Looking into Teeth with Ultrasound

Abstract. *Ultrasound is readily conducted across a boundary when the specific acoustic impedances of the two media are about equal. The specific acoustic impedance of dental enamel is about that of aluminum. A longitudinal sonic pulse, less than 250 nanoseconds in duration, conducted to the tooth through an aluminum rod, has positively detected the enamel-dentin junction as well as the dentin-pulp interface.*

Dental diagnosis is made by two principal techniques: visual and radiographic. These are supplemented by simple mechanical aids, mostly sharp-pointed explorers. Ultrasonic diagnosis has long been attractive because it can be employed as a noninvasive, nondestructive test and because, unlike x-rays, it is genetically safe. Smirnow (*1*) used water for coupling the ultrasonic transducer to the tooth, and it can be shown that most of the ultrasonic energy is reflected at the water-tooth interface; his reports are inconclusive, and he has been unable to demonstrate internal structure. Using solid-to-solid contact between transducer and tooth, we have positively located two major dental interfaces by ultrasound: the enamel-dentin junction and the dentin-pulp interface.

Several investigators have measured the acoustical properties of hard dental tissues within the last 2 years, but only the results of Kossoff and Sharpe (*2*) have been published. The specific acoustic impedance of dental enamel for longitudinal waves is between 15 and 18×10^6 kg/sec·m^2; the values for dentin and water are between 7 and 9 and 1.5×10^6 kg/sec·m^2, respectively.

The intensity-reflection ratio at the plane boundary of two media for normally incident radiation is expressed

$$R = [(Z_2 - Z_1)/(Z_2 + Z_1)]^2$$

where R is the reflection ratio, Z_1 is the specific acoustic impedance in medium 1, and Z_2 is the specific acoustic impedance in medium 2.

The intensity of the reflected wave at the water-tooth interface, with Z equal to 17 for enamel and 1.5 for water, is 0.7 of the incident wave. Thus only 30 percent of the incident power penetrates the enamel. The same ratio applies for the radiation emerging from the enamel into the water, so that at most only 9 percent of the initial intensity can return to the transducer from the interior of the tooth if there are no other losses. It seems doubtful whether Smirnow could observe much detail of the interior of teeth by his technique when one considers the many other sources of attenuation and the noise of high-gain amplifiers.

Another constraint in the application of longitudinal ultrasound to teeth is a consequence of the sonic velocity in hard tooth tissues. The velocity in dental enamel has been found to be between 5400 and 6100 m/sec by the investigators who measured the specific acoustic impedance; that for dentin, between 3600 and 4000 m/sec. The enamel layer is approximately 1 mm thick, so that the sonic transit time for a sound pulse through it is about 170 nsec. An echo from the enamel-dentin interface will appear about 340 nsec after the pulse has penetrated a 1-mm-thick layer. The two surfaces of the enamel layer may be distinguished if the pulse duration is significantly shorter than the pulse-echo time.

Aluminum has a specific acoustic impedance of 17.3×10^6 kg/sec·m^2 for longitudinal waves, which is close to that of dental enamel. A transducer was fabricated with a thin wafer of PZT-5 (a piezoelectric ceramic) used as the piezoelectric element, with a backing of bismuth alloy cast onto the wafer to ensure perfect contact. The front surface was coupled by epoxy bonding to an aluminum rod, 1 cm in diameter and 2 cm long. The thickness of the wafer was reduced by grinding, before assembly, until sonic pulse output was of less than 250-nsec duration. Electrical excitation was provided by a pulse source generating an asymmetric pulse shape having a rise time of about 15 nsec and a very slowly decaying tail. The output of the transducer was coupled to a Tektronix-547 oscilloscope with a type-W plug-in unit. The only signal amplification was provided by the oscilloscope. The shape of the generated pulse is identical with that identified as *Transducer-tooth* interface in Fig. 1. The sonic pulse is not rectified, but it has the form (Fig. 1) of the letter W with an exaggerated central peak.

We used a bovine incisor because it has a broad surface with an enamel layer of fairly uniform thickness. Weidmann *et al.* (*3*), for example, found very little variation in the density of the labial surface of human incisors, and much greater variation in other kinds of teeth, especially molars. Since ours was the first demonstration of the performance of the transducer, as well as the first test of our concepts, we took considerable care to ensure ultrasonic reflections from both the dentin-enamel junction and the dentin-pulp interface. The bovine tooth was fixed in 5 percent formalin for several weeks before transfer to water; it was always kept moist and was tested in the moist state. The tooth was prepared by a grinding of a flat surface in the midlabial area to ensure a good area of contact between it and the transducer. A thin layer of oil was applied to complete the sonic path.

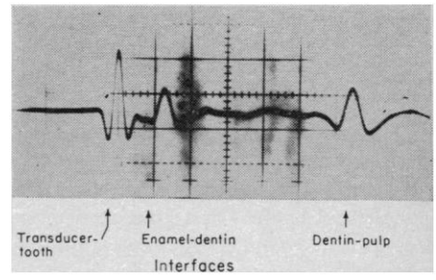

Fig. 1. Typical sonic echogram from a prepared incisor observed midlabially.

The results (Fig. 1) are the first demonstration known to us of the dentin-enamel junction by ultrasound. The time scale is 250 nsec per major division; vertical calibration, 20 mv per major division. There are three pulses: (i) the reflection from the interface between the aluminum coupling rod and the tooth; (ii) one, 300 nsec later, indicating the dentin-enamel junction; and (iii) another, 1.6 μsec after the first, indicating the dentin-pulp interface. It is interesting that the initial pulse is greatly diminished when coupling to the tooth occurs and the other pulses appear. It is very evident that most of the sound is crossing the aluminum-enamel interface in confirmation of the theory and demonstrating that the specific acoustic impedance of the enamel is close to that of aluminum.

In order to confirm that the reflections came from the surfaces to which we ascribed them, we drilled a flat-bottomed hole from the rear side of the tooth. When the bottom of the hole was detected ultrasonically, the timing of the pulse echo corresponded to the known position of the bottom. As the hole was deepened, its pulse echo moved forward in time correspondingly; when the depth of the hole corresponded to that of the dentin-enamel junction, the two pulse-echo times were equal.

The equipment used by us was the first successful transducer fabricated in our laboratory with the capability of injecting short ultrasonic pulses into teeth and recovering the pulse echoes. While improved transducers have been developed more recently, a diagnostic tool has not yet been produced. We have demonstrated that one must use solid-to-solid contact for detection of the internal structure of teeth, and that the loss of sonic energy is negligible for the required depth in hard dental tissues. However, the transducer must be improved so that it may be applied to the tooth surface without the need to grind a flat area to ensure a sonic path. We are continuing our efforts to shorten the sonic pulse, aiming at a pulse duration no longer than 30 nsec and a transducer tip no greater than 1 mm in diameter. We believe that the ultrasonic transducer will be employed differently from x-rays for diagnostic purposes, and that new techniques will be necessary for its successful utilization.

SIDNEY LEES
FRANK E. BARBER
Bioengineering Department,
Forsyth Dental Center,
Boston, Massachusetts 02115

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2. G. Kossoff and C. J. Sharpe, *Ultrasonics* **4**, 77 (April 1966).
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Escherichia coli: High Resistance or Dependence on Streptomycin Produced by the Same Allele

Abstract. *Mutations to streptomycin resistance in* Escherichia coli *K12, when transferred to a C strain, can confer dependence on streptomycin. These alternatives in expression of the allele are probably a result of interaction between two ribosomal proteins.*

We have found in strains of *Escherichia coli* that one allele can confer either resistance to streptomycin or dependence on the drug. The alternative expressions of this allele depend, we believe, on another closely linked gene that, like the *str* gene, specifies a ribosomal protein.

The alternatives in expression of the allele were observed in a transduction experiment when STR-R (streptomycin-resistant) strains, derivatives of *E. coli* K12, were donors and a strain of *E. coli* C (N873) was the recipient. A large proportion of the streptomycin-resistant transductants were dependent on streptomycin (Table 1).

To be able to conclude that the expression of the allele (dependence or resistance) is a function of strain background in *E. coli* C, we carried out the following controls.

Donor strains K12 *str*-HR (streptomycin high resistance) do not harbor either a masked *str*-HD (streptomycin high dependence) allele that is occasionally separated from a *str*-HR allele during transduction, or a *str*-HD allele and a modifier that change the phenotype to streptomycin resistance. We repeated the transduction experiment, using two K12 strains as recipients, one (N4) directly related to the donor strain and the other (AB258) from a different strain collection. In both experiments not a single STR-D (streptomycin-dependent) recombinant was detected (Table 1). [In notations, symbols in capital letters refer to phenotype; italicized letters, to genotypes; for further detail, see (*1*).]

In another control, *E. coli* B strains were used as recipients. Again, only STR-R recombinants were isolated (Table 1), which again indicates that the donor strain did not harbor a *str*-HD allele, and that the observed change in phenotype was characteristic of the C strain.

The STR-R transductants were not due to an interaction between elements that do not involve the *str*-HR allele of the donor strain, since, in an experiment in which the streptomycin-sensitive parental strain JC12 was used as a donor and the C strain (N873) was the recipient, neither STR-R nor STR-D recombinants were isolated. (Here, as in the other transduction experiments, transfer of auxotrophic markers was measured to ensure that transduction took place.)

The recipient strain N873 can give rise to *str*-HR and *str*-HD derivatives by mutation, like any other *E. coli* strain. The STR-D transductants (Table 1) were compared and found to be equivalent to *str*-HD mutants of N873 in their pattern of resistance to different levels of streptomycin.

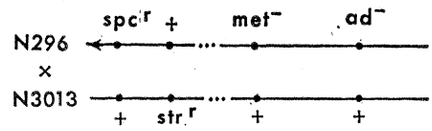

Fig. 1. Diagram of the cross of N296 and N3013.

The STR-D transductants had indeed received and maintained the *str*-HR allele, for the allele can once again be expressed as STR-R in recombinants of a cross with a K12 strain.

For example, N3013, one of the STR-D transductants of the C strain N873, was crossed to K12 strain N296, a spectinomycin-resistant (*spc^r*) (*2*) derivative of strain JC12 (further details about strains are given in the legend to Table 1). From the cross of N296 with N3013 (Fig. 1), 8500 STR-D SPC-R recombinants and 94 STR-R SPC-R recombinants were isolated, showing that the *str*-HR allele had been retained in N3013. The number of STR-R SPC-R recombinants is probably a low estimate, since this class is apparently selected against during the transition from dependence to resistance, when cells are relatively sensitive (*3*). Examination of the 94 STR-R

Table 1. Transduction experiments. In all experiments, the donors were the K12 strains N316, N321, and N707, derivatives of JC12, an Hfr strain auxotrophic for methionine and adenine, which transfer the chromosome clockwise starting somewhere before *arg* G (obtained from Dr. B. Low, New York University). Strain N316 is a *str*-HR derivative, N321 is a spectinomycin-resistant (*2*) *str*-HR derivative of JC12, the *str* mutation being independent from the one in N316; and N707 is a *nek* derivative (a strain resistant to neomycin and kanamycin) of N316. In all experiments at least 25 transductants obtained in the cross of the donor N316 and one of the recipient strains were analyzed. The C recipient was N873, a *gal*$^-$ derivative of strain C obtained through Dr. J. Eigner from this department, originally from Dr. R. L. Sinsheimer. The K12 recipients were N4 and AB258. Strain N4 is a methionine prototroph obtained from a transduction experiment in which strain JC12 was the recipient; AB258 is a threonine, leucine, thiamine auxotroph obtained from Dr. E. Adelberg, Yale University. The B recipients were J961, a *thy*$^-$ *gal*$^-$ strain obtained from Dr. N. Melechen, St. Louis University, and strain B148, a *ura*$^-$ strain from this laboratory. The transduction conditions with phage Plkc were similar to those of Hashimoto (*7*), with the modification described by Apirion and Schlessinger (*1*).

Donor	Recipient	Transductants (No.)	
		STR-R	STR-D
K12 *str^r*	C *str^s*	36	40
K12 *str^r*	K12 *str^s*	65	0
K12 *str^r*	B *str^s*	72	0