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NOTES

Crystalline Inclusions of Bacterium 22M

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Crystalline inclusions occur in bacterium 22M. Arrays consist of parallel rows of polyhedral subunits (7.5 nm in diameter), 2.0 nm apart, with a distance of 3.0 nm between rows.

In an occasional cell of the bacterial strain 22 M [isolated from a case of lepromatous leprosy by Claude Reich (12)], crystalline inclusions have been observed. They have not been found in fresh cultures (up to 18 hr of incubation) but can be seen in cells derived from the centers of colonies after prolonged incubation.

Inoculum was seeded on plates of Brain Heart Infusion agar (Difco), containing 4.5% rabbit blood, and was incubated for 4 days at 37 C and for an additional 5 days at room temperature. Samples of the colonies were taken from the center and from the margins, fixed in glutaraldehyde and osmium (6, 14), and embedded in Epon (8). Sections were cut on an LKB 4800 A Ultratome with a diamond knife, transferred to Formvar-covered, lightly carbon-coated grids, and stained with uranyl acetate and lead citrate (4). Micrographs were taken with a Siemens Elmiskop 1A equipped with a decontamination device and employing a 35-μm objective aperture and an accelerating voltage of 80 kv.

Inclusions were found in about 10% of the cells taken from the centers of the colonies but in none of the cells from the edges of the colonies. The inclusions varied in shape from rectangular to irregular (Fig. 4 to 9, 12, and 13). Although these elements have sometimes been found at the periphery of the cytoplasm (Fig. 5), they most commonly occur in its interior (Fig. 4, 9, and 13), sometimes closely associated with the nucleus (Fig. 6, 7, and 12). The subunits of these crystalline arrays appear to be polyhedral (Fig. 10 and 11) with a diameter of 7.5 nm. They are arranged in parallel rows, with a distance of 2.0 nm between each polyhedron in the row and 3.0 nm between each of the parallel rows. Sometimes the overall pattern is distorted, either because growth has been hindered by other subcellular structures, such as polyphosphate granules (Fig. 8) or finely granulated bodies (Fig. 4, 7, and 8), or because of some disorientation during the growth of the lattices (Fig. 9).

In a separate study on the growth and physiology of strain 22 M, it was possible, on the basis of ultrastructure, to distinguish cells of actively growing populations (log phase) from cells found in aging (stationary phase) cultures. In actively growing cells, in which the crystal inclusions are not found, one sees numerous ribosomes, spheroidal polyphosphate granules (Fig. 3; demonstrable with toluidine blue O), laminated lipoidal masses (Fig. 2; demonstrable with Sudan black B), and a mesosome which is associated with both the nucleus and a well-organized developing septum (Fig. 1). In aged cells containing crystalline inclusions, there is a marked reduction in, or absence of, ribosomes (Fig. 4–13), and the nuclei exhibit puffy electron-opaque areas (Fig. 12). The lamellated lipoidal structures and the mesosomes are no longer seen, polyphosphate granules are without characteristic size and shape, and the septal initials appear disorganized (Fig. 4, 5, 12, and 13). Thus, the formation of crystalline arrays occurs in cells exhibiting degenerative processes. Whether the crystalline inclusions are derived from such pre-existing reserves as lipid, protein, and phosphate is not known. The chemical nature of these inclusions is yet to be determined.

Undoubtedly, several combinations of substances assume crystalline forms similar in appearance to those noted here. Structures similar in size and organization of subunits have been reported in pyrenoids of the diatom *Achnanthes brevipes* (5), in the parenchyma cells of *Avena* coleoptiles (3), in the mature ballistospores of *Basidiobolus ranarum* (1), and in the germinating sporangiospores of *Rhizopus stolonifer* (2). In these cases, the basic material(s) comprising the crystals has been reported as protein with small amounts of ribonucleic acid (5) or protein and lipid (2, 3). The intramitochondrial crystals found

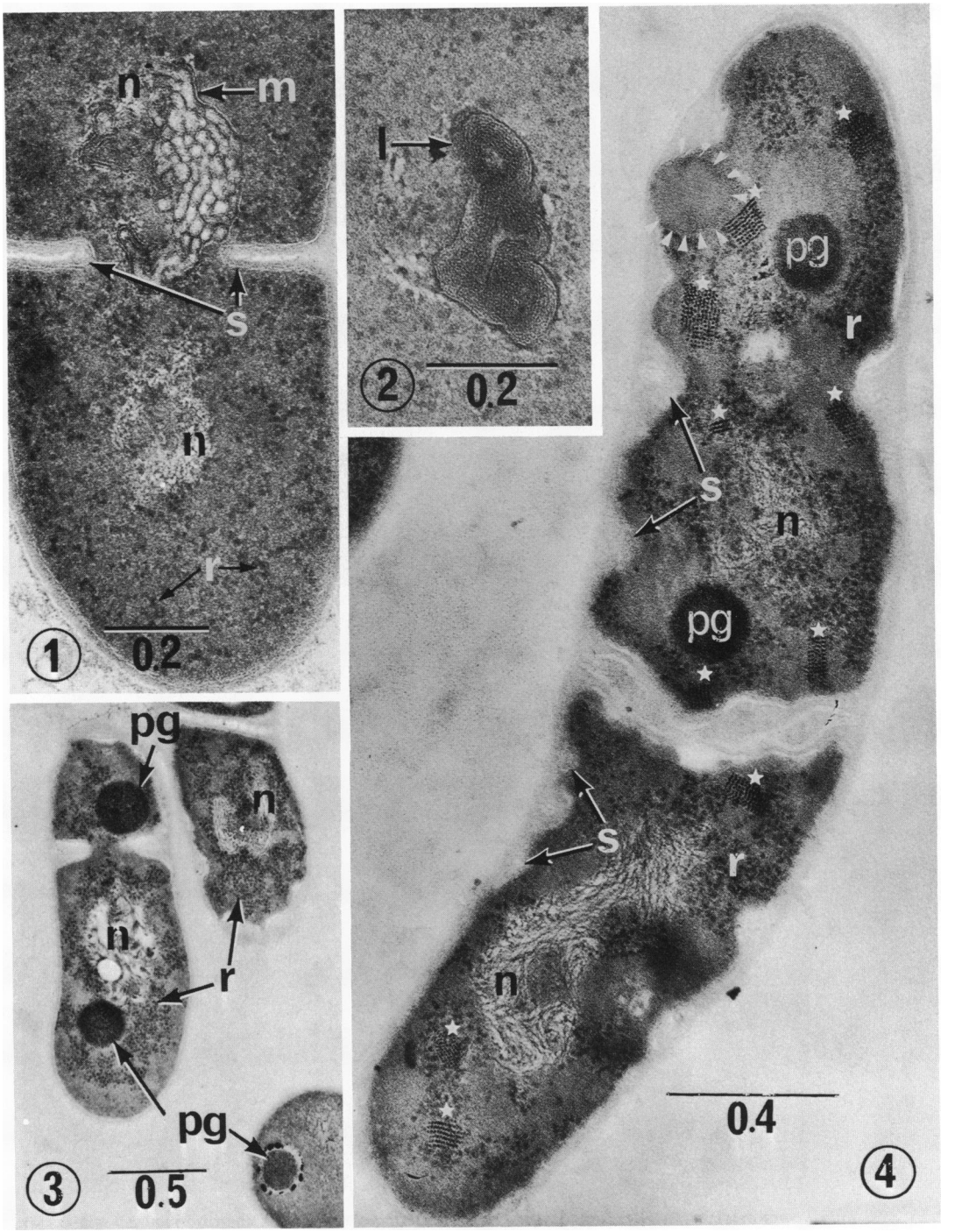

FIG. 1. *Thin section of actively growing cell showing mesosome (m) closely associated with both nucleus (n) and septum (s). Ribosomes (r) are uniformly distributed in cytoplasm. × 78,000. In this and all of the following figures, markers are measured in terms of micrometers.*

FIG. 2. *Concentrically lamellated structure (l), thought to be lipoidal. × 100,000.*

FIG. 3. *Thin section of slightly aged cell: two large polyphosphate granules (pg) and localized ribosomes (r). × 30,000.*

FIG. 4. *Unusually large number of crystals (*) in two adjacent cells; disorganized septal initials (s) and fine granular structure (arrowheads). × 65,000.*

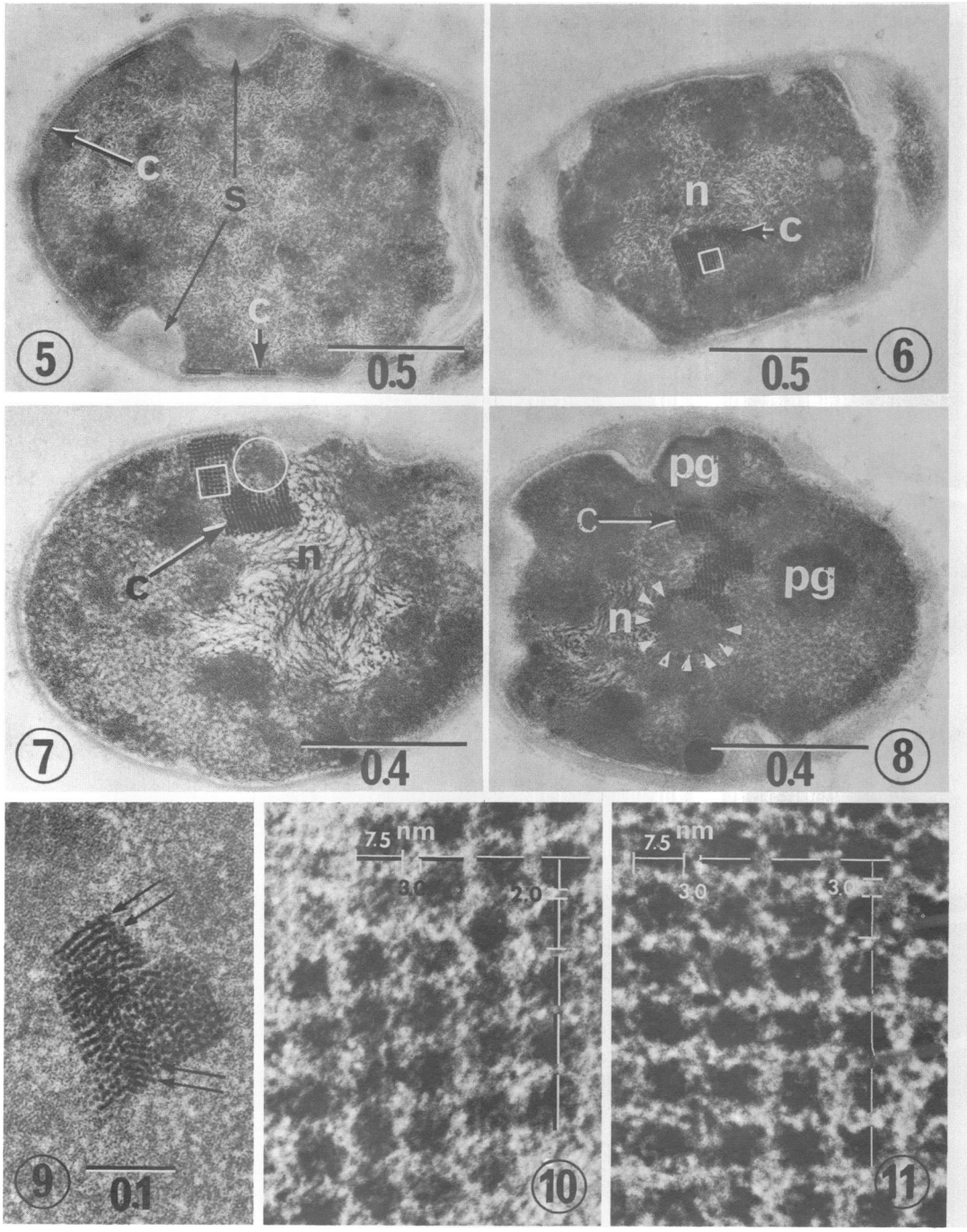

FIG. 5. *Thin layers of crystals (c) located underneath plasma membrane. Note the disorganized septum (s).* × *41,000.*

FIG. 6. *Crystal (c) associated with nucleus (n).* × *46,000.*

FIG. 7. *Crystal (c) associated with nucleus (n) and distorted by the fine granular structure (circled).* × *60,000.*

FIG. 8. *Distorted crystalline arrays developed between the polyphosphate granules (pg) and the fine granular structures (arrowheads).* × *57,000.*

FIG. 9. *Distorted crystalline arrays in the cytoplasm. The arrows indicate directions of the arrays.* × *132,000.*

FIG. 10. *High magnification of a squared portion of the crystal in Fig. 6, showing a longitudinal view of arrays.* × *870,000.*

FIG. 11. *High magnification of a squared portion of the crystal in Fig. 7, showing transverse view of crystalline arrays.* × *870,000.*

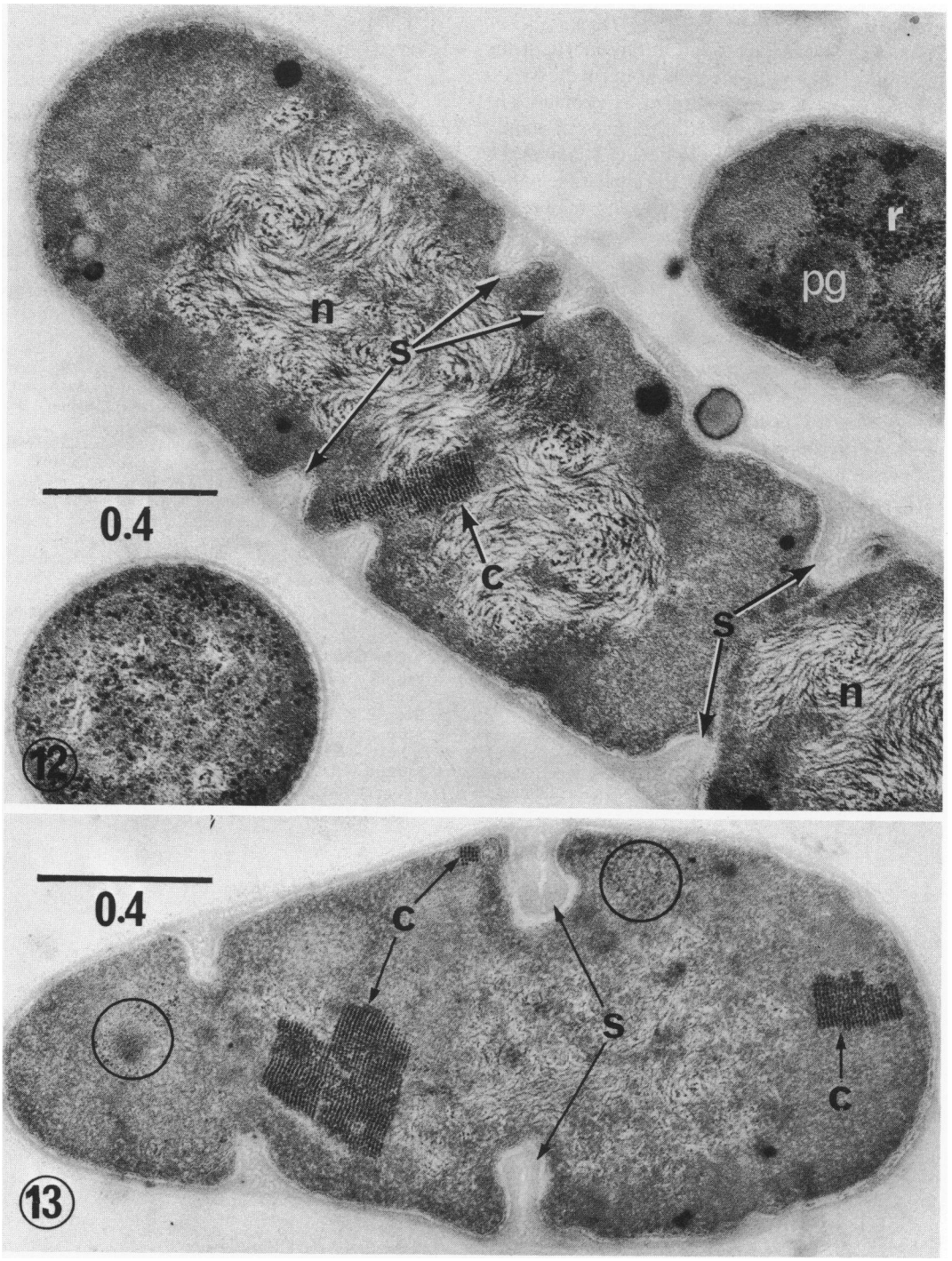

FIG. 12. *Longitudinal section of large cell exhibiting a crystal (c), extensive feathery nucleus (n), and disorganized septa (s).* × *65,000.*

FIG. 13. *Thin section of an aged cell showing one of the largest and one of the smallest crystals found in this study. Two circled areas were presumably occupied previously by polyphosphate granules.* × *65,000.*

in the root meristematic cells of *Pisum sativum* (7) and the inclusions found in the oocyte of the mosquito (13) and in the cells of dormant potato tubers (9) have been described as protein. The cells in the cortical collecting tubules of mouse kidney (10) contain lattices having subunits 4.8 nm in diameter, with center-to-center spacings of 9.8 nm. These cells are said to be lipoprotein crystallized within a membrane-bound vacuole. Most crystalline arrays associated with viral infections, plant, animal, or bacterial, differ in complexity and size from those illustrated here. Recently, intracellular bodies having, in cross section, an appearance similar to those found in strain 22 M have been observed in *Clostridium cochlearium* (11). In longitudinal section, however, the crystalline inclusions appear to be made up of parallel rods, without clear differentiation of subunits. It was suggested, in this case, that there might be some relation between defective bacteriophage and the inclusions observed. This kind of explanation for the origin of the inclusions reported by us seems unlikely. Strain 22 M is lysogenic and when induced with ultraviolet light does produce bacteriophage. In induced cells, however, we have observed no crystalline inclusions.

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