

ULTRASONIC EFFECT ON STAPHYLOCOCCUS CULTURES

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Ultrasound waves find a wide application in bacteriology, immunology and enzymology. In compliance with the data reported by Elpiner, Ruban and Skard (1, 2, 3), desintegration of gram-positive and gram-negative, aerobic and anaerobic, pathogenic and non-pathogenic bacteria occurs within the field of ultrasound waves. The various bacteria are distinguished by their sensibility to the effect of ultrasound waves. According to Elpiner (1), the filament shaped bacteria appear to be particularly sensible to ultrasound treatment, the rod-like are less sensible and the coccoid — staphylococci and streptococci — are the most resistant.

The goal of the present work is to investigate the morphological and biochemical changes and sensibility to antibiotics of staphylococcus cultures, treated with ultrasound waves with frequency 800 kHz.

Material and Method

Staphylococcus culture — Staphylococcus albus, strain 393 and Staph. aureus, strain 775 — was used for the purpose. A two-million suspension of a 24-hour culture, 10 ml volume, was treated with ultrasound for periods of 15 and 45 minutes with the aid of an ultrasound apparatus with intensity 3 Watt/cm². Four consecutive ultrasonic applications were performed on 6-day intervals. Each subsequent ultrasound treatment was carried out with culture, previously treated with ultrasound. Since the 24-hour suspension was placed for ultrasound treatment into glasses with polyethylene bottoms, sterilized by means of ultra-violet irradiation, the culture purity was preserved, duly tested on blood agar.

Change in the pigmentogenesis was not noted. The specimens prepared after Gram, revealed disruption of the protoplasmic bridges connecting the individual cells after each ultrasound treatment; thus, they failed to present the characteristic grapelike (aciniform) shape. The electron-microscope examination corroborated the latter findings (Fig. 1). Desintegration of single pictures was not established on the electron microscopic image.

Biochemical properties — After each culture exposure to ultrasound, lasting 15 and 45 min, biochemical investigations were carried out, which are illustrated in tables 1 and 2. It is evident from the data presented therein that following repeated ultrasound exposure, some of the proteolytic and saccharolytic properties of the staphylococcus are altered.

1) After four-fold ultrasonic treatment the mannitol-negative strains begin to decompose (split up) the mannitol (+—).

2) After the primary treatment with ultrasound, the Staphylococcus aureus, strain 775, causes the coagulation of milk.

3) Whereas strain 775 loses its capacity to dilute gelatin with water but partially, strain 393 displays a complete loss of this property.

4) The plasmocoagulase reaction is also negative. Initially, both strains are plasmocoagulase-positive. On the second hour after the final ultrasonic treatment, strain 775 shows no plasma changes whatsoever till the 48th hour, whilst strain 393 coagulates it not earlier than the 24th hour.

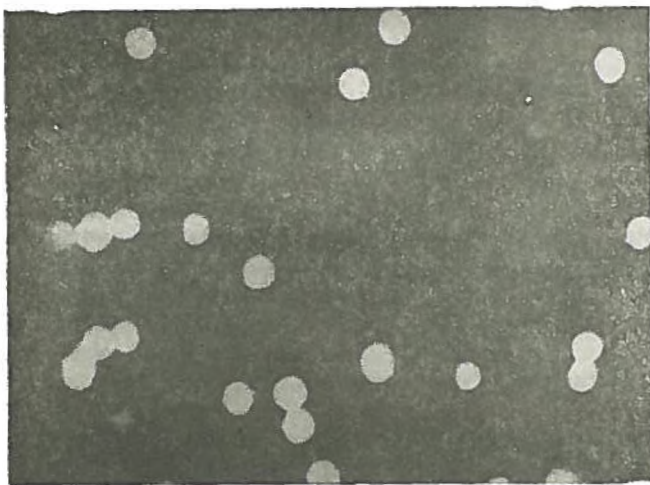


Fig. 1

5) After three-fold ultrasonic treatment, the indole-negative strain 393 is converted into indole-positive, which is evident from tables 1 and 2.

In the course of 10 days, the tests set for proving amylase in the presence of *amylum solubile* were negative.

The results of the antibiograms, made after each ultrasonic treatment with a series of 10 antibiotics, presented in tables 3 and 4, show that in some of the antibiotics the depression zone is increased. Strain 393 intensifies its sensibility to the erythran (initial sensibility with depression zone 30 mm, whilst subsequent to the final ultrasonic treatment — 36 mm), to chlornitromycin (initial depression zone — 26 mm, and after the final one — 34 mm), to biomycin (from 22 mm initial depression zone to 30 mm) and to riverin (from 15 to 32 mm). The originally sensible to tetracycline strain displays a 32 mm depression zone after the last ultrasonic treatment. The sensibility to rovamycin, syntomycin and vulkamycin is likewise augmented.

The changes in the sensibility to antibiotics after ultrasound treatment of strain 775 are illustrated in table four. The initial sensibility to penicillin from 30 mm rises to 42 mm, to erythran — from 30 mm to 38 mm and to biomycin — from 20 mm to 28 mm. Although in lesser degree, an increased sensibility is also established to chlornitromycin, riverin, tetracycline and vulkamycin. It is worth to note that with 45-minute-duration of the ultrasonic treatment, the sensibility to some of the antibiotics is higher as compared to those treated 15 min. The levels reported in tables 3 and 4 are the result of the mean arithmetical values of seven measurements. During

Table 1

	Glucose		Mannitol		Maltose		Saccharose		Lactose		Milk		Nitrates		Indole		H ₂ S		NH ₃		Gelatin		Plasmo-coagulase		
	15	45	15	45	15	45	15	45	15	45	15	45	15	45	15	45	15	45	15	45	15	45	15	45	
Duration of ultrasonic treatment																									
Strain 775 treated with ultrasound	I session	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	+
	II session	+	-	-	+	+	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	+
	III session	+	-	-	+	+	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	+
	IV session	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+
Strain 775 not treated with ultrasound	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+

Table 2

	Glucose		Mannitol		Maltose		Saccharose		Lactose		Milk		Nitrates		Indole		H ₂ S		NH ₃		Gelatin		Plasmo-coagulase		
	15	45	15	45	15	45	15	45	15	45	15	45	15	45	15	45	15	45	15	45	15	45	15	45	
Duration of ultrasonic treatment																									
Strain 393 treated with ultrasound	I session	+	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	+
	II session	+	-	-	+	+	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	+
	III session	+	-	-	+	+	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	+
	IV session	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
Strain 393 not treated with ultrasound	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+

Table 3

Antibiotic	Penicillin	Streptomycin	Erythran	Chloritromycin	Blomycin	Rovamycin	Reverin	Tetracycline	Vulkamycin	Synthomycin
Duration of ultrasonic treatment	15 45	15 45	15 45	15 45	15 45	15 45	15 45	15 45	15 45	15 45
Strain 775 treated	30 30	23 30	30 30	30 30	30 30	25 25	30 30	30 30	30 30	25 25
with ultrasound	30 30	30 30	30 30	30 30	30 30	— 30	24 30	24 30	30 30	30 30
	38 40	28 28	32 34	32 32	24 28	26 24	28 30	28 28	32 32	24 20
	38 42	20 24	32 38	34 36	26 28	18 25	30 33	30 35	34 34	22 25
Strain 775 not treated with ultrasound	30	30	30	30	20	25	30	30	30	25

Table 4

Antibiotic	Penicillin	Streptomycin	Erythran	Chloritromycin	Blomycin	Rovamycin	Reverin	Tetracycline	Vulkamycin	Synthomycin
Duration of ultrasonic treatment	15 45	15 45	15 45	15 45	15 45	15 45	15 45	15 45	15 45	15 45
Strain 393 treated with ultrasound	—	—	26 25	30 25	28 26	25 26	18 18	15 20	29 32	19 18
	— 14	—	28 30	30 30	28 30	24 30	14 24	14 24	30 30	19 26
	—	—	28 34	30 32	24 24	18 26	28 30	26 32	28 32	24 24
	— 14	—	34 36	35 34	30 30	27 27	32 32	32 32	34 35	30 26
Strain 393 not treated with ultrasound	—	—	30	26	22	20	15	30	30	20

their statistical elaboration, the deviation, related to the value of the culture not treated with ultrasonics, does not exceed the permissible error, which is $\approx 6\%$.

Inference

1) The experiments performed are demonstrative of certain changes occurring in the staphylococcus during treatment with ultrasonic waves, with frequency 880 kH, intensity 3 watts/cm² and duration of the treatment 15 and 45 minutes.

2) The duration of the ultrasonic treatment herein referred to accounts for certain biochemical changes; gross derangements of the cellular structure were not established.

REFERENCES

1. Эльпинер, И. Е. Ультразвук. Физико-химическое и биологическое действие
2. Рубан, Е. Л. Микробиология, 1953, 22, 23.
3. Скард, И. Изв. АН Латв. ССР, 1953, 2, 49.

ВОЗДЕЙСТВИЕ УЛЬТРАЗВУКА НА СТАФИЛОКОККОВЫЕ КУЛЬТУРЫ

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РЕЗЮМЕ

При помощи озвучения ультразвуковым аппаратом с интенсивностью 3 ватта/см² в течение 15 минут и 45 минут обрабатывалась 2-миллиардная суспензия в объеме 10 мл от суточной культуры *Staph. albus* № 393 и *Staph. aureus* № 775.

При проведении четырех последовательных сеансов были установлены известные биохимические изменения, однако грубые морфологические изменения установить не удалось. Были установлены также и некоторые изменения в чувствительности культур к ряду антибиотиков.