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Influence of Ultrasonic Radiation in the Medical Therapeutic Range on the Fine Structure of the Liver Parenchymal Cell*

An Electron Microscope Study on Mice

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Der Einfluß von Ultraschallwellen im therapeutischen Bereich auf die Feinstruktur der Leberzelle

Eine elektronenmikroskopische Untersuchung an Mäusen

Zusammenfassung. Ultraschallenergie in einer Intensität von 0.5 bis 3 Watt/cm² wurde auf den Oberbauch nicht-anaesthesierter Mäuse aufgebracht. Danach wurden Leberstückchen elektronenmikroskopisch untersucht. Das Aussehen der Leberzellen schwankte von fast normal (1 Watt/cm² für 1 min) bis zu völligem Verlust (Coagulation) der Feinstruktur der Zellorganellen (3 Watt/cm² für 3 min). Außerdem kam es zur Kavitation und Zerreißungen im Cytoplasma der Leberzellen. Die Veränderungen sind wahrscheinlich bedingt durch die sowohl thermalen wie mechanischen Wirkungen des Ultraschalls; allerdings mag auch die relative Anoxie eine Rolle bei der Entstehung der intracellulären Vacuolen spielen.

Summary. Ultrasonic energy of an intensity from 0.5 to 3 watts/cm² was applied on the upper abdomen of unanaesthetized mice and specimens of the liver were examined with the electron microscope. The appearance of the fine structure of the liver cells ranged from nearly normal (intensity of 1 watt/cm² for 1 minute) to complete loss (coagulation) of the fine structure of the cell organelles (intensity of 3 watts/cm² for 3 minutes). In addition, cavitation and disruption of the cytoplasm occurred in the hepatic cells. The changes are probably due to both the thermal and mechanical effects of the ultrasound, but also the relative anoxia may play some role in causing the formation of intracellular vacuoles.

The biologic effects of ultrasound can be divided into two categories, thermal and non-thermal. Once the absorption coefficient of the tissues and the reflection at tissue interfaces are known, it is possible to calculate the so-called pattern of relative heating. The depth of penetration of the ultrasonic energy is very satisfactory. One-half of the intensity at the muscle surface is still available at a depth of 3 cm. Although most of the biologic reactions are due to the temperature elevation, the entire reaction cannot in some instances be explained on the basis of heating alone. The non-thermal effects of ultrasound include, e.g., alteration of the permeability of the biologic membranes and the phenomenon of gaseous cavitation (LEHMANN, 1965).

There has been considerable investigation of the histological effect of ultrasonic radiation using the ordinary light microscopic methods (SOUTHAM et al., 1953; BEIER and DÖRNER, 1954; HUGHES and NYBORG, 1962; GODFREY et al., 1963; COWDEN and ABELL, 1963). In the following, however, the influence of

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ultrasonic radiation on the ultrastructure of a typical parenchymal cell, the liver cell, was studied with the electron microscope.

Materials and Methods

The ultrasonic unit used throughout these experiments was the Megason XII (manufactured by The Birtcher Corporation, Los Angeles, Calif.). The soundhead has a surface of 5.0 cm^2 and emits a cylindrical beam of sound waves of 1 megacycle frequency. The generator has a rated maximum output of 15 total watts, or 3 watts/cm^2.

The experimental animals were young adult mice varying from 22 to 25 g in weight. For direct application of the soundhead on the upper abdomen of the unanaesthetized animals, paraffin oil was used as a coupling medium. The animals were treated in groups of five and the following intensities of ultrasonic energy and durations of treatment were used: 0.5 watt/cm^2 for 2 minutes, 1 watt/cm^2 for 1 minute, 1 watt/cm^2 for 2 minutes, 2 watts/cm^2 for 1 minute, 2 watts/cm^2 for 2 minutes, 3 watts/cm^2 for 1 minute, and 3 watts/cm^2 for 3 minutes. During treatment the soundhead was slowly moved in small overlapping circles on the upper abdomen of the animal (the circular method).

The animals were decapitated and exsanguinated 30 minutes after the treatment with ultrasound. Specimens of the liver were immediately fixed in 3 per cent glutaraldehyde (SABATINI et al., 1963) in phosphate buffer (pH 7.2) for 1 hr. at 4°C and removed into phosphatesucrose (PALADE, 1952). The samples were then fixed for 1 hr. in 1 per cent osmium tetroxide solution buffered with veronal. After rinsing in distilled water, the specimens were dehydrated in ethyl alcohol in an ascending series of concentrations and embedded in the epoxy resin Epon 812 (LUFT, 1961). Polymerization to a suitable degree of hardness was effected in an oven at + 45°C for about 48 hours. Thin sections were cut with the Porter-Blum MT-2 ultramicrotome using glass knives, poststained with lead citrate solution (REYNOLDS, 1963) and examined in the Siemens Elmiskop I electron microscope at original magnifications of 2,000 to 15,000 and enlarged as desired.

Results

The animals had no cutaneous lesions after treatment. No visible visceral lesions were observed in the animals treated with 1 watt and 2 watts/cm^2. In the animals treated with 3 watts/cm^2 for 3 minutes there were small haemorrhagic areas on the surface of the liver and the intestines were congested and swollen.

Electron microscopic examination of the liver specimens revealed the following. The intensity of 0.5 watt/cm^2 caused no significant changes in the fine structure of the liver cells. The intensity of 1 watt/cm^2 given for 1 minute caused only very weak changes such as slight swelling of the rough endoplasmic reticulum. When given for 2 minutes the same intensity caused marked dilation of the endoplasmic reticulum and clear vesiculation of the cytoplasm. The internal cristae of the mitochondria were somewhat decreased in number and glycogen had disappeared nearly completely.

The intensity of 2 watts/cm^2 applied for 2 minutes caused clear changes in the fine structure of the liver cells. The cytoplasm was oedematous and there was extensive vesicle formation throughout the cell. Glycogen had completely disappeared (Fig. 1).

After the application of an intensity of 3 watts/cm^2 for 3 minutes there were spotty areas in the liver which represented coagulation necrosis with haemorrhage. In these areas there was complete loss of the fine structure of the cell organelles. These changes were not uniform throughout the entire liver and corresponded only to the coagulated areas detected in the gross examination. Erythrocytes in

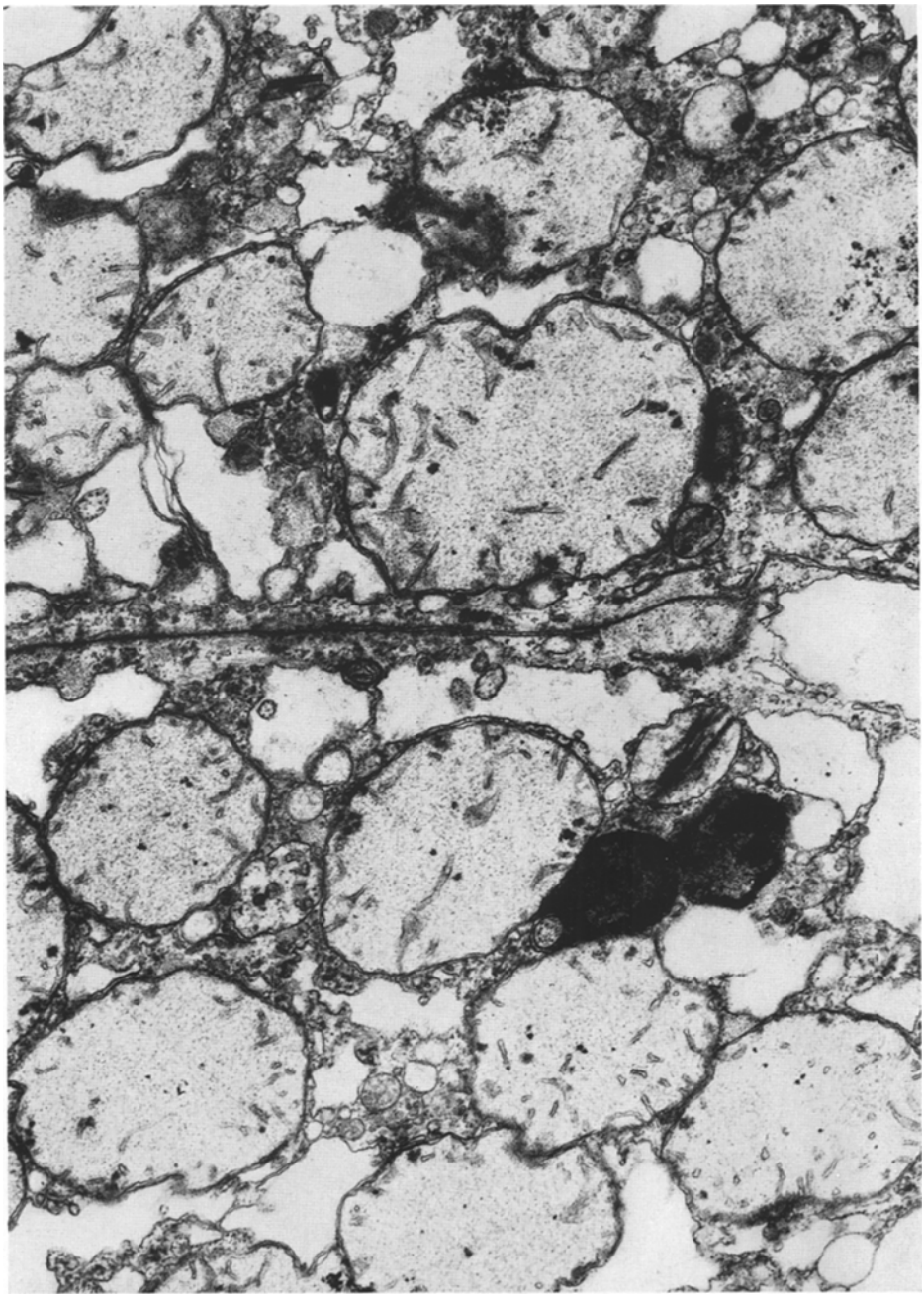

Fig. 1. Portions of two adjacent liver cells of a mouse after treatment with 2 watts/cm^2 of ultrasound for 2 minutes. There is no glycogen, the cytoplasm is oedematous and the formation of vesicles is rich. × 21,000

the liver parenchymal cells were also a common finding indicating irreversible damage to the liver (Fig. 4).

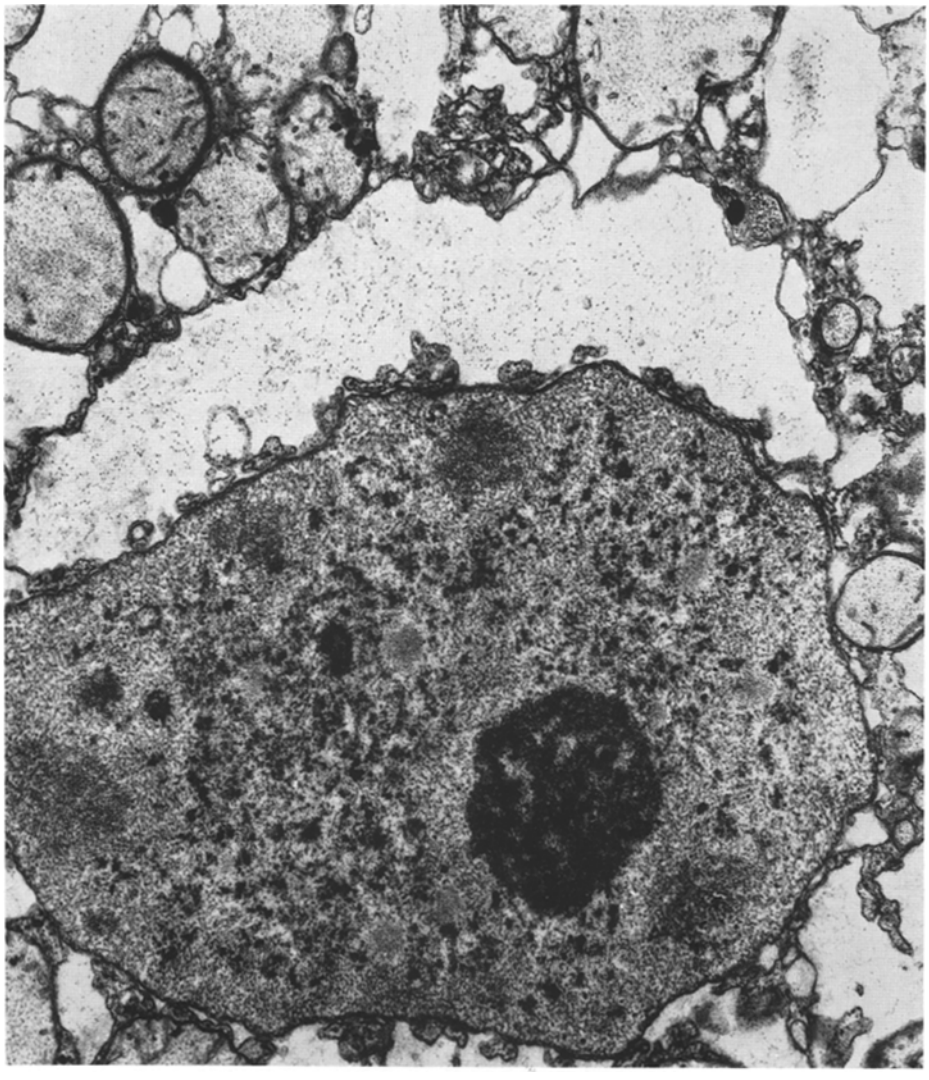

Fig. 2. Portions of a nucleus and cytoplasm of a parenchymal liver cell of a mouse after treatment with 3 watts/cm² of ultrasound for 1 minute. The cytoplasm is oedematous and there is an intracellular vacuole curved around the nucleus. × 16,000

The intensity of 3 watts/cm², when applied for 1 minute, caused somewhat lesser changes. Oedema of the cytoplasm, vesicle formation and pyknotic nuclei were a usual finding. Sometimes also cavitation, consisting of bubble formation in the intracellular spaces, and disruption of the cytoplasm occurred. These phenomena took place most often at the borderline between the nucleus and cytoplasm (Figs. 2 and 3).

Discussion

There are few reports in the literature concerning histological changes in the liver under the influence of ultrasonic treatment.

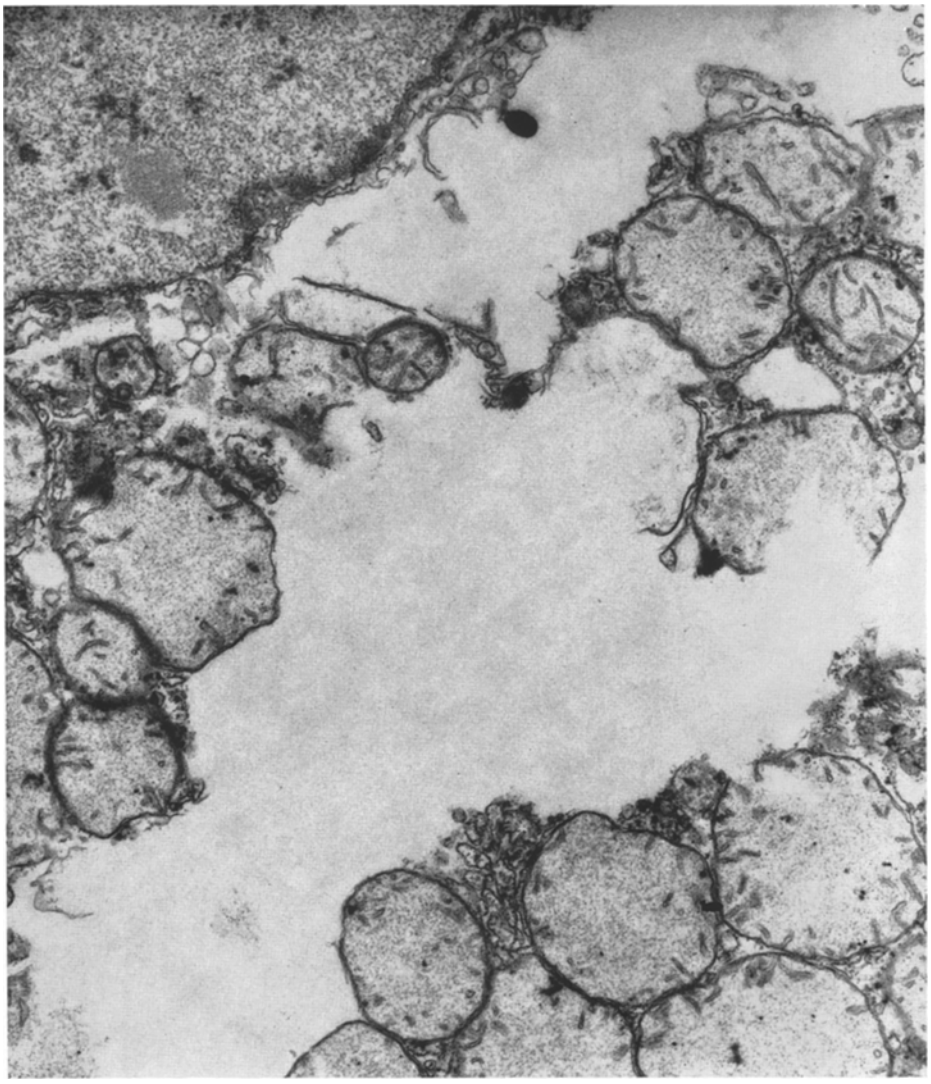

Fig. 3. Portions of a nucleus and cytoplasm of a parenchymal liver cell of a mouse after treatment with 3 watts/cm² of ultrasound for 1 minute. There is disruption of the cytoplasm occurring mainly at the borderline between the nucleus and the cytoplasm. The organelles show no signs of coagulation. × 16,000

JANKOWIAK et al. (1958) observed after ultrasonic irradiation passive hyperaemia, fatty degeneration and a partial loss of glycogen. Among the changes increasing quantitatively with the duration of the ultrasonic treatment there were necrosis and dissociation of the cells. BELL (1957, 1958) noted that a dose of 12 watts/cm² for 15 seconds produced a necrotic focus in mouse liver. BEIER and DÖRNER (1954) found marked hyperaemia of the liver immediately below the capsule. With a treatment period of 5 minutes at an intensity of 3 watts/cm² no changes were found in the liver cells, but with an intensity of 5—6 watts/cm² there was extensive vacuolization, haemorrhagic effusions and necrosis. DÖNHARDT and PRESH (1953) also observed necrosis of cells in the livers of rats and changes in the lipoids of the blood serum after ultrasonic irradiation. SOUTHAM et al. (1953) did not find the above mentioned changes in the cells at an intensity of 1 watt/cm². Serious lesions and necrosis of

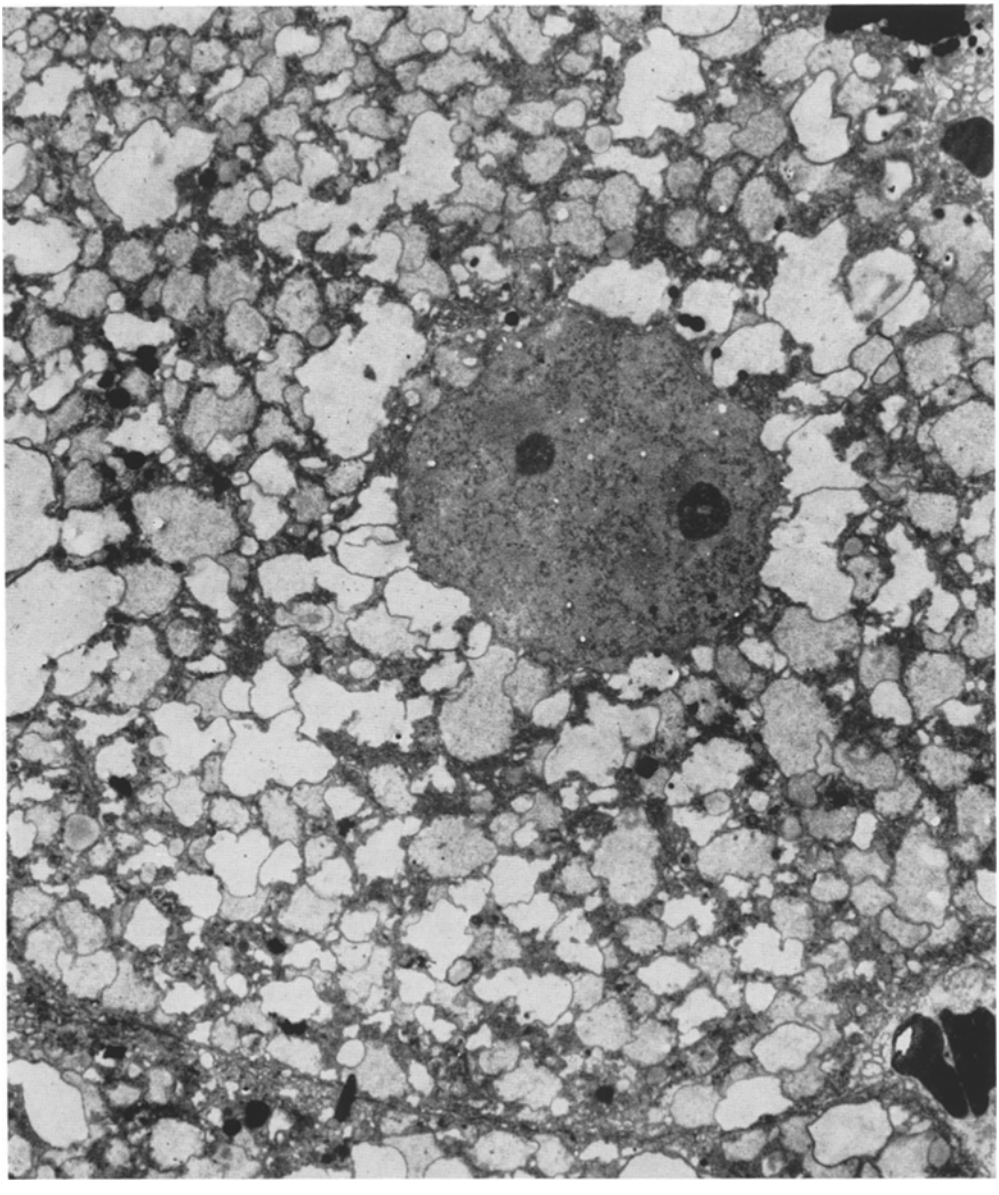

Fig. 4. Parts of three liver cells of a mouse after treatment with 3 watts/cm² of ultrasound for 3 minutes. There is complete coagulation of the cells and the cell organelles have for the most part lost their fine structure. × 8,100

the liver cells appeared for the first time at an intensity of 10 watts/cm². According to BEJDL (1954) there were no changes in the liver cells at low intensities, but at high intensities cell deaths occurred due to tearing of the nucleus, its coagulation and breaking.

As is shown also in the present study, it is possible to cause serious damage to the liver without producing any lesion of the skin, because the skin is much more resistant to the damaging action of the ultrasound (BELL and ARGYRIS, 1957) than the liver. As to the ultrasound intensity that can cause destruction of the

tissues, it is, of course, dependent on the size of the experimental animal because of the thermal factor of the ultrasonic energy. In the present study, in which mice served as experimental animals, the intensity of 1 watt/cm^2 was the first to cause slight changes, and only 3 watts/cm^2 caused severe damage when applied for 3 minutes. In addition, the technique of the application is of great importance. In this study the soundhead was slowly moved in the upper abdomen. Because the moving of the soundhead was slow and occurred over a relatively small area, this technique of application was closely related to the stationary technique in which the soundhead is held in one position. The stationary technique produces a rapid rise of temperature in a very small area ("hot spots"), while the rest of the tissues are not heated markedly. The appearance of "hot spots" is the most probable explanation for the occurrence of completely coagulated haemorrhagic small areas in the livers of the mice treated with an intensity of 3 watts/cm^2.

The changes in the fine structure of the cytoplasm of the liver parenchymal cells are degenerative in character. The first changes in the fine structure are similar to the changes caused by several hepatotoxic substances, which for the most part are still reversible (ROUILLER, 1964). They include, e.g., changes in the endoplasmic reticulum, disappearance of the glycogen, vesicle formation in the cytoplasm, and minor changes in the mitochondria. The more marked changes, as pyknosis of the nuclei, extensive intracellular oedema and vesicle formation, are probably already irreversible changes, as are disruption of the cytoplasm and coagulation of the intracellular organelles.

It is impossible to say whether the changes in the fine structure of the hepatic cells are due to the thermal or non-thermal effect of ultrasound. The primary reactions occurring within an ultrasonic beam at therapeutic intensities of the order of 1 to 4 watts/cm^2 are directly related to the particle movement as a result of wave propagation. The amplitude of displacement of the particles is of the order of $1 \cdot 10^{-6}$ to $6 \cdot 10^{-6}$ cm. The maximum velocity of the particles is approximately 10 to 26 cm/sec and the accelerations to which the particles are subjected are about $5 \cdot 10^7$ to $16 \cdot 10^7$ cm/sec^2. The pressure amplitude in the waves is approximately 1 to 4 atmospheres, and thus a great difference in pressure occurs over a relatively short distance (LEHMANN, 1953). By cavitation is meant that empty spaces or gas bubbles are formed in the tissues during the phase of low pressure. The distance between the region of maximal rarefaction and that of maximal compression is about 0.1 cm. Therefore the spotty formation of intracellular gas bubbles or the intracellular disruption of organelles can be explained also on the basis of mere mechanical ultramassage. The spotty elevation of temperature in the tissues can, however, be an explanation of the formation of gas bubbles, although the disruption of the cytoplasm as is presented in the Fig. 3 seems to be more probably the result of mechanical disruptive forces.

The perinuclear disruption of the cytoplasm and the formation of perinuclear vacuoles is a peculiar phenomenon which can be explained in two ways. Because the reflection of ultrasound can occur at interfaces between tissues of different acoustic impedance, a part of the ultrasonic energy may be reflected from the nucleus and lead to an increase of ultrasonic energy close to the nucleus, i.e., in the perinuclear space. On the other hand, BREWER and HEATH (1965) have shown that anoxia causes formation of intracellular vacuoles in liver cells. These vacuoles

are big, round or oval and sometimes curved around the nucleus and they contain no remnants of cytoplasmic bodies. It is therefore possible that at least some of the vacuoles observed in the hepatic cells after application of ultrasound are due to the relative anoxia of the liver cells caused by the increased metabolism due to temperature elevation.

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