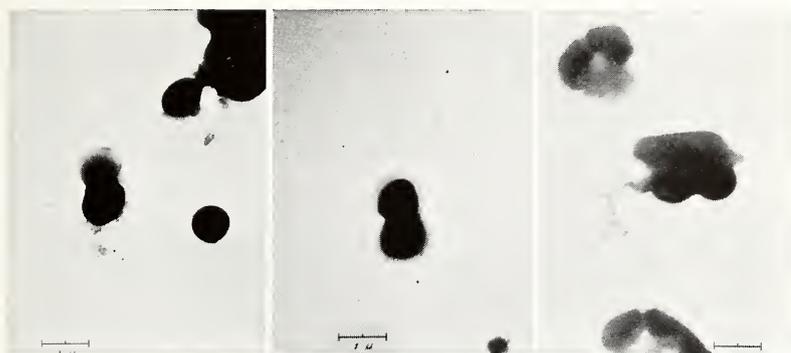


## Electron Micrographs of Bacteria Medicated with Penicillin

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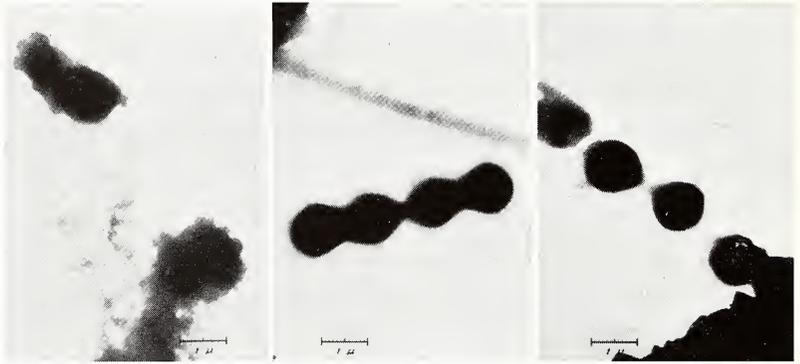
**Introduction.** A voluminous literature attests the interest of bacteriologists in all kinds of substances having antiseptic action. These substances might, for convenience, be classified as "chemical" and "biological." The chemical antiseptics include the well-known commercial antiseptics, including, in the last decade, the sulfonamides. The relatively enormous literature on this subject need not be considered here. The biological antiseptics, on the other hand, include bacteriophage; gramicidin and its allies; and penicillin and similar substances. Mechanism of action and visible effects on vulnerable bacteria have been dealt with in the case of bacteriophage (1) and gramicidin (2) but not thus far with penicillin.



This report concerns the appearance of bacteria, as revealed by micrographs taken with the electron microscope, with and without medication with penicillin. The penicillin used in this study was that referred to by Powell and Jamieson (3, 4). The cultures used comprised *Staphylococcus aureus* No. 209, obtained from the Food and Drug Administration, (5) *Streptococcus hemolyticus* C-203, commonly used in this country for experimental chemotherapy, and *Clostridium welchii*, obtained from the Antitoxin Laboratories of this company.

**Experimental.** All tests of action of penicillin were of the usual bacteriostatic type, including inoculation of one standard loop of live culture into culture media containing varying dilutions of penicillin and incubated at 37° C. for 8 to 48 hours. All cultures were grown in appropriate broth media, centrifuged, the supernatant fluid removed, and the bacteria resuspended in saline solution. A drop of this suspension was then placed on the small screen disc holding the usual collodion film. On drying, the disc was placed in the specimen holder and introduced into the evacuated chamber of the electron microscope.

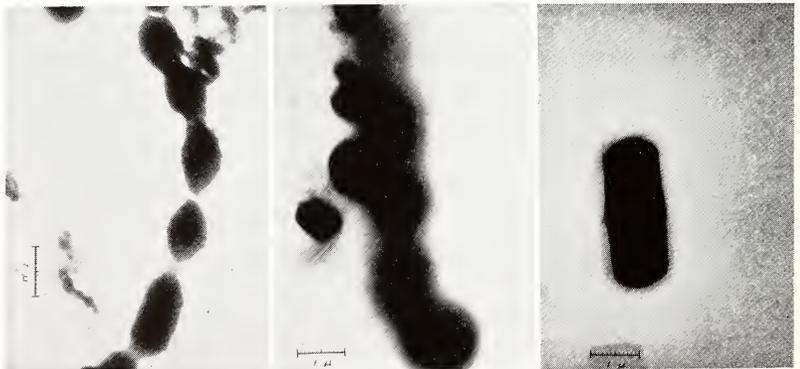
The micrographs in all instances show normal and medicated bacteria subjected to electron microscope magnification of 6,000 times and then photographically enlarged to 21,000 times. In most cases, the best micrographs of medicated bacteria were made from the strongest dilution of penicillin plus culture in which there was some visible bacterial growth present. Many observations showed the penicillin-medicated bacteria as greatly enlarged, and in many cases fission was incomplete and long chains of organisms were observed.



Figures 1 and 2 show untreated *Staphylococcus aureus* No. 209 grown in broth and subsequently washed to remove extraneous material clinging to the cells. Figures 3 and 4 show the same strain of *Staphylococcus* following medication with penicillin. In Figure 3 the cell in the upper left hand corner appears to be extruding cellular substance.

Figures 5 and 6 show untreated *Streptococcus hemolyticus* C-203 as grown in broth and prepared as described above. Figures 7 and 8 show the same strain of *Streptococcus* medicated with penicillin. The greatly increased size of the cells may be noted, and also the cell walls of many organisms appear irregular and ragged.

Figures 9 and 10 show 48-hour cultures of *Clostridium welchii* unmedicated and medicated, respectively, with penicillin. The medicated



organisms appear at this time quite regularly in filaments and chains, and in nearly all instances the separate cells in the chains are scarcely distinguishable.

**Discussion.** In the last couple of years, penicillin has attained considerable importance because of its in-vitro and in-vivo antibacterial action. As a "biological" antiseptic, tested in vivo, it appears to fulfill the necessary criteria of a truly chemotherapeutic substance considerably better than gramicidin, which in turn shows more promise in this respect than bacteriophage. Attention has been called to the great number of reports dealing with the first two of these biological antiseptics, namely bacteriophage and gramicidin. These studies include data on such critical properties as specificity, potency, mode of action, and also visible effects produced on medicated organisms.



In this report an attempt has been made to examine the visible effects, if any, which penicillin produces on vulnerable bacteria, using for this purpose suitable electron micrographs. Such pictures appear to show that penicillin does not cause lysis of medicated bacteria (as does the bacteriophage) but on the other hand inhibits or kills vulnerable bacteria, following which minor cellular changes, including enlargement (hypertrophy?) as indicated above, may follow. In this respect, the action of penicillin resembles that of gramicidin or even that of the sulfonamides, in which visible cellular changes are not so profound as in bacteriophagy.

In concluding, I want to thank Mr. W. A. Jamieson, Dr. H. M. Powell, and Dr. J. M. McGuire for making this paper possible and for their cooperation and advice.

#### References

1. D. M. Hetler and J. Bronfenbrenner, *Proc. Soc. Exp. Biol. and Med.*, **29**:806-808, 1932.
2. R. J. Dubos and R. D. Hotchkiss, *J. Exp. Med.*, **73**:629-640, 1941.
3. H. M. Powell and W. A. Jamieson, *Proc. Soc. Exp. Biol. and Med.*, **49**:387-389, 1942.
4. H. M. Powell and W. A. Jamieson, *Jour. Indiana State Med. Assn.*, **35**:361-362, 1942.
5. G. L. A. Ruehle and C. M. Brewer, *U. S. Dept. of Agri. Circular*, **198**, 1931.