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NOTES

Arrays of Hemispheric Surface Projections on *Chlamydia psittaci* and *Chlamydia trachomatis* Observed by Scanning Electron Microscopy

WILLIAM W. GREGORY,† MOREY GARDNER,‡ GERALD I. BYRNE,§ AND JAMES W. MOULDER*
Department of Microbiology, University of Chicago, Chicago, Illinois 60637

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Scanning electron microscopy of two strains of *Chlamydia psittaci* and four strains of *Chlamydia trachomatis* representative of the wide diversity in origin and behavior of members of the genus revealed patches of regular arrays of hemispheric projections on the surfaces of elementary bodies of all six strains. These distinctive and perhaps unique surface structures are probably present in all populations of chlamydiae.

Matsumoto and his associates (10, 11) and Stokes (15) saw regular arrays of hemispheric projections on the surfaces of two closely related strains of *Chlamydia psittaci*. By scanning electron microscopy, we have observed similar arrays of surface structures on two strains of *C. psittaci* and four strains of *Chlamydia trachomatis* selected as representative of the diverse origins and different biological properties of members of the genus *Chlamydia* (12) (Table 1).

The 6BC and feline pneumonitis strains of *C. psittaci* and the mouse pneumonitis and 440L strains of *C. trachomatis* were grown in L cells by the method of Hatch (6). The G-17 and UW-57 strains of *C. trachomatis* were propagated in McCoy cells as described by Wentworth and Alexander (18). Freshly prepared chlamydial suspensions were allowed to settle onto 12-mm-diameter glass cover slips that either had well-spread monolayers of L cells growing on them or had been immersed overnight in 10 mM poly-D-lysine to promote adhesion of the chlamydial cells to the glass substrate. After 1 to 5 h of incubation at 37°C, specimens were prepared for scanning electron microscopy as described by Byrne (3).

Chlamydiae multiply by a unique developmental cycle marked by alternation of two morphological types (5). The smaller elementary body has a diameter of about 0.3 μm. It is the infectious form of chlamydiae and is specialized for gaining entry into host cells. The larger reticulate body may be up to 1.0 μm in diameter and is specialized for reproduction by binary fission within host cells. When populations of the 6BC strain of *C. psittaci*, the strain that we studied in greatest detail, were examined by scanning electron microscopy, most of the elementary bodies and only a very few reticulate bodies exhibited patches of regularly spaced hemispheres on their surfaces (Fig. 1A). When viewed in profile, these structures clearly projected outward from the cell surface. The diameter of an average projection was 25 nm, and the center-to-center spacing between projections was about 65 nm. The structures were grouped in regular arrays which were best visualized as a set of hexagons with one projection in the center of each. Only one patch was observed on any single chlamydial cell. The average number of projections visible in each patch was 19, and the largest number ever seen was 28. These measurements agree well with those reported by Matsumoto and his associates (10, 11) on the surface structures of the meningopneumonitis strain of *C. psittaci*.

Similar arrays of surface projections were also seen on the feline pneumonitis strain of *C. psittaci* and four strains of *C. trachomatis* (Fig. 1B to F). Although all the chlamydial strains so far examined exhibited nearly identical surface architecture, the projections were less frequently

† Present address: Department of Microbiology, University of Rochester Medical Center, Rochester, NY 14642.
‡ Present address: Division of Infectious Diseases, Department of Medicine, Jewish Hospital of St. Louis, St. Louis, MO 63110.
§ Present address: Division of Infectious Diseases, Department of Medicine, New York Hospital-Cornell Medical Center, New York, NY 10021.

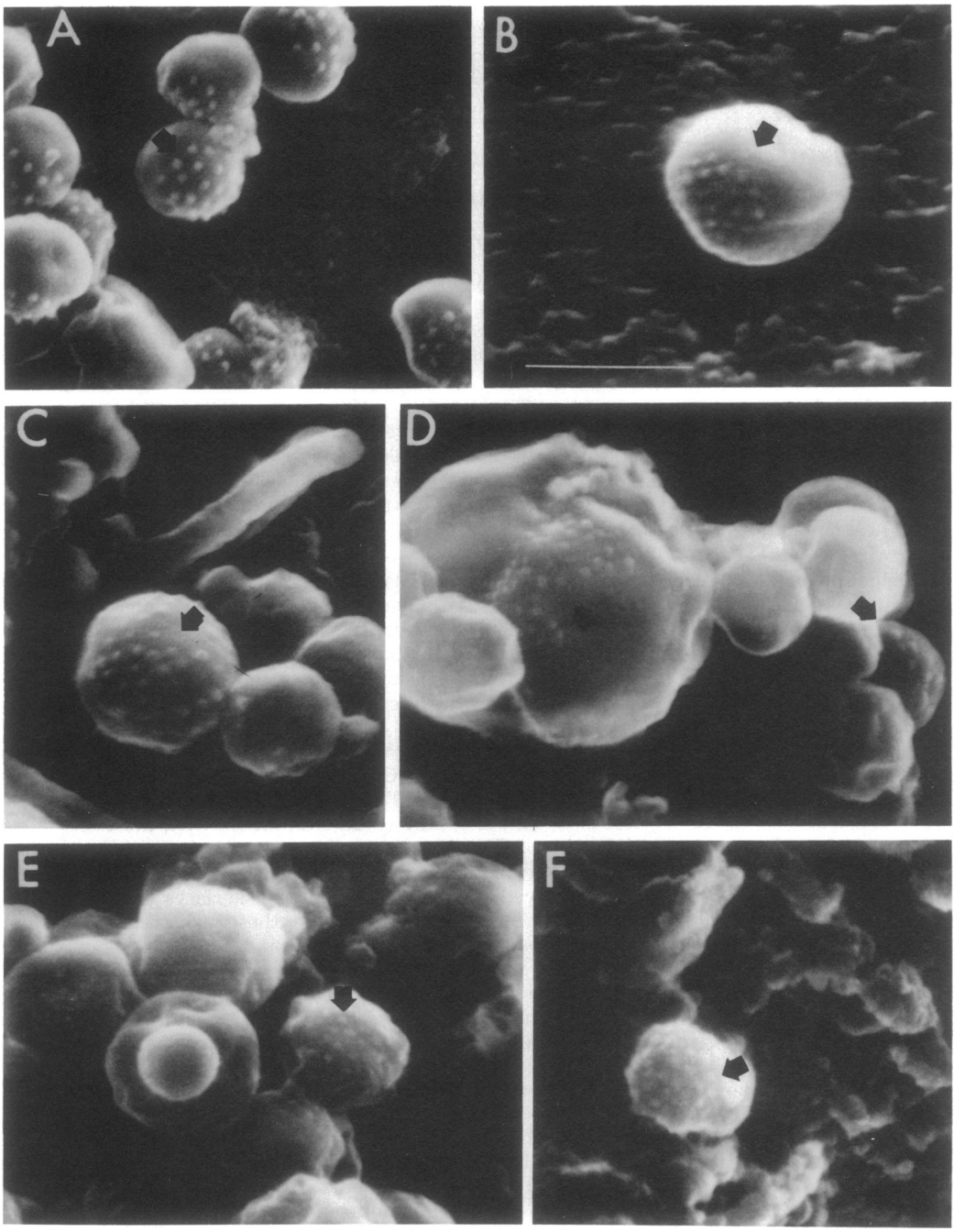

FIG. 1. *Scanning electron micrographs of two strains of C. psittaci and four strains of C. trachomatis settled onto L cells. (A) C. psittaci (6BC). (B) C. psittaci (feline pneumonitis). (C) C. trachomatis (mouse pneumonitis). (D) C. trachomatis (440L). (E) C. trachomatis (G-17). (F) C. trachomatis (UW-57). Arrows point to prominent arrays of projections. The bar in Fig. 1B represents 0.5 μm. × 50,000.*

observed and were less prominent in strains of *C. trachomatis* than in the strains of *C. psittaci*, even when the samples compared were prepared in the same batch and viewed on the same day.

The consistent occurrence of similar arrays of hemispheric projections on six chlamydial strains of widely varying origin makes it likely that these structures will be found in all popu-

TABLE 1. *Designation and origin of the chlamydial strains examined by scanning electron microscopy*

Species	Strain	Serotype[a]	Origin	Reference
C. psittaci	6BC		Parakeet	—[b]
C. psittaci	Feline pneumonitis		Cat	2
C. trachomatis	Mouse pneumonitis		Mouse	7
C. trachomatis	440L	L1	Human: lymphogranuloma venereum	13
C. trachomatis	G-17	A	Human: endemic trachoma	1, 14
C. trachomatis	UW-57	G	Human: cervicitis	17

[a] Only *C. trachomatis* strains of human origin have been serotyped systematically.
[b] Isolated in 1941 by B. Eddie at the Hooper Foundation, University of California, San Francisco, Calif. (M. R. Ross, personal communication).

lations of chlamydiae. The ubiquitous distribution of this unusual and perhaps unique morphological feature throughout the genus *Chlamydia* affords a distinctive phenotypic marker for recognizing members of this genus.

The occurrence of regular arrays of hemispheric surface projections on strains of both *C. psittaci* and *C. trachomatis,* which show little or no interspecific genetic homology (8), suggests an important function for these structures that either has been strongly conserved during divergent evolution from a remote common ancestor or has been selected independently during convergent evolution of the two *Chlamydia* species. They apparently play no role in the attachment of chlamydiae to host cells (4, 9) because there was no difference in the percentage of elementary bodies with visible patches of hemispheric arrays between populations of *C. psittaci* (6BC) that were allowed to settle onto L cells or onto poly-D-lysine-coated glass. If the elementary bodies specifically attached to L cells by means of their hemispheric projections, then there should have been a significantly lower proportion of elementary bodies with exposed patches in populations that settled onto L cells. If the hemispheric projections play a role in the successive reorganizations of the chlamydial cell envelope that are essential elements of the chlamydial developmental cycle (16), then they should be found on transitional cell types intermediate between the elementary body and the reticulate body (6). Figure 1D shows a preparation of the 440L strain of *C. trachomatis* containing a large cell which appears to be a dividing reticulate body with a cluster of projections bordering what is probably the plane of cleavage between two daughter cells of unequal size. The patches on this and on other putative transitional cells were less regular in arrangement and size of projection than those usually seen on elementary bodies.

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