INVOLVEMENT OF ULTRASONICALLY INDUCED CAVITATION IN MAMMALIAN TISSUES IN VIVO

Revised

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I. INTRODUCTION

The widespread use of ultrasound in clinical medicine necessitates an effort to understand the mechanism of interaction of the acoustic radiation with biological systems. Acoustic cavitation is one phenomenon which has the potential to cause damage to a biological system, assuming that the cavitation occurs within or in close proximity to the system. Therefore, extensive studies are necessary to fully assess the role of acoustic cavitation in producing biological damage. A typical experimental study would involve investigating the conditions under which cavitation is most likely to occur in a certain biological system, confirming that cavitation occurs under these conditions, and verifying that the observed bioeffect, if any, is due to cavitation. There have been many studies done in the past under various experimental conditions involving a wide range of biological systems. A brief general review of these studies and their findings is presented.

In studies involving ultrasonic irradiation of biopolymers and cells in liquid suspensions, where cavitation nuclei are believed to exist, cavitation was suggested to be involved in the production of the observed bioeffects. These bioeffects included decrease in the antigenicity and enzyme activity of dilute solutions of protein (Edwards et al., 1976), degradation of biological macromolecules (Pritchard et al., 1966), and cell disruption (Hughes and Nyborg, 1962). In the latter two studies, the investigators claimed that ultrasonic irradiation under conditions of stable cavitation was able to produce the observed bioeffects. These investigators proposed that large shear forces associated with cavitation induced microstreaming were responsible for the damage observed.

Many plant tissues contain gas bodies stabilized in the spaces between cells. Miller (1979) concluded that activation of the intercellular gas bodies present in leaves of the aquatic plant Elodea during ultrasonic irradiation produced cell death. Reduced growth rate of plant roots where entrapped gas bodies are known to exist intercellularly was observed upon irradiation with ultrasound (Carstensen et al., 1979), and a cavitation-like mechanism has been postulated to be responsible for the observed effect.

In animal tissues, naturally occurring gas bodies are more difficult to observe. But there have been some studies which indicate the possible existance of cavitation nuclei in human and animal tissues leading to cavitation effects. Intracardiac echoes, suggesting circulating microbubbles, were derived from the blood in human cardiac chambers using pulse-echo ranging techniques (Gramiak and Shah, 1971). Ter Haar et al. (1982) claimed that free gas bubbles have been induced in mammalian tissue in vivo by 0.75 MHz ultrasound. The guinea pig hind limb was scanned with a pulse-echo ultrasonic imaging system during irradiation with the cw ultrasound. A considerable number of new echoes arose during irradiation, of which the majority were caused to disappear by increasing the ambient pressure. The observed echoes were postulated to be from gas bubbles larger than 10μm in diameter. In vivo irradiation of the cat brain (Fry et al., 1970) and the cat liver (Chan and Frizzell, 1977) with ultrasound intensities well above 1000 W/cm^2 produced lesions believed to be due to cavitation. Lele (1978) suggested that with ultrasound intensities above 1500 W/cm^2, transient cavitation was involved in producing tissue damage in the calf liver in vitro and the cat brain in vivo after monitoring subharmonic and anharmonic emissions during insonation.

Although these past studies and their findings offer insight into the interaction of acoustic cavitation with biological tissues, much more needs to be learned in order to understand the role of ultrasonically induced cavitation as a mechanism of damage to mammalian tissues in vivo in the megahertz frequency range. Unfortunately far fewer in vivo studies have been conducted than studies involving cell suspensions or plant tissues. The proposed research will investigate further and offer additional insight into this area of interest and concern.

The purpose of the proposed research is to study the role of ultrasonically induced cavitation as a mechanism of damage to the mammalian central nervous system (CNS) in vivo when the CNS tissue is irradiated by moderate to high intensity ultrasound in the megahertz frequency range. The mammalian specimen to be used is the mouse neonate, the irradiation site is the third lumbar region of the spinal cord, and the observed bioeffect is the functional motor paralysis of the hind limbs.

II. PREVIOUS RESEARCH

The irradiation procedure for this study was originally developed by Dunn (1958). A detailed description of the irradiation system and experimental methods can be found elsewhere (Frizzell et al., 1983; Lee, 1982).

Previously, mouse neonates within 24 hours of birth were irradiated with 1 MHz unfocused cw ultrasound in a $10^{\circ}C$ bath of degassed, Ringer's solution at atmospheric pressure. The transducer was a 3.18 cm diameter aperture quartz disk. The specimen was placed in the farfield of the ultrasound source and positioned so that the ultrasound beam was centered on the third lumbar vertebral section, which contains a high density of the neurons and nerve fibers associated with hind limb motor function. The observed bioeffect was the functional motor paralysis of the hind limb and the intensity range used was from 86 W/cm^2 to 289 W/cm^2. Similar irradiations were performed at intensities of 86 W/cm^2, 144 W/cm^2, and 289 W/cm^2 and at an ambient pressure of 16 atm. In these studies the effective dose (ED) for paralysis in 10, 50 and 90 percent of the specimens was determined and specified in terms of the incident intensity and the corresponding exposure duration (t) as determined from probit analysis of the data.

During the irradiations acoustic emissions from the irradiation site, which might possibly be due to cavitation, were monitored. A focused spherical segment of ceramic PZT8 (Channel Industries, Inc., Santa Barbara), resonant at 0.5 MHz with a diameter of 5.1 cm and a radius of curvature of 8.9 cm, was used to monitor the subharmonic and anharmonic signals. Also a broadband focused transducer 19 mm in diameter (KB Aerotech, Lewistown, PA) with a center frequency of 1.65 MHz and half-power frequencies at 1.2 and 2 MHz was used to monitor the supraharmonic signals and broadband noise. The 0.5-MHz narrowband monitor was designed to be useable at both atmospheric pressure and at 16 atm. The broadband monitor, however, could only be used at atmospheric pressure.

Table I gives the values for t_{10}, t_{50}, and t_{90} at 1 MHz, $10^\circ C$, and 1 and 16 atm hydrostatic pressure for the intensity range 86 to 289 W/cm^2. Figure 1 shows the data for the ED50 exposure conditions of Table I plotted as intensity versus exposure duration. The bars for each data point indicate 95% confidence intervals on the computed t_{50}. Comparison of the 1 atm and 16 atm data shows a large difference in the t_{50} at 289 W/cm^2 and a smaller difference in the t_{50} at 86 W/cm^2. These differences are significant at the $p<.05$ level. There is no significant difference at 144 W/cm^2.

The difference in half-harmonic signals received, referenced to the 1 MHz received signal, with and without the specimen and holder in place at exposure conditions for 90% paralysis and at 1 atm is shown in Table II. The supraharmonics and broadband noise levels at 1 atm are given in Table III. The narrowband 0.5 MHz monitor observation of the subharmonic signal levels at the 90% paralysis exposure conditions for intensities of 86, 122, 192, and 289 W/cm^2 were barely detectable at 16 atm.

These results may be interpreted as follows. First, based upon the measurement of the subharmonic signal levels, cavitation may be present at 1 atm for all intensities employed in this study. The observation that there was a substantial decrease in these signal levels upon pressurization of the system to 16 atm further supports this possibility. Second, based upon the effect of pressurization on the t_{50} for paralysis, cavitation appears to contribute to the observed paralysis at 289 W/cm^2 but does not appear to contribute significantly at 144 W/cm^2 and lower intensities. The second and third harmonic level can not be taken as evidence for the presence of cavitation since these are expected to be associated with finite amplitude effects.

To determine if thermal effects were responsible for the observed paralysis at the lower intensities, namely 144 W/cm^2 and below, the t_{50} from pulsed ultrasound was determined. The irradiation system and experimental methods used were identical to the cw study except that pulsed 1 MHz unfocused ultrasound at 20, 40, 67, and 100 percent duty cycles was used at atmospheric pressure. The pulse width and the temporal average intensity (TAI) were held constant at 1 ms and 45 W/cm^2 respectively. Therefore for the lower duty cycles, higher peak intensities were used.

The observed t_{50} for each of the duty cycles is given in Table IV. As can be seen, the value for t_{50} decreases for a decrease in duty cycle and correspondingly for an increase in peak intensity. Assuming (1) that a linear acoustic model applied and (2) that the production of heat in the mouse neonate spinal cord was responsible for the paralysis at a TAI of 45 W/cm^2, then the values for t_{50} should have been the same for all duty cycles. Since this was clearly not the case, the role of harmonic generation by finite amplitude propagation on the heat generation rate in the irradiated tissue was investigated.

Using a numerical method developed by Haran and Cook (1983), the harmonic content at the location of the specimen, 9.5 cm from the source, was computed for each duty cycle used. Figures 2 and 3 show the harmonic content for a plane nonlinear acoustic wave propagating in the Ringer's solution as a function of distance from the source. Comparison of Figures 2 and 3 shows increased harmonic distortion at the location of the specimen for the higher peak intensity irradiation. Since greater harmonic distortion results in increased absorption of the ultrasonic energy incident upon the specimen, the ratio of the heat generation rate at the dorsal surface of the specimen with the nonlinear effects considered, $\dot{q}_{NL}$, to that with no harmonic generation, $\dot{q}_L$, was computed for each of the irradiation conditions used and is provided in Table V. Thus for a peak intensity of 224 W/cm^2, the rate of heat generation at the dorsal surface of the specimen would be nearly twice that which would exist if no harmonic generation occurred, and about 1.8 times that occurring at 45 W/cm^2. Similarly for a peak intensity of 112 W/cm^2, the heat generation rate would be 1.2 times that at 45 W/cm^2.

The effective TAI of the pulsed irradiations can be computed by multiplying 45 W/cm^2 by these correction factors. In Figure 4, ED50 exposure conditions from pulsed irradiations for the different duty cycles with adjustments to the TAI are compared to those without any adjustments. The adjusted ED50 points are seen to fit fairly well into a smooth curve with the cw ED50 points. Therefore when nonlinear effects are taken into account, the ED50 exposure conditions for the pulsed irradiations are consistant with a thermal model of damage.

III. PROPOSED RESEARCH

A. CW Irradiation Experiment at 37°C

From the cw irradiation study discussed previously, cavitation was suggested to be involved in producing the observed effects at 289 W/cm^2, based primarily upon the effects of pressurization. Here the ambient pressure was varied to bring about a change in the threshold for cavitation, which would result in a change in the onset of the observed effects of cavitation. Likewise, variations in the ambient temperature can affect the cavitation threshold. Therefore the effects on the ED50 of temperature variation can also be used as an indicator of the possible occurrence of effects associated with cavitation.

The following experiment will be performed in order to investigate the behavior of the cw ED50 curve shown in Figure 1 with temperature. The ambient temperature will be raised to 37°C, and irradiations will be performed for the intensity range from 45 W/cm^2 to 289 W/cm^2 at 1 atm ambient pressure and then at 16 atm ambient pressure. Prior to irradiation the specimen will be anesthetized with intramuscular injections of ketamine hydrochloride and xylazine (approximately 25 mg/kg body weight each).

Figure 5 shows the ED50 curves for the 10°C and 37°C cw irradiations at 1 atm ambient pressure. The 37°C ED50 data were obtained using specimens anesthetized with halothane. It is predicted that similar curves will be obtained when both 10°C and 37°C cw irradiations are performed using ketamine hydrochloride and xylazine to anesthetize the specimens. However this result alone will not be sufficient for conclusive evidence for cavitation since an increase in the ambient temperature is expected to also increase the heat generation by ultrasound in the specimen during irradiation. However, if cavitation is involved, the data for ED50 for the 37°C irradiations at 16 atm are expected to differ from the 1 atm data at a lower intensity than found at 10°C irradiations. Such a result would be indicative of acoustic cavitation involvement since the threshold for cavitation is lowered with an increase in temperature.

Acoustic emissions from the specimen will be monitored during irradiations. If cavitation is present at 1 atm for all intensities employed for the cw irradiation study at 10°C, then it is expected that acoustic emission levels present at 10°C will be increased when the ambient temperature is increased to 37°C. Upon pressurization to 16 atm the signals are expected to decrease significantly.

In summary, this experiment will test the hypothesis that the observed effects of cavitation for the cw irradiations are enhanced with an increase in ambient temperature.

B. Pulsed Irradiation Experiment I

The results obtained thus far from cw irradiations suggest that cavitation may contribute to the observed paralysis at the higher intensity levels used. In order to further investigate the role of cavitation as a mechanism by which the observed effects occur, irradiations will be performed using pulsed ultrasound.

Irradiations with pulsed ultrasound using sufficiently high temporal peak intensities can induce cavitation even if the corresponding TAI is very low. If the TAI is kept far below the level where significant heating occurs for the exposure duration range used, then it can be assumed that any observed effects are due to nonthermal effects. Here it is postulated that the nonthermal effects are mainly due to cavitation.

The following experiment will be performed in order to investigate the dependence of the ED50 on temporal peak intensity for pulsed irradiations where the TAI is kept constant at a very low value. The TAI and pulse width will be kept constant at 45 W/cm^2 and 100 μs, respectively. The ambient temperature will be 10°C. ED50 values will be determined for duty cycles in the range of 100% to 5%. Correspondingly, the peak intensity range will be from 45 W/cm^2 to 900 W/cm^2. Irradiations will be performed for both 1 atm and 16 atm ambient pressures.

From the previous pulsed irradiation study, discussed under Previous Research, it was shown that finite amplitude effects cannot be neglected when interpreting the results of high amplitude pulsed exposures. Therefore, as before, the increased absorption of the ultrasonic energy must be taken into account. It is postulated that above a certain peak intensity level. thermal effects will not fully explain the observed values of ED50. If cavitation is involved at the higher intensities, the exposure durations for ED50 exposures will be much smaller than would be predicted on the basis of thermal effects alone. The intensity at which this deviation begins to occur would define the threshold for effects due to a nonthermal mechanism. presumably cavitation. Further, it would be expected that this intensity would coincide with the intensity at which pressurization has an effect on the ED50 curve.

Acoustic emisssions from the specimen will be monitored, and it is predicted that they will offer additional information on the onset of cavitational effects. It is also predicted that there exists a threshold peak intensity level where a sudden increase in the level of subharmonic and broadband noise signals occurs. This observation may define a threshold for the onset of transient cavitation.

From this study, three peak intensity levels will be derived using three different methods. If these levels all indicate a threshold level for the onset of the observed effects of cavitation, then they should all be the same. In summary, this experiment will attempt to define a threshold peak intensity level where the onset of cavitation with the production of the observed effects is strongly indicated.

C. Pulsed Irradiation Experiment II

Up to this point, no attempt has been made to distinguish between the observed effects of transient cavitation and stable cavitation. In general, the effects of stable cavitation are enhanced if the irradiation conditions allow for sufficient growth of preexisting cavitation nuclei toward their resonant size by rectified diffusion. However this is not necessarily true for transient cavitation. In a pulsed irradiation experiment where the TAI, duty cycle, peak intensity and the exposure duration are kept constant, and only the pulse width is increased, the observed effects will be enhanced with increased pulse width if they are due to stable cavitation. Significant changes may not be observed for the effects due to transient cavitation.

The following experiment will be performed to determine the role of stable cavitation in the production of the hind limb paralysis. From the results of the previous irradiations at 1 atm, three intensity levels will be chosen for this experiment. The first intensity level will be the highest peak intensity that will have been used in the previous experiment and will correspond to the condition where transient cavitation is most likely to produce the effects. The second intensity level will be slightly above the threshold peak intensity for the onset of the effects of cavitation, which will have been determined in the previous experiment. If stable cavitation can produce the observed effects, then it is most likely indicated at this level. The third intensity level will be far below the threshold peak intensity value and will correspond to the condition where effects due to cavitation are unlikely to occur. The TAI will be kept constant at 45 W/cm^2 as previously, and the duty cycles for the three irradiation conditions will correspond to the peak intensities used. The pulse width will be varied from 10 μs to 10 ms. All other experimental parameters will be unchanged from the previous experiment.

The following results are predicted. At the third intensity level, no significant changes in the ED50 are expected. At the second intensity level, where stable cavitation is indicated, there will be a gradual decrease in the value of ED50 as the pulse width is increased and vice versa. At the first intensity level, no significant changes in the ED50 are expected. However, some slight changes in the value of ED50 like those observed at the second intensity level may occur. This may be due to the fact that those cavities which oscillate for a number of cycles before collapse require additional exposure time than those that collapse after one or a few cycles. Therefore a longer pulse width may allow additional collapse events to occur resulting in enhanced effects of transient cavitation. These results may suggest that stable cavitation is involved in the production of the observed effects under appropriate irradiation conditions.

The results from these combined experiments should provide a clear indication of the role of cavitation in the production of hind limb paralysis in the mouse neonate.

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Table I. Exposure conditions for 10, 50 and 90% of the specimens developing hind limb paralysis at 1 MHz and 10°C. I is the ultrasonic intensity, P is the hydrostatic pressure, and t_n are the exposure durations where n is the percentage of specimens paralyzed.

I (w/cm^2)	P (atm)	t_{10} (s)	t_{50} (s)	t_{90} (s)
86	1	3.44	5.04	9.40
86	16	2.59	4.50	18.70
105	1	2.01	2.56	3.53
122	1	1.37	1.53	1.72
144	1	0.84	0.97	1.14
144	16	0.85	0.97	1.12
192	1	0.36	0.44	0.58
256	1	0.23	0.30	0.45
289	1	0.20	0.26	0.37
289	16	0.30	0.50	1.54

Table II. Comparison of monitored half harmonic signal levels with, and without, the specimen and holder present in the irradiation chamber, at the exposure conditions for 90% paralysis and at 1 atm.

0.5 MHz Signal Level

Intensity (W/cm2)	Specimen present (dB)	Specimen absent (dB)	Difference (dB)
86	-30	-42	12
105	-22	-40	18
122	-20	-37	17
144	-18	-37	19
192	-16	-42	26
256	-18	-37	19
289	-22	-38	16

Table III. Harmonic and noise signal levels, referenced to the fundamental, as a function of the incident intensity with (W) and without (WO) the specimen and holder present. Exposures are for the 50% paralysis level.

Intensity (W/cm^2)	2nd Harmonic (dB)			3rd Harmonic (dB)			Broadband Noise (dB)		
	WO	W	Diff	WO	W	Diff	WO	W	Diff
86	—	-20	—	—	-17	—	—	-62	—
105	-30	-21	9	-48	-15	33	-60	-57	3
122	—	-23	—	—	-11	—	—	-62	—
144	-30	-21	9	-48	-13	35	-60	-55	5
192	—	-20	—	—	-12	—	—	-50	—
256	-22	-15	7	-8	-8	0	-65	-50	15
289	-16	-20	-4	—	-8	—	-60	-50	10

Table IV. ED50 exposure conditions for pulsed ultrasound irradiations of the mouse neonate spinal cord at 1 MHz. The ambient temperature and pressure are 10^{o}C and 1 atm respectively.

Duty Cycle (%)	I_P (W/cm^2)	t_{50} (s)
20	224	4.7
40	112	7.4
67	67	32.7
100	45	50.0

Table V. The ratio of the heat generation rate with the nonlinear effects considered to that with no harmonic generation for each of the peak intensities used in the pulsed ultrasound irradiations.

Peak Intensity (W/cm^2)	Duty Cycle (%)	N	$\dot{q}_{NL}/\dot{q}_L$
45	100	10	1.10
67	67	10	1.16
112	40	10	1.34
224	20	15	2.01

Note: The ratio of the heat generation rate with the nonlinear effects considered to that with no harmonic generation is approximated as the following truncated series,

$$\dot{q}_{NL}/\dot{q}_L = \sum_{n=1}^{N} \frac{\alpha_n}{\alpha} I_n/I_o = \sum_{n=1}^{N} n^{1.2} I_n/I_o.$$

where α is the fundamental absorption coefficient, α_n is the absorption coefficient of the nth harmonic, I_n is the intensity level of the nth harmonic, and I_o is the source intensity. N was chosen such that those harmonics whose relative values were at most 0.2% of the source intensity were truncated.

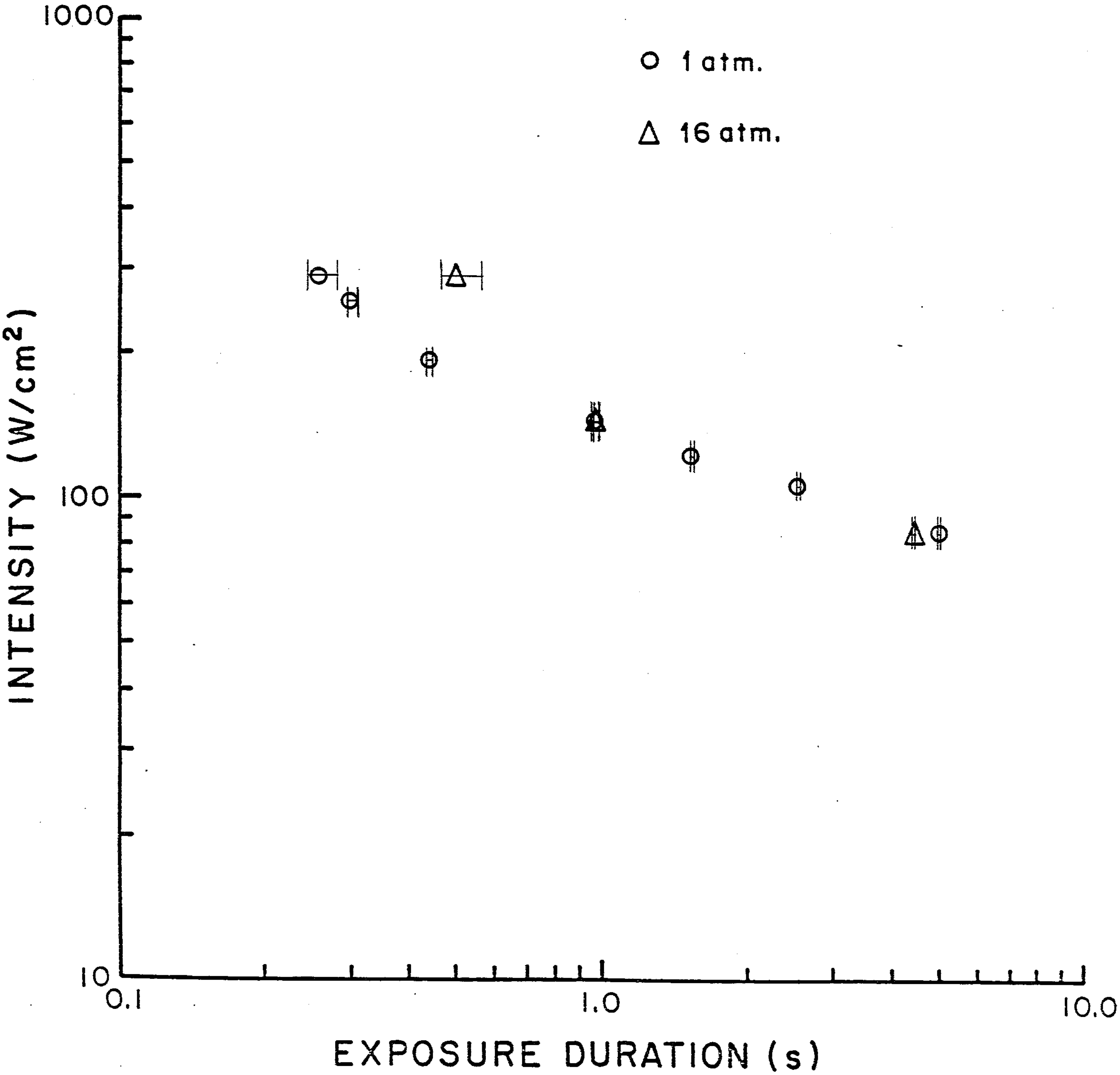

Figure 1. ED50 exposure conditions for hind limb paralysis in the mouse neonate with 1 MHz ultrasound at 10^{o}C, viz., intensity versus exposure duration.

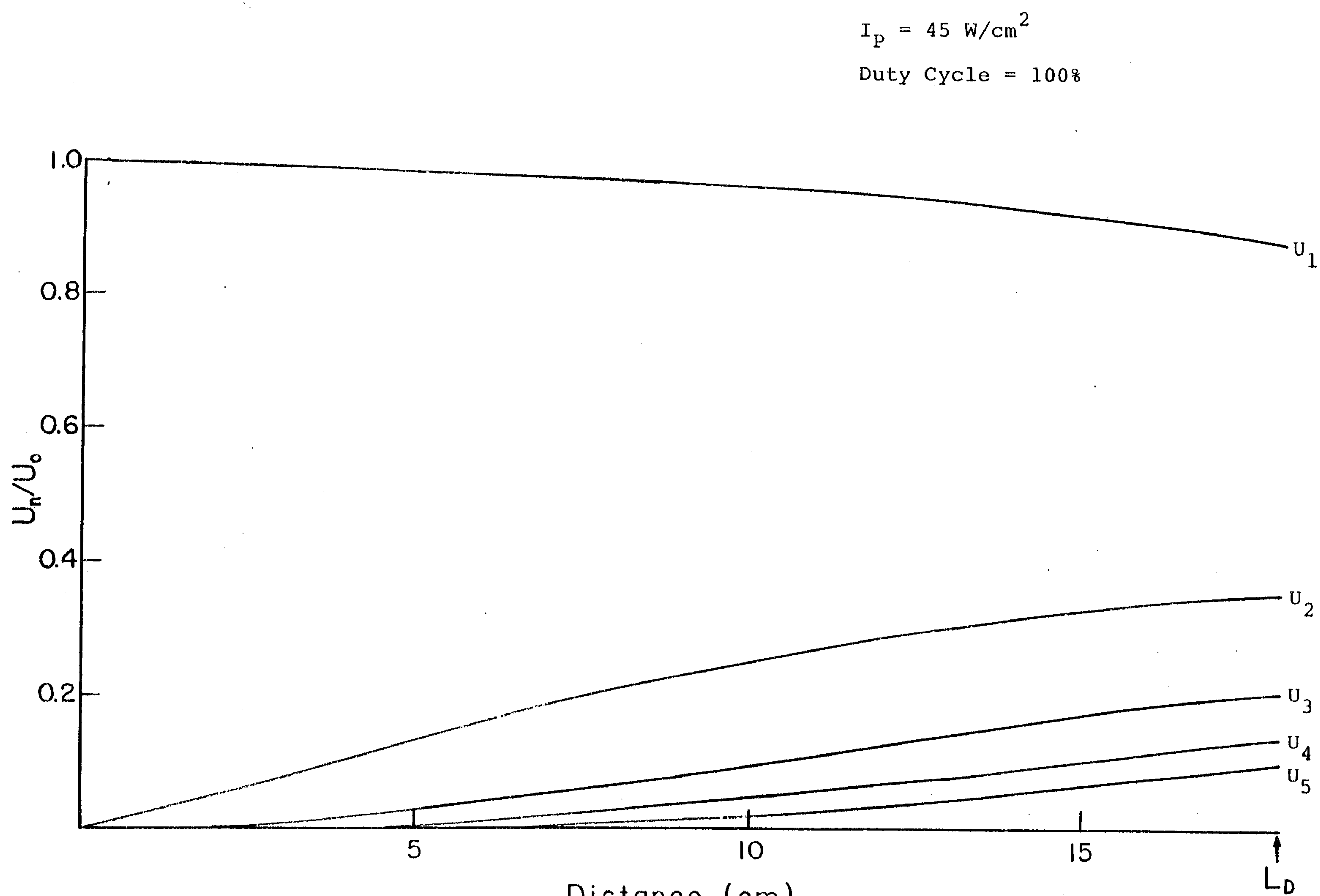

Figure 2. Normalized harmonic content versus propagation distance for a 1 MHz sound wave in 10°C Ringer's solution: $I_{source} = 45\ W/cm^2$.

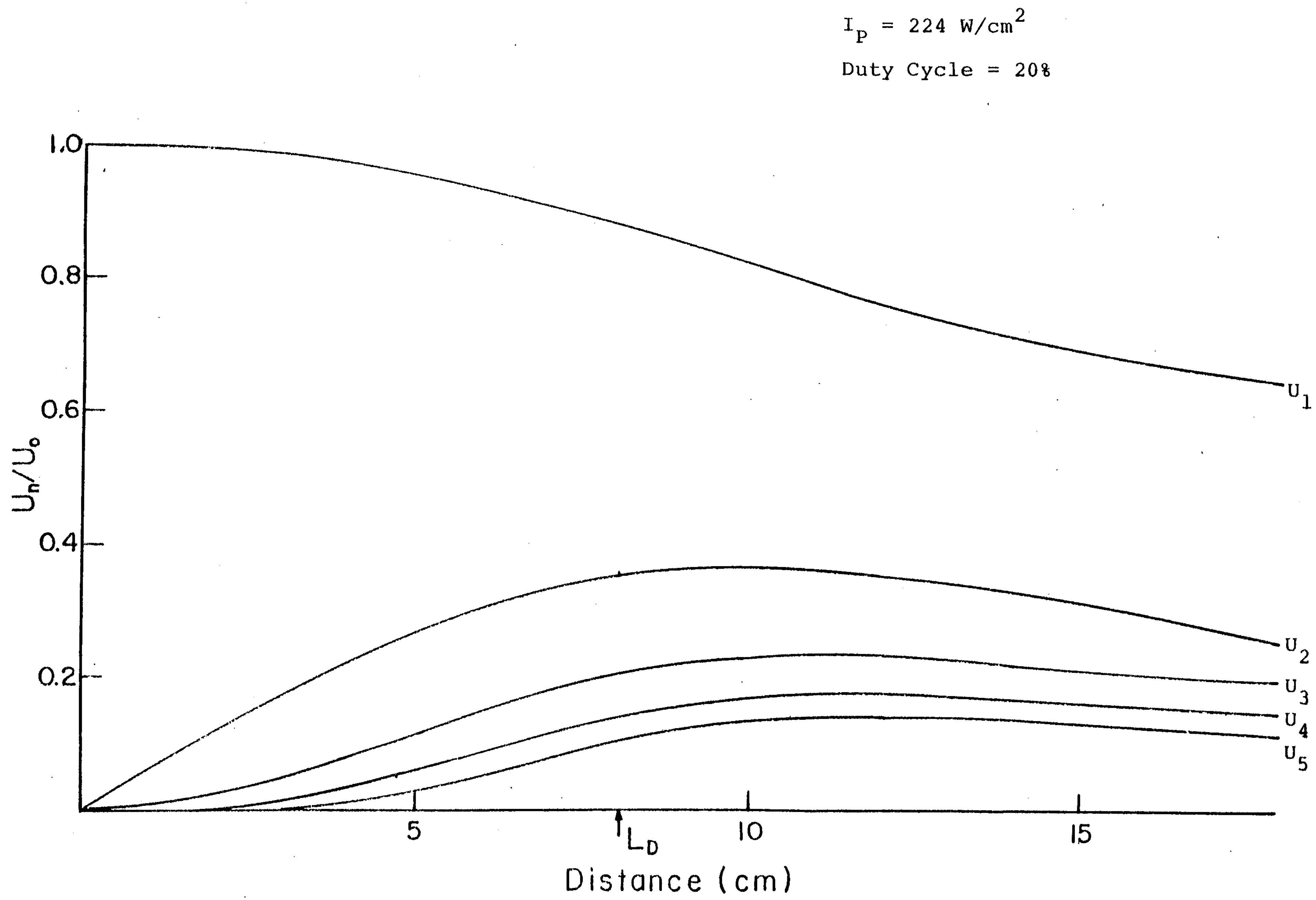

Figure 3. Normalized harmonic content versus propagation distance for a 1 MHz sound wave in 10°C Ringer's solution: $I_{source} = 224\ W/cm^2$.

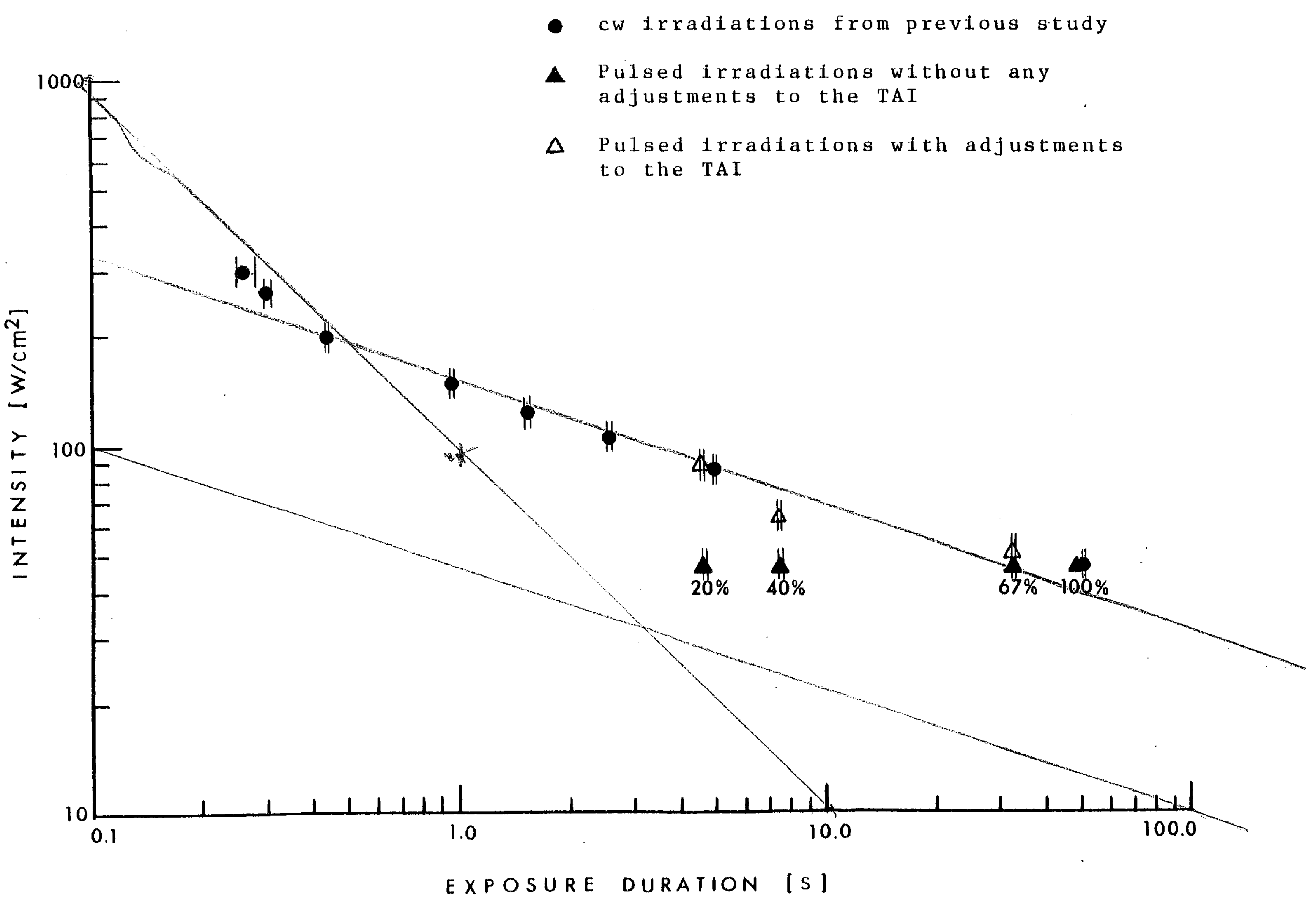

Figure 4. Comparison of the ED50 exposure conditions for hind limb paralysis in the mouse neonate with 1 MHz ultrasound at 1 atm and 10°C.

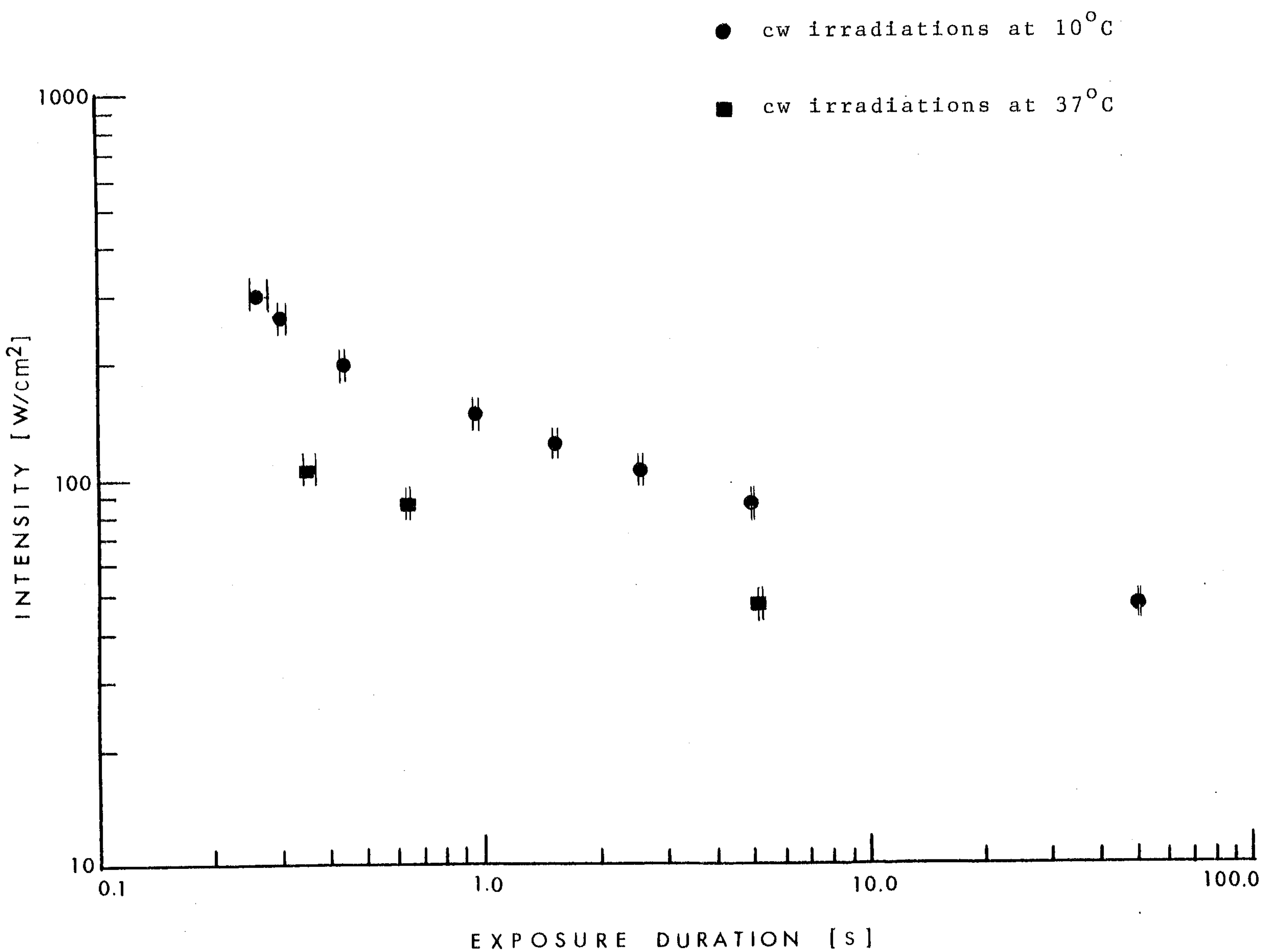

Figure 5. ED50 exposure conditions for hind limb paralysis in the mouse neonate with 1 MHz ultrasound at 1 atm.