Table 1. Course of the clinically inapparent mumps virus infection of the nervous system of suckling hamsters that had been inoculated intracerebrally with 100 TCD<sub>50</sub>'s. +, Definite but mild reaction; ++, moderate; and +++, severe reaction.

Assay method	Days after inoculation							
	1	2	3	5	7	9	11	14
Virus content in $log_{10}$ TCD <sub>50</sub> per gram of brain	< 1.5	3.0	3.7	42	35	< 1.5	< 15	< 1.5
Intensity of perivascular inflammatory reaction	0	0	0		++		·++	+
Intensity and extent of virus antigen in ependymal cells	0	0	+	++	+++	+++	++	0
Neutralizing antibody (titer)				<1:4	<1:4	1:4	1:20	

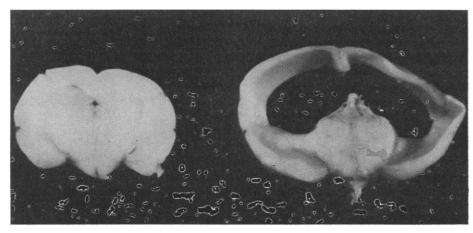


Fig. 2. Coronal sections of hamster brains 4 weeks after inoculation of mumps virus. Brain on left, inoculated with virus plus antiserum, appears normal; brain on right, inoculated with virus alone, shows typical hydrocephalus.  $(\times 3)$ 

Severe stenosis or total occlusion of the aqueduct of Sylvius was found in all cases; occlusions consistently occurred in the rostral portion of the aqueduct just caudal to the posterior commissure at the level of the superior colliculi.

Control animals, inoculated with virus mixed with antiserum against the Enders strain of mumps virus, developed neither perivascular inflammatory response nor antigen detectable by fluorescent antibody staining, and subsequently they developed neither clinical nor pathological evidence of hydrocephalus. These observations indicate that the infection and the sequela of hydrocephalus were not due to nonspecific effects of inoculation or to the presence of a simian agent in the inoculum. Two other strains of mumps virus, recently isolated from human cerebrospinal fluid, also induced hydrocephalus after intracerebral inoculation of 100  $TCD_{50}$ 's, which indicates that this phenomenon was not peculiar to one strain of mumps virus.

The similarity of this disease to the aqueductal stenosis observed as a common cause of hydrocephalus in children and adults (3) suggests that the clinically inapparent infection of ependymal cells with a common respiratory

virus might result in subsequent noninflammatory occlusion or stenosis of the aqueduct of Sylvius in man.

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## **References and Notes**

- C. Levaditi and R. Schoen, Compt. Rend. Soc. Biol. 122, 876 (1936); H. Jones, G. Rake, B. Stearns, J. Infect. Dis. 76, 55 (1945); C. P. Li and W. G. Jahnes, Virology 9, 489 (1959); M. Vandeputte, Rev. Belge Pathol. Med. Exp. 28, 178 (1961); R. J. Huebner, W. P. Rowe, W. T. Lane, Proc. Nat. Acad. Sci. U.S. 48, 2051 (1962); Y. Yabe, L. Samper, G. Taylor, J. J. Trentin, Proc. Soc. Exp. Biol. Med. 113, 221 (1963); A. Holtz, G. Borman, C. P. Li, ibid. 121, 1196 (1966).
   R. T. Johnson, J. Neuropathol. Exp. Neurol.
- 2. R. T. Johnson, J. Neuropathol. Exp. Neurol., in press.
- W. E. Dandy, Surg. Gynecol. Obstet. 31, 340 (1920); J. H. Giobus and P. Bergman, J. Neuropathol. Exp. Neurol. 5, 342 (1946); D. S. Russell, Observations on the Pathology of Hydrocephalus (Her Majesty's Stationery Office, London, 1949); R. S. Beckett, M. G. Netsky, H. M. Zimmerman, Amer. J. Pathol. 26, 755 (1950); D. A. Drachman and E. P. Richardson, Arch. Neurol. 5, 552 (1961). Arguments for the developmental versus the acquired etiologies of aqueductal stenosis in man are summarized in these articles.
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## **Enclosed Bark as a Pollen Trap**

Abstract. Counts were made of pollen in traps formed by enclosed bark in two remnants of bristlecone pine, Pinus aristata Engelm., from the White Mountains of east-central California. The traps, dated by tree-rings at A.D. 350 and 1300 B.C., contained a major complex of pine-sagebrush pollen and traces of other species, representing the equivalent of the present vegetation.

Sediments have been the primary source of pollen for palynological studies. Dating of sediments has been by radiocarbon determinations, extrapolation of sedimentation rates, or other methods. Another source of pollen of recent millenia is the enclosed bark or healed scars disclosed when bulk remnants of ancient bristlecone pine, Pinus aristata Engelm., are sectioned for treering studies. The initiation and closure of such pollen traps can be dated by the tree-ring method. An analysis has been made of pollen from two such traps found in bristlecone pine trees from the White Mountains of eastcentral California.

Sample 1 was found in a section from a dead and downed snag in Methuselah Walk, a small valley containing 9 of the 17 trees over 4000 years old reported by Schulman (1). This remnant (TRL 63-43) grew at an elevation of about 2900 m (37°23'N, 118°10'W), in an area nearly devoid of understory vegetation. On adjacent slopes, however, grow relatively dense stands of curl-leaf mountain mahogany (Cercocarpus ledifolius Nutt.), big sage brush (Artemisia tridentata Nutt.), and Mormon tea (Ephedra viridis Coville).

The specimen contains tree rings dated from 3080 B.C. to A.D. 850. This high-quality ring record has been incorporated into the master chronology for the area, and units of dated wood have been distributed to three radiocarbon laboratories.

Bark formed over a scar area on the tree was subsequently trapped by the thumb-like lobe of wood which grew over it. The scar was formed about A.D. 311, and the bark grew and was trapped during the following century. Hence, any pollen included in the bark sample must date from A.D. 300 to A.D. 400.

Sample 2 was exposed in the cross section of a snag (VL 100) cut in 1963. The dead tree was located 150 m from the western margin of the bristlecone pine stand near the head of the North

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Table 1. Pollen grains recovered from two pollen traps dated at approximately A.D. 350 (sample 1) and 1300 B.C. (sample 2). One diatom was found in Sample 2, while none was found in sample 1.

Pollen type	Sample 1	Sample 2		
Pinus	53	76		
Artemisia	22	12		
Juniperus	1	0		
Quercus	3	0		
Cyperaceae	0	1		
Gramineae	2	3		
Compositae	.6	2		
Cheno-ams	5	3		
Sarcobatus	1	1		
Eriogonum	4	0		
Urtica	1	0		
Ephedra viridis	1	0		
Rumex	0	1		
Unknown	1	1		

Fork of Crooked Creek (37°30'N, 118°10'W). It stood on a rocky knoll of dolomite on the crest of a high ridge at an elevation of 3160 m. Only a sparse ground cover is associated with this stand of bristlecone pine, but a granite area to the west supports a dense cover of big sagebrush, and curlleaf mountain mahogany grows on a slope to the south.

Crossdating with the master tree-ring chronology for bristlecone pine established a dated sequence from 200 B.C. to the outermost ring at A.D. 1385. A simple ring count back from 200 B.C. continued the series to 2100 B.C., the innermost ring on the specimen.

The unit of bark in which the pollen was deposited was preserved in a longitudinal fissure extending above and below a dead branch, which was subsequently engulfed by radial stem growth. As the two lobes flanking the dead branch grew together, the bark was compressed between them, effectively sealing the sample. Thus, dating indicates that the pollen in the sample was trapped between 1500 B.C., when the branch was formed, and 1100 B.C., when the area became closed to additional deposits of air-borne pollen.

Abundant pollen was recovered from both samples (Table 1). Sample 1, at A.D. 350, contained about 108,000 pollen grains per gram of bark, while sample 2, at 1300 B.C., contained about 40,000 grains per gram. We made these estimates by adding a known amount of Corylus and Alnus pollen to the samples before extracting the trapped pollen and noting the number of these grains which were recovered with the fossil pollen grains.

The bark in which the pollen was embedded could not readily be dissolved without the use of techniques which also destroy pollen. Consequently, the pollen had to be counted while still mixed with a much larger amount of fine organic debris.

The pollen recovered from the samples is representative of the vegetation in the area today (2). Pine is the most abundant pollen type in both samples. The second most abundant type, sagebrush, represents a species that also reaches its maximum age in the White Mountains; plants over 200 years old have been reported from three major areas (3). Gramineae, Compositae, cheno-am (Chenopodiaceae plus Amaranthus) and Eriogonum pollen are only moderately abundant, and all other types are scarce. Since we know from the ages of living trees and remnants on the ground that there was a bristlecone pine forest at least 6600 years ago, the agreement between the pollen samples at the time the traps were formed and the modern vegetation is expected.

The two samples described here are intended to be illustrative rather than conclusive; our purpose is to point out the potential of the method. Before such samples can provide valid conclusions about the vegetation which they represent, many more pollen grains must be counted from each sample, and the resulting pollen counts must be compared to modern pollen samples from soil surface and bark. But even though old trapped bark samples apparently are comparatively rare, they should not be ignored as a source of paleoecological information.

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## References and Notes

- E. Schulman, Nat. Geogr. 113, 355 (1958).
   H. A. Mooney, G. St. Andre, R. D. Wright, Amer. Midland Natur. 68, 257 (1962); R. A. Kuehner, thesis, University of Michigan, 1966.
   C. W. Ferguson, Annual Rings in Big Sage-brush (Univ. of Arizona Press, Tucson, 1964).
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## Viral Inhibition of Lymphocyte **Response to Phytohemagglutinin**

Abstract. The response to phytohemagglutinin by lymphocytes from eight of fourteen patients with congenital rubella was inhibited, whereas that of lymphocytes from patients with other diseases was not. The response of normal lymphocytes infected in vitro was also inhibited. The results suggest that early association of lymphocytes with virus inhibits the function of the cell and contributes to persistent carrying of virus in congenital rubella. This phenomenon may be a means of detecting viruses not now recognizable by routine methods of tissue culture.

Lymphocytes from some babies with the congenital rubella syndrome do not respond when stimulated with phytohemagglutinin (PHA) in vitro; the possibility that viral invasion of the lymphocytes interferes with the mitogenic effect of PHA has been considered (1). Exposure of lymphocytes to PHA in vitro provokes the synthesis of protein, RNA, and DNA, and stimulates mitosis (2). In certain human diseases of unknown etiology the responsiveness of lymphocytes to this mitogenic influence may be impaired. These diseases include Hodgkin's disease (3), sarcoidosis (4), and chronic lymphatic leukemia (4, 5), as well as the immunologic deficiency disorders, primary agammaglobulinemia acquired (6). ataxia-telangiectasia (7), and diseases that are associated with thymic deficiency (8).

We have demonstrated that lymphocytes from babies with congenital rubella syndrome fail to respond to PHA and that this characteristic does not appear to be shared by children with certain other viral diseases. This abnormality of lymphocytes apparently disappears with the passage of time and with a decrease in the clinical manifestations of the disease. Further, exposure in vitro of lymphocytes from a normal adult to either rubella virus or Newcastle disease virus (NDV) interferes with their capacity to respond to PHA.

Peripheral leukocytes were separated from the plasma of heparinized blood samples, centrifuged, and washed once in Hanks balanced salt solution. The cells were resuspended in Eagle's medium containing fetal bovine serum (20 percent), 100  $\mu$ g of streptomycin