the cerci aid in the control of equilibrium and compensatory reflexes during flight. While the suggested roles of predator and sound detector remain possibilities, our findings lend support to the view that the cerci also function as equilibrium organs.

Since *Arenivaga* is a burrowing cockroach and some desert species have been found at depths of 60 cm (*11*), one might think that the tricholiths would be broken off during burrowing. The cerci of larvae and adult female specimens are contained within cavities in the abdomen. Those of adult males protrude prominently from the abdomen, but are afforded protection on the dorsal side by wings that overhang them. The tricholiths of the males are protected from soil contact on the ventral side by being located within a concave depression surrounded by trichobothria and sensilla chaetica. While there is some variation in the number of these pendulous sensilla normally found on the cerci of adult animals, missing or broken-off tricholiths are rare.

Tricholiths are not unique to *Arenivaga*. Similar pendulous sensilla have been identified on the cerci of three genera of crickets—*Achaeta*, *Gryllus*, and *Gryllotalpa* (*2*)—and several species of cockroaches in the family Polyphagidae (*3*). The insects listed are nocturnal, fossorial species that dig or live in impermanent burrows or "swim" through quasi-fluid soil or litter without leaving a tunnel. Such forms are less able to assess asymmetric tensions of the limbs in order to derive equilibrium information since the substrate collapses about them and does not provide a firm foothold. A discrete receptor system detecting spatial position would be a particularly desirable adaptation for these insects. Behavioral experiments should indicate the extent to which *Arenivaga* is dependent on the system for geotactic adjustments.

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12. This report is dedicated to Dr. L. M. Roth on the occasion of his 60th birthday. We thank F. Bailey, V. Hamilton, and B. W. Leander for technical assistance. Supported by grants from the Institute for University Research of Texas Tech University, the National Science Foundation (BNS 7722283), and the National Aeronautics and Space Administration (NSG-7435).

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Platelet Aggregation Induced by Ultrasound Under Specialized Conditions in vitro

Abstract. *Human platelets were induced by 2.1-megahertz ultrasound to form aggregates around gas-filled pores in membranes immersed in platelet-rich plasma. The spatial peak intensities required were only about 16 to 32 milliwatts per square centimeter. Ultrasound generated by a medical Doppler device, whose intensity exceeded this, induced aggregate formation under the same conditions.*

The findings reported here resulted from a search for systems and methods sensitive enough to detect biological effects of ultrasound under conditions characteristic of medical diagnostic practice. A procedure that yields reproducible results and is convenient to use would be valuable for investigating factors that determine any potential for hazard posed by medical use of ultrasound. It is not easy to find systems sensitive enough because of the low intensities typically used for diagnostic equipment.

In the approach reported here, we utilized hydrophobic membranes with straight-through pores a few micrometers in diameter (Nuclepore Corp., Pleasanton, California). The pores retain gas when the membranes are immersed in aqueous media. The gas pockets or bubbles are stable in the pores; they are small in size and, if they were formed as free gaseous bodies in the medium, would quickly shrink and disappear. When sonicated at megahertz frequencies, these stablized gas bubbles pulsate and thus provide a controlled form of acoustic cavitation in the medium. Cavitation has been cited as a major mechanism for the biological effects of ultrasound, but its occurrence in low-intensity experiments is often problematic. In our experiments this uncertainty was obviated by exposing stable bubbles to the medium via the porous membranes.

The biological subject in these studies was the human blood platelet. Platelets are present in large numbers in blood, and they contribute to blood coagulation by sticking to surfaces and aggregating to form a hemostatic plug at a site of blood vessel damage. During these processes they change in shape, release certain biochemicals from their cytoplasmic granules, and fuse together to form a semisolid mass. Platelets may be important in the pathogenesis of some thrombotic diseases. Agents that might cause unwarranted platelet aggregation in vivo may have a potential for producing serious effects. Williams *et al.* (*1, 2*) found that ultrasonic exposure can modify platelet morphology and function in vitro. In our experiments, suitable exposure of human platelet-rich plasma (PRP) to the ultrasonically activated gas-filled pores resulted in the formation of platelet aggregates near the pores.

The general procedures have been described (*3*). The PRP was prepared by centrifugation of fresh human whole blood (from an apparently healthy male donor) collected into trisodium citrate as anticoagulant. The PRP, containing roughly 300,000 platelets per microliter, was pipetted directly into a sample chamber, which had a 1-mm-wide strip of porous membrane mounted in it. The chamber was formed by two 15-μm-thick sheets of polyvinyl chloride film clamped between two circular plastic rings (inner diameter, 3 cm) and held about 0.5 ml of PRP. For this work, the porous membranes were 12 μm thick and contained pores 4 μm in diameter (standard deviation, 0.3 μm) of number density 720 mm^{-2}. After sonication, the strip was in-

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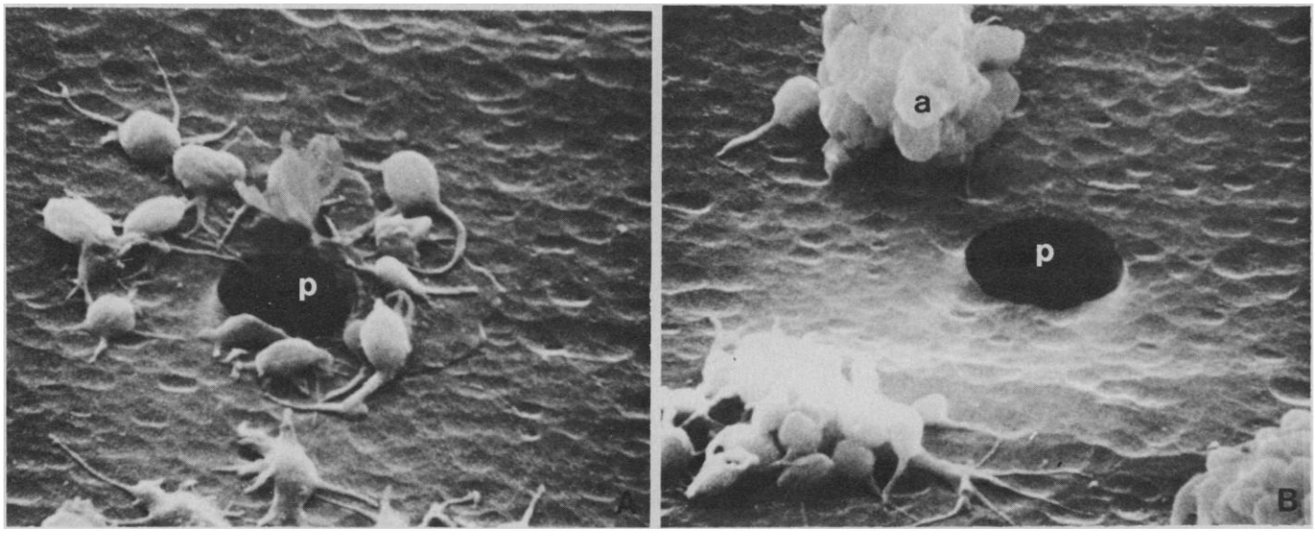

Fig. 1. Scanning electron photomicrographs of platelets that have remained attached to the membrane strip after sonication. (A) Platelets gathered near a pore (*p*) but individually attached to a membrane strip exposed to 8 mW/cm² for 10 minutes (pore diameter, 4.8 μm); (B) aggregates (*a*) associated with a pore after exposure to 125 mW/cm² for 10 minutes (pore diameter, 4.2 μm).

spected by optical microscopy while still in the chamber. The strip was then removed, together with adhering aggregates, fixed in 4 percent paraformaldehyde, and prepared for scanning electron microscopy (SEM) by critical-point drying in Freon and then by gold coating.

Two ultrasonic generators were employed: a laboratory system and a commercial Doppler device used in medicine. The ultrasonic fields produced were measured by a standard method (*4*). The laboratory system consisted of an air-backed transducer, 1.9 cm in diameter, with a signal generator and amplifier. The transducer was mounted at one end of a 40 by 40 by 120 cm water-filled tank, maintained at 37°C, so that the ultrasonic beam was directed horizontally. An absorber made of rubber wedges was placed at the far end of the tank to intercept the transmitted beam and reduce standing-wave formation by echoes. At the fundamental resonance frequency of 2.16 MHz, this system produced a beam with a spatial peak intensity at the sample holder (12 cm from the transducer) of 35 mW/cm² for 1-V (root-mean-square) continuous excitation and with 3-dB beamwidth of 10 mm at this position.

The Doppler device was a Hewlett-Packard Cardiotocograph (model 8030A) with a split-disk, narrow-beam transducer (model 15274A). It is representative of a class of instruments widely used for long-term monitoring of fetal heart rate during labor. The 2.1-MHz transducer was mounted in the laboratory tank and produced a spatial peak intensity of 80 mW/cm² at the position of the sample chamber (4 cm from the transducer). The spatial average intensity at the transducer face was found to be 21 mW/cm², in approximate agreement with the manufacturer's specification of 18.8 mW/cm² (this instrument had no front-panel intensity adjustment).

We could not visually discern any difference between samples sonicated at intensities differing by less than a factor of 2 (3 dB) (*3*). For this work, samples in freshly assembled chambers were sonicated at spatial peak intensities of 250, 125, 64, 32, 16, and 8 mW/cm² for 10 minutes with the laboratory system. This entire experiment was repeated on three different occasions, with blood from the same donor, but with the sonication intensities applied in a different order in each experiment. As observed by optical microscopy immediately after sonication, the results of all three experiments were similar. Distinct platelet aggregates were seen near the pores after sonication at or above 32 mW/cm², and the aggregates increased in size with increasing intensity. At 8 mW/cm², platelets were gathered around the pores, but these gatherings gradually dispersed and were not considered to be aggregates. At 16 mW/cm², the results were not consistently describable as aggregates or gatherings for the three repetitions. From these observations, we conclude that the aggregation phenomenon has a threshold of about 16 to 32 mW/cm² (spatial peak) for these conditions. Above this threshold, distinct, irreversible platelet aggregates were produced, while at lower intensities only reversible gatherings of platelets were seen around the pores.

Several of the strips sonicated with the laboratory system was subsequently observed by SEM. Many of the aggregates formed at the higher intensities do not adhere to the membrane throughout the fixing and critical-point drying procedures; hence the SEM observations do not reliably determine the presence or distribution of aggregates on the strips as they existed immediately after sonication. Nevertheless, several interesting observations were made. A few platelets adhere individually to the strips even in controls. However, in the controls they were distributed randomly, while at intensities suitable for gathering to be observed they were concentrated around the pores. Figure 1A is an SEM photomicrograph of individual platelets adhering to a membrane sonicated at 8 mW/cm². Note that the platelets are concentrated near the pore. When aggregates were observed by SEM, the platelets composing them appeared to be bound to one another (to form a semisolid mass) rather than discretely bound to the membrane. Figure 1B shows an example of small aggregates found on a strip sonicated at 125 mW/cm². Note that the aggregates are separated from the pore but still associated with it. We found larger aggregates (up to 30 μm across) on strips sonicated at 125 mW/cm², but they were not as clearly associated with a single pore as those shown in Fig. 1B.

The roughly defined threshold of 16 to 32 mW/cm² is less than the spatial peak intensity produced by the Doppler device. It was therefore not surprising to find that sonication of similar PRP samples for 10 minutes with this device also produced aggregates. Figure 2 is an SEM photomicrograph of a small portion of one strip sonicated with the Doppler de-

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.

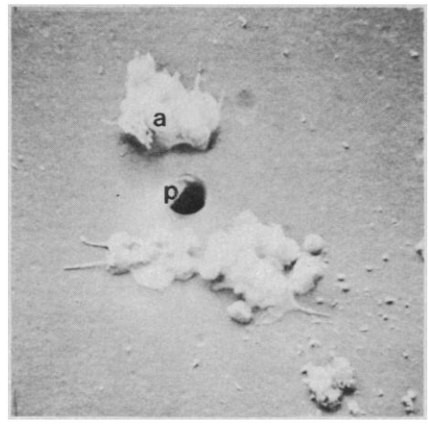

Fig. 2. Scanning electron photomicrograph of aggregates (*a*) associated with a pore (*p*) after exposure to ultrasound generated by a Doppler ultrasound device used in medicine (pore diameter, 4.5 μm).

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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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