Radiat Environ Biophys (1986) 25:235–240

Radiation and Environmental Biophysics
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# Low-kilohertz-water-borne ultrasound biological effects

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Received February 4, 1986 / Accepted July 22, 1986

**Summary.** The effects of 25 kHz ultrasound on murine testes was studied, as mimicking possible World WAR II SONAR exposure to swimming maintenance personnel. Very few specimens were found to exhibit morphological tissue alterations, depending upon length of exposure time and proximity of the source to the tissue. Thermal processes seem to be eliminated, but microstreaming may be implicated, as the physical mechanism(s) of interaction.

## Introduction

Interest has been exhibited in the health and safety of marine divers who must on occasion swim in the neighborhood of emitting SONAR projectors (Smith and Hunter 1980). Concern has emerged as these personnel are required to spend appreciable time, as long as six hours, in the fields of transducers radiating acoustic power at near cavitation threshold levels at significant depths. For example, at 200 m depth the cavitation threshold is estimated to be increased, from the approximately 0.33 $W/cm^2$ at 1 atm at the surface of the sea, to approximately 20 $W/cm^2$ at 7 atm ambient pressure (Hueter and Bolt 1955). Further, reports occasionally emerge alleging that World War II (WWII) sonarmen, who were required to swim near emitting units to inspect the transducer surface and to perform other maintenance duties, have developed physical disorders including atrophy of limbs and sterility (Youngstrom 1977).

The study reported herein was undertaken as a preliminary inquiry of the possible effects of water-borne kilohertz ultrasound. Because sterility is alleged to have resulted from exposure to WWII SONAR emissions and because this laboratory has studied megahertz ultrasound effects on the mammalian testis (Bailey et al. 1981), the established biological procedures and ultrasound experience could be brought to the problem with little difficult.

A result of earlier studies was that because of the high water content, and consequent low ultrasonic absorption coefficient value, testicular tissue can experience high amplitude incident acoustic waves before evidence of morphologic alterations are detected (Goss et al. 1978; Bailey et al. 1981; O'Brien et al. 1979). Noting that these studies were conducted at 1 MHz and that the ultrasonic absorption coefficient of soft tissues varies nearly linearily with frequency (Goss et al. 1978, 1980; Dunn and Goss 1984), any effects produced in the low kilohertz frequency range are not likely to result from thermal mechanisms.

## Materials and methods

Hap:ICR male mice two to four months of age were anesthetized with methoxyflurane (Metofane, Pitman-Moore). The lower abdominal and scrotal areas were shaved, depilated, wetted with a mild detergent solution and then the animal mounted in a specially designed holder (Fry et al. 1978) such that the ventral side faced the energy source. A loose ligature was tied around the base of the scrotum to prevent the animal from retracting the testes into the abdominal cavity during the exposure.

Following preparation, the animal and holder were placed in a Plexiglas tank containing highly degassed 37° C mammalian Ringers solution as the acoustic coupling medium. The probe was aligned such that the tip was normal to the coronal plane, of the spread specimen, and positioned at the center of the scrotum.

A Biosonik IV (Bronwill Scientific, Rochester, NY) tissue homogenizer was used as the acoustic energy source. The operating frequency of the instrument is 25 kHz and the intensity employed for this study was 15 W/cm$^2$, as determined in this laboratory by a calorimetric method (Sokollu 1966). The standard probe of the instrument was used, which is 36 cm in length and 1.0 cm in diameter at the tip.

Specimens were exposed in groups of six with each group containing four exposed and two sham animals. The entire scrotum, i.e., both testes, was exposed with a single irradiation regime schedule. Sham animals were treated in an identical fashion as the exposed animals except that the sound source was not energized. The exposure time and the distance of the probe from the scrotum were varied for each group. Exposure times ranged from 30 s to 6 min and distances ranged from the probe touching the scrotum to the probe placed 3.75 cm from the scrotal surface.

Following exposure, the animals were placed in individual cages and allowed to recover. They were sacrificed 24 h after exposure by cervical dislocation. The testes were surgically excised, fixed in Bouin's fixative, and processed according to routine dehydration and paraffin embedding procedures. The blocks were cut in 6 μm serial sections and every tenth section was mounted on glass slides. The slides were subsequently stained with periodic-acid-Schiff reaction with a hematoxylin counterstain (McManus 1948). Following staining, the slides were examined under light microscopy for morphological alteration of the tissue.

## Results

Of the nearly 150 animals exposed in this study only four showed morphological alteration to the testes which could be attributed to ultrasonic energy. In addition, the damage was confined to only one testis of the animal, the other testis exhibited no alteration. For those four specimens/testes showing changes, the area of alteration generally involved only a few seminiferous tubules in a small area except for one animal in which approximately one-third of the testis was involved in the most severely damaged area. In all cases, the remainder of the seminiferous tubules in the damaged testis was normal and the tubules appeared functional as evidenced by the presence of normal appearing sperm in the lumen of the epididymis.

The tissue alteration was similar to that previously described following exposure to megahertz ultrasonic energy (Bailey et al. 1981) and was manifested in the form of cellular disruption, vacuoles in the cells and tissues, pycnosis of cell nuclei, thickening of the tunica albuginea, presence of polymorphonuclear leukocytes, and distortion of the normal round shape of the seminiferous tubules involved.

The exposure parameters for those animals affected were as follows: (*1*) a distance of 3 mm at an exposure time of 150 s; (*2*) the probe touching the scrotum at an exposure time of 60 s (two animals); and (*3*) a distance of 1.5 mm with a 75 s exposure time. One animal exposed at a distance of 3 mm for 150 s showed an area of damage to the epididymis but both testes were normal. Three other animals in the group with the probe touching, and at an exposure time of 60 s, showed petechial hemorrhages on the scrotal skin after exposure but no alteration to the testes or surrounding tissues.

## Discussion

The findings of this experiment suggest that it is unlikely that the alleged sterile condition reported by the WWII SONAR technician resulted from exposure to the SONAR beam. This conclusion is reached in the following.

Firstly, the intensity of the acoustic power source was significantly greater than that of WWII SONAR devices. The intensity of the tissue homogenizer used for the reported exposures was determined to be 15 W/cm$^2$ whereas the SONAR transducers in question operated at approximately 1 W/cm$^2$ maximum output (R. Woollett, personal communication). The acoustic intensity produced by WWII SONAR projectors was below the cavitation level and, for sea water at the surface and at low kilohertz frequencies, this is approximately 0.33 W/cm$^2$ (Hueter and Bolt 1955). The designed purpose of the tissue homogenizer, on the other hand, is to disrupt cells and tissues by means of cavitation and thus is designed to operate at intensities sufficient to produce cavitation in aqueous-like media.

Secondly, it is necessary to consider the differences between experimental preparation of the specimen scrotum, viz., intimate contact with the sound propagation medium, versus the immediate environment of a diver's scro-

tum clothed, and otherwise enclosed. The experimental animals were shaved, depilated around the scrotal area, and wetted with solution to insure intimated acoustic contact of the highly degassed coupling medium, allowing an unimpeded exposure of the scrotum to the acoustic energy. Thus, it can be estimated that reflected energy was minimal and virtually all incident energy propagated through the testis. The situation for the diver is, of course, very different. It can be assumed that WWII divers wore one or more layers of clothing which, together with the presence of scrotal hair and epidermal wrinkles, which would trap air on the scrotal skin surface, would produce an acoustic impedence mismatch serving to decrease significantly the amplitude of the acoustic wave reaching the testicular tissue. Thus, it can be concluded that the amplitude of the wave propagating through the testis of the diver could be orders of magnitude less than that emitted by the SONAR transducer.

Thirdly, the difference between the experimental situation and the diver's "natural" situation, as regards movement of the subject, needs to be considered. The experimental animals were anesthetized and restrained such that the scrotum received the full magnitude of the acoustic energy for the entire exposure period. The situation is quite different for the diver who must be considered to be in constant motion due to the swimming activity associated with the purpose for being in the neighborhood of the SONAR transducer. Such motion reduces the amount of time the acoustic energy is directed toward the scrotal area, as well as providing for partial shielding of the scrotum due to the body movement, etc.

Although great care must be exercised in extrapolating from animal experiments conducted under laboratory conditions to the human diver situation, for which the exact conditions prevailing can not be known, it does appear that, based upon the study reported herein, irreversible effects due to exposure to SONAR emissions during routine inspections is unlikely. This conclusion emerges from the lack of observed effects in our study which represented optimal exposure conditions and opportunity for morphological alteration of testicular tissue. Further, as regards the effects observed in the laboratory exposures, it must be noted that they would have shown only slightly decreased fertility due to the relatively small area and small number of seminiferous tubules involved. Depending upon the extent of the involvement of the stem cell population within the tubules, the decrease in fertility would have been temporary, lasting until the stem cells replicate and repopulate the area. Repopulation would take at least one entire spermatogenic cycle, which in man represents a minimum of 75 days (Monesi 1972).

It may be instructive to consider the mechanisms involved in the production of the effects observed in this study. The thermal mechanism is not particularly likely because of the presumed low value of the absorption coefficient at the low frequency of 25 kHz. Extrapolating measurements from the 0.5 to 7 MHz frequency region to 25 kHz yields the acoustic amplitude absorption coefficient value of $2.6 \times 10^{-4}$ cm$^{-1}$ (Goss et al. 1978). Thus, exposure to 15 W/cm$^2$ ultrasound for 6 min would produce a tempera-

ture elevation, in the absence of any thermal conduction or convection phenomena operating, of less than 1° C. Clearly, thermal events can be ruled out.

Of the few specimens exhibiting observable morphological effects in this study, it is clear that length of exposure time and distance of the sound source to the specimen surface played important roles. Thus, microstreaming, small scale steady fluid motion in the neighborhood of the vibrating source, may be considered as the physical mechanism of interaction of those exhibiting petechial hemorrage on the scrotal skin surface (Nyborg 1965). A significant feature of such microstreaming is the establishment of a boundary layer of thickness $\delta$ over which a velocity gradient is developed. The boundary layer and the concomitant shearing stress are given, respectively, by

$$\delta=\sqrt{\frac{2\eta}{\omega\rho}}$$

and

$$\sigma=\eta G=\eta\,\frac{\omega\xi^2}{\delta a}$$

where $\eta$ and $\rho$ are the shear viscosity and density of (in the case herein) the coupling fluid, $\omega$ is the angular frequency of the vibrator, $G$ is the velocity gradient and $a$ is the radius of the vibrator (Nyborg 1965). Using the values for water and the transducer employed in this study, the boundary layer $\delta=3.6$ µm and the shear stress $\sigma=1{,}100$ dynes/cm$^2$. The latter value is of the same order of magnitude as those found necessary to hemolyse erythrocytes in suspension by a single sonically driven bubble (Rooney 1970) and by a vibrating wire system (Williams et al. 1978). Thus, credence is given to this mechanism for this case.

The irreversible effects observed in the interior of the testis are more difficult to discuss. Testis is unusual in having such a significant fraction of its volume occupied by fluid-filled tubules. Indeed, the low value of the ultrasonic absorption coefficient of testis is attributable to its average high water content associated with these fluids. Thus, the opportunity for cavitation nuclei to be present may be greater than for those tissues less vascularized, though further details cannot be obtained from this preliminary study.

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