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RESEARCH PAPERS

Dose-dependent Effect of Ultrasound on Fetal Weight in Mice

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Outbred non-Swiss albino mice (CF_1) were time-mated, exposed on the eighth day of gestation to 1 MHz continuous wave ultrasound, and examined on the eighteenth day of gestation. Seven exposure conditions (spatial average intensity versus exposure time) were employed for the 272 litters: 0 W/cm² (sham); 0.5 W/cm², 300 sec; 0.7 W/cm², 300 sec; 2.0 W/cm², 20 sec; 3.0 W/cm², 20 sec; 3.0 W/cm², 10 sec; and 5.5 W/cm², 10 sec. Relative to the sham group, every exposed group exhibited a reduced average fetal weight, ranging from 5.3 to 17.5 per cent, and the nonparametric Kruskal-Wallis one-way analysis of variance by ranks indicated that the average fetal weight varied significantly, at the 0.001 level, with exposure condition. In addition, a linear dose-effect dependence of exposure condition versus average fetal weight was observed, in which the dose parameter was defined as I^2t, where I is the spatial average exposure intensity and t is the exposure time. (Key words: Ultrasound, biological effects, fetal growth, fetal development, fetal weight, dose effect)

The first statistically based study that suggested that in utero exposure to ultrasound affected prenatal growth and development was reported by O'Brien.[1] Time-mated CF_1 mice (outbred non-Swiss albinos) were exposed (at spatial average intensities from 0.5 to 5.5 W/cm² and exposure times up to 300 sec) to continuous-wave (1 MHz) ultrasound on the eighth day of gestation. The fetuses were removed by laparotomy on the 18th day of gestation and were individually weighed. A statistically significant fetal weight reduction, from 5.3 to 17.5 per cent relative to the sham, was observed.

The observation that in utero ultrasonic exposure of mice can cause weight reduction in fetuses compared with the sham has since been confirmed by two other groups using two different strains of mice, namely, LAF_1/J mice[2,3] and CFW Swiss-Webster mice.[4] In the earlier study,[2] relatively high-level pulsed ultrasound conditions (center frequency around 1 MHz) were employed, and a significant reduction in fetal weight was reported for spatial peak, temporal average intensities above 50 W/cm² (spatial peak, temporal peak intensity of 2,936 W/cm²) and exposure times of 20 sec when fetuses were irradiated on the eighth day of gestation. The later report by Fry et al.[3] indicated that the highest exposure conditions (spatial peak, temporal peak intensity of 1,936 W/cm²; spatial peak, temporal average intensity of 51 W/cm²; and exposure time of 20 sec) produced a statistically significant 18.8 per cent fetal weight reduction (relative to the sham), whereas at a spatial peak, temporal average intensity of 45 W/cm² and lower (spatial peak, temporal peak intensity of 1,936 W/cm² and exposure time of 20 sec), there was no change in the fetal weight relative to the sham. Stolzenberg et al.[4] produced fetal weight reductions ranging up to 25 per cent relative to the sham under continuous-wave exposure conditions (frequency of 2 MHz, spatial average intensity of 1 W/cm², exposure times up to 200 sec) at gestation ages of 0, 7, and 12 days.

Recent investigators,[5,6] in attempts to further understand the mechanisms of fetal weight reduction in mice, have been unable to duplicate these findings. Thus, there have been conflicting observations on the fetal weight effects, the causes of which have been speculated to be strain differences.[6]

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This report is the detailed account of my initial findings[1] and an extension of the data analysis to show that a dose-effect response was observed.

MATERIALS AND METHODS

Twenty-five- to 35-g CF_1 (outbred non-Swiss albino) female mice were time-mated by placing males and females together for a two-hour period in the morning and then examining each female for the presence of a vaginal plug as an indication of successful mating. The female mice were exposed to continuous-wave ultrasound at an ultrasonic frequency of 1 MHz during the first quarter of the eighth day of gestation, based on time of mating (day 0 of gestation is defined as the day of mating). In preparation for the exposure procedure, the time-mated mice were weighed and anesthetized with sodium pentobarbitol diluted to 10 per cent, with 0.9 per cent sodium chloride administered intraperitoneally, typically 0.37 ml per 30.[7] The whole abdominal surface was shaved with a small animal clipper and the ears were punched to code the animal. The sides and back were not shaved, and a depilatory was not used on the abdominal surface. The purpose here to maximize the effect, if any, since this type of study had not been performed in the past under such well-known and well-controlled conditions.

Five to 10 min after the mouse had been injected with anesthetic, it was mounted in a spread-eagle manner on a support structure, which in turn was immersed in a tank of degassed isotonic saline in such a manner that the mouse's abdominal surface was on axis, 24 cm (in far field) from the 3.18-cm-diameter, unfocused ultrasonic transducer surface. The saline was maintained at 30° ± 0.2°C, because a mouse's body temperature drops to about that temperature following administration of anesthesia. The abdomen was carefully wiped free of visible bubbles, and the operator commenced the computer-controlled exposure procedure, which randomly determined the ultrasonic exposure condition. After the exposure procedure, the mouse was dried with a towel and placed in a cage. The daily exposure procedure, which sometimes involved up to 35 mice, was completed within three hours and was conducted under such conditions that the person who prepared and handled the animals during the exposure procedure and who ran the exposure system did not know the specific exposure condition of any animal.

The equipment and dosimetric details of the ultrasonic exposure system have been described in detail.[7] Briefly, spatial distribution plots of the ultrasonic field, made with a hydrophone in a plane perpendicular to the beam axis, 24 cm from the transducer surface, yielded a 3 dB intensity beam width of 1.3 cm. The "exposure intensity" (accuracy, ±15 per cent; reproducibility, ±5 per cent) reported here is the spatial average intensity determined by dividing the total ultrasonic power by the transducer area (11.34 W/cm^2) as determined under free-field conditions. The ratio of the spatial-peak (on-axis) intensity to spatial average intensity was 2.8. The total ultrasonic power was determined by the radiation pressure principle by using the buoyant float technique or the chemical microbalance procedure, both of which are primary methods.[8] Immediately before the animal exposure procedure was performed, the suspended ball technique,[9] also a primary method, was used to determine the axial spatial peak intensity and so verify the proper calibration of the system.

The mice were examined during the first quarter of the 18th day of gestation. The examination procedure consisted of sacrificing the mouse by cervical dislocation, performing a laparotomy, and conducting a gross morphologic analysis of the uterus and its contents. The uterus was examined for live, dead, and abnormal fetuses and for resorptions. Each live fetus was removed from the uterus and weighed to an accuracy of ±5 mg. At the time of this examination, the examiner did not know under which ultrasonic exposure condition each mouse had been exposed.

RESULTS AND DISCUSSION

The total number of implants consisted of the sum of live fetuses, dead fetuses, resorptions, and exencephalies from 272 litters for each of the seven ultrasound exposure conditions. The results are summarized in table 1. Exencephaly, or brain hernia, is an easily observed gross congenital anomaly in which the midbrain protrudes through the roof of the skull, probably owing to an interference in cranial development. The average number of implants per litter should be independent of the specific exposure group; the data supports this in that a chi-square test[10] did not reveal a significant difference between results for the sham and for each exposure group.

Dead fetuses, sometimes referred to as late resorptions, were fetuses that exhibited structural features such that the gestational age at which development ceased could be approximated, the earliest being about 11 days of gestation. A chi-square test[10] did not suggest any apparent effect upon fetal death from ultrasound exposure. Table 2 summarizes the number of early resorptions in the 272 litters. For example, the sham group consisted of 77 litters, of which 28 (36.3 per cent) had no resporptions, 21 (27.3 per cent) had one resorption, and so on, for totals of 96 resorptions, 1.3 resorptions per litter, and 2.0 resorptions per litter with resorptions. The distribution of resorptions, resorptions per litter, and resorptions per litters with resorptions does not appear to suggest a trend in the exposure groups compared with the sham group.

Table 1. Number of Implants, by Type, in 272 Litters of Fetal Mice Exposed in Utero to 1 MHz Continuous-wave Ultrasound

Exposure Intensity (W/cm^2)	Exposure Time (sec)	No. of Litters	No. of Implants				Total No. of Implants	Total No. of Implants per Litter
			Live Fetuses	Dead Fetuses	Resorptions	Exencephalies		
0 (sham)	–	77	836	12	96	3	947	12.3
0.5	300	42	411	4	62	0	477	11.4
0.7	300	25	263	5	22	2	292	11.7
2.0	20	40	441	7	41	2	491	12.3
3.0	20	18	210	8	14	0	232	12.9
3.0	10	50	498	8	75	0	581	11.6
5.5	10	20	206	6	27	3	242	12.1

Table 3 summarizes the weight of the 2,865 live fetuses at the 18th day of gestation for each of seven exposure conditions. Each fetus was weighed, and the average fetal weight was calculated as the sum of fetal weights divided by the number of live fetuses for the given group. Relative to the sham group, each of the exposure groups had a reduced average fetal weight, which ranged from 5.3 to 17.5 per cent. The nonparametric Kruskal-Wallis one-way analysis of variance by ranks[10] was applied to the individual fetal weights, indicating that the average fetal weight varied significantly, at the 0.001 level, with exposure condition. The Kruskal-Wallis test aids in deciding whether several independent samples (in this study, individual fetal weights) come from the same population (exposure groups). This nonparametric test was utilized because the particular exposure conditions did not permit interval scaling. The test was applied for larger samples with correction for ties.

Histograms (figs. 1–7) show the distribution of individual fetal weights for each of the seven exposure conditions. The vertical axis represents the percentage of live fetuses, relative to the total number of live fetuses for the specific exposure condition, that fall into each of 0.05-g weight increments. Weight values that fell at the histogram breakpoints were included in the higher weight range. Each of the histograms shows the average fetal weight for reference. No fetus weighed more than 1.50 g.

Qualitatively, the sham group histogram (fig. 1) represents the most symmetric distribution of fetal weights, with a standard deviation of 0.12 g. The distributions for the six exposure groups appear to be skewed to lower fetal weights. Table 4 supports this observation, in that the moment coefficients of skewness, applied to the data represented in figures 1 through 7, indicate that the fetal weight distributions in all seven groups have negative skewness. For a perfectly symmetric curve, such as a normal distribution, g_1 is zero. The distributions shown in figures 3, 4, and 5 reveal a greater negative skewness than the other four distributions, apparently owing to the greater number of quite low fetal weights. The moment coefficient of kurtosis, a measure of the peakedness of a distribution, indicates that five distributions have relatively high peaks ($g_3 > 3$) and two distributions are flat-topped ($g_3 < 3$). For a normal distribution, $g_3 = 3$.

To investigate further the skewness and kurtosis of the fetal weight distributions, the fetal weights that fell outside the range of three standard deviation units of the sham group were eliminated from the subsequent analysis; that is, those fetuses that weighed less than 0.80 g and greater than 1.5 g were eliminated from analysis. The purpose was to eliminate the potential bias from low and very low fetal weights. Deletion of these data that fell outside three standard deviation units was, admittedly, somewhat arbitrary. The aim here was simply to gain further insight into the fetal weight distribution for each exposure condition. Table 5 summarizes the fetal weight data for these fetuses with weights between 0.80 and 1.50 g. The average fetal weight for every exposure condition increased, but relative to the sham group, each exposure group had an average fetal weight ranging from 5.2 to 11.3 per cent lower than that of the sham. Kruskal-Wallis one-way analysis of variance by ranks was performed as before, and showed that the average fetal weight varied significantly, at the 0.001 level, with exposure condition. From table 4 it should be noted that, in each exposure group, the distribution became more symmetric and more closely approached a normal distribution with the deletion of the fetal weights less than 0.80 g.

A dose-effect response of the exposure condition versus fetal weight was examined by defining the dose parameter I^2t, where I is the exposure intensity (W/cm^2) and t is the exposure time (sec), as listed in table 3 for each of the seven exposure conditions. The choice of this ultrasonic dose parameter is not completely arbitrary.

A weak frequency dependence[12] on threshold ultrasonic dosages for irreversible structure changes in the adult mammalian central nervous system[13,14] has been ascribed to the brain meninges.[15] Taking into account the effect of the subarachnoid space on the ultrasound transmission into the brain, the le-

Table 2. Number of Early Resorptions

Exposure Intensity (W/cm^2)	Exposure Time (sec)	Total No. of Litters	No. of Litters in Which the Following No. of Resorptions Were Found: 0	1	2	3
0 (sham)	–	77	28 (36.3)	21 (27.3)	17 (22.1)	7 (9.1)
0.5	300	42	13 (31.0)	13 (31.0)	6 (14.3)	5 (11.9)
0.7	300	25	10 (40.0)	11 (44.0)	2 (8.0)	1 (4.0)
2.0	20	40	14 (35.0)	17 (42.5)	6 (15.0)	2 (5.0)
3.0	20	18	8 (44.0)	6 (33.3)	4 (22.2)	0
3.0	10	50	13 (26.0)	19 (38.0)	9 (18.0)	4 (8.0)
5.5	10	20	8 (40.0)	5 (25.0)	2 (10.0)	3 (15.0)

Percentages are shown in parentheses.

sion threshold "delivered intensity" becomes a frequency-independent function over the frequency range 1 to 10 MHz. Mathematically, this function has been described by an I^2t dependency, where I is the ultrasound intensity at the lesion site and t is the exposure time of a single pulse. A similar I^2t dependency has been observed for irreversible functional changes, namely, hind-limb paralysis of neonatal mice.[14,16] This same I^2t dependency has been shown for threshold intensities for focal lesions produced in cat liver,[17] and an approximate I^2t dependency has been shown for focal lesions produced in rabbit liver, kidney, and testes.[18] In comparison with other forms of energy, a similar dose dependency has been observed for mammary neoplasms at low ionizing radiation doses in which two x-ray secondary particles (produced by a single neutron) are required to elicit the effect.[19] In photochemical and photobiologic studies, biphotonic excitation has been observed at high-energy concentrations.[20] Basically, a linear dependent effect on the dose parameter I^2t suggests that two energy events are required to produce the observed effect. Johnston and Dunn[21] have shown that a hysteresis model can explain an I^2t dosage parameter, although there is insufficient information to speculate whether the reduced fetal weight observations reported here can be explained by this model.

Figure 8 shows the average fetal weight data as a function of the dose parameter I^2t for the seven exposure groups. The method of least squares,[22] applied to the individual fetal weights, was employed to investigate a functional relationship between fetal weight and the ultrasonic dose parameter I^2t.

First, the normality and equal variance assumptions of least squares were investigated. The Kolmogorov-Smirnov one-sample test[10] was applied to each exposure group to assess the normality assumption. The test was applied to cumulative distributions divided into 10-mg increments. For the seven groups, the fetal weight data represent a normal distribution at the 5 per cent significance level. Bartlett's test was applied to determine whether the variance among the seven groups differed significantly and for any reasonable significance level and whether the population variances corresponding to the seven exposure groups were unequal. Even though the equal variance assumption is not satisfied, it is instructive nonetheless to examine the potential for a trend between fetal weight (FW) and I^2t. Assuming a linear model,

$$FW = -0.000645\ (I^2t) + 1.136 \qquad (1)$$

with the regression significant at the 0.0001 level. The standard errors of the slope and intercept, 0.000034 and 0.004, respectively, yield the respective 95 per cent confidence limits of -0.000645 ± 0.000067 and 1.136 ± 0.008. Examination of the lack of fit and pure error sources for this linear regression model suggested that there was no reason to doubt the linear model.

Fisher's least significant difference test[23] was used to examine more closely the manner of the fetal weight reduction. The test was applied to the seven fetal weight groups to identify a significant difference among the averages, as shown directly below.

I^2t:	0	75	80	90	147	180	302.5
Average fetal weight:	1.14	1.08	1.07	1.07	1.04	1.05	0.94

in 272 Litters of Fetal Mice

No. of Litters in Which the Following No. of Resorptions Were Found:				Total No. of Resorptions	No. of Resorptions per Litter	No. of Resorptions per Litters with Resorptions
4	5	6	7			
2 (2.6)	0	2 (2.6)	0	96	1.3	2.0
3 (7.1)	2 (4.8)	0	0	62	1.5	2.1
1 (4.0)	0	0	0	22	0.9	1.5
0	0	1 (2.5)	0	41	1.0	1.6
0	0	0	0	14	0.8	1.4
2 (4.0)	1 (2.0)	1 (2.0)	1 (2.0)	76	1.5	2.0
1 (5.0)	1 (5.0)	0	0	27	1.4	2.3

There appear to be four distinct groupings based on I^2t ordering: the sham ($I^2t = 0$), the next three groups ($I^2t = 75$, 80, and 90), the next two groups ($I^2t = 147$ and 180) and the highest dose group ($I^2t = 302.5$). The average fetal weights within each of these four groups were not significantly different from one another, whereas those within each group were significantly different, at the 5 per cent level, from those within the other groups.

COMMENT

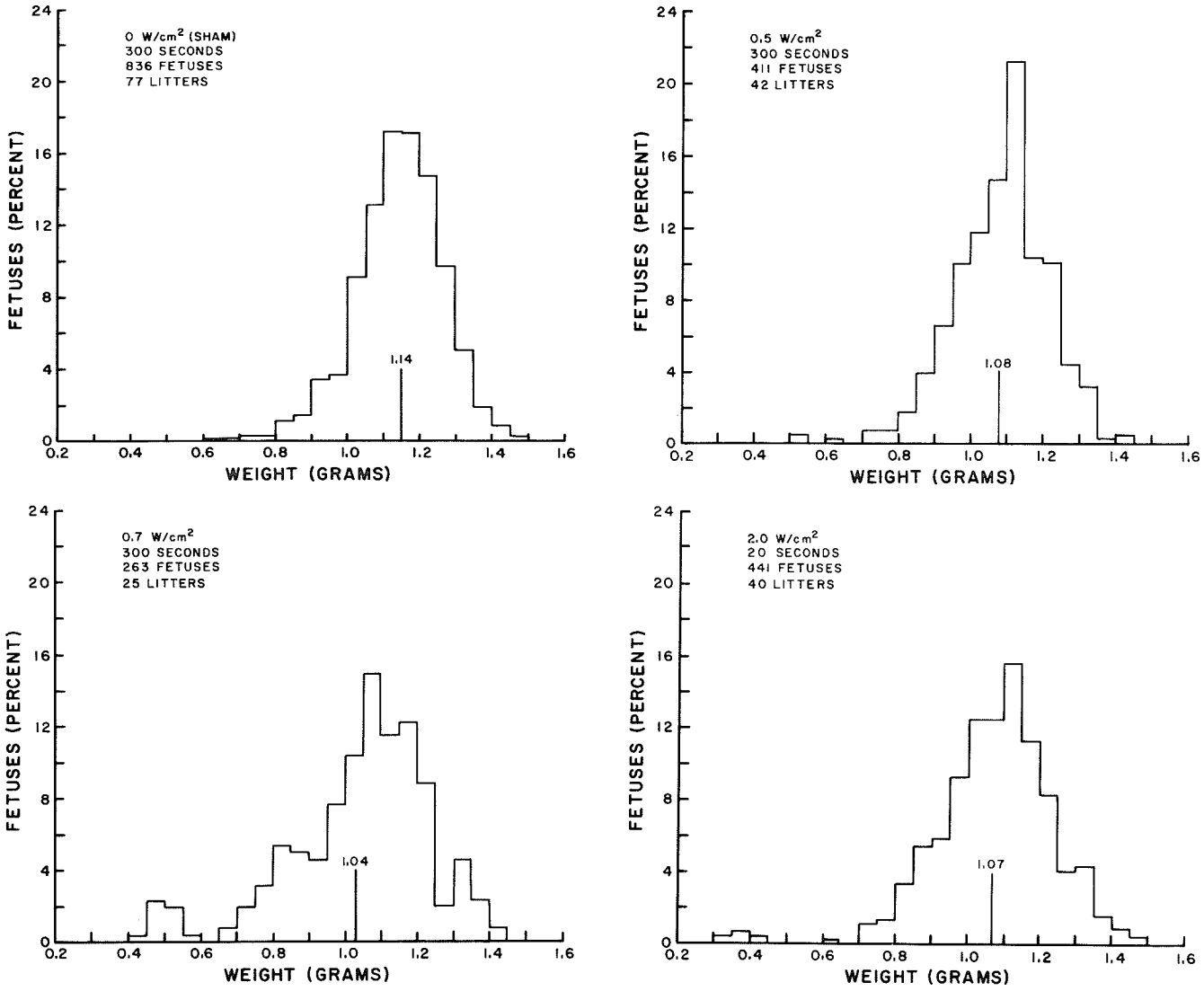
Dose-effect studies are invaluable for assessing risk.[24] Too often, a biologic effect is reported under a single exposure condition. When the exposure condition arises from a diagnostic device, we tend to question whether the effect is, in fact, real or whether the experimental set-up produced extraordinary conditions to elicit the effect. Under such conditions, however, there is a strong tendency to believe that the diagnostic exposure conditions do represent a risk to the patient. When the exposure condition is at levels much in excess of diagnostic conditions, we tend to discount the applicability of such biologic effect studies to the clinical situation. The overall problem is that non-dose-effect studies are difficult to apply to assessment of risk. They do, however, identify biologic end-points to which dose-effect experimental regimens should then be applied.

Consider, for example, this fetal weight study, in which equation 1 is a dose-effect relationship. If we were to apply this relationship to a clinical exposure condition, then we would examine the upper value of the dose parameter I^2t for static pulse-echo scanners.[25] Here, the intensity parameters are those measured in water, not in situ where the ultrasonic attenuation of tissue would decrease the intensity. For a single pulse, the spatial peak, pulse average intensity is about 300 W/cm^2 and the exposure time (here, the pulse duration) is about 1 μsec, yielding an I^2t around 0.09. For the time-average case, the spatial peak, temporal average intensity is about 200 mW/cm^2 and the exposure time (here, the length of the examination for maximal effect) is about 30 min, yielding an I^2t around

Table 3. Weights of 2,865 Live Fetal Mice at 18 Days' Gestation

Exposure Intensity (W/cm^2)	Exposure Time (sec)	No. of Live Fetuses	Fetal Weight (g; average ± SD)*	Weight Change (%) (relative to sham)
0 (sham)	–	836	1.14 ± 0.12 SD	–
0.5	300	411	1.08 ± 0.13 SD	−5.3
0.7	300	263	1.04 ± 0.20 SD	−8.8
2.0	20	441	1.07 ± 0.17 SD	−6.1
3.0	20	210	1.05 ± 0.16 SD	−7.9
3.0	10	498	1.07 ± 0.13 SD	−6.1
5.5	10	206	0.94 ± 0.18 SD	−17.5

* Kruskal-Wallis test indicated that the average fetal weight varied significantly at the 0.1 per cent level ($P_\alpha \leq 0.001$) with exposure condition.

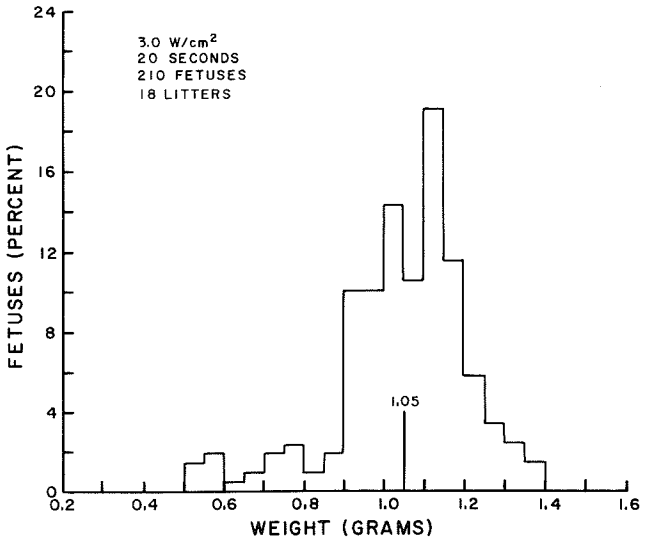


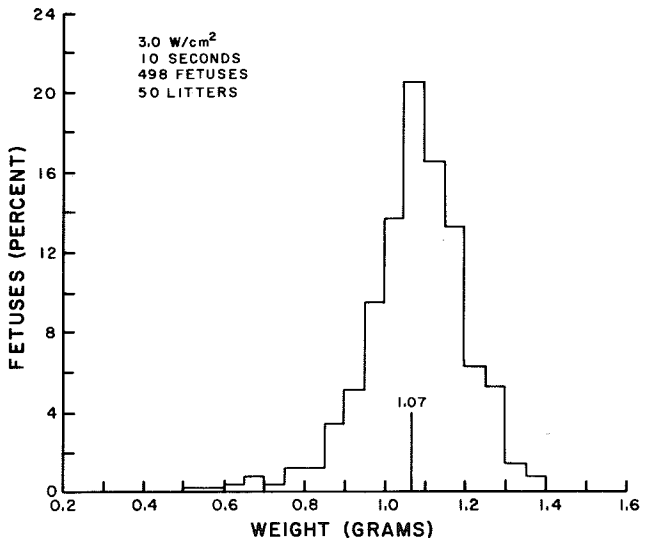
Figures 1 to 4. Distributions of fetal weights under different exposure conditions. *Fig. 1 (top left);* 0 W/cm², 300-sec exposure. *Fig. 2 (top right),* 0.5 W/cm², 300-sec exposure. *Fig. 3 (bottom left),* 0.7 W/cm², 300-sec exposure. *Fig. 4 (bottom right),* 2.0 W/cm², 20-sec exposure.

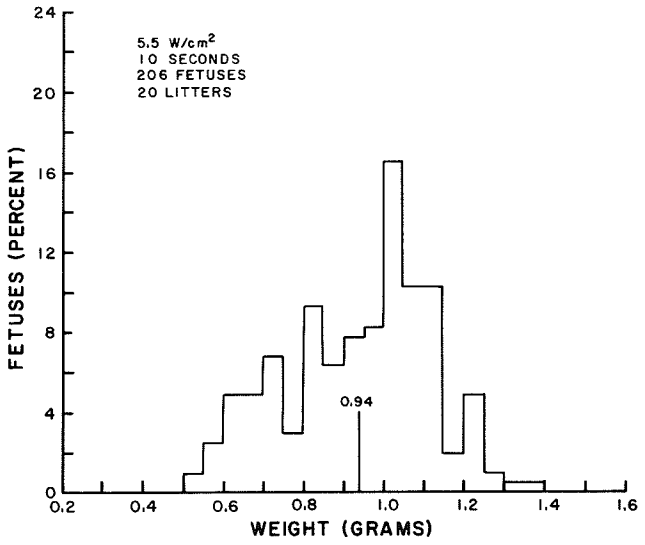
Table 4. Moment Coefficients of Skewness (g_1) and Kurtosis (g_2)* for Weight Data 1) From All Live Fetal Mice and 2) From Only Live Fetal Mice Weighing Between 0.80 and 1.50 g at 18 Days' Gestation

Exposure Intensity (W/cm²)	Exposure Time (sec)	All Live Fetuses			Live Fetuses Weighing Between 0.80 and 1.50 g		
		No.	g_1	g_2	No.	g_1	g_2
0 (sham)	–	836	−0.51	3.86	828	−0.25	3.18
0.5	300	411	−0.57	18.96	402	−0.01	2.76
0.7	300	263	−0.90	3.97	235	0.002	2.60
2.0	20	441	−1.09	1.71	422	0.13	2.71
3.0	20	210	−0.95	4.30	191	0.18	2.62
3.0	10	498	−0.79	4.76	482	−0.04	2.90
5.5	10	206	−0.31	2.38	159	0.22	2.72

* $g_1 = m_3 m_2^{-3/2}$ and $g_2 = m_4 m_2^{-2}$, where m_r is the rth moment about the mean.







Figures 5 to 7. Distributions of fetal weights under different exposure conditions. *Fig. 5 (top left)*, 3.0 W/cm², 20-sec exposure. *Fig. 6 (top right)*, 3.0 W/cm², 10-sec exposure. *Fig. 7 (right)*, 5.5 W/cm², 10-sec exposure.

72. Of course, this latter case would require examination of the same tissue volume for the entire length of time, which might not be necessary with a static pulse-echo scanner but possibly would be with a Doppler fetal monitor, in which the spatial peak intensity is about 75 mW/cm². For an exposure time of one hour, the I^2t is about 20. The point is that, with a dose-effect model, one is in a better position to examine the possible effect under clinical conditions. The model would have to be validated for such applicability, of course. There is a long way to go with respect to ultrasound.

Table 5. Summary of Weights of 2,719 Live Fetal Mice Weighing Between 0.80 and 1.50 g at 18 Days' Gestation

Exposure Intensity (W/cm²)	Exposure Time (sec)	No. of Live Fetuses	Average Fetal Weight (g)*	Weight Change (%) (relative to sham)
0 (sham)	–	828	1.15	–
0.5	300	402	1.09	−5.2
0.7	300	235	1.09	−5.2
2.0	20	422	1.09	−5.2
3.0	20	191	1.08	−6.1
3.0	10	482	1.08	−5.2
5.5	10	159	1.02	−11.3

* Kruskal-Wallis test indicated that the average fetal weight varied significantly at the 0.1 per cent level ($P_\alpha \leq 0.001$) with exposure condition.

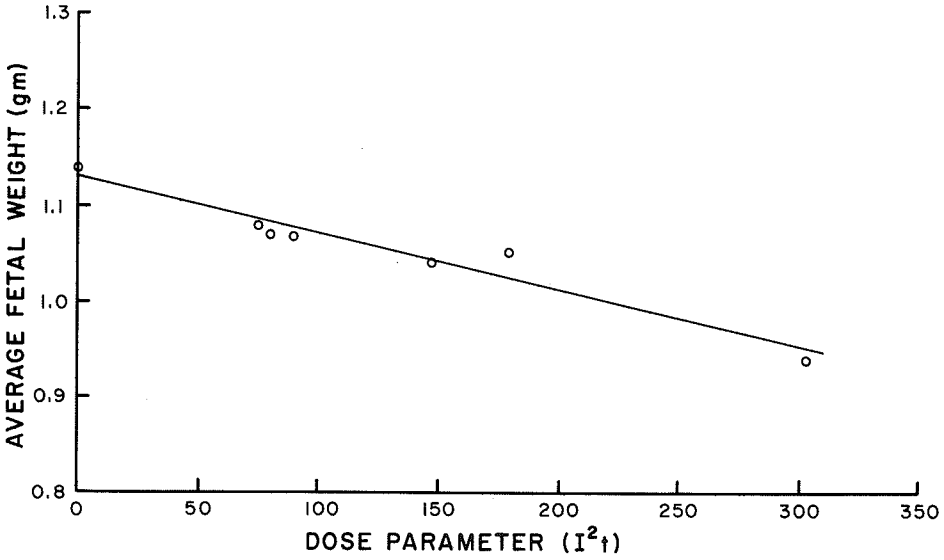


Figure 8. Average fetal weight as a function of the dose parameter I^2t, utilizing all live fetal weight data (table 3). The solid line is described by equation 1.

In the examples given here, the *maximal* intensity value was employed. The dose-effect model is valid only for the CF_1 mouse under the specific exposure conditions. There is even a hint that fetal weight reduction of some other strains of mice might not be influenced by ultrasound.[6]

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REFERENCES

1. O'Brien WD Jr: Ultrasonically induced fetal weight reduction in mice. *In* White DN, Barnes R (eds): Ultrasound in Medicine, 2. New York, Plenum Press, 1976
2. Fry FJ, Johnson LK, Erdmann WA, et al: Ultrasonic toxicity in the mouse. *In* Hazzard DG, Litz ML (eds): Symposium on Biological Effects and Characterizations of Ultrasound Sources (HEW publication no. FDA 78-8048). Washington, DC, US Government Printing Office, 1977, p 153
3. Fry FJ, Erdmann WA, Johnson LK, et al: Ultrasonic toxicity study. Ultrasound Med Biol 3:35, 1978
4. Stolzenberg SJ, Torbit CA, Edmonds PD, et al: Effects of ultrasound on the mouse exposed at different stages of gestation: acute study. Rad Environ Biophys 17:245, 1980
5. Kim HL, Picciano MF, O'Brien WD Jr: The combined effect of ultrasonic exposure and protein restriction on maternal and fetal mice. Ultrasound Med Biol, in press
6. O'Brien WD Jr, Januzik SJ, Dunn F: Ultrasound biological effects: a suggestion of strain specificity. J Ultrasound Med 1:000, 1982
7. O'Brien WD Jr, Christman CL, Yarrow S: Ultrasonic biological effect exposure system. *In* deKlerk J (ed): 1974 Ultrasonics Symposium Proceedings (IEEE catalog no. 74-CHO-896-1SU). Piscataway, NJ, Institute of Electrical and Electronics Engineers, Inc, 1974, p 57
8. O'Brien WD Jr: Ultrasonic dosimetry. *In* Fry FJ (ed): Ultrasound: Its Application in Medicine and Biology, part 1 (Methods and Phenomena Series, no. 3). New York, Elsevier–North Holland Pub Co, 1978, p 343
9. Dunn F, Averbuch AJ, O'Brien WD Jr: A primary method for the determination of ultrasonic intensity with the elastic sphere radiometer. Acustica 38:58, 1977
10. Siegel S: Nonparametric Statistics. New York, McGraw-Hill Book Co, 1956
11. Spiegel MR: Statistics (Schaum's Outline Series). New York, McGraw-Hill Book Co, 1961
12. Dunn F, Lohnes JE, Fry FJ: Frequency dependence of threshold ultrasonic dosages for irreversible structural changes in mammalian brain. J Acoust Soc Am 58:512, 1975
13. Fry FJ, Kossoff G, Eggleton RC, et al: Threshold ultrasonic dosages for structural changes in the mammalian brain. J Acoust Soc Am 48:1413, 1970
14. Dunn F, Fry FJ: Ultrasonic threshold dosages for the mammalian central nervous system. IEEE Trans Biomed Eng 18:253, 1971
15. Johnston RL, Dunn F: Influence of subarachnoid structures on transmeningeal ultrasonic propagation. J Acoust Soc Am 60:1225, 1976
16. Fry WJ, Dunn F: Ultrasonic irradiation of the central nervous system at high sound levels. J Acoust Soc Am 28:129, 1956
17. Chan SK, Frizzell LA: Ultrasonic thresholds for structural changes in the mammalian liver. *In* deKlerk J, McAvoy BR (eds): 1977 Ultrasonics Symposium Proceedings (IEEE catalog no. 77-CH1-264-1SU). Piscataway, NJ, Institute of Electrical and Electronics Engineers, Inc, 1977, p 153
18. Frizzell LA, Linke CA, Carstensen EL, et al: Thresholds for focal ultrasonic lesions in rabbit kidney, liver and testicle. IEEE Trans Biomed Eng 24:393, 1977
19. Rossi HH, Kellerer AM: Radiation carcinogenesis at low doses. Science 175:200, 1972
20. Wang SY: Introductory concepts for photochemistry of nucleic acids. *In* Wang SY (ed): Photochemistry and Photobiology of Nucleic Acids, 1. New York, Academic Press, 1976, p 1
21. Johnston RL, Dunn F: Hysteresis as an interaction mechanism of ultrasound and tissues. *In* Wagai T, Omoto R (eds): Proceedings of the Second Meeting of the World Federation for Ultrasound in Medicine and Biology. Amsterdam, Excerpta Medica, 1979, p 17
22. Draper NR, Smith H: Applied Regression Analysis. New York, John Wiley & Sons, Inc, 1966
23. Fryer HC: Concepts and Methods of Experimental Statistics. Boston, Allyn and Bacon, Inc, 1966
24. O'Brien WD Jr: Safety of ultrasound. *In* deVlieger M, Holmes JH, Kossoff G, et al (eds): Handbook of Clinical Ultrasound. New York, John Wiley & Sons, Inc, 1978, p 99
25. AIUM/NEMA: Safety Standard for Diagnostic Ultrasound Equipment. (AIUM/NEMA Standards publication no. UL 1-1981). Bethesda, MD, American Institute of Ultrasound in Medicine/National Electrical Manufacturers Association, 1981