

# Degradation of DNA by Intense, Noncavitating Ultrasound

S. A. HAWLEY, R. M. MACLEOD, AND F. DUNN

*Biophysical Research Laboratory, University of Illinois, Urbana, Illinois*

(Received 3 May 1963)

Previous research leads to the conclusion that intense, noncavitating ultrasound affects tissue at the level of macromolecular structures. As an initial study of the interaction of intense sound waves and macromolecules, solutions of DNA were irradiated with noncavitating, 1-Mc/sec ultrasound at intensities in the neighborhood of 30 W/cm<sup>2</sup>. Sedimentation-velocity analysis shows that the molecular weight of the DNA is reduced. Cavitation and thermal processes can be eliminated as possible degradation mechanisms. It is suggested that relative motion between the macromolecules and the suspending medium may be the mechanism by which degradation occurs.

IT is well established that properly controlled, noncavitating, high-intensity ultrasound produces unique effects on the central nervous system<sup>1</sup> and provides a versatile method for modifying brain structures.<sup>2</sup> Although considerable effort has been expended in attempts to elucidate the physical mechanism(s) of the interaction of intense ultrasound and biological structures (and much useful information has resulted), the details of the physical interaction remain in an unsatisfactory state of understanding.

The following statements review briefly the reactions observed relative to complex biological specimens. (1) High-intensity, noncavitating ultrasound, under appropriate dosage conditions, produces selective irreversible changes in tissue components. This has been demonstrated by extensive histological studies on the tissue components of the mammalian central nervous system.<sup>1</sup> (2) Appropriate dosage of noncavitating, high-intensity ultrasound induces physiological changes observable immediately after the minimum dosage required to produce the effect is delivered.<sup>1,3-5</sup> The duration of exposure, to produce a given change, is dependent upon the acoustic intensity and may be of the order of 10<sup>-1</sup> sec for intensities in the neighborhood of 10<sup>3</sup> W/cm<sup>2</sup>.

Physiological changes following exposure are evident as soon as an appropriate test can be made and, for example, may be within a few tenths of a second in cases where electrical activity is observed. (3) There are no delayed effects, as is the case for x-ray and nuclear radiation, indicating that ultrasound acts at a level of structure closely associated with physiological function and not at levels of structure which result in physiological changes after a time delay. (4) The effects of high-intensity ultrasound on tissue structure, at dosages which produce selective irreversible changes, appear to be produced at submicroscopic sites. This has been concluded from detailed histological studies which show that the subsequent changes, in response to the primary action(s), are observable as visible lesions approximately fifteen minutes and longer after irradiation.<sup>6</sup> (5) The effects produced by ultrasound, at dosages which result in selective action, are not the result of thermal mechanisms, although the primary process possesses a temperature coefficient.<sup>3,5,7</sup> Dosage studies indicate that different processes are important over different ranges of the acoustic-field variables.<sup>5</sup>

This paper constitutes an initial progress report on a study prompted by the finding, indicated in (4) above, that the initial site of action of ultrasound on tissue is a submicroscopic structure—perhaps a macromolecule. Thus investigation of the interaction of ultrasound and macromolecular species in solution may lead to clues

<sup>1</sup>W. J. Fry, in *Advances in Biological and Medical Physics*, edited by J. H. Lawrence and C. A. Tobias (Academic Press Inc., New York, 1958), Vol. 6, p. 281.

<sup>2</sup>W. J. Fry, R. Meyers, F. J. Fry, D. F. Schultz, L. L. Dreyer, and R. F. Noyes, *Trans. Am. Neurol. Assoc.* 16 (1958).

<sup>3</sup>F. Dunn, Ph.D. thesis, University of Illinois, Urbana, Illinois (1956).

<sup>4</sup>W. J. Fry and F. Dunn, *J. Acoust. Soc. Am.* 28, 129 (1956).

<sup>5</sup>F. Dunn, *Am. J. Phys. Med.* 37, 148 (1958).

<sup>6</sup>J. W. Barnard, W. J. Fry, and R. F. Krumins, *J. Comp. Neurol.* 103, 459 (1955).

<sup>7</sup>W. J. Fry, D. Tucker, F. J. Fry, and V. J. Wulff, *J. Acoust. Soc. Am.* 23, 364 (1951).

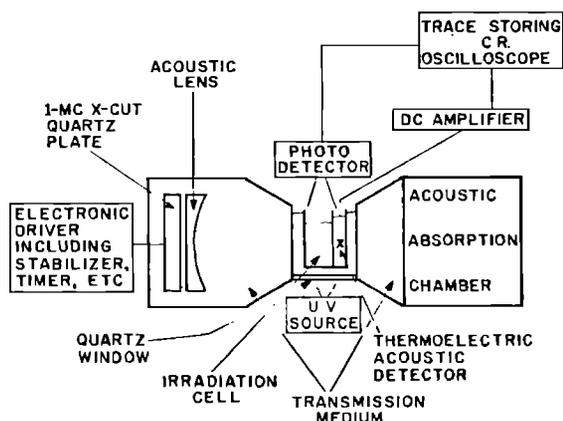


Fig. 1. Schematic illustration of experimental arrangement.

regarding the physical mechanisms of the interaction with tissue structures. DNA (deoxyribonucleic acid) was selected as the macromolecular structure to be investigated in the initial phases of this study because of the ease of obtaining material and because the methodology of preparation and analysis, while not entirely settled questions, is fairly well delineated.

Figure 1 is a schematic illustration of the experimental arrangement. The ultrasound was produced by an X-cut quartz plate excited electrically at the resonant frequency of the system 981 kc/sec by an electronic driver possessing a feedback arrangement to insure a constant, predetermined voltage applied to the quartz plate and consequently a constant acoustic intensity in the irradiation cell. A plano-concave polystyrene lens was employed to focus the sound into the sample chamber. The irradiation cell, rectangular in shape, consisted of two chambers—one containing the sample to be irradiated and the other supporting a small copper-constantan thermocouple imbedded in castor oil, i.e., a transient thermoelectric acoustic detector.<sup>9</sup> Thin polyethylene membranes (0.003 in. thick) formed the forward and rearmost walls of the cell, as well as the inter-chamber partition. The sample chamber was also fitted with quartz side windows to enable transmission of ultraviolet light through the sample (for detection of cavitation) during the acoustic-irradiation period. The irradiation cell was supported on a three-dimensional coordinate system (not shown in Fig. 1), inserted in a larger compartment, and coupled to the sound source by distilled, degassed water, the acoustic transmission medium. The acoustic-absorption chamber, containing degassed castor oil, prevented the production of standing waves throughout the acoustic-transmission path. The output of the thermoelectric acoustic detector was fed through a dc amplifier and displayed on a trace-storing CR oscilloscope. The ultraviolet light emerging from the sample chamber was detected by a photomul-

tiplier, the output of which was also presented to an oscilloscope. The acoustic detector and the three-coordinate positioning system allowed the sample chamber to be positioned at the focal volume of the acoustic lens (the irradiation cell comprised approximately 8% of the embracing volume). The distribution of the acoustic intensity over the sample chamber did not exceed 10%. The transient thermoelectric acoustic detector (described in detail elsewhere<sup>9-10</sup>) was employed also to measure the incident acoustic intensity with a total uncertainty of less than 10%.

DNA from *Bacillus subtilis* was used in this study and prepared according to the method described by Marmur.<sup>11</sup> The concentration of the stock solution from which samples to be irradiated were prepared was 250  $\mu\text{g}/\text{ml}$ , the buffer being degassed standard saline citrate (0.15  $M$  NaCl + 0.015  $M$  sodium citrate, pH 7.0). The irradiation samples were prepared by diluting the stock solution to 20  $\mu\text{g}/\text{ml}$  in degassed standard saline citrate. The sedimentation coefficient,  $S_{20,w}$ , for each irradiated sample (and controls) was determined by sedimentation-velocity analysis, using a Spinco Model E ultracentrifuge equipped with ultraviolet absorption optics. (The rotor speed was 35 600 rpm, and the centrifuge cell was fitted with a Kel-F centerpiece.)

Figure 2 illustrates the molecular degradation of *B. subtilis* DNA resulting from ultrasonic irradiation at two intensities. The ordinate is the sedimentation constant  $S_{20,w}$  (the velocity per unit field of force relative to water at 20°C) in svedbergs (1 svedberg =  $10^{-13}$  sec), and the abscissa is irradiation time in minutes. It is seen that the dependence of the sedimentation constant upon irradiation time possesses two distinct phases, viz., an initial rapid decrease resulting from appreciable decrease in the molecular weight of the DNA molecules, and a subsequent leveling-off, implying that further reduction in the molecular weight virtually ceases. It is observed further that the initial time rate of change of the sedimentation constant, as well as the final value reached, exhibits marked dependencies upon the acoustic intensity. Observation of the 259- $m\mu$  optical density of the DNA solution during the acoustic-irradiation period revealed no discernible increase in absorbance, implying that molecular degradation occurs principally as backbone scission, i.e., breakage transverse to the molecular axis. The results illustrated in Fig. 2 were obtained at the base temperature of 26°C, and the observed maximum temperature rise was approximately 5°C.

Several methods were employed simultaneously during the irradiation procedure to detect the presence of cavitation. (1) The response of the thermoelectric detector is altered drastically at the onset of cavitation and

<sup>9</sup> W. J. Fry and R. B. Fry, *J. Acoust. Soc. Am.* **26**, 294, 311 (1954).

<sup>10</sup> F. Dunn and W. J. Fry, *IRE Trans. Ultrasonics Eng.* **5**, 59 (1957).

<sup>11</sup> J. Marmur, *J. Mol. Biol.* **3**, 208 (1961).

<sup>8</sup> W. J. Fry and F. Dunn, in *Physical Techniques in Biological Research*, edited by W. L. Nastuk (Academic Press Inc., New York, 1962), Vol. 4, Chap. 6, p. 261.

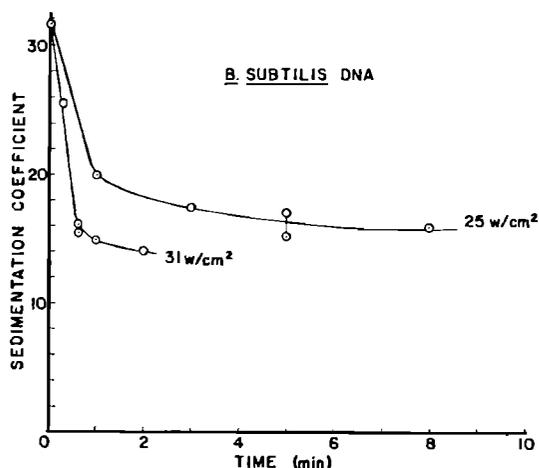


FIG. 2. Sedimentation constant of *B. subtilis* DNA vs irradiation time for two acoustic intensities.

exhibits an erratic response thereafter.<sup>12</sup> For cavitation occurring at the thermocouple-junction boundary, the thermoelectric response increases sharply, and initial time rates of temperature rise of the order of  $10^2$ °C/sec, in water exposed to 1-Mc/sec sound of  $100$  W/cm<sup>2</sup>, can be produced with favorable cavitation conditions, i.e., the presence of sufficient nuclei. For cavitation occurring in the region between the sound source and the detector, the cavitating volume represents a region with an appreciably different acoustic impedance from that of the noncavitating fluid. Consequently, a portion of the acoustic energy incident upon the cavitating volume is reflected back to the sound source, a portion is absorbed in the cavitating volume, and a diminished amount of energy reaches the detector. For this case, the onset of cavitation results in an abrupt decrease in the thermoelectric response of the detector followed by erratic behavior. (2) The energy reflected from the cavitating volume serves to alter the electrical-input impedance at the terminals of the quartz plate, thereby causing the voltage stabilizer to respond in order to maintain the predetermined transducer voltage. Such a corrective re-

sponse of the voltage stabilizer is observed as a deflection on a meter in the feedback circuit. (3) The presence of a cavitating volume alters sufficiently the index of refraction of the fluid such that the transmission of ultraviolet through the fluid is affected appreciably. During the irradiation of the DNA samples, the responses of the thermoelectric detector, the voltage stabilizer, and the transmission of  $259\text{-m}\mu$  uv were all monitored simultaneously. The results presented above were obtained in the absence of any phenomena suggestive of the presence of cavitation. It should be recalled that the acoustic-intensity threshold of cavitation for carefully prepared liquids is quite high. For example, well-degassed, distilled water at 1 Mc/sec exhibits a cavitation threshold in the neighborhood of  $10^3$  W/cm<sup>2</sup>.<sup>8,13,14</sup> Also the cavitation threshold of liquids is known to increase with increasing shear viscosity<sup>15</sup>; the shear viscosity of the samples irradiated in this study was approximately two times that of water, i.e., of the order of 0.02 P. It is thus felt that cavitation can be eliminated as the mechanism by which molecular degradation of DNA was produced in these experiments.

It is considered that degradation of DNA by intense, noncavitating ultrasound results from stresses established within the molecule due to relative motion between the molecules and the suspending fluid (solvent). The relative motion, which results in a viscous interaction, arises because of the density differences between the molecules and the fluid—the molecular density being approximately 70% greater than the density of the fluid. The frictionlike forces existing between the molecules and the fluid produce stresses which, if sufficiently great, rupture chemical bonding. This work is being continued to establish the mechanism of degradation on a firm basis.

#### ACKNOWLEDGMENTS

The authors gratefully acknowledge contributions to this research by Mrs. C. K. Rhodes. This research was supported in part by the Acoustics Branch of the U. S. Office of Naval Research.

<sup>12</sup> F. Dunn and W. J. Fry, in *Ultrasound in Biology and Medicine*, edited by E. Kelly (American Institute of Biological Sciences, Washington, D. C., 1957), p. 226.

<sup>13</sup> R. Esche, *Akust. Beih.* 4, 208 (1952).

<sup>14</sup> M. G. Sirotyuk, *Soviet Phys.—Acoust.* 8, 201 (1963).

<sup>15</sup> H. B. Briggs, T. B. Johnson, and W. P. Mason, *J. Acoust. Soc. Am.* 19, 663 (1947).