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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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

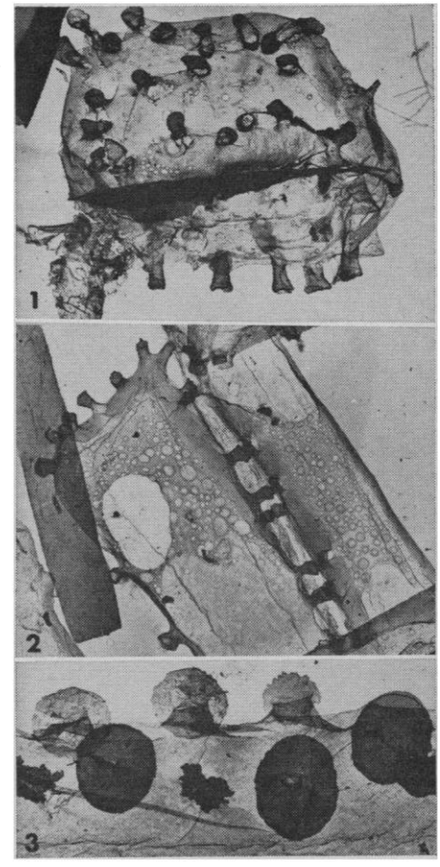

Fig. 1. Electron micrograph of a carbon replica of a silica replica of a *Betula* xylem parenchyma cell. Projections from the replica surface represent simple pits (× 2700). Fig. 2. This replica demonstrates paired simple pits between a ray parenchyma cell and a tracheid (× 1700). Fig. 3. In this carbon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

projections from ray parenchyma cells often adjoin similar projections from adjacent parenchyma or tracheid cells (Fig. 2). These projections represent simple pit pairs, the only type of pit pairs occurring between parenchyma cells (*4*). Some xylem vessels and tracheids have large umbrella-shaped projections which probably represent bordered pits and pit connection replication (Fig. 3). Light-microscopic examination of silica replicas shows the pit pair projections, but the accompanying chromatic aberration caused by the silica renders the images indistinct. Consequently, carbon replicas of the silica replicas were prepared for electron-microscopic examination as previously described for sponge spicules (*5*). Carbon replicas worked well for masses of silica replica of one to ten cells, but with larger pieces the replicas collapsed or were confusingly superimposed.

This technique seems to have great potential for the three-dimensional examination of cellular spaces, intercellular connections, and the morphology of woody cells. More rigorous control of experimental conditions will provide information about siliceous petrifaction of plant tissues and possibly the biogenesis of siliceous structures in living plants and animals.

Cornus, Smilax, Morus, Osmunda, Rosa, Quercus, Acer, and *Pinus,* as well as *Betula* wood, have been silicified. To date, I have been unable effectively to silicify leaf tissue or various nonmineralized animal tissues (human hair, feathers, and insects).

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Lymphocytes: Circulation Altered by Trypsin

Abstract. *Rat thoracic duct lymphocytes altered by trypsin in vitro do not circulate normally. At early intervals after transfusion of lymphocytes labeled with chromium-51 selective accumulation of radioactivity in the lymph nodes is abolished, while uptake in the spleen is not reduced. Later, the cells appear to "home" to lymph nodes and recirculate to the lymph.*

Small lymphocytes circulate through the body by a unique route—they selectively emigrate from the blood stream into lymphoid tissue (except the thymus) and then recirculate to the blood via the lymphatics (*1–5*). The properties of lymphocytes which control their fate in the body are poorly understood. There is evidence, however, that lymphocytes must be viable in order to recirculate to the lymph (*1*). Also, it is known that lymphocytes need not be identical genetically with their host in order to "home" to lymphoid tissue and recirculate to the lymph (*1, 2, 4, 6, 7*). More recently it has been found that incubation of lymphocytes with a mixture of glycosidases (*7*) or with neuraminidase (*8*) before transfusion into recipients markedly altered the fate of the cells, although the cells appeared to be viable at the time they were transfused. In the present investigation lymphocytes were incubated in vitro with trypsin and the effect of this treatment on their fate in recipients was studied.

Lymphocytes obtained during the first 24 hours after cannulation of the main lymphatic duct of a donor rat (*1, 9*) were separated from the lymph by centrifugation at 4°C for 5 minutes at 150*g*. The cell pellet, containing 350 to 600 $\times$ 10^6 lymphocytes, was resuspended in 5 to 8 ml of saline, 100 μc of radioactive chromium (*10*) was added, and the mixture was incubated for 1 hour at room temperature. At the end of the incubation period the cells were washed three times with 40 ml of saline, counted, asasyed for radioactivity, and resuspended in saline so as to provide 100 $\times$ 10^6 lymphocytes per milliliter.

Lymphocytes labeled with Cr^{51} were dispensed in 0.5-ml samples, containing 50 $\times$ 10^6 lymphocytes, in separate tubes. Immediately after incubation for 5 minutes, with or without trypsin (*11*), in a 37°C water bath without shaking, each cell suspension was drawn into a syringe and transfused into the tail vein of a recipient rat lightly anesthetized with ether. Each injection was completed within 30 seconds. All transfusions were completed within 30 minutes after the labeled cells had been washed and resuspended in saline. Recipients were killed by cervical dislocation, and various organs were removed, weighed, and assayed for radioactivity. The superficial and deep cervical nodes and the mesenteric nodes from each recipient were carefully dissected free of fat, pooled, and assayed together. Usually a total of about 0.4 to 0.8 g of nodal tissue was obtained from one recipient and the counts per minute per gram were calculated. The spleens usually weighed 0.4 to 1.2 g. For this mixed lymphoid and reticuloendothelial organ, values are presented as counts per minute per organ since among recipients of untreated lymphocytes values more closely coincided by this measure than by comparing counts per minute per gram. Sprague-Dawley rats, a closed but not inbred stock, were used as donors and recipients unless otherwise indicated. In some experiments Fisher rats, members of a highly inbred strain, were used. All donors and recipients were males and they weighed from 250 to 300 g.

During the 5-minute incubation in vitro, prior to transfusion into recipients, there was no greater release of radioactivity from lymphocytes incubated with trypsin (50 $\times$ 10^6 lymphocytes and 0.01 mg of trypsin in 0.5 ml of saline) than from suspensions incubated without the enzyme; 96 percent of the total radioactivity in the samples was found in the cell pellet. Even after 30 minutes of incubation with trypsin, about 95 percent of the total radioactivity remained associated with the cells.

Lymphocytes incubated with trypsin for 5 minutes under these conditions showed no decrease in the percentage of motile cells (when resuspended in medium 199 containing 15 percent calf serum and observed for 3 hours by means of phase-contrast microscopy at 37°C) and no decrease in the percentage of cells excluding trypan blue (when resuspended in saline and incubated for 1 hour at 37°C); 90 to 98 percent of the lymphocytes incubated with or without the enzyme showed these characteristics of viability. During the 5-minute incubation period in vitro with trypsin, a fine mucinous material was released from the cells. This material coalesced to form a larger pre-