

Ultrasound Biologic Effects:

A Suggestion of Strain Specificity

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Pregnant hybrid LAF₁/J mice were exposed to continuous-wave ultrasonic energy at a frequency of 1 MHz at a spatial peak intensity of either 2.5 W/cm² or 0 W/cm² (sham) for 20 seconds on the eighth day of gestation. Fetal weight (day 18 of gestation) and postpartum pup weight (21, 29 and 42 days post conception) were determined. No significant differences in weight were observed. This result differs from findings in others studies using outbred mouse strains. It is suggested that hybrid mice exposed to ultrasonic energy in utero may be more resistant to alterations in fetal and pup weight progression as compared with outbred strains. (Key words: experimental model; fetal weight, mouse strain specificity.)

In recent years, studies have been conducted on animal systems to obtain information believed useful for estimating possible effects of ultrasound on human patients undergoing clinical diagnostic procedures. It is our view that these studies can be divided into two general categories. In the first category are those studies for which some aspect of the exposure conditions may have been chosen to approximate, in some way, an aspect of prevailing clinical conditions, but with no specific pathologic endpoint fore-identified (or specified) to be observed. The general demeanor of these studies is the lack of finding any effect attributable to the ultrasonic exposure(s), with the attendant conclusion that the clinical application of ultrasound is either with or without some risk. This argument is often considered strengthened by the fact that the duplication of clinical conditions for animal exposures in the laboratory involved either continuous-wave or long pulse regimens, rather than the extremely short pulse regimens employed in clinical diagnostic procedures. The following studies, though not exhaustive, are considered to fall into the above-described category: Takeuchi et al.¹; McClain et al.²; Mannor et al.³; Warwick et al.⁴; Woodward et al.⁵; Akamatsu⁶; Smyth.⁷ There have also been studies, which could be included in this category, for which effects were observed following ultrasonic exposure but which involved greatly

exaggerated conditions, such as many hours of exposure time with attendant difficulty in animal restraint.⁸⁻¹⁰ The problems in distinguishing between effects of ultrasonic exposure and effects resulting from the necessary experimental conditions associated with the exaggerated treatment render these studies difficult to interpret.

The second category of studies was conducted after detectable effects (or alterations in the animal system) had been identified in pilot studies, and it became the principal task of these studies to determine the quantitative exposure-response relationships. A secondary task may also have been to determine, if possible, some type of threshold conditions for each effect. A structure often used for such studies has been the pregnant uterus and other portions of the reproductive system of the mouse. In this experimental model, O'Brien¹¹ observed a statistically significant fetal weight reduction, from 6 to 18 per cent, on the eighteenth day of gestation, resulting from in utero ultrasonic irradiation on the eighth day of gestation, with spatial average intensities in the range of 0.5 W/cm² to 5.5 W/cm² for durations in the range of 300 sec to 10 seconds, respectively. Curto¹² has reported a small but significant neonatal mortality increase for specimens exposed on the eighth day of gestation to 0.125 W/cm² for 3 minutes, as compared with that of the sham-exposed animals. Both of these studies were conducted at 1 MHz. In a series of experiments in which there were differences from the above-mentioned two studies as regards frequency of ultrasound, bath temperature, use of dipilatory agent in the specimen preparation, anesthetic, day of gestation of irradiation, location of specimen in the ultrasonic field, and specimen orientation in the field, Stolzenberg et al.^{13,14} were able to confirm the

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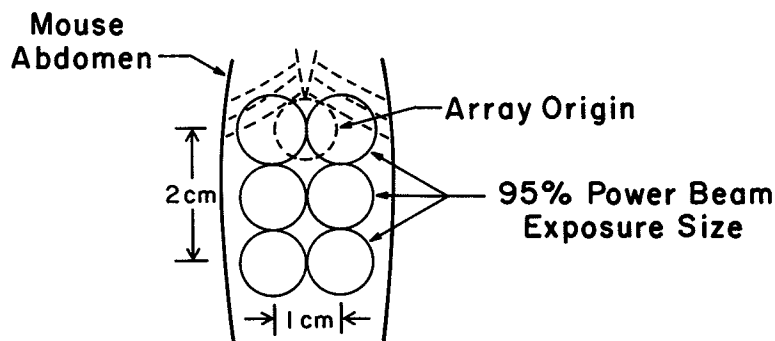


Figure 1. Mouse exposure array.

fetal weight reduction (9 to 10 per cent weight loss in the exposed mice relative to the sham-exposed animals), but Edmonds et al.¹⁵ were not able to confirm the neonatal mortality findings under the 2 MHz–0.5 W/cm² exposure conditions on day 14 of gestation.

Common among these studies, however, was the use of outbred (offspring derived from same strain of parents that are not closely related) mice; CF₁ in the first two studies and CFW Swiss-Webster in the latter three studies. The investigation reported herein was undertaken to examine the dependence of a particular ultrasonic effect, *viz.*, fetal and postpartum weight change, utilizing a hybrid (offspring derived from parents of different strains) strain of mice.

MATERIALS AND METHODS

We employed a hybrid, rather than an outbred, strain of mouse, the LAF₁/J (Jackson Memorial Laboratories, Bar Harbor, Me.), which is derived from two inbred (offspring derived from same strain of parents which are very closely related, such as brother–sister) strains, a C57L/J female and an A/HeJ male. The animals were proved as breeders at age 13 weeks by caging one male with three females for approximately two weeks. Any females determined pregnant in that group were, along with the male, considered proven and eligible for the study. For the mating procedure, approximately ten proven females and five proven males were caged together for two hours in the morning, after which the females were inspected for the presence of a vaginal plug, suggestive of coitus.

On the eighth day of gestation (day zero being the day of mating), the females were anesthetized with methoxyflurane, an ether-based liquid sufficiently volatile to yield a gaseous mixture. Rate and depth of respiration were monitored visually as subjective indicators of anesthetic level. The specimens were then shaved from the sternum posteriorly on the ventral and dorsal surfaces, excluding the legs, and a commercial depilatory was applied for about three minutes to remove stubble. To assure a more complete wetting of the skin surface by the transmitting medium of 37° C degassed mammalian Ringer's so-

lution, the specimens were immersed in a dilute detergent solution and rinsed in warm tap water immediately prior to mounting in the animal holder. This holder supports the animal in a spread-eagled fashion, securing the limbs and tail. Provision is made to supply the anesthetic mixture continuously via a hood that fits over the animal's muzzle. The holder with animal is then secured in the irradiation chamber.

An alignment fixture attached to the transducer (and removed during irradiation) is employed to define the origin of the two-dimensional array of exposure locations, as shown schematically in figure 1. The center of the array origin is located 5 mm caudad from the xiphoid process.

An unfocussed quartz transducer having an aperture 9.5 mm in diameter and operating at 1 MHz was employed. It was calibrated against a ball radiometer^{16,17} and the spatial distribution of the acoustic field was determined by the transient thermoelectric method.¹⁸ The calibrations were determined in the far field at a distance from the transducer of 12.5 cm, the distance at which the animal would be located but without it in the field. The half-power beam width at this position in the field is 20 mm and the 95 per cent power beam width is approximately 10 mm. The animal exposure intensity reported herein is the free-field spatial-peak (axial) intensity at this position, that is, without the animal in the field.

The exposure parameters are programmed into the laboratory minicomputer, which contains a library of exposure arrays from which the user can select to be implemented for a specific irradiation procedure. The 2 × 3 array chosen for this study (fig. 1) provides for a relatively uniform ultrasonic exposure over the entire abdominal region. Six shots per exposure were employed. The ultrasonic exposure parameters reported are the spatial peak intensity and the exposure time per shot. The automated system allows for blind irradiation by selecting, in a pseudo-random fashion, the animals to be exposed and those to be sham-exposed. The computerized system controls the voltage across the transducer and the specimen position within the ultrasonic field (relative to the array origin). The operator initiates the procedure by selecting the ultrasonic in-

tensity of exposure, the exposure time of each individual exposure in the chosen array, the number of mice to be treated in the procedure, and the fraction to be pseudo-randomly selected for ultrasonic exposure. For the present study, the spatial peak intensities for individual exposures were 2.5 W/cm² and 0 W/cm² (sham exposure) for a duration of 20 sec. Thus, the sham-exposed animals received preparations and exposures identical to those of the irradiated mice, but they were not exposed to ultrasonic energy, and the investigator handling and preparing the mice did not know which animals were in which group. A control group of animals was also included in the study. They were randomly chosen after mating and placed in individual cages like the irradiated and sham-treated groups, but did not leave the animal room except for weighing. Cage controls are routinely used to assure that no other agent or illness has affected the mice, and since their treatment was different from that received by the sham-treated and ultrasonically exposed animals, their data are not included here.

After the irradiation procedure, mice were removed from the animal holder, wrapped in tissue to prevent chilling during recovery from the anesthesia, and placed in their individual cages.

The exposed, sham-exposed, and cage control groups were then each divided randomly into two groups, one in which the females would be sacrificed at the eighteenth day of gestation and the other in which the females were allowed to deliver their litters. In the former group, following sacrifice, the fetuses were obtained by laparotomy and individually weighed immediately. Also recorded were the positions of the fetuses, resorptions, and fetal abnormalities. For the latter group, the postpartum data were obtained by weighing pups 21, 29, and 42 days after conception. The time of day at weighing was maintained nearly constant so that all animals were weighed at very nearly the same point of development, i.e., between 10 AM and 2 PM.

RESULTS

The data, shown in table 1, were analyzed using two statistical tests to determine significance of effects of ultrasonic exposure in utero on the weight of developing fetal mice. A two-factor mixed design (repeated measures on one factor), which tested the significance of differences of overall weight gain and of rate of weight gain between groups, did not indicate a significant difference.¹⁹ Here, the standard deviations, average fetal and pup weights, and number of pups included in each litter at the time of weighing were considered. The number of pups is an important aspect to be followed through successive weighings as it includes the involvement of cannibalism, which could possibly be the result of handling procedures and/or developmental problems introduced by ultrasonic irradiation.

The question regarding significant weight differ-

Table 1. Data and Analysis

	Weighing Day (dpc*)	Fetal Weight (g)		Number of Pups	Number of Litters
		Average	SD		
Irradiated	18	1.053	0.163	91	13
	21	1.778	0.245	184	21
	29	5.780	0.904	179	21
	42	12.409	1.909	179	21
Sham-treated	18	1.045	0.118	71	10
	21	1.861	0.249	152	17
	29	5.884	0.768	152	17
	42	12.739	1.695	152	17

* dpc = days post conception.

ences at the four specific weighing dates for weight differences between exposed and sham-exposed groups was examined using the t test for a difference between two independent means.¹⁹ Here the average fetal and pup weights per litter, as well as the individual specimen weights, were considered, and it was found that of all the weighing days, *viz.*, 18, 21, 29, and 42 days post conception (dpc), the only statistically significant weight difference between exposed and sham-exposed groups occurred for the 21 dpc weighing, and then only for the individual specimen weighings.

DISCUSSION

An aspect of the above-described findings that may have considerable impact on the way the risk of clinical ultrasound is viewed in response to results of animal studies is associated with the animal model and, in this study, the apparent strain-specific tolerance of mice. Stolzenberg et al.¹⁴ examined fetal weight reductions (14 and 17 dpc) following ultrasonic exposure in utero (spatial average intensity of 1 W/cm² and exposure times ranging from 80 to 200 seconds at various days of gestation. They used the outbred CFW Swiss-Webster mouse and found a significant weight reduction, which was in general agreement with O'Brien's¹¹ observation. Stratmeyer et al.²⁰ recently observed a statistically significant difference between fetal weights (examined at 18 dpc) of the outbred ICR strain of mouse when ultrasonically exposed at 0 (sham) and at 0.075 and 0.75 W/cm² (spatial average intensity) for 120 seconds on day 4 of gestation.

The results of the present study, compared with those of O'Brien,¹¹ Stolzenberg et al.,¹⁴ and Stratmeyer et al.,²⁰ suggest that the more wild type LAF₁/J strain may be more resistant to weight reduction than are the outbred strains. Lending further support to this hypothesis is the work of Fry et al.,²¹ in which they utilized the hybrid LAF₁/J mouse. They subjected pregnant females to high

levels of pulsed ultrasound conditions and found that up to 50 W/cm^2 (spatial peak, temporal average intensity) for 20 seconds, no fetal weight reduction was observed. Above 50 W/cm^2 , however, they did observe such reductions with a 20-second exposure duration. With the general human population being hybrid, it would appear either that experimental animal models employed for human analog studies also should be hybrid, to aid in making extrapolations more meaningful, or that the "safety factor" obtained by the use of outbred animals be estimated prior to application of results of such investigations.

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