Electron Microscopy of *Staphylococcus aureus* Treated with Tetracycline

Abstract. After exposure to tetracycline, *Staphylococcus aureus* was fixed with formaldehyde and osmium tetroxide, thin-sectioned, and examined with the electron microscope. Compared with control cells, the tetracycline-treated cells had no transverse septa, were larger, had a greater electron density, and had thicker cell walls.

Although the tetracycline antibiotics have been in clinical use for over a decade, their mode of action has not been elucidated. Early evidence, reviewed by Eagle and Saz (*1*), indicated that this class of antibiotics caused a variety of metabolic disturbances. Recent evidence indicates that the mechanism of action may be the result of inhibition of cell-wall synthesis (*2*).

Penicillin exerts its action through inhibition of cell-wall synthesis (*3*). Electron microscopy of sectioned *Staphylococcus aureus* treated with penicillin revealed that the cell walls were thinner than normal (*4*). We now report results of electron microscopy of sectioned *S. aureus* treated with tetracycline.

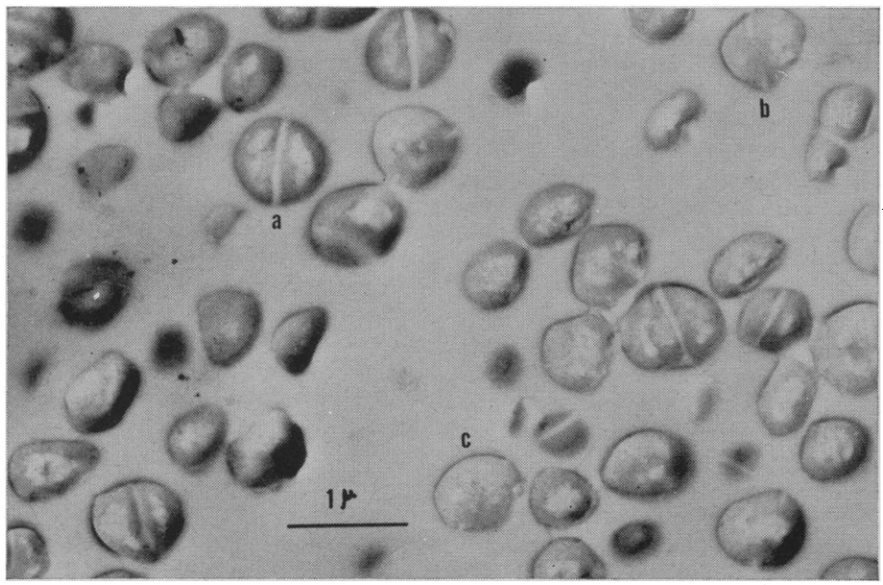

Fig. 1. Sections of normal *Staphylococcus aureus*. *a*, Cell sectioned in plane perpendicular to transverse septum; *b*, Cell sectioned in plane perpendicular to transverse septum that is just beginning to form; *c*, Cell sectioned in plane parallel to transverse septum.

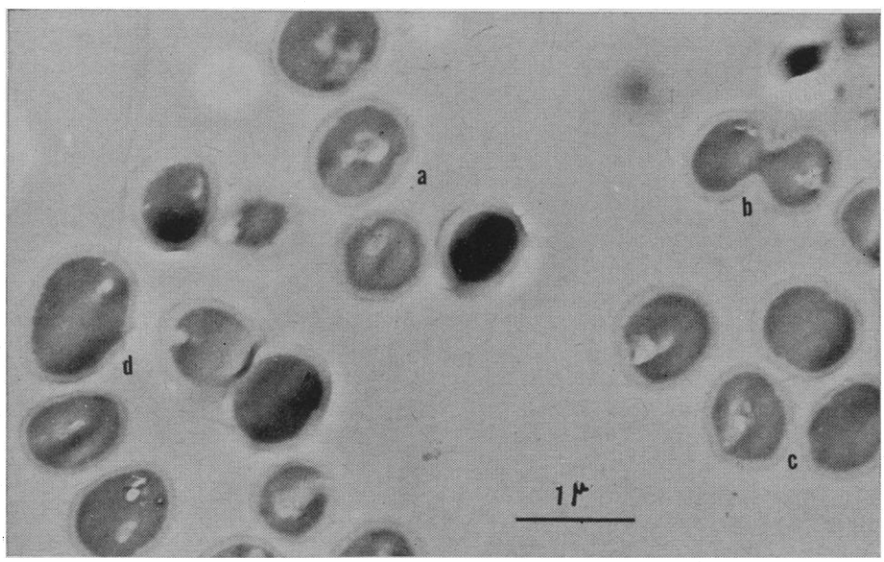

Fig. 2. Sections of tetracycline-treated *Staphylococcus aureus*. *a*, Typical swollen cell with greatly thickened cell wall; *b*, Cell arrested in late stage of cell division with cytoplasm still connected; *c*, Cells after division but with cell walls still connected; *d*, Cell arrested in early stage of cell division with prominent groove in cell wall.

Staphylococcus aureus H was grown in a medium containing glucose, peptone, yeast extract, and K_2HPO_4 at concentrations of 5, 5, 2, and 0.3 g/liter, respectively. Cultures were grown at 37°C on a reciprocating shaker in flasks equipped with side arms of optical glass. Growth was measured turbidimetrically at 610 mμ.

Duplicate flasks were inoculated with a 16-hour broth culture and incubated until the cultures were at the beginning of the exponential phase of growth. At this time, tetracycline hydrochloride was added to a concentration of 1 μg/ml to one of the cultures; the other served as a control. Incubation was continued for 135 minutes. The cells were harvested, washed twice, fixed by treatment with 10 percent formaldehyde for 10 hours and for 1 hour in buffered (*p*H 7.3) 1 percent osmium tetroxide. After dehydration in graded concentrations of ethanol, the cells were embedded in a mixture of 80 percent butyl methacrylate and 20 percent methyl methacrylate. After the polymer became set (70°C for 16 hours), sections were cut on a Porter-Blum ultramicrotome equipped with a diamond knife. Sections were collected on 200-mesh copper grids with a carbon film support. The sections were viewed and electron micrographs were taken with an RCA EMU 3C electron microscope.

Sections of control cells, shown in Fig. 1, were quite similar in appearance to previously published sections of *S. aureus* (*4*, *5*). Transverse septa, corresponding to the line of cellular cleavage, are visible in cells that were sectioned in a plane perpendicular to the septum.

Cells which were treated with tetracycline, shown in Fig. 2, differed markedly from the control cells. The treated cells were larger and had a greater electron density than the untreated cells. Transverse septa are not visible in any sections, even though some of the cells (*b* and *d*) were clearly sectioned in a plane perpendicular to the cleavage line. Most conspicuous is the fact that the cells have greatly thickened cell walls.

These results are in contrast to the thinner cell walls caused by the action of penicillin (*4*). These results are in-

terpreted to mean that, in the presence of physiological concentrations of tetracycline, cell wall synthesis is not inhibited, and may even be accelerated. The increase in size and electron density is interpreted to mean that at least some of the internal synthetic processes have continued. The electron-dense material most likely could be nucleic acid. There is no ready explanation for the absence of transverse septa (*6*).

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References and Notes

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6. We thank Mary Englert and Marcia Wishnick for technical assistance. Chemical evidence that cell-wall synthesis is unimpaired by physiological concentrations of tetracycline has been obtained.

4 September 1962

Penetration of Fallout Fission Products into an Indiana Soil

Abstract. Although most of the radioactive fallout reaching the earth stays in the leaf layer and the top inch of soil, some is carried further into the soil and can be detected to a depth of several inches. This report describes a gamma-ray spectrometric study of fallout activity as a function of depth of soil. Total gamma activity deposited from recent tests was found to be over 1.5 curies per square mile at a site in eastern Indiana during July, 1962.

Nuclear bomb tests currently being carried on and previous tests, especially during the 1954–58 period, have produced a variety of radioisotopes. A portion of these, the delayed fallout, is carried great distances in the atmosphere and eventually is returned to the earth's surface, primarily by the scavenging effect of rain and snowfall. Because these radioisotopes are associated with small fallout particles ranging in size from a few microns on down, they are initially deposited in a thin surface layer of leaves, grass, and soil. Eventually, penetration into deeper layers of soil takes place.

The gamma spectrum and activities of three fallout radioisotopes in the 0- to 2-inch layer of soil samples in 1960 have recently been described by Mortensen (*1*). In our work we were concerned with learning more about which isotopes enter the soil most easily and the rate at which penetration takes place, particularly for the longer-lived isotopes.

Soil samples were collected in 1-inch layers, dried, and ground to give them uniform mixing. The counting cans were designed with an inner cylinder to fit around a 3- by 3-inch thallium-activated sodium iodide scintillation crystal. The sample can was filled to the same level each time. Thus by reproducing the shape and volume of the sample, counting was carried out under constant geometry conditions. The pulse-height analysis was carried out with a 512-channel multichannel analyzer. Background was minimized by means of a lead shield surrounding the crystal and can.

Gamma-ray spectra of surface samples indicated the presence of several unnatural gamma-emitting isotopes. The peak energies from these isotopes, however, were primarily below 1 Mev, a region which also shows gamma radiation from the naturally radioactive elements in the soil. The latter consist essentially of the uranium decay series, the thorium decay series, and potassium-40. To separate the fallout counts, we made use of the ability of the pulse-height analyzer to subtract one spectrum from another. By this means it was possible to "peel off" the natural radioisotope contribution to the composite spectrum. The method has been described in some detail by Gustafson *et al.* (*2*). First, background was subtracted. Then a thorium mock soil was made by thoroughly mixing monozite sand of known thorium content (*3*) into a blend of $NaSO_4$ and $NaSO_3$, thus reproducing the soil density and counting geometry. This mock soil was used to "peel off" the gamma spectrum of an equilibrated thorium series by subtracting counts from the soil spectrum until the 2.62-Mev peak of the thorium series was reduced to zero. Similarly, a uranium mock soil was made with a 4 percent uranium ore calibration sample (*4*) in order to subtract the 1.76-Mev peak of Bi^{214}, a member of the uranium series (*5*). A potassium mock soil consisting of KCl was used to "peel off" the K^{40} counts occurring at the 1.46-Mev peak. The remaining spectrum then corresponded to gamma counts from the fallout isotopes.

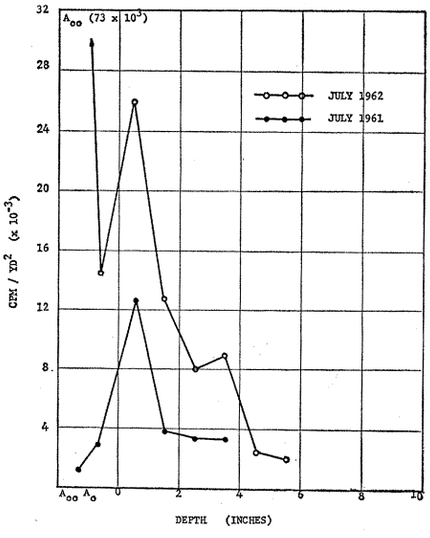

Fig. 1. Total gamma activity of fission products versus soil depth.

Two sets of Miami silt loam samples were run; one set was collected in July 1961, and the other in July 1962; both sets were collected from the same location. Because we wished to study an undisturbed soil profile, sampling was done in a virgin forest. The 1961 samples contained only the fallout products from the earlier testing series, while the 1962 samples included isotopes from the recent testing. The A_{oo} layer refers to the undecomposed and the A_o to the decomposed vegetable matter on the surface.

The gamma counts remaining after compensating for natural radioactivity

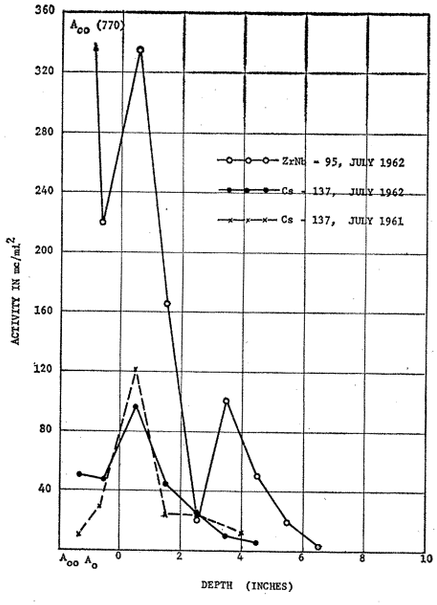

Fig. 2. Cs^{137} and $ZrNb^{95}$ concentrations in soil.