Concurrent Morphological and Chemical Events in Staphylococcus aureus Exposed to Penicillin

Abstract. Disruption and lysis as well as accumulation of cell-wall precursor material have been demonstrated in several strains of Gram-positive cocci as results of the action of penicillin. The concurrence of these phenomena in typically penicillin-sensitive bacteria lends further support to the hypothesis that penicillin exerts its antibiotic action through interference with the formation of cell walls.

A widely accepted hypothesis explains the antimicrobial action of penicillin in terms of an inhibition of the formation of a certain basal polymer of bacterial cell walls (*1*). Biochemical evidence for this type of action has been provided by the findings that a muramic acyl pentapeptide is a ubiquitous constituent of bacterial cell walls and that in penicillin-sensitive *Staphylococcus aureus* large quantities of this monomeric substance, linked to uridine diphosphate, accumulate during penicillin action (*2*). Lysis of *S. aureus* under the influence of penicillin has rarely been mentioned (*3*). On the other hand, morphological evidence of damage to cell walls, such as spheroplast formation and ultimate lysis, is readily observed in Gram-negative bacteria (*4*). These organisms are insensitive to penicillin and require excessive concentrations of the antibiotic before they exhibit cytological changes; accumulation of cell-wall precursor material is not a prominent phenomenon in these bacteria.

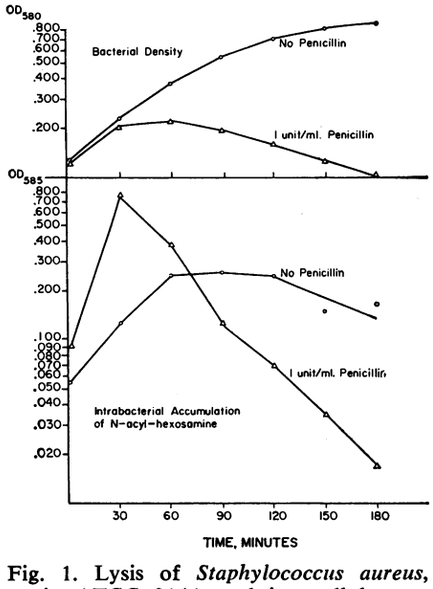

Fig. 1. Lysis of *Staphylococcus aureus*, strain ATCC 9144, and intracellular accumulation of N-acyl aminohexose during growth in the presence of penicillin.

Thus, two lines of evidence derived from the study of different categories of bacteria have been assembled into a unified hypothesis of penicillin action (*1*). This hypothesis would be strengthened further if it could be shown that all the essential events caused by penicillin occur in one and the same typically penicillin-sensitive organism. We wish to report the demonstration of this occurrence in strains of *S. aureus*.

Several strains of *S. aureus* (strain H, strains SA-13 and SA-9, and standard strains ATCC 9144 and 9996) (*5*) were grown in a medium consisting of yeast extract and peptone (*6*); growth and penicillin-induced lysis were followed spectrophotometrically at 580 mμ (Fig. 1). The lowest concentrations of penicillin which caused lysis of the different test strains were similar in magnitude to the minimal growth-inhibitory concentrations that were determined by a previously published method (*7*). No turbidimetric indication of lysis was obtained when sucrose (0.64*M*) was supplied to the experimental medium for osmotic protection.

Accumulation of N-acyl-aminohexose was determined as described previously (*7*). Such analytical results are indicative of the quantities of uridine-diphosphate-muramic acyl peptides present in bacteria (*8*). Figure 1 shows the typical increase in the intracellular amounts of this material in penicillin-exposed *S. aureus*. A seeming decrease in N-acyl-aminohexose coincided with the onset of lysis and thus with the decrease in the number of intact organisms. Penicillin-free control cultures accumulated smaller amounts of N-acyl-aminohexose.

Microscopic observation of penicillin-exposed *S. aureus* employing specific staining of the cell walls after Robinow's method (*9*) produced the results shown in Fig. 2. After 90 minutes' exposure to penicillin and immediately prior to the onset of lysis, the bacteria were enlarged and the cell walls appeared to be extended (Fig. 2*b*) by comparison with the cell walls of the normal organisms (Fig. 2*a*). After 180 minutes, that is, when lysis had been under way for 90 minutes, numerous folded-up and wrinkled objects were seen which in our opinion represented empty cell walls remaining after the lysis of bacteria (Fig. 2*c*).

Our study closes a gap in the current hypothesis of penicillin action in that the biochemical consequences of inhibition of cell-wall formation and the cytological consequences of cell-wall damage have been shown to occur in each of five penicillin-sensitive strains of *S. aureus*, among them strain H, which has been used in the classical studies of Park and Strominger (*2*). Although the ultimate question of the mechanism by which penicillin interferes with the integration of muramic acyl pentapeptide into the cell-wall polymer still remains to be answered, we believe our work lends additional support to the current hypothesis (*1*) of penicillin action (*10*).

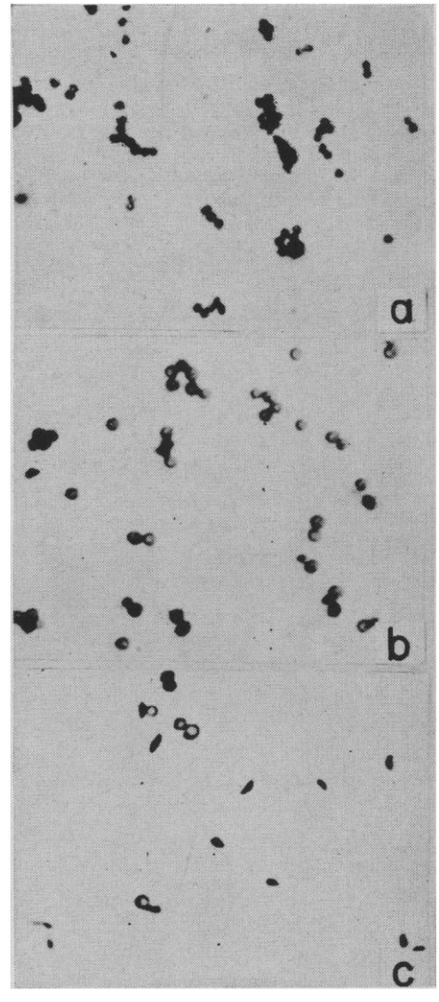

Fig. 2. Cytological changes of *Staphylococcus aureus*, strain ATCC 9144, growing in the presence of 0.075 unit of penicillin per milliliter: *a*, penicillin-free control; *b*, 90 minutes' exposure to penicillin; *c*, 180 minutes' exposure to penicillin.

JENNIE CIAK
FRED E. HAHN
Department of Molecular Biology, Walter Reed Army Institute of Research, Washington 12, D.C.

References and Notes

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10. We thank Herbert Whitfield for his skilled technical assistance.

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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

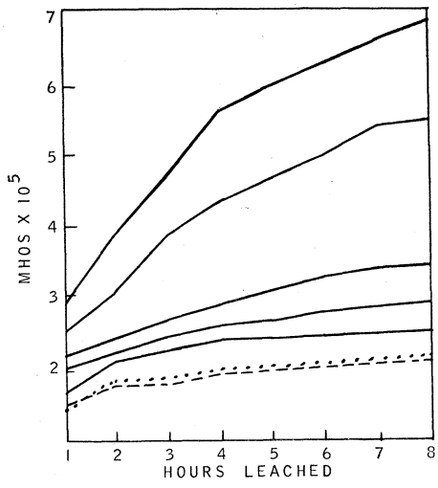

Fig. 1. Changes in electrical conductivity, expressed in reciprocal ohms (mhos), of distilled water in which oat tissues susceptible to *H. victoriae* were suspended and shaken. Solid lines represent results with tissues which had taken up victorin solutions diluted 10, 10^2, 10^3, 10^4, 10^5, times, 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).

HARRY WHEELER
HOMER S. BLACK

Department of Botany and Plant Pathology, Louisiana State University, Baton Rouge