BACTERIAL MORTALITY IN CULTURE AND IN SEPTIC WOUNDS

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By means of electron-microscopic autoradiography it is possible to compare the morphology of bacterial cells with the intensity of various metabolic processes taking place in them. Because of the high resolving power of the method, it is possible to determine not merely statistical, but also "individual" structural and metabolic characteristics of bacteria. Information of this kind cannot be obtained by any other methods of morphology, biochemistry, or bacteriology. Individualization of the structural and functional features of bacteria has helped to discover a phenomenon described below, which is masked on statistical analysis.

EXPERIMENTAL METHOD

Two strains of *Staphylococcus aureus*, isolated from patients with traumatic and burn wounds, were investigated in culture. Test tubes with a suspension of bacteria containing $5 \cdot 10^7$ cells in 1 ml of medium 199 were incubated for 24 h at 37°C. The electron-autoradiographic investigation of DNA and RNA synthesis by bacteria was carried out 2 and 24 h after incubation. For this purpose, ^{3}H-thymidine was added to a sample of bacteria in a dose of 100 μCi/ml, or ^{3}H-uridine was added in a dose of 50 μCi/ml, and the samples were incubated for 1 h. The bacteria were then sedimented, washed with cold isotonic solution, fixed with glutaraldehyde and osmium tetroxide, and embedded in epoxide resins [6]. Material from septic wounds consisted of fragments removed during debridement and diagnostic biopsy on 14 patients with thermal burns of the IIIa-IV degree, affecting 20-55% of the body surface, and in four patients with extensive septic soft tissue wounds. Material from traumatic wounds was investigated 8-18 days after trauma, and from burn wounds 0.5-3 months after burning. Pieces measuring 1 mm^3 were excised from the material and incubated at 37°C for 1.5 h in medium 199 containing 100 μCi/ml of ^{3}H-uridine or 20 μCi/ml of ^{3}H-thymidine. At the end of incubation the material was washed with cold medium 199, fixed with glutaraldehyde and osmium tetroxide, and embedded in Epon. Electron-microscopic autoradiographs were prepared from blocks including cultures of bacteria and wound tissue [3, 4].

EXPERIMENTAL RESULTS

The electron-autoradiographic study of staphylococcal cultures 2 h after the beginning of incubation showed that more than half of the bacterial cells synthesized DNA and that nearly all cells synthesized RNA. Many bacteria in the cultures were diploforms, i.e., two cells completely separated by a septum of the cell wall, but not yet having separated from each other. Both cells of a single diploform were always similar in morphology and in content of radioactivity. The latter was expressed in the fact that either no grains of silver were present above the two cells of the diploform, or the concentration of grains was about the same above each of the two cells. Similarity of the two cells as regards intensity of incorporation of the precursor was observed in preparations incubated both with ^{3}H-thymidine and with ^{3}H-uridine (Fig. 1a).

In cultures incubated for 24 h the nutrient medium was exhausted, metabolic products accumulated, and the pH fell. These changes caused death of most bacteria. The structural disturbances in them were manifested mainly as reduction of the electron density of the cyto-

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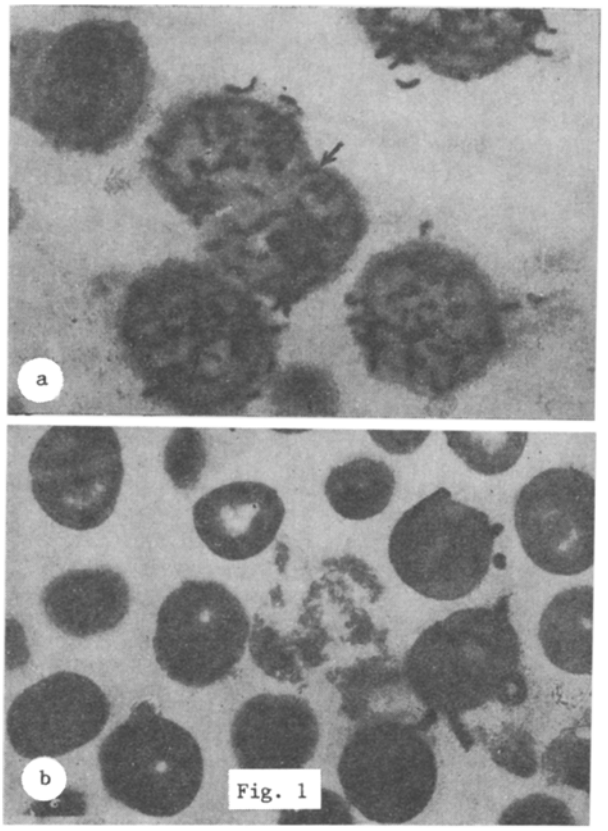

Fig. 1

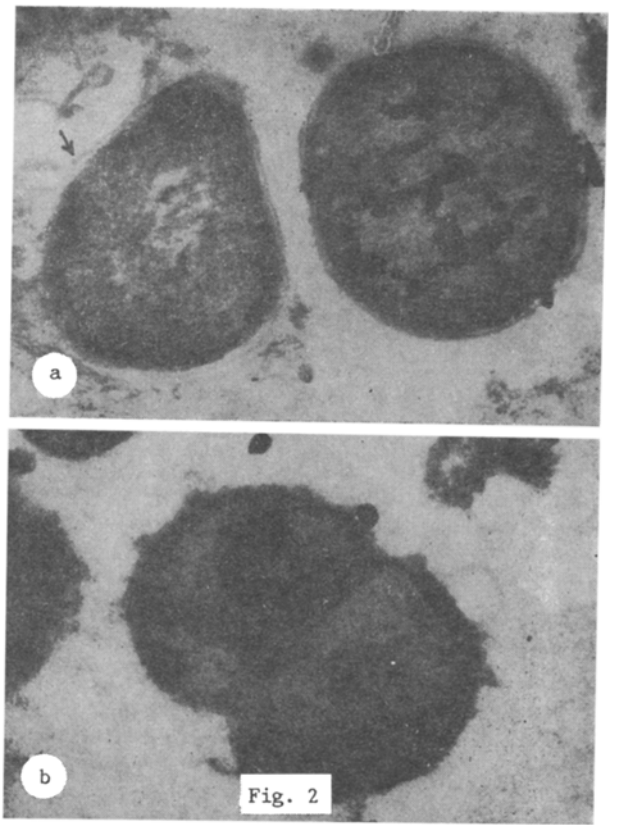

Fig. 2

Fig. 1. Staphylococcal culture labeled with ^{3}H-uridine: a) incubation for 2 h. Two cells of a diploform (arrow) are virtually indistinguishable in the number of grains of silver above them. 25000 ×; b) incubation for 24 h. Among the many dead bacteria two cocci lie close together and are intensively synthesizing RNA: they are perhaps daughter cells of a recently dividing bacterium. 20,000 ×.

Fig. 2. Difference between nucleic acid synthesis in isolated bacterial cells and in cells of diploforms from biopsy material from burn wound: a) incubation with ^{3}H-uridine. Structure and intensity of RNA synthesis are preserved in one of two bacteria lying close together, in the other synthesis cannot be detected and the cell wall is ruptured (arrow). 35,000 ×.

plasm. Cells labeled with ^{3}H-thymidine were very rare, and cells labeled with uridine were rather more frequently seen, although they accounted for not more than 1-2% of the number of dying cells. Just as at the beginning of incubation, in each diploform discovered the two cells were always virtually identical in structure and function. In preparations with exhaustive nutrient medium sometimes there was the unique possibility of monitoring the fate of the living daughter cells even after separation of the diploform into two isolated individuals. We often found two living cells (intensively synthesizing RNA) lying close together and surrounded by a large number of dead bacteria (Fig. 1b). The extremely small total number of living bacteria in these preparations made it unlikely that living cells would lie side by side by chance. Undoubtedly, at least in some cases, two living cells lying close together were recently separated cells of a diploform. It is important to note that in each such pair the difference in the concentration of grains of silver above the cells was not significant, whereas the concentration of grains above some pairs was several times higher than that above others.

In biopsy material from wounds in the region of the demarcation barrier and, in particular, in the zone of necrosis, many bacteria were found. In this paper only nonphagocytosed micro-organisms will be described. Among them were some which were partially destroyed and did not synthesize nucleic acids, and others which preserved their structure and intensive function (Fig. 2a). Many of the diploforms found in the wound, just as in culture, were two individuals similar in structure and in the state of their nucleic acid turnover. However, unlike in culture, in the wounds diploforms consisting of cells similar morphologically,

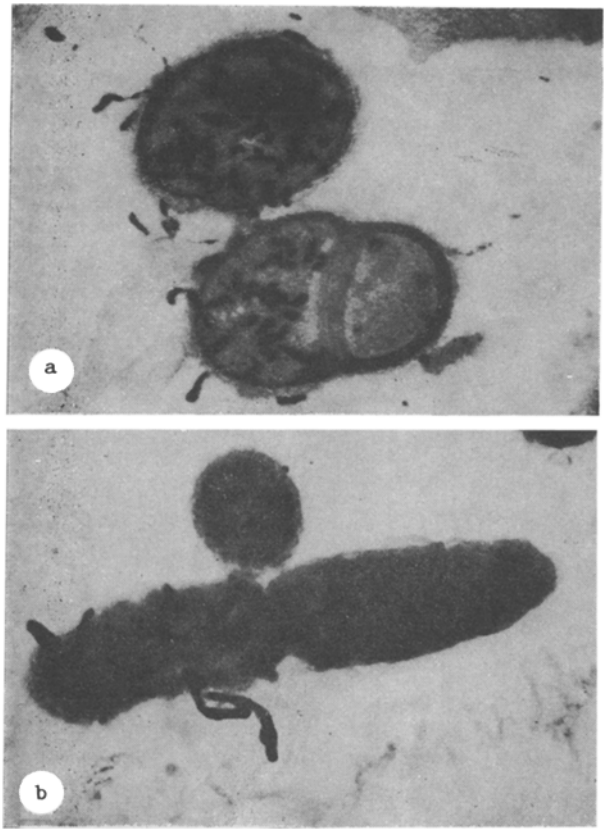

Fig. 3. Nucleic acid synthesis by diploforms of bacteria in wounds: a) tissue from burn wound incubated with ^{3}H-uridine. Marked difference between two cells of diploform in intensity of RNA synthesis. 32,000 ×; b) Tissue from traumatic wound incubated with ^{3}H-thymidine. DNA synthesis found only in one rod-shaped cells of a diploform. 32,000 ×.

but differing sharply with respect to DNA (Fig. 2b) and RNA (Fig. 3a) synthesis, could frequently be found. These discoveries were made among both cocci and rod-shaped bacteria (Fig. 3b).

Division of bacteria has been described as the formation of two completely identical cells with synchronized DNA synthesis, at least until the cells have completely separated and come under different conditions [2, 5]. The results of the present experiments confirm this view and show that this is the state of affairs not only in a fresh culture, but also under the influence of damaging factors. The latter disturb different metabolic processes and cause death of many bacterial cells. However, those infrequent individuals which remain capable of division under these conditions leave behind two offspring identical as regards the features studied. The many bactericidal factors of the body cells and tissues may have the same action on viability and division of bacteria. Meanwhile, the presence of diploforms consisting of cells differing sharply in their nuclei acid turnover in a wound shows that, besides the factors mentioned above, there are also conditions in the body with a direct effect on the bacterial genome: either its duplication, or its distribution among the daughter cells is disturbed, or extensive parts of the genome are blocked. As a result one of the daughter cells, which is morphologically normal, since its cytoplasm was created by a normal maternal cell, either has no genome whatever, or receives a defective, nonfunctioning genome, in which neither replication nor transcription could be seen by the method used. The nonviability of such a cell will be evident.

The phenomenon described above may be the cause of the difference, long known to microbiologists [1], in the number of diploforms observed in the same species of bacteria in vitro and in vivo. Differences in the strength of connection between two living bacteria or one

living and one dead may be responsible for the greater or lesser accumulation of diploforms in culture and in vivo.

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RECEPTORS FOR GROUP A STREPTOCOCCAL POLYSACCHARIDE ON HUMAN THYMUS LYMPHOCYTES. STIMULATION OF THEIR EXPRESSION BY ADENOSINE, THEOPHYLLINE, AND THYMOCYTE SUPERNATANT

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KEY WORDS: receptors for polysaccharide; group A streptococcus; human thymus lymphocytes; adenosine; theophylline; thymocyte supernatant.

In previous investigations the writers demonstrated the presence of antigens characteristic of epithelia of epidermal type in the epithelial tissue of the thymus [2, 10], and also showed that one of the epidermal antigens represented in the thymus has a common determinant with the group A streptococcal polysaccharide [1, 15].

Besides antigens of the skin epithelium, the thymus also contains factors characteristic of cells of the secretory epithelium. For instance, it contains numerous cells producing lactoferrin [3], and the membrane system of the gland contains a secretory component [4]. Receptors for lactoferrin and the secretory component are found on thymocytes, and their expression is stimulated by adenosine, theophylline, and by thymocyte supernatant, but levamisole has no action on it [5, 6].

The aim of this investigation was to study the ability of thymocytes to bind group A streptococcal polysaccharide (A-PSC), which has a crossed determinant with a heterophil antigen of the thymus characteristic of the basal layers of skin epithelia; to study the effect of the above-mentioned preparations and of thymocyte supernatant on binding of A-PSC by thymocytes.

EXPERIMENTAL METHOD

Immunofluorescence experiments were carried out on thymus lymphocytes from children undergoing surgery at the age of 7-14 years for congenital heart defects (13 cases). The thymocytes were washed twice in Eagle's medium with the addition of 10% inactivated bovine serum, a suspension containing 10^7 cells/ml was prepared, poured into test tubes with an excess of medium, and allowed to stand overnight at 4°C. Next day the cells were washed and the percentage of viable lymphocytes was determined with the aid of trypan blue. This parameter varied in different individuals from 98 to 90%; its fluctuations did not affect the ultimate result. To detect lymphocytes binding A-PSC the cells were incubated consecutively for 1 h at 37°C in 0.1

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