# Effects of Ultrasonic Irradiation upon *Amoeba proteus*

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*Amoeba proteus* was ultrasonically irradiated, the ultrasonic mean output intensity being about 0.4 W/cm² at 1 MHz. Contractions of amoebae, rotations and aggregation of intracellular particles and cell membrane damages were observed. At even lower ultrasonic intensities sodium pinocytosis was found to increase after application of ultrasound.

## Introduction

The irradiation of living organisms with ultrasound causes effects in several ways (El'piner [1]). High intensities damage the cells markedly, causing irreversible cell changes, rupture of cell walls etc. But even low intensities of ultrasound have been shown to affect cell parameters (see review by Hill [2]). In this paper we will show some qualitative results of ultrasonic irradiation of *Amoeba proteus* when rather low intensities of ultrasound are used.

## Methods

*Amoeba proteus* were cultured on *Tetrahymena*. Cells were starved for three days ("normal" cells) or ten days ("starved" cells) before taken to an experiment. The growth medium was a modified Pringsheim-solution (Chapman-Andresen [3]).

An examination of cellular events during the ultrasonic irradiation was facilitated by the following film technique. The amoeba to be studied was put on a glass plate in contact with the circular ultrasound crystal (Brush Clevite PZT 5). A glass slip covered liquid and amoeba. A hole was drilled through the crystal, allowing a beam of light to pass through and illuminate the amoeba. The whole set up was mounted on a normal microscope desk. The ultrasound frequency used was close to 1 MHz except in experiments on pinocytosis (see below).

Pinocytosis channels in the cell periphery were counted by eye inspection (as described by Josefsson [4]). In experiments on pinocytosis no glass cover slip was used during irradiation.

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The peak voltage applied to the ultrasound crystal did never exceed 20 V. With reasonable assumptions of the impedance of the piezo-electric ceramic crystal, the power output from the crystal into the Pringsheim-solution including the amoeba will be about 1 W (Randeraat [5]). With a crystal area of about 2.8 cm² this makes a mean power output of about 0.4 W/cm². The ultrasound intensity affecting structures in the amoeba can, however, not be calculated, nor is it possible to measure it in the present experimental set up.

Intensities of the order of 0.4 W/cm² are rather low, compared with intensities mostly used in experiments on the biological effects of ultrasound. Hill [2] claims 0.4 W/cm² to cause cavitation at 1 MHz, (the threshold being estimated to about 0.2 W/cm² according to Hill's criteria). The effects observed in the irradiated amoeba might therefore be cavitation effects.

## Observations

### *a. The cytoplasm*

1. The normal movements of the amoeba relative to the glass surface requires an intimate contact with the surface. After the start of the ultrasonic irradiation one often finds a small but very rapid contraction of the amoeba. This can be interpreted, *e. g.*, as a loss of glass contact when the cell surface is put into motion.

2. Rotation of intracellular particles and structures occurs during irradiation also when the intensity is below 0.4 W/cm². Especially clearly visible are the rotations of crystal bodies in the cytoplasm.

This phenomenon has been studied in *Helodea* cells mechanically stimulated by a needle put into contact with the cell wall and then excited (Dyer and

Nyborg [6, 7]. The phenomenon can be explained, according to these authors, by so-called "acoustical streaming". This streaming can, very simplified, be attributed to stresses built up by vibratory motions in the cell medium. Theoretical treatment of acoustical streaming involves taking second order effects into consideration when calculating the motion of fluids (see Dyer and Nyborg [6, 7]).

In our experimental set up the cell membranes were never touched by a vibrating needle. Yet the acoustical streaming can occur, due to inhomogeneities in the membrane (the membrane can, for example, be in contact with subcellular particles at some place). The inhomogeneities cause locally changing vibratory motions of the membrane, the conditions for local acoustical fields are fulfilled and acoustical streaming can arise. The rotatory motion can be enhanced if the ultrasound also affects the visco-elastic properties of the cell (Johnsson and Lindvall [8]).

3. A local aggregation of cellular particles is often observable as darker bands across the cells (arrows 1 in Fig. 1 B *). These bands may indicate regions where the oscillatory motion in the cytoplasm is comparatively slow (in analogy with the Kundt-experiment in which particles can be used to indicate nodes in a standing wave pattern – the nodes being indicated by the larger amount of particles). These regions are sometimes found in the middle of the cells and sometimes close to the membrane – the precise location is likely to be dependent on many variables, *e. g.* the resonance frequency of the crystal and the location of the amoeba in the ultrasonic field.

4. When the low intensity irradiation has proceeded for some minutes it often happens that the cell membrane (arrow 2 in Fig. 1 B) bursts and the cytoplasm poures out (arrow 3 in Fig. 1 B). This stage is preceded by a contraction of the amoeba which results in a spherical shape (*cf.* Fig. 1 B). The spherical contraction is a reversible reaction; if the irradiation stops before the membrane bursts the amoeba will start moving and will eventually regain an irregular shape.

*b. Pinocytosis*

In the experiments on ultrasonic effects upon pinocytosis in *Amoeba proteus* the voltage applied to the crystal was 15 V and the crystal was operated outside the resonance region, *i. e.* at 850 kHz. This means, that the intensity is much lower than 0.4 W/cm$^2$ and therefore it is highly unlikely that cavitation can play a role in these experiments. Rupture of the cells never occurred in the pinocytosis investigation.

The number of pinocytosis channels induced by extracellular sodium ions is markedly affected after the ultrasonic irradiation. The pinocytosis intensity was estimated by counting the number of channels for 20 min immediately after the irradiation. The effect of ultrasonic irradiation, lasting for 15 sec, is demonstrated for amoebae in a concentration chain of NaCl (Fig. 2 A). The number of pinocytosis

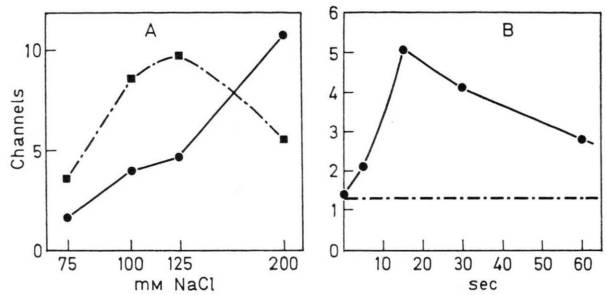


Fig. 2. A. Frequency of pinocytosis in normal cells. The ordinate gives the mean number of channels observed per minute during immersion in sodium chloride at pH 6.0. The abscissa gives the concentration of the inducing solution. Frequency curves are given for non-irradiated (squares, interrupted line) and irradiated (circles, solid line) aliquots of cells taken from one cell-culture. Exposure of ultrasound for 15 sec. B. Pinocytosis induced by 100 mM sodium chloride in cells starved for 10 days. Ordinate as in Fig. A. The abscissa gives the duration of ultrasound irradiation before the assay period. The interrupted line indicates the pinocytosis level of cells not exposed to ultrasound.

channels induced by low concentration of sodium has decreased for the irradiated amoebae (solid line) as compared with the normal non irradiated amoebae (interupted line). This indicates that, after a low dose of ultrasound, membrane properties regulating the sensitivity to sodium ions have been altered.

The ultrasonic action therefore inhibits the sodium induced (75 – 125 mM) pinocytosis in normal amoebae (see Fig. 2 A). There is a striking similarity between cation induced pionocytosis inhibited by calcium ions (Cooper [9], Josefsson [4]) and inhibition after ultrasonic irradiation. In both instances inhibition is maximal at low concentrations of the inducer. Cell *starved* for 8 – 10 days display few pinocytotic channels in 100 mM NaCl. Recent observations

* Fig. 1 see Table on page 608 a.

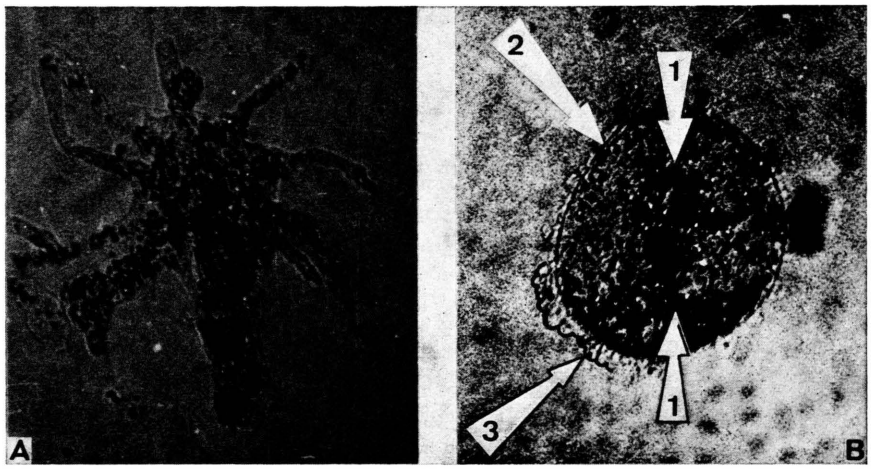


Fig. 1 A. A non-irradiated amoeba photographed one minute after the positioning on a glass plate under the microscope.

Fig. 1 B. The same amoeba as in 1 A but after about 2 min ultrasonic irradiation. Arrows 1 shows a band of high concentration of crystal bodies in the cytoplasm. Arrow 2 shows an intact part of the cell membrane while arrow 3 shows a region where the disrupted membrane allows cytoplasm (indicated) to pour out.

(Josefsson *et al.*[10]) indicate that sodium induced pinocytosis was restored in starved cells when different kinds of physical and chemical stimuli have been applied. It was therefore of interest to examine the effects of ultrasound under these circumstances.

As shown in Fig. 2 B sodium pinocytosis was increased after application of ultrasound. This effect was increasing with irradiation times up to 15 sec but longer irradiation times did not cause an increase in the number of channels.

## Discussion

The observations on morphological characteristics reported above are valid for *Amoeba proteus*, but are likely to be present in other uni- or multicellular organisms. The irradiation by ultrasound has been shown to be a physical tool with which characteristic streaming in the cytoplasm as well as the number of pinocytotic channels in the membrane can be affected. The latter finding indicates that change of membrane function is prevalent for a long time after the irradiation.

The character of a membrane change after physical and chemical stimulation might be an occurrence of calcium binding ligands in the surface of the cell. Since rupture of the cell membrane was excluded in the pinocytosis experiments, a tentative explanation would be that some secretory mechanism is stimulated, increasing the calcium binding properties in the cell surface until it is optimal for starved cells but supraoptimal for normal cells (with respect to pinocytosis induced by 100 mM NaCl). It might therefore be reasonable to investigate whether ultrasound affects calcium binding properties of the membrane and thereby decreases the affinity of sodium ions for membrane ligands.


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