trical stimulation of this region of the hypothalamus produced an increase in plasma thyrotropin concentration (*19*), and pituitary implants in this area show thyrotrope differentiation and stimulate thyroid function (*20*). Measurements of TRH remaining in the hypothalamus after lesions were also compatible with its being localized in the thyrotrophic area (*21*). In our study TRH was found in highest concentrations within nuclei of the thyrotrophic area, but was found outside this area as well.

The distribution of TRH differs from that of the luteinizing hormone releasing hormone, which is found almost exclusively in the arcuate nucleus and median eminence (*22*). The fact that TRH is distributed among several nuclei suggests that more detailed anatomical, physiological, and pharmacological studies of these nuclei are needed to ascertain their individual roles in generating the neuroendocrine (*4, 23*) and behavioral (*24*) effects that have been attributed to TRH. In addition, it has recently proved possible to measure norepinephrine (*7*), dopamine (*7*), serotonin (*8*), histamine (*9*), and choline acetyltransferase (*10*) in isolated hypothalamic nuclei. It is hoped that the part played by one or more of the biogenic amines in the control of TRH release can be established.

MICHAEL J. BROWNSTEIN
MIKLOS PALKOVITS
JUAN M. SAAVEDRA
Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20014
RABIM M. BASSIRI
ROBERT D. UTIGER
Endocrine Section, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia 19104

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Lectins: A Possible Basis for Specificity in the Rhizobium–Legume Root Nodule Symbiosis

Abstract. *Soybean lectin labeled with fluorescein isothiocyanate combined specifically with all but 3 of 25 strains of the soybean-nodulating bacterium* Rhizobium japonicum. *The lectin did not bind to any of 23 other strains representative of rhizobia that do not nodulate soybeans. The evidence suggests that an interaction between legume lectins and* Rhizobium *cells may account for the specificity expressed between rhizobia and host plant in the initiation of the nitrogen-fixing symbiosis.*

Roots of many legume plants form symbiotic nitrogen-fixing relationships with soil bacteria of the genus *Rhizobium*. A considerable degree of specificity often is manifest between bacteria and legume host. Rhizobia that initiate the symbiotic root nodule structure in soybeans, for example, are incapable of forming root nodules with clover, alfalfa, garden beans, or others. Such specificity is the basis for cross-inoculation classification in legume production and for species differentiation in the genus *Rhizobium*. Management of the legume root–*Rhizobium* interaction provides the main means by which biological nitrogen fixation is harnessed for agricultural production (*1*).

A major unresolved problem related to *Rhizobium*-legume specificity is the mechanism whereby this specificity is expressed. Hamblin and Kent (*2*) explored the hypothesis that lectin may bind the bacteria to the roots. They worked with a single strain of *R. phaseoli* and found that lectin-treated bacteria were capable of agglutinating erythrocytes, but they did not address the question of specificity. Our investigations were based on a similar hypothesis and led to techniques whereby reactions between soybean lectin and soybean rhizobia (*R. japonicum*) could be observed by direct microscopy. We sought to examine the specificity of the lectin-*Rhizobium* interaction.

Lectins were prepared from ground and defatted seeds and roots of soybean (variety 'Chippewa') according to the procedures of Liener and Pallansch (*3*) leading to their fraction II. The seed lectin had a protein content of 12 mg/ml (*4*) and a hemagglutination titer of 6400 (*5*), as compared to 1.5 mg/ml and 128 for the soybean root lectin. The low titer of the soybean root preparation nonetheless reflected substantial lectin activity since controls gave no hemagglutination.

Soybean seed lectin was conjugated (*6*) with the fluorochrome fluorescein isothiocyanate for use as a stain in the microscopic examination of rhizobia. Bacteria were observed by fluorescence microscopy (*7*) after staining with the fluorochrome-labeled lectin to detect the

Table 1. Binding of soybean lectin labeled with fluorescein isothiocyanate to different strains of several species of *Rhizobium*. Smears from cultures 6 to 10 days old were air-dried, heat-fixed, and stained for 20 minutes with labeled lectin diluted 1 : 4 in phosphate-buffered saline. The stained smears were washed with the buffer for 15 minutes, a cover slip was mounted on the preparation, and the slides were examined by fluorescence microscopy with a Zeiss Universal microscope. The extent of the lectin-binding reaction is indicated by the following symbols: (−) negative; (+) occasional cells (fewer than 1 percent) positive with 4+ fluorescence, the remainder negative; (++) some cells (1 to 5 percent) positive with 4+ fluorescence, the remainder negative; (1+ to 4+) most of the cells positive, with these increasing intensities of fluorescence.

Rhizobium strain	Reaction
R. japonicum	
USDA 24	4+
USDA 29	+
USDA 31	+
USDA 38	4+
USDA 62	+
USDA 71	+
USDA 110	4+
USDA 117	+
USDA 123	3+
USDA 126	++
USDA 135	+
USDA 138	4+
USDA 140	4+
Nitragin 61A24	4+
Nitragin 61A72	−
Nitragin 61A89	4+
Nitragin 61A92	1+
Nitragin 61A93	−
R. japonicum	
Hill 1	4+
Hill 3	4+
Hill 5	4+
R54A	4+
Wa-16-1	+
W-80	4+
Wisc 505W	−
R. leguminosarum	
Nitragin 128C19	−
Nitragin 128C50	−
Nitragin 128C53	−
Nitragin 175P2	−
Nitragin 9201	−
R. meliloti	
Nitragin 102F28	−
Nitragin 102F32	−
Nitragin 102F33	−
Nitragin 102F34	−
Nitragin 102F49	−
R. phaseoli	
Nitragin 127K17	−
Nitragin 127K29	−
Nitragin 127K30	−
Nitragin 127K31	−
Nitragin 127K32	−
R. trifolii	
Nitragin 16284	−
Nitragin 162P17	−
Nitragin 162S24	−
Nitragin 162X6	−
Nitragin 162Z3	−
Miscellaneous	
Jackbean 22A1	−
Lima bean 127E17	−
Cowpea 176A22	−

lectin-rhizobia reaction. Two reactions typical of lectin binding by strains of *R. japonicum* are illustrated in Fig. 1. Figure 1A is a photomicrograph of *R. japonicum* strain USDA 110, one of several positively reactive strains which bound copious amounts of labeled lectin. The diffuse, often fan-shaped, white zone associated with the cells represents bound lectin (this zone has strong yellow-green fluorescence under the microscope). Figure 1B shows *R. japonicum* USDA 138, which like several others was strongly positive for fluorescence, but the lectin (arrow a) was localized and well defined about the cell. The typical negative reaction is seen here in the form of certain cells (arrow b) which did not combine with the lectin. The unstained (negative) cells were detected by use of transmitted dark-field white light, while the fluorescent (positive) cells were illuminated by incident, near-ultraviolet light.

Table 1 summarizes our observations on the specificity of the soybean lectin–rhizobia interaction. All rhizobia that bound the soybean lectin were soybean rhizobia, *R. japonicum*; all other species of *Rhizobium* included in the experiment were completely negative. Among the 25 strains of *R. japonicum*, 14 reacted with the maximum fluorescence (4+) shown by most of the cells in a field. In one strain, Nitragin 61A92, most cells were reactive but bound very little lectin, giving a 1+ reaction. Of the remainder, three strains were entirely negative, and seven others were positive only with respect to occasional lectin-binding cells among a vastly greater population of negative cells.

We have found no way in preliminary experiments to induce the specific lectin-binding reaction in the three frankly negative strains of *R. japonicum*, or to increase the proportion of reactive cells in the several other *R. japonicum* strains with a low incidence of lectin binding. The differences in lectin binding seen microscopically among the various positive strains suggest that more than one lectin could be present in our crude ammonium sulfate fraction, and hence that other fractionation procedures should be tried with negative strains. Moreover, bacterial nutrition and growth stage might be expected to influence the polysaccharide chemistry of the cell surface in relation to lectin binding.

Much attention has been focused on lectins in view of their many unusual chemical and biological properties, but virtually nothing is known of their role in nature (*8*). It is most attractive to consider that legume lectins may present a site on the legume root surface which

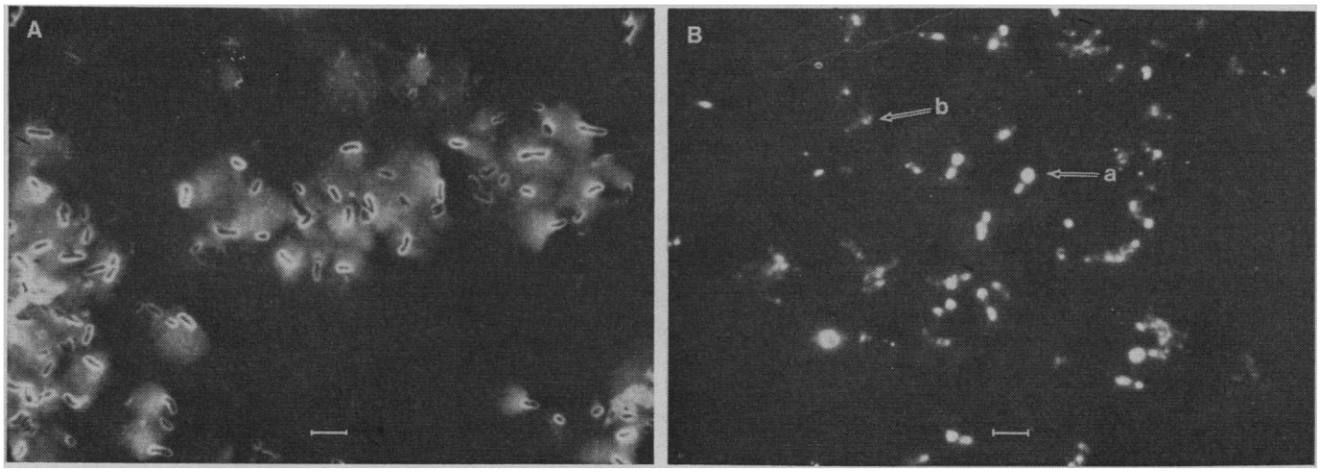

Fig. 1. Microscopic appearance of the specific reaction between *R. japonicum* cells and soybean lectin labeled with fluorescein isothiocyanate. (A) *R. japonicum* strain USDA 110. Note the heavy concentrations of diffuse lectin bound to nearly every cell. (B) *R. japonicum* strain USDA 138. Lectin was bound by most cells, but in a compact, well-defined zone (arrow a). Cells which bound no lectin can be seen in the field (arrow b). (Scale bar, 10 μm.)

interacts specifically with a distinctive polysaccharide on the surface of the appropriate *Rhizobium* cell as a prelude to nodulation. These data demonstrate that the possibility is a real one which merits thorough investigation.

B. B. BOHLOOL
E. L. SCHMIDT
Departments of Microbiology and Soil Science, University of Minnesota, Minneapolis 55455

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6. The protein content of fraction II was adjusted to 1 percent with saline; to 10 ml of this solution was added 4 ml of 0.1*M* sodium phosphate buffer (*p*H 7.0) and then 4 ml of 0.1*M* sodium phosphate buffer (*p*H 8.0) containing 5 mg of fluorescein isothiocyanate (isomer I, Baltimore Biological Laboratories). The resulting solution was adjusted to *p*H 9.0, merthiolate (1 : 10,000) was added, and the reaction proceeded for 24 hours. Unreacted dye was removed by dialysis against saline buffered with 0.02*M* sodium phosphate to *p*H 7.2 until the dialyzate was completely colorless. The fluorescent lectin conjugate was distributed into tubes and held frozen at −20°C.
7. Microscopy was performed with a Zeiss Universal microscope equipped for double lighting with incident illumination from an HBO-200 (Osram) light source and transmitted dark-field tungsten illumination. Zeiss filter No. 1 was used in the path of the transmitted light and Zeiss No. 50 barrier filter was used throughout. Most observations were made by using the two lighting systems in combination.
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Microbodies (Peroxisomes) Containing Catalase in Myocardium: Morphological and Biochemical Evidence

Abstract. *Microbodies characterized by a single limiting membrane and finely granular matrix occur in mouse myocardium and appear in close spatial relation to mitochondria and sarcoplasmic reticulum. The presence of catalase in the microbodies is revealed cytochemically and confirmed biochemically by direct measurement of its activity in myocardial tissue fractions. It is suggested that the microbodies may play an important role in myocardial lipid metabolism.*

Peroxisomes (microbodies) are cytoplasmic organelles, characterized morphologically by a single limiting membrane and a finely granular matrix (*1*). They contain catalase and a variety of H_2O_2-producing oxidases (*2, 3*). In germinating bean seedlings, peroxisomes [also referred to as glyoxysomes (*4*)] are abundant and play a key role in the conversion of lipids to sugars (*4, 5*). The exact biological function of peroxisomes in mammalian cells, however, remains unknown. Possible participation in gluconeogenesis has been suggested by de Duve and Baudhuin (*2*) and link to lipid metabolism was originally postulated by Novikoff and Shin (*6*). Indeed, application of a cytochemical method in which alkaline diaminobenzidine (DAB) is used to demonstrate the peroxidatic activity of catalase (*7*) has revealed large numbers of microbodies in animal cells actively involved in lipid metabolism, such as brown and white fat cells and steroid-secreting cells (*8*). The heart uses lipids and fatty acids as an energy source (*9*). To our knowledge, this is the first report of microbodies in this tissue, specifically in mouse myocardial fibers.

Hearts of male albino mice were fixed, either by vascular perfusion for 10 minutes or by immersion for 2 hours, with 2.5 percent glutaraldehyde in 0.1*M* cacodylate buffer (*p*H 7.4). Sections of the left ventricle (30 μm) were prepared with a tissue chopper (*10*) and were incubated for 2 hours at room temperature in a medium containing 1 mg of 3.3′-diaminobenzidine tetrahydrochloride in 1 ml of 0.1*M* NaOH-citrate-phosphate buffer (*p*H 10.5) and 0.02 percent H_2O_2 (*11*). After incubation, the tissue was fixed with OsO_4 and processed for light and electron microscopy.

Examination of unstained sections 1 μm thick by light microscopy revealed numerous distinct dark brown granules, which appeared smaller and darker than mitochondria. By electron microscopy, electron-opaque reaction product was localized in round and oval particles 0.2 to 0.5 μm in diameter and located in the sarcoplasm of myocardial cells (Figs. 1 and 2). The particles often appeared close to mitochondria and membranes of the sarcoplasmic reticulum and were mostly

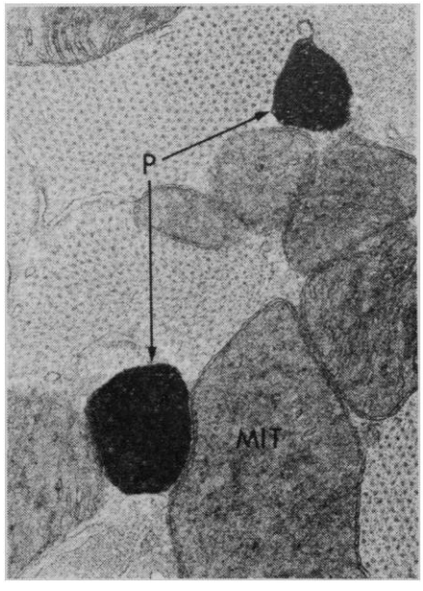

Fig. 1 (left). Electron micrograph of transverse section of mouse myocardial fiber. Tissue was fixed by immersion and incubated as described in the text for cytochemical demonstration of catalase (*11*). Electron-opaque reaction product is found in particles (*P*) 0.2 to 0.5 μm in diameter which appear in close association with mitochondria (*MIT*) (× 42,000).

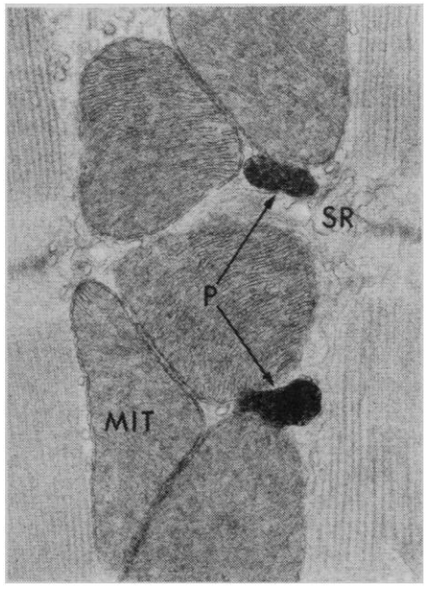

Fig. 2 (right). Longitudinal section of mouse myocardial fiber. Tissue was fixed by vascular perfusion and incubated as described in the text for demonstration of catalase (*11*). Reaction product is found in particles (*P*), which are mostly localized at the junction of I and A bands. The particles appear in close association with mitochondria (*MIT*) and elements of sarcoplasmic reticulum (*SR*) (× 30,000).