Ultrasonically Induced, In Vivo Morphological Damage in Mouse Testicular Tissue*

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Mouse testes were exposed in vivo to 1 MHz continuous wave ultrasonic energy for 30 sec at spatial peak intensities of either 2 or 5 W/cm^2. Following exposure they were surgically removed at times ranging from immediate (within 60 sec) to 63 days, and histologically prepared for optical microscopy examination. The observed alterations can be classified according to the number and type of affected cell layers in the tubule. Leydig cells were unaffected, and some alterations differed from those previously seen at higher ultrasonic exposure intensities or at much longer exposure times.

Key Words: Mouse; Testes; Ultrasound; Seminiferous tubule; Morphology; Leydig Cells; Sertoli cells; Non-ionizing radiation.

INTRODUCTION

A recent study showed that ultrasonically, in vivo irradiated mouse testes, exposed for 30 sec at a spatial peak intensity of 25 W/cm^2 at an ultrasonic frequency of 1 MHz, exhibited two types of damage [6], viz., seminiferous tubule damage, with a suggestion of minor intertubule space involvement, and a more severe form of tubule damage, with significant interstitial involvement. Spermatocytes appeared to be affected earlier than spermatogonia, contrary to the sequence of events following ionizing radiation [2]. The presently reported study was undertaken with the view toward the identification of more subtle and/or alternate forms of morphological changes, at the lower spatial peak intensities of 2 and 5 W/cm^2, as well as classification of types of intratubular damage resulting from ultrasonic exposure.

MATERIALS AND METHODS

Details of the experimental procedures have been previously described in detail [3, 6]. Each testis was exposed to 1 MHz continuous wave ultrasonic energy for 30 sec with spatial peak intensities of 2 or 5 W/cm^2. The in vivo testicular irradiation of the 5–11 month LAF/J_1 mice (Jackson Labs, Bar Harbor, ME) followed anesthetization with methoxyfluorane [4], shaving the scrotum and neighboring inguinal areas, bathing the shaved region in a mild detergent to affect wetting of the surface by the acoustic coupling medium (37°C), viz., degassed mammalian Ringer's solution, and mounting the animal in the supporting structure with the muzzle extending above the liquid surface. A ligature, which did not impede testicular blood flow, insured that the testes remained suspended in the scrotum and did not retract into the abdominal cavity.

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Experimental groups consisted of three or four mice, of which one was a sham exposure wherein all steps of the procedure were identical to that for the exposed animals except no acoustic exposures occurred. Following the exposure procedure, testes of each experimental group were surgically removed at times ranging from immediate (within 60 sec of last exposure) to 63 days after exposure. Testes were placed in 10% formaldahyde solution, or in Bouin's fixative, and then processed by routine histological dehydration and embedding in paraffin. Each pair of testes was cut in 6-μm serial sections with every tenth section mounted on glass slides, yielding twelve sections—six left and six right, per side. The periodic-acid-Schiff reaction with a Harris' hematoxylin counter stain was employed [5].

The mounted and stained sections were examined under optical microscopy at 10× and 25× for morphological changes and abnormalities. All slides for each animal were examined at 10× for presence, if any, and extent of damage. Three to four slides were then selected, from sections equidistant from each other throughout the teste, for a more detailed study at 25× and for scoring abnormalities.

RESULTS AND DISCUSSION

Observed morphological changes could be categorized according to the cellular organization of the seminiferous tubules, the presence of polynucleated cells in the tubules, and the presence of abnormal cells or debris in the lumen. The general shape and condition of the section, as well as the presence of increased amounts of interstitial fluid, could also be noted. Tubular changes were classified according to the definitions listed in Table 1.

Figure 1a is a normal seminiferous tubule from a sham irradiated animal showing normal cell layers. Light damage is classified as one layer of cells missing, or disrupted, in part or in whole. The shape of the tubule is usually normal, but may be slightly altered. Figure 1b shows all cell layers present except for the late spermatid layer, which should be in stage 14–15 of spermatid development and lined-up around the edge of the lumen preparatory to release. In an alternate form of light damage, termed light disorganization, all the cell layers are present but one layer is randomly scattered, rather than being arranged in the normal pattern.

Moderate damage is classified as more than one layer of cells missing or disrupted. The shape of the tubule may vary from normal to greatly altered. All of the late spermatid layers (stage 15) and most of the early spermatids (stage 5) are missing in the example shown in Fig. 1c and the tubule is kidney-shaped, as opposed to the normal oval shape. Moderate

TABLE 1 Damage Classifications for Tubular Morphological Changes

CLASSIFICATION	SHAPE	CELL LAYERS	CELLULAR ORGANIZATION
Normal	Normal round or ellipsoidal	All layers present	All layers in proper organization
Light	Usually normal, may be slightly altered	One layer (usually late spermatid) may be missing	One layer, in part or in whole, disrupted
Moderate	May range from normal to greatly altered	Two layers missing, in part or in whole	Two layers disrupted
Heavy	Almost always altered—sometimes severely	All layers missing or stem cells and/or Sertoli cells present	Organization of all layers completely disrupted

disorganization has all cell layers present, but two of the four layers are scattered throughout the tubule instead of in their appropriate concentric rings.

Heavy damage is characterized by all cell layers missing or the stem cell–Sertoli cell layer may be present. The shape may range from normal to severely altered and, as seen in Fig. 1d, all cells are missing except some remaining stem cells and Sertoli cells. Three vacuoles are also visible, as well as some debris in the lumen. The interstitial fluid may also be affected, viz., increasing or decreasing in amount according to the severity of damage. The Leydig cell population, however, remains stable. Another characteristic frequently observed in heavily damaged tubules is the presence of a densely PAS positive fluid within the tubule, generally seen in completely denuded tubules and filling the entire lumen.

Occasional damaged tubules have been observed to occur naturally in normal specimens and may fall into any of the above classifications. Also, the rete testes appear as heavy damaged tubules in normal, sham irradiated, and irradiated testes and should not be confused with tubules altered due to the ultrasonic exposure procedure.

In addition to effects of the cellular organization of the tubules, other abnormalities have also been observed. It appears that the number of polynucleated cells present is linked to the presence of damage. Though it is common in a normal testis to see an occasional polynucleated cell, more than approximately one per histological section may be considered abnormal and indicative of damage. Polynucleates and giant cells are most often seen as spermatids in a common membrane, usually while still round, but other cells may also be involved. Another indication of alteration due to ultrasonic exposure is the presence of abnormal cells or of debris in the lumen of otherwise normally appearing tubules, which may be due to sloughing off of cells in a damaged area further up the tubule. Though sloughing usually involves spermatids and spermatocytes, spermatogonia are occassionally seen in this debris. Damaged tubules often exhibit debris or cells in the lumen, but it is generally material from that point in the tubule. In addition to these recordings of damage, the number of polynucleated cells in both damaged and normal tubules, as well as the number of tubules with abnormal cells or debris in the lumen have also been recorded.

Other less frequently occurring abnormalities include large intratubular vacuoles, abnormally large and empty lumens, small intratubular spots of PAS positive material, broken basement membranes, vacuolated spermatids, and cells with picnotic nuclei.

Morphological alterations due to exposure to ultrasound at the low intensities presented in this paper appear to differ in some respects from effects of higher intensities previously reported by [6]. Firstly, no gradation of tubular involvement was seen, but altered tubules were randomly scattered throughout the sections and no visible estimate could be made of which side of the testis was facing the source of ultrasound. Secondly, the lacework appearance of tubules and gapping between cells reported at 25 W/cm^2 was rarely seen at the lower intensities of 5 and 2 W/cm^2. This may be due to a decrease in the effect of ultrasound on intratubular fluid. Finally, PAS positive materials, acrosomes in particular, were present and normal in all but heavily damaged tubules, which suggests a continuance of spermiogensis not seen at higher intensities [6] or at much longer exposure times [1]. Observed differences in extent and in type of alteration between the two intensities of exposure used were considered not significant.

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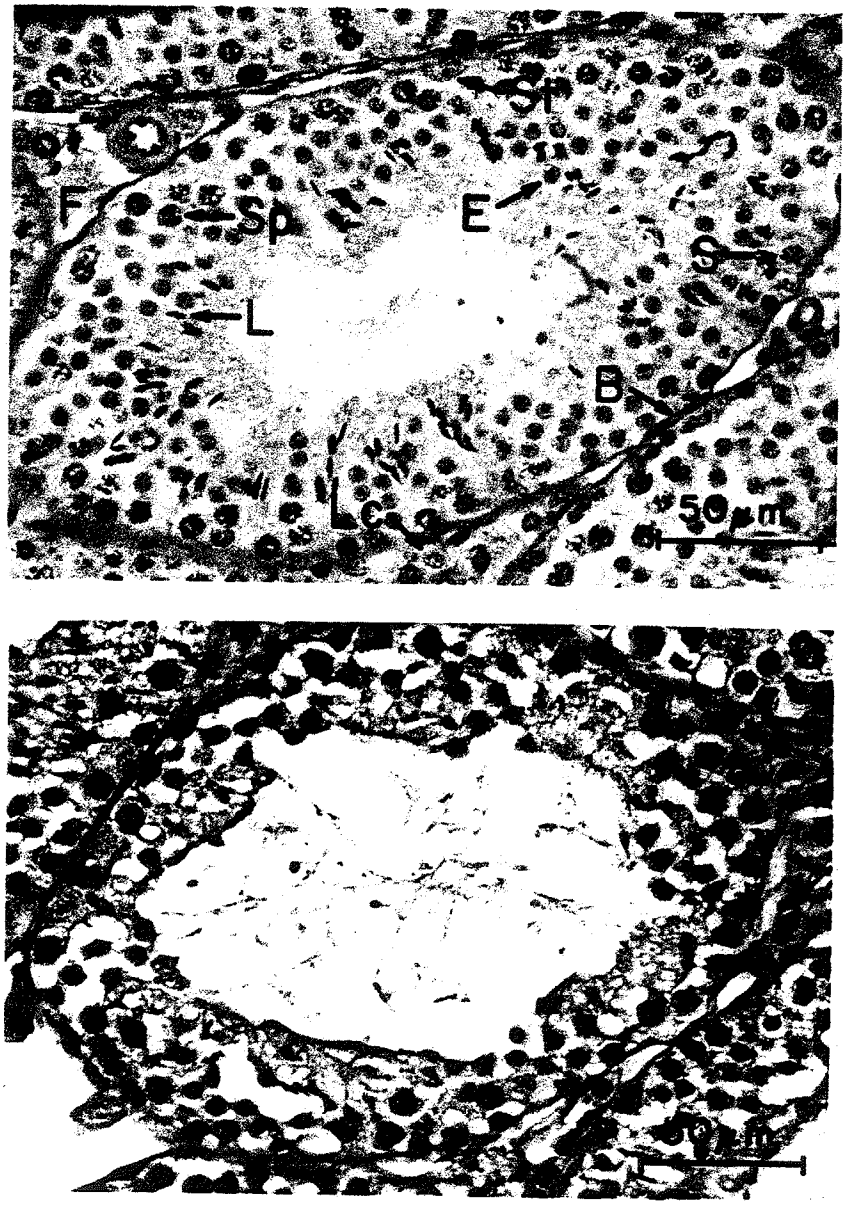
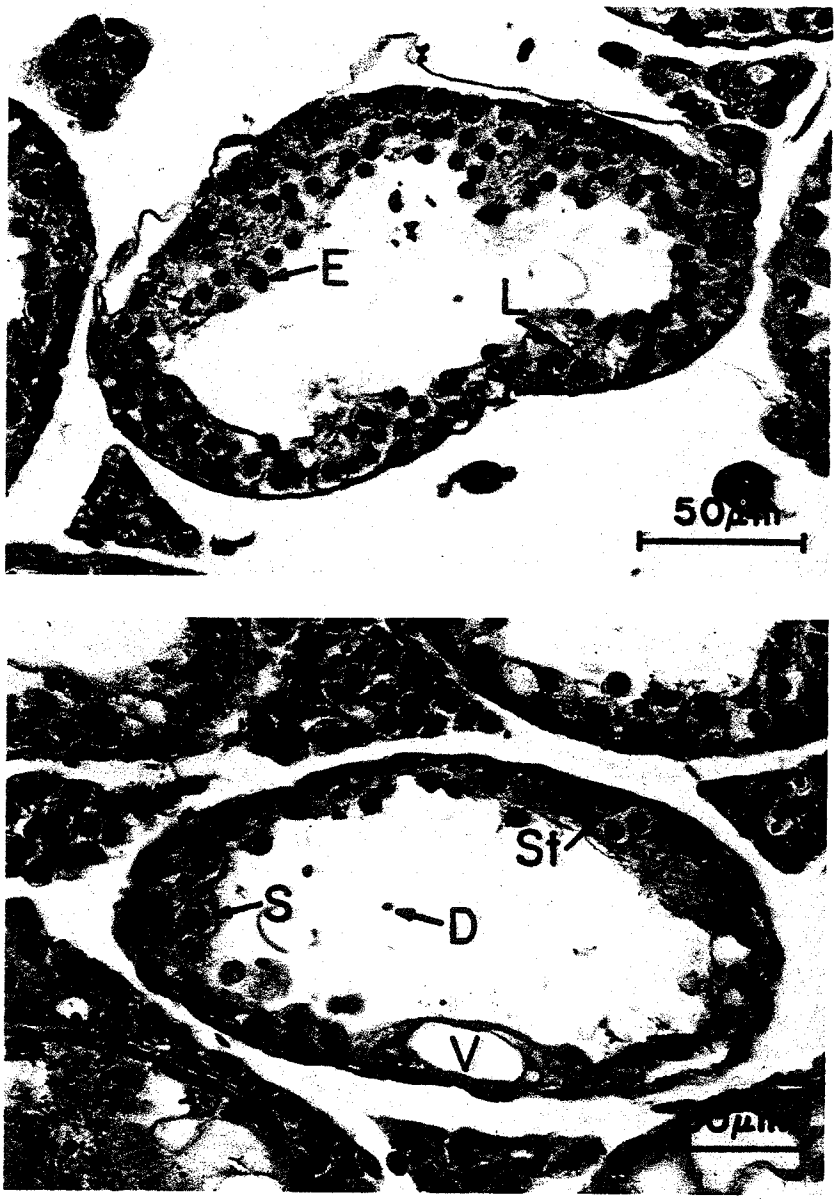


FIGURE 1. a. Sham exposed specimen exhibiting normal mouse seminiferous tubule showing *Lc*-Leydig cells: *F*-interstitial fluid, *B*-basement membrane, *St.-Sertoli cells*, *S*-spermatogonia, *Sp*-spermatocytes, *E*-early spermtids, and *L*-late spermatids with PAS positive acrosomes. b. Irradiated (2 W/cm^2) mouse testis 5 weeks post-exposure. An example of light damaged tubule showing absence of late spermatid layer. c. Irradiated (5 W/cm^2) mouse testis 5 weeks post-exposure exhibiting



moderate damage of tubule. Portions of both the *L*—late spermatid and *E*—early spermatid layers are missing. Shape of tubule has also been slightly altered. d. Irradiated (5 W/cm^2) mouse testis 5 weeks post-exposure exhibiting heavy damage. All cells are missing except for some S-spermatogonia and a few *St*—Sertoli cells. *V*-vacuoles are present as well as *D*—debris in the lumen.

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