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EFFECT OF GENTAMICIN ON NEUTROPHIL–STAPHYLOCOCCUS

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The autoradiographic investigation of neutrophil–microorganism interaction [2, 3] has enabled the intra- and extracellular bactericidal ability of neutrophils and its change under the influence of humoral factors to be demonstrated and compared [6] under normal conditions and in burns. There is no doubt about the fact that the principal method of treatment of infection at the present time, namely with antibiotics, modifies the natural course of interaction of neutrophils with bacteria. The method suggested has enabled these changes to be analyzed by comparing the degree of viability of bacteria existing in various topographic relationships with neutrophils with the ultrastructural changes taking place in the bacteria themselves and in the neutrophils.

This communication gives the results of experiments with gentamicin, an antibiotic which does not penetrate [8] into neutrophils.

EXPERIMENTAL METHOD

Blood samples were obtained when blood was taken from donors at a blood transfusion station (14 samples) and also in the course of bacteriologic and other diagnostic analyses on patients in the burns department (10 samples). The blood was collected in tubes containing 1 ml of 3% neutralized EDTA solution, to prevent clotting, and 1 ml of 10% gelatin solution, to accelerate erythrocyte sedimentation, to 10 ml of blood. After sedimentation of the erythrocytes for 30 min in an incubator, the layer of plasma with leukocytes was withdrawn, and the leukocytes were washed twice with medium 199 and added to a suspension of bacteria in the same medium. The bacteria consisted of a clinically isolated strain of *Staphylococcus aureus*, sensitive to gentamicin. During work with this strain the maximal allowable concentration (MAC) of gentamicin was determined 5 times, and with a concentration of bacteria of 10^7 cells/ml it was found to vary from 0.156 to 1.56 μg/ml. A culture seeded 24 h before the experiment on agar and washed off with 0.45% sodium chloride solution was used. After sedimentation in hypotonic solution the bacteria were resuspended in medium 199 and the concentration adjusted to the required value. A mixture of leukocytes and bacteria in the ratio of 1/10 was incubated in the presence of autologous serum (1/10 of the volume of the mixture) or without serum at 37°C, the tubes being inverted every 5 min. Before the beginning of incubation gentamicin (2 μg/ml) was added to the medium. After 60 min of incubation ^{3}H-uridine was added in a dose of 5 μCi/ml and incubation continued for a further 5 min. The incubation mixture was then

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TABLE 1. Effect of Gentamicin on Viability of Bacteria in Incubation Medium, on Their Ingestion by Neutrophils, and on Intra- and Extracellular Bactericidal Activity of Neutrophils

Group of subjects	Number of nonviable bacteria after incubation, %				PI, %				Number of nonviable bacteria							
									in phagosome (intracellular bactericidal activity)				in intercellular space (extracellular bactericidal activity)			
	medium 199		medium 199+ 10% serum		without serum		with serum		without serum		with serum		without serum		with serum	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Control	9±2	11±3	5±0,9	21±4,8	12±4,2	9±2,9	29±7,3	22±5,3	25±5,7	53±11,4	28±6,4	57±11,9	18±4,9	35±6,7	19±5,2	31±6,4
p		>0,05		<0,01		>0,05		>0,05		<0,01		<0,01		<0,01		<0,05
Patients with burns	10±2,2	14±3,8	5±1,1	24±5	9±2	9±2,2	64±13	69±13	2,0±0,4	8±1,7	85±10	89±10	3±10,6	51±8	33±6,7	50±9
p		>0,05		<0,01		>0,05		>0,05		<0,01		>0,05		<0,01		<0,05

Legend. a) Without gentamicin, b) with gentamicin.

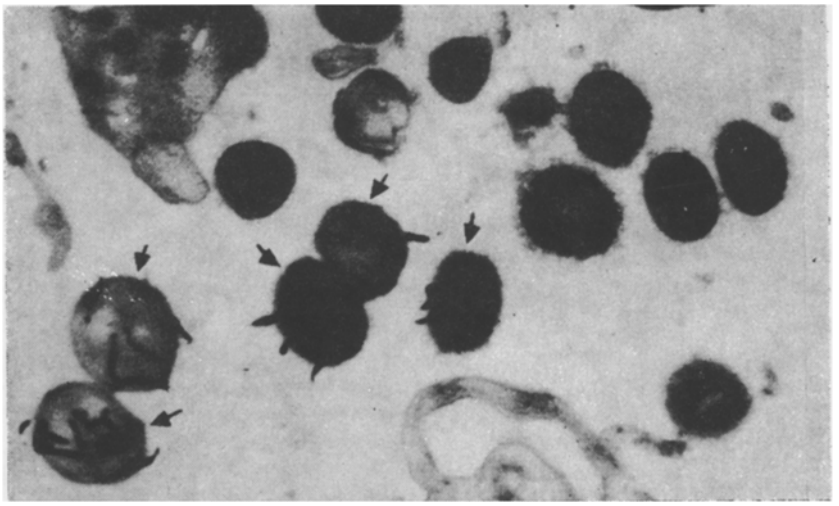

Fig. 1. State of extracellular bacteria on incubation with neutrophils in medium not containing gentamicin. 15,000 ×. Many cocci preserve their ability to synthesize RNA (arrows).

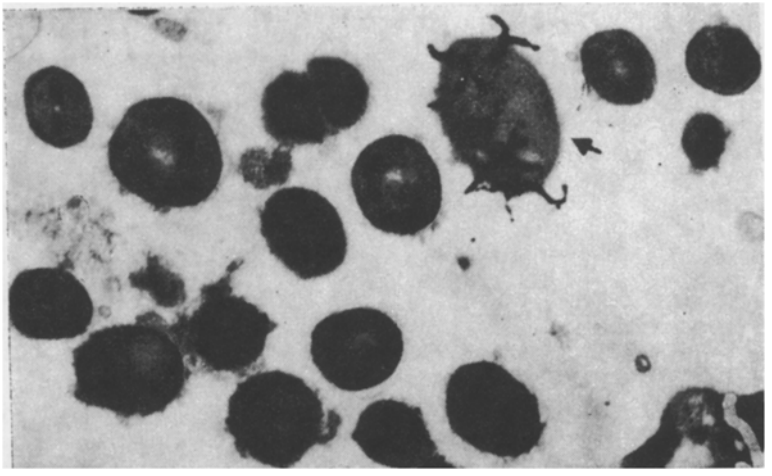

Fig. 2. State of extracellular bacteria on incubation in medium containing gentamicin. 15,000 ×. Only one of 17 bacteria visible in the photograph remained able to synthesize RNA (arrow).

cooled by immersing the tubes in running water. The cooled incubation mixture was centrifuged at 3000 rpm for 20 min. These conditions led to sedimentation both of neutrophils (containing and not containing bacteria) and of free bacteria. The bacteria also were incubated without neutrophils in medium 199 containing 2 μg/ml of gentamicin, with or without serum. The residues were fixed with 1% glutaraldehyde solution in cacodylate buffer, washed twice with Ringer's solution, followed by resuspension and sedimentation under the above conditions. The residue was mounted initially in gelatin, and later embedded in epoxide resins by the method described previously [1]. Autoradiographs of semithin and ultrathin sections were prepared [4, 5]. The semithin sections were exposed in darkness for 4 days and the ultrathin sections for 30-40 days. Both were developed with paraphenylenediamine.

The effect of gentamicin was determined on the following parameters of the state of the bacteria and their interaction with neutrophils: 1) the number of nonviable bacteria after incubation in medium 199; 2) the number of nonviable bacteria after incubation in medium 199 with 10% donor's or patient's serum; 3) the phagocytic index (PI), namely the percentage of neutrophils with bacteria in phagosomes after incubation in the presence or absence of serum; 4) the number of nonviable bacteria in phagosomes of 60 active phagocytes (intracellular bactericidal activity) after incubation in the presence or absence of serum; 5) the number of

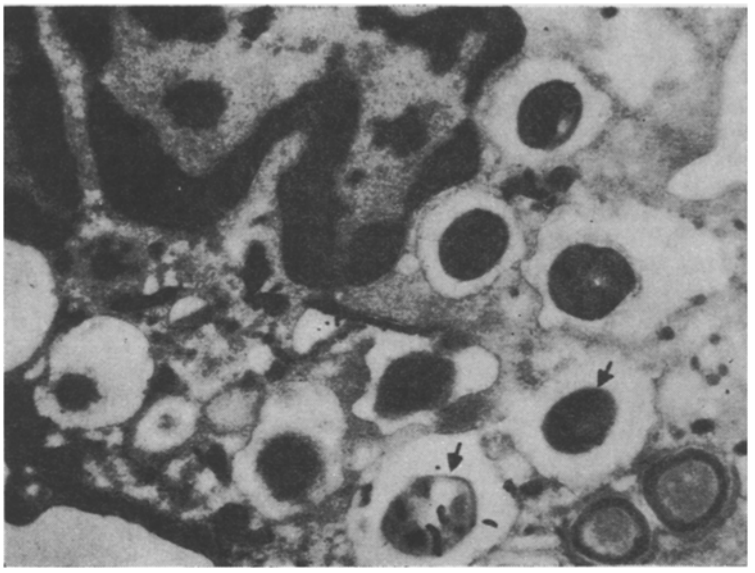

Fig. 3. State of staphylococci ingested in the presence of serum by neutrophil from a burned patient. 12,000 ×. Weak labeling found in only two bacteria (arrows), the remainder (seven) are unlabeled.

nonviable bacteria among 1000 micro-organisms located in the intercellular space (extracellular bactericidal activity) after incubation in the presence or absence of serum. All numerical data are compared with values obtained in corresponding controls, differing only in the fact that the incubation mixture did not contain gentamycin.

EXPERIMENTAL RESULTS

The quantitative experimental results are given in Table 1. They show that gentamicin had no significant inhibitory action on the staphylococcal culture in medium 199. The reason for this result, it may be supposed, was that the duration of incubation of the culture with gentamicin was too short. Like all antibiotics, gentamicin acts more strongly, other conditions being the same, the higher the rate of multiplication of the micro-organisms. This rule was clearly exhibited for cultures incubated with donors' and patients' sera, which, if present in the nutrient medium, intensified multiplication of the staphylococci. Gentamicin had a significant inhibitory action on cultures incubated in the presence of serum. We found no significant effect of gentamicin on the ingestive capacity of the neutrophils, in agreement with data in the literature [7]. In samples with donors' blood, gentamicin reduced PI a little both in the absence and in the presence of serum, whereas in tests with patients' blood it either did not change the value of PI or increased it very slightly; however, none of these changes were statistically significant.

Gentamicin significantly increased the bactericidal activity of neutrophils from healthy blood donors in all versions tested (with and without serum, intra- and extracellular). The distinct effect of gentamicin, which does not penetrate into cells, on intracellular killing of staphylococci may be attributable either to inclusion of the antibiotic during phagosome formation or to the more rapid death of bacteria inhibited by gentamicin before ingestion.

Significant enhancement of the antibacterial action of the neutrophils by gentamicin was obtained in all except one version of the tests conducted with leukocytes from burned patients (Figs. 1 and 2). The exception was intracellular killing in the presence of serum, which was enhanced but not significantly by gentamicin. Most probably in this case the mechanism of the weak action of the antibiotic on bacteria, whose viability had been depressed by some other factor (natural bactericidal activity of the neutrophil—immune serum complex), already mentioned above, may have been triggered. However, a conclusion of practical importance emerged from this result: the antibiotic is highly effective only when activity of the natural defensive mechanisms is inadequate, but if their activity is high (Fig. 3) it does not give rise to any significant enhancement of antimicrobial protection.

Gentamicin thus has a marked inhibitory action on a strain of *Staph. aureus* sensitive to it, growing in medium with serum. In medium without serum, the inhibitory action of gentamicin is reduced. In medium without serum, the inhibitory action of gentamicin is reduced. The antibiotic does not significantly change the ingestive capacity of neutrophils from healthy blood donors or patients with burns but enhances natural bactericidal (killing) activity of the neutrophils. If the level of the natural bactericidal action of the neutrophils is high, the inhibitory action of gentamicin is manifested only weakly.

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ULTRASTRUCTURAL ANALYSIS OF NEUTROPHIL–MACROPHAGE INTERACTION IN AN INFLAMMATORY FOCUS

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KEY WORDS: leukocyte, macrophage, phagocytosis

Interaction of leukocytes with macrophages (Mph) in an inflammatory process in the course of phagocytosis has not been adequately studied. The generally accepted view that the function of polymorphonuclear leukocytes (polymorphs) is concerned entirely with the control of infection [2] has recently been disputed. Recent investigations [3-5] have shown that many polymorphs, especially in a burn wound, contain wound debris in their phagosomes much more often than bacteria. Meanwhile, phagocytosis of some species of bacteria by Mph has been quite widely reported in the literature [8, 10, 13]. More complex interactions between polymorphs and Mph during phagocytosis evidently arise than was hitherto supposed. After contact with microorganisms and toxins polymorphs quickly perish, but Mph are more resistant. Their role is more varied, for it embraces demarcation of the inflammatory focus, neutralization of toxic breakdown products, and regulation of various cellular systems [2].

The aim of the present investigation was to analyze polymorph–Mph interrelations in an inflammatory focus and the particular features of the morphological picture of phagocytosis in these cells and to examine the process of phagocytosis from the dynamic point of view.

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