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Changes in Permeability Induced by Victorin

Abstract. When susceptible oat tissues were treated with victorin, the toxin produced by the fungus *Helminthosporium victoriae*, and suspended and shaken in water, they lost electrolytes much more rapidly than untreated controls. Similar results were obtained with susceptible plants infected with *H. victoriae* but not with victorin-treated or inoculated, resistant plants. These results provide further evidence of the specificity of victorin and its ability to produce all the symptoms of Victoria blight of oats. They also suggest that changes in permeability, by affecting the salt balance of cells, may play a role in the augmented respiration characteristic of diseased plants.

Most toxic agents produced by plant pathogens fail to induce all the symptoms characteristic of the disease or to show the specificity exhibited by the pathogen. This has made the role of such toxins in plant diseases difficult to evaluate. However, *H. victoriae* and its product, victorin, both cause severe damage only to oat varieties of Victoria lineage; the visible symptoms induced by the two agents are indistinguishable (*1*, *2*).

Furthermore, both the fungus and the toxin cause similar metabolic abnormalities (augmented respiration, decreased sensitivity to the uncoupling agent 2,4-dinitrophenol, and increased oxidation of ascorbic acid); these effects are obtained only with tissues susceptible to the pathogen (*3*). Victorin therefore provides an excellent tool for investigations of biochemical changes in diseased plants. Since changes in cell permeability are characteristic of many plant diseases, we studied the effects of *H. victoriae* and victorin on permeability.

Changes in permeability were estimated, in most cases, from variations in electrical conductivity of distilled water in which the tissues were suspended and shaken. Cut stems of resistant and susceptible plants were placed in diluted solutions of a crude victorin preparation which when undiluted had an activity of 500 units per milliliter (*2*). After 2 hours of uptake, 1-g samples of leaf tissue were washed five times with distilled water and then shaken (120 strokes per minute) in 100 ml of distilled water. Controls were allowed to take up distilled water or diluted solutions of deactivated victorin (*2*). A conductivity bridge was used to measure changes in the specific conductance of the water in which the tissues were shaken.

The results of a typical experiment with susceptible plants are shown in Fig. 1. The baseline values, taken after 1 hour, show that during this period victorin-treated tissues lost electrolytes to the ambient solution more rapidly than the controls, and that the effect varied with the concentration of the victorin solutions applied. Tissues treated with the two highest concentrations of victorin continued to lose electrolytes more rapidly than the controls during the remainder of the test period.

Similar tests with resistant plants showed no significant differences between victorin-treated and control tissues. Thus, in this as in its other effects, victorin is specific for tissues susceptible to *H. victoriae*. Tests of susceptible plants, naturally infected with *H. victoriae*, gave results comparable to those obtained with victorin-treated susceptible tissues.

Tests in which permeability changes were estimated by a modification of Thatcher's deplasmolysis method (*4*) confirmed the results of conductivity tests. Isotonic sucrose solutions of double strength, rather than urea or the salts used by Thatcher, were used to plasmolyze cells; deplasmolyzation time was determined in half isotonic sucrose solutions. With this method, infection with *H. victoriae* resulted in an average increase in permeability of 40 percent.

Changes, usually increases, in cellular

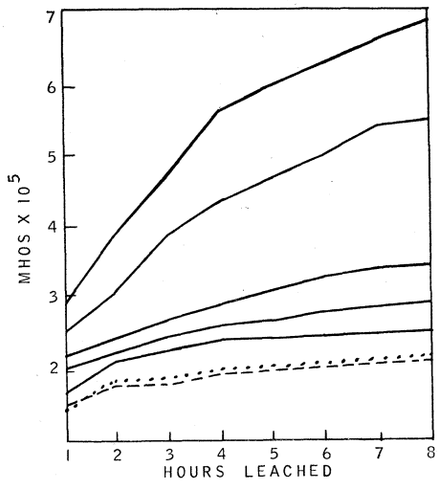

Fig. 1. Changes in electrical conductivity, expressed in reciprocal ohms (mhos), of distilled water in which oat tissues susceptible to *H. victoriae* were suspended and shaken. Solid lines represent results with tissues which had taken up victorin solutions diluted 10, 10^2, 10^3, 10^4, 10^5, times, reading down from the top. The dotted line is a control which had taken up water and the dashed line a control which had taken up deactivated victorin diluted tenfold.

respiration and permeability are probably the two most characteristic features of plant diseases. This plus the fact that both are induced by victorin raises the question of a possible relation between the two phenomena. The nature of increased respiration in diseased plants has received much attention in recent years and several mechanisms—increased activity of normal metabolic pathways, shifts to new pathways, activation of specific enzymes, and uncoupling of oxidative phosphorylation—have been advanced to account for the increased rates (*5*). Under certain conditions, various inorganic salts may cause marked increases in respiratory rates in plant tissues. Although the nature of salt-stimulated respiration is, at present, a subject of controversy (*6*), it seems clear that changes in permeability will result in changes in rates of salt uptake and transfer as well as in concentration in various parts of the cell. The possibility that permeability changes, by affecting the salt balance or by other means, may bring about respiratory changes in diseased plant tissue is currently under investigation (7).

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Blood Groups and Splenomegaly in Chick Embryos

Abstract. The B blood group locus in chickens is shown to be associated with the graft-against-host reaction in chick embryos.

In a recent paper Schierman and Nordskog (*1*) have shown that when chicken donors and hosts have the same B blood group allele, survival of skin grafts is prolonged. A similar relationship exists in the mouse, where strains differing in alleles of the H-2 locus not only manifest accelerated rejection of tissue grafts but also differ in respect to red cell antigens (*2*). In addition Billingham (*3*) has reported more severe "runting" symptoms in baby mice made tolerant with cells from strains differing at the H-2 locus. It appears, then, that genes of the H-2 locus are involved in histocompatibility and blood group difference and that they also play a role in the graft-against-host reaction.

Information about the physiological function of cell antigens is of profound biological significance and the purpose of this investigation is to add to this knowledge by exploring the relationship between red cell antigens and the induction of splenomegaly in non-inbred lines of chickens.

Sterile blood was obtained from adult hens of three B locus genotypes, designated B 19/19, B 21/21 and B 19/21. Fifteen-day-old embryos of all three genotypes each received 0.1 ml of blood intravenously from a single donor. The weights of the spleens removed 4 days later, which were used as a quantitative indication of the graft-against-host reaction (*4*), are shown in Table 1.

Table 1. Average spleen weights and standard errors for chick embryos injected with blood from blood-grouped adult female donors.

Donor hens		Av. wt. (mg)	Embryos (No.)
Blood type	No. used		
Blood type of hosts 19/19			
19/19	1	16.9 ± 1.2	24
19/21	2	15.5 ± 0.9	24
21/21	3	98.8 ± 6.8	37
Controls		11.8 ± 0.7	12
Blood type of hosts 19/21			
19/19	1	81.3 ± 6.7	9
19/21	2	19.3 ± 1.5	3
21/21	3	118.9 ± 12.7	10
Controls		14.8 ± 0.9	13
Blood type of hosts 21/21			
19/19	1	96.1 ± 6.9	28
19/21	2	16.4 ± 0.9	22
21/21	3	18.7 ± 1.0	27
Controls		12.2 ± 0.6	11
Ungrouped			
19/19	1	76.3 ± 5.7	27
19/21	2	65.4 ± 9.0	20
21/21	3	84.1 ± 8.7	19
Controls		13.8 ± 0.7	11

From these figures it is clear that where host and donor are of the same B locus genotype, little or no splenomegaly is produced, whereas considerable enlargement of the spleen occurs where they differ. The figures from the B 19/21 donors are particularly relevant to the graft-against-host nature of this phenomenon, in that although the donors' blood type differs from that of two of the host groups, no enlargement occurs in any of them, since the hosts do not possess any B group antigens foreign to the donors. The slight enlargement seen in the groups where none would be expected if only the B locus were involved, presumably indicates that other antigens play an additional, if minor, role.

Whether the underlying genetic situation here resembles that of the H-2 region in the mouse or that of other antigen complexes in man and cattle must remain in doubt until time has afforded further opportunities for the detection of recombinations. In any event, the variety of effects associated with the B locus (see also *5* for the relationship with reproductive fitness) suggests that this region of the fowl chromosome exerts a profound effect on cell functions.

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Calcium and Other Ions in Blood and Skeleton of Nicaraguan Fresh-Water Shark

Abstract. The bull shark, *Carcharhinus leucas*, employing archaic but effective means of regulating the physical-chemical composition of its body fluids, thrives in tropical fresh-water rivers and lakes. The ionic strength of the serum and the concentrations of total solutes, calcium, urea, and other ions are below the levels found in marine elasmobranchs but higher than the levels in teleosts. The patterns of the calcium deposits of the vertebrae are identical in marine and fresh-water subspecies.

Migrations into fresh water of the bull sharks of Lake Nicaragua in Central America, Lake Sentani of western Dutch New Guinea, and the Ganges and other Asiatic rivers have been reported by Herre (*1*) and Boeseman (*2*). The physiology of these fishes presents extraordinary mechanisms of ion and osmotic regulation. Smith (*3*) reported a decrease in the serum concentration of urea with relatively little change in phosphate or chloride in small sharks and sawfish found in rivers of Malaya and Siam; no data were recorded about the other solutes of the blood. My previous research (*4*) has included work on the chemistry of calcium and the major components of the blood and skeleton of the bull shark of the Atlantic, *Carcharhinus leucas*, and 13 other species of marine elasmobranchs, but none were fresh-water migrants.

On 27 March 1962, one tarpon (*Megalops atlanticus*) and four bull sharks, three females and one male, ranging in length from 5 feet 11 inches to 7 feet 1 inch, were taken in southeastern Nicaragua from Rio San Juan (*5*) near El Castillo. Small fishes, alligators, and viscera of swine were used as bait, but only freshly caught tarpon brought results. The fish were photographed after exsanguination by cardiac puncture and section of the caudal artery. Blood was collected in thermos