

Fixation of Bacterial L Forms for Electron Microscopy

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Electron micrographs of sectioned bacterial L forms have been published by Hofschneider and Lorek (p. RR-9 *In* S. S. Breese, Jr. [ed.], *Electron Microscopy*, vol. 2, 1962, Academic Press, Inc., New York), Tulasne, Minck, and Kirn (Ann. Inst. Pasteur **102**:292, 1962), and Ryter and Landman (J. Bacteriol. **88**:457, 1964). When working with stable L forms of *Proteus mirabilis* (Weibull and Lundin, J. Bacteriol. **81**:812, 1961) and *Staphylococcus albus* (Weibull, Proc. Soc. Exp. Med. **113**:32, 1963), we found that the nature of the growth medium was of importance

for the outcome of the fixation procedure. When the *Proteus* L form was grown in the liquid medium described by Abrams (J. Bacteriol. **70**:251, 1955), it was rapidly lysed when solutions of osmium tetroxide or formaldehyde were added to the culture. However, when this organism was grown in Albimi Brucella Broth (Albimi Laboratories, Inc., New York N.Y.), the addition of osmium tetroxide or formaldehyde caused practically no lysis, as judged from turbidity measurements on the bacterial cultures before and after fixation, or from visual inspection. The staphylococcal L form, grown in meat broth supplemented with 1.5% NaCl, had to be prefixed in formaldehyde to prevent its lysing when osmium tetroxide was added to the culture.

Figures 1 and 2 illustrate the findings described above. Specimens to be sectioned were fixed with osmium tetroxide for 16 hr by the method of Kellenberger, Ryter, and Séchaud (J. Biophys. Biochem. Cytol. **4**:671, 1958) after prefixation with 4% formaldehyde for 1 hr, and were embedded in Vestopal W. Sections were studied in a Hitachi model HS-6 microscope working at instrumental magnifications of 4,000 to 7,000. Spray preparations were obtained by fixing liquid bacterial cultures with 4% formaldehyde and dialyzing the fixed culture against distilled water.

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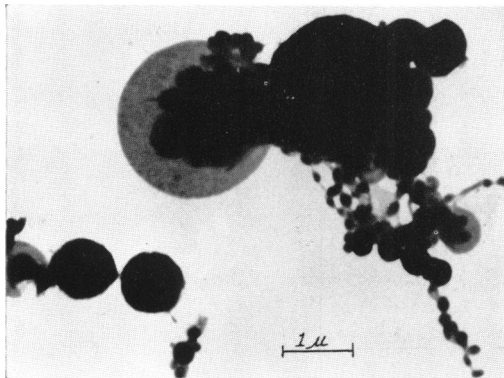


FIG. 1. Spray preparation of a *Proteus* L form (*P. mirabilis* strain L9). The L bodies consist of approximately spherical subunits connected with each other by thinner portions of cell material. $\times 9,000$.

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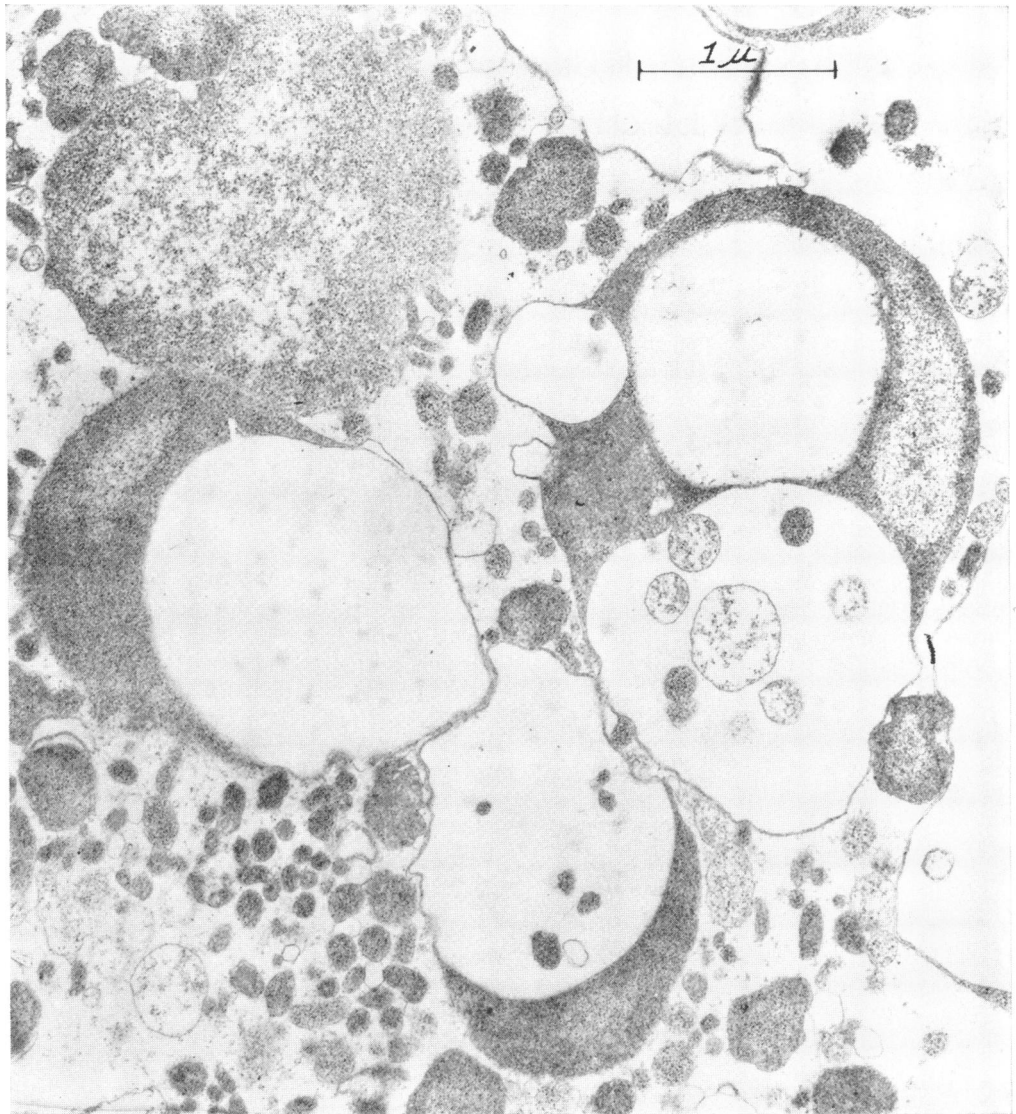


FIG. 2. Section of a staphylococcal L form derived from *Staphylococcus albus* ATCC 6538 P. Note three large L bodies in the center containing vacuoles and nuclear regions. Inside the vacuoles, small cytoplasmic elements, surrounded by membranes, can be seen. $\times 26,000$.