Plant Physiol. (1979) 64, 575–580
0032-0889/79/64/0575/06/$00.50/0

Distribution of Lectins in the Jumbo Virginia and Spanish Varieties of the Peanut, *Arachis hypogaea* L.[1]

Received for publication March 22, 1979 and in revised form June 4, 1979

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ABSTRACT

Peanut lectin was purified from seed meal of the Spanish and Jumbo Virginia varieties of peanut (*Arachis hypogaea* L.) by affinity chromatography on lactose coupled to Sepharose 4B. Polyacrylamide gel isoelectric focusing resolved the lectin preparation from Jumbo Virginia seeds into seven isolectins (pI 5.7, 5.9, 6.0, 6.2, 6.3, 6.5, and 6.7). Seed meal from the Spanish variety contained six isolectins which were indistinguishable from the pI 5.7, 5.9, 6.2, 6.3, 6.5, and 6.7 isolectins from Jumbo Virginia. Quantitative, lactose-specific hemagglutination was used to examine the lectins in tissues of both peanut varieties. In young (3- to 9-day-old) seedlings of each variety, more than 90% of the total amount of lectins detected in the plants was in the cotyledons. Most of the remainder was in hypocotyls, stems, and leaves; young roots contained no more than 4 micrograms of lectin per plant. Lectins were present in all nonroot tissues of 21- to 30-day-old seedlings, except 27-day-old Spanish hypocotyls. As cotyledons of each variety senesced, several of the more basic isolectins decreased to undetectable levels, but the acidic isolectins remained until at least 15 days after planting. Some of the seed isolectins and several apparently new lactose-binding lectins were also identified in affinity-purified extracts of 5-day-old roots and hypocotyls. Rabbit antibodies raised against the Jumbo Virginia seed isolectin preparation reacted with seed, cotyledon, and hypocotyl lectin preparations from both varieties. Analysis of seed lectin preparations from seven varieties of *A. hypogaea* and of a related species (*A. villosulicarpa*) indicated that isolectin composition in *Arachis* may be a characteristic of both the species and the subspecies (botanical type) to which the variety belongs.

It has been known for 20 years that seed extracts of peanut (*Arachis hypogaea* L.) agglutinate neuraminidase-treated human erythrocytes (2). The hemagglutinating factor was purified by affinity and gel filtration chromatography and found to be a lectin with specificity for molecules containing terminal D-galactose (12, 24). The mol wt of PNL[3] was found to be about 110,000, and it was apparently composed of four identical subunits. More recently, Newman (14) reported that PNL was not a single molecular species, but rather a mixture of up to six isolectins, all of which are erythroagglutinins.

Several characteristic seed lectins, such as those from soybean and white clover, have aroused considerable interest because of their putative roles as recognition molecules for symbiotic rhizobia (4). Although there is a body of evidence suggesting that variation in lectin content and composition in legumes is common (3), isolectins have not been carefully examined in terms of possible recognition functions. Hence, preliminary to investigating the peanut-*Rhizobium* symbiosis with respect to a possible involvement of PNL, it became necessary to standardize methods to purify and separate the peanut isolectins reproducibly, to examine the distribution of PNL in vegetative parts of peanut plants, and to investigate whether there is varietal variation in peanut lectins. The results of these studies are presented here.

MATERIALS AND METHODS

Source of Seeds. Jumbo Virginia and Spanish peanut (*Arachis hypogaea* L.) seed was purchased from W. Atlee Burpee, Riverside, Calif. Red Tennessee peanut seed was from George W. Park Seed Co., Greenwood, S.C. Dr. A. J. Norden, Department of Agronomy, University of Florida, Gainesville, provided peanut seed of the following varieties: Starr, New Mexico Valencia A, Florunner, Florigiant. Seed of the related species, *A. villosulicarpa* Hoehne (PI 336985), was the gift of Dr. R. O. Hammons, USDA, Tifton, Ga.

Purification of PNL. Peanut seeds were crushed and defatted with petroleum ether in a Soxhlet apparatus. Defatted meal (5 g) was homogenized in extraction buffer (100 ml) for 30 s with a Polytron homogenizer (Brinkmann Instruments). Extraction buffer (final pH 7.2) consisted of PBS (0.43 g KH_2PO_4, 1.48 g Na_2HPO_4, and 7.2 g NaCl/l containing 50 mM ascorbate, 1 mM $MgSO_4$, 5 μg phenylmethane sulfonylfluoride/100 ml (Sigma Chemical Co.), and 2 g insoluble PVP/100 ml (Sigma Chemical Co.). The extract was centrifuged at 12,000*g* for 15 min, filtered through Whatman GF/A glass fiber paper, and added to 9 ml of affinity adsorbent, which had been prepared by the oxirane coupling of lactose to Sepharose 4B (25). The slurry was rotated in a round bottom flask for 2 h at 4 C, and the adsorbent was collected on a sintered glass filter and washed exhaustively with PBS. The adsorbent was transferred to a column and the eluate monitored at 280 nm. Elution with PBS was continued until the A_{280} of the eluate was negligible, and then elution with 100 mM lactose in PBS was initiated. The eluted protein was collected as a single fraction, dialyzed overnight against PBS, and stored frozen at −20 C.

Growth of Plants. Peanut seed was planted in wooden flats containing Vermiculite and maintained at 30 C in a controlled environment chamber. Lighting was constant and from a cool-white fluorescent source. At 3-day intervals from 3 to 30 days after planting, seedlings were harvested, washed, and dissected into cotyledons, roots, hypocotyls, stems, and leaves. The plant tissues were frozen and lyophilized, and the lyophilized tissues were weighed and ground to fine powders. Cotyledon powders were defatted as described above and reweighed prior to storage. All tissue powders were stored at room temperature.

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[3] Abbreviations: PNL: peanut lectin; PBS: phosphate-buffered saline; IgG: immunoglobulin G.

Hemagglutination Method. Tissue samples (25–500 mg) were homogenized as described above in extraction buffer containing 100 mM lactose. After centrifugation and filtration, extracts were dialyzed overnight against PBS and stored at −20 C. Human type O blood cells were prepared by treating a 3% cell suspension in PBS (v/v) for 1 h with *Vibrio cholerae* neuraminidase obtained from Calbiochem (25 units of enzyme per 50-ml blood cell suspension).

For the assays, 25-μl aliquots of each extract were transferred to a well in a polystyrene hemagglutination plate (Cooke microtiter plate, Fisher Scientific) and diluted in 2-fold increments to a final dilution of 2,048 in PBS and PBS containing 100 mM lactose. Standards, consisting of affinity-purified PNL solution (1 mg/ml in PBS) from the appropriate peanut variety diluted similarly, were included. A 3% (v/v) suspension of neuraminidase-treated blood cells in 25 μl PBS was then added to each well. After 3 h at room temperature, the titer, which was defined as the reciprocal of the greatest dilution giving visible agglutination, was recorded for each extract. PNL in the extract was then calculated based on the titer of the standard PNL solution.

Immunological Methods. Three New Zealand white rabbits were immunized with affinity-purified Jumbo Virginia PNL in PBS using Freund's incomplete adjuvant according to the procedure of Howard and Shannon (10). A fourth animal received PBS and adjuvant emulsion only, and served as a control. Each rabbit had been bled prior to immunization. After 30 days, each rabbit received the appropriate booster injection (10), and 10 days later the animals were bled. IgG was isolated from the control and from the anti-Jumbo Virginia PNL sera (10) and the fractions were purified by passage through columns of Bio-Gel P-100 (30 × 0.9 cm) in PBS. The IgG fractions were eluted as symmetrical protein peaks, and were stored at −20 C in 50% saturated ammonium sulfate. Prior to use, stored IgG fractions were dialyzed against PBS, and protein was adjusted to 4.8 mg/ml. The Ouchterlony double diffusion assays were performed as described earlier (5).

Analytical Methods. Polyacrylamide gel electrophoresis in 7.5% gels was performed according to the methods of Reisfeld *et al.* (18) and Davis (6). Gels (5 mm i.d.) were run at 3 mamp per gel and stained with Coomassie Brilliant Blue R-250 (26). Discontinuous electrophoresis in 10% polyacrylamide gels containing SDS was done according to the procedure of Laemmli (11) with mol wt markers from Boehringer Mannheim. Isoelectric focusing in 5% polyacrylamide gels containing 2% ampholytes (Bio-Lyte 3/10, Bio-Rad) was performed according to the directions of the manufacturer. Gels were focused at 200 v for 20 h at 4 C and stained with Coomassie Brilliant Blue R-250 (27). When necessary, the pH gradients in the gels were measured with a Bio-Rad Gel Pro-pHiler. Protein was determined by the method of Lowry *et al.* (13) using BSA as a standard.

RESULTS

Purification of PNL. Affinity chromatography of peanut seed extract from Spanish or Jumbo Virginia yielded from 1.5 to 2.5 mg of PNL/g of defatted meal (meal was about 55% lipid). The lectin from Jumbo Virginia appeared as a single diffuse band after electrophoresis at pH 4.3 (Fig. 1B) and after electrophoresis in the presence of SDS (Fig. 1A). At pH 9.5, three poorly resolved bands were visible (Fig. 1C). The Jumbo Virginia lectin was separated into seven bands by isoelectric focusing (from five runs, mean isoelectric points ± SD were 5.7 ± 0.13, 5.9 ± 0.12, 6.0 ± 0.08, 6.2 ± 0.08, 6.3 ± 0.08; 6.5 ± 0.07, and 6.7 ± 0.08) (Fig. 2). One additional band at pI 7.0 was observed occasionally, but was not investigated. Further purification of Jumbo Virginia PNL by affinity chromatography did not remove any of the seven bands, which were labeled 1 to 7 starting from the most acidic and progressing to the most basic. When the bands were sliced from focusing gels and eluted into PBS, each was capable of agglutinating neuraminidase-treated red blood cells in a lactose-specific manner.

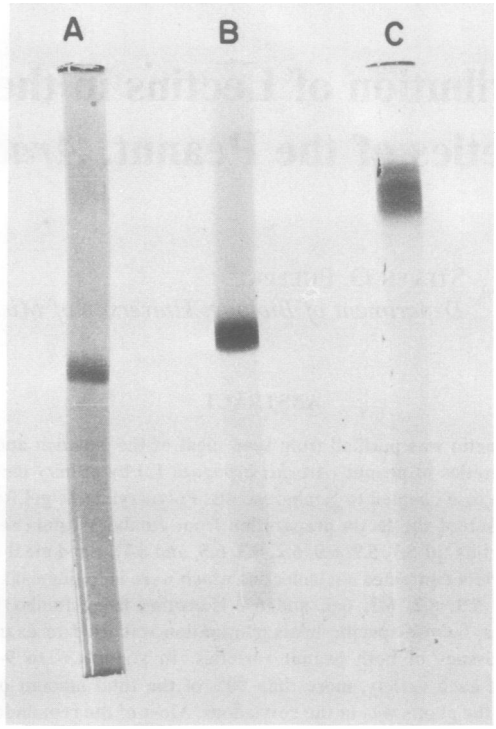

FIG. 1. Polyacrylamide gel electrophoresis of affinity-purified PNL from Jumbo Virginia seed meal. Each gel was loaded with about 40 μg of protein. A: 10% polyacrylamide disc gel containing SDS (15); B: 7.5% polyacrylamide gel (pH 4.3) (22); C: 7.5% polyacrylamide gel (pH 9.5) (6).

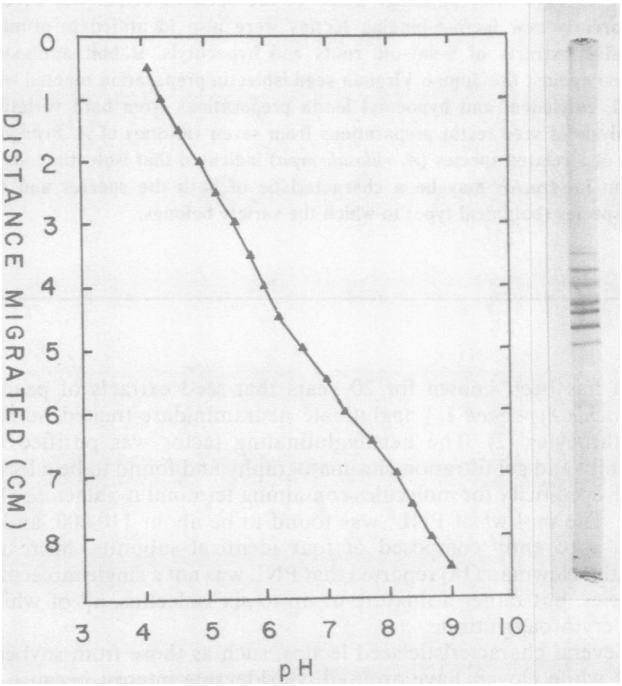

FIG. 2. Determination of isoelectric points of Jumbo Virginia seed isolectins. Jumbo Virginia PNL (about 40 μg) was isoelectric focused in 5% polyacrylamide gels containing 2% Bio-Lyte 3/10 ampholytes. The pH gradient was determined with a Bio-Rad pH Pro-pHiler.

The migration of Spanish PNL in acidic and basic gels and in gels containing SDS was the same as that of Jumbo Virginia PNL

(data not shown). In contrast to Jumbo Virginia, Spanish PNL contained only six isolectins, and their pI values were indistinguishable from those of the pI 5.7, 5.9, 6.2, 6.3, 6.5, and 6.7 Jumbo Virginia isolectins (see Fig. 8B). Further evidence that the six Spanish and Jumbo Virginia isolectins were identical was provided by experiments in which individual focusing gels were loaded with a mixture of Jumbo Virginia and Spanish PNL. Regardless of the proportions of the two mixtures, only seven bands were resolved. Moreover, in double diffusion tests both the Spanish and the Jumbo Virginia lectin preparations formed precipitates with anti-Jumbo Virginia PNL IgG (Fig. 3). Precipitates did not form with the control rabbit IgG or with preimmune serum.

When affinity-purified PNL from either source was electrophoresed in 10% gels containing SDS, a single component at mol wt = 27,000 relative to BSA (mol wt = 67,000), ovalbumin (mol wt = 45,000), chymotrypsinogen (mol wt = 25,000), and Cyt *c* (mol wt = 12,500) was observed. These results thus indicate that PNL from Jumbo Virginia seeds was a mixture of seven isolectins with similar mol wt and receptor specificity and that Spanish seed PNL contains only six of the Jumbo Virginia isolectins.

Lectins in Peanut Seedlings. The temporal distribution of lectins in roots, stems, hypocotyls, and leaves was similar for both peanut varieties (Figs. 4 and 5), and in no case was hemagglutination in the presence of 100 mM lactose observed. The amount of lectins in 3- and 6-day-old Jumbo Virginia roots appears to be large (Fig. 4A), but the hypocotyl-root axes of these young seedlings were not extracted separately as roots and hypocotyls. Based on analogy with Spanish (Fig. 5A), where 3- and 6-day-old hypocotyls and roots were extracted separately, it is likely that the hypocotyl part of the hypocotyl-root axes contained most of the lectins in noncotyledon tissues of 3- and 6-day-old Jumbo Virginia plants.

At any particular time, the absolute amount of lectins (as μg/plant in a given tissue) was usually greater in the Spanish than in the Jumbo Virginia plants, even though the Spanish plants consistently weighed less than their Jumbo Virginia counterparts. In both varieties, the greatest amounts of lectins were in hypocotyls of young seedlings (Figs. 4A and 5A). By 9 to 12 days after planting, lectins were no longer detected in roots (<20 μg lectin/g dry weight of tissue), and levels in hypocotyls were declining. Conversely, lectin levels in stems and leaves of both varieties appeared to be variable (Figs. 4B and 5B). Most of these variations can probably be ascribed to unavoidable imprecision in the assays. This arises because the data points are derived from dilution end points, where the lectin concentration in each extract is decreased by a factor of 50% with increasing dilution. This drawback of the procedure, however, is counterbalanced by the very low detection limit of the assay for PNL.

Table I summarizes the results of the hemagglutination assays with peanut plants, including cotyledons. In both varieties, lectin levels were greatest in very young seedlings containing cotyledons

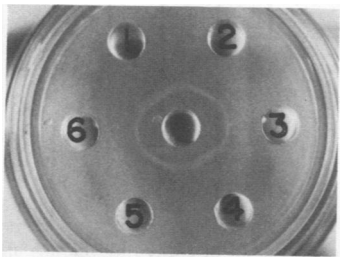

FIG. 3. Double diffusion gel. Rabbit anti-Jumbo Virginia PNL IgG (960 μg/200 μl PBS in the center well) was reacted with 300-μg aliquots of affinity-purified PNL preparations from: (1) Jumbo Virginia seed meal; (2) 6-day-old Jumbo Virginia cotyledons; (3) Spanish seed meal; (4) 6-day-old Spanish cotyledons; (5) Jumbo Virginia seed meal; and (6) Spanish seed meal. Preimmune serum and serum from a control animal immunized with PBS did not react with the PNL preparations.

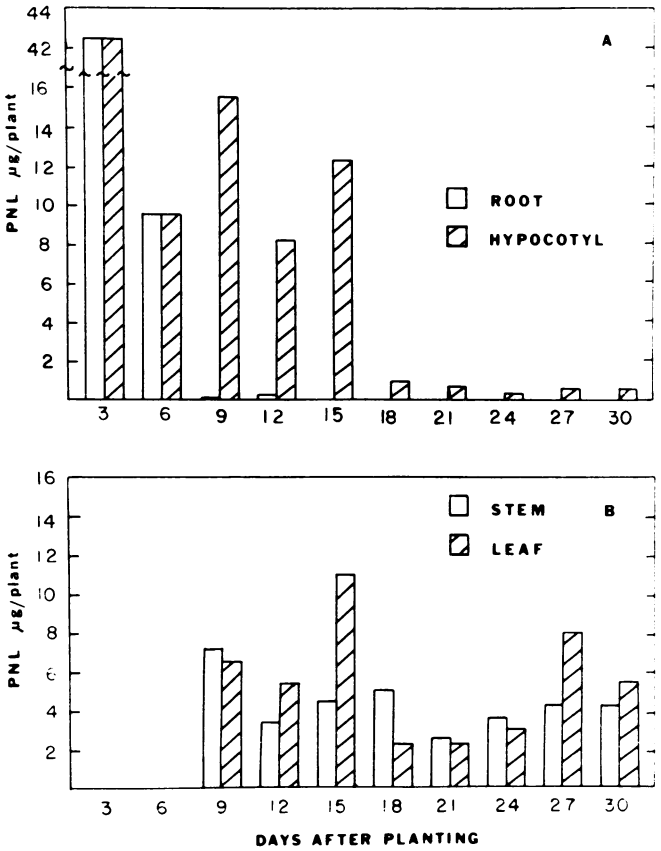

FIG. 4. Time course changes in lectin levels in roots, hypocotyls, stems, and leaves of Jumbo Virginia peanut seedlings. Lyophilized tissue powders were homogenized in extraction buffer containing 100 mM lactose, and the extracts were dialyzed against PBS. Lectin levels were determined by quantitative hemagglutination using an affinity-purified Jumbo Virginia PNL standard. The hypocotyl-root axes of 3- and 6-day-old seedlings were not separated into roots and hypocotyls, but were extracted intact.

rich in PNL. Thereafter, lectin levels in cotyledons and in whole plants decreased substantially. In Jumbo Virginia cotyledons, a particularly sharp decrease in lectin level occurred between the 15th and 18th days after planting. During this interval, cotyledon senescence was rapid, and cotyledon dry weight per plant decreased by 65%. In the Spanish variety, where the initial amount of lectins was relatively small, there was a sudden decrease between day 3 and day 6, and then a continued gradual decline.

To determine the PNL extraction efficiency, affinity-purified PNL (5–10 μg/ml of extraction buffer) was added to representative samples prior to homogenization. Recovery of exogenous PNL was quantitative, and hence it was not necessary to correct for extraction efficiency. Nevertheless, it is important to note again that in spite of the great sensitivity of hemagglutination assays (detection limit <0.5 μg PNL/ml), inherent precision is not great.

Isolectin Distribution. To determine the fate of the seed isolectins, cotyledons were harvested at 3-day intervals as they senesced on growing seedlings. Each lectin preparation was purified twice by affinity chromatography and then subjected to isoelectric focusing. The band patterns of Jumbo Virginia (Fig. 6) indicate that all seven isolectins were present on days 3 and 6, although band 7 stained lighter than the others. On the 9th day and thereafter, only bands 1 and 2 were stained darkly; the others were either indistinct or not visible. In each of the cotyledon preparations, several diffusely staining areas, which were more acidic than band 1 and which were not observed in seed extracts, were detected.

As cotyledons of the Spanish variety senesced, the more basic bands also lost intensity relative to the more acidic bands 1, 3, and 4 (photo not included). By day 9, band 7 was not detected, and

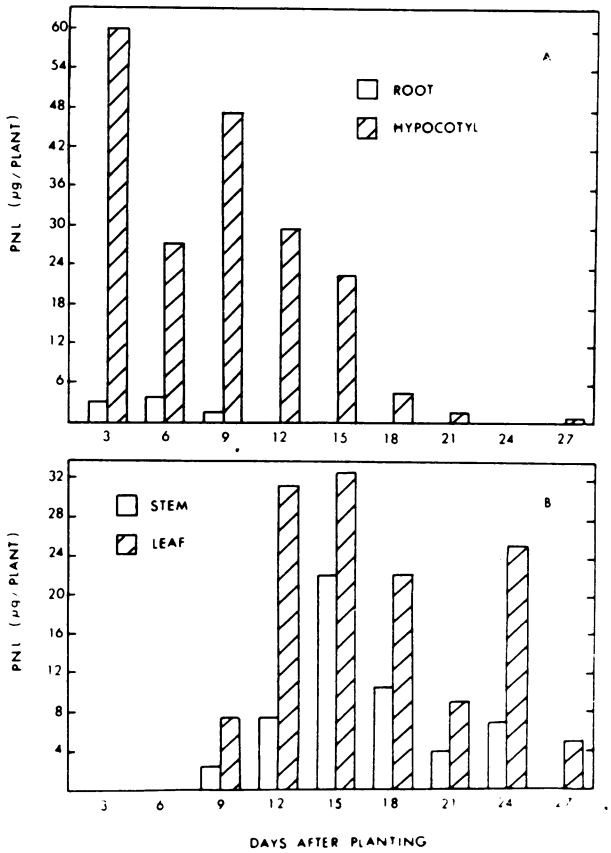

FIG. 5. Time course changes in lectin levels in roots, hypocotyls, stems, and leaves of Spanish peanut seedlings. Lyophilized tissue powders were homogenized in extraction buffer containing 100 mM lactose, and extracts were dialyzed against PBS. Lectin levels were determined by quantitative hemagglutination using an affinity-purified Spanish PNL standard.

Table I. *Lectin Levels in Cotyledons and in Entire Jumbo Virginia, and Spanish Peanut Seedlings*

Lyophilized, defatted cotyledon powders were extracted in PBS containing PVP, lactose, and phenylmethane sulfonylfluoride, and levels of PNL in the extracts were determined by quantitative hemagglutination. Total protein in the extracts was determined by the method of Lowry *et al.* (13). PNL levels in entire seedlings were calculated by summing the PNL/plant in cotyledons, roots, hypocotyls, stems, and leaves.

Plant Age	PNL		PNL	
	Spanish	Jumbo Virginia	Spanish	Jumbo Virginia
days	*mg/plant in cotyledons*		*mg/plant*	
3	2.01	4.02	2.07	4.06
6	0.84	4.64	0.87	4.65
9	0.64	3.90	0.70	3.93
12	0.21	2.62	0.27	2.65
15	0.08	2.46	0.15	2.52
18	0.01	0.12	0.03	0.13
21	Trace	0.04	0.02	0.05
24	0.01	0.10	0.04	0.11
27	0.01	0.05	0.01	0.06
30		0.05		0.06

bands 5 and 6 were comparatively indistinct. In contrast to Jumbo Virginia, however, no new bands were detected. All of the lectin preparations (isolectin mixtures) from cotyledons of both varieties reacted with anti-Jumbo Virginia PNL IgG (see Fig. 3). In addition, upon electrophoresis in the presence of SDS, all preparations gave a single band at mol wt = 27,000.

The isolectins of 5-day-old hypocotyls of each variety were also compared to one another and to seed isolectins (Fig. 7). Based on experimentally determined pI values, visual comparisons, and experiments with mixtures of various affinity-purified preparations, it was clear that Jumbo Virginia hypocotyls consistently contained bands 1, 2, 5, and 7. Band 1 invariably appeared as a doublet, and several additional lightly staining bands were present. In Spanish, all six seed isolectins were present in hypocotyls, but bands 5 and 6 were of relatively low intensity (Fig. 7). Again, nonseed bands were present. Anti-Jumbo Virginia PNL IgG

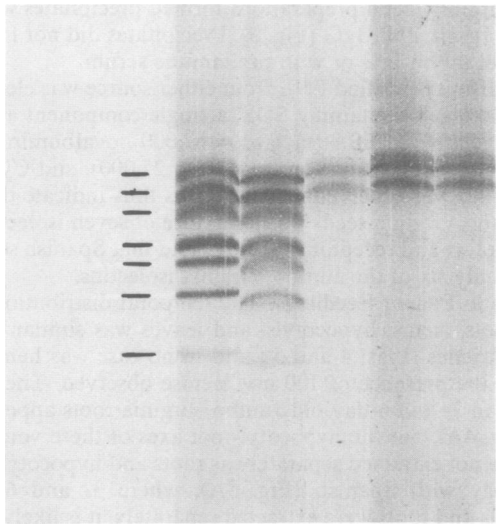

FIG. 6. Isolectins in Jumbo Virginia cotyledons. PNL from each lyophilized cotyledon powder was purified twice by affinity chromatography, and a 50-μg aliquot of each preparation was isoelectric focused in a 5% polyacrylamide gel containing 2% Bio-Lyte 3/10 ampholytes. The bars mark the seven Jumbo Virginia seed isolectin bands, which were identified from measured isoelectric points.

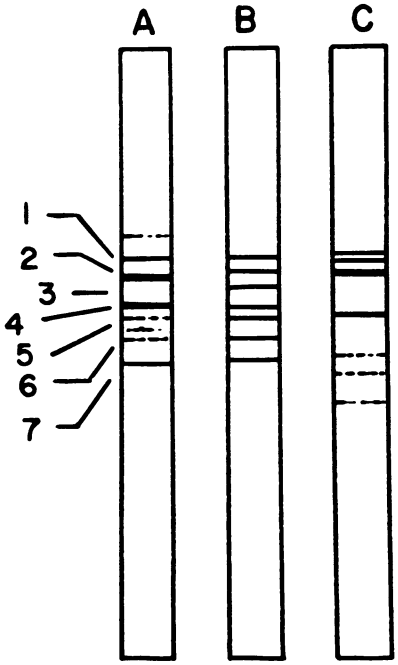

FIG. 7. Map of isolectins from 5-day-old Spanish and Jumbo Virginia hypocotyls. PNL from each tissue was purified twice by affinity chromatography, and 50 μg of protein from each preparation was isoelectric focused in a 5% polyacrylamide gel containing 2% Bio-Lyte 3/10 ampholytes. Positions of bands 1 through 7 are marked on the left. A: Spanish hypocotyl; B: Jumbo Virginia seed meal control; C: Jumbo Virginia hypocotyl. Gels were aligned by the measured pH gradients, and the thickness of each line represents its relative staining intensity.

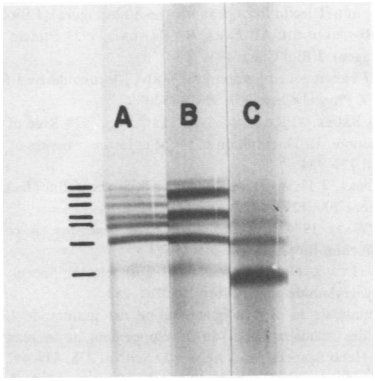

FIG. 8. Isolectin arrays in *A. hypogaea* and *A. villosulicarpa.* PNL from each seed meal was purified twice by affinity chromatography, and 50 μg of protein from each preparation was isoelectric focused in a 5% polyacrylamide gel containing 2% Bio-Lyte 3/10 ampholytes. A: Red Tennessee, a Virginia-type with seven isolectins; B: Spanish, a Spanish-type with six isolectins; C: *A. villosulicarpa,* with three isolectins. Gels were aligned by measured pH gradients; bars mark isolectin bands 1 through 7.

reacted with hypocotyl preparations from each variety, and both hypocotyl lectin preparations gave a single mol wt = 27,000 band in SDS gels.

Obtaining affinity-purified root lectins was difficult because of their extremely low concentration in plants. The use of up to 4 g (dry weight) of tissues afforded affinity-purified lectin preparations which yielded dim bands in focusing gels. For both varieties, the band patterns from roots were indistinguishable from those of hypocotyls, but low recovery of root lectins precluded diffusion tests with antibodies.

Isolectins in Other Peanut Varieties. In a preliminary screen of isolectin composition in the genus *Arachis,* affinity-purified lectin preparations from seeds of seven peanut varieties and a related species were isoelectric focused. The samples yielded three characteristic arrays of isolectins, which are shown in Figure 8. The seven Jumbo Virginia isolectins were present in both varieties of the Valencia-type (Red Tennessee and New Mexico Valencia A), the Runner-type (Florunner), and the Virginia-type (Florigiant and Jumbo Virginia). However, both Spanish-types (Starr and Spanish) contained only the six Spanish isolectins. *A. villosulicarpa* contained bands 5, 6, and 7, the latter being much more intense than the others. In *A. villosulicarpa* there was also a generalized diffuse staining in the region of the acidic isolectins, but bands were never resolved.

DISCUSSION

Isolectins in Peanut Seeds. Lectins from peanut seeds have been purified previously by both affinity and gel filtration chromatography (12, 24). PNL initially appeared to be a homogeneous protein, but recently it has been resolved into several isolectins by gel electrophoresis and isoelectric focusing (14). Since the PNL isolectins were purified from seeds of unknown genetic makeup, it was unclear whether various peanut varieties produced different lectins, as is the case for *Lens culinaris* (7) and *Glycine max* (8), or whether genetically uniform seeds produced several isolectins, as in *Pisum sativum* (21) and *Phaseolus vulgaris* (17). A lectin with the subunit, binding, and electrophoretic characteristics attributed previously to PNL (12, 14, 24) was identified in Jumbo Virginia seeds and could be reproducibly separated by polyacrylamide gel isoelectric focusing into seven agglutinating isolectins (Fig. 2). The acidic isoelectric points of the isolectins are consistent with the high Glu and Asp content of PNL (12, 24), and reasonably approximate those of the isolectins isolated earlier from genetically undefined seed (pI 7.0–7.4) (14). The isolectin array of Spanish seeds was the same as that of Jumbo Virginia (Fig. 8B), except for the lack of band 3. In addition, the Spanish isolectins were immunologically cross-reactive with anti-Jumbo Virginia PNL IgG and had the same subunit mol wt as that of the Jumbo Virginia isolectins.

Analysis of the seed isolectins from several peanut varieties indicated that seeds of Spanish-type peanuts have six isolectins and that the Runner, Virginia, and Valencia types of *A. hypogaea* have seven (Fig. 8). A related species, *A. villosulicarpa,* has only the three most basic *A. hypogaea* isolectins. Possible varietal differences in peanut lectins, as have been determined recently for soybean lectin (16), may aid in identifying the natural function of lectins. Moreover, several inconsistencies in the literature, such as the observation that some PNL preparations are mitogenic and others are not (9), may simply reflect variation in content of isolectins in PNL preparations (14).

Seed Isolectins in Growing Plants. In the present study, the peanut isolectins did not decline at the same rates in cotyledons of growing plants (Fig. 6). Similar results have been described for two pea isolectins in *P. sativum* (21). In both peanut varieties, the more basic isolectins decreased rapidly relative to acidic isolectins, with a concomitant appearance of new acidic bands. Since the isoelectric focusing technique is not particularly sensitive, it is not possible to state unequivocally that several of the isolectins in fact disappeared. Rather, it is apparent that there was a great decrease in levels of the more basic isolectins relative to those of the acidic isolectins. Although the origin of the newly appearing isolectin bands was not investigated, it is tempting to suggest that they represent fragments of the more basic isolectins which retained binding activity, but had more acidic pI values. It is equally possible, however, that the new isolectins were synthesized *de novo.*

Whether any of the isolectins were artifacts of extraction procedures or storage conditions is also germane to the discussion. Variations in several extraction parameters, such as length of homogenization and composition of the extraction medium, had no discernible effect on isolectins. Omission of PVP, ascorbate, and phenylmethane sulfonylfluoride from the extraction buffer did not decrease the recovery of PNL from seed meals or alter the isolectin composition. Likewise, length of storage of purified preparations and cycles of freezing and thawing of isolectin solutions did not diminish the biological activity of samples or affect isolectin composition.

Distribution of Lectins in Plants. The distribution of lectins in Spanish and Jumbo Virginia peanut seedlings was determined by quantitative hemagglutination. Most of the lectins in young seedlings were in the cotyledons (Table I), as are the lectins of *Dolichos biflorus* (23), *L. culinaris* (19, 20), and *P. sativum* (21, 22). Peanut hypocotyls initially contained high levels of lectins, but these decreased substantially about 2 weeks after planting. Thus, in 18- to 30-day-old seedlings, most noncotyledon lectins were in the shoot. Levels of root lectins of both varieties were very low, and as is the case for other legumes (15, 20, 22, 23), root lectins were detectable for relatively few days after germination. Since in controls added lectin could be quantitatively recovered from extracts of all types of tissues, including older roots, the lack of detectable lectins in such roots was not due to loss of lectins during extraction. Bowles *et al.* (1) reported recently that roots of 35-day-old peanut seedlings of an undetermined variety (or varieties) contained extraordinarily high levels of a PBS-soluble lectin, which may be PNL. In the Jumbo Virginia and Spanish varieties, where PNL is not detected in 30-day-old roots, it is doubtful that such relatively great concentrations could be attained during an additional 5-day growing period.

Although the isolectin composition of the shoot lectins is unknown, three lines of evidence indicate that at least some of the hypocotyl, and probably also the root isolectins, are equivalent to isolectins found in seeds. First, isoelectric focusing permitted the resolution of root and hypocotyl isolectins having isoelectric points indistinguishable from those of seed isolectins. Second, antibodies

prepared against the Jumbo Virginia seed isolectins reacted with hypocotyl lectin preparations. Third, the subunit mol wt of hypocotyl lectins were identical to those of seed isolectins.

Acknowledgments—I thank R. O. Hammons and A. J. Norden for seed, Lee-Chin Su for skilled technical help, and Harvey P. Friedman for preparation of the antisera and advice on immunological methods.

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