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Polysaccharides in Soil Fabrics

Abstract. *Treatment of ultrathin sections of natural soil fabrics with heavy metal stains, specific for carbohydrates, showed that polysaccharides are widely distributed in soils. In addition to being associated with living cells and dead remains of plants and microbes, carbohydrates also coat clay platelets and occur in crevices of submicron size within mineral aggregates. The determination of the precise location of polysaccharides in soils explains in part why some carbohydrates are resistant to microbial degradation and why small quantities of microbial polysaccharides are able to stabilize clay aggregates.*

Between 50 and 80 percent of the dry weight of plants consists of carbohydrates (*1*), the most abundant organic materials entering soils (*1, 2*). Carbohydrates constitute between 5 and 10 percent of the soil organic matter (*3*), and they are important in the maintenance of aggregate stability, in the ion-exchange properties of soils, and in the nutrition of heterotrophic soil microorganisms (*2, 3*). Although chemical analyses of carbohydrates extracted from soils have provided qualitative and quantitative data on the total amount of carbohydrates (*1, 2*) and on the relative proportions of individual monosaccharides in soils (*1*), the precise location of most carbohydrate in situ in natural soil fabrics is unknown.

With the solution of the technical problems arising from the preparation of ultrathin sections of soils, it is possible to locate soil components in situ, provided that the components are electron-opaque (*4*). Soil minerals have a natural intrinsic electron density, and many organic components associated with living plant, animal, and microbial cells can be made electron-dense if one modifies the techniques commonly used in the preparation of biological tissues for electron microscopy (*4–6*). Using these methods, one can identify fragments of decomposing tissues down to submicron sizes (*4*) and recognize humified amorphous materials almost to their macromolecular dimensions. Unfortunately, however, many carbohydrates do not react with the fixatives and heavy metal stains normally used in electron microscopy (*4, 6, 7*). Thus the location of carbohydrates in ultrathin sections of soil fabrics has been inferred on morphological rather than histochemical grounds. Direct localization of polysaccharides in situ in undisturbed soil fabrics has largely been restricted to those materials such as root or microbial mucilages whose position in the fabric is already known (*4–6*) and whose slight electron density may be attributable to the traces of noncarbohydrate materials such as proteins and polyphenols that they contain (*7*). The presence of carbohydrates near biological objects can sometimes be inferred from a lack of electron density in an otherwise electron-opaque fabric. For example, in soil fabrics containing microorganisms (Fig. 1) there is usually an electron-transparent space surrounding bacteria into which the clay platelets do not penetrate. Since soil organisms are known to secrete carbohydrate slimes and capsule materials, it is assumed that the apparent "void" is filled with electron-transparent microbial polysaccharide, but the nature of the majority of electron-transparent spaces of submicron size (for example, *V* in Fig. 1) cannot be determined with the same confidence. Roots that secrete carbohydrate gels may occupy less than 1 percent of the bulk soil volume, and microbial cells constitute only a small percentage of the soil biomass (*8*). Thus, the polysaccharide associated with living cells does not account for all the carbohydrate found as a result of chemical analysis of soils. It is therefore of particular importance to locate carbohydrate in situ in natural soil fabrics by positive and specific histochemical reactions.

An extensive literature has accumulated on methods for positively staining polysaccharides at the ultrastructural level (*9–12*). Of these, the ruthenium red/OsO_4 (Ru/Os) stain for acidic polysaccharides and the silver methenamine and silver proteinate methods for carbohydrates reactive to periodic acid–Schiff bases (PA/Ag) have proved to be the most reliable and least controversial (*9, 10*).

The Ru/Os stain of Luft is generally assumed to stain carbohydrates containing polyuronides and has been widely used to demonstrate microbial slimes and capsule materials in natural environments including tissues and soil fabrics (*4*). Figure 2 shows Ru/Os-reactive fibrous materials in a natural soil fabric containing microorganisms. The fibers are about 8 to 10 nm in diameter and of indeterminate length, but they stretch across fine pores of submicron diameter to form an open mesh in which small stacks of clay platelets become entangled. These fibrous networks are commonly observed in crumbs from A horizons of soils under pasture, and the fibrils may persist long after the death of the microorganisms that secreted them (*4*).

Not all Ru/Os-reactive carbohydrates in soils are fibrous, however. Both roots (*5*) and bacteria (*4, 6*) secrete gels that are granular in texture, and small pockets of granular material are often observed in soils in the absence of living cells. These deposits are small but widespread and are found in crevices between stacks of clay platelets (Fig. 3). This means that not only are the fine pores occluded, but that the clay stacks are bound together by the gels.

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Polyuronide-containing carbohydrates probably constitute less than 10 percent of the polysaccharides in soils (*1*), and so, even after Ru/Os staining, up to 90 percent of the carbohydrate may be undetected. Recently a number of reliable histochemical techniques have been introduced for the location of carbohydrates which contain 1,2-diol groups (*vic*-glycol groups) (*10–12*). These methods depend on the generation of aldehyde groups by oxidation with periodic acid, and silver is attached to the aldehyde group by means of a bridging organic complex. These reactions have been shown to be quite specific for 1,2-diol groups, provided that the tissues have already been treated with OsO_4 to block other PA/Ag-reactive sites such as sulfhydryl groups (*11*). These methods have been widely used in biomedical investigations and to a lesser extent in preparations of plant materials and microorganisms (*9–12*). Humified materials constitute up to 90 percent of the soil organic matter (*1, 2*), and they stain densely with OsO_4 because of the polyphenolic and proteinaceous materials they contain (*7*). However, treatment with periodic acid removes osmium from the sections (*11*), and so after the PA/Ag treatments any electron density not due to soil minerals must be derived from silver-reactive carbohydrate.

Using the periodate–silver methenamine method, I have been able to locate carbohydrate in soil fabrics not only in fragments of cell walls of bacteria (*b* in Fig. 4) and plants (*w* in Fig. 4) but also in micropores between clay platelets where morphologically recognizable material cannot be seen. Some of the carbohydrate-filled pores are less than 0.05 μm in diameter. Silver is also located at the surface of the majority of clay platelets.

The silver grains produced by the silver methenamine reagent are 10 to 15 nm in diameter and may therefore obscure fine morphological detail remaining in the polysaccharides they detect. The silver proteinate reagent of Thiery (*12*) produces a much finer electron-dense deposit and may therefore be used to localize polysaccharides at much higher resolution. In Fig. 5 it can be seen that the silver proteinate has labeled the cell wall and granules of carbohydrate of a soil bacterium (*b*) while the storage product (presumably polyhydroxybutyrate) of an adjacent cell is unaffected. Also revealed are fine threads of capsule materials (*c*), which bind clay particles together across a void about 0.1 μm in

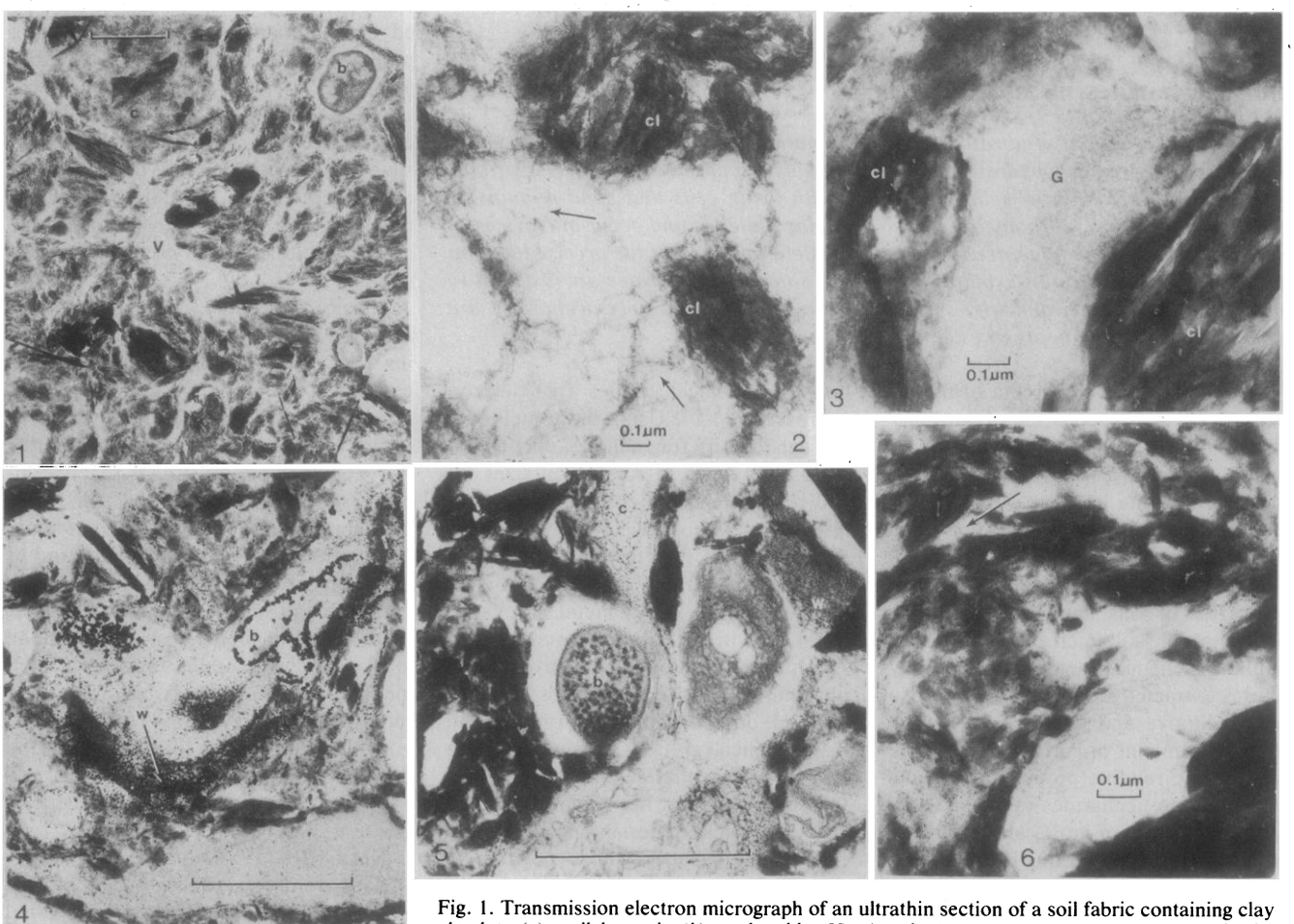

Fig. 1. Transmission electron micrograph of an ultrathin section of a soil fabric containing clay platelets (*c*), soil bacteria (*b*), and voids (*V*). An electron-transparent space surrounds the bacterium after conventional fixation. The soil was fixed in 3 percent glutaraldehyde-acrolein in 0.05*M* phosphate buffer, *p*H 7.3, for 3 hours at 4°C. After 3 hours the buffer was washed off at 4°C, and the soil was fixed in 1 percent OsO_4 for 15 hours, then dehydrated in a graded series of *tert*-butyl alcohol, and embedded in Spurr's medium. Fig. 2. Preparation as in Fig. 1, except that 0.01 percent ruthenium red was added to the fixatives. Clay bundles (*cl*) are linked by Ru/Os-reactive fibrils (arrows). Fig. 3. Preparation as in Fig. 2. Micropores between the clay platelets were filled with granular Ru/Os-reactive carbohydrates (*G*). Fig. 4. Fixation as in Fig. 1. Sections were treated with periodic acid and silver methenamine to demonstrate carbohydrates with 1,2-diol groups (*11*). Carbohydrates are associated with plant cell wall material (*w*) and old bacterial cell wall (*b*) and occur in voids and at the surface of clay minerals. Fig. 5. Fixation as in Fig. 1. Sections were treated with periodic acid, thiocarbohydrazide, and silver proteinate (*12*). Carbohydrates are demonstrated in the bacterial cell wall (*w*), bacterial reserves (*b*), and on fibrous material and clay minerals (*c*). Fig. 6. Fixation and staining as in Fig. 5. Carbohydrates are in microvoids (arrow) and on clay minerals. (The scale bar in Figs. 1, 4, and 5 is 1 μm.)

diameter. Humified cell wall material, which can be identified by its characteristic ultrastructure, also contains some carbohydrate (*W*). Small deposits of amorphous carbohydrate fill pores less than 0.03 μm in diameter in the clay fabric, as shown in Fig. 6 (arrow), and silver-reactive material coats many of the clay platelets as in the silver methenamine–treated soils.

The use of polysaccharide-specific stains on ultrathin sections provides new information on the morphology and distribution of organic matter in natural soil fabrics. As expected, carbohydrates are seen to be associated with roots and soil microorganisms and with dead tissue fragments and humified materials, but some is associated with the clay fraction. The precise morphology and location of polysaccharides in clay fabrics explains in part why some carbohydrates are protected from microbial attack and why small quantities of added polysaccharide have so profound an effect on soil stability. Some carbohydrates may be immune to decay simply because they are physically separated from soil microorganisms.

Although bacterial populations in soils are large (10^9 cells per cubic centimeter), they are mainly aggregated in rhizospheres and in colonies near cell wall fragments (*5*, *6*). Very few bacteria lie in the clay fabric as in Fig. 1, and only the carbohydrate in their immediate vicinity will be available to them. Since most bacteria are ≧ 0.3 μm in diameter, they are unable to enter the finer crevices and lyse the more substantial deposits of polysaccharide inside. Bacteria isolated in clay fabrics are therefore frequently devoid of stored material (Fig. 1), in marked contrast to those in adjacent rhizospheres (*6*). Much carbohydrate associated with clays will remain inaccessible to the microflora until the crevices are opened by the mechanical action of soil animals or until the bacteria are brought in contact with carbohydrate deposits by the effects of experimental manipulations (*13*). It is unlikely that normal agricultural practices open such small pores.

As soil dehydrates, surface tension effects will tend to concentrate polysaccharides in crevices between adjacent clay platelets and these will be bonded strongly where their edges overlap. In this manner a small amount of carbohydrate may give rise to strong boxlike cardhouse structures (*14*), which will be resistant to dispersion or collapse, especially if the polysaccharides become irreversibly denatured on dehydration. Since the individual threads of the fibrous polysaccharides produced by microorganisms are so small in cross-sectional area, a small mass will contain a large length of fiber and so will enmesh large volumes of fabric. This explains why quantities as small as 0.02 to 0.2 percent of added microbial carbohydrate markedly stabilize clay aggregates (*3*).

Because they are electron-dense, Ru/Os and PA/Ag deposits produce bright backscatter electron images, and they may therefore be used to demonstrate carbohydrates in uncoated fabrics in the scanning electron microscope as well as in the transmission electron microscope mode. Treatment with hydrolytic enzymes specific for particular carbohydrate linkages in advance of Ru/Os or PA/Ag treatment allows individual types of carbohydrate to be located and identified in soil fabrics (*15*).

R. C. FOSTER

Division of Soils, Commonwealth Scientific and Industrial Research Organization, Private Bag No. 2, Glen Osmond, South Australia 5064

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Hemin Lyses Malaria Parasites

Abstract. *Malaria parasites isolated from mouse erythrocytes are lysed by ferriprotoporphyrin IX chloride (hemin) or by a chloroquine-hemin complex in amounts that could be produced by release of less than 0.1 percent of the heme in erythrocytic hemoglobin. This effect of hemin may explain the protection against malaria provided by thalassemia and other conditions causing intracellular denaturation of hemoglobin. The toxicity of the chloroquine-hemin complex may explain the selective antimalarial action of chloroquine.*

Recently it was found that ferriprotoporphyrin IX chloride (hemin) lyses *Trypanosoma brucei* (*1*) and normal erythrocytes (*2*) and that the formation of a chloroquine-hemin complex in erythrocytes does not inhibit hemolysis (*2*). These observations have important implications for the biology and chemotherapy of malaria. Erythrocytic malaria parasites exist in an environment rich in hemoglobin and could be exposed to toxic amounts of heme when hemoglobin undergoes denaturation, as occurs spontaneously in hemolytic anemias associated with the production of Heinz bodies (*3*). Malaria parasites also degrade hemoglobin and produce a large amount of heme, which normally is sequestered in

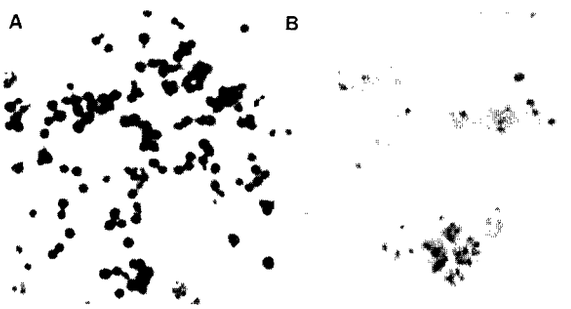

Fig. 1. Microscopic appearance of isolated *P. berghei* (×1100). (A) Control view. The parasites were suspended (10^7 per milliliter) in the standard medium and incubated. At the end of the incubation period glutaraldehyde was added to achieve a final concentration of 2 percent and the mixture was left at room temperature for 20 minutes. The mixture was centrifuged and the pellet was washed once with standard medium. Smears of the washed pellet were made on albumin-coated glass slides, air-dried, and stained. (B) Parasites from a portion of the same suspension used in (A), incubated in the presence of 20 μ*M* hemin. Treatment of the parasites with a complex formed from 20 μ*M* hemin and 5 μ*M* chloroquine produced results similar to those seen in (B); chloroquine alone caused no change.

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