to determine whether the site of resistance is in the needles, which are the primary infection courts, or in the bark. Both tissues are potential sites of resistance in other white pines, and the type of resistance they confer is apparently inherited independently (*1*, *3*). Thus, either one or both mechanisms, if controlled by single genes in sugar pine, could have been responsible for resistance in these progenies and could have produced the ratios observed. In any case, a simple mode of inheritance of resistance seems indicated. This finding has important implications not only for improved rust resistance in sugar pine, but, through hybridization, for other commercial white pine species as well.

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Peroxidase-Mediated Virucidal Systems

Abstract. *Peroxidase (myeloperoxidase or lactoperoxidase), hydrogen peroxide, and a halide such as iodide, bromide, or chloride form a potent virucidal system that is effective against polio and vaccinia virus, particularly at a low pH. The peroxidase–halide–hydrogen peroxide system may contribute to the host defense against certain viral infections.*

The inactivation of viruses by the host organism is achieved through complex mechanisms which are poorly understood (*1*). Interest has centered on the role of antibody and interferon. A peroxidase–halide–hydrogen peroxide system, which has bactericidal and fungicidal activity (*2–4*), is shown here to have potent virucidal properties and thus may contribute to the host defense against certain viral infections.

Myeloperoxidase (MPO) was purified from canine leukocytes (*5*), and lactoperoxidase (LPO) was purified from bovine milk (*6*). Peroxidase activity was determined by the *o*-dianisidine method (*7*). Type I poliovirus (LSc-2ab strain) and vaccinia virus (Wyeth calf lymph strain), grown in tube monolayer cultures of rhesus monkey kidney cells (Flow Laboratories) and stored at −20°C, were diluted in water to an infectivity titer of 10^6 $TCID_{50}$ per milliliter (*8*), dialyzed against water for 1 hour at 4°C, and filtered through a Millipore filter (0.22 μm pore size) just prior to use. A portion of the virus suspension (0.1 ml) was incubated with the components of the reaction mixture (Table 1, Fig. 1) in a water bath and shaken for 1 hour at 37°C in an atmosphere of air. The reaction was stopped by the addition of 0.1 ml of 0.1*M* sodium thiosulfate, tenfold serial dilutions of the reaction mixture were made in phosphate-buffered saline (*p*H 7.0) containing penicillin (1000 unit/ml), streptomycin (1000 μg/ml), and amphotericin B (5 μg/ml), and 0.1 ml portions were added to duplicate monolayer cultures for determination of infectivity.

Infectivity of poliovirus and vaccinia virus was determined by cytopathic effect in tube monolayer cultures of rhesus monkey kidney cells grown in minimum essential medium (MEM) with Hanks salts (Flow Laboratories) supplemented with penicillin, streptomycin (Grand Island), and 10 percent fetal calf serum (Hyland). The cells were maintained in minimum essential medium with Earle salts supplemented with penicillin, streptomycin, and 2 percent calf serum. The monolayers were incubated at 37°C in a roller drum (New Brunswick) until no further cytopathic changes were observed (usually 5 days). The $TCID_{50}$ was calculated by the method of Spearman-Karber (*9*). Infectivity of poliovirus also was determined by plaque assay on monolayers of HeLa cells. Following adsorption for 30 minutes at room temperature, the monolayers were overlaid with MEM, supplemented with 2 percent fetal calf serum, penicillin, streptomycin, and 0.3 percent Agarose (Bausch and Lomb). After 2.5 days the monolayers were fixed with 5 percent formalin, stained with 1 percent gentian violet in 20 percent alcohol, and the plaque-forming units were counted.

Incubation of poliovirus with the complete myeloperoxidase–iodide–hydrogen peroxide system produced at least a reduction of 10,000-fold in infectivity titer as measured either by cytopathic effect or plaque assay (Table 1). Vaccinia virus also was inactivated by this system (Table 1). Comparable results were obtained if acetate was substituted for lactate buffer at *p*H 4.5. Bromide or chloride could be substituted for iodide although a higher concentration of chloride was required. The complete system was virucidal when human MPO was substituted for the canine enzyme (*10*). Lactoperoxidase could substitute for MPO when iodide or bromide was employed as the halide. However, a virucidal effect was not observed when chloride was used in the lactoperoxidase–halide–hydrogen peroxide system (Table 1). The lactoperoxidase–chloride–hydrogen peroxide system was reported to have low bactericidal activity (*3*). Deletion of the peroxidase or H_2O_2 from the reaction mixture or heat treatment of the enzyme (100°C for 30 minutes) completely prevented the antiviral effect when either iodide, bromide, or chloride was the halide employed. A significant, but reduced, antiviral effect was noted in experiments with vaccinia virus (but not with poliovirus) when the halide was absent. The optimum *p*H was distinctly acid (Fig. 1). Although the virucidal activity decreased sharply as the *p*H approached neutrality, a virucidal effect could be demonstrated at *p*H 7.0 with both the myeloperoxidase and lactoperoxidase systems.

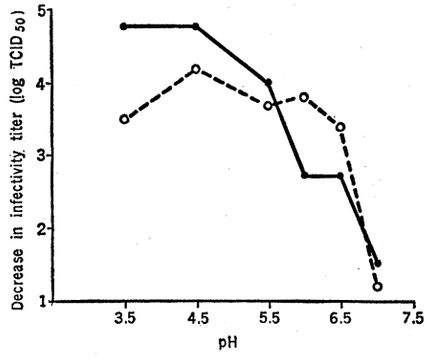

Fig. 1. Effect of *p*H on virucidal activity of peroxidase system. Poliovirus was assayed by cytopathic effect after incubation with iodide, hydrogen peroxide, and myeloperoxidase or lactoperoxidase as described in Table 1. The *p*H was varied as indicated with lactate (*p*H 3.5 to 5.5) or phosphate (*p*H 6.0 to 7.0) buffers.

Iodination of bacteria by the peroxidase–iodide–hydrogen peroxide system

Table 1. Virucidal effect of the peroxidase–halide–hydrogen peroxide system. The reaction mixture (final volume 1.0 ml) contained 20 μmole of sodium lactate buffer (*p*H 4.5) for poliovirus and 20 μmole of sodium-potassium phosphate buffer (*p*H 6.0) for vaccinia virus, either poliovirus [$10^{5.2}$ $TCID_{50}$/ml or 3.0×10^6 plaque-forming units (PFU) per milliliter, as indicated] or vaccinia virus ($10^{4.2}$ $TCID_{50}$/ml), and the supplements as follows: MPO, 30 units; LPO, 30 units; H_2O_2, 0.1 μmole; NaI, 0.1 μmole; NaBr, 0.1 μmole; and NaCl, 10 μmole. The total number of monolayer cultures inoculated at each virus dilution is shown in parentheses. Statistical analysis was performed as described by Finney (*9*).

Supplements	$TCID_{50}$/ml ± S.E.		PFU/ml poliovirus
	Poliovirus	Vaccinia virus*	
None	$10^{5.2\pm0.07}$ (48)	$10^{4.2\pm0.2}$ (12)	3.0×10^6 (8)
MPO+H_2O_2+NaI	$10^{0.8\pm0.09}$ (24)†	$10^{1.4\pm0.1}$ (8)†	< 100 (10)†
MPO(heated)+H_2O_2+NaI	$10^{4.9\pm0.18}$ (8)		2.7×10^6 (6)
MPO+H_2O_2	$10^{4.8\pm0.2}$ (8)	$10^{3.1\pm0.2}$ (12)†	2.8×10^6 (4)
MPO+NaI	$10^{5.1\pm0.27}$ (8)	$10^{3.7\pm0.1}$ (8)	2.6×10^6 (10)
H_2O_2+NaI	$10^{5.1\pm0.08}$ (8)	$10^{4.0\pm0.2}$ (12)	2.5×10^6 (10)
MPO+H_2O_2+NaBr	$10^{0.8\pm0.14}$ (12)†		
MPO+H_2O_2+NaCl	$10^{1.2\pm0.22}$ (12)†		
LPO+H_2O_2+NaI	$10^{0.7\pm0.10}$ (20)†	$10^{0.8\pm0.1}$ (8)†	< 100 (6)†
LPO(heated)+H_2O_2+NaI	$10^{5.3\pm0.23}$ (8)		2.8×10^6 (4)
LPO+H_2O_2	$10^{5.0\pm0.21}$ (12)	$10^{2.6\pm0.1}$ (12)†	2.5×10^6 (6)
LPO+NaI	$10^{5.4\pm0.21}$ (8)	$10^{4.1\pm0.2}$ (8)	3.6×10^6 (4)
LPO+H_2O_2+NaBr	$10^{1.1\pm0.38}$ (8)†		
LPO+H_2O_2+NaCl	$10^{5.0\pm0.19}$ (8)		

* Vaccinia virus was used at *p*H 6.0 due to its inactivation in buffer alone at a more acid *p*H.
† Value significantly different from that of the control ($P < .01$).

occurs under conditions in which a bactericidal effect is observed, and thus the two may be causally related (*2*). A similar mechanism may explain the virucidal effect of the peroxidase–iodide–hydrogen peroxide system since iodination of virus particles can occur and may result in a loss of infectivity (*11*). Myeloperoxidase and H_2O_2 have an antimicrobial effect in the absence of halide under certain conditions (*3, 12*). A significant but reduced virucidal effect on vaccinia virus (but not on poliovirus) in the absence of halide was observed here. The addition of a halide with the virus preparation or with the other reagents cannot be excluded.

The components of the peroxidase–halide–hydrogen peroxide virucidal system are present in mammalian tissues and extracellular fluids. Peroxidases are found in the mammary, salivary, lacrimal, and harderian glands and their secretions (lactoperoxidase), in neutrophils (myeloperoxidase), in eosinophils, and in the thyroid. Hydrogen peroxide is formed in the polymorphonuclear leukocyte after phagocytosis (*13*), in certain extracellular fluids (for example, by the xanthine–xanthine oxidase system in milk), and by microbial metabolism either within leukocytes or extracellularly. The halides are widely distributed in extracellular and intracellular fluids although their concentration varies.

Many viruses are engulfed by neutrophils, mononuclear cells, and tissue macrophages (*14–16*). Some investigators have emphasized the protection afforded by intraleukocytic residence of viruses against specific antibodies and other nonspecific viral inhibitors of blood and have pointed to phagocytosis as a means of disseminating viral particles (*15*). However, others have reported a rapid decrease in the titer of certain viruses following ingestion by leukocytes (*16*). The nature of the intraleukocytic virucidal systems is not known. Peroxidase-mediated systems may exert a virucidal effect in leukocytes and in certain extracellular fluids (for example, saliva, milk, tears, and areas of inflammation) and may thus contribute to the host defense against viral infection.

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Antigen Competition: Antigens Compete for a Cell Occurring with Limited Frequency

Abstract. *Experimentally altered ability of transferred spleen cells to generate hemolytic plaque-forming cells provided evidence that antigens compete for a type of multipotential cell that contributes to the formation of immunologically competent units. Delay of exposure of transferred spleen cells to antigen provided results which suggest that different types of cells interact to form competent, antigen-reactive units even in the absence of antigen.*

Concurrent primary antibody formation against an unrelated antigen quantitatively reduces the primary immune response to a given test antigen. Immunologists have known of antigen competition for many years (*1*; for review, see *2*) but have no satisfactory explanation of the manner in which antigens compete. Competition occurs between soluble antigens (*3*), particulate antigens (*4–6*), a soluble and a particulate antigen (*7*), and between chemically defined haptens (*8–10*). Response to a particulate antigen may prolong survival of allografts (*5, 7*). Sev-