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 curl-leaf 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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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 acquired agammaglobulinemia (*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 μg of streptomycin

and 100 units of penicillin per milliliter and 0.3 g of bicarbonate per 100 ml and adjusted to a final concentration of 1×10^6 leukocytes per milliliter. Cultures were prepared in quadruplicate with 2 ml of cell suspension per culture. Phytohemagglutinin-M (Difco, 0.05 ml) was added to appropriate cultures at various times.

Rubella virus (R-3 strain) (*9*) was added to selected cultures to a final concentration of 1 plaque-forming unit (PFU) (*10*) per cultured leukocyte. Rubella virus from throat washings was isolated in primary cultures of African green-monkey kidney cells (*11*). Newcastle disease virus was obtained from American Type Culture Collection, grown in chorioallantoic sacs of embryonated hens' eggs, purified according to the procedure described by Wheelock (*12*), and assayed by plaque formation on primary cultures of embryonic chick fibroblasts. This virus was added to the cultures at various times at a ratio of 25 PFU per leukocyte. All cultures were incubated at 37°C for 72 hours. C^{14}-Thymidine (0.064 μc; specific activity, 30 mc/mmole) was added to three culture tubes of each set 5 hours before termination of the culture by addition of excess unlabeled thymidine and centrifugation to obtain a cell pellet. The cells were resuspended and washed twice in cold Hanks solution, twice in cold 5-percent trichloroacetic acid, and twice in cold methanol. The resulting precipitate was dissolved in 0.25 ml of "NCS solubilizer" (Nuclear-Chicago), mixed with scintillation fluid, and counted in a Packard Tri-Carb scintillation counter. Data were expressed as average counts per minute for three cultures of each set, and the response was considered positive when radiolabel was tenfold or greater in cultures with PHA. We used the fourth culture to determine cell morphology and viability with the dye exclusion method (*13*). There was a marked unresponsiveness to PHA of lymphocytes from eight to fourteen children with the rubella syndrome. The majority of these patients were 3 months old or less and were in that phase of the disease characterized by failure to thrive, recurrent infections, thrombocytopenia, hepatosplenomegaly, lymphadenopathy, pneumonitis, and a generalized macular skin rash. The normal responsiveness to PHA appeared with the passage of time and in association with a disappearance of these findings. Although persistent excretion of virus is part of the congenital rubella syndrome, we could not demonstrate a relationship between PHA unresponsiveness and ability to isolate virus from simultaneous throat cultures. Approximately equal percentages of positive virus cultures were obtained for all patients with congenital rubella.

Table 1. Effect of addition of virus on the response of lymphocytes to phytohemagglutinin (PHA).

Additions (hr)			Viable cells at termination (%)	Thymidine incorporation (counts/min per culture)	Inhibition (%)
PHA	Rubella virus	NDV			
No previous treatment					
			72	73	
		0	80	73	
	0		91	34	
0			83	4,836	0
0	0		72	337	93
0		0	70	72	98
0	24		80	1,566	68
0		24	48	131	97
Previous treatment with antiserum from convalescent patients					
0			84	20,591†	0
0	0		75	1,327	94
0	0*		78	29,839	0

* Rubella virus plus rubella antiserum. † C^{14}-Thymidine (0.2 μc) was used in these experiments.

Lymphocytes from six patients with diseases such as varicella, rubeola, pertussis, and undiagnosed febrile illnesses were tested, and all responded normally to PHA in vitro.

Our data indicate that exposure of lymphocytes from a normal subject to rubella virus or NDV in vitro interferes with the capacity of these cells to respond to PHA, resulting in a 68 to 98 percent inhibition of thymidine incorporation (Table 1). These viruses interfere with the responsiveness whether they are added to the culture at the time of, or even as late as 24 hours after, addition of PHA. The inhibitory effect of rubella virus on the response of lymphocytes to PHA can be neutralized with antiserum to rubella (*14*). That the effect was due to the virus was further shown by the failure of virus inactivated with ultraviolet light to interfere with PHA responsiveness. Control cultures treated with tissue culture fluid from noninfected cultures, with normal human serum, and with serum from convalescent patients showed no inhibition of PHA responsiveness.

Our experiments show that lymphocytes of some children with congenital rubella syndrome cannot respond to the mitogenic stimulus of PHA in vitro. The finding that human leukocytes infected in vitro with rubella virus or with a paramyxovirus (NDV) display similar impairments of responsiveness indicates that viral influence upon the cell may be responsible for this phenomenon. Other viral diseases such as rubeola and varicella, a bacterial infection pertussis, and fevers of undetermined origin failed to interfere with lymphocyte responsiveness.

The impairment of reactivity to PHA in diseases such as Hodgkin's disease, chronic lymphatic leukemia, and others mentioned above, may be the result of the diverse effects of genetic or acquired influences on the normal development and function of lymphocytes. The outstanding correlation in these clinical syndromes has been that between generally impaired cellular immunity and unresponsiveness of lymphocytes to PHA. The fact that lymphocyte responsiveness appears with the passage of time in congenital rubella indicates that developmental abnormality in lymphoid cell differentiation does not account for this observation.

The explanation of these results must be found in the relationship of the virus to the lymphocyte. The virus had been isolated at least once from the throat, urine, stool, or spinal fluid of each patient with congenital rubella syndrome, or significant neutralizing antibody titers had been demonstrated, before the studies of in vitro lymphocytes. Unresponsiveness to PHA, however, was not always correlated with the concurrent ability to isolate virus from the throat. The success or failure to demonstrate virus in the throat may have little or no bearing on the rela-

tionship between virus and lymphocyte existing at the time of examination. The association of rubella virus with the lymphocytes of infants with the congenital rubella syndrome has not been demonstrated (*15*), but the problem is worthy of continued investigation. The difficulty encountered in isolating the virus from lymphocytes may result from the inability to remove cell-associated neutralizing antibody or from the relationship of the virus and cell (*16*).

A possible basis for the abnormal lymphocyte responsiveness produced by rubella virus and NDV might be an alteration of receptor function at the lymphoid cell surface. Gesner and Ginsberg (*17*) showed that glycosidases at the cell surface contribute to the recognition functions controlling traffic of lymphoid cells within the lymphoid system. Borberg *et al.* (*18*) interfered with the agglutinating effect of PHA on red cells by administering *N*-acetylgalactosamine before treatment with PHA. Furthermore, treatment of lymphocytes with certain enzymes that act on cell surfaces abolishes responsiveness to PHA (*19*). The effect of PHA on lymphocytes is to produce rapid changes in nucleic acid metabolism (*20*); this indicates that the surface action is quickly translated to an influence on cytoplasmic and possibly nuclear metabolism. Our results show that rubella virus and NDV can exert their inhibiting influence even when virus is added to the lymphocyte cultures as late as 24 hours after addition of PHA, which suggests that this inhibition occurs at a site other than the cell surface.

Another explanation for the abnormal lymphocyte responsiveness is the alteration of metabolic pathways by virus thus preventing the mitogenic effect of PHA. Nonlymphoid cells chronically infected with rubella virus have a reduced growth rate (*9, 21*). Although it has been reported that synthesis of protein and ribonucleic acid of cells infected with rubella was unimpaired (*22*), the virus may still produce sufficient alterations to inhibit stimulation of synthesis. In fact infection of human lymphocytes with rubella virus in vitro interferes with protein and ribonucleic acid synthesis induced by PHA (*23*). The finding that virus irradiated with ultraviolet light does not inhibit the PHA responsiveness of lymphocytes supports this hypothesis and suggests that the intact virus genome is required.

The inhibition by these viruses of the response of lymphocyte to PHA suggests that a similar alteration of cellular function may contribute to the persistent shedding of virus in the congenital rubella syndrome. This alteration of lymphocyte responsiveness might also prove a valuable factor in the detection of viruses that cannot be identified by presently available methods. Mella and Lang (*24*) have reported that the number of cells undergoing mitosis was reduced in preparations of leukocytes from patients with infectious hepatitis and, furthermore, that this effect can be produced by the addition of serum from patients with hepatitis to normal leukocytes. Mycoplasma organisms also inhibit mitosis (*25*). The spectrum of agents which might be revealed by this abnormality of lymphocyte responsiveness awaits analysis.

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Gene Activation without Histone Acetylation in Drosophila melanogaster

Abstract. *Chromosomal puffing, generally believed to represent gene activation in* Drosophila, *was induced in the presence of radioactively labeled acetate. There is no preferential uptake of these labeled molecules in regions of gene activation. It is concluded that the acetylation of histones does not play a general role in the regulation of RNA synthesis in* Drosophila melanogaster.

The view has recently been offered that histone acetylation brings about a change in the structure of the chromatin and is a necessary prerequisite for the synthesis of RNA's at previously repressed gene loci (*1*). This viewpoint derives, in part, from studies on the human lymphocyte. For example, when RNA synthesis in suspended lymphocytes is increased following addition of phytohemagglutinin (a kidney bean protein fraction), the increase in the uptake of uridine-2-C^{14} into RNA is preceded by an impressive augmentation in the uptake of sodium acetate-2-C^{14} into histones (*1*).

Although these findings support the view that histone acetylation serves to change the fine structure of the chromatin and thus to allow DNA to serve as a template for RNA synthesis, there is little evidence to show that histone acetylation is spatially correlated with the genetic loci which are activated. The present investigation attempts to determine whether histone acetylation occurs at the site of gene activation in a system, the Dipteran polytene chromosome, in which gene activation is morphologically manifested by chromosomal puffing.

In these experiments, two adjacent puffs (86F and 87A-B of the standard