

tensinases is inhibited by only 50% [8]. Cold preincubation, however, evidently neutralizes activity of the unbound part of the angiotensinases, although reversibly [5]. In the method of determination of renin in the blood adopted in the USSR [4], only EDTA is also used to inhibit angiotensinase activity, and this is no obstacle to the work. The modifications now described can therefore be regarded as completely satisfactory, especially for comparative studies. Its advantage lies in the several essential simplifications of the techniques of analysis, the most important of which is overcoming the difficulty of obtaining the substrate. During the choice of appropriate conditions, this modification, it seems, may prove to be suitable for the investigation of single glomeruli in biopsy specimens of human kidneys under clinical laboratory conditions.

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METHOD OF SONICATION OF CELL SUSPENSIONS

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UDC 612.014.2.014.45

A simple chamber is suggested for the sonication of small quantities of a cell suspension. It consists of a transparent plastic cylinder, which fits on the top of a UTP-1 ultrasonic generator, the top of which serves as the floor of the chamber. Losses of ultrasonic energy are thus eliminated and it is possible to determine the intensity of the ultrasound acting on the cells of the suspension with fair accuracy.

KEY WORDS: ultrasound; cells; suspension.

Progress in the most rational use of ultrasound in medicine is hampered by the lack of study of its action on cells and tissues [6]. The most accessible form for the study of the direct action of ultrasound on cells is in suspension, for in that case both free-living cells (such as blood cells or microorganisms) and cells forming organs and tissues of animals or plants can be treated in this way.

Attempts to study the action of ultrasound on cell suspensions has been undertaken for a long time [1, 5, 10], but the methods used for these purposes have had many disadvantages. The most important of these was inability to determine with any degree of accuracy the intensity of the ultrasonic energy acting on the cells. The reason for this was usually that the suspension was kept in glass flasks, immersed in an oil fountain formed by the action of powerful ultrasound [4, 7-10]. The presence of intermediate media between the ultra-

Kiev Research Institute of Orthopedics. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 84, No. 11, pp. 635-637, November, 1977. Original article submitted June 6, 1977.

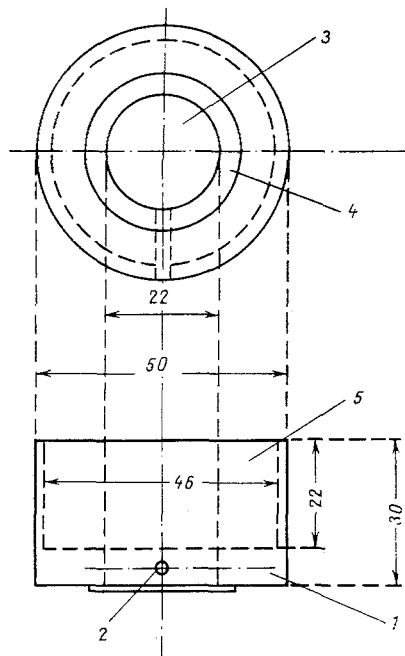


Fig. 1. Diagram of arrangement of chamber for sonication: 1) floor of cylinder; 2) channel for introducing suspension; 3) chamber; 4) polyethylene film; 5) place for head of apparatus with detector.

sonic detector and the suspension made it impossible to determine the intensity of ultrasound reaching the cells accurately because of the considerable losses of energy by reflection, scatter, and the formation of static waves [2, 11], since the acoustic resistances of these media differ and their spatial parameters are not standard.

Subsequent modifications of these methods using thin films of polyvinyl or polyethylene [1, 3, 5] reduced these disadvantages to some extent, but did not enable the intermediate media to be dispensed with.

In order to develop a rational method of sonication of suspensions the writer has made a comparatively simple chamber whereby intermediate media can be eliminated, and more precise information can accordingly be obtained on the intensity of the ultrasound acting directly on the objects in suspension. This chamber can be used for sonication not only of suspensions, but also of solutions or emulsions.

The chamber consists of a hollow cylinder with a transparent plastic space. In the center of the base a round hole is drilled of the same diameter (4.5 cm^2) as the piezo-quartz detector of the UTP-1 ultrasonic generator. The cylinder rests firmly on the head of the apparatus containing the detector. Because of this construction part of the surface of the head (the projection of the quartz plate of the detector) serves as the floor of the chamber. The other side of the hole in the floor of the cylinder is covered with thin polyethylene film, fixed by waterproof glue. The film provides for rapid loss of heat by the suspension undergoing sonication into a water bath, in which the head of the generator is immersed together with the chamber; the path is lined inside with rough rubber to prevent the formation of static waves; a thin channel is drilled in the side of the cylinder for introduction of the suspension into the chamber (about 4 ml) by means of a syringe and needle. The hole is then sealed with a piece of adhesive tape (Fig. 1).

By virtue of this construction, no intermediate solid medium is present between the surface of the head of the detector and the liquid in the chamber. The layer of cells of the suspension directly adjacent to the head is exposed virtually completely to the action of ultrasound of the intensity assigned by the apparatus. Since under the influence of ultrasonic waves the cells of the suspension are in constant movement, they are all in the immediate vicinity of the detector (the floor of the chamber). All these remarks also apply to suspensions of cells prepared from organs and tissues. During sonication of whole tissue, however, the ultrasonic energy falls away exponentially during penetration in depth.

The resistance of the cells to the destructive action of ultrasound in high intensities (from corresponding generators) can be determined from the difference in their concentrations in the suspension before and after sonication. By this method the writer was able to establish changes in the resistance of erythrocytes to ultrasound of varied intensity in the case of diseases of the endocrine organs. This method can thus be used also to determine the resistance of erythrocytes to the mechanical action of high-frequency waves.

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