ACOUSTIC MICROSCOPY OF MAMMALIAN KIDNEY*

by **L. W. KESSLER, Ph.D.; †S. I. FIELDS, B.S.; and F. DUNN, Ph.D.

ABSTRACT

Acoustic micrographs of unstained specimens of mouse kidney were produced at frequencies of 100 MHz and 220 MHz with resolutions of 20μm and 9μm, respectively. The acoustically revealed structure has been examined in relation to established microanatomy. High contrast details corresponding to connective tissue boundaries of supporting elements of the nephron are exhibited. In addition, various regions of the kidney such as the cortex and the three medullary regions can be differentiated. At these frequencies, the acoustically exhibited structures are considered to result from scattering at connective tissue interfaces, resulting from local acoustic impedance discontinuities, rather than from intrinsic acoustic absorption within the microstructures of the tissue.

Indexing Words

Acoustic Micrographs Ultrasonic Microscope Kidney Microanatomy

INTRODUCTION

The field of acoustic microscopy is becoming an integral part of ultrasonic imaging *(1)*. The high acoustic frequencies employed make possible the imaging of detail in the micron range, thereby providing knowledge of the physical state of tissue microstructure. It is suggested that the transmission patterns displayed in acoustic micrographs identify distributions of the elastic properties and components of the specimen similar to findings which relate to the echographic visualizability of tissues for macroscopic studies at lower frequencies *(2)*. Such novel information of the acoustic propagation characteristics of tissues and organs, and the attending physical properties, *at the microscopic level*, has both relevance and application in biology and medicine.

The instrument employed in the present study operates at 100 MHz and 220 MHz *(1, 3)* with resolutions in aqueous media, including many soft tissues, of 20μm and 9μm, respectively. Details of its operation have been reported elsewhere *(3, 4)*. In brief, the principle of operation, illustrated schematically in Figure 1, involves the propagation of high frequency sound waves through the specimen to be viewed where they produce a ripple pattern upon striking a mirrored surface (coverslip). The ripple pattern represents the transmitted acoustic amplitude which is then reconstructed electronically by a scanning laser beam and a photodiode. The acoustic image is made to appear in real time on a TV monitor with a 3mm x 3mm field of view and a 60μm depth of field.

OBSERVATIONS AND DISCUSSION

Preparation of the mouse kidney tissue specimens for the acoustic micrography included 10% formalin perfusion and subsequent frozen sectioning. Other studies have suggested that relative acoustic properties prevail whether tissues

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**Present address: Sonoscope, Inc., 752 Foster Avenue, Bensenville, Illinois 60106.

†Present address: Medical Student, University of Chicago Pritzker School of Medicine, 950 East 59th Street, Chicago, Illinois.

From the Bioacoustics Research Laboratory, University of Illinois, Urbana, Illinois 61801.

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For reprints contact: Professor F. Dunn, Bioacoustics Research Laboratory, University of Illinois, Urbana, Illinois 61801.

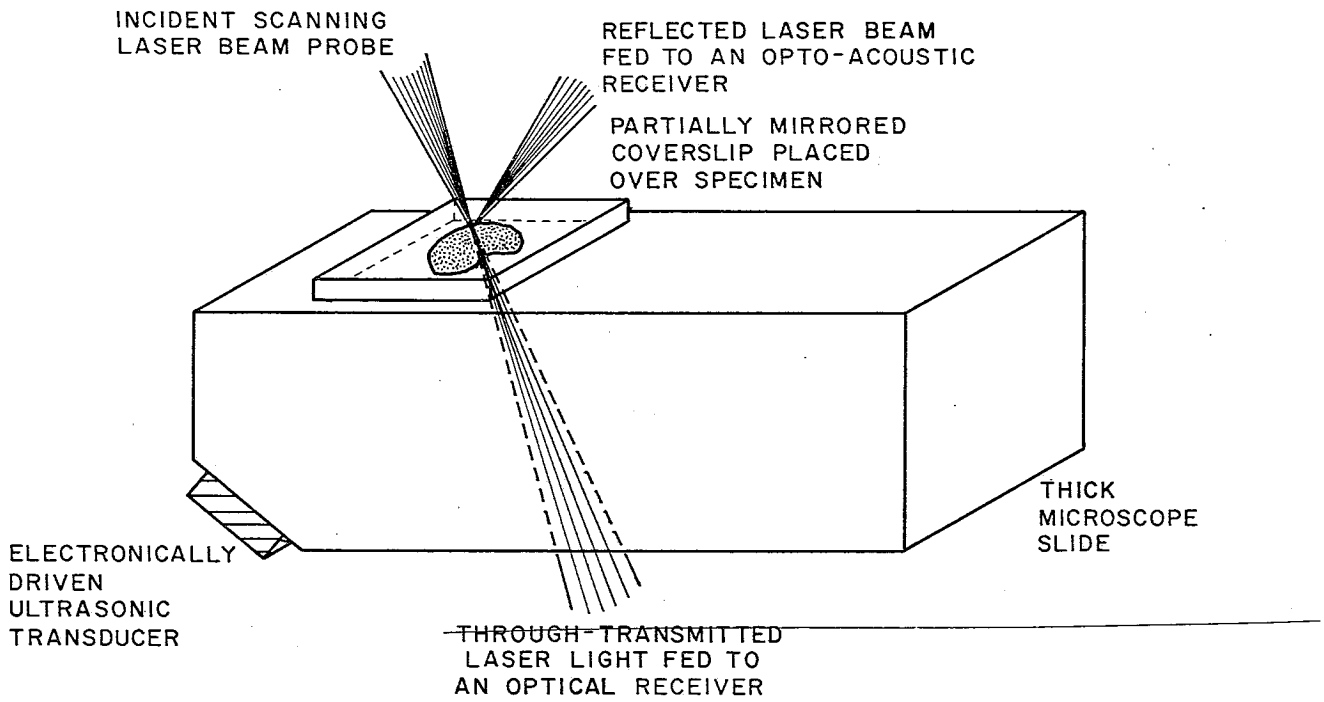

FIGURE 1. Schematic diagram of the acoustic microscope stage. A moistened specimen is placed on the stage and covered with a partially mirrored coverslip. Ultrasonic energy produced by the transducer "insonifies" the specimen and produces a slight mechanical perturbation of the coverslip surface. These disturbances, which are proportional to the local acoustic displacement amplitudes, are detected by the reflection of a focused, scanning laser beam probe, which drives the optoacoustic receiver. An optical image of the specimen is produced simultaneously by detection of the through-transmitted laser light.

employed are fresh, fixed, or frozen fresh and later thawed *(5, 6, 7)*. The high acoustic attenuation of the kidney tissue at these frequencies; viz. 10dB/mm at 100 MHz and 45dB/mm at 220 MHz *(7)*, as well as the desire to identify optically the acoustically revealed structure, guided the choice of thickness of tissue specimens employed.

The mouse kidney is unilobular with a nephron structure similar to the human kidney. The proximal tubules, located in the outer stripe of the medulla, have the following approximate dimensions: external diameter 50μm, lumen diameter 10μm, and wall thickness 20μm *(8)*. The intertubular spacing within each triad of tubules is about 10μm. The tubule walls are composed of one cell thickness surrounded by basement lamella. The intertubular spaces contain capillaries surrounded by basement lamellae, kidney interstitial cells, and a relatively high concentration of connective tissue elements.

Figure 2 is a composite acoustic micrograph of a portion, approximately 1 sq cm, of the midlongitudinal section of a mouse kidney. Lighter areas within each individual picture of the composite identify regions of greater acoustic transmission through the specimen and darker areas identify those regions of lesser transmission as would be the case for greater absorption or an impedance mismatch producing

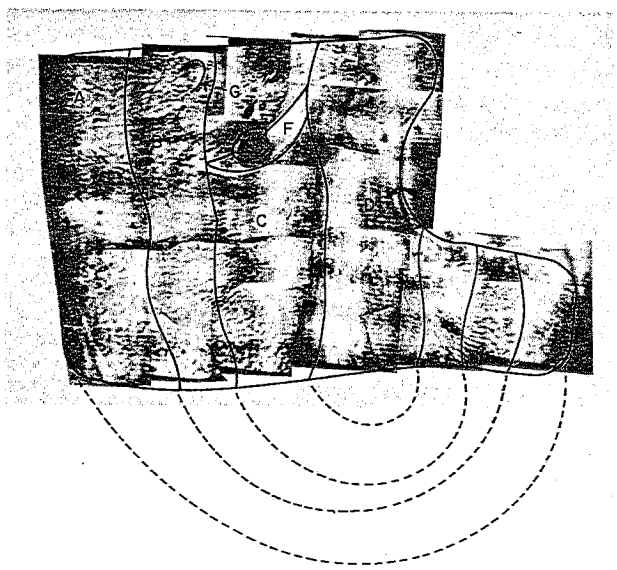

FIGURE 2. Composite ultrasonic micrograph of a section of mouse kidney. A, cortex; B, outer stripe-outer medulla; C, inner stripe-outer medulla; D, inner medulla; E, air bubble artifact; F, vessel, oblique section; G, vessels, transverse section; H, papilla.

energy reflection or scattering. By using the known dimensions of specific structures, as indicated above, it can be determined that the dark stripes represent intertubular regions rather than the uriniferous tubules or their lumina. These structures, representing regions of high attenuation or appreciable impedance mismatch appear at the limit of resolution and therefore cannot be the uriniferous tubules. The tubule lumen can be ruled out because of the low acoustic attenuation and low impedance mismatch expected therein. Low frequency (low megaherz region) examination has suggested that it is the elastic properties of most soft tissues, determined mainly by the collagen (and other structural proteins) content, that are largely recognizable for the acoustic impedance inhomogeneities visualized in the echographic mode *(2)*. The average acoustic intensity transmission loss of 2.5 dB for this specimen thickness of 240μm is not believed to be the result of intrinsic absorption alone, because of the very significant deviation from the average attenuation in any particular localized area of structure. However, the detail appearing in the acoustic micrographs as acoustic contrast may be attributed partially to scattering at interfaces of appreciable acoustic impedance mismatch. These acoustic impedance discontinuities are thought to arise from the enormous difference in elastic properties of the supporting and supported tissues *(2)* and thus allow visualization of these structural details.

The different regions of the kidney can be characterized from the acoustic micrographs and the bordering regions between them identified according to the acoustic image of the intertubular structure and the changes that occur in them. Collagen fibers are abundant in the outer stripe of the medulla, but less so in the cortex and inner medulla. The regions of low acoustic transmission in the micrograph are correspondingly most prominent in the outer stripe of the medulla. The borders between the outer stripe and the cortex, as well as between the outer and inner stripes, are evident by the increased transmission by the intertubular regions, corresponding to a lesser quantity in collagenous connective tissue occurring at these boundaries.

Intertubular spaces are observed reversing and terminating in the cortical region, corresponding to the highly coiled nature of the uriniferous tubules therein. Round structures which may indeed be Bowman's capsule appear in the cortex, beside the reversing and terminating structures, as they are observed under optical microscopy.

CONCLUSION

The observations of this study suggest the following:

1) Acoustic impedance interfaces resulting from the differences in elastic moduli between the microstructural connective tissue components, and those they support, may act as acoustic scatterers and thus be visualized as attenuating structures in the acoustic micrograph.

2) The acoustic contrast appears to be related directly to the relative amounts of connective tissue components of greater elastic moduli present at the interface.

3) As it appears to be the physical state of the elastic components of microanatomical structure that reveals the physiological state of the tissue or organ being investigated, future studies should include acoustic micrographic characterization of various pathologies for application to differential diagnosis of various disease states.

4) Acoustic microscopy may be the first technique that allows visualization of the distribution of the elastic properties of tissues *in vitro*. Since there is no staining involved, it is also applicable *in vivo (4)*.

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