< 30	38	< 30	14	252	18	135	136
H	< 30	19	> 810	31				
I	< 30	16	60	19			270	64
J	< 30	35	> 810	18			810	90
K	< 30	25						
L	< 30	4	425	23	30	42	150	45

Table 2. Antibodies in serums from patients with infectious mononucleosis. HT, heterophile antibody; FA, fluorescent antibody; CF, complement-fixing antibody.

Patient	Test	Before onset		During acute and convalescent stages							
				Sample 1		Sample 2		Sample 3		Sample 4	
		Titer	Day	Titer	Day	Titer	Day	Titer	Day	Titer	Day
1	HT	< 10	24	640	52	< 10	94				
	FA	< 10		10–20		10–20					
	CF	< 30		90		90					
2	HT	< 10	144	< 10	9	< 10	36				
	FA	< 10		40–80		40					
	CF	< 30		330		193					
3	HT	< 10	477	< 10	10						
	FA	< 10		80–160							
	CF	< 30		70							
4	HT	< 10	480	160	23	80	47	40	68		
	FA	< 10		160–320		80–160		160			
	CF	< 30		376		1200		820			
5	HT	< 10	30	640	6	640	32	640	54	80	172
	FA	< 10		160–320		160		160		80	
	CF	< 30		480		470		120		108	
6	HT	< 10	131	160	14	80	33	40	47	20	77
	FA	< 10		160		160		160		160	
	CF	< 30		820		1200		900		550	
7	HT	< 10	141	40	9	640	19	160	35	< 10	125
	FA	< 10		40		320		160		160	
	CF	< 30		< 30		122		137		335	
8	HT			40	12	640	29	< 10	1060		
	FA			10		160		40			
	CF			< 30		90		53			
9	HT	< 10	231	640	9	160	27	< 10	320		
	FA	< 10		80		160		40			
	CF	< 30		30		360		80			

antibodies persist for many years is demonstrated by their presence in serums of two patients who had infectious mononucleosis 12 and 37 years ago, respectively. Furthermore, we have observed that these complement-fixing antibodies persisted at constant levels in 26 normal adults whose paired serums were collected 10 years apart (*6*). Patients 2 and 3 failed to develop heterophile antibodies, 36 and 10 days after onset, but showed significant amounts of fluorescent and complement-fixing antibodies. Heterophile-antibody tests remained negative in serums obtained from these patients at 69 and 43 days, respectively (*7*).

Complete removal of heterophile antibodies by absorption of serums with washed red blood cells from sheep had no effect on the complement-fixing antibody titers, and, conversely, antiserum to sheep red blood cells prepared in rabbits failed to react with HLV antigen.

We also sought to determine whether antibodies to other common viral infections are stimulated in the course of infectious mononucleosis. Accordingly, serums obtained before and after illness were tested for complement-fixing antibodies to herpes simplex and cytomegaloviruses and for hemagglutination-inhibiting antibodies to reoviruses types 1, 2, and 3. There was no evidence of any immunological response to these antigens in any of the patients studied.

Our results and the findings of others (*1*, *2*) provide good evidence for a possible relation between HLV and infectious mononucleosis. However, additional evidence is required to ascribe an etiologic role to this virus.

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References

1. G. Henle, W. Henle, V. Diehl, *Proc. Nat. Acad. Sci. U.S.* **59**, 94 (1968).
2. J. C. Niederman, R. W. McCollum, G. Henle, W. Henle, *J. Amer. Med. Ass.* **203**, 205 (1968).
3. P. Gerber and S. M. Birch, *Proc. Nat. Acad. Sci. U.S.* **58**, 478 (1967).
4. These serums were made available by Drs. R. W. McCollum and J. C. Niederman who performed the heterophile- and fluorescent-antibody tests. Duplicate fluorescent-antibody tests were also carried out by Drs. Henle.
5. J. L. Sever, *J. Immunol.* **88**, 320 (1962).
6. P. Gerber and E. N. Rosenblum, *Proc. Soc. Exp. Biol. Med.*, in press.
7. R. W. McCollum, personal communication.

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Silicification of Betula Woody Tissue in vitro

Abstract. *Pieces of* Betula *twigs were placed in solutions of sodium metasilicate (5,000 to 10,000 parts per million) and allowed to remain for 12 to 24 hours, after which they were washed and wet-ashed with chromic acid. Opaline silica was deposited on inner surfaces of cell walls so that silica replicas of the various types of cell lumens were produced. Entire twigs were not replicated intact, but macroscopic replicas of cell aggregates were common. Carbon replicas of the silica replicas provide an unusual view of cellular spaces in woody tissue. The pits were viewed as projections from cells rather than holes in cell walls. This technique offers a new way of examining woody tissue and a method for deliberate petrification in a relatively short period of time under laboratory conditions.*

Plant parts, especially twigs, which fall into waters of high silica content (750 parts per million) allegedly become impregnated with opaline silica so that a siliceous replica of the twig remains after ashing; similar silicification of plant tissue occurs after several years of immersion in jars of such water (*1*). Grasses deposit opaline phytoliths as a regular growth function (*2*). Spongillid sponges deposit opaline silica in the form of characteristic spicules. Organisms from both groups quickly deposit opaline silica on polysaccharide substrates (*3*). Furthermore, petrified wood is formed by the deposition of opaline silica on polysaccharide surfaces. Unaware of any prior concerted effort to silicify or petrify plant tissues, I attempted the laboratory silification of twigs. Fresh (living when collected, immediately prior to treatment) *Betula papyrifera* twigs were placed in 100-ml polyethylene beakers containing sodium metasilicate solutions (5,000 to 10,000 parts per million) and allowed to remain for 12 to 24 hours at room temperature (15° to 20°C). They are then washed several times with distilled water and wet-ashed 48 to 72 hours with chromic acid (Chromerge) to remove all organic material (*2*). Complete removal of organic material is verified by examination with polarization microscopy and in empty carbon replicas (*3*). Entire twigs do not remain intact, but separate into many pieces, from parts of single cells to aggregates as large as 10 to 12 mm (*3*). The silica seems to have replicated the cell lumens. The replicas were generally very fragile: multicellular arrays are easily broken with microprobes or sonic vibration. The silicate solution apparently moves freely through the wood, and silica is deposited on the inner surfaces of the cell wall to form replicas or casts. Because the respective cellular spaces are variously interconnected by the intercellular pits, many of these replicas remain attached to each other after wet-ashing, whereas others do not (Figs. 1–3).

If silica deposits on the inner surfaces of the cell walls and then these cell walls are completely removed by wet-ashing, the length of the replicated pit projections on xylem parenchyma cells indicates the thickness of the primary and secondary wall material which originally surrounded them (Fig. 1). Pit

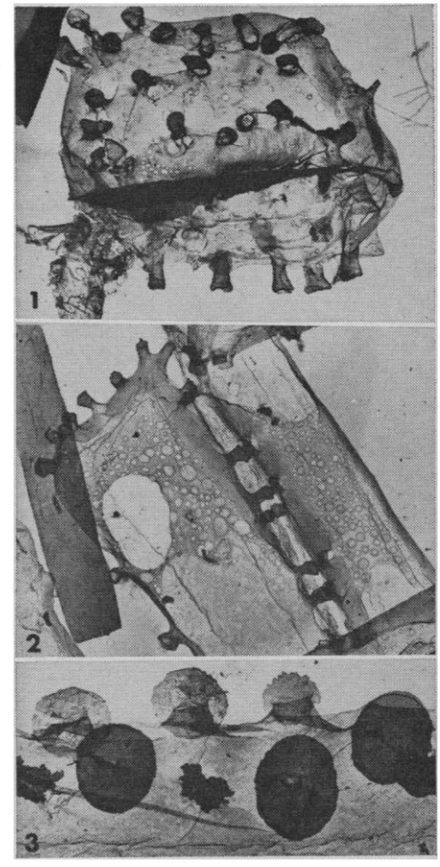

Fig. 1. Electron micrograph of a carbon replica of a silica replica of a *Betula* xylem parenchyma cell. Projections from the replica surface represent simple pits (× 2700). Fig. 2. This replica demonstrates paired simple pits between a ray parenchyma cell and a tracheid (× 1700). Fig. 3. In this carbon replica of the cellular space, the large round structures are probably replicas of bordered pits (× 2500).