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MORPHOLOGICAL CHANGES TO MOUSE TESTICULAR TISSUE FROM *IN VIVO* ULTRASONIC IRRADIATION (PRELIMINARY REPORT)

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Abstract—Mouse testes have been ultrasonically irradiated *in vivo* and subsequently examined morphologically. Each testis was sequentially exposed for 30 sec at a spatial peak intensity of 25 W/cm^2 at a frequency of 1 MHz. The testes were removed at varying times post irradiation, from immediate to 19 days, fixed and histologically prepared and stained with hematoxylin and eosin and periodic acid Schiff. The results suggest that two types of ultrasonically induced damage occur wherein, for different specimens under identical exposure conditions, there is either semeniferous tubule disruption with a suggestion of minor intertubule space involvement or a more severe form of tubule damage with significant interstitial tissue involvement. It appears that spermatocytes are affected earlier than spermatogonia, contrary to the situation following ionizing radiation.

Key words: Ultrasound, Acoustic, Ultrasonic damage, Testes, Semeniferous tubules, Spermatocytes, Spermatogonia.

INTRODUCTION

Use of diagnostic ultrasound on, or in close proximity to, the male reproductive organs has been shown to yield useful clinical information (Miskin and Bain, 1974; Albright and Harris, 1975). However, conflicting reports have appeared from animal studies wherein ultrasonic energy has been found to affect spermatogenesis and fertility (Kamocsay *et al.*, 1955; Fahim *et al.*, 1975) and where no such effects were observed (Lyon and Simpson, 1975; Urry *et al.*, 1978). Thus, the few data available for basing an assessment of risk to testicular tissue from ultrasonic energy are contradictory.

A study has been initiated for the purpose of providing basic information regarding the interaction between ultrasound and mouse testicular tissue. A portion of this work concerning the ultrasonic absorption coefficient of testicular tissue has been reported (Brady *et al.*, 1976). This paper deals with a continuation of that work in which ultrasonically induced morphological changes in the testis are examined.

Testes are the male organs which produce spermatozoa. The production of spermatozoa is called spermatogenesis and consists of spermatocytogenesis and spermiogenesis. In the mouse spermatocytogenesis begins with a stem cell or type A spermatogonium which gives rise to a smaller type B spermatogonium through the intermediate spermatogonium. Type B spermatogonia either divide or metamorphose into primary spermatocytes which have two sets of chromosomes. The first meiotic division of the primary spermatocyte yields two haploid secondary spermatocytes. The second meiotic division yields four spermatids, each with one set of monovalent chromosomes, and signals the beginning of spermiogenesis. Spermiogenesis, the transformation of a spermatid into a spermatozoon, involves no divisions. At any given cross-sectional segment of the mouse seminiferous tubule, type A spermatogonia are initiating the process which will liberate spermatozoa some 34.5 days later. Spermatocytogenesis requires approximately 25 days and spermiogenesis 9.5 days. This process is initiated every 8.6 days (Monesi, 1972; Rugh, 1968; Nalbandov, 1976).

METHODS AND MATERIAL

In vivo testicular irradiation of LAF$_1$/J mice (Jackson Labs, Bar Harbor, Maine) proceeded by anesthetizing the 5–7 month old males with methoxyfluorane (Hall, 1971), shaving the scrotum and surrounding inguinal regions, bathing the shaved region in a mild detergent to assure wetting of the surface by the coupling medium and mounting the animal in a specially designed supporting structure (holder), virtually the same as that des-

cribed by Fry *et al.* (1978). A ligature which did not impede testicular blood flow was employed to insure that the testes remained suspended in the scrotum. Observations post irradiation showed that the testes did not retract into the abdominal cavity. The holder was positioned in the Lucite(R) irradiation tank and 37°C degassed mammalian Ringer's solution served as the coupling medium between the 1 MHz ultrasonic source and the scrotum. Acoustic absorbing material lined portions of the tank to minimize reflections.

Intrascrotal temperature was determined by inserting a 0.075 mm diameter copper-constantan thermocouple under the scrotal skin and recording, from a digital telethermometer, the peak temperatures obtained from ultrasonic exposures at 25 W/cm^2 for the various exposure times.

The spatial peak intensity was 25 W/cm^2 at the site where the testis was placed, but without the specimen in the field. The half-power beam width at that position was 6 mm as determined by the transient thermoelectric technique (Dunn and Fry, 1972; O'Brien, 1978). Exposure time for both sham and irradiated groups was 30 sec and both testes were sequentially exposed by positioning the beam axis in the center of each. No attempt was made to shield acoustically the adjacent testis. Ample time (several minutes) was allowed for testicular tissue to reach thermal equilibrium with the coupling medium prior to ultrasonic exposure. Normal mouse testicular temperature ranges from around 29°C for the anesthetized animal to around 33°C for the unanesthetized animal (Waites, 1970). The higher than normal testicular temperature was not contributory to the effects observed in that each irradiated specimen has a parallel sham for comparison. The reported ultrasonic intensity represents the free field value determined by the Bioacoustics Research Laboratory calibration facility to a precision of ±3% (Dunn *et al.*, 1977).

The testes were removed at varying times post irradiation, from immediately (within 60 sec post irradiation) to 19 days post irradiation, and placed in 10% formalin in Ringer's solution for a minimum of 48 hr. A sham irradiation specimen was employed for every histological preparation. Standard histological dehydration and paraffin embedding was employed (Thompson, 1966). Six micron sections were prepared to minimize possible confusion concerning the specific stage of spermatogenesis. Adjacent section sets were stained with hematoxylin and eosin (H & E) and periodic acid Schiff (PAS). H & E stains nuclear material black and cytoplasm and connective tissue various shades of gray while PAS stains carbohydrates, a main constituent of the acrosome, black and the remaining cellular components various shades of gray. All micrographs included in this paper are H & E stained.

The stained sections were analyzed first at low power (40×) to detect and classify gross damage to, for example, intra-tubular, extra-tubular, and the connective tissues. The sections than were examined at higher magnifications (100× – 1000×) to detect the specific cell types damages, alteration of spatial organization and alteration of temporal sequences. This last criterion applied most particularly to the spermiogenic cycle and was evaluated principally from the PAS sections.

RESULTS

Figures 1 and 2 show normal mouse testis in cross sections. Normals and shams were morphologically indistinguishable. Figure 1 shows the seminiferous tubules embedded in a matrix of interstitial cells which are encapsulated in the tunica albuginea, a tough, connective tussue shell. Figure 2 illustrates in greater detail the structure of two adjacent seminiferous tubules and a variety of different germ cell stages. Here two stem cells are visible, adjacent to, and just below the basement membrane which forms a diagonal in the figure. On the opposite side of this membrane the curving line of deeply stained cells are the type B spermatogonia, which are the mitotic offspring of the stem cells *via* the intermediate stage. The mitotic figures visible in the lower tubule are also type B spermatogonia dividing to form primary spermatocytes. The remaining cells visible in the lower tubule are pachytene primary spermatocytes which have a mottled appearing nucleus, and the thin, tapered slopes of stage 15–16 spermatids (Rugh, 1968) which will be released into the tubule lumen as immature spermatozoa.

The lacework appearance in the upper part of Fig. 3 from a specimen is found to be characteristic of the early stages of gross, acoustically induced damage and results from vacuolization to the tubule wall and sloughing of the germinal epithelium from the basement

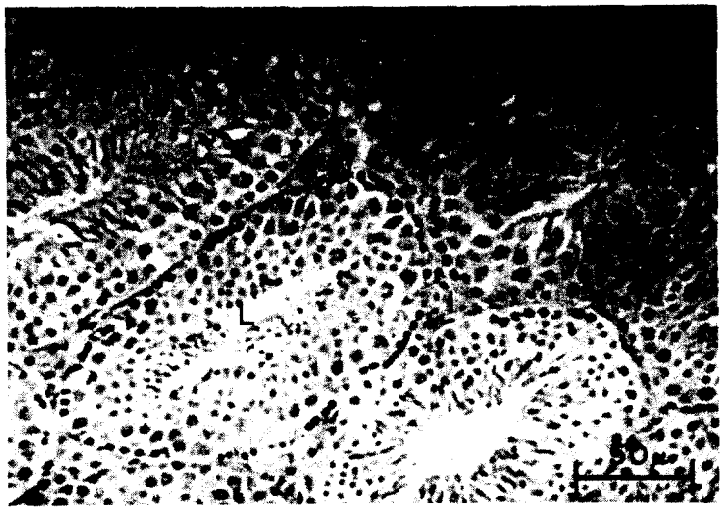

Fig. 1. Normal mouse testis. L-lumen, W-tubule wall, I-interstitial tissue. (100×).

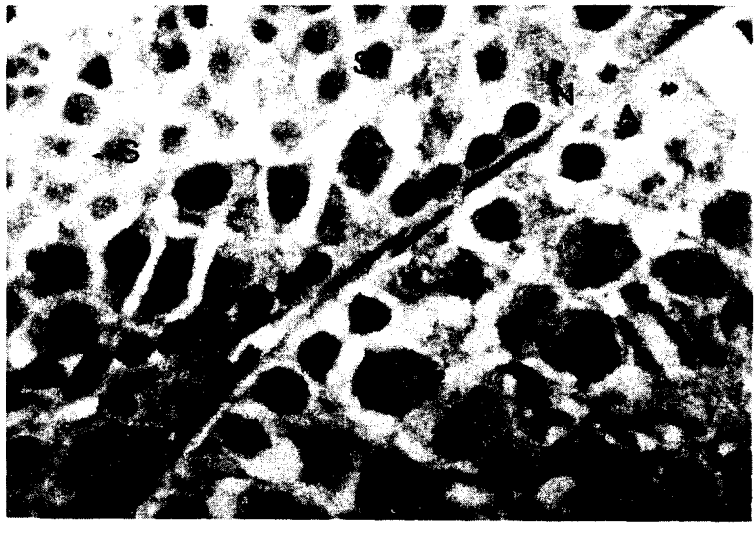

Fig. 2. Normal mouse testis. A and B-type—A and B spermatogonia, respectively, P-primary spermatocytes, N-Sertoli cells, S-spermatids (450×).

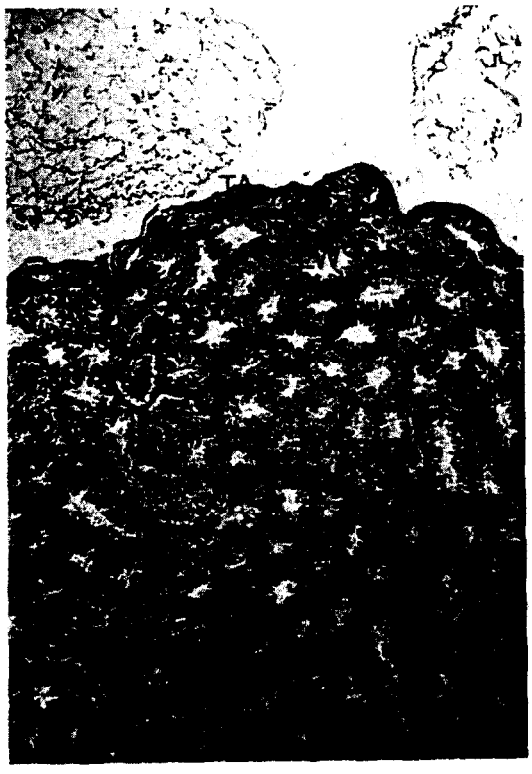

Fig. 3. Irradiated mouse testis sacrificed immediately post irradiation. 4 and 6-general areas of Figs 4 and 6, respectively, TA-tunica albuginea, T-seminiferous tubule. LW-lacework appearance (40 ×).

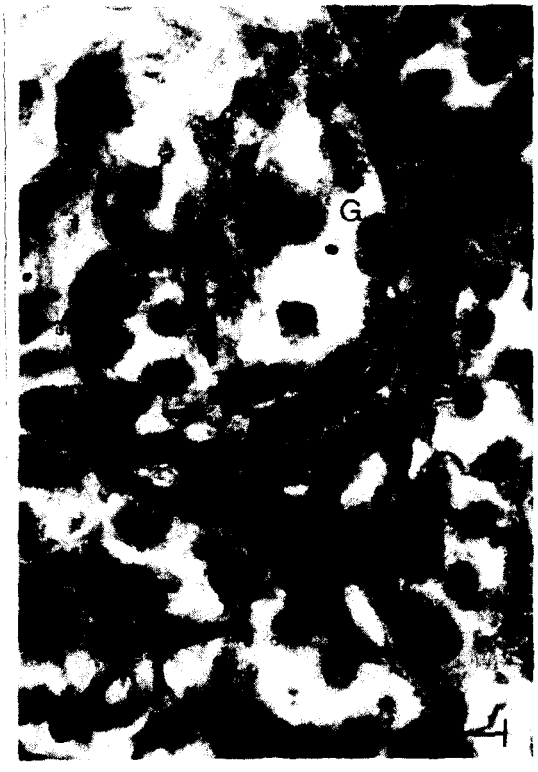

Fig. 5. Irradiated mouse testis sacrificed immediately post irradiation. G-gap. (1000 ×).

Fig. 4. Irradiated mouse testis sacrificed immediately post irradiation. G-gap. (400 ×).

Fig. 6. Irradiated mouse testis sacrificed immediately post irradiation. G-gap. (250 ×).

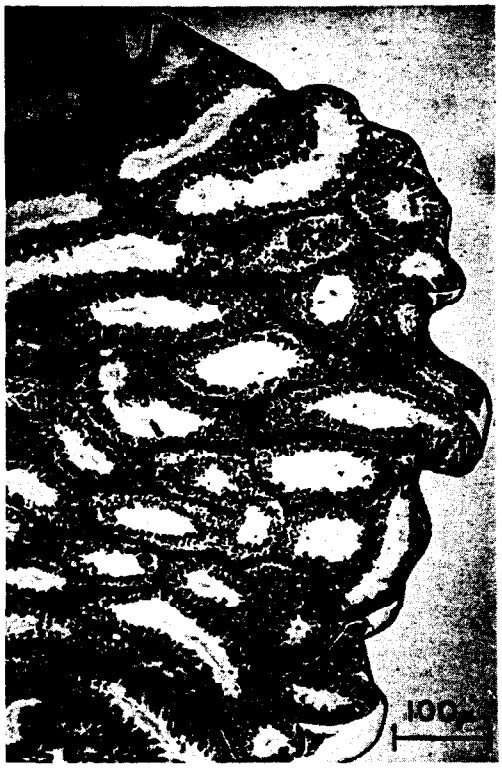

Fig. 7. Irradiated mouse testis sacrificed 48 hr post irradiation. 8-general area of Fig. 8. (40 ×).

Fig. 9. Irradiated mouse testis sacrificed 48 hr post irradiation. (400 ×).

Fig. 8. Irradiated mouse testis sacrificed 48 hr post irradiation. (250 ×).

Fig. 10. Irradiated mouse testis sacrificed 7 days post irradiation. D-disrupted interstitial tissue. (250 ×).

Fig. 11. Irradiated mouse testis sacrificed 7 days post irradiation. D-disrupted interstitial tissue, V-vacuolization. (400 ×).

Fig. 12. Irradiated mouse testis sacrificed 7 days post irradiation. D-disrupted interstitial tissue. (250 ×).

membrane. This specimen was placed in formalin fixative within 60 sec of irradiation, the disruption is seen in greater detail in Figs 4 and 5 wherein areas abnormally devoid of cells within the tubule appear as gaps along the basement membrane. In addition, cells at various stages of development, which normally are in contact with the Sertoli cells to provide at least mechanical support if not various metabolites (Monesi, 1972), are seen to be apparently free from such contact.

Under the low power observation of Fig. 3, a gradation of tubule disruption is evident ranging from a normal appearance to one of disruption comparable to that shown in the upper part of the figure. The principal feature seen in the lower part of the figure is the gap between the basement membrane, to which the germ cells remain adjacent, and the cells, which are in various first stages of meiosis, as seen in greater detail in Fig. 6.

The interstitial tissue generally appears morphologically normal. There are a few areas which suggest that the intertubule space has become thickened, perhaps due to fluid exudation.

At 48 hr post irradiation (Fig. 7) an overall increase in the disruption in the seminiferous tubule with fluid accumulation is seen. Figures 8 and 9 indicate, in greater detail, the appearance of spermatids free from contact with the Sertoli cells, the gap along the basement membrane where stem cells appear to have deteriorated and giant or aggregated cells. Other than selected areas of swelling of the intertubule space, and areas where the tubule wall appeared to have ruptured, the interstitial tissue and cells appeared normal.

The general nature of testicular disruption seen in Figs 3 and 9 has been seminiferous tubule involvement with a suggestion of minor alterations to the intertubule space and represents one class of observed damage. However, as early as 6 hr post irradiation under identical exposure conditions in other specimens, a much more severe class of damage is evident with significant interstitial tissue involvement. At 6 days post irradiation, this damage is characterized by the lack of tissue continuity between tubules. That is, the interstitial tissue area has become fluid filled with Leydig cells floating within this fluid. Also numerous tubules appear to be fluid filled shells or clogged with cellular debris. At 48 hr post irradiation, testicular damage is similar to that seen at 6 hr post irradiation.

By seven days post irradiation, on some specimens, testicular damage has progressed to exhibit groups of fluid filled tubules which show no signs of spermatogenic activity and other groups which demonstrate less severe damage but only to the tubules, as seen in Figs 10–12. The damage shown in these three figures is similar to that described for the more severe damage at 6 and 48 hr post irradiation. The interstitial tissue is severely disrupted, being fluid filled and giving the appearance of Leydig cells floating about. Seminiferous tubule damage is varied, ranging from fluid filled lumen in Fig. 10 to vacuolization and generalized disruption in Fig. 11 to debris filled with no evidence of spermatogenic activity in Fig. 12. The three figures are from different areas of the same histological section, which is representative of damage at seven days post irradiation.

The damage to testicular tissue from mice sacrificed up to 19 days post irradiation shows evidence of increasing damage, but even at day 19 there is evidence of spermatogenic activity on sections stained with H and E. However, adjacent sections stained with PAS suggests that beyond the seventh day post irradiation spermiogenesis has ceased. The evidence for this is the absence of PAS positive material, i.e. absence of carbohydrate content of the acrosomes.

DISCUSSION

This preliminary report suggests that ultrasound is capable of markedly disrupting testicular tissue by affecting both spermatocytogenesis and spermiogenesis and by disrupting the interstitial tissue. The lack of PAS positive material beyond the seventh day post irradiation suggests that spermiogenesis has ceased.

The spatial distribution of damage over any single preparation is highly variable, and in some cases does not correspond to the free field intensity distribution which is believed to be uniform within 5% across the breadth of the testis owing to the significantly low ultrasonic absorption in testicular tissue (Brady *et al.*, 1976; Goss *et al.*, 1978). Such a distribution of damage, and specifically with regard to nearly adjacent tubules where one area appears normal and another area appears damaged, may be unique to ultrasonically induced damage to testis. The closeness of the tubules would provide a basis for suggesting some non-thermal mechanism, such

as cavitation-like activity, for producing this highly localized damage.

The damage at the cellular level is varied, depending upon the type of cell examined. In this case of ionizing radiation, the order of sensitivity of germ cells is known (Oakberg, 1956b; Mandl, 1964): the type B spermatogonia are most sensitive, followed by intermediate and type A spermatogonia. These are then followed by primary spermatocytes, secondary spermatocytes, spermatids and finally spermatozoa. The Sertoli cells and interstitial cells are very resistant to damage by ionizing radiation (Oakberg, 1956a). This order appears, at least initially, to be different for damage caused by ultrasound. Observations of the damage produced at 1 MHz shows spermatocytes to be the earliest influenced cell types in the tissue. Evidence for this is the liquefaction and breaking up of these cells in large numbers as the first signs of damage.

The physical mechanisms responsible for the observed testicular damage are not well understood, but the field of possibilities may be somewhat narrowed. Cavitation can occur under certain circumstances in aqueous liquids at this intensity. Although the disruption of the tissue is severe, it is not grossly destroyed as has been observed in intact tissue from transient cavitation (Fry *et al.*, 1970). This, however, does not preclude stable cavitation although never demonstrated in intact tissue. The possibility of a thermal mechanism is necessary to consider owing to the thermal sensitivity of testicular tissue. At an ultrasonic intensity of 25 W/cm^2 the intrascrotal temperature is measured to be 47–50°C at the end of a 30 sec exposure. The effect of a transient temperature increase on testicular morphology is not known. Generally, temperature effects are studied for longer periods of time, around 20–30 days, at elevated temperature which are in equilibrium with surroundings, i.e. gradients are not conspicious (Van Damark and Free, 1970). As of now, however, there is no evidence to favor any particular mechanism.

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