Dental Enamel: Detection of Surface Changes by Ultrasound

Abstract. *Evidence indicates that the tooth surface differs in structure from the enamel immediately beneath it, and particularly that the enamel rod type structure is minimal in the true natural surface. Furthermore, the rod ends appear to disappear with age after the eruption of the tooth. The thickness of the surface layer may be as much as 25 micrometers. Studies of caries incidence show a peak in the attack curve 2 to 4 years after eruption and a decline thereafter for all teeth. This information indicates that the mechanical structure of the tooth surface should be carefully studied. A highly useful means appears to be ultrasound since the specific acoustic impedance of highly mineralized tissue like enamel is strongly dependent on fraction volume mineralization and since nondestructive test techniques can be based on ultrasonics. An experimental demonstration of ultrasonic detection in vitro of tooth surface demineralization is given.*

The tooth surface is generally regarded as the critical interface in preventive dentistry, and the thrust of research is to keep the surface intact. Physical methods for inspecting the tooth surfaces have been limited to a few techniques, and most of these are for in vitro applications. Visual inspection either with the unaided eye or with a microscope, reinforced by mechanical probing with a sharp pointed explorer, is the most common technique for discovering changes in the tooth surface. Hardness testing (*1*) has been employed as a means for testing the state of mineralization in "artificial mouth" experiments, to quantitatively evaluate induced changes of the tooth surface. We show here that a small change in the condition of the natural tooth surface in vitro can be sensed by ultrasound. By contrast, hardness testing techniques require the tooth surface to be ground flat, thereby removing the natural tooth surface.

The natural tooth surface is apparently different in structure from the subsurface enamel layer. Dental enamel is described in textbooks as a structure of parallel prisms with the prism axes normal to the surface. However, Mannerberg (*2*) and Orban (*3*) present pictures which show a progressive loss of surface structure with advancing age. The surface of a recently erupted tooth exhibits well-defined perikymata with prism ends in the valleys. In many individuals all prism ends disappear with age and the tooth surface displays none of the underlying prismatic structure. Poole and Silverstone (*4*) and Hoffman *et al.* (*5*) used the scanning electron microscope to produce illustrations which show that the surface is quite plainly a layered structure atop the prisms. The thickness of the aprismatic surface layer is not known but presumably is of the order of micrometers. Poole (*6*) has estimated that the thickness of the aprismatic layer at the crests of the ridged perikymata is 25 μm.

Carlos and Gittelsohn (*7*) in their study of caries incidence in permanent teeth show a peak in the attack curve occurring 2 to 4 years after eruption and a decline thereafter for all teeth and for both sexes. These authors note: "From time to time hypotheses have been entertained regarding the acquisition of immunity to caries during the late adolescence and early adulthood. These data suggest that if such a mechanism is operative it is related to tooth age rather than chronologic age, and is common to all types of teeth." The two sets of observations point up the need to investigate the dynamics of the mineralization process at the natural tooth surface, particularly under conditions that lead to carious lesions.

Gilmore (*8*) employed the Hashin-Shtrikman composite theory to compute the elastic properties of calcified tissues. He used a two-component model in which one component is the mineral hydroxyapatite and the other is an organic substance much like collagen. Figure 1, adapted from Gilmore, shows the longitudinal wave velocity and the associated specific acoustic impedance as a function of mineral volume concentration. The curves marked I span the total possible regime for enamel and show that the sonic properties are very sensitive to composition. Curves marked II give the local slope of the main curves expressed in percentage. The specific acoustic impedance varies by 2 percent or more for each 1-percent change in mineral volume concentration, an indication that a change in the surface mineralization of the tooth should strongly affect ultrasound.

Ultrasound has been employed for many years in pulse-echo systems as a means for visualizing subsurface structure in biological media. Recently Lees and Barber demonstrated that pulse-echo ultrasonography can be used to detect and locate the dentino-enamel junction beneath the tooth surface (*9*). Since in pulse-echo systems the transit time of the pulse is the basic means of measurement, the resolution capability is limited by the duration of the sonic pulse. It is not practical to use such a direct approach to sense changes in the tooth surface where the layer is only a few micrometers thick.

Lately it has been shown that a layer of one substance, say, water, as thin as 1 μm, embedded between two solids, affects the wave shape of the ultrasonic pulse echo reflected from the thin layer (*10, 11*). Under suitable

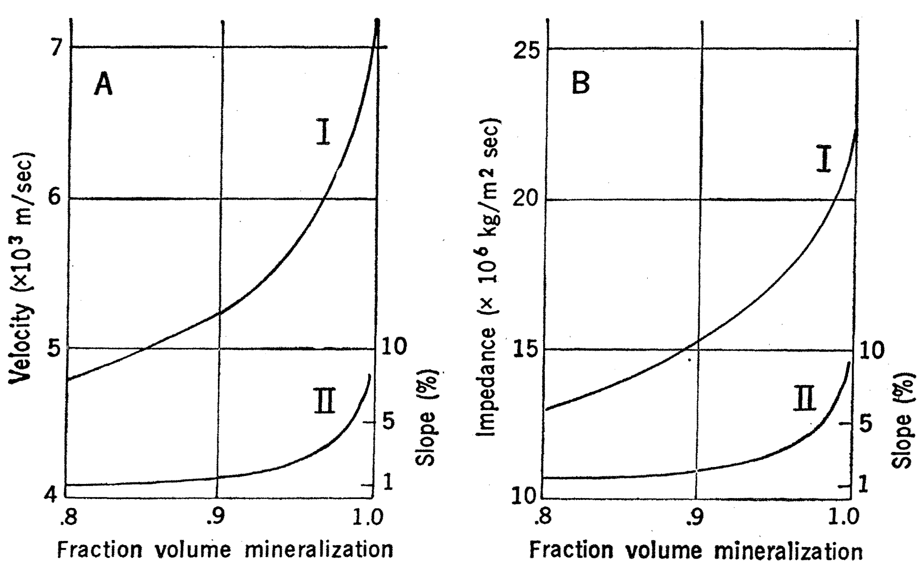

Fig. 1. Computed sonic properties of composite hydroxyapatite-collagen. (A) Longitudinal velocity. (B) Specific acoustic impedance.

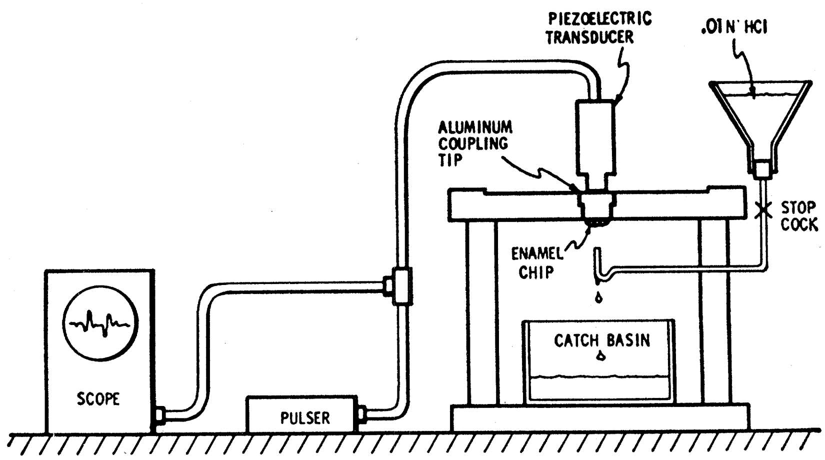

Fig. 2. Experimental apparatus.

conditions the thickness of the film can be determined by the change in the wave shape because of the variation in the specific acoustic impedance encountered by the ultrasound. Similar ultrasonic effects due to changes in the surface of metals have been observed by Rollins (*11*). The effect of demineralization at the tooth surface is more evident in a change of the pulse shape than of the transit time.

Our experiment was designed to show the capability of ultrasonics to detect surface changes of a tooth exposed to a demineralizing environment. The experimental arrangement is shown in Fig. 2. A slab of tooth enamel 1 mm thick, cut from an extracted human incisor, was bonded with epoxy cement to the tip of the transducer. The natural untouched tooth surface lay exposed. In this experiment the transducer tip carrying the enamel chip could be removed from the instrument for examination by use of a scanning electron microscope or other instruments. This arrangement also permitted several samples to be studied in succession so that the observed effect could be verified. The tooth sample was bonded under pressure to minimize the bond layer thickness, estimated to be no more than 1 μm. It is necessary to use a thin bonding layer to maximize transmission of sound across the boundary.

In the experiment a flask filled with $0.01N$ HCl was fitted with a glass tube leading to a nozzle directed at the exposed tooth surface. When the stopcock was opened, a jet of dilute acid bathed the tooth and the period of exposure could be readily controlled. The transducer was designed and fabricated at Forsyth Dental Center to yield a well-damped pulse of short duration. The pulser (Fig. 2) was an avalanche transistor functioning as a switch to apply a 20-volt electrical pulse to the piezoelectric transducer. The active element was made from a PZT-5 piezoceramic disk 12.7 mm in diameter and 0.25 mm thick. The well-damped characteristic of the sonic wave was due to the backing. Cerrobend alloy 158 F (a bismuth alloy) was cast directly on the silvered face of the piezoceramic wafer. The other face was polished flat and smooth, after which a chrome-gold electrode 2 mm in diameter was vacuum-deposited in the center. When the face of the piezoceramic with the gold electrode was pressed against the flat surface of the aluminum coupling rod carrying the enamel chip, and the pulser was used to excite the transducer, ultrasonic echoes from the interface between the coupling rod tip and the dental enamel and from the free enamel surface could be detected on the oscilloscope. A typical wave shape for the received echo signals is shown in Fig. 3A.

The effect of dilute acid on the tooth surface is a slight change in the wave shape. A relatively simple artifice was adopted to enhance the visibility of this change with available test equipment. Two signals are marked in the echograms of Fig. 3. The signal

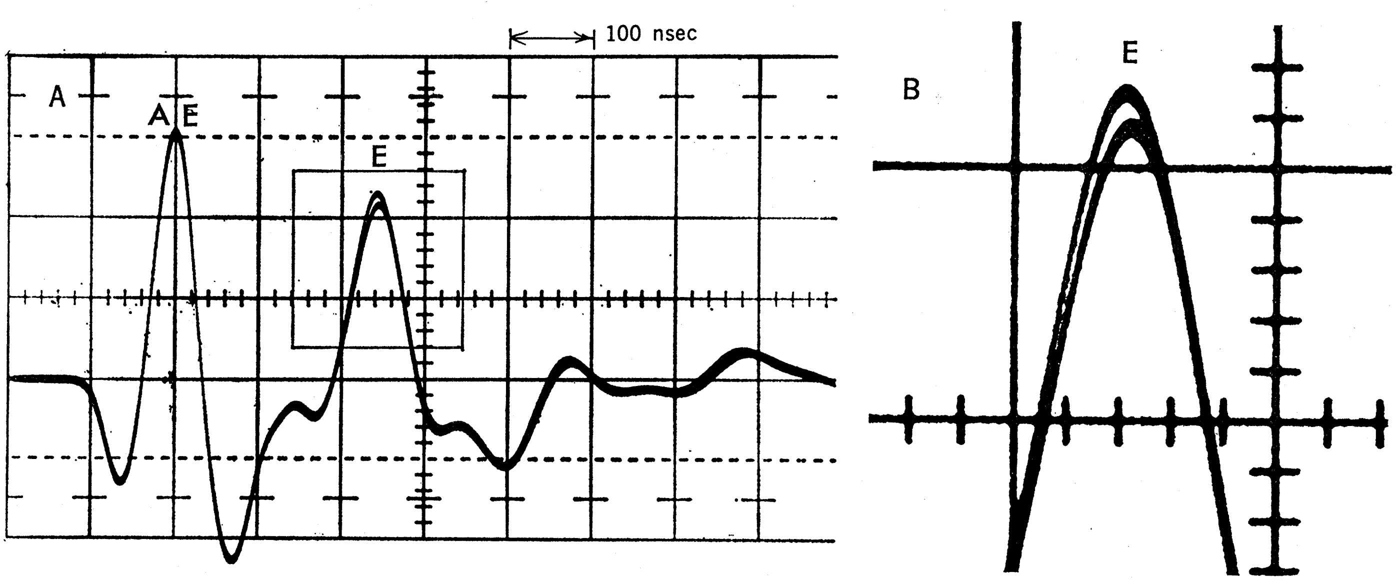

Fig. 3. Ultrasonic echograms of enamel during demineralization. (A) Time-lapsed double exposure showing the superposition of received signals before and after 10-minute exposure of tooth surface to $0.01N$ HCl. *AE* refers to the echo from the aluminum-enamel interface, and *E* refers to the echo from the unbonded natural enamel surface. The time scale was 100 nsec per division. (B) Photographic enlargement of the echoes from the enamel surface [outlined portion of the wave form in (A)]. The two curves show the effect of etching.

marked E is a doubled curve. Figure 3B is an enlarged view of the outlined box of Fig. 3A. The smaller inner curve (Fig. 3B) is the received echo before acid treatment, and the larger outer curve is the echo after acid treatment. The picture was obtained by double exposure. The scope image was adjusted so that the echo from the transducer tip, AE, in the second exposure was superimposed on that of the first exposure.

If there were no effect on the echogram due to the surface demineralization, both images would appear as one. Since there are two images, it is clear that the change in the surface condition did modify the wave shape of the echo. The picture in Fig. 3 was the result after a 10-minute exposure of the tooth surface to dilute acid.

Although the change in shape is small, it is easily detected. Changes due to additional exposure of the tooth surface to the acid caused the echogram to change in the same sense, an indication of a definite correlation between the exposure to acid and the response in the wave shape. It is presumed that a means for measuring the state of mineralization of the tooth surface by ultrasound has been established.

The change in the wave shape of the ultrasonic echo may be due to a change in the sonic propagation characteristics of the surface, as suggested by Gilmore's work (*8*). Or it may be due to the observed changes in the surface structure or even possibly to the actual mechanical displacement of the tooth surface. The effect is probably attributable to all three causes but it is not known which is dominant.

An instrument system has been designed to reduce the observed effect to quantitative terms (*12*). In this system the complex ultrasonic wave form is reduced to digital form and the digital signals are stored in the core memory of a computer for transfer to magnetic tape. The digital transformation process employs a sampling process and takes advantage of the fact that the echo pattern of the ultrasonic system changes very slowly. In the instrument system the design calls for a pulse sampling rate of 10 khz. The part of the wave under investigation can be adequately represented by 100 to 200 points. In 1 second between 50 and 100 repeated observations can be made of each sample; thus signal averaging may be employed. Since the data are in digital form it will be possible to process the data on-line, with all available theory being used to extract information. The instrument system is being built and should be operable by 1 October 1970.

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Limits of Microbial Existence: Temperature and pH

Abstract. *A microscopic survey made to detect the presence of bacteria in hot springs of varying temperature and* pH *characteristics revealed that in neutral and alkaline hot springs bacteria are found at temperatures up to the boiling point of water (92° to 100°C, depending on the altitude). In hot springs of increasing acidity the upper temperature limit at which bacteria are found decreases; at* pH *2 to 3 the upper temperature limit is 75° to 80°C. Bacteria have thus been able to evolve with the ability to grow at either high temperature or high acidity, but not at both high temperature and high acidity. These results suggest that there are physicochemical limitations of the environment beyond which life is impossible.*

Our earlier studies (*1–3*) have shown that in neutral or alkaline hot springs bacteria live and grow in boiling water. Virtually every neutral or alkaline hot spring examined at Yellowstone National Park showed fairly high population densities, and measured growth rates (*3*) were rapid. Since at the altitude of Yellowstone Park water boils at 92.5° to 93°C, even superheated springs have temperatures which rarely exceed 95°C. Recent studies in New Zealand and Iceland, where the boiling springs are at lower altitudes and have temperatures of 99° to 101°C, have shown that at these locations also virtually every spring in the neutral and alkaline *p*H range has high populations of bacteria.

In acid hot springs, on the other hand, bacteria often appear to be absent (*4*). Only at temperatures around 60° to 65°C do macroscopically visible accumulations of bacteria appear in acid springs, whereas in neutral and alkaline springs such accumulations are seen at 85° to 90°C (*2*). It seemed, therefore, that acid might add an additional environmental stress which could prevent bacteria from evolving with the ability to grow at the highest temperatures. To document this possibility in some detail, we have surveyed for the presence of bacteria a large number of hot springs of various temperatures and *p*H characteristics in Yellowstone Park, in other thermal areas of the western United States, in New Zealand, and in Iceland. The results of this survey show clearly that as the environmental *p*H decreases, the upper temperature limit at which bacteria are observed also decreases. In addition to a general survey of springs, we have also studied the thermal gradients of several acid springs, in which bacteria appeared to be absent from the source, to determine the highest temperature at which bacteria could be found. These studies show that there is nothing intrinsically unfavorable for bacterial growth in acid springs. In fact, we have detected the presence of a number of bacteria that actually require acid conditions for growth.

Almost 300 individual springs were examined for the presence of bacteria. Of these, 163 were in Yellowstone National Park, 21 were in other parts of the western United States (Lassen Volcanic National Park, California; The Geysers, California; Steamboat Springs, Nevada; and Beowawe, Nevada), 68 were in the central volcanic region of the North Island of New Zealand, 40 were in Iceland, and 2 were in Japan.