# SOME FEATURES DISTINGUISHING LOCAL LESIONS PRODUCED BY FOCUSED ULTRASOUND IN BRAIN TISSUES

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By means of focused ultrasound (US) it is possible to create local lesions of any required size in the deep brain structures without injuring surrounding tissues or tissues located along the course of the ultrasonic beam, so that this is a promising technique for use in experimental neurophysiology and neuroanatomy and also in clinical neurosurgery. Knowledge of the mechanisms of interaction of US with nerve tissue will allow the results of ultrasonic action to be predicted.

The appearance of local foci of destruction after the use of sufficiently high intensities of the order of several hundred watts per square centimeter has been shown to be due mainly to heating of the tissues through absorption of ultrasonic energy up to a certain critical temperature [13]. It is considered that with intensities of over 1000 W · $cm^{-2}$ an essential role in tissue destruction begins to be played by mechanical factors and, in particular, by cavitation (cavitation here means effects due to the appearance of gas bubbles in the ultrasonic field and their activity [7]). Cavitation is linked with the formation of cavities, bubbles, and local hemorrhages in nerve tissue [2, 3, 10].

Brain tissue is known to differ in its sensitivity to ultrasonic irradiation [2, 9, 14] depending on its acoustic and thermal properties, and also on its biochemical composition.

The aim of this investigation was to study the effect of the volume velocity of the blood flow, one of the important factors which determine differences in sensitivity, on the local rise of temperature and, consequently, on the size of the destructive lesions produced by ultrasonic action under heat-generating conditions. The role of cavitation in the destruction of brain tissue under these conditions also was studied.

## EXPERIMENTAL METHOD

Various rabbit brain structures with the following values of the velocity of the blood flow v (in ml/min/100 g tissue) were chosen as test objects [1]: gray matter of the cerebral cortex (CGM) 42-236; white matter of the semilunar centers (SLWM) 22-64, thalamus 27-81; caudate nucleus 27-70.

Local destructive lesions in the above-mentioned structures were produced in stereotaxic operations on adult rabbits, anesthetized with pentobarbital, by means of a spherical US generator with resonance frequency $f_0$ = 1.98 MHz and with angle of convergence of the beam of 20-72° (the calculated value of the radius of the focal spot in this case $r_0$ = 0.077 cm). The intensity, averaged for the area of the focal spot ($\bar{I}_F$) was 270-1000 W · $cm^{-2}$ in different experiments, and the duration of irradiation (t) was 0.5, 1.0, and 5.0 sec. Irradiation was applied through a burr-hole and the intact dura mater. To verify the presence or absence of cavitation, the method in [4] was used; it is based on recording the subharmonic component of acoustic noise with a frequency of $f_0/2$.

For the histological investigation series of sections were stained by Nissl's method. The length of survival of the animals after ultrasonic irradiation was 24 h.

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For the theoretical calculation of the expected size of the lesions the hypothesis was adopted that the mechanism of destruction is purely thermal, and the maximal critical temperature $T_{cr}$ on the boundary of the focus of destruction at t = 0.5, 1.0, and 5.0 sec was taken to be 67, 65, and 60°C respectively [13].

The rise of temperature in the region of exposure to a single pulse of US was determined by solution of the nonstationary equation of heat conduction for an infinite cylinder of circular cross section with an internal heat source Q(r) [5]:

$$\frac{dT(r,t)}{dt} = \frac{k}{\rho C_p}\left[\frac{d^2T(r,t)}{dr^2} + \frac{1}{r}\frac{dT(r,t)}{dr}\right] + \frac{Q(r)}{\rho C_p}, \quad Q(r) = 2\cdot\alpha_0\cdot I(r),$$

where I(r) is the intensity of US in the focal plane. This value was determined by the equation in [6]:

$$I(r) = 0.273\cdot I_0\cdot I_1^2\left(3.83\, r/r_0\right)/\left(r/r_0\right)^2,$$
$$I_0 = I_0^0\cdot l^{-2\alpha h} = 4.35\cdot I_F,$$

where $I_0^0$ denotes the intensity in the center of the focal region not allowing for absorption; h denotes the depth of the center of the focal region from the surface of the hemisphere; r is the cylindrical coordinate. The parameters of the tissue were taken to be as follows: density $\rho = 1$ g · cm$^{-3}$, specific heat $C_p = 4.17$ W · sec · g$^{-10}$ · °C$^{-1}$. Since there are no unambiguous data in the literature on values of extinction ($\alpha$) and absorption ($\alpha_0$) coefficients in different brain structures, we used the following averaged values in the calculations: $\alpha = 0.07\ f^{1.14}$ · cm$^{-1}$ and $\alpha_0 = 0.024\ f^{1.18}$ · cm$^{-1}$ [11], where f is the frequency of ultrasound, in MHz.

Convective heat loss from the heated region on account of the blood flow was allowed for by introducing (according to the empirical method in [12]) an effective coefficient of thermal conductivity $k^* = k^0 + 5.8 + 10^{-3}$ v, where $k^0 = 5.8 \cdot 10^{-3}$ W · cm$^{-1}$ · °C$^{-1}$ is the thermal conductivity of the tissue in the absence of blood flow.

## EXPERIMENTAL RESULTS

The results of calculations of the rise of temperature $\Delta T_{max}$ in the center of the focal region in different brain structures depending on the duration of irradiation are given in Fig. 1a. (Here and subsequently, continuous curves with identical numbers bound regions of possible change of the calculated value depending on the variability of v in the given structure; the bottom curve corresponds to the higher value of v, the top curve to the lower value. The broken curve demonstrates the rise of temperature when v = 0.)

It will be clear from Fig. 1a that in CGM, where the velocity of the blood flow is high, the smallest rise of temperature must be expected, and this ought to lead to greater resistance of the cortex to ultrasonic irradiation. In SLWM the value of v is below the mean value in the cortex and, consequently, the expected rises of temperature, other conditions being the same, are greater, i.e., there should be a fall in the threshold intensity for the appearance of destructive lesions and an increase in the size of those lesions.

Analysis of the distribution of the rise of temperature in the focal region showed that changes in the velocity of the blood flow within the same structure may lead to indeterminacy in the value of the expected rise of temperature and, consequently, to variability of the dimensions of the foci of destruction. Distribution of temperature in the focal plane during exposure of SLWM of the rabbit brain to US with an intensity of 450 W · cm$^{-2}$, with t = 5.0 sec, is shown in Fig. 1b as an example. Curves 1 and 2 correspond to a rise of temperature for maximal and minimal values of v, and curve 3 was obtained at v = 0. The graph shows that the expected diameter of the focus of ultrasonic destruction, depending on the realized value of v, can lie within the range of 2r = 0.064-0.94 cm.

Histological investigation revealed local foci of destruction, oval in shape and with distinct outlines, in the assigned areas of the cortex and thalamus (Fig. 2). During irradiation of the head of the caudate nucleus* destruction also extended to SLWM and the internal capsule. Cavities, gaps, and local hemorrhages observed in some cases, which were associated with the action of cavitation, did not essentially distort the shape of the destructive lesions.

The results of comparison of the diameters of the foci of destruction obtained experimentally with the calculated data are given in Fig. 3 in which values of the intensity of US are plotted along the abscissa and the diameter of the foci of destruction for an exposure of t = 5.0 sec along the ordinate. The dimensions of the focal

*To destroy the head of the caudate nucleus in chinchilla rabbits, the sagittal coordinate used had to be 2 mm less than indicated in the atlas [8].

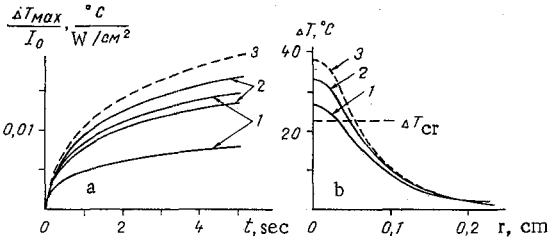


Fig. 1. Rise of temperature in focal region obtained by theoretical calculation. a) In center of focal region depending on duration of exposure: 1) CGM, v = 42-236 ml/min/100 g tissue, 2) SLWM, v = 22-64 ml/min/100 g tissue, 3) v = 0; b) in focal plane in SLWM (r denotes distance from center): 1) v = 64 ml/ min/100 g tissue, 2) v = 22 ml/min/ 100 g tissue, 3) v = 0; f = 1.98 MHz, $I_F$ = 450 W · $cm^{-2}$, t = 5.0 sec.

lesions located in CGM, SLWM, and the thalamus are in good agreement with the results of calculations based on a purely thermal model of destruction. The greater diameter of the local foci in cases when destruction also extended to the internal capsule can be explained by the influence of one of the following factors: the higher coefficient of absorption of US, the slower velocity of the blood flow, and the lower temperature at which irreversible changes take place in this structure. The dimensions of the foci of destruction in all structures investigated during irradiation by a single pulse of US with a duration of 0.5 and 1.0 sec exceeded the calculated values.

The experiments thus showed that the velocity of the blood flow has a significant effect on temperature distribution in the focal region and that allowance for it when "thermal" modes of ultrasonic irradiation are used can partly explain both the difference in threshold intensities and size of the foci of destruction in different brain structures and the variability of their size within the same structure. However, for more accurate quantitative calculations of the dimension of the destructive lesions further data on the acoustic parameters of the various structures are needed, and in particular, the coefficient of absorption; the biochemical characteristics of these structures must also be taken into account.

The onset of cavitation, recorded as the subharmonic component of cavitation noise, was distinctly threshold in character and in most cases corresponded to intensities of between 230 and 580 W · $cm^{-2}$, depending on the structure irradiated. For instance, the lowest values of cavitation thresholds were obtained during irradiation of the region included the boundaries of the caudate nucleus, SLWM, and the internal capsule: activity of insignificant amplitude was recorded at $\bar{I}_F$ = 330 W · $cm^{-2}$. No tissue destruction was found under these circumstances. With an increase in the intensity of US the amplitude of the subharmonic component increased. In this case gaps and slits and, in some cases, hemorrhages, located mainly on the boundaries between SCWM and the caudate nucleus, appeared in the foci of destruction (Fig. 2). During irradiation of the thalamus local foci of destruction without gaps and cavities were obtained at $\bar{I}_F$ = 350 W · $cm^{-2}$, t = 5.0 sec, whereas the subharmonic component appeared at an intensity of not below 430 W · $cm^{-2}$. Its amplitude increased sharply (Fig. 4) when destruction spread also to the fimbria hippocampi, and under these circumstances gaps and cavities appeared close to the boundaries between the above-mentioned structures. The cavitation strength of CGM was found to be exceptionally high. In a focus of destruction, consisting of a zone of total tissue necrosis, no gaps and hemorrhages characteristic of the action of cavitation were found. Here no cavitation was recorded when at $\bar{I}_F$ = 1000 W · $cm^{-2}$. When destruction spread to the subjacent white matter a subharmonic signal was observed at $\bar{I}_F \geq$ 580 W · $cm^{-2}$. Histological investigation revealed gaps and cavities, not in the center of the focal region, but in the white matter, where the intensity was substantially below that indicated. As a rule they were situated along the boundaries of the lateral ventricles of the brain or along fibers, apparently displacing them (Fig. 2).

It can be tentatively suggested that the threshold phenomena observed correspond to the formation and subsequent pulsations of "stable" cavitation bubbles. With an increase in the intensity of US, cavitation activity of the pulsating bubbles and, consequently, the amplitude of the subharmonic components, increased significantly. The lowest cavitation strength was found on the boundaries separating different structures and on the boundaries of the cerebral ventricles.

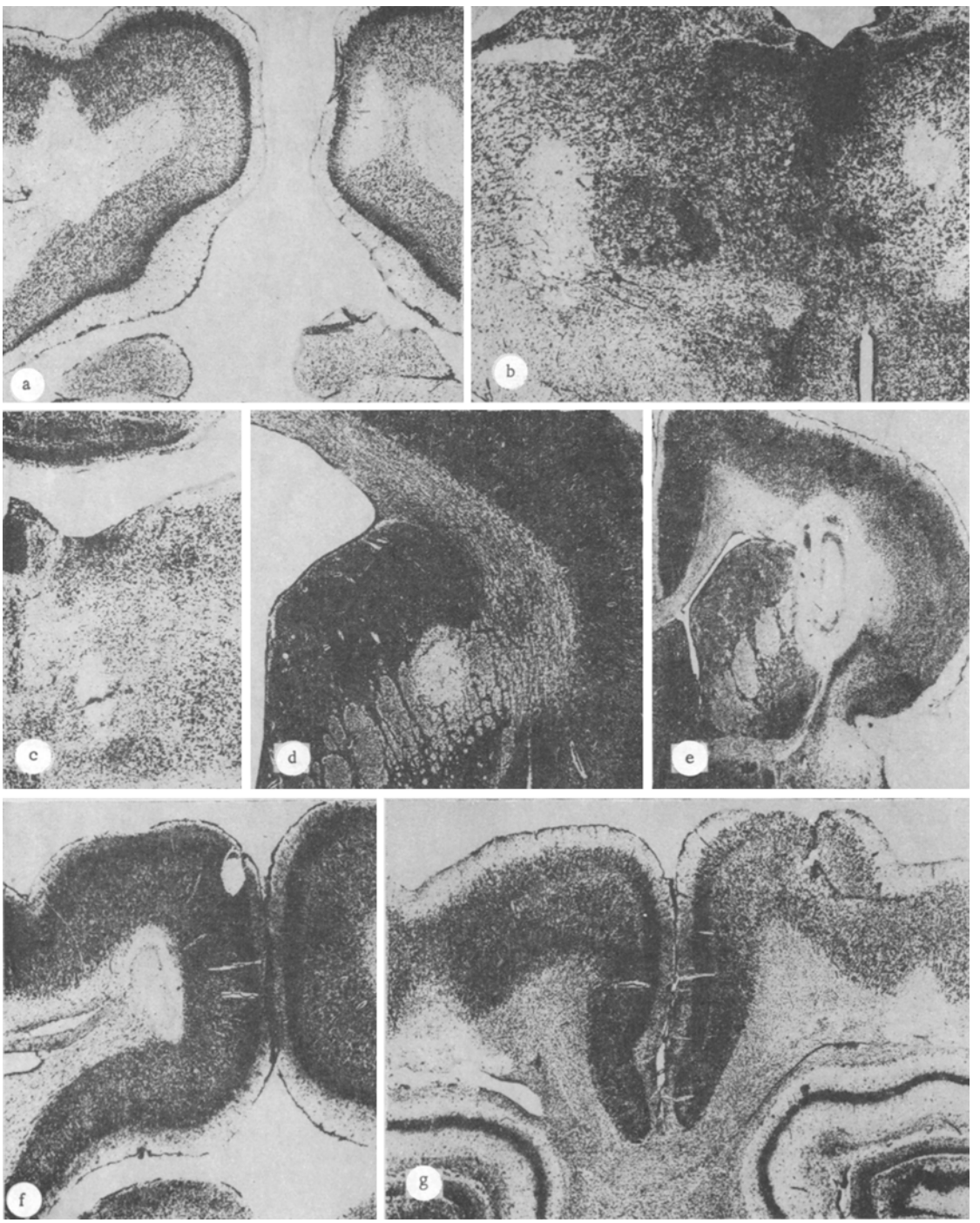


Fig. 2. Local foci of destruction created in various rabbit brain structures by focused US.

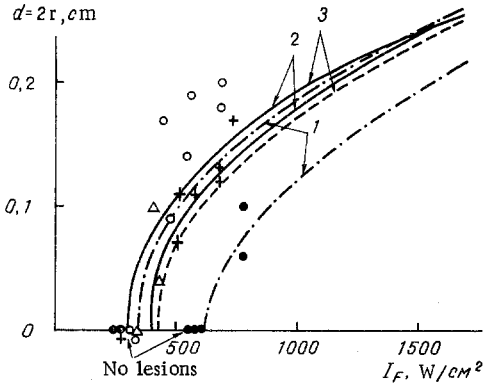


Fig. 3. Diameters of foci of destruction in different brain structures depending on intensity (curves show calculated values, t = 5.0 sec). 1) Cerebral cortex, 2) thalamus, 3) SLWM. Asterisks indicate internal capsule.


Fig. 4. Subharmonic component of cavitation noise recorded during irradiation of thalamus (a) and region including boundary separating heterogeneous structures (b). f = 1.98 MHz, $I_F$ = 430 W · cm$^{-2}$, t = 5.0 sec.

The main cause of the appearance of local foci of destruction after the use of the above-mentioned doses of irradiation, as was shown above, was not cavitation. This is also shown by the disparity between doses with which a subharmonic component of cavitation noise was recorded and doses with which local foci of destruction appear. However, definite correlation was observed between the appearance of gaps and slits and cavitation activity. During the practical use of focused US for tissue destruction, data of their cavitation strength must be taken into account. In this case the boundaries separating heterogeneous structures, together with gas bubbles, the boundaries of the cerebral ventricles, and of blood vessels may evidently be "weak points" (cavitation centers) in biological tissues. The mechanism of preferential appearance of cavitation on boundaries between heterogeneous structures demands additional study.

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# EFFECT OF THE ANTIOXIDANT DIBUNOL ON EPR SIGNALS IN RAT TISSUES AT DIFFERENT AGES

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Since the work of Harman [8] and Émanuél' [6] it has been known that the antioxidant butylated hydroxytoluene (ionol, dibunol) has a geroprotective action, i.e., it lengthens the life span of laboratory animals if added regularly to their diet.

In model reactions of free-radical peroxidation of lipids and other natural compounds dibunol exhibits antioxidant properties: It inhibits these reactions and intercepts active free radicals (FR), but the nature of its action in vivo has not yet been explained [5, 6].

The aim of this investigation was to study the effect of dibunol on function of enzymes of tissue oxidative metabolism and also on the blood proteins transferrin and ceruloplasmin in rats of different ages. Electron paramagnetic resonance (EPR), a direct method of detection and study of paramagnetic centers and electron-transport chains in the tissues [1], was chosen as the experimental method.

## EXPERIMENTAL METHOD

Experiments were carried out on the tissues of adult (5-6 months) and old (28-30 months) male Wistar rats. Dibunol (4-methyl-2,6-di-tert-butylphenol), dissolved in a 10% aqueous solution of the solubilizer Tween-80, was injected intraperitoneally in a dose of 10 mg/100 g body weight. The rats were decapitated 6 h after injection of dibunol. The dose of dibunol and time of its administration were chosen on the basis of data showing the dynamics of the effect of dibunol on tissue antioxidative activity [3]. All animals were killed at the same time (3-4 p.m.). Tissue samples for EPR measurements were prepared in the form of cylindrical columns, frozen at 77°K, with a length ($l$) of 20 mm and a diameter (d) of 4.7 mm (in the case of heparinized whole blood), $l$ = 20 mm and d = 3.0 mm (for liver, kidneys, myocardium, skeletal muscles, thyroid gland), and $l$ = 5 mm and d = 3.0 mm (for adrenal cortex) [4]. EPR signals were recorded on an E-109 spectrometer (Varian, France), equipped with an internal standard, at a temperature of 77°K and with an amplitude of high-frequency modulation of the magnetic field of 10 G. The microwave power was kept constant at 50 mW for recording EPR signals of blood, 0.2 mW for recording signals with a g-factor of about 2.0, and 10 mW when recording all other signals. In each experiment eight experimental and eight control animals were used. The results were subjected to statistical analysis by the usual methods [2].

## EXPERIMENTAL RESULTS

The EPR spectra of the tissues corresponded in shape of lines and average intensity to those observed previously [1, 4, 7]. To determine the quantitative characteristics of the spectra, the intensities (amplitude

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