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Silver Sulfadiazine: Effect on the Ultrastructure of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa exposed to silver sulfadiazine (AgSu) were examined in an electron microscope. The treated cells were distorted in shape, and structures (blebs) protruded from the cell surface. These "blebs" appeared to arise from the cell wall. A strain of *P. aeruginosa* resistant to AgSu did not display these changes. Upon exposure of *P. aeruginosa* to silver nitrate, none of these changes was seen; rather, such cells are characterized by large, central aggregations of nuclear material. The results are consistent with previous findings which suggested that AgSu acted at the cell surface.

Although complexes between silver sulfadiazine (AgSu) and deoxyribonucleic acid (DNA) can be formed in vitro (12), there is no evidence that such complexes result in bacteria treated with AgSu. Rather, the biochemical and metabolic evidence suggests (10) that the drug acts at the external cell structures. The present report deals with an electron microscopy study of the effects of AgSu on *Pseudomonas aeruginosa*. The effects of silver nitrate were also examined. The results appear to confirm the conclusions based on metabolic findings.

MATERIALS AND METHODS

Bacterial strains. The *P. aeruginosa* strain (no. 686) used in this study, was described previously (10) *P. aeruginosa* R-1 is a silver sulfadiazine-resistant strain isolated in a burn ward in which AgSu was used.

Experimental procedure. Bacteria in medium HA (11) were brought to the early exponential phase of growth, at which time they were supplemented either with AgSu (2.8×10^{-5} M) or $AgNO_3$ (3.7×10^{-5} M). (The actual final concentration of $AgNO_3$ was probably less because of the formation of insoluble AgCl. AgSu, on the other hand, does not ionize (12), and therefore no insoluble AgCl is formed). At the end of 1 h of incubation, cells were prepared for electron microscopy. At the beginning as well as at the end of each experiment viable cells were enumerated.

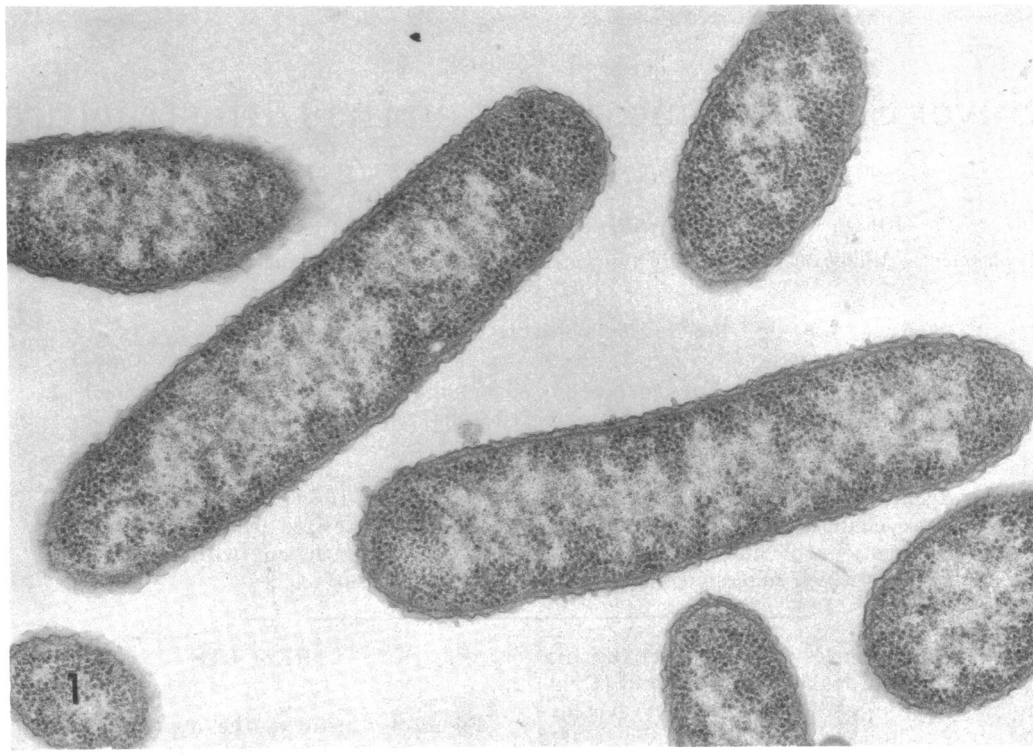
Electron microscopy. Bacteria were fixed as previously described (17) in 1.5% glutaraldehyde for 2 h, washed thoroughly, and postfixed overnight in 1% osmium tetroxide. After fixation, specimens were dehydrated in ethanol and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips 200 electron microscope.

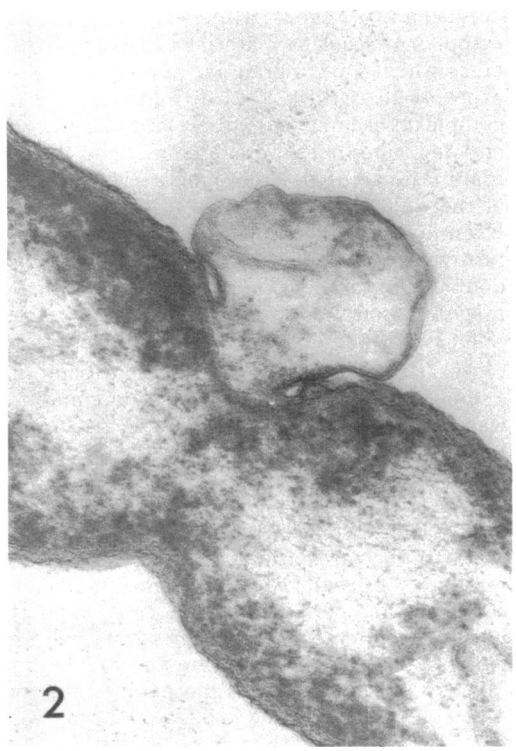
RESULTS

Figure 1 illustrates the appearance of untreated *P. aeruginosa*. Ribosomes are well-defined and are evenly dispersed throughout the cytoplasm. Nuclear material is seen as lighter areas which have a granular appearance. After exposure to AgSu (2.8×10^{-5} M) for 1 h, the cytoplasm became more finely granular (Fig. 2), and few ribosomes were present. The nuclear material increased, occupying a larger volume of the cell, and it contained fibrillar material. Examination of the cell surface revealed large protruding structures, or "blebs," limited by a double membrane (Fig. 2 and 3). At higher magnification, it often appeared that these "blebs" arose from the cell wall (Fig. 3). In most cases, "blebs" contained material resembling cytoplasm, but frequently nuclear-like material was observed. After treatment with AgSu most bacteria were morphologically distorted, and occasional elongated cells were noted (Fig. 4).

Treatment with $AgNO_3$ (3.7×10^{-5} M) caused the formation of large, central aggregates of nuclear material (Fig. 5) composed of numerous fibers. The cytoplasmic matrix resembled that of AgSu-treated cells, with the normal complement of ribosomes greatly diminished. The cell surface appeared normal with no "blebs" present. Cellular morphology was not noticeably altered, and elongated forms were not observed after exposure to $AgNO_3$.

Previous studies had shown that certain strain of *P. aeruginosa* were partially resistant to the action of AgSu (unpublished data).





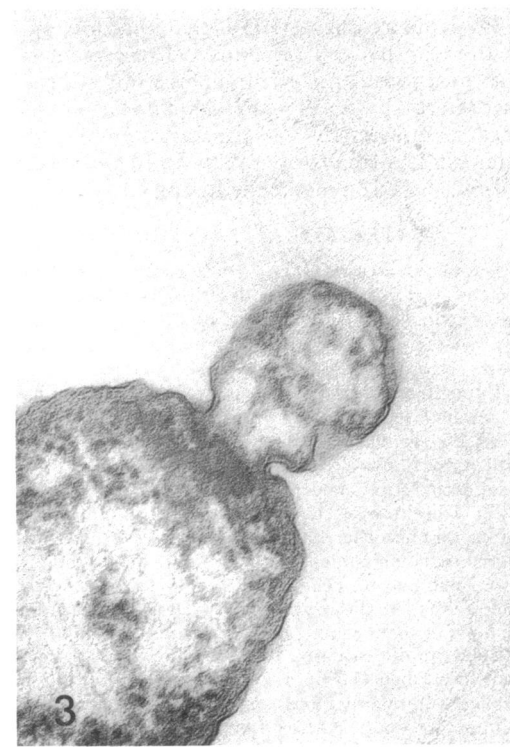
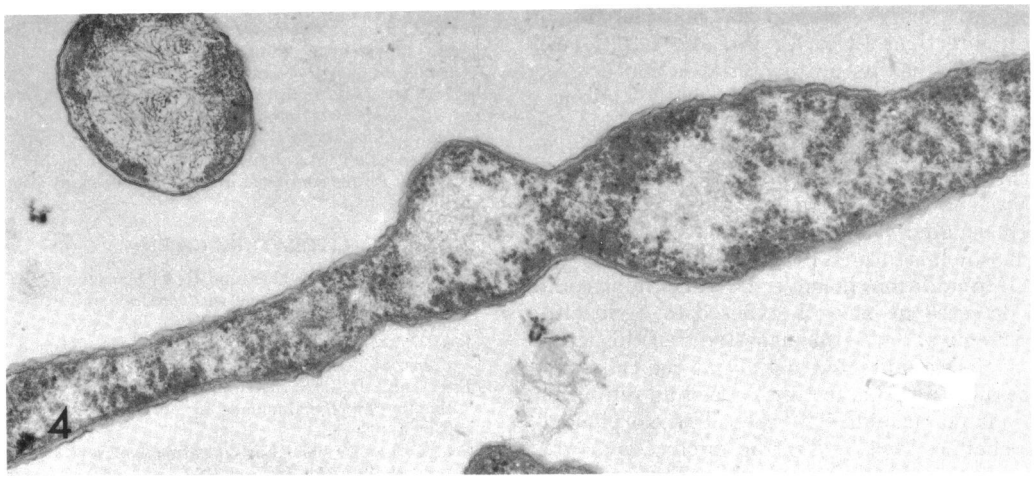
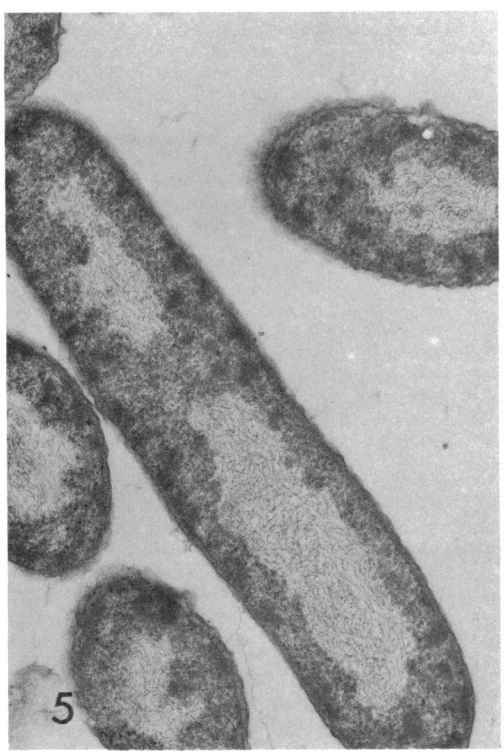


FIG. 1. *Untreated P. aeruginosa.* ×*40,000.*

FIG. 2. *P. aeruginosa 686 after exposure to AgSu* (2.8×10^{-5} *M*) *for 1 h. The cytoplasm is almost completely devoid of ribosomes. Survival rate was 0.027%.* ×*100,000.*

FIG. 3. *"Bleb" arising from the cell surface of P. aeruginosa treated with AgSu for 1 h. Fibrillar components within lighter-staining nuclear areas are clearly defined.* ×*100,000.*





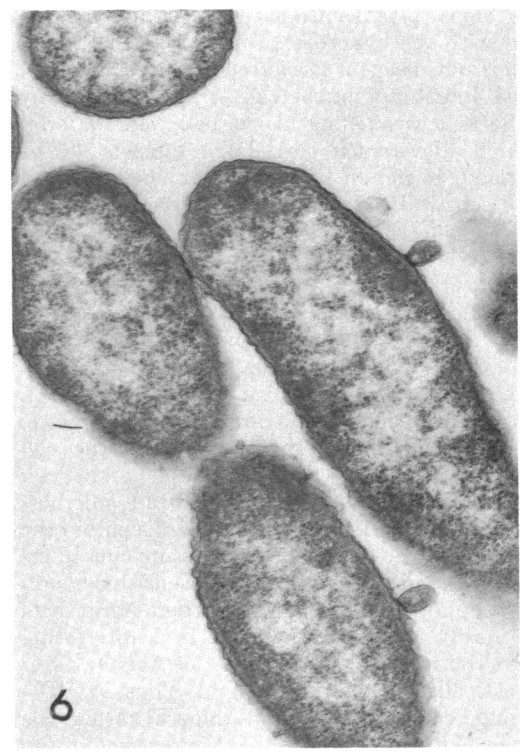


FIG. 4. *A typical elongated form of P. aeruginosa after 1 h of exposure to AgSu.* ×*40,000.*

FIG. 5. *P. aeruginosa treated with* $AgNO_3$ (3.7×10^{-5} *M*) *for 1 h. Survival rate was 0.038%. Central aggregate of nuclear material is fibrillar in appearance.* ×*40,000.*

FIG. 6. *P. aeruginosa R-1, a strain resistant to AgSu, after exposure to AgSu* (2.8×10^{-5} *M*) *for 1 h. Survival rate was 73%. A few small "blebs" are seen at cell surface.* ×*40,000.*

Figure 6 illustrates the effect of AgSu on one such strain (R-1). The amount of nuclear material increased slightly after treatment, but the cytoplasm remained unchanged with ribosomes normal in appearance and distribution. Occasional "blebs" were observed at the cell surface (Fig. 6), but these apparently were fewer in number and much smaller than similar structures seen in sensitive cells after treatment with AgSu.

DISCUSSION

AgSu is very useful in the management of burn infections (2–7, 13, 15), and it has been suggested that its antibacterial action derived from its ability to interfere with hydrogen bonding of the DNA (5). This conclusion was based upon the finding that in vitro silver nitrate reacted with purified DNA (1, 8, 14, 16). It was shown that although AgSu does interact with isolated DNA, the complex differs from that which is formed between $AgNO_3$ and DNA (12). In addition, no silver-DNA complex could be detected in bacteria exposed to AgSu (10). Biochemical data (10) suggested that the action of AgSu was directed mainly at the cell envelope and that this in turn caused an inhibition of macromolecular syntheses. The morphological changes seen by electron microscopy correlate with the biochemical events. The formation of "blebs" after treatment with AgSu is consistent with the hypothesis that the antimicrobial effect of this agent is exerted at the cell surface. The "blebbing" of the cell surface presumably reflects a weakening of the cell wall material which allows such partial "protoplasts" to be formed. It should be noted that "blebbing" of the cell wall has also been observed in *Escherichia coli* whose cell wall was weakened by lysine deprivation (9). Current studies on the composition of the cell wall of AgSu-treated bacteria will probably clarify this point. The secondary changes involving a diminution in the number of ribosomes presumably reflect the ribonucleic acid degradation that has been observed previously (10) and which is thought to result from the effects of AgSu on the cell membrane.

The finding that an AgSu-resistant microorganism exposed to AgSu does not exhibit the changes in the external cell structure characteristic of AgSu-treated and AgSu-sensitive bacteria supports the conclusion that AgSu acts primarily at the cell surface. It is interesting that the morphological effects of $AgNO_3$ differ drastically from those of AgSu. Thus, $AgNO_3$-treated cells do not exhibit "blebs"; rather, the main feature of such cells is aggregation of the nuclear material into filaments. This would suggest that $AgNO_3$ does indeed alter the cellular DNA. It remains to be elucidated whether this in vivo effect is similar to that observed in vitro (1, 8, 14, 16).

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