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CHAPTER 15

The Role of Bacterial Exopolysaccharides in Nature and Disease

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All bacteria growing in natural or pathogenic environments elaborate at their cell surfaces an exopolysaccharide glycocalyx. This consists most often of a highly hydrated matrix of fibrillar polyanionic polymers that constitute an ion exchange resin and surround the cell in a layer. This surface matrix mediates the adhesion of bacterial cells to surfaces and the subsequent formation of surface-associated biofilms in all aquatic systems. The glycocalyx matrix uses its inherent ion exchange properties to trap and concentrate nutrients in the biofilm and this property also protects the cells within the biofilm from chemical antibacterial agents that must first saturate the matrix before reaching the innermost bacterial cells.

INTRODUCTION

The production of exopolysaccharides is a significant energy cost to bacteria, and yet direct observations of bacterial cells in a wide variety of natural and industrial environments show, unequivocally, that all such cells are surrounded by structured exopolysaccharides and that many produce very large amounts of extracellular glycocalyx material. Exopolysaccharides escaped detection by electron microscopy for many years because of their low affinity for heavy metal stains and because of their pronounced tendency to collapse during the dehydration stages of preparation for this type of observation. However, the development of a specific stain (ruthenium red) for organic polyanions (Luft 1971) and the development of stabilizing agents, such as specific antibodies (Bayer and Thurow 1977) and lectins (Birdsell et al. 1975), have allowed us to demonstrate their ubiquity, their remarkable dimensions, and their complex organization (Fig. 1). In this review, we consider the various roles of bacterial glycocalyces in bacterial growth and persistence in natural, medical, and industrial environments. It is useful, in this context, to remember that the only environment in which these structures do not have a positive survival value is in the *in vitro* monospecies culture. When bacteria are shielded from antibacterial agents and competition and are fed a predigested nutrient "compost," they often conserve metabolic energy and dispense with glycocalyx production (Doggett et al. 1964). This profound cell surface difference between cultured cells and wild cells makes extrapolation from *in vitro* data to environmental situations especially hazardous.

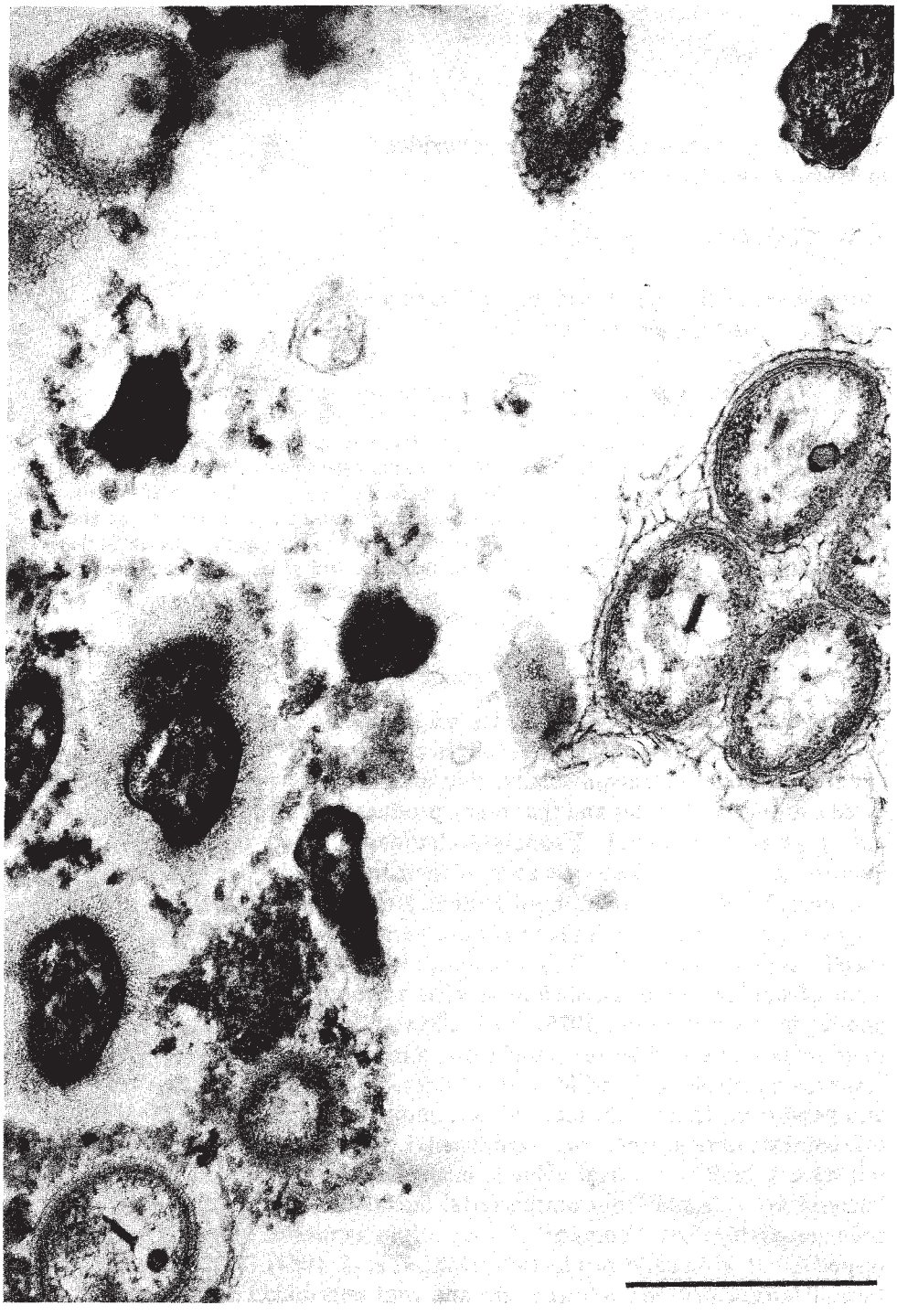

FIG. 1. Transmission electron micrograph (TEM) of a natural aquatic population of bacteria demonstrating their exopolysaccharide structures. Extracellular glycocalyces are seen to surround all of the cells in these mixed microbial populations and a wide variety of glycocalyx morphologies are seen. Bar 1.0 μm.

Discussion

Role of Exopolysaccharides in Ecological Positioning and Nutrition

Perhaps the simplest bacterial strategy for ecological positioning to obtain optimal nutrition is seen in pristine aquatic environments, such as alpine streams (Geesey et al. 1978), where bacteria adhere avidly to available surfaces by means of their glycocalyces. These adherent bacteria rapidly develop adherent biofilms in which they are bound to each other, and to the inert substrate, by a fibrous exopolysaccharide matrix that is usually very extensive (Fig. 2). Bacterial exopolysaccharides are usually composed of uronic acids and other anionic molecules (Sutherland 1977), and they act as ion exchange resins that extract charged organic molecules and inorganic ions from the passing water. This ion exchange effect, added to the natural concentrations of organic molecules at surfaces in aquatic environments (Zobell 1943), produces a favorable concentration of bacterial nutrients within the bacterial biofilm, and even in very oligotrophic waters, the adherent bacterial biofilms thicken to macroscopic dimensions.

Within the microbial biofilm, a complex population depends on many other nutrient factors such as the recycling of the components of cells that die and lyse within the matrix and on association with primary producers such as algae. Locke et al. (1984) have developed a biofilm concept that visualizes the adherent biofilm as a coherent community within which cell-cell association and nutrient cycling are fully operative. In our laboratory, well-developed stable biofilms are "fed" daily for as little as 15 min, and they thrive, using nutrient "trapping" and nutrient recycling. Bacteria usually form metabolically functional consortia in natural environments (Costerton et al. 1981) in which a primary colonizer attaches at an ecologically favorable site and a secondary colonizer often associates, by glycocalyx interaction, with the primary organism (Fig. 3) to begin a functional metabolic consortium that may finally include several species. A consensus is developing that such complex endergonic processes as the production of methane and H_2S actually require bacterial consortia (Hamilton 1984).

In the more complex case in which the substratum is not inert, bacteria show great specificity in their colonization of surfaces, and both starch and cellulose have been shown to be avidly colonized by amylolytic and cellulolytic organisms, respectively (Minato and Suto 1978). Thus, when a nutrient substratum (e.g., a leaf) is introduced into an aquatic environment, it will be colonized by specific organisms (Cheng et al. 1980) that will begin a sequence of monospecies and consortial developments until the nutrients are exhausted or the available space is filled (Fig. 4). Animal tissues may themselves be sources of nutrient for bacteria (e.g., urea, keratin, etc.), and many animal tissues rapidly develop commensal bacterial communities on their surfaces (Marrie et al. 1978) by mutual attraction of their glycocalyces by tissue lectins. In some cases the animal tissue and the bacterial community may develop a physiological cooperation based on shared enzymes and metabolic complementation (Cheng and Costerton 1979). The role of the bacterial glycocalyx is paramount in all of these interactions because this structure constitutes the functional external bacterial surface and provides the matrix that holds the members of the consortia in close juxtaposition.

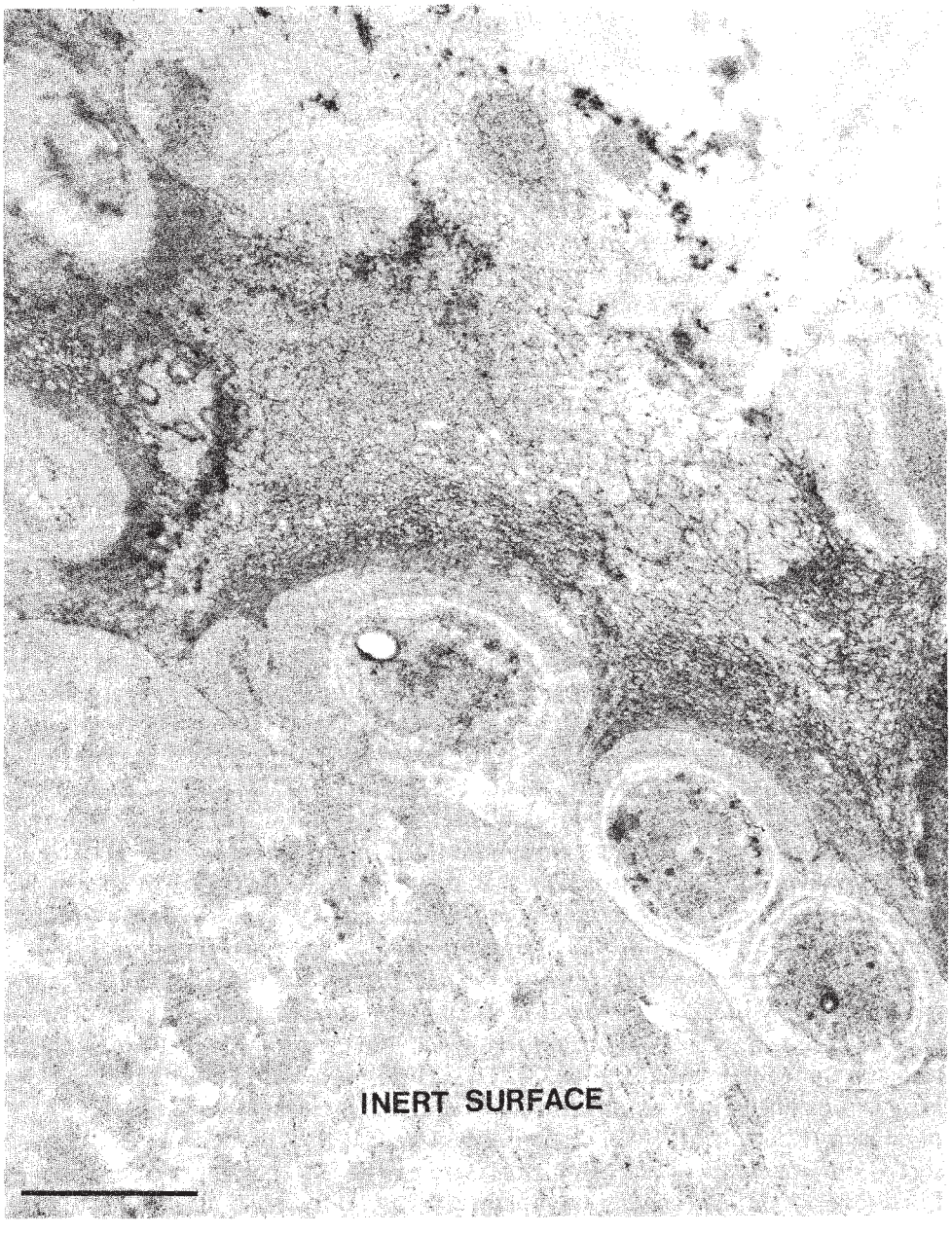

FIG. 2. TEM of the microbial biofilm that developed on a submerged surface in the Athabasca River. The sessile bacterial cells are adherent to the colonized surface and are embedded in an extensive matrix of their own fibrous anionic exopolysaccharide glycocalyces. Bar 1.0 μm.

Role of Exopolysaccharides in Protection from Antibacterial Factors

In any natural environment, bacteria operate at the interface between organic nutrients and the biological food chain. Bacteria are the only organisms capable of using dissolved organic nutrients in low concentrations (Costerton et al. 1981), and their cells constitute the first stage in the natural biological predation series.

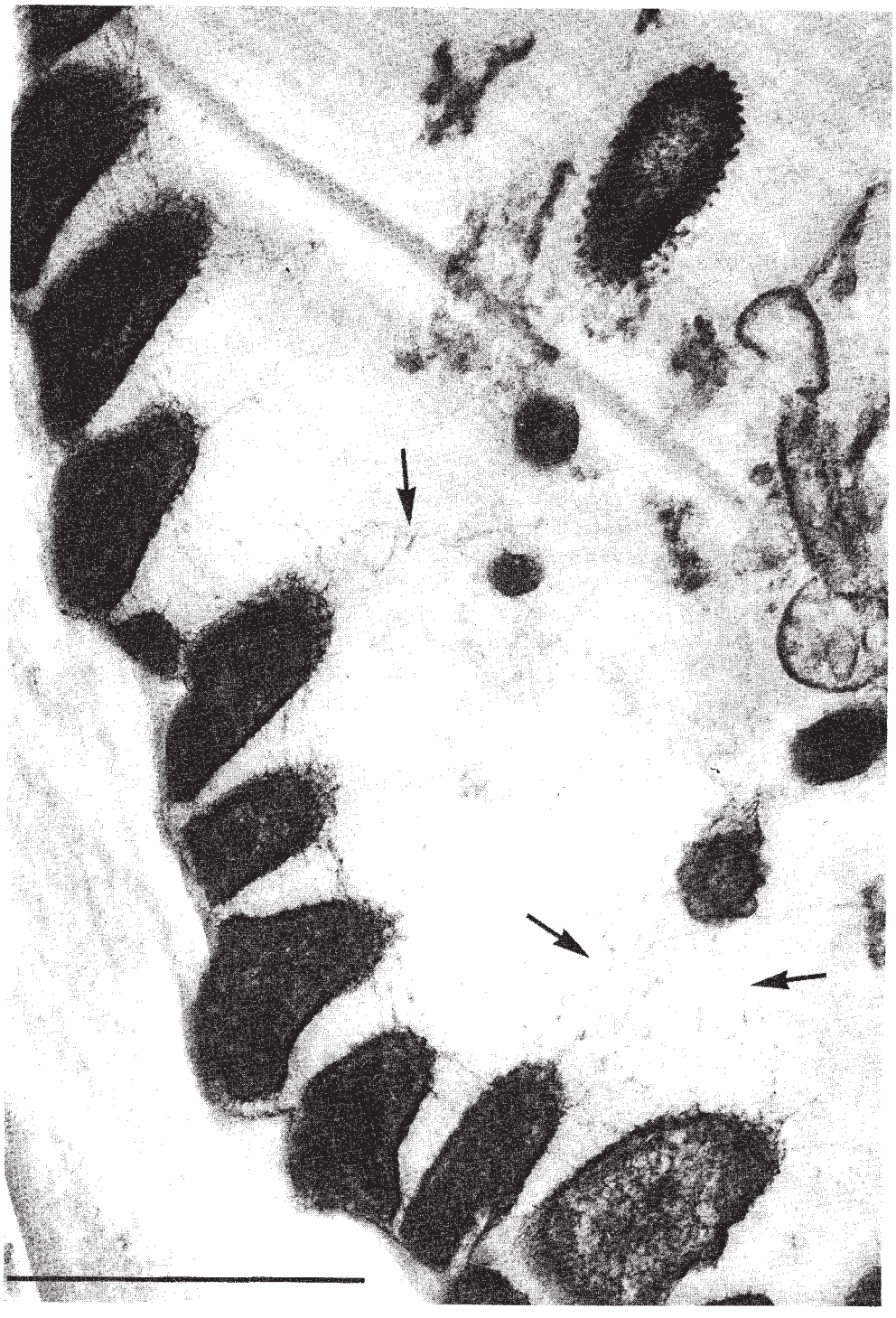

FIG. 3. TEM of a cellulose fragment colonized and partly digested by primary cellulolytic bacteria that adhere to the cellulose and to each other by means of glycocalyx fibers. A functional consortium is set up when the very small secondary colonizers adhere to the layer of primary colonizers, also by means of their fibrous glycocalyces (arrows). Bar 1.0 μm.

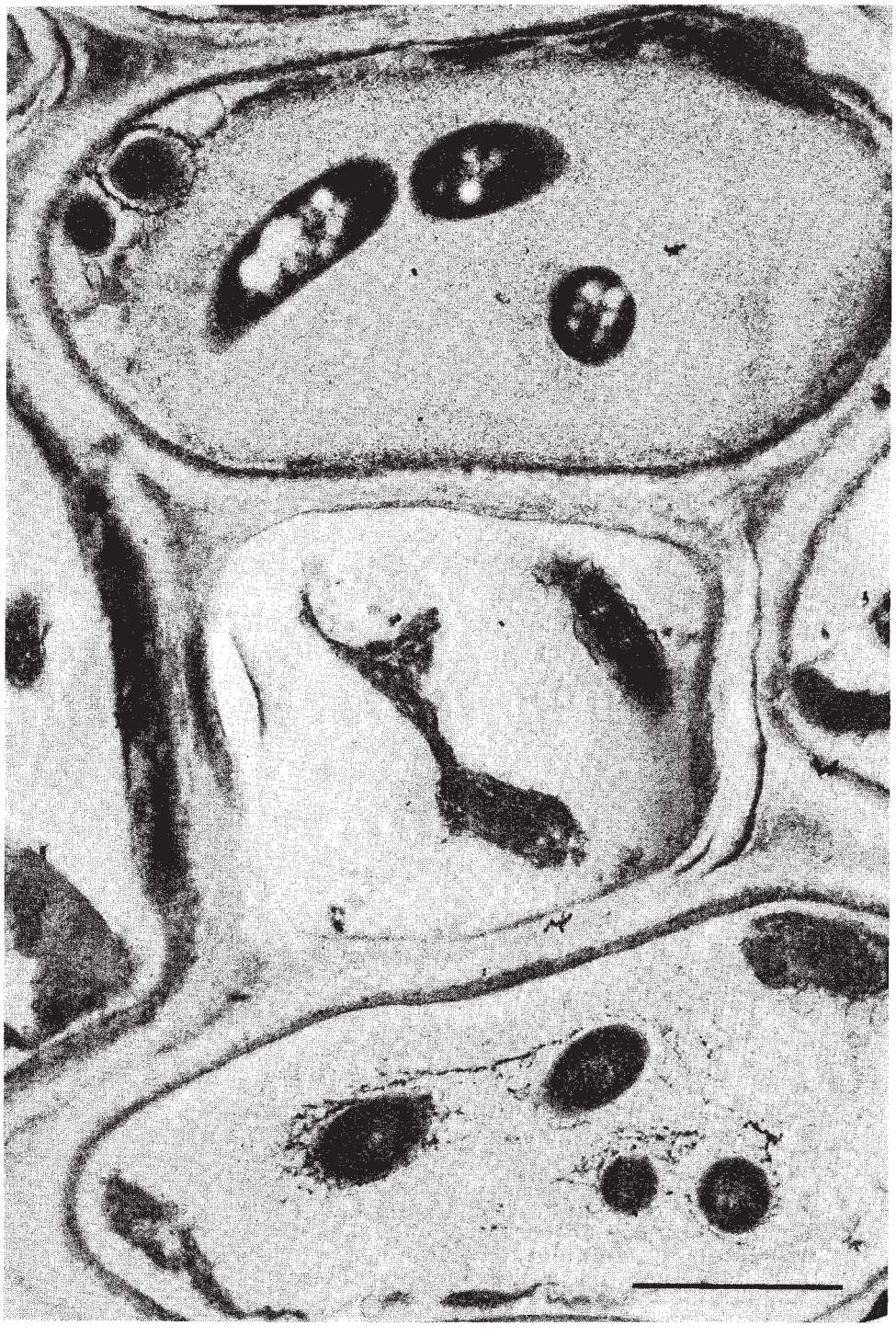

FIG. 4. TEM of a partially digested legume leaf showing that different bacteria have come to predominance in different niches and have filled the available space with their cells and their fibrous glycocalyces. Bar 1.0 μm.

Thus, a very wide variety of eukaryotic organisms seek to consume bacteria by engulfment, sieving, vortex feeding, rasping, etc., and the bacterial counter strategy is the formation of exopolysaccharide-enclosed adherent biofilms (Costerton et al. 1981). Similarly, the defensive phagocytic cells of animals seek to engulf bacteria, following opsonization by specific antibodies, but this attack is frustrated by simple capsule formation (Baltimore and Mitchell 1980) and entirely obviated by adherent biofilm formation (Marrie and Costerton 1983; Mayberry-Carson 1984). The corollary of this bacterial strategy is that these microorganisms are often confined to a dental plaque-like biofilm on a surface, and while they can persist indefinitely, they cannot launch a disseminating invasion of the ecosystem until the predatory cells are weakened or withdrawn. These surface-associated bacteria are very difficult to recover and even more difficult to quantitate by conventional microbiological methods, and they usually remain undetected until the development of the *in situ* biofilm sampler—the Robbins Device.

Biofilm bacteria are also protected from subcellular and molecular antibacterial agents. Adherent matrix-enclosed cells are protected from bacteriophage, specific antibodies (Schwarzmann and Boring 1971), and surfactants (Govan 1975), and all of these agents can be used to favor the growth of biofilm-producing bacteria, even in *in vitro* culture conditions (Govan 1975).

The bacterial glycocalyx that constitutes the matrix of the bacterial biofilms is an anionic ion exchange resin, and one cannot expect that molecular antibacterial agents, such as biocides and antibiotics, would have the same access to a deep biofilm cell as to a free-floating uncoated cell. As a molecular antibacterial agent penetrates a biofilm, it is bound to its target in superficial cells and is ionically bound to the matrix components so that it is depleted as it penetrates. Biofilms that are about 200 cells (1 mm) in thickness are routinely found in medical (Marrie and Costerton 1984) and industrial (Costerton and Lashen 1984) systems, and it is naive to expect an antibacterial agent to penetrate the biofilm and to kill its innermost cells at a concentration that will kill free-floating naked cells in culture. We have shown that biocides must be used in substantially higher concentrations than have heretofore been used (Ruseska et al. 1982) and that a long-term "soaking" dose of biocide yields maximum cost effectiveness. We have also shown that 1,000 μg/ml of tobramycin fails to kill biofilm cells of *Pseudomonas aeruginosa* when the same cells in suspension have an MIC of 0.6 μg/ml (Nickel et al. 1984). Clearly, we cannot predict an effective biofilm-killing dose from *in vitro* test data, because biocides differ markedly in their penetrating power. We must test biocides and antibiotics against sessile bacterial populations, under realistic conditions, in order to determine appropriate doses and dosing patterns for effective control of biofilm bacteria. It is sobering to realize that the bacteria involved in corrosion and in fouling in industrial systems are, by definition, the innermost cells of thick bacterial biofilms. The flow-through Robbins Device (Fig. 5) provides a system in which biofilms can be developed, monitored, and challenged with antibacterial agents to establish effective doses for the control of biofilm bacteria.

Factors Affecting Exopolysaccharide Production

Nutritional conditions have a profound effect on exopolysaccharide production by bacteria and factors such as divalent cation concentration, carbon-nitrogen

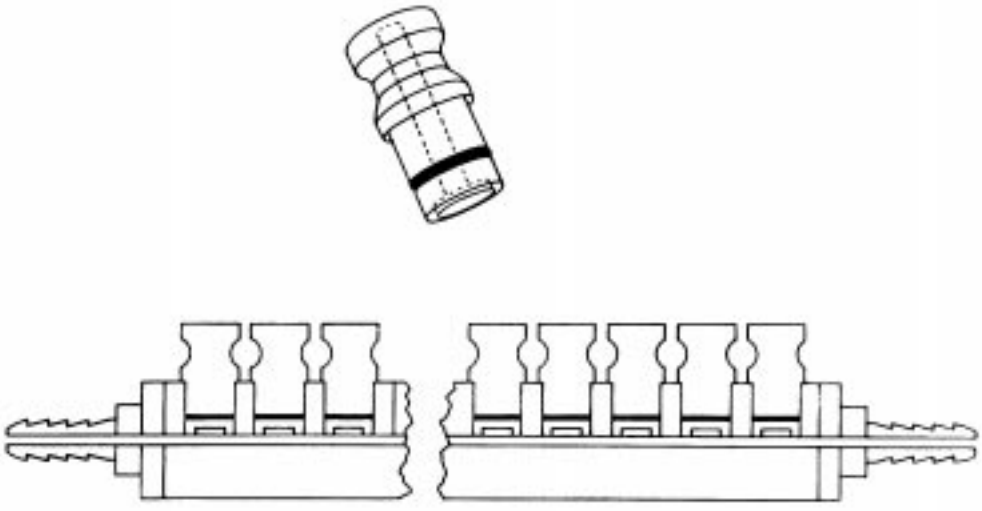

FIG. 5. Diagrammatic representation of the modified Robbins Device in which discs of various materials can be exposed to flowing fluids containing bacteria, and biofilms can be built up and aseptically sampled by removing specimen "plugs."

ratio, and specific substrate (e.g., gluconate) availability have been shown to be important in this regard (Chan et al. 1984). Further, the physical nature of the medium (solid vs. liquid) has been shown to affect exopolysaccharide production in different ways in different strains. Bacteria in batch culture have been shown to continue to produce very large amounts of exopolysaccharide even after the culture had entered the stationary phase of growth (Chan et al. 1984). However, the sine qua non of exopolysaccharide production is the availability of sufficient organic carbon and energy for this very endergonic process. Recent fascinating observations by Amy and Morita (1983) have confirmed that starvation radically reduces bacterial cell size and also reduces exopolysaccharide production. This observation confirms earlier reports (Novitsky and Morita 1976) that deep sea bacteria, most of which are starved ultramicrocells, show a sharply reduced tendency to adhere to available surfaces in comparison to the bacteria in more nutritionally favorable estuarine environments.

These data allow the hypothesis that a point source of organic nutrients in a flowing aquatic system would stimulate the development of especially thick metabolically active biofilms on adjacent submerged surfaces, and that this burgeoning biofilm would trap a large proportion of the organic nutrients for conversion into biomass and exopolysaccharide (Locke et al. 1984). Studies of the sessile and planktonic bacterial populations of receiving streams (Osborne et al. 1983) have supported this hypothesis in that point source nutrient stimulation has been shown to produce thickening and activation of biofilms on adjacent surfaces and very limited downstream effects (Ladd et al. 1979). In contrast to streams, in which bulk flow of water occurs, groundwater is exposed to very large amounts of the surface area of soil particles, and proportionately larger biofilm populations develop on these surfaces and sharply reduce the nutrient content of the flowing water (Ladd et al. 1982). This nutrient withdrawal from flowing groundwater is so effective that both bacterial numbers and total organic content

decrease sharply downward through the soil horizons, but starved ultramicrocells are still present in groundwater in deep soil horizons. These small but effective replicating units can be restored to full size, complete metabolic activity, and effective exopolysaccharide production and adhesion by the simple provision of suitable nutrients. When water containing bacteria is flowed through the pore spaces of a solid matrix (such as a sand pack, or sandstone) a similar pattern of inlet face biofilm plugging is seen (Gupta and Swartzendruber 1962; Hart et al. 1960). We have examined bacterial growth in glass bead "cores" (Shaw et al. 1984) that provide a constant pattern and size of pores ($\sim 33\mu$m), and mimic a very porous sandstone (~ 8 darcys), and we note very extensive adhesion, proliferation, exopolysaccharide formation, and plugging of the inlet face of this solid matrix by bacteria (Fig. 6). This phenomenal biofilm formation sharply reduces both the hydraulic flow and the total organic carbon content of the water. The internal surfaces of the core can be exposed by simple fracturing of the fragile glass structure and show no bacterial adhesion or biofilm formation (Fig. 7). Thus, the basic bacterial "strategy" governing the growth in solid matrices is identical with that seen in bulk flow systems, in that these microorganisms form exopolysaccharide-enclosed biofilms on the first available surfaces, reduce the nutrient content of the percolating water, and penetrate the porous matrix in the form of nonadherent, starved ultramicrocells. The practical sequellae of this basic pattern of bacterial growth in solid matrices are that the bacteria introduced into oil-bearing formations will normally express metabolic activity only very near the injection face, but that metabolic activity (including exopolysaccharide production and pore plugging) can be induced at considerable depth if nutrients can be made to penetrate and to stimulate preexisting ultramicrocells.

Conclusions

Ecological studies of bacterial growth in natural systems have discovered certain patterns, or "strategies," that are used by bacteria in whatever environment they find themselves. Unlike test tube bacteria, wild strains produce large amounts of exopolysaccharide and they use this external component to colonize specific surfaces, to mediate specific associations with other bacteria and with tissues, to produce the matrix of a protective biofilm, and to trap and concentrate nutrients from flowing fluids. In ecosystems with a finite supply of nutrients, these molecules are adsorbed and metabolized by bacteria within adherent biofilms until starvation overtakes any cells that penetrate past this nutrient-rich zone. These starved cells reduce their cell size and their exopolysaccharide production and remain as minute, nonadherent propagules until they encounter sufficient concentrations of nutrients to resume normal metabolic activity. Bacteria are relatively primitive cells that employ these basic strategies of growth and survival, in all environments, and their growth and distribution in any particular system may be anticipated if we bear in mind their predictable responses of growth and exopolysaccharide production in response to the availability of surfaces and of nutrients.

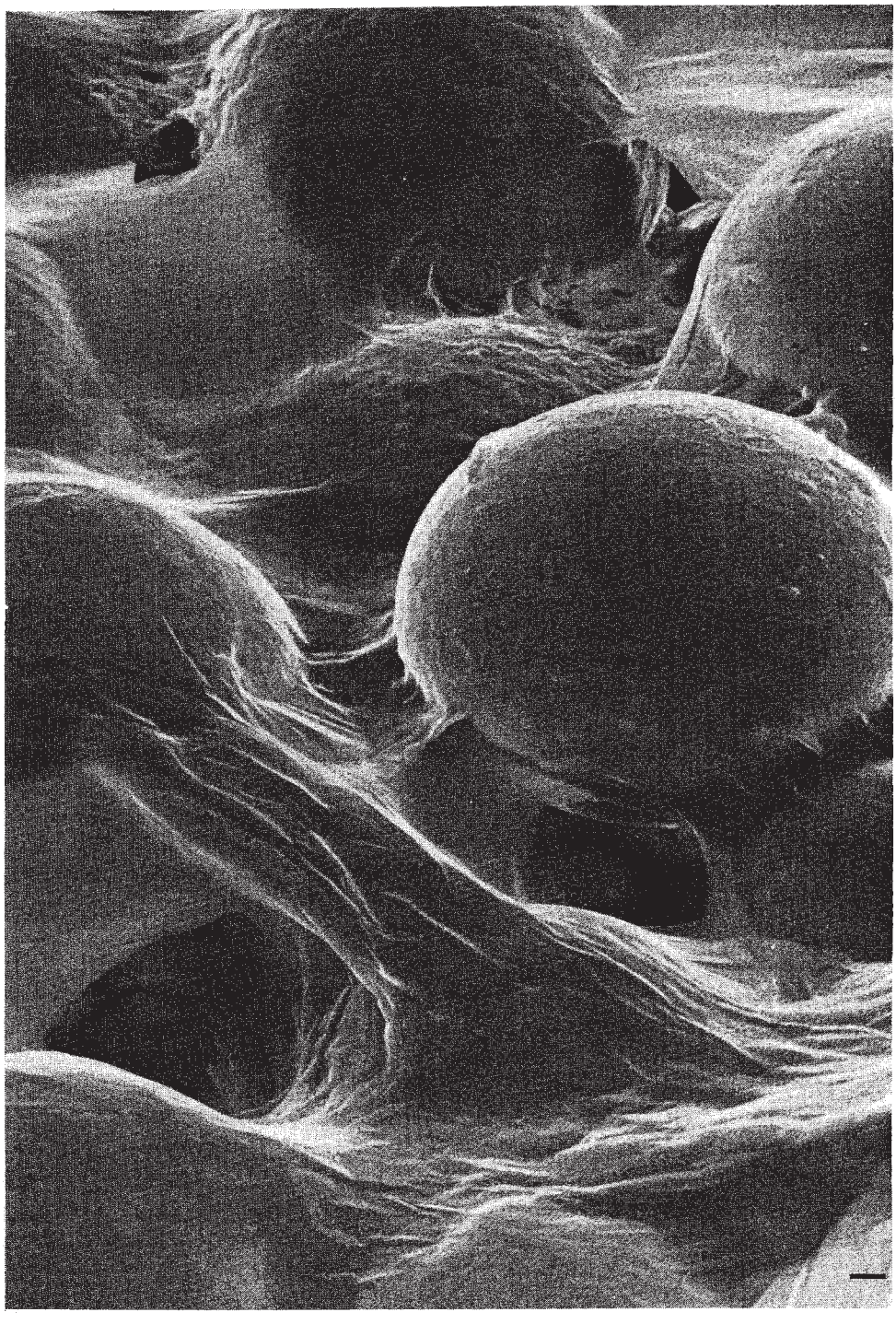

FIG. 6. Scanning electron micrographs (SEM) showing bacterial growth and glycocalyx production that has occluded the inlet surface of a glass bead "core," which mimics an open sandstone rock, with a "skin" of biofilm within which the bacteria are virtually buried. The inlet surface of the core is nutrient-rich and bacteria adhere and proliferate. Bar 5 μm.

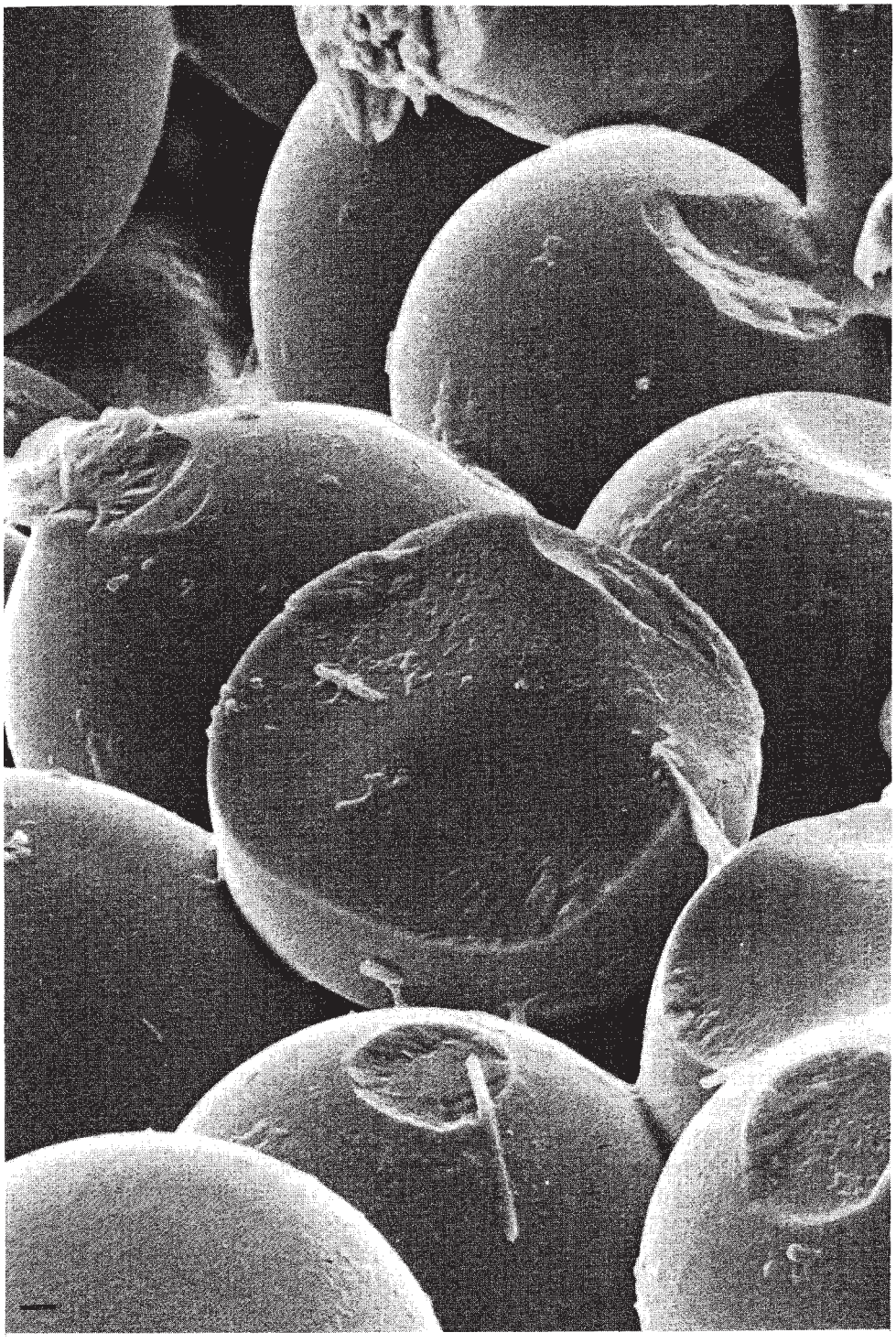

FIG. 7. SEM of the same core seen in Fig. 6 that has been broken 2 cm below the heavily colonized inlet face. Note the absence of adherent bacterial growth in this nutrient-depleted zone of the glass bead core. Bar 5 μm.

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