

intermediate-disturbance hypothesis, our study provides experimental evidence of a keystone species that enhances local diversity by actually decreasing the overall intensity of predation relative to areas where that species is absent (Fig. 1C). Thus, two general kinds of keystone species exist. The first type enhances diversity directly by increasing disturbance from point 1 toward point 2 in Fig. 1C. Such predators are presumably prevented from normally driving the system close to point 3 by density-dependent factors, although they apparently can do so under certain conditions (16). The second type of keystone species enhances diversity not only directly, but also indirectly by decreasing disturbance by other predators from point 3 toward point 2 in Fig. 1C. In the case of the yelloweye damselfish, this indirect effect is a result of defending a feeding territory against more intensive predators.

MARK A. HIXON

Department of Ecology  
and Evolutionary Biology,  
University of California, Irvine 92717

WILLIAM N. BROSTOFF  
Department of Botany,  
University of Hawaii, Honolulu 96822

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8. Each block supported four of each type of settling plate: flat slabs cut from sun-dried coral rock and squares of roughly sanded gray polyvinyl chloride plastic, both of which measured 50 cm<sup>2</sup>, and naturally contoured coral rock slabs, which were approximately the same size but had irregular contours. Each of these three substrates had specific advantages as settling surfaces relative to the others (10). All plates were mounted horizontally with their upper surfaces on the same plane. Each block was sampled four times during the main experiment.
9. Cages were constructed of 1.3 by 1.3 cm galvanized wire mesh and measured 60 by 60 by 30 cm. One block was placed in each cage, and each settling plate was at least 15 cm from the wall of the cage. Exterior cage surfaces were prevented from fouling by intense grazing of fishes; interior surfaces were periodically cleaned by divers. All cages were placed just outside territories.
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11. The algae on the plates grew in mixed-species stands. All erect and prostrate noncoralline algae were scraped from each plate, spread uniformly within a glass petri dish, and the number

- of species was counted. Randomly selected points (100) within the dish were then examined under  $\times 100$  magnification. The alga in the central point of each ocular field provided data on relative abundances.
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  17. Grazing intensity was measured as the "standing crop" of visible fish bite marks (mostly by parrotfish) counted on each polyvinyl chloride settling plate sampled during the first 10 weeks of the experiment. Afterward, bite marks on plates exposed outside territories became too

dense to quantify accurately, while those on plates inside territories remained nearly constant. No bite marks were found on caged plates. The flat and natural coral rock plates showed the same relative pattern of grazing intensity, but individual bite marks were not readily countable on these substrates.

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## Slow Compressional Wave Propagation in Wet Human and Bovine Cortical Bone

Abstract. *Ultrasonic wave propagation in bovine plexiform and human Haversian bone was studied in the range 0.5 to 15 megahertz. A new longitudinal wave was observed which traveled more slowly than the ordinary longitudinal wave. The slow wave was associated with the dynamics of fluid motion in the pores of bone.*

In two papers (1) published in 1956, Biot treated the problem of elastic wave propagation in a fluid-saturated porous medium. He showed that in addition to a shear (transverse) wave, two dilatational (longitudinal) waves were propagated in such media: a higher velocity wave (designated as a wave of the first kind) whose fluid and solid component amplitudes are in phase, and a lower velocity wave (designated as a wave of the second kind) in which these amplitudes are in opposite phase. Waves of the first kind are the usual bulk waves, exhibiting neg-

ligible dispersion; waves of the second kind are highly attenuated.

The slow compressional waves have been observed in sintered glass beads at ultrasonic frequencies by Plona (2) and in fluid-filled sands and glass beads in the audible frequency range by Paterson (3). Plona's results were confirmed analytically by Berryman (4). Plona and Johnson (5), in a follow-up study, demonstrated the existence of slow compressional waves in three fluid-filled commercially available porous structures as well. The commercial materials included

Table 1. Slow wave amplitudes and velocities.

Specimen	Frequency (Mhz)	$A_s/A_r$	$V_s$ (km/sec)	$V_s/V_r$
Bovine longitudinal	0.73	0.50	2.3	0.54
Bovine circumferential	0.73	0.22	1.53	0.43
Bovine longitudinal	1.0	0.32	2.30	0.55
Bovine circumferential	1.0	0.22	1.53	0.44
Human la longitudinal	1.0	0.82	2.34	0.62
Bovine longitudinal	2.0	0.12	2.32	0.54
Human la longitudinal	2.0	0.53	2.34	0.62
Bovine longitudinal	3.0	0.044	2.33	0.55
Human lb longitudinal	3.0	0.058	1.89	0.49
Bovine longitudinal	4.0	0.030	2.32	0.55
Human lb longitudinal	4.0	0.042	1.88	0.49
Bovine longitudinal	5.0	0.064	2.32	0.50
Human lb longitudinal	5.0	0.028	1.90	0.55
Bovine longitudinal	6.0	0.0038		
Human lb longitudinal	6.0			

a stainless steel porous disk, a Coors Porous Ware brand ceramic disk, and a 3M brand porous structure. Vacuum impregnation was used to saturate all the samples with water. Plona and Johnson concluded that the slow compressional waves described by Biot are "readily observable in a wide variety of porous materials provided certain requirements are met."

We report here on the observation of such slow compressional waves in a naturally occurring porous material, bone. Our laboratory has long been involved in examining anisotropic ultrasonic wave propagation in bone as related to structure (6).

Figure 1, a to c, shows the type of porosity observed in bovine plexiform bone. The larger voids are the main canals for blood supply; the smaller pores are the osteocytic lacunae, which are also interconnected by even smaller channels, the canaliculi. It has been estimated that such pores in human Haversian bone result in about 7 to 12 percent

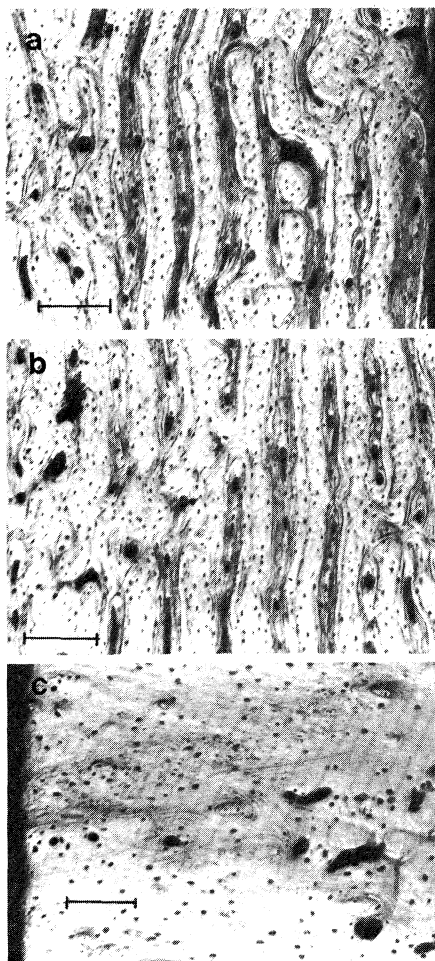


Fig. 1. Microstructure of bovine plexiform bone B1 (scale bar, 0.20 mm). (a) Cut perpendicular to longitudinal direction; (b) circumferential direction; (c) radial direction.

void space (7); the amount for bovine cortical bone appears to be somewhat smaller. The present experiment was aimed at measuring the attenuation of such waves in wet bovine plexiform and human Haversian bone in the range of 1/2 to 15 MHz (8). During these recent experiments, a new wave propagating more slowly than the usual compressional wave was observed in water-saturated bone.

A Matec 6600 pulser-receiver was used to generate the electric pulses, which were fed into a broadband piezoelectric transducer with a resonant frequency of 1.0, 2.25, 5.0, or 10.0 MHz. All experiments were performed at  $23.5^\circ \pm 0.5^\circ\text{C}$ ; water was used as the couplant. A pulse transmission technique involving two poly(methyl methacrylate) (PMMA) buffer rods was employed at all frequencies (8).

While the slow waves were observed over a range of frequencies, up to 6 MHz, it was only at the lower frequencies, principally below 2 MHz, that these waves were excited at significant amplitudes. In order to check that these waves were not experimental artifacts, the same experimental configuration was used with PMMA and with dry bone. No such slow waves were observed in either case. Waveforms of both the fast and slow waves in a longitudinally oriented specimen of bovine plexiform bone are shown in Fig. 2, a and b. In Fig. 2a the ultrasonic pulse had a center frequency of 1 MHz; in Fig. 2b the pulse center frequency was 3 MHz. When a 5-MHz pulse was used the attenuation was so great that the slow wave was not seen on this scale (Fig. 2c). The velocities and amplitudes for all three specimens measured are given in Table 1;  $V_s$  and  $V_f$  are the group velocities of the slow and fast waves, respectively, and  $A_s$  and  $A_f$  are the corresponding amplitudes. Error estimates for the group velocities are  $\pm 2$  percent. At the frequencies for which slow waves were observable, little distortion of the waveforms was evident. This observation is consistent with the smallness of the frequency dependence of velocity of slow waves in this domain. It was necessary to use short pulses in order to achieve adequate separation of the wave packets. Such pulses contain a wide distribution of frequencies; they provide useful data only when the dispersion is small. That is the case in the range considered above.

A new kind of longitudinal wave has been observed in bone. These waves travel more slowly than the usual longitudinal waves and they appear only at

the lower ultrasonic frequencies. The slow waves do not represent shear waves generated by mode conversion since shear waves in bovine bone have a velocity of about 2.0 km/sec (9). Slow waves represent a new dynamical phenomenon associated with the microstructure, since the classically anisotropic, viscoelastic continuum does not admit two kinds of longitudinal waves. Several kinds of media with microstructure, including continua with microstructure, can propagate more than one kind of longitudinal wave. For example, granular materials (10) and solids with voids (11) fall into this category. Dry bone, however, has voids but does not propa-

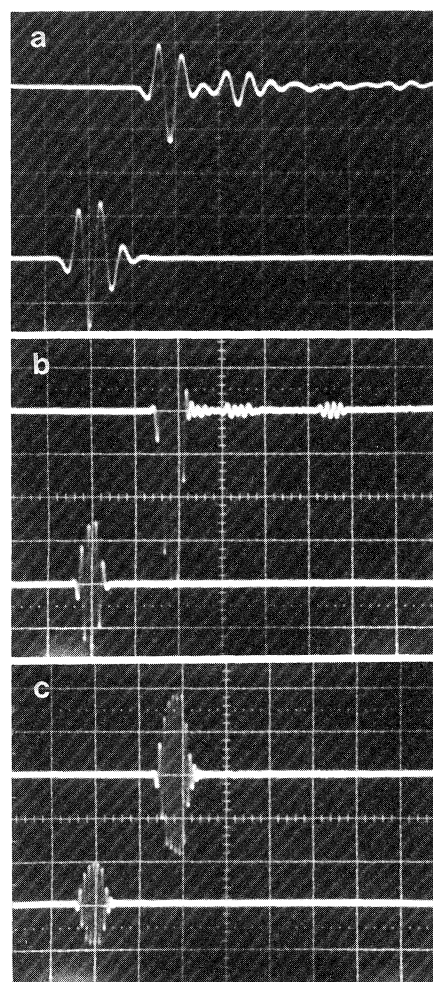


Fig. 2. Oscilloscope traces of slow waves in bovine bone. (Bottom trace) Transmitted signal through PMMA buffers, with 20 dB of electrical attenuation. (Top trace) Transmitted signal through buffers and a bone specimen. In the top trace the first pulse is the fast longitudinal wave, the second is the slow wave, and the third, where visible, is the first echo through the bone. The buffers are 1 inch and 2 inches long, so that buffer echoes are delayed and do not appear in the photographs. Vertical axis, 10 mV per division; horizontal axis, 2  $\mu\text{sec}$  per division. Pulse center frequency: (a) 1 MHz; (b) 3 MHz; (c) 5 MHz.

gate slow waves. In addition, the critical frequency associated with voids should be well above the region considered here, probably above 40 MHz, the frequency at which the wavelength becomes comparable to the largest voids, the Haversian canals. The theory of Biot (1) for wave propagation in a fluid-saturated porous solid, by contrast, predicts effects of the sort observed here.

The full significance of this observation is not yet understood. However, study of these slow waves is important because such waves may be useful for probing the dynamic fluid-solid interaction in bone. Piekarski and Munro (12) suggested that fluid pumping through the canaliculi in response to physiological stresses may be the mechanism for providing nutrition and biological information to cells as well as for the removal of waste products. Johnson and co-workers (13) considered streaming potentials responsible for the electromechanical effects in saturated bone. However, these are all modeled as occurring in the range of physiological frequencies, from approximately 0.1 to 100 Hz. The information contained in, or the physiological role of, such waves at megahertz frequencies should be investigated.

RODERIC LAKES\*

HYO SUB YOON

J. LAWRENCE KATZ

Department of Biomedical Engineering,  
Rensselaer Polytechnic Institute,  
Troy, New York 12181

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\* Permanent address: College of Engineering,  
University of Wisconsin

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## Cloned Fragment A of Diphtheria Toxin Is Expressed and Secreted into the Periplasmic Space of *Escherichia coli* K12

**Abstract.** An 831-base pair segment of the corynebacteriophage  $\beta^{tox-45}$  genome encoding fragment A of diphtheria toxin was cloned into plasmid pUC8 in *Escherichia coli* K12. Strains containing recombinant plasmids expressed the adenosine diphosphate ribosyl transferase activity characteristic of fragment A; this activity could be inhibited by polyvalent antiserum to fragment A as well as by the appropriate monoclonal antibodies to diphtheria toxin. The transferase activity was secreted into the periplasmic space of *E. coli*. These findings have implications for the future construction of genetically engineered chimeric toxins.

Diphtheria toxin production depends on a complex association between a family of closely related toxigenic corynebacteriophages and their sensitive *Corynebacterium diphtheriae* host (1-5). Lyso- genic conversion with toxigenic phages or with phages that carry mutations within the *tox* gene permits *tox* gene products to be expressed and studied (6, 7); however, the inability to ob-

Table 1. Inhibition of ADPR-transferase activity of CRM45 (2  $\mu$ g) and the periplasmic fraction of *E. coli* SM529 (pDT201) (50  $\mu$ l) by monoclonal antibody to diphtheria toxin and polyvalent antiserum to fragment A (18). Purified preparations of monoclonal antibody to fragment A (1.7 mg/ml) and of monoclonal antibody to fragment B (1.5 mg/ml) were used. Antibody preparations were incubated with an equal volume of purified CRM45 (40  $\mu$ g/ml) or periplasmic fraction for 1 hour at 25°C before being assayed for ADPR-transferase activity. The ADPR-transferase assay was performed in duplicate with 50  $\mu$ l of a standard assay mixture containing 10 mM tris-HCl pH 8.0, 2  $\mu$ M [adenine-<sup>14</sup>C]NAD (~ 2.5  $\mu$ Ci/mmol) and elongation factor 2 as a crude extract of wheat germ. The sample (50  $\mu$ l) was added and the mixture was incubated at 37°C. After 30 minutes, trichloroacetic acid was added, the precipitate was filtered and washed, and the radioactivity was determined. The background was approximately 200 count/min, and maximal incorporation was approximately 12,000 count/min.

Treatment	Radioactivity (count/min)	
	CRM 45	Periplasmic fraction
None	10,544 $\pm$ 265	10,458 $\pm$ 294
Polyvalent antiserum to fragment A	899 $\pm$ 115	877 $\pm$ 3
Monoclonal antibody to fragment A	2,315 $\pm$ 277	3,867 $\pm$ 242
Monoclonal antibody to fragment B	8,354 $\pm$ 552	9,059 $\pm$ 126

Table 2. Distribution of glucose-6-phosphate dehydrogenase, alkaline phosphatase, and ADPR-transferase activity in *E. coli* SM529 (19) carrying plasmid pDT201. Cells to be fractionated were grown at 37°C in 250 ml of M9 minimal medium plus 1 percent glucose, 1 percent casamino acids, 0.0002 percent thiamine, and 10  $\mu$ g/ml ampicillin to obtain an absorbance at 590 nm of 0.4 to 0.57 (~ 2  $\times$  10<sup>8</sup> cells per milliliter). The culture supernatant fluid obtained after centrifugation was concentrated tenfold by ultrafiltration on an Amicon YM-10 membrane before being assayed. Cells were washed in 30 mM tris-HCl (pH 8.0) and 20 percent sucrose and suspended in 5 ml of the same buffer. The periplasmic fraction was obtained by lysozyme-EDTA treatment (20). Spheroplasts were lysed in 10 mM tris-HCl (pH 8.0) by freeze-thawing three times in the presence of deoxyribonuclease T (0.1 mg/ml). The pellet obtained after centrifugation for 15 minutes at 13,000g was suspended in 10 mM tris-HCl (pH 8.0), and 0.1 percent Triton X-100 and designated the membrane fraction. Samples to be assayed for alkaline phosphatase were dialyzed extensively against 10 mM tris-HCl (pH 8.0). The assays for alkaline phosphatase and glucose-6-phosphate dehydrogenase were performed in triplicate as described (21). Total activity in a typical assay for alkaline phosphatase was 600.7 units per 10<sup>8</sup> cells (units expressed as the amount giving a change in absorbance at 420 nm of 1.0 per hour). Typical values for the total activity of glucose-6-dehydrogenase was 10 moles of nicotinamide adenine dinucleotide phosphate reduced per hour per 10<sup>8</sup> cells. The ADPR-transferase assay was performed as described (legend to Table 1), except that the standard assay mixture contained 100 mM dithiothreitol. *Escherichia coli* SM529, both untransformed and transformed with pUC8, is devoid of ADPR-transferase activity.

Fraction	Enzymatic activity (%)		
	Glucose-6-phosphate dehydrogenase	Alkaline phosphatase	ADPR-transferase
Supernatant fluid	0	1.9 $\pm$ 1.3	0
Periplasmic	0	92.1 $\pm$ 5.0	86.6 $\pm$ 3.3
Cytoplasmic	96.5 $\pm$ 2.7	4.6 $\pm$ 4.6	8.9 $\pm$ 2.1
Membrane	1.6 $\pm$ 1.6	2.9 $\pm$ 2.7	3.5 $\pm$ 0.6