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Observations on the Effect of Different Intraperitoneal Media on Degree of Tissue Destruction Caused by Ultrasonic Radiation in the Rat Mesentery

ERKKI J. VALTONEN *

Department of Physical Medicine, University Central Hospital, Helsinki, and The Electron Microscope Laboratory, University of Helsinki, Finland

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Beobachtungen über die Wirkung von intraperitonealen Zwischenmedien auf den Grad der Gewebszerstörung im Mesenterium durch Ultraschall

Zusammenfassung. An Ratten wurde die Wirkung von intraperitoneal eingebrachter wässeriger Salzlösung und Luft auf den Grad der Gewebszerstörung (gemessen am Zerfall der Mastzellen) nach Ultraschall untersucht. Es ergab sich, daß die Gewebszerstörung am größten bei denjenigen Tieren war, denen Luft injiziert worden war; sie entsprach auch der Größe der Zunahme der Körpertemperatur infolge Ultraschallwirkung. Obwohl wässerige Salzlösung ein gutes Zwischenmedium darstellt, war nach ihrer Injektion die Gewebszerstörung und die Zunahme der Körpertemperatur geringer infolge ihrer kühlenden Wirkung.

Summary. The effects of intraperitoneal salt solution (water) and air on the degree of tissue destruction produced by ultrasonic radiation in the rat mesentery were studied. The degree of tissue destruction was greatest in the group of animals having injected air in the peritoneal cavity, and also corresponded to the increase of body temperature caused by the ultrasound. Salt solution (water), although a good transmitting medium, had a cooling effect; therefore with it the destruction of the tissue and the increase of the body temperature were less.

The propagation of ultrasonic energy in tissues depends mainly on two factors, absorption characteristics of the biologic media and reflection of ultrasonic energy at tissue interfaces. Reflection can occur at interfaces between tissues of different acoustic impedance. The absorption of sound energy by various media converts sound into heat (HERFORTH and WINTER, 1958; PAUL, 1958).

Consequently, the quality of the coupling medium is of primary importance as far as the effects of the ultrasound on the tissues are concerned. Because water (salt solutions) and air play a certain role under many therapeutic and experimental conditions, their effects, when used as intraperitoneal coupling mediums on the reaction of the mesentery to ultrasonic radiation are worth studying.

Materials and Methods

The method used in this study is based on the extreme sensitiveness of the mast cells of the mesentery to all kinds of changes in their environmental factors. Because the number of injured mast cells increases in proportion to the dose of ultrasonic energy applied, this method

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is based on the determination of the percentage of intact mast cells in the mesentery of the rat (VALTONEN).

Adult male Sprague-Dawley rats weighing 220—240 g were used as experimental animals. The animals were divided into three groups. Those in the first group were injected intraperitoneally with 8 ml of a buffered salt solution (154 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 4.0 mM Na_2HPO_4, 2.7 mM KH_2PO_4; pH 7.0) to which had been added 1 mg glucose, 1 mg human serum albumin and 10 I.U. heparin (Messrs. Medica, Helsinki) per ml. The animals in the second group were injected intraperitoneally with 8 ml of air. The third group served as controls. Each of the three groups were further divided into two subgroups of six animals. Half the subgroups were treated with ultrasound, the other subgroups served again as controls.

The ultrasonic unit used was the Megason XII (manufactured by The Birther 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. For direct application of the soundhead on the abdomen of the unanaesthetized animals, paraffin oil was used as a coupling medium. The intensity of ultrasonic radiation was 3 watts/cm^2 and the treatment lasted two minutes. During treatment the soundhead was held in one position throughout the duration of the treatment (the stationary technic). The body (rectal) temperature of the animals was registered with an alcohol thermometer immediately after the treatment with ultrasound. The animals were decapitated and exsanguinated 30 minutes after the treatment.

As much as possible of the mesentery of the whole intestine was taken and gently spread on the slides with the intestines hanging over the edges, and air dried. The strands of fat were dissected away, leaving on the slides only the fat-free translucent mesentery windows. Very thin preparations were thus obtained, which could be examined with oil immersion objectives (GUSTAFSSON and CRONBERG, 1957; CRONBERG, 1961; VALTONEN, 1966). The preparations were stained for 1 minute in a 1 per cent physiological saline solution of toluidine blue. In the preparations of each animal 200 consecutive mast cells were studied and the percentage of completely intact cells was calculated. Swollen mast cells, mast cells with ejected granules, and disrupted mast cells were considered to have been injured by the treatment with ultrasound (VALTONEN). Standard statistical methods were used in the analysis of the data. The mean values with standard errors were calculated and Student's *t*-test was used for testing the significance of differences between the means of the treated and control animals.

Results

The results are summarized in the Table. It is to be seen that merely the intraperitoneal injection of liquid or air did not cause significant changes in the

Table. *Percentage of intact mesenterial mast cells and average body temperatures of the experimental animals after treatment with ultrasound*

Intraperitoneal coupling medium	No treatment	Treatment with ultrasound	
	Percentage of intact mast cells Mean ± SE	Percentage of intact mast cells Mean ± SE	Average body temperature °C
Controls	82 ± 4	62 ± 3	37.5
Liquid	84 ± 4	80 ± 5	37.0
Air	85 ± 2	55 ± 4	38.0

"normal" percentage of intact mast cells, which was somewhat over 80 per cent. Treatment with ultrasound diminished the number of intact mast cells in the group of control animals to 62 per cent, which change is statistically significant

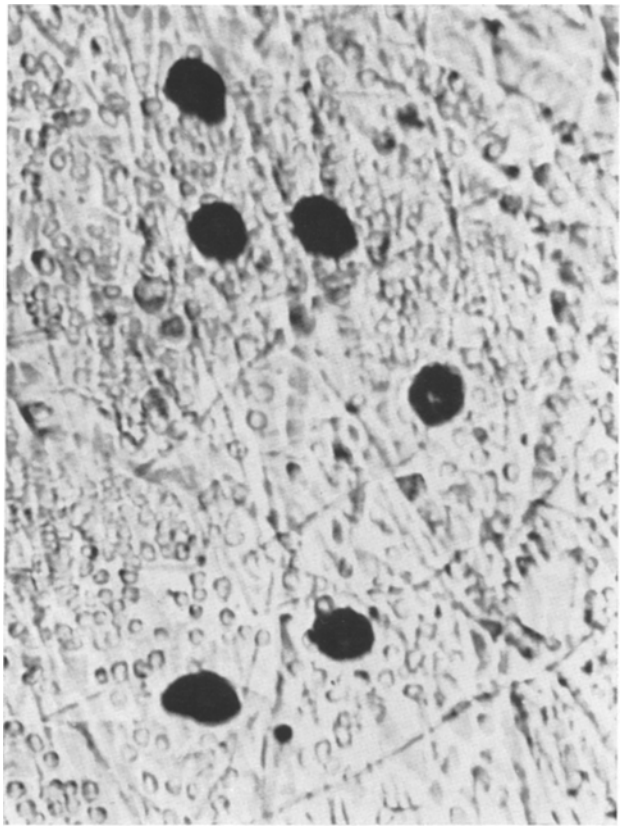

Fig. 1. Six intact mast cells in the mesentery of a control rat. Toluidine blue staining. ×600

($0.01 > P > 0.001$). In the group of animals having air in the peritoneal cavity the number of intact mast cells was still smaller, being 55 per cent ($0.01 > P > 0.001$). On the other hand, mesenteries of the group having liquid in the peritoneal cavity showed no significant changes in the number of intact mast cells after treatment with ultrasound. As far as the changes in the body temperature are concerned, the treatment with ultrasound caused a rise in the average temperature of the control animals up to 37.5°C, while in the group injected intraperitoneally with liquid the mean body temperature rose only to 37.0°C. On the other hand, in the group of animals with air in the peritoneal cavity the rise in the body temperature was greatest, being on the average 38.0°C. The increase of the body temperature caused by treatment with ultrasound consequently corresponds to the degree of injury to the tissue.

Discussion

The results obtained were somewhat unexpected. The primary belief was that the destruction to the tissue would be much greater in the group of animals having liquid in the peritoneal cavity than in the group injected intraperitoneally

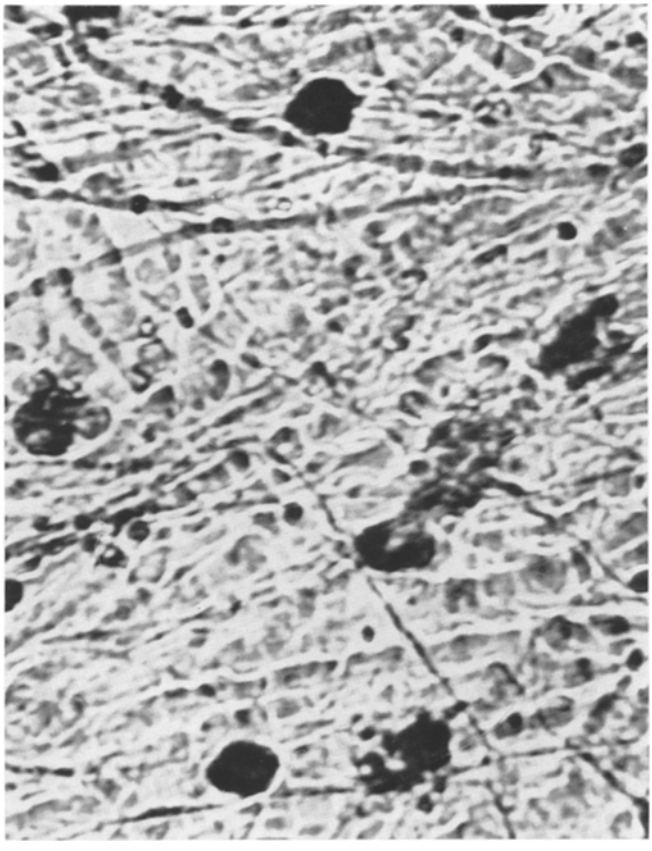

Fig. 2. Rat mesentery 30 minutes after application of ultrasound at the intensity of 3 watts/cm^2 for 2 minutes. Salt solution (water) served as intraperitoneal coupling medium. Two intact and four disrupted mast cells are visible in this field. Toluidine blue staining. ×600

with air, because water is a very good coupling medium while air, on the contrary, prevents the propagation of the ultrasound.

The explanation for this occurrence is, of course, the thermal effect of the ultrasound. There is a rise in tissue temperature which is directly proportional to the power of the input and the time of exposure or application, and inversely proportional to the bulk of the insonated tissue. In animals which have a large amount of liquid in the abdominal cavity the local heat distribution is conditioned by the cooling effect of the liquid (water). In addition, it has been shown that insonation will raise the temperature of water 20—50 times less than that of living tissue (Summer and Patrick, 1964).

The high rise in the body temperature of the animals with air in the peritoneal cavity is caused by the reflexion of ultrasound. Reflexion occurs at boundaries or interfaces, which are described as the surfaces common to two different media. The reflected power is always smaller than the incident power, and the coefficient

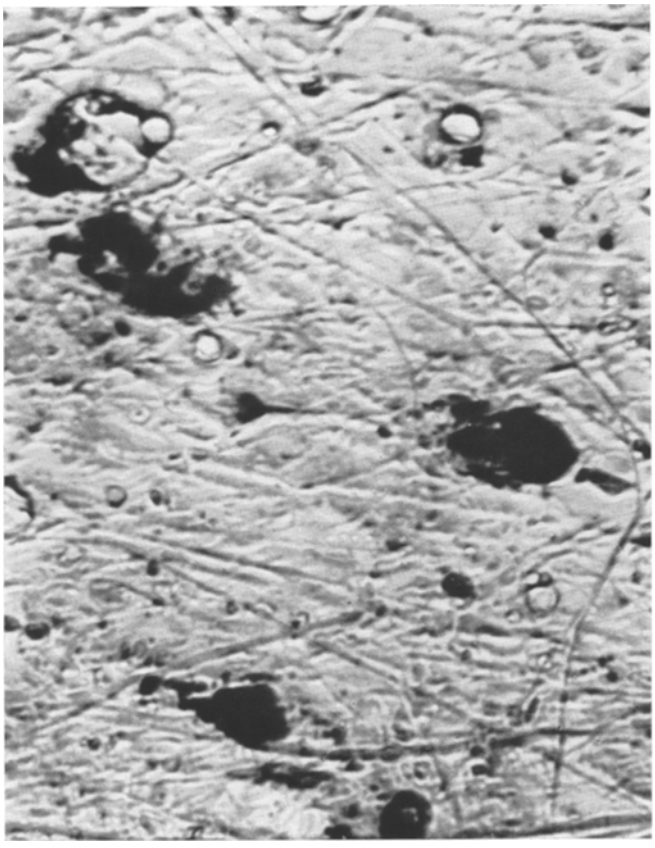

Fig. 3. The effect of intraperitoneally injected air. Rat mesentery 30 minutes after application of ultrasound at the intensity of 3 watts/cm^2 for 2 minutes. One intact and four severely disrupted and swollen mast cells with ejected granules are visible. Toluidine blue staining. ×600

of reflexion is always smaller than 1.0. Because air acts as a nearly perfect reflector (between tissue and air the coefficient of reflexion is about 0.999) the ultrasonic beam emerging from the tissue into the surrounding air is deflected back and absorbed at the point of reflexion causing heat (SUMMER and PATRICK, 1964). Heating of the tissues progresses further at a considerable speed because no cooling effect of the liquid is available to diminish the heat.

Although there is still much research to be undertaken before all the effects of the ultrasound in the peritoneal cavity are fully understood, the results obtained in this study give some advice for clinical practice with ultrasonic therapy. The abdomen should not normally be insonated by a physiotherapist. If ultrasound is used, a medium intensity is required since air is usually present in the viscera. In addition, insonation must never be given to the abdomen of a pregnant woman.

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Dr. ERKKI J. VALTONEN
Department of Physical Medicine
Meilahti Hospital
Helsinki, Finland