

# ELECTRON MICROSCOPIC STUDY OF *ESCHERICHIA COLI* TREATED WITH GIEMSA PRIOR TO FIXATION\*

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(With 4 text-figures)

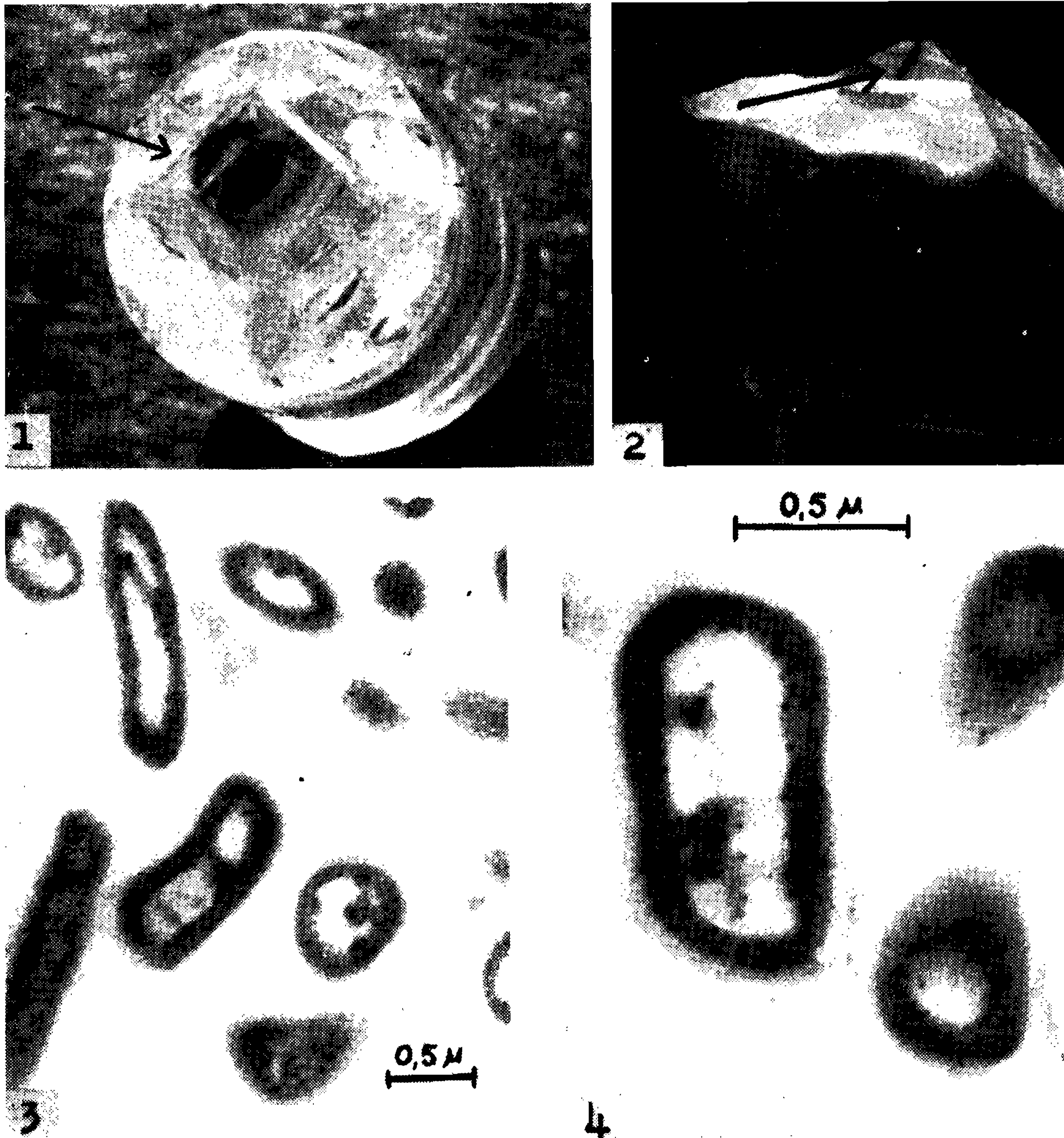
Colonies of *Escherichia coli* (strain 9637 ATCC) were overlaid with melted, tempered, agar (HASHIMOTO & NAYLOR, *J. Bacteriol.*, 75: 640, 1958). Blocks of about 1mm<sup>3</sup>, each including one colony, were cut out and immersed in Giemsa (Merck) solution (1:9 ml distilled water) for 30 minutes. This was followed by fixation in 1% buffered OsO<sup>4</sup> (PALADE, G. E., *J. Exp. Med.*, 95: 3: 285-297, 1952), for 2 hr at room temperature. Dehydration and embedding of the material were carried out according to standard procedure. The last change of methacrylate, and the embedding methacrylate contained 0,3% uranyl-nitrate. The blocks were arranged in order to put the colony axis along the same axis as the capsule. Polymerization was carried out at 55°C during 16 hr.

Treatment of the blocks with Giemsa enhances markedly their visualization since the agar acquires a faint purplish colour and the colony, sandwiched between the agar halves, is deep blue (figs. 1, 2).

Cells of *E. coli* thus treated, sectioned with a porter-Blum microtome and examined with a Philips electron microscope (magnification 20000 X and 40000X) show small electron dense granules distributed in the bacterial cytoplasm bordering the nuclear zone (figs. 3,4).

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Figs. 1-2 — Colonies of *Escherichia coli* treated with Giemsa, fixed in 1% buffered OsO<sub>4</sub> and embedded in methacrylate under standard conditions. Figs. 3-4 — Electron micrographs of ultrathin sections of *Escherichia coli* treated with Giemsa, fixed in 1% buffered OsO<sub>4</sub> and embedded in methacrylate. The cells show small electron dense granules distributed in bacterial cytoplasm bordering the nuclear zone. Magnification: 20000x and 40000x.