dysfunction rather than chronic alcohol abuse.

Further support was given to this suggestion by the results in 77 patients with nonalcoholic liver and biliary tract disease (Table 3). The mean A/L ratio was raised in the groups of patients with active chronic hepatitis, primary biliary cirrhosis, cryptogenic cirrhosis, and acute type A hepatitis, but not in the group with primarily biliary tract disease with little or no hepatocellular damage, as judged from percutaneous needle biopsy.

A rise in the plasma A/L ratio would thus seem to indicate liver damage. In order to gain some idea of the sensitivity of this ratio as an index of hepatocellular dysfunction, we studied in more detail the 43 patients with alcohol-related liver disease. Plasma samples were taken for bilirubin, aspartate transaminase, alkaline phosphatase, prothrombin time, total protein, albumin, bile acids, and amino acids, and the degree of histological damage was assessed by two independent observers and graded as mild (1) moderate (2) and severe (3).

We found no significant correlation between the plasma A/L ratio and the standard liver function tests, or the degree of hepatocellular damage as assessed histologically (Fig. 2). Thus, although the A/L ratio is raised in patients with liver disease, it is not a very sensitive index of hepatocellular dysfunction.

It has also been suggested that the ratio does not alter appreciably between the fasting and nonfasting state (*1*). We looked at the fasting and the 2-hour postprandial levels in ten patients (Fig. 3) and found that the value of the A/L ratio was not consistent and, in several of the patients, only one reading was abnormal.

In view of this variation in value of the plasma A/L ratio, we undertook a further study in ten patients with liver disease of varying etiology and severity. Plasma samples for bilirubin, aspartate transaminase, alkaline phosphatase prothrombin time, total protein, albumin, bile acids, and amino acids were taken before breakfast after an overnight fast, 2 hours after breakfast, and 2 hours after lunch. The degree of histological damage was assessed and graded as before.

We found no consistently significant correlation between the plasma A/L ratio and the standard liver function tests, fasting, and 2-hour postprandial bile acids, or the degree of histological damage (Fig. 4).

We have not been able to confirm the results of previous workers. In our hands, a raised A/L ratio does not indicate long-term alcohol abuse. It would seem to indicate hepato-dysfunction although it is not a very sensitive index of this. Its value can vary considerably throughout the day, and a single estimation is probably of limited use. There appears to be no justification for using the plasma A/L ratio as an empirical biochemical marker of alcoholism.

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Lectin Release by Soybean Seeds

Abstract. *Lectin is released from soybean seeds during water uptake. Hemagglutination activity data show that the lectin is a preferential release product within the first 8 hours of hydration. A qualitative filter-paper assay for detection of lectin released by single seeds is used to show that the release phenomenon is independent of seed viability and insensitive to azide.*

Proteins or glycoproteins collectively classed as lectins and capable of reversible binding interactions with specific carbohydrates or carbohydrate residues are present in relatively high concentrations in leguminous seeds. Their detection and purification are aided by their ability to agglutinate erythrocytes, a phenomenon probably involving bivalent binding of specific carbohydrate residues of the oligosaccharide components of cell membrane glycoproteins. In addition, lectins of different carbohydrate-binding specificity have been isolated by the use of erythrocytes of different serological types (*1*). While these molecules have proved useful as probes in the study of cell membrane structure and function, their physiological significance to the plant is largely unknown. In this regard, it has been suggested that the presence of lectins in legume seeds is related to the ability of the mature plants to fix atmospheric nitrogen within root nodules formed after infection of root tissues by appropriate strains of *Rhizobium* (*2, 3*). Plant lectins may provide the means by which the rhizobia are recognized and bound to the external surfaces of the plant root (*2*). It has also been suggested that lectins function in a protective (antibiotic) role as inhibitors of fungal polysaccharases (*4*), hyphal cell wall synthesis (*5*), and spore germination (*5*). In addition, it has been suggested that they may mediate cell-extension growth in plants (*6*) and provide membrane recognition sites for regulation of the dynamics of internal membrane compartmentation (*7*).

The possible utilization of lectin of legume seeds, either as a recognition-binding mechanism for attachment of rhizobia to the root-hair surface or as an antibiotic mechanism, presupposes the presence of free lectin outside of the plant symplasm, that is, in association with the cellulosic cell wall or in the rhizosphere or potential rhizosphere. Here we provide evidence that lectin activity can be detected outside soybean seeds during the earliest phases of soybean seed hydration.

Soybean seeds (cultivars 'Tracy,' 'Verde,' or 'D68-127') were surface-sterilized and incubated at 18°C in a minimal volume of sterile glass-distilled water (but sufficient to allow several milliliters in excess of that required for full hydration) for periods of 4, 8, 16, 24, and 48 hours. At the end of each period, the water remaining around the seeds was removed, the seeds were gently rinsed, and the solutions were combined and lyophilized to dryness. The seeds were extracted for total ammonium sulfate–precipitate nonglobulin protein; this extract and the lyophilizate were assayed for protein content (*8*) and hemagglutination activity.

Figure 1 shows the time course of seed hydration and concurrent appearance of protein in the water surrounding seeds. At 4 hours, the earliest time tested, the seeds were more than 60 percent hydrated, and the hydration was complete by 16 hours. During this period, appearance of protein was detected in the medium, the quantity being less than 2 mg per gram of dry seed in the first 4-hour period to more than 8 mg per gram of dry seed in 48 hours. The rate of protein appearance was approximately linear (0.26 mg per gram of dry seed per hour) over the first 24 hours and slightly less in the 24- to 48-hour period. When tested with rabbit erythrocytes, all lyophilizates showed hemagglutination activity. The hemagglutination activity of the lyophili-

zates was specifically inhibited by inclusion of 10 m*M* *N*-acetylgalactosamine or saccharides containing D-galactose in the erythrocyte suspension, and also could be removed by prior immunoprecipitation with rabbit antiserum against purified soybean lectin, thus indicating its identity as the released lectin from the soybean seeds. The specific activity of the released soybean lectin (expressed as hemagglutination units per milligram of protein) rises from a detectable level at 4 hours to a maximum at 8 hours, declines slightly at 16 and 24 hours, and rises moderately at 48 hours (Fig. 2a). This pattern of hemagglutination activity suggests that soybean lectin is a preferential export molecule within the first 8 hours of germination and that it is subsequently subject to dilution by additional nonlectin protein. The soybean lectin, however, is not the exclusive exported protein during these initial 8 hours of hydration. The hemagglutination titer at this time is low compared with previously reported values for purified lectins (*1*). Analyses by sodium dodecyl sulfate–polyacrylamide gel electrophoresis showed the presence of about ten components in the 8-hour lyophilizate. The most prominent of these, however, was estimated at 30,000 daltons (or 23,000 daltons in the case of 'D68-127'), which corresponds to the reported subunit size of purified soybean lectins (*1*, *9*).

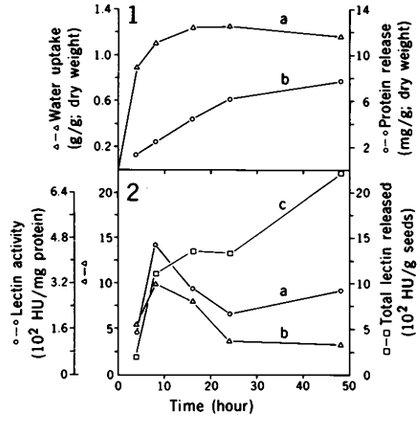

Fig. 1. Time course of water uptake (a) and protein release (b) by hydrating soybean seeds. Curves are mean of at least two determinations. Fig. 2. Time course of hemagglutination activity of (a) lyophilizate of solution that bathes seeds, and (b) nonglobulin soluble protein extracted from seeds. Curves are means of at least two determinations. (c) Total accumulated lectin in the solution bathing the seeds. Each point represents total hemagglutination units accumulated during the period of time indicated. Dry soybean seeds (20 g) were surface-sterilized [5-second immersion in 50 percent (by volume) ethanol-water, followed by extensive washing in sterile distilled water; 5-minute immersion in 5 percent hypochlorite solution and extensive washing in sterile water] and then incubated, partly immersed in water, at 18°C for periods up to 48 hours. At the end of each incubation period, the solution remaining around the seeds was removed, the seeds were rinsed with sterile water with a syringe, and these solutions were combined and lyophilized. The rinsed seeds were blotted dry, weighed, and extracted for nonglobulin protein by homogenization in 1.0*M* NaCl–0.1*M* sodium acetate buffer (*p*H 4.5). After precipitation of globulins overnight at 4°C, remaining nonglobulin protein was precipitated by ammonium sulfate saturation (100 percent), then redissolved in distilled water and extensively dialyzed against water. Hemagglutination activity of this seed nonglobulin protein extract and of the bathing solution lyophilizate (taken up in 5 ml of distilled water) was determined by a standard, visually determined hemagglutination assay. Serial dilutions of protein in phosphate-buffered saline were made in a total volume of 0.2 ml in siliconized glass tubes. A further 0.2 ml of a washed 4 percent suspension of rabbit red blood cells was added, and the tubes were incubated at 37°C for 30 minutes. The macroscopic agglutination pattern was observed after 15 hours at 4°C. One hemagglutination unit (*HU*) represents the lowest protein quantity required to cause a positive agglutination pattern in the 0.4-ml mixture.

The specific hemagglutination activity of the nonglobulin protein extractable from hydrated seeds by 1.0*M* NaCl–0.1*M* sodium acetate buffer (*p*H 4.5) is illustrated in Fig. 2b. Although the seed extracts showed a generally higher specific activity than the lyophilizates of water bathing the seeds, the similarity in the change of specific activity with time between seed extracts and the released protein lyophilizates is remarkable. These data provide no evidence for an export of lectin outside the seed at the expense of depletion of an internal lectin pool, but instead suggest that an equilibrium situation may exist between soluble lectin pools inside and outside the seed. Total lectin activity accumulated outside the seed at various times after initiation of hydration is shown in Fig. 2c. While such data may represent a complex turnover situation (for example, loss of activity due to protein denaturation or protease degradation over longer incubation periods), there is a rapid accumulation of lectin outside the seed during the active hydration process and a gradually increased accumulation in the 24- to 48-hour period.

Visual evidence of lectin release by soybean seeds is shown in Fig. 3. Here lectin released by individual seeds incubated for varying times at room temperature is demonstrated by taking advantage of the manner in which rabbit erythrocytes settle from suspension onto filter paper. In the absence of lectin a uniform "lawn" of red cells is obtained when the filter paper is removed from the suspension. The presence of lectin in a localized area causes agglutination of red cells above that area. The agglutinated cells are washed free on removal of the paper, leaving a white patch in the uniform background (Fig. 3a). Lectin release by single seeds incubated for 4, 24, and 48 hours on the same filter-paper disk is shown in Fig. 3b. A hydrated soybean is included to demonstrate the relation of the area containing the released lectin to the seed originally located at that position. By this method, lectin release by a single seed incubated for only 4 hours can be detected, and the diameter of the clear area obtained increases with the time of incubation.

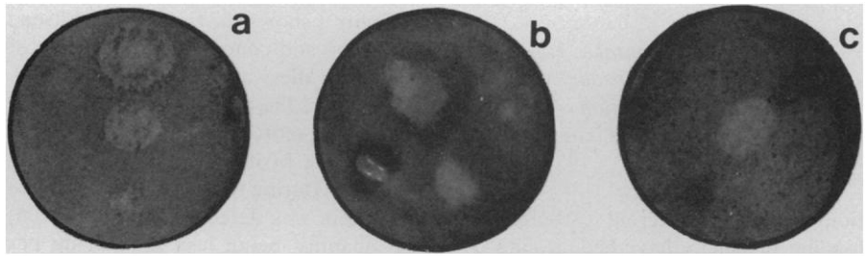

Fig. 3. Detection of lectin released into filter paper. (a) Control. Purified soybean agglutinin (from left to right) 2, 5, and 10 μg. (b) Lectin released by single soybean seeds incubated for 4 hours (lower right), 24 hours (lower left), and 48 hours (upper right). Upper left shows a hydrated soybean seed. (c) Lectin released by a single soybean seed in the presence of 0.2 percent sodium azide. Surface-sterilized seeds were hydrated on filter paper (Whatman No. 5), wetted with distilled water (or 0.2 percent aqueous solution of sodium azide), and incubated at room temperature. The seeds were removed, and the filter paper was submerged in a 4 percent suspension of washed rabbit red blood cells in phosphate-buffered saline (*p*H 7.2). The red cells were allowed to settle from suspension for 30 minutes at 37°C; the paper was then agitated briefly to wash agglutinated cells free of the paper, blotted from beneath, and stored at −60°C before photography. Purified soybean agglutinin was obtained from defatted soybean flour as described (*1*, *21*).

While these experiments show that lectin is released from the hydrating tissues of soybean seeds, the mechanism of the release is not known. But some simple experiments have yielded results deserving of closer study. The phenome-

non of lectin release appears to be independent of seed viability. Soybean seeds characteristically lose their capacity to germinate relatively rapidly within a few years of storage (*10*). Using the qualitative filter-paper assay, we have tested such viable and nonviable seeds of the soybean varieties noted earlier for lectin release with identical results—lectin is released regardless of germination capacity.

Perhaps the most significant aspect of early lectin release by soybean seeds is an observed insensitivity to sodium azide, a potent inhibitor of oxidative phosphorylation and cellular metabolism. At azide levels of 0.02 percent (3.08 m*M*) and 0.2 percent (30.8 m*M*), which completely inhibit the germination response of viable soybean seeds and which far exceed azide levels that produce anomalous germination effects in some legume seeds (*11*), the lectin-release phenomenon is still evident (Fig. 3c). Such azide concentrations had no effect on the endpoint (hemagglutination titer) of agglutination induced by purified soybean agglutinin. Azide insensitivity may suggest that the mechanism by which soybean lectin is released from the seed in the early phases of hydration may differ radically from other known seed protein export systems. In the barley aleurone system, for example, where certain hydrolases are exported from the aleurone layer of the endosperm in response to gibberellic acid, the appearance of enzyme activity is sensitive to inhibitors of oxidative phosphorylation (*12*) and requires de novo protein synthesis (*13*), activation of phospholipid biosynthesis (*14*), and elaboration of endoplasmic reticulum (*15*) and membrane-bound polyribosomes (*16*), ostensibly for the purpose of compartmentation and transport of the enzymes to be secreted. Soybean lectin, in contrast, is detectable in dry seeds, and its appearance during hydration is presumably the result of activation of a preexisting stored protein fraction. While its localization within the seed is probably chiefly confined to cotyledonary tissue (*17*), it is apparently not associated with the protein bodies in cotyledonary cells where the 7*S* and 11*S* "glycinin" storage proteins reside (*18*). Our observations of an insensitivity of lectin release to azide, and a similarity in changes in lectin activity between seed-associated lectin and lectin outside the seed, may indicate that soybean lectin is not a cellular component as has been suggested (*19*). It may instead be localized in the cell walls or apoplastic portion of seed tissues, and its release upon seed hydration may be a result of simple diffusion of the protein from the hydrating apoplasm. This hypothesis is consistent with the recent suggestion (*6*) that, of the lectin activity associated with mung-bean hypocotyl tissue, most is a non-covalently bound component of the cell-wall fraction, and also with the demonstration (*20*) that lectins of cotyledons from jackbean and red kidney bean are localized in clusters of spherical bodies in the intercellular spaces, on the cell walls, and at the periphery of the cytoplasm associated with the cell membrane.

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Aluminum Absorption and Distribution: Effect of Parathyroid Hormone

Abstract. *In rats, gastrointestinal aluminum absorption and tissue distribution were altered by parathyroid hormone; the resultant tissue concentrations were similar to those observed in dialysis patients with a fatal encephalopathy. In dialysis patients, serum aluminum and endogenous parathyroid hormone concentrations are significantly correlated. These data suggest that aluminum toxicity in dialysis patients results from oral aluminum ingestion in the presence of hyperparathyroidism.*

Aluminum has been historically regarded as nonessential (*1*) and nontoxic (*2*). Environmental exposure is virtually universal as aluminum constitutes a substantial part of the earth's crust and is commonly found in food, medicine, and cosmetics. Nevertheless, aluminum toxicity has been observed following the direct application of aluminum salts to cerebral tissues (*3–5*), and there have been increasing reports of aluminum toxicity from environmental exposure. An aluminum ball-mill operator with progressive encephalopathy was found to have brain aluminum concentrations 20 times normal (*6*). High concentrations of aluminum in brain have also been demonstrated in Alzheimer's disease (*3*) and in patients on long-term hemodialysis (*7*). Among these hemodialysis patients was a group with a fatal encephalopathy and brain aluminum concentrations that were 12 times normal. Dialysis patients without encephalopathy had four times the normal concentration of aluminum in the brain.

Patients with dialysis dementia, those with Alzheimer's disease, and the aluminum ball-mill operator all exhibited a progressive encephalopathy characterized by severe motor and behavioral abnormalities. Increased respiratory alumi-