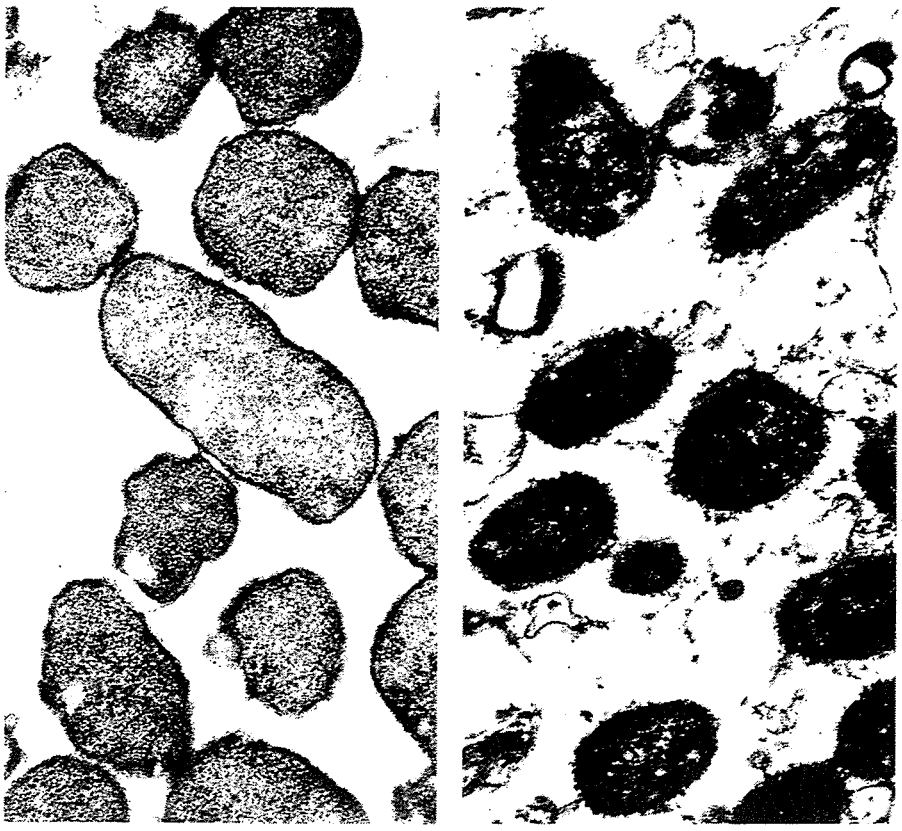
How Bacteria Stick

In nature (but not in laboratory cultures) bacteria are covered by a "glycocalyx" of fibers that adhere to surfaces and to other cells. Adhesion might be prevented by a new kind of antibiotic

by J. W. Costerton, G. G. Geesey and K.-J. Cheng

Bacteria stick, tenaciously and often with exquisite specificity, to surfaces ranging from the human tooth or lung and the intestine of a cow to a rock submerged in a fast-moving stream. They do so by means of a mass of tangled fibers of polysaccharides, or branching sugar molecules, that extend from the bacterial surface and form a feltlike "glycocalyx" surrounding an individual cell or a colony of cells. The adhesion mediated by the glycocalyx determines particular locations of bacteria in most natural environments; more specifically, it is a major determinant in the initiation and progression of bacterial diseases ranging from dental caries to pneumonia.

These major—and, with the benefit of hindsight, rather obvious—facts about the bacterial cell surface have become known only within the past decade. Ironically the main reason for the late discovery of the bacterial glycocalyx and its functions was the long reliance by microbiologists on an otherwise eminently effective investigative system: the pure laboratory culture of an individual bacterial strain. To generate and maintain a glycocalyx a bacterial cell must expend energy, and in the protected environment of a pure culture the glycocalyx is a metabolically expensive luxury conferring no selective advantage; cells that fabricate these elaborate coatings are usually eliminated from pure cultures by uncoated mutants that can devote more of their energy budget to proliferation. Microbiologists have largely studied such naked mutants.

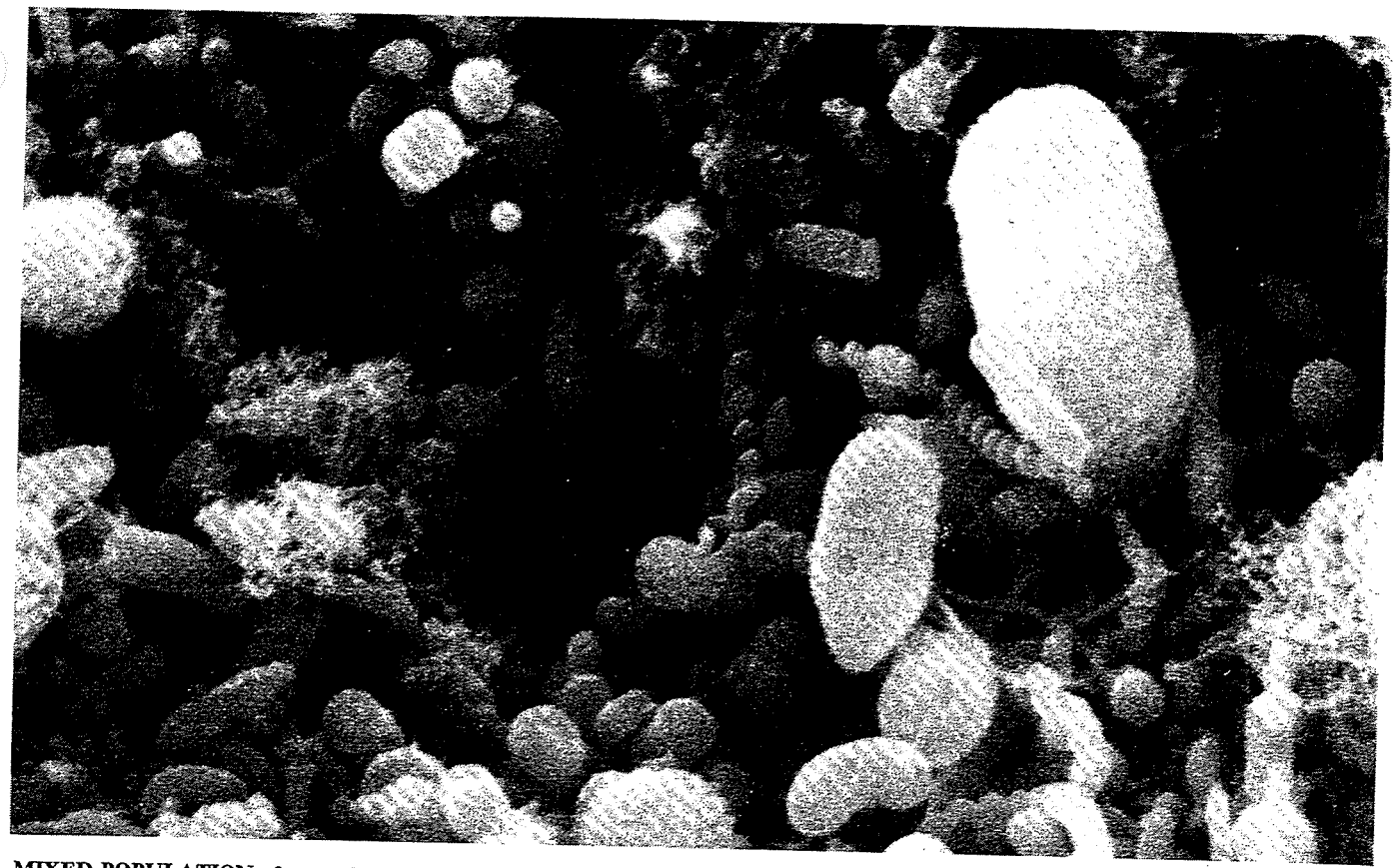


NAKED BACTERIA (*left*) **are from a typical pure laboratory culture of** *Escherichia coli;* **the glycocalyx-coated bacteria** (*right*) **are** *Pseudomonas* **cells from an infected human bladder. In both preparations the cells were stained with ruthenium red, which is taken up by any polysaccharide glycocalyx fibers that are present. The bacterial glycocalyx was ignored until recently because the familiar pure laboratory strains do not need it and therefore do not fabricate it.**

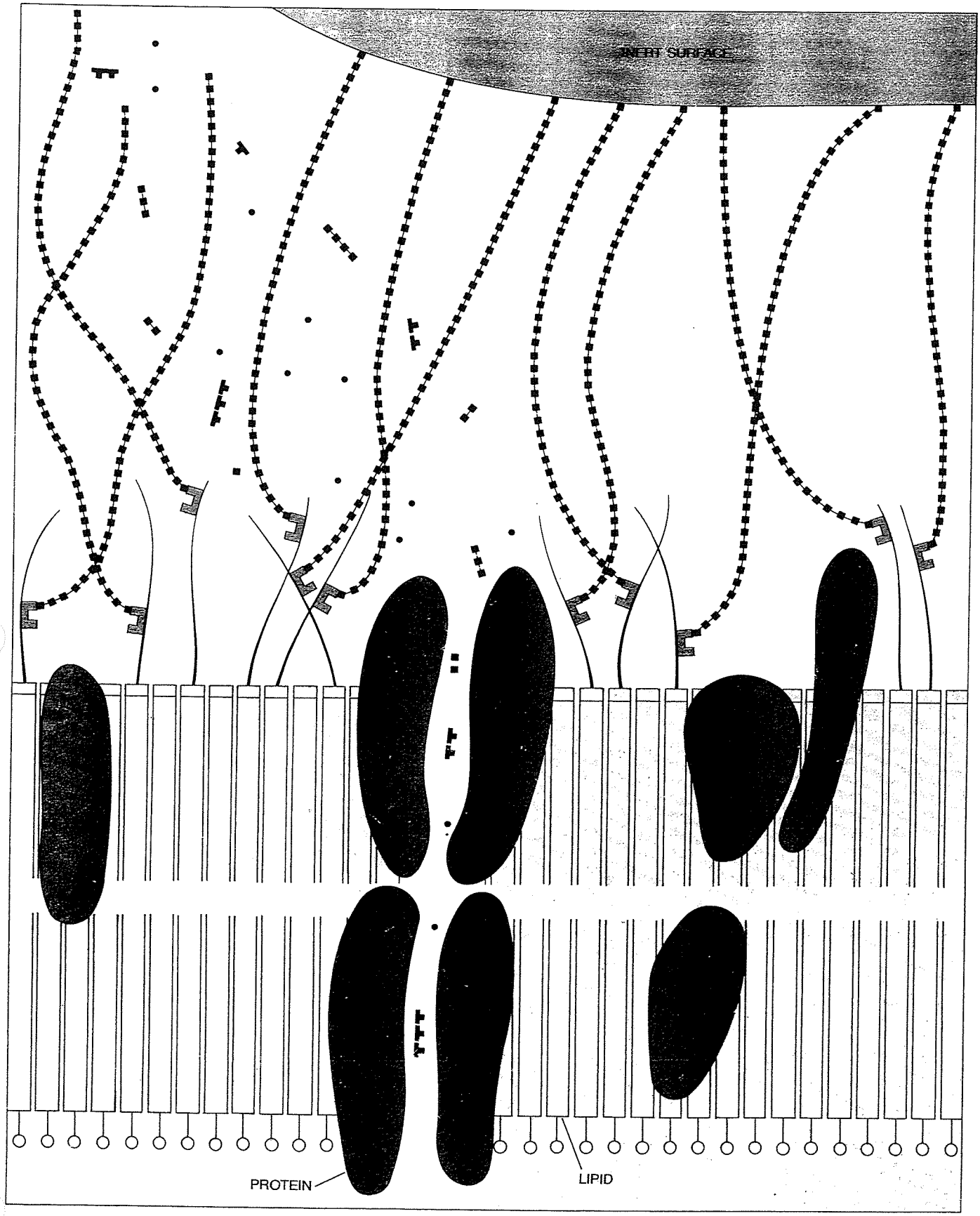
In a competitive natural environment populated by several kinds of bacteria, on the other hand, selection favors cells that are protected, and enabled to adhere to a desirable surface, by a glycocalyx. It was only in 1969 that Ivan L. Roth of the University of Georgia demonstrated carbohydrate fibers surrounding bacteria in an aquatic system and Ian W. Sutherland of the University of Edinburgh Medical School characterized the surface polysaccharides of bacteria taken from natural environments, thus drawing attention to the universality of what we now know as the glycocalyx. Since then studies in our laboratories at the University of Calgary and at the Agriculture Canada Research Station at Lethbridge in Alberta, and in a number of other laboratories, have made it clear that the glycocalyx is essential to the biological success of most bacteria in most of the varied natural environments in which they are observed.

The polysaccharide-coated surface is not a peculiarity of the bacterial cell. The more rigid polysaccharide cell wall of higher plants was among the first microscopic structures described by Robert Hooke in 1665. The analogous surface of animal cells—a glycocalyx like that of bacteria—was described only in 1971, by Vincent T. Marchesi and his colleagues at the National Institute of Arthritis, Metabolism, and Digestive Diseases. They isolated and identified glycoproteins arrayed in the membrane of animal cells and showed that the polysaccharide fibers they bear extend outward from the membrane to form

BACTERIAL GLYCOCALYX is a network of fibers that extend from the bacterial surface. The fibers adhere to one another and to inert or animal-cell surfaces nearby. This electron micrograph made in the laboratory of one of the authors (Costerton) shows a mixed population of bacterial cells that have been attached by their glycocalyxes to one another and to the surface cells of a cow's rumen.



MIXED POPULATION of a number of different kinds of bacteria and some yeast cells adhering to the lining of a cow's rumen is seen in a scanning electron micrograph. Here the individual polysaccharide fibers by means of which the bacteria are held to the rumen cells and to one another are not resolved, but some of the glycocalyx fibers have coagulated and are seen as masses of branching foamy material.



GLYCOCALYX extends from the outer membrane of a bacterium as is indicated in this generalized and highly schematic diagram. The membrane is a bilayer of lipid molecules (*forked structures*) in which protein molecules (*gray shapes*) are embedded. Lipopolysaccharide molecules (*black hairlike structures*) extend from the membrane. The glycocalyx is a mass of long polysaccharide fibers (*chains of colored squares*). The fibers are chains of sugar molecules that are generated by bacterial enzymes called polymerases (C-*shaped structures*) affixed to the lipopolysaccharides. The glycocalyx fibers adhere to nearby surfaces, in this case an inert surface (*top right*). In addition to mediating bacterial adhesion the fibers channel toward the bacterium various nutrients such as sugars (*rectangles*), amino acids (T-*shaped objects*) and inorganic ions (*dots*), which enter the cell through channels in the membrane formed by arrays of proteins.

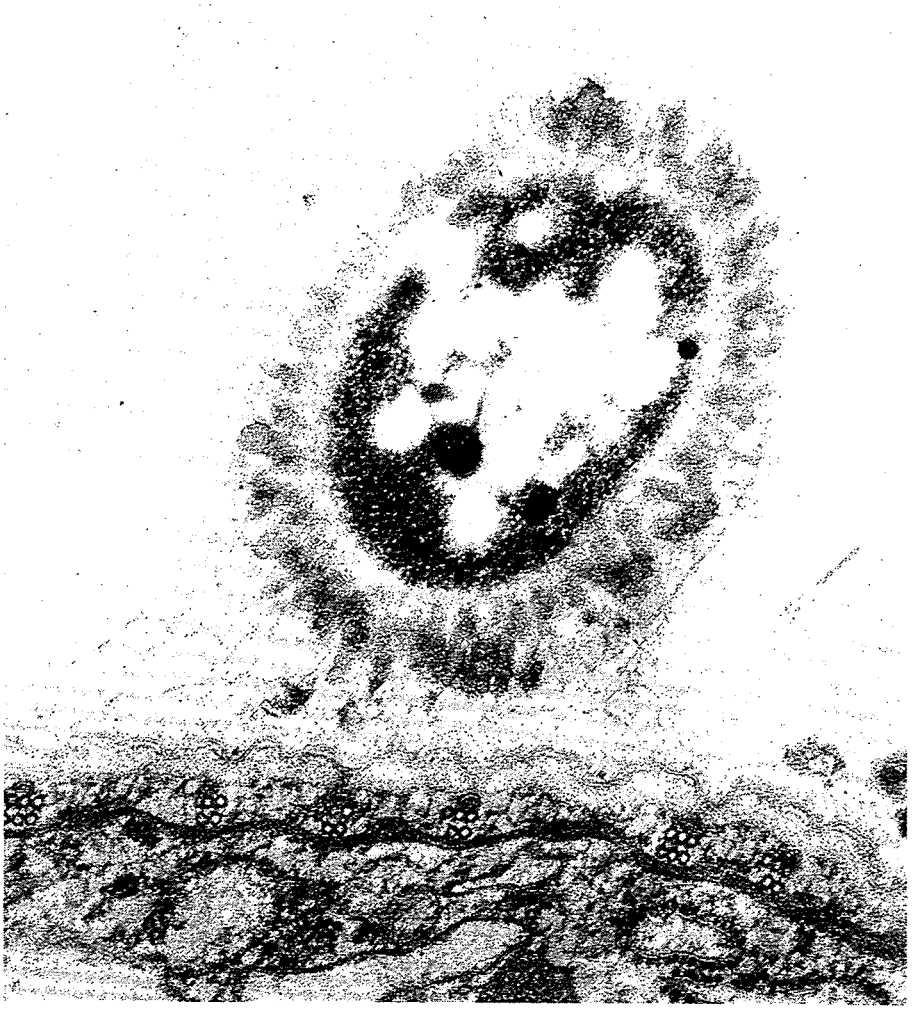
not just projections but a matted glycocalyx that is itself a continuous surface, actually the functional surface of the cell. The precise chemical nature of this ubiquitous glycocalyx varies with the sugar composition of the fibers, so that it differs from cell to cell within the body of the animal and probably also at different stages in the life of the individual cell.

The striking conclusion to be drawn from these observations is that the real functional surface of all cells—bacterial, animal and higher-plant—is a tangled mat of polysaccharide fibers fabricated and oriented by the cell itself. A bacterial cell adheres to a plant cell, an animal cell or another bacterial cell by juxtaposing its own glycocalyx to the surface of the cell it adheres to. In many cases the link is supplied by the simple proteins called lectins, which bind very specifically to polysaccharides with a particular molecular structure [see "Lectins," by Nathan Sharon: SCIENTIFIC AMERICAN, June, 1977].

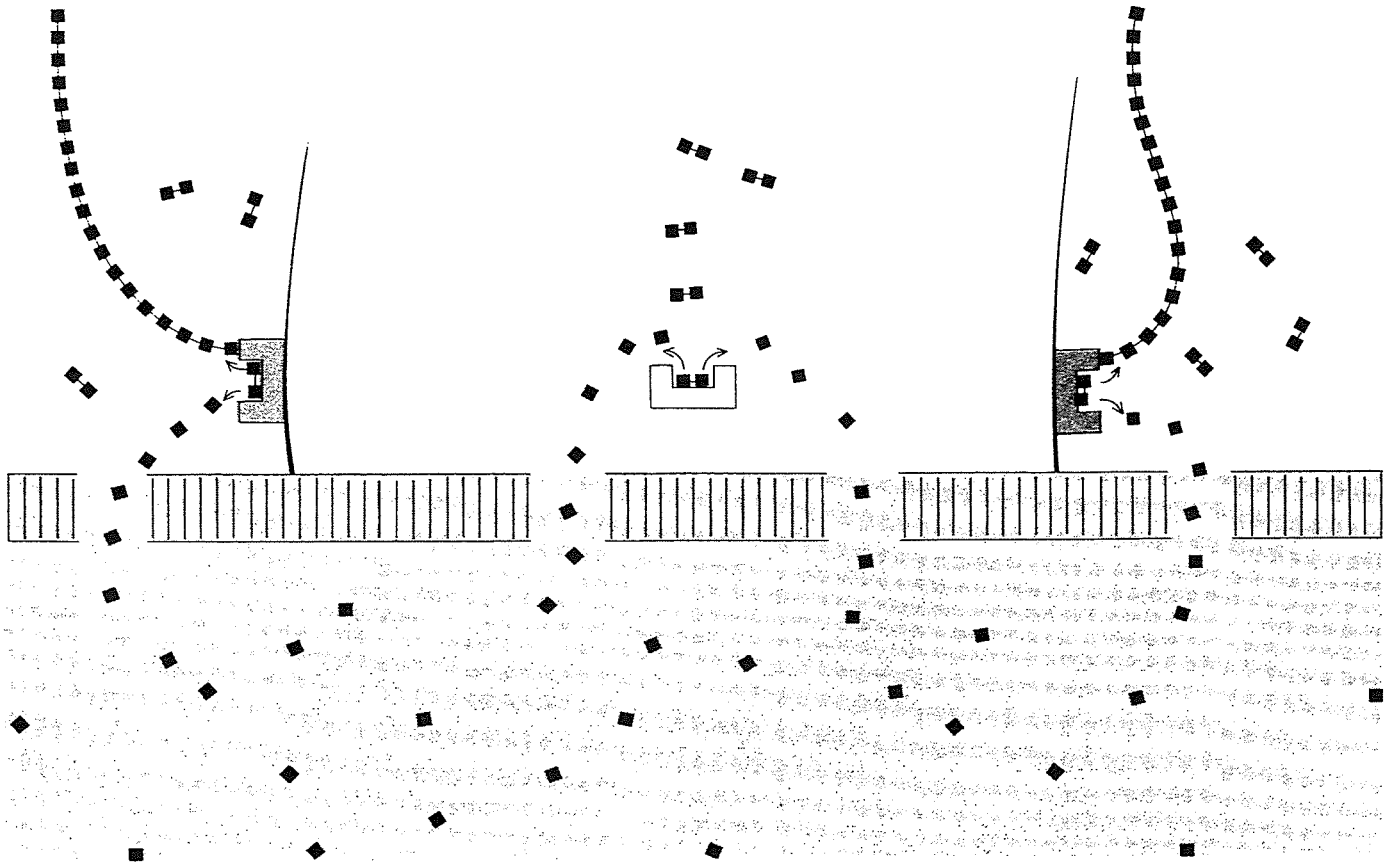
The pioneering investigation of glycocalyx formation in bacteria was actually a study of *Streptococcus mutans,* an organism that colonizes the human tooth, by Ronald J. Gibbons of the Forsyth Dental Center in Boston and other workers. Between 1960 and 1967 they reported that three enzymes at the bacterial surface deal in particular ways with the common sugar sucrose, which is composed of one molecule of glucose and one of fructose. The enzyme invertase splits the sucrose into its two components, both of which are released to become sources of energy for the cell. A second enzyme, glucosyltransferase, splits the sucrose and releases fructose as a nutrient but polymerizes the glucose into a long polysaccharide called a glucan, which is insoluble in water. A third enzyme, fructosyltransferase, builds the fructose into a similar but water-soluble polysaccharide and liberates glucose.

The important finding is that the glucan is somehow able to adhere to the inert enamel surface of a tooth and thus to attach the bacterial cell to the tooth. Since glucosyltransferase is present throughout the glucan network of the glycocalyx, the glucans it continues to produce keep thickening the glycocalyx, entrapping more bacterial cells of the same or different species and building up the yellowish film known as plaque. Another human oral bacterium, *Streptococcus salivarius,* colonizes not the tooth itself but the gum. It liberates free glucans that migrate to adjacent tooth surfaces and, with their accompanying glucosyltransferase, build a polysaccharide mat that traps a mixed population of bacteria to form plaque. It is within the plaque that the bacterial enzymes re-

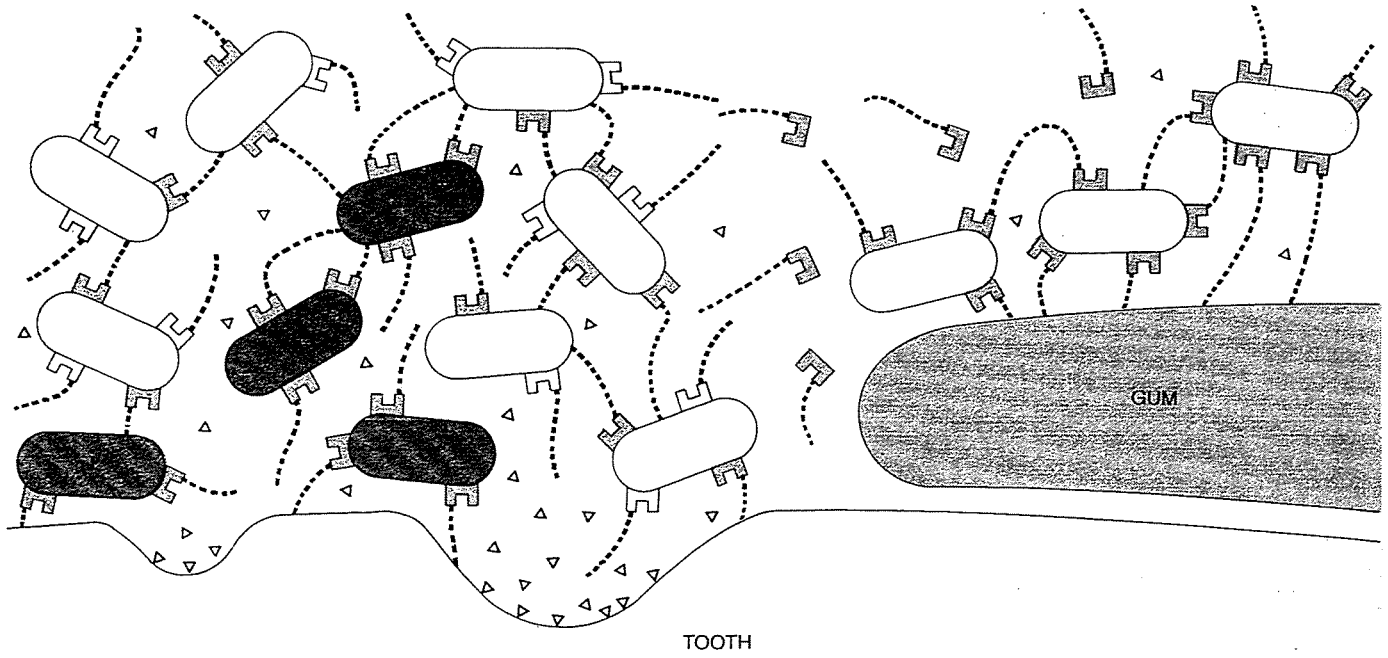
SINGLE BACTERIAL CELL attached itself to a plastic surface submerged in a mountain stream and was anchored against the flow of rushing water by the fibers of its glycocalyx.



ELABORATE GLYCOCALYX attaches a single bacterium (*top*) to an animal-cell glycocalyx, a mat of fibers that is visible above the larger cell's double-layered plasma membrane.



DENTAL PATHOGEN *Streptococcus mutans* **deals with the common sugar sucrose as is shown here, according to Ronald J. Gibbons of the Forsyth Dental Center in Boston. Sucrose is composed of one molecule of glucose (*colored square*) linked to one of fructose (*black square*). The enzyme invertase (*middle*) splits sucrose, releasing the two simple molecules to enter the cell as nutrients. Glucosyltransferase (*left*) splits sucrose and releases the fructose, linking the glucose units to form a long polymer called a glucan, which is insoluble in water and is the major glycocalyx component. Fructosyltransferase (*right*) releases glucose and polymerizes fructose.**



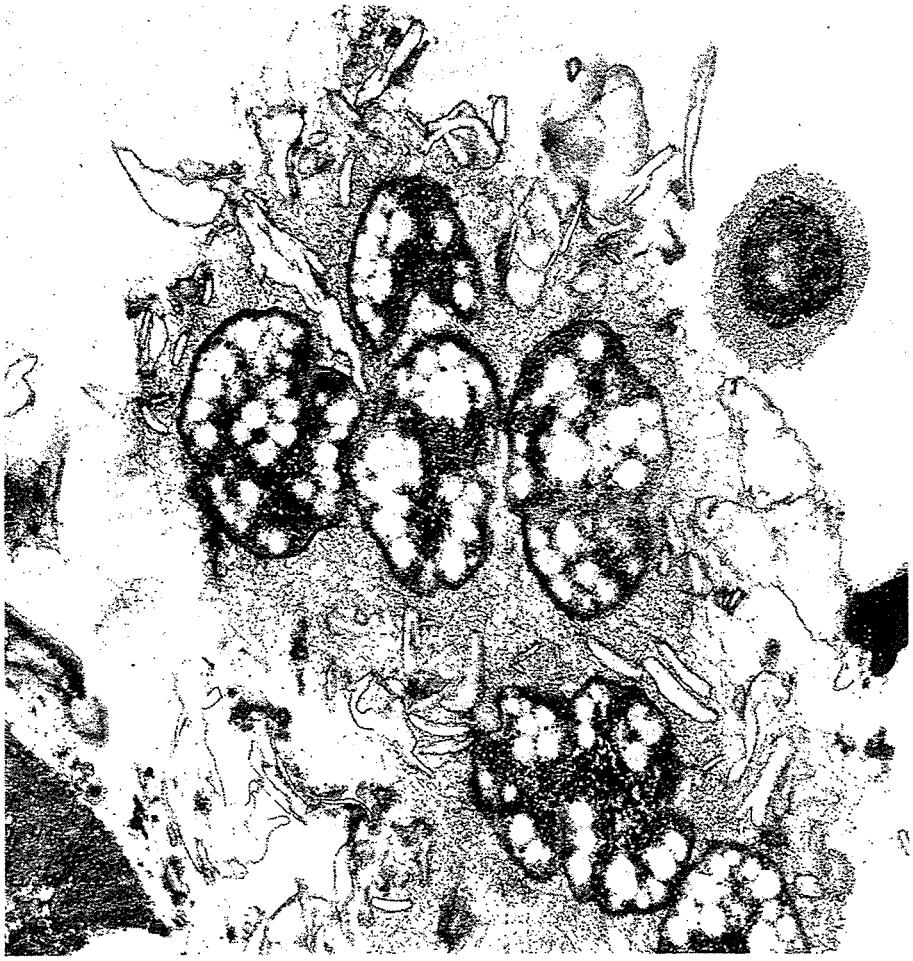
DENTAL PLAQUE, the yellowish film that forms on teeth, is composed of bacteria fixed in a network of glycocalyx material. Some oral bacteria (*dark gray cells at left*) fabricate their own adherent glucans. Others, such as *Streptococcus salivarius* (*light gray*), which colonizes the gum (*right*), make glucans and liberate some of the fibers, which travel, with their polymerizing enzymes, to a nearby tooth surface. There the free glucans can contribute to the formation of a mat that traps various nonspecific bacteria (*white*), building up plaque. Within the plaque the bacterial enzymes (*triangles*) that attack deutal enamel are directed against the tooth, causing cavities.

sponsible for tooth cavities are concentrated against the tooth enamel.

Gibbons' report on dental pathogens directed the attention of investigators to other natural bacterial environments. Roth and other workers looked at various surfaces submerged in water and found they were covered with a film of bacterial polysaccharide. We undertook to investigate the process that creates such a film by placing plastic disks in a rushing mountain stream. Examining the disks with the electron microscope, we found that a bacterium can anchor itself to the inert plastic by spinning a mat of polysaccharide fibers that withstands enormous shear forces. The initial colonization may be accomplished by either bacteria or algae (which have similar polysaccharide fibers), and in time a complex mixed population of cells builds up within a network of fibers. Often one sees within these populations microcolonies of cells of one type, the products of cell division within a common glycocalyx.

It is still not known by just what glue-like bond a glycocalyx fiber is linked to a rock or a plastic. In contrast to inert surfaces, the cells of plants and animals present to bacteria a cell wall or a glycocalyx that is chemically defined by the composition of its own particular polysaccharides. The polysaccharide fibers of the bacterial glycocalyx, which are for the most part negatively charged, can form a polar bond with a higher-cell polysaccharide by way of divalent positive ions in the medium. Lectins with a specific attraction for the glycocalyx fibers and for the higher-cell polysaccharides can also form a bridge between them. Bacteria whose fibers can bind neither directly to those of the higher cell nor to a suitable lectin present in the system simply do not adhere. As a result of the specificity of adherence a higher-cell surface may often be colonized by bacteria of a single species, which may then proliferate to form a microcolony enclosed and anchored by the fibers of its component cells. At that point other bacteria that are not specific for the higher cell may adhere to the glycocalyx of the initial colonizers, forming a mixed population.

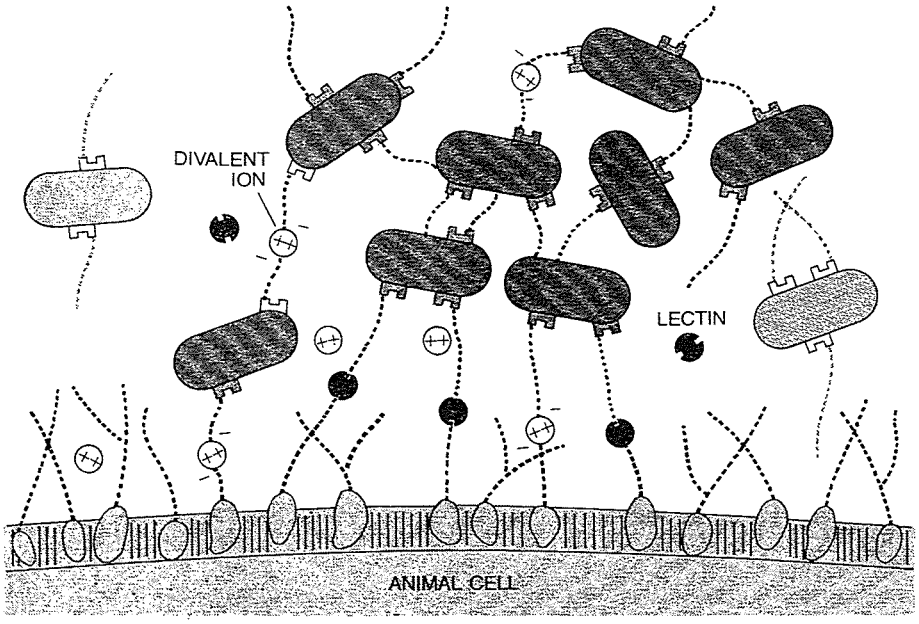
The extent to which a given bacterium will adhere to a particular tissue can be assessed by admitting the bacteria, in a suitable medium, to an experimental chamber with the tissue to be studied as the "floor." After a stated interval the tissue is examined to determine how many bacteria have adhered to the glycocalyx of the tissue cells. Andrew B. Onderdonk of the Veterans Administration Hospital in Boston and his colleagues showed that in such a situation cells of a *Bacteroides fragilis* strain with a well-developed glycocalyx adhere well to the peritoneum lining the body cavity of a rat and that mutant cells that do not produce a glycocalyx fail to adhere. Similar experiments suggest that the nature of the higher-cell glycocalyx changes as the cell ages, which may explain why some cells in the intestine of the cow may be highly colonized whereas other cells in the same tissue are not colonized at all. The recent finding that the glycocalyx is altered in cells that have been infected by a virus may explain the increase in susceptibility to bacterial disease that is often observed to follow viral infection.



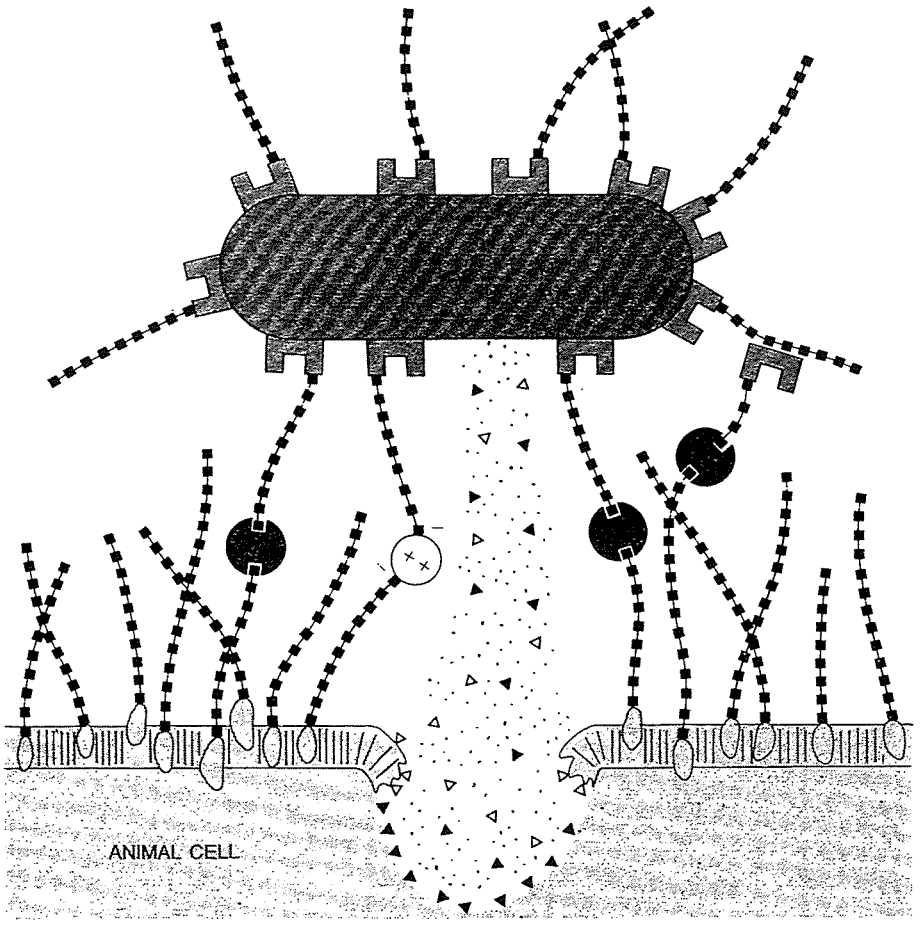
DIVISION OF BACTERIA within a glycocalyx tends to produce a microcolony of sister cells of the same species. Here a microcolony is seen in an enveloping common glycocalyx that also contains remnants of dead cells whose contents have been recycled as nutrients for the cells that survive. A nonmember cell outside the microcolony has its own glycocalyx (*top right*).

If the bacteria native to a rushing stream were not adherent, the stream would be virtually sterile because the bacteria would be swept away much faster than they could swim against the current. We find that a square centimeter of a submerged surface may typically have as many as a million bacteria attached to it, whereas a cubic centimeter of the water flowing over that surface contains only 1,000 bacteria. The adaptive value of adherence in this situation is not hard to understand. The bacteria live on the organic molecules they extract from the passing water. Life in a stationary location with a continuous supply of organic nutrients, and with vigorous aeration and excellent waste removal also provided by the stream of water, clearly agrees with the bacteria: in streams enriched by pollution we have recorded populations as large as 10 billion attached cells per square centimeter. (Such a population forms a thick slimy layer on rocks in the streambed, but it also serves to remove significant amounts of organic pollutants from the water.)

Much as adhesion to a rock benefits the bacteria in a stream, adhesion to a particular tissue provides bacteria that colonize animal or plant cells with a constantly renewed supply of organic nutrients and with physical conditions conducive to growth. For example, Rolf Freter of the University of Michigan has shown that *Vibrio cholera* cells adhere specifically to the "brush border" of human intestinal cells. *Neisseria gonorrhoeae* adhere to the lining of the urethra, and other pathogen-host pairs also exhibit specific adhesion. Adhesion enables infecting bacteria to resist removal, particularly in a system that is nor-



ANIMAL-CELL GLYCOCALYX is also a fringe of polysaccharide fibers, the branching sugar chains on the glycoprotein molecules of the cell membrane (*bottom*). The bacterial and animal-cell glycocalyxes interact as is shown here. The sugar units at the ends of the fibers can interact directly through polar attraction, the negatively charged sugars being linked by a divalent positive ion such as magnesium. The bridge may also be supplied by a lectin (*dark gray*), a protein that carries receptor sites for which the fibers' sugar units have an affinity. The interaction is specific: bacteria (*light gray*) with a different polysaccharide chemistry do not adhere.



BACTERIAL INFECTION may begin with the specific adhesion of a bacterium to a particular animal-tissue cell. The closely packed fibers of the two glycocalyxes provide an environment within which the bacterial toxins and enzymes (*triangles*) diffuse without loss to attack the animal-cell membrane and to digest the contents of the animal cell. The nutrient molecules (*black dots*) thus released from the animal cell are channeled back to the infecting bacterium.

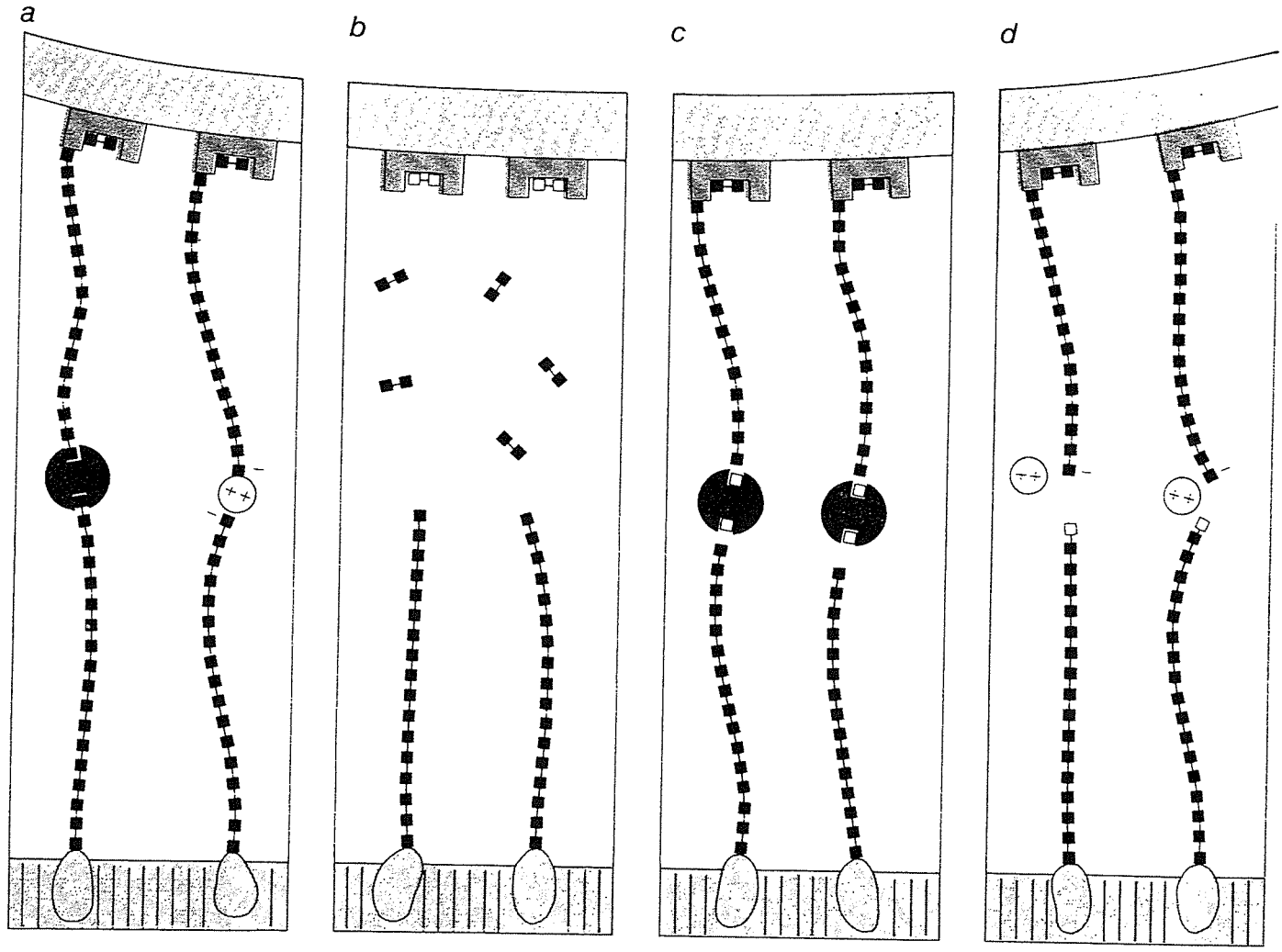
mally sterile. We find that persistent pathogens in the urinary tract are surrounded by well-developed glycocalyxes that keep the cells from being carried away in the urine. The pronounced specificity of some bacteria and viruses that attack only a particular host tissue (as *Salmonella typhimurium* attacks lymphoid patches in the intestine and as the poliovirus attacks nerve cells) may well be explained by the specificity of the glycocalyx of the host-tissue cells.

Among other examples of specificity there are starch-digesting bacteria in the rumen of the cow that adhere to grains of starch so specifically that the best way to separate such cells from the mixed bacterial population of the rumen is to recover the starch grains from the rumen fluid. Again, the luminescent bacteria that populate the light organs of deep-sea fishes such as *Photoblepharon* are selected from the vast population of marine bacteria by their ability to bind to the glycocalyx of the cells that line the cup-shaped luminous organ. In this symbiotic relation the bacteria provide a light source for the fish and in return enjoy a favorable nutritive situation, feeding on secretions of the fish.

The fibers of the glycocalyx may not only position bacteria but also conserve and concentrate the digestive enzymes released by the bacteria and direct them against the host cell. Our micrographs show that enzymes from a bacterium attached to a food source, such as cell-wall material from hay in the cow's rumen, may dig a cavity into which the bacterium slowly works its way. Before aging surface cells of the skin and similar tissues are sloughed off they may be invaded by adherent bacteria that digest pits in them. This suggests that the troublesome persistence of *Staphylococcus aureus* on the skin of health professionals could be due to the fact that these bacteria are hard to eliminate from the deep pits they dig in the skin cells from which they derive their nutrients.

A glycocalyx can also function as a food reservoir for bacteria. The polysaccharide fibers are for the most part negatively charged. Somewhat in the manner of an ion-exchange resin in the laboratory, they can bind nutrient ions and molecules that wander into the immediate environment or are produced by bacterial digestive enzymes, and thus keep them available to the cell.

In nature bacteria are subjected to many sources of stress, against most of which the glycocalyx offers protection. Attachment to a surface protects the bacteria from certain protozoans. The glycocalyx is a physical barrier against predatory bacteria and bacterial viruses, and its binding capacity traps even small harmful ions and molecules in the environment. The protective capacity of the



PATHOGENIC ADHESION might be blocked, in order to prevent or treat infection, by a new kind of antibiotic. The adhesion of a bacterium (*top*) to an animal cell (*bottom*) by means of a polar bond or a lectin (*a*) might be disrupted in one of three ways. An analogue (*white squares*) of the units that are polymerized to form the bacterial glycocalyx might be supplied, occupying the active sites of the polymerizing enzyme and preventing the synthesis of a polysaccharide fiber (*b*). The active sites of the lectin might be blocked by a similar analogue (*c*), or a blocking agent that mimics the glycocalyx material could be supplied to block the animal-cell glycoprotein receptors (*d*).

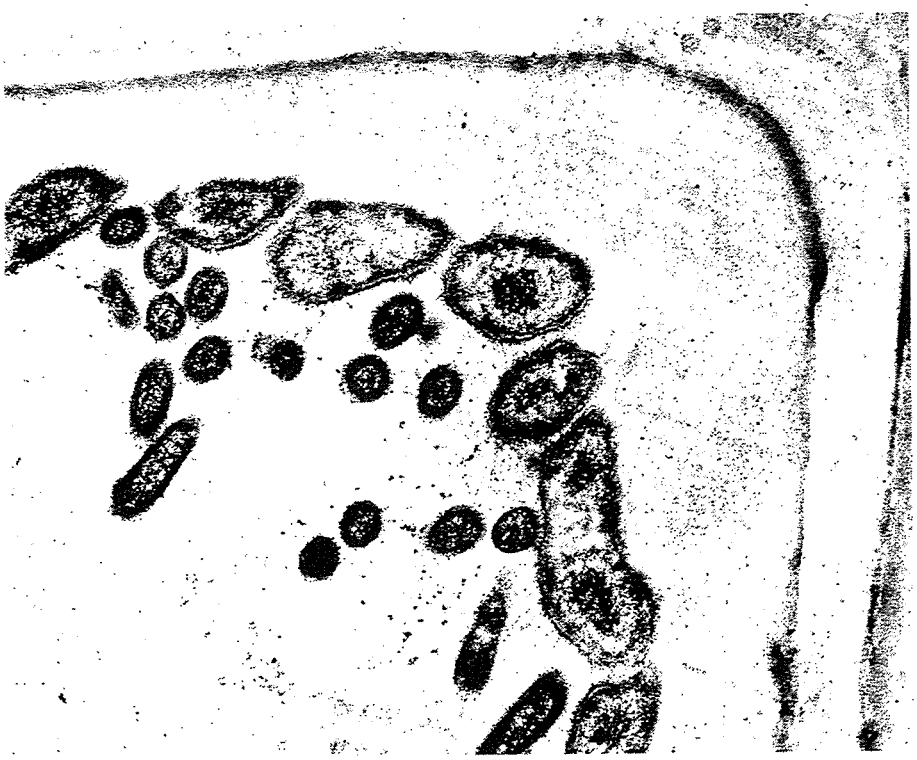
glycocalyx is of particular interest in pathogenic bacteria. We have found that single, uncoated *Pseudomonas aeruginosa* cells introduced into the lung of a rat are quickly phagocytized by the rat's white blood cells but that the defending cells cannot handle microcolonies of the same bacterium enclosed in a glycocalyx. Other experiments indicate that the glycocalyx prevents host antibodies from reacting with the surface of *P. aeruginosa* in the urinary tract.

In some diseases the manufacture of bacterial glycocalyxes, and thus the virulence of the bacteria, seems to be enhanced by excessive concentrations of certain nutrients; this may be the case in cystic fibrosis, in which there may be an excess of sodium and magnesium ions in the affected lung. Some of our preliminary results indicate that antibiotics may not be able to overcome the binding capacity of the glycocalyx in order to reach their bacterial target. Since infecting bacteria in many diseases are surely grouped in microcolonies protected by glycocalyxes, it is clear that antibiotics should be designed with the penetration of those thickets of polysaccharide in mind.

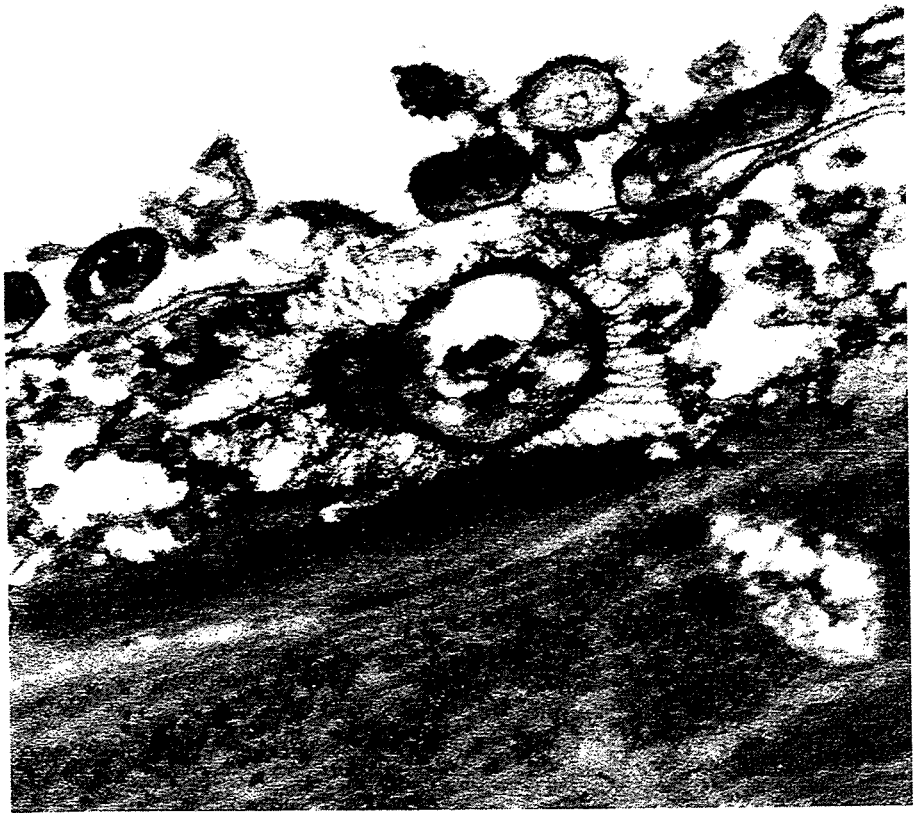
In addition to maintaining bacteria in an advantageous position and protecting them the glycocalyx may at times group bacteria in something approaching an organized community. In lake sediments and in other ecosystems two or more species of bacteria may act together in a "consortium" to carry out physiological processes. Ralph S. Wolfe of the University of Illinois at Urbana-Champaign has described consortiums in which one species of bacterium releases hydrogen from organic compounds and passes it on to another species, which uses it to reduce carbon dioxide to methane (CH_4). A physiological transfer of this kind requires that the members of the consortium be held close together, and that may be accomplished by chemical affinity between the glycocalyxes of the associated cells. Such a physiological consortium seems to be represented in some micrographs we have that show bacteria digesting the cellulose of a plant-cell wall while bacteria of another species are ranged along the cellulose-digesting bacteria's "free" sides.

These bits of evidence suggest a community structure, with cells of a particular species adhering in a favorable niche close to the source of a necessary nutrient. Such adherent populations could respond with unique plasticity to changes in nutrient conditions or in other environmental conditions: bacteria in the "wrong" niche would simply die, leaving space and nutrients for other bacteria more suited to the location. The entire mixed population of cells would have some of the characteristics—but none of the vulnerability—of a multicellular higher organism.

If adhesion has a central role in the success of pathogenic bacteria, then the prevention of adhesion should be an effective way to prevent or combat bacterial infection. It should be possible to develop a new class of antibiotics that interfere with glycocalyx formation or function in specific pathogens. There are at least three ways in which such inhibition might be achieved. One way would be to disrupt the synthesis of glycocalyx



CELLULOSE-DIGESTING BACTERIA (*larger cells*) **adhere to the wall of a plant cell in animal feed. An associated population of smaller bacteria adheres to the large ones, presumably through a specific interaction of the two bacterial glycocalyxes. The two bacterial species may be an example of a "consortium," a cooperative association in which a metabolic product of one bacterial population provides a nutrient that is required by the second population.**



DEAD CELL at the surface of an animal tissue has been invaded by an adherent bacterium, which has broken through the cell membrane and has sunk into a cavity produced by its own digestive activity. A population of other bacteria adheres to the animal cell's membrane.

fibers. The bacterial polymerase that links sugar molecules to form these fibers should be inhibited if it is presented with a compound that mimics its normal substrate and therefore occupies the enzyme's active site, but that cannot be processed to build the normal polysaccharide fiber. In the absence of such fibers there would be no glycocalyx, no adhesion and no resistance to white cells. One might also find a compound that would occupy and block the active site of a lectin mediating the adhesion of bacterial glycocalyx fibers to the fibers of host cells.

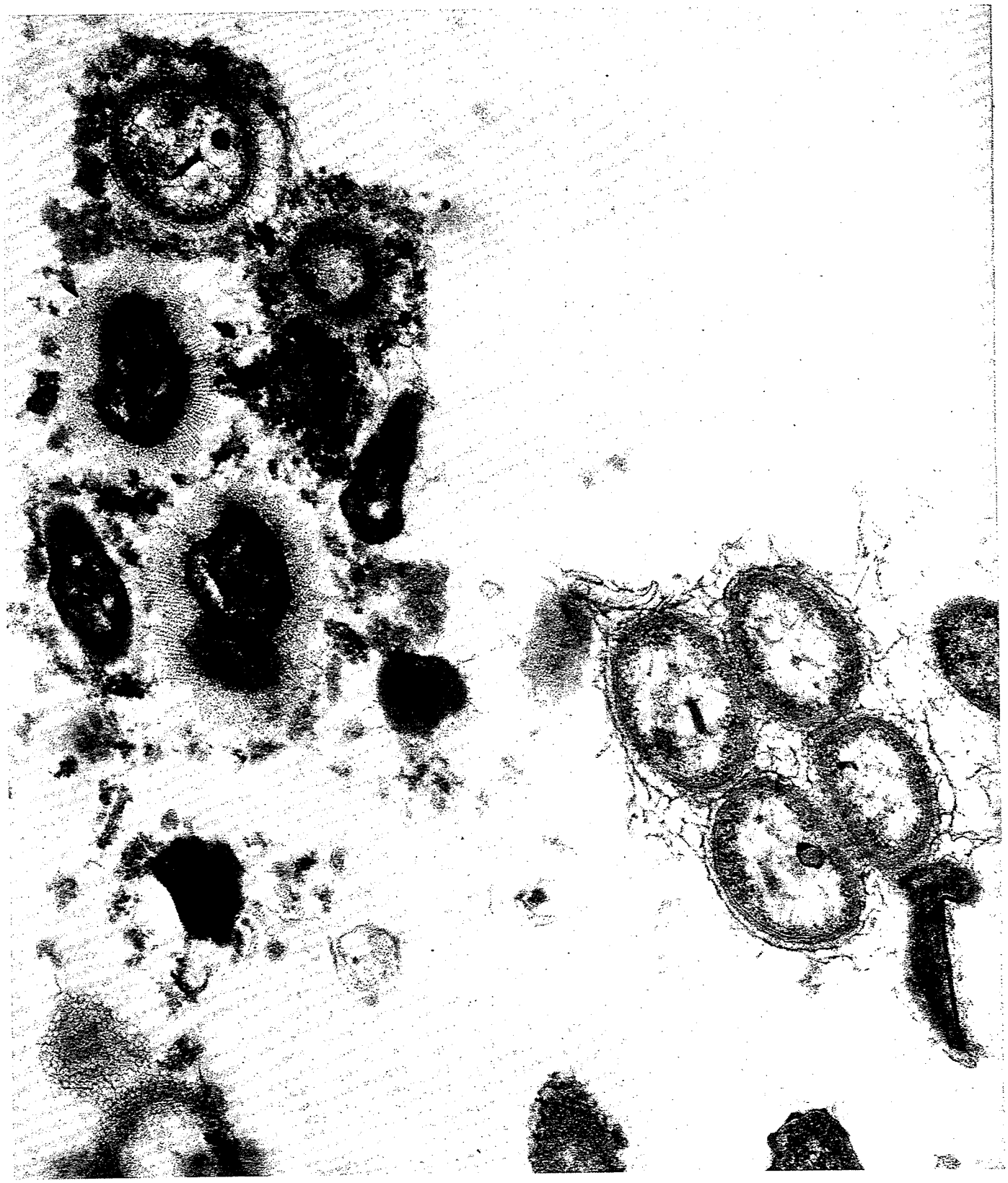
Finally, it should be possible to block the "receptor" sites on host cells, that is, the glycoprotein fibers to which bacterial fibers adhere directly. One attractive aspect of an antibiotic directed against the glycocalyx is that it need not enter the host cells or the bacterial cells, thereby avoiding two common problems in antibiotic therapy: toxicity to host cells and the induction of bacterial resistance based on changes in the permeability of the bacterial-cell membrane.

Perhaps the best initial approach would be the last one listed above. Since polysaccharides are rather simple chains of sugar molecules and since specific adhesion depends on the chemical affinity of bacterial polysaccharides for host-cell polysaccharides, adhesion should be blocked by bits of the glycocalyx material, by its sugar subunits or by chemical analogues of those subunits. Onderdonk has already found that treating mouse peritoneal tissue with glycocalyx material from *Bacteroides fragilis* prevents the attachment of *B. fragilis* cells to the mouse tissue; he attributes this effect to the blockage of the receptor sites on the mouse cells by the glycocalyx polysaccharide. Other workers have reported a similar effect in the crop of the chicken, which normally comes to be covered by a layer of lactobacilli. Treating the crop of germ-free chickens with the polysaccharide of the lactobacillus glycocalyx prevents the formation of the bacterial layer.

Presumably the cells of the human throat have receptors for the streptococci that cause "strep throat" and its more serious aftereffects, including rheumatic heart disease. It should be possible to block those receptors with simple sugar molecules that are analogues of the polysaccharide units of the streptococcal glycocalyx. The task of identifying those polysaccharides and developing analogues would be particularly worthwhile in the case of streptococcal infection and other diseases where repeated infections can have serious effects. The practice in such conditions is to avoid reinfection by long-term prophylactic treatment with a conventional antibiotic, and that can be dangerous. The antibiotic kills many of the nonpathogenic

adherent bacteria that are normally present and that occupy many of the receptor sites, thus opening the way to colonization by drug-resistant strains of the pathogenic bacteria, strains that tend to be selected by the very treatment designed to suppress the pathogen. An antiglycocalyx therapy would make it unnecessary to maintain the patient on a conventional antibiotic.

The development of new ways to control bacterial infections will require a much more detailed knowledge than we now have of the polysaccharide constituents of particular pathogens and host tissue cells. That will take time. The basic perception that bacteria colonize their environments by adhering tenaciously and specifically is a first step toward an ability to manipulate and control bacteria more effectively.



FIBERS OF GLYCOCALYX display a wide variety of patterns in electron micrographs of thin sections. The fibers of this mixed population from a cow's rumen form (*clockwise from lower right*) patterns designated random, honeycomb, radial and radial-concentric.