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EFFECT OF ULTRASOUND ON ADHESIVENESS OF *Escherichia coli*

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Ultrasound has been widely used recently to destroy bacterial cells and to isolate intracellular components and various antigens [5]. However, despite many investigations into the action of ultrasound on microorganisms, the problem of how low-frequency ultrasound, not causing destruction of bacteria, as reflected in the activity of the bacterial cell, has not been adequately discussed in the recent literature. Since low-frequency ultrasound is being used more and more frequently [2, 3] in the treatment of some pathological processes accompanied by bacterial invasion (peritonitis, suppurative wounds), it is important to discover whether such treatment affects the virulence of bacteria.

The object of this investigation was to study one of the factors of virulence of *Escherichia coli* responsible for its adhesiveness during treatment with low-frequency ultrasound of low amplitude, such as is used at the present time to sterilize the peritoneal cavity of patients with suppurative peritonitis in the Department of Clinical Surgery, I. M. Sechenov First Moscow Medical Institute.

EXPERIMENTAL METHOD

The effect of ultrasound on adhesiveness of bacteria was studied with particular reference to *E. coli*, one of the most common pathogens causing suppuration in peritonitis [4].

Strain *E. coli* 815 was obtained from the L. A. Tarasevich State Research Institute for Standardization and Control of Medical Biological Preparations and specially selected for the presence of type I pili. A 24-h culture of bacteria was diluted to a concentration of

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10^7 bacterial cells/ml and treated with ultrasound with the URSK-7P-18N apparatus under the same conditions as those currently used for the treatment of the infected peritoneum during operations: frequency 26.5 kHz, amplitude of oscillations 30 μ, duration of treatment 15 min [3].

The adhesiveness of *E. coli* was determined by the direct hemagglutination test and also by electron microscopy.

Cells for electron-microscopic study were fixed with 2% formalin and applied to Formvar-coated copper grids. The specimens were examined in the HU-7 electron microscope (Hitachi, Japan) after all-round spraying with palladium [9].

The hemagglutination test with guinea pig erythrocytes was set up by the method in [8] at room temperature.

EXPERIMENTAL RESULTS

In the course of an infectious condition caused by several pathogenic bacteria current opinion distinguishes an initial phase during which the agent becomes fixed on the surface of the host's cells. This stage is due to the presence of special structures of the surface membrane of bacteria responsible for adhesion of the bacteria to receptors of the host's susceptible cells. These components have a protein structure and under the electron microscope they appear as tiny hairs or pili [6, 7]. The increased interest in the study of pili can be explained on the grounds that recently they have come to be regarded as one of the factors of bacterial virulence. Adhesiveness caused by the presence of pili is characteristic of strains isolated from patients [6].

In the present investigation the effect of treatment with low-frequency ultrasound of low amplitude on the adhesiveness of *E. coli* due to the presence of type I pili was studied. Much evidence has recently accumulated to show that correlation exists between the ability of bacteria to agglutinate certain types of erythrocytes and the presence of particular types of pili [6, 8]. Type I pili, the commonest form, enable bacteria to adhere to many cells of the body. In particular, this is how *E. coli* cells, shigellas and salmonellas adhere to epithelial cells of the intestinal tract and the urinary tract, and how *Klebsiella pneumoniae* cells adhere to cells of the respiratory tract [6, 10].

Electron-microscopic examination of *E. coli* 815 cells not treated with ultrasound revealed numerous pili about 7-10 nm thick and under 2 μ in length, similar to the description of type I pili (Fig.1a). The presence of type I pili on the bacteria was confirmed by the direct hemagglutination test with guinea pig's erythrocytes (Table 1). Agglutination of the erythrocytes by bacteria was observed in a dilution of 1:128. In the presence of 1% D-mannose agglutination of the erythrocytes was completely absent, and they were accordingly identified as type I pili [7, 10].

Electron-microscopic investigation of bacteria treated with ultrasound showed various changes in cell morphology. Just as after treatment with high-frequency ultrasound [1], some of the microorganisms had become spherical in outline, and part of the contents of some bacterial cells had been expelled. The most characteristic change in "sonicated" bacteria was disintegration of the flagella and pili. Even those bacteria which appeared outwardly unchanged had lost most of these filaments, and fragments of pili and flagella were visible in the preparation (Fig. 1b). Loss of pili was accompanied by a decrease in adhesiveness of the cells: The titer in the hemagglutination test fell to 1:16-1:8 compared with 1:128 in the control (Table 1).

Treatment of the bacteria with low-frequency ultrasound thus led to loss of their pili, and, consequently, to loss of one of the factors of virulence, responsible for adhesiveness. These data can be considered from the standpoint of clinical practice: To sterilize the peritoneal cavity in various forms of peritonitis during operations, low-frequency ultrasound of low amplitude, insufficient to cause destruction of the peritoneal cells, is used. At the same time, treatment with ultrasound of this kind cannot cause total destruction of microorganisms. Nevertheless, according to data in the literature [3], maximal sterilizing effect is observed with a combination of antibiotic therapy and ultrasound treatment, and the effectiveness of such treatment is much greater than that of antibiotics alone. Among the different mechanisms of the sterilizing action of ultrasound, an important role is played by reduction of virulence as a result of reduced adhesiveness of the microorganism. It is evident that with loss of the pili, the ability of the microorganisms to become fixed to sensitive cells

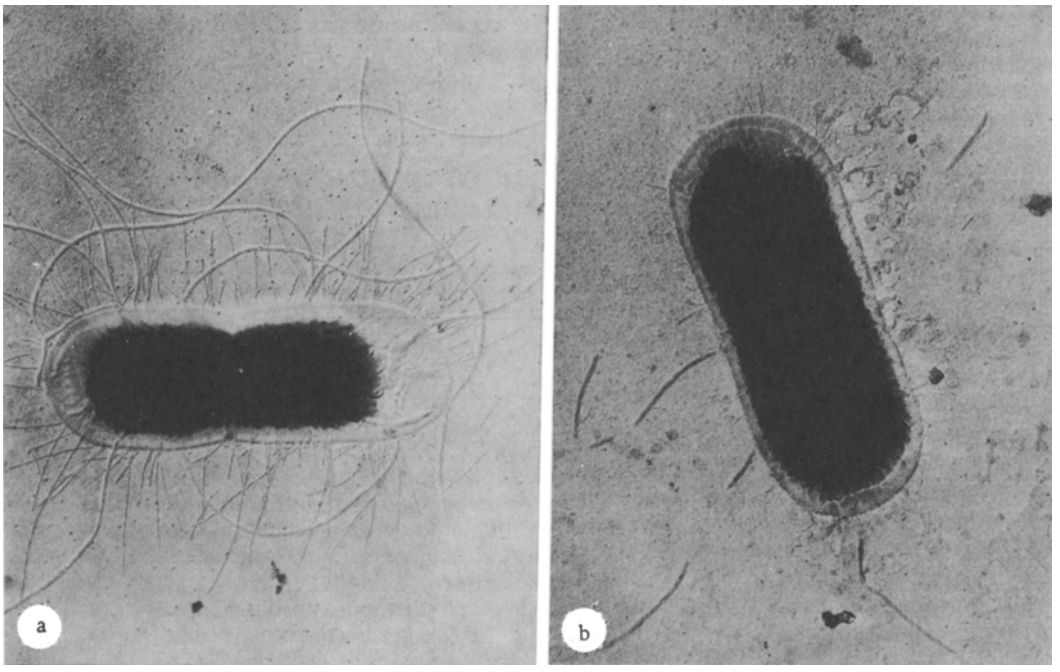

Fig. 1. Effect of ultrasound on morphology of *E. coli* strain 815. a) *E. coli* cell before treatment with ultrasound. Type I pili and flagella can be seen; b) *E. coli* cell after treatment with ultrasound. Fragments of flagella and pili are visible. Arrows indicate partial release of cell contents. Sprayed with platinum and palladium. 30,000 ×.

TABLE 1. Direct Hemagglutination Test of *E. coli* 815 with Guinea Pig Erythrocytes

Dilution of original bacterial suspension	Control cells		Cells treated by ultrasound	
	without addition	with addition of 1% mannose	without addition	with addition of 1% mannose
1:2	++++	—	++++	—
1:4	++++	—	++++	—
1:8	++++	—	+++	—
1:16	++++	—	++	—
1:32	++++	—	—	—
1:64	+++	—	—	—
1:128	++	—	—	—
1:256	—	—	—	—

Legend. The initial suspension of bacteria in all cases was diluted to $OD_{600} = 3.0$ (OD = optical density).

is disturbed and elimination of the pathogenic agent from the host is accelerated. Blocking of receptors of the epithelial cells by fragments of pili [6, 11], broken off as a result of ultrasonic treatment, evidently also plays a definite role.

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