revealed in the graph of labeled mitoses by depressions in the plateaus of the curves at approximately their midpoints. Hence the curves, though comprising two peaks, represent only one population of cells whose cell-cycle time is expressed by the horizontal distance between two consecutive curves at the deepest points of their depressions.

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Immunochemistry of Newly Found Substituents of Polysaccharides of Rhizobium Species

Abstract. *Acetyl and pyruvic acid groups were detected and estimated in the extracellular polysaccharides of* Rhizobium trifolii *and* R. meliloti. *Pyruvic acid was found also in the specific polysaccharide of pneumococcal type 27, and is believed to explain the cross-reactivity between antiserum to pneumococcal type 27 and these and other pyruvate-containing bacterial polysaccharides. Removal of pyruvate from a* Rhizobium *polysaccharide rendered it inactive with its homologous antiserum and with antiserum to pneumococcal type 27, but it then reacted with several antiserums to other pneumococcal types with which the intact material was unreactive.*

Extracellular polysaccharides of strains of *Rhizobium trifolii* exhibited anomalous behavior. The polysaccharide of strain TA1 (hereinafter referred to as TA1), after exhaustive methylation by usual standard techniques showed a methoxyl content of only 31 percent, yet no free hydroxyl groups were detectable in the infrared spectra (*1*). There was also a large discrepancy in the content of uronic acid found in TA1 by decarboxylation (23 percent), by the carbazole reaction (23 percent), and by titration with dilute alkali (42 percent) (*1*). Although the polysaccharides of five strains of *R. trifolii* contained glucose, galactose, and glucuronic acid in the same relative proportions, as judged by chromatographic comparisons, they displayed little serological cross-reactivity, but reacted well with their homologous antiserums by double diffusion in agar. Moreover, when tested with antiserums to pneumococcal types 1 to 29, they failed to cross-react significantly except in antiserum to pneumococcal type 27. This was also one of the antipneumococcal serums that reacted with polysaccharides of *R. meliloti*, which are known to contain very little uronic acid (*2*). Partial hydrolysis of TA1 with 0.01*N* HCl for 20 hours at 100°C eliminated some oligosaccharides, but 65 percent of the polysaccharide was recovered with apparently unchanged composition with respect to the sugars. This degraded TA1 no longer precipitated antiserum to TA1 or to pneumococcal type 27 but now showed precipitation with antiserums to types 6, 7, 8, 9, 10, and 14.

Table 1. Acetyl and pyruvic acid substituents in polysaccharides of *Rhizobium*. Results shown are for air-dried samples.

Polysaccharide	Acetyl (%)	Pyruvic acid (%)
R. meliloti strain B	4.5	4.7
strain F	4.1	5.0
R. trifolii strain TA1	4.1	9.0
strain UNZ29	3.7	10.4
strain WA67	3.3	9.1
strain CC10	2.1	8.8
strain 2480a	4.0	9.2
R. radicicolum	2.2	7.8
Pneumococcal type 27	7.0*	8.7
R. trifolii strain TA1		
Acid-degraded	4.4	0.3
NaOH-treated	0	6.9

* From R. Brown (*14*).

All these results suggested the presence of hitherto undetected "masking" groups or side chains. Accordingly, the polysaccharides were analyzed for phosphate, *O*-acetyl (*3*), and pyruvic acid (*4*). Both acetyl and pyruvate were found in all of the *Rhizobium* polysaccharides examined (Table 1); they were free of phosphate. The acetyl of TA1 and the pyruvate of all the *Rhizobium* polysaccharides were identified chromatographically as the hydroxamate and the 2,4-dinitrophenylhydrazones, respectively, by comparison with authentic standards. The hydroxamates were separated in an ethyl acetate, acetic acid, formic acid, water system (18:3:1:4, by volume) and in an ethyl acetate, pyridine, water system (5:2:5, by volume) and detected with alcoholic acidic ferric chloride; chromatograms of the 2,4-dinitrophenylhydrazones were developed in a butanol, ethanol, water system (5:1:4, by volume) (*5*). These substituents, which the amounts found in TA1 indicate to be present on approximately one-third of the sugar residues, provide an explanation for the low methoxyl and high titratable acid values obtained with this polysaccharide.

Mild treatment of TA1 with acid, as above, resulted in the selective removal of the pyruvate while 0.2*N* NaOH at room temperature for 22 hours displaced the acetyl groups (Table 1). Loss of pyruvic acid abolished serological activity with antiserums to TA1 and to pneumococcal type 27 while removal of acetyl led to loss of reactivity only with antiserum to TAl.

Because pyruvate groups could be implicated in the specificity of pneumococcal type 27, we analyzed the specific polysaccharide of this pneumococcal type for pyruvic acid, and it was found to contain 8.7 percent. Antiserum to type 27 had already been shown to react strongly with the polysaccharides of *Rhizobium radicicolum* and *Xanthomonas campestris* (*6*). The former, which contains glucose and glucuronic acid (*7*), was analyzed and found to contain pyruvate and acetyl (Table 1); the presence of both groups (approximately 10 percent acetyl, 3.0 to 3.5

percent pyruvic acid) in the latter polysaccharide was known (*4*, *8*).

These results show that pyruvate groups, which have also been found in agar (1 percent) (*9*) and in polysaccharides of *Corynebacterium insidiosum* (*10*), *Klebsiella rhinoscleromatis* (8.3 percent (*11*), a *Pseudomonas* strain (4.3 percent) (*8*), and in the polysaccharides of all *Xanthomonas* species so far examined (1.0 to 7.6 percent) (*8*), are also important substituents in the polysaccharides of *Rhizobium trifolii* and *R. meliloti* and function as a major determinant in their serological specificity. Pyruvate groups or pyruvylated sugars also appear to account for the cross-reactivity between antiserum to pneumococcal type 27 and the diverse polysaccharides containing pyruvate substituents; the type 27 polysaccharide had been shown to contain glucose, galactose, rhamnose, glucosamine, and phosphate (*12*). However, despite the presence of pyruvate and acetyl groups and the similarity of their sugar composition, it is still surprising that the polysaccharides of *R. trifolii* show very little cross-reactivity with each other.

In that current views ascribe a large part to polysaccharides in the specificity of the interaction between *Rhizobium* species and their plant hosts (*13*), our findings raise questions as to whether the newly demonstrated acyl groups or the corresponding acylated sugars are mediators of these, as well as of immunological specificities.

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Visualization of Nucleolar Genes

Abstract. *The presence of extrachromosomal nucleoli in amphibian oocytes has permitted isolation and electron microscopic observation of the genes coding for ribosomal RNA precursor molecules. Visualization of these genes is possible because many precursor molecules are simultaneously synthesized on each gene. Individual genes are separated by stretches of DNA that apparently are not transcribed at the time of synthesis of precursor rRNA in the extrachromosomal nucleoli.*

During early growth of the amphibian oocyte, the chromosomal nucleolus organizer is multiplied to produce about a thousand extrachromosomal nucleoli within each nucleus (*1*). There is convincing evidence that these nucleoli function similarly to chromosomal nucleoli in the synthesis of rRNA precursor molecules (*2*). In thin sections of fixed oocytes, each extrachromosomal nucleolus typically shows a compact fibrous core surrounded by a granular cortex (Fig. 1). Previous studies have shown that only the core region contains DNA, whereas both components contain RNA and protein (*3*). The large size of the amphibian oocyte nucleus (*4*) allows rapid isolation and manipulation of the extrachromosomal nucleoli before extensive denaturation and cross-linking of proteins occurs. If saline of low molarity or deionized water is used as the isolation medium, nucleolar cores and cortices can be separated and the DNA-containing cores dispersed for electron microscopy (*5*).

Each unwound isolated nucleolar core consists of a thin axial fiber, 100 to 300 Å in diameter, that is periodically coated along its length with matrix material (Figs. 2 and 3). The axial fiber of each core forms a circle, and treatment with deoxyribonuclease breaks the core axes. The diameter of trypsin-treated axial fibers (about 30 Å) suggests that the core axis is a single double-helix DNA molecule coated with protein (*6*). The matrix segments along a core axis exhibit thin to thick gradations, and show similar polarity along the axial fiber. Each unit is separated from its neighbors by matrix-free axis segments.

Nucleolar core axes are stretched to variable degrees depending on preparative procedures. For example, drying preparations out of deionized water before staining causes little or no stretching of axial cores (Fig. 2), whereas precipitating preparations with acetone staining solution before drying stretches the core axes to varible degrees over the grid surface (Fig. 3). When regions of core axes appear unstretched or uniformly stretched, the matrix units along a specific region are similar in length; unstretched matrix units are 2 to 2.5 μ long but can be 5 μ long after severe stretching. The matrix-free segments between matrix units also show variations in length due to stretching, but, in addition, exhibit differences in length independent of stretching (Fig. 3). Most matrix-free segments are about one-third the length of adjacent matrix units, but bare regions up to ten times as long as neighboring matrix

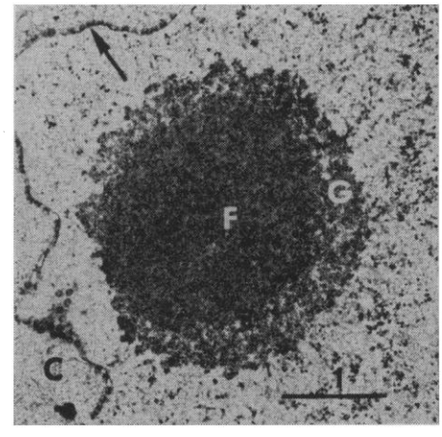

Fig. 1. Thin section of extrachromosomal nucleolus from *Triturus viridescens* oocyte. A granular cortex (*G*) surrounds a compact fibrous core (*F*). Portions of the nuclear envelope (arrow) and cytoplasm (*C*) are visible. Conventional osmium tetroxide fixation, Epon embedding, and uranyl acetate staining. Scale, 1 μ.