vice. Several aggregates are associated with the pore.

The platelets that make up the aggregates in Figs. 1 and 2 have apparently undergone a change in shape, and have coalesced irreversibly into semisolid masses. Work in collaboration with A. R. Williams of the University of Manchester has shown that the platelets in such aggregates have also released the contents of their cytoplasmic granules. The luciferin-luciferase enzyme system of fireflies was used to detect adenosine triphosphate released by the platelets during sonication (*5*). This technique may eventually allow more accurate quantification of our results than is possible with simple microscopic inspection.

Two physical mechanisms appear to be responsible for the effect (*6*). First, the pulsating gas in a pore intensifies the acoustic field near the pore, and within this field platelets are attracted by radiation force to the pore. Therefore, during sonication the platelets gather and become concentrated near the pores. This concentrating action in itself may be sufficient to cause irreversible aggregation; Massini and Lüscher (*7*) showed that close cell contact can induce the release reaction in platelets. Second, the local acoustic field around a pore produces an acoustic boundary layer at the membrane surface, which results in acoustic microstreaming flow around the pore. This fluid flow includes velocity gradients near the membrane surface, which can damage platelets by subjecting them to shear stress as they approach the pore and are caught up in the flow. Brown *et al.* (*8*) demonstrated that platelets are exceptionally sensitive to shear and may be induced to form aggregates by shear stresses as low as 50 dyne/cm^2. Because both mechanisms—concentration of the platelets and shear stress—act near the ultrasonically activated gas-filled pores, both mechanisms must be considered important in the etiology of platelet aggregation in our experiments.

Under our specialized experimental conditions, we have demonstrated platelet aggregation by low-intensity ultrasound. This significant effect apparently requires the presence of some form of stable cavitation, which was provided in this work in the form of air-filled pores in hydrophobic membrane strips. The aggregation phenomenon appears to have a threshold intensity of 16 to 32 mW/cm^2 for 10 minutes of sonication at a frequency of 2.16 MHz with pores 4 μm in diameter. A commercial ultrasound instrument used in diagnostic medicine was found to have a spatial peak intensity greater than this threshold, and platelet aggregates were produced when a membrane strip immersed in citrated PRP was exposed to the ultrasonic beam generated by this device. At this time it is not known whether these results indicate that any risk is associated with the application of such devices in medicine, because it is not known whether any form of stable cavitation, such as was required here for production of platelet aggregates, occurs in vivo under conditions characteristic of medical practice. This technique does, however, provide sufficient sensitivity to demonstrate a biological reaction to low-intensity ultrasound, and may be suitable for evaluating the relative safety of medical procedures involving ultrasound.

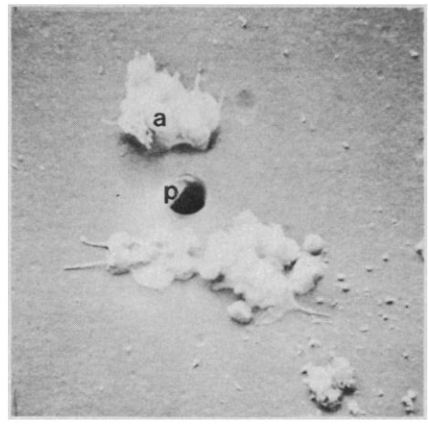

Fig. 2. Scanning electron photomicrograph of aggregates (*a*) associated with a pore (*p*) after exposure to ultrasound generated by a Doppler ultrasound device used in medicine (pore diameter, 4.5 μm).

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Prevention of Fungal Infection of Plants by Specific Inhibition of Cutinase

Abstract. *Specific antibodies prepared against cutinase from* Fusarium solani pisi *and diisopropylfluorophosphate, a potent inhibitor of this enzyme, prevented infection of the host (pea epicotyl) by this organism, without affecting the viability of the spores. This finding shows that enzymatic penetration of cuticle is involved in pathogenesis.*

Fungal infection of plants constitutes one of the major problems in agriculture. If, as suggested before (*1*, *2*), fungal penetration of plants involves enzymatic digestion of the cuticular polymer cutin, it might be possible to prevent fungal infection by chemicals targeted against this enzyme. We present results indicating that infection of pea epicotyl by the pathogen *Fusarium solani pisi* can be prevented by (i) specifically inhibiting the fungal cutinase with rabbit antibodies prepared against this enzyme, and (ii) by diisopropylfluorophosphate (DFP), an inhibitor of this enzyme. This evidence opens the way to the development of a novel, specific, enzyme-directed weapon against fungal attack of plants.

Fusarium solani f. pisi (ATCC 38136) was grown on potato dextrose agar; a suspension of micro- and macroconidia was prepared as described (*3*). Cutinase I from the extracellular fluid of cutin-grown *F. solani pisi* was isolated in electrophoretically homogeneous form (*4*), rabbit antiserum against the enzyme was prepared, and the immunoglobulin fraction was isolated from the serum (*5*). The conidial suspensions were mixed with appropriate portions of the immunoglobulin fraction or DFP, and 5-μl droplets of the suspension were placed on the surface of 1.2-cm sections of epicotyl from 6-day-old etiolated seedlings of *Pisum sativum* cv. Perfection. Each treatment consisted of 45 sections of the epicotyl resting on moist filter paper in petri dishes. After incubation of the inoculated tissue sections at 22°C for 72 hours in the dark, the infected sections, as shown by

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Table 1. Effect of antibodies and an inhibitor of cutinase on infection of pea epicotyl by *F. solani pisi*. The inoculum (5 μl) contained 1.2×10^6 spores and the indicated levels of immunoglobulin (IgG) per milliliter. Infection is expressed as the percentage of epicotyl segments infected.

Treatment	Amount	Infection (%)
None		86
Rabbit IgG from serum	4 mg/ml	90
Rabbit IgG from serum	8 mg/ml	87
Rabbit IgG from serum	6 mg/ml	93
IgG from antiserum	1.75 mg/ml	20
IgG from antiserum	3.5 mg/ml	0
Diisopropylfluorophosphate	0.01 m*M*	0
Diisopropylfluorophosphate	1.0 m*M*	0

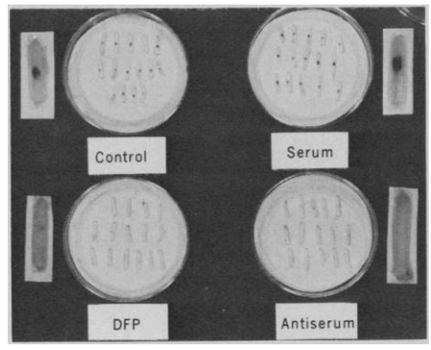

Fig. 1. Photograph of pea epicotyl segments 72 hours after inoculation with 5 μl of conidial suspension of *F. solani pisi* in water (*Control*), water containing 16 mg of protein per milliliter of immunoglobulin fraction from rabbit serum (*Serum*), water containing 1.75 mg of protein per milliliter of rabbit immunoglobulin from antiserum prepared against cutinase (*Antiserum*), and water containing 10 μ*M* DFP. In each case a magnification of a representative tissue segment is also shown.

the dark spot (Fig. 1), were counted; infection is expressed as the percentage of epicotyl segments infected.

Diisopropylfluorophosphate, which is a potent inhibitor of cutinase (*4*), virtually prevented infection of pea epicotyl by *F. solani pisi* (Table 1 and Fig. 1). That the lack of infection was not due to a general toxicity of this chemical to the organism was indicated by microscopic examination of the inoculated region, which showed profuse growth of germinated conidia on the tissue surface, at all of the concentrations of the inhibitor used in this experiment.

Antibody prepared against cutinase prevented infection of the tissue by *F. solani pisi*, whereas the immunoglobulin fraction from rabbits, not immunized with cutinase, had no effect on infection (Fig. 1 and Table 1). Microscopic examination of the inoculated area showed profuse growth of germinated conidia, indicating that immunoglobulin did not adversely affect the germination or growth of the organism. These results clearly show that the specific inhibition of cutinase, known to be caused by the antibodies (*5*), prevented fungal entry into the plant, and thus prevented infection.

Chemical examination of the cuticular polymer of the epicotyl segments by the combined gas-liquid chromatography-mass spectrometry techniques described (*6*) showed that the cutin was composed of chiefly dihydroxypalmitate, ω-hydroxypalmitate, and palmitic acid. These results confirm the previous electron microscopic evidence concerning the presence of cutin on this tissue. The previous finding that *F. solani pisi* excretes cutinase during penetration of its host (*3*) and our finding that specifically blocking the action of this enzyme prevents infection constitute convincing evidence that cutinase is involved in the entry of this pathogen into its host. As was pointed out before (*2*), the tendency to ignore the cuticle, and particularly cutin, as a barrier to pathogen penetration is not justified on the grounds that many pathogens enter the plant via natural openings, because the pathogen must penetrate a cutin layer even when they enter through such channels. Thus, our findings are likely to have widespread application to the understanding of fungal pathogenesis of plants. Even though cuticular penetration is not likely to be involved in the specificity of host selection by fungal pathogens, prevention of penetration would prevent pathogenesis. Since cutinase is not involved in the normal metabolism of either plants or animals, it might be feasible to devise chemicals that are specifically targeted against this enzyme and do not interfere with the metabolism of the host plant or animals which consume the plant products. Thus the present results could be the foundation for the development of enzyme-targeted fungicides ("antipenetrants").

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A Relationship Between DNA Helix Stability and Recognition Sites for RNA Polymerase

Abstract. *The RNA polymerase binding sites on the DNA of (i) the* aroE-trkA-spc *segment of the* Escherichia coli *genome, (ii) transposon Tn3, (iii) plasmid ColE1, and (iv) coliphage* λ *were mapped by electron microscopy, with the use of the BAC technique; these maps were compared with the maps of the early-melting regions for the same genomes. The results indicate that in all these cases the binding sites for the* E. coli *RNA polymerase lie preferentially in the early melting regions of DNA. These data indicate that helix stability may be an important feature of the multipartite nature of the promoter structure.*

Although the nucleotide sequences of many different promoters and promoter-flanking DNA regions are now available (*1-7*), the analysis of these sequences does not fulfill the original hope of leading to a detailed understanding of the interaction between RNA polymerase and DNA (*1*). There are indications of a relationship between the helix stability along the DNA molecule and the location of those DNA regions that preferentially bind RNA polymerase. For example, we found for the genome of coliphage λ a remarkable correlation between the DNA regions which preferentially melt (*8*) and sites specifically binding *Escherichia coli* RNA polymerase (*9*), as detected by direct visualization by the BAC (benzyldimethyl-C_{12}-C_{14} ammonium chloride) technique (*10*). Our findings are in agreement with analogous results obtained for phage λ by indirect techniques (*11, 12*). To determine whether this correlation holds more generally, we mapped by