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Investigations on the
Effects of Ultrasonics on Bacteria

With Special Consideration of its
Bactericidal Effect

By
I. HESSELBERG

A.S JOHN GRIEGS BOKTRYKKERI
BERGEN

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FOREWORD

The experimental work on which the present publication is based, was carried out during my service as assistant physician at The Gade Institute, Department of Bacteriology and Serology, University of Bergen.

This study was undertaken on the initiative of the director of the Institute, professor dr. med. Th. M. Vogelsang. I am deeply indebted to him for providing me with excellent working facilities and for following my work with inspiring interest. I also wish to express my gratitude to professor dr. med. P. Oeding for his kind help and guidance during the entire work. Further I want to thank the staff of the Institute for faithful assistance in the bacteriological controls.

The ultrasonic exposures are carried out with an apparatus constructed by cand. real. A. Rekaa, who undertook the physical calculations. I am very thankful for his friendly and close cooperation during the experimental part of this study. I am also grateful to professor dr. techn. B. Trumpy, for providing me with working facilities at the Physical Institute, University of Bergen.

From *Premicobligasjonsfondet* I received a scholarship to visit Institut Pasteur, Paris, for discussion of my results with experts in ultrasonics. I thank the committee for this grant.

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Bergen, September 1958.

Ivar Hesselberg.

INTRODUCTION

Ultrasonics is the term commonly employed to designate sound waves having greater frequencies than 20,000 cycles per second. The term is often defined as sound imperceptible by the human ear.

The latter definition, however, is both arbitrary and vague. Moreover wide individual variations exist and age plays a role inasmuch as younger persons, especially children, are able to discern tones produced by higher frequencies. Thus it was known in ancient times that animals could be affected by sounds which escaped the human ear. Dog whistles making use of ultrasounds are commonplace. More recent investigations have revealed that other animals beside dogs are sensitive to sounds with high frequencies. Thus mice are known to react to sound waves approaching 50,000 cycles/sec. Certain nocturnal animals, such as the bat, emit ultrasonic waves the echo of which serves them as a sort of radar for orientation in the dark. In this connection it is interesting to note that night prowlers in particular react to ultrasound waves ranging from 40,000 to 200,000 cycles/sec. In this manner they should be enabled to «hear» insect-hunting bats inasmuch as these emit sound waves ranging from 30,000 to 70,000 cycles/sec.

Already in the experimental phases of the echo-depth-recorder in 1917 attention was drawn to the killing impact of ultrasound waves on smaller submarine organisms. A series of fundamental experiments were conducted during and after the first world war on the biological effects of ultrasound waves although most research on these phenomena were carried out in the period following the second world war.

Concerning the practical application of ultrasonics, the weightiest results have been obtained in the purely technical fields, such as with the use of the echo-depth-recorder and control of materials. Attempts to sterilize drinking water and food articles have not materialized. In the technical field of agriculture ultrasonics have been employed in attempts to enhance the growth potential of plants and vegetables and also to kill parasites, but hitherto these procedures have failed to yield indisputably positive results.

Marked progress has been recorded in recent years on the therapeutic application of ultrasonics. Quite satisfactory results were initially reported in sundry disorders, such as in particular skin diseases, scar contractures, various types of infections and rheumatic indispositions. Subsequent employment of ultrasonics for this purpose would seem meanwhile to fall short of the results obtained by other available therapeutic procedures. By exposing suspensions of bacteria and viruses to ultrasonics one has occasionally succeeded in producing extracts having immunizing properties. Continued research along this line might possibly yield up practical results.

PART A

REVIEW OF ULTRASONICS AND ITS EFFECTS

I. Definition and Physical Properties

Ultrasonic waves are physically of the same nature as ordinary sound waves, i. e. mechanical, elastic frequencies in solids, liquids or gases (8, 18). The origin of ultrasonic waves is always a vibrating body or surface bordering on an elastic medium. Such vibrations produce compressions and rarefactions, transmissible as waves throughout the medium. Individual particles in the wave will vibrate back and forth along the same lines as the transmission direction. These waves are called longitudinal waves in contrast to the transversal waves wherein the individual particles vibrate vertically on the transmission direction. The transmission velocity is dependent upon the elastic qualities of the medium.

The number of vibrations per second are called frequencies, which for ultrasonic waves exceed 20,000 cycles/sec. The pressure which arise within the compression waves exceeds that of the medium's equilibrium pressure, while stretch-tension occurs during rarefactions. This alternating sound pressure vibrates between several atmospheres overpressure and underpressure, at the same time as the pressure variations shift with enormous speed. At sufficiently large underpressures the liquid particles are torn apart, and thereby forming cavities in the liquid. This phenomenon is called cavitation. When during the subsequent pressure phase the cavities collapse, a transient localized pressure of several thousand atmospheres will be produced within the cavities. These cavities will be filled with the available type of gas (in most instances consisting of air) and eventually attain such magnitude that they can be detected as visible bubbles (cavitation bubbles) in the liquid. The cavitation phenomenon depends on the intensity and frequency of the waves as well as the viscosity of the liquid.

The absorption of an ultrasound wave may depend on the friction within the transmission medium. The greater the viscosity, the greater becomes the absorption. The energy which is dissipated by the wave becomes converted into heat.

The reflection usually takes place on the interface between two media and is conditioned by the difference in the product of sound velocity and density (the acoustic impedance) of the two media. When a wave proceeds vertically against a reflecting surface, the reflected wave will interfere with the invading wave and thus a standing wave is produced. If the wave proceeds obliquely against a reflecting surface, then the reflected wave will obey the same laws for direction as those which are valid for light waves reflected from a mirrored surface.

II. Production of Ultrasonics

Ultrasound waves have been known since 1880 when GALTON & REMAGEN (117 p. 4) produced a tone generator, the so-called Galton-pipe, with frequencies exceeding those discerned by the human ear. The Galton-pipe produced sound vibrations having frequencies up to 40,000 cycles/sec. (40 kilocycles per second: 40 kc/s). HARTMANN (8 p. 5) subsequently constructed a sound generator capable of producing ultrasound in air, which exceeded in frequencies and effect those produced by the Galton-pipe.

In works dealing with ultrasound both in gases, liquids and solids, two other types of ultrasound generators have been used almost exclusively, namely the piezoelectric and the magnetostrictive generators. Both are essentially similar in this respect that mechanical vibrations with high frequencies are produced upon a surface in such a manner that a wave is set up in an adjoining medium.

Piezoelectric generators. When a solid body is subjected to mechanical tension, pressure or stretch, it is said to be piezoelectric (pressureelectric) if the electric charge thus is set free. The charge changes sign when a contraction (compression) proceeds to expansion (dilatation) and vice versa. Piezoelectric materials may even have converse piezoelectric effect, i. e. that the material contracts or expands when exposed to an electric field. Several types of crystals possess this capacity, but the quartz crystal is the one most commonly used. This effect is present in the crystal only along certain axis directions which are called electric axes. A quartz crystal is cut into a circular disc whose end facets are perpendicular to one of the electric axes.

The disc is placed in an alternating electric field with perpendicular direction on the end facets and thus mechanical depth vibrations are produced. Piezoelectric ultrasound generators were put into use improved by the French investigator LANGEVIN and co-workers in 1917 (117 p. 4). They used an electric arc to operate the electric oscillation circuit. A marked improvement took place, however, when electron tubes could be used to produce sustained electric vibrations.

Piezoelectric generators in which the quartz crystal vibrates with its fundamental frequency, can be used up to several million vibrations per second (megacycles per second: Mc/s). A 1 mm thick disc has a fundamental frequency of approximately 3 Mc/s. Discs considerably thinner than 1 mm may become troublesome because they break easily. Higher frequencies can be attained by exciting the crystal with the uneven overvibrations. By means of overvibrations one may generate ultrasound with a frequency of 100 Mc/s with a 1—2 mm thick disc. CARLIN (18) reported in 1949 that he attained a frequency of approximately 500 Mc/s.

Magnetostrictive generators. If one brings a metal rod or a tube of ferromagnetic material into an alternating electric field with lines of force parallel the rod's axis, it gets periodic length alterations as a result of magnetization and demagnetization. This effect is called magnetostriction. The upper frequency limit for magnetostrictive transmitters is of the order 100 kc/s.

III. Effects of Ultrasonics

A. MODE OF ACTION

When an ultrasound wave passes through a medium or passes from one medium to another, quite particular chemophysical changes take place in the media. These changes are partly of an entirely different type than those produced by ordinary audible sound waves. Nevertheless it has been ascertained that audible sound with a sufficiently great intensity gives rise to similar effects as ultrasound waves (118).

≈ In spite of innumerable investigations the problem of the ultrasound's mode of action is still far from clarified, even though a series of experiments would seem to lend support to available theoretical considerations. By and large one may say that the chemophysical effects of ultrasound on biological material may be attributed to three main causes: 1. Heat production. 2 Mechanical factors. 3. Chemical factors.