Pineal timekeeping ability can account for phase setting. Restoration of a rhythm with a nonoscillating gland would require an interaction between the pineal gland and another structure to produce an oscillation. Another possible explanation is that the pineal gland permits expression of the innate rhythm.

3) The pineal gland measures the length of the dark-time. We have concluded from our previous investigations that the pineal gland may function to measure the length of the dark-time against internal standards established by a combination of prior photoperiod and an internal circadian oscillation (*2*). From the experiment reported here, we suggest that pineal gland timekeeping ability could provide an internal standard. Further experimentation following a variety of photoperiodic treatments is necessary to establish whether the glands contain information as to prior photoperiod.

Our current view of the circadian organization in vertebrates is that the pineal gland plays a crucial role and has at least part of the timekeeping mechanism. At this point we view the system as requiring other structures, perhaps the suprachiasmatic nucleus, which is involved in circadian rhythms in mammals (*7*), and other sites of melatonin synthesis, such as the retina (*8*). We also expect that the exact organization of the system will vary in detail from one species to another in such ways as to account for behavioral differences (nocturnal versus diurnal) and physiological differences (photoperiodism) associated with the environmental adaptations of each species.

SUE BINKLEY
JEROME B. RIEBMAN
KATHLEEN B. REILLY
Biology Department,
Temple University,
Philadelphia, Pennsylvania 19122

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Ratio of Plasma Alpha Amino-*n*-Butyric Acid to Leucine as an Empirical Marker of Alcoholism: Diagnostic Value

Abstract. *The ratio of plasma alpha amino-*n*-butyric acid to leucine was raised in patients with both alcohol-related and nonalcohol-related liver disease. This ratio appears to act as a relatively nonsensitive index of hepatocellular dysfunction rather than an index of alcoholism.*

It has been shown that the plasma ratio of alpha amino-*n*-butyric acid to leucine (A/L ratio) is raised in chronic, heavy drinkers and the suggestion has been made that this ratio might serve as an objective, empirical marker of alcoholism (*1*).

In order to test this hypothesis we have looked at the A/L ratio in the plasma of 50 control subjects, 43 alcoholics with various degrees of liver damage, and 77 patients with nonalcoholic liver and biliary tract disease. We have also studied the relation between the A/L ratio and several parameters of liver cell damage, including histology, and have observed the changes that occur in the ratio postprandially and diurnally.

Venous samples of blood were taken between 9 and 10 a.m. and placed in heparinized tubes. The plasma was separated and deproteinized with 70 percent sulfosalicylic acid, 50 μl per milliliter of plasma. Norleucine was added to the su-

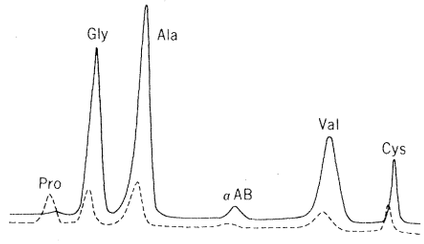


Fig. 1 (left). Part of a plasma amino acid tracing from a control subject showing the small peak of α-amino-*n*-butyric acid. Abbreviations: Pro, proline; Gly, glycine; Ala, alanine; αAB, α-amino-*n*-butyric acid; Val, valine; and Cys, cysteine. The peak area is proportional to the amino acid concentration, the proportionality constant being similar for all peaks shown. Fig. 2 (right). Relation between the plasma A/L ratio and liver histology in 43 patients with alcohol-related liver disease. Histological grading : 1, mild; 2, moderate; and 3, severe liver damage.

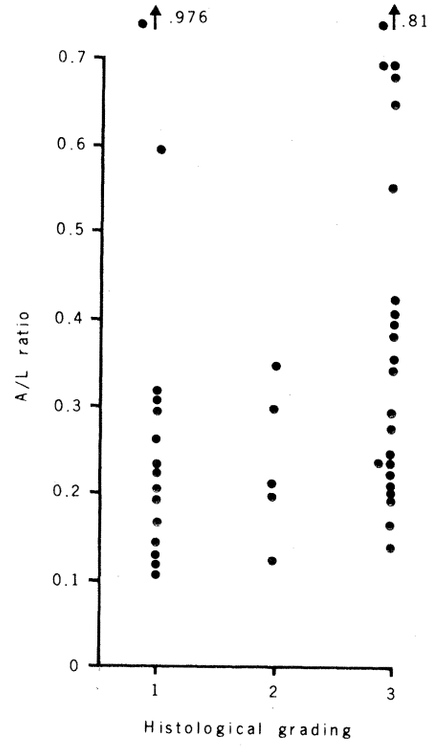


Table 1. A/L ratio in control subjects and in the alcoholic group: a history of recent alcohol abuse is accounted for.

Group	Patients (No.)	A/L mean ± 1 S.D.	Significance from control*
Control†	50	0.181 ± 0.058	
Total alcoholic group	43	0.225 ± 0.110	$P < .025$
Alcoholics recently drinking	22	0.226 ± 0.135	$.1 > P < .05$
Alcoholics not recently drinking	21	0.219 ± 0.080	$P < .05$

*Student's *t*-test. †Ten laboratory staff, 20 neurological inpatients, 10 dermatology inpatients, and 10 gastroenterology inpatients.

Table 2. A/L ratio in control subjects and in the alcoholic group, taking account in the alcoholics of (i) a history of recent alcohol abuse and (ii) the severity of the liver damage as judged histologically.

Group	Patients (No.)	A/L mean ± 1 S.D.	Significance from control*
Controls	50	0.181 ± 0.058	
Fatty change only: *drinking*	13	0.169 ± 0.065	NS†
Fatty change only: *not drinking*	9	0.177 ± 0.036	NS†
Alcoholic hepatitis ± cirrhosis: *drinking*	9	0.308 ± 0.170	$P < .001$
Alcoholic hepatitis ± cirrhosis: *not drinking*	12	0.250 ± 0.090	$P < .005$

*Student's *t*-test. †Not significant.

Table 3. A/L ratio in control subjects and patients with nonalcoholic liver and biliary tract disease.

Group	Patients (No.)	A/L mean ± 1 S.D.	Significance from control*
Controls	50	0.181 ± 0.058	
Active chronic hepatitis	24	0.289 ± 0.092	$P < .001$
Biliary cirrhosis (degree 1)	20	0.262 ± 0.085	$P < .001$
Cryptogenic cirrhosis	12	0.298 ± 0.118	$P < .001$
Type A hepatitis	9	0.266 ± 0.150	$P < .005$
Biliary disease†	12	0.210 ± 0.085	NS‡

*Student's *t*-test. †Patients with cholelithiasis ± cholangitis. ‡Not significant.

pernatant after deproteinization to a final concentration of 0.25 m*M* to act as an internal standard. All samples were stored at −20°C prior to analysis with a Technicon TSM amino acid analyzer. We used a 6-hour taped program for the two-column method for physiological solutions. The alpha amino-*n*-butyric acid peak is one of the smallest (Fig. 1) and hence measuring the peak area is subject to considerable error among observers; consequently all values were calculated by one observer to obviate this error as far as possible. The overall reproducibility of results was consistent within ± 5 percent.

In the control subjects, the mean A/L ratio ± 1 S.D. was 0.181 ± 0.058. The mean A/L ratio ± 1 S.D. in the 43 alcoholic patients was significantly higher than in the control subjects, 0.225 ± 0.110 ($P < .025$). However, in these alcoholics the A/L ratio was raised whether or not there had been recent alcohol ingestion (Table 1). This suggested that a rise in the ratio might indicate alcohol-related liver damage rather than alcoholism per se.

All 43 alcoholics had had a recent liver biopsy and were grouped into those with fatty change only, and those with alcoholic hepatitis or cirrhosis, or both. In the alcoholics with fatty change only, the A/L ratio was not raised regardless of recent alcohol abuse. By contrast, in the alcoholics with more advanced liver damage the ratio was raised both in patients who had been drinking recently and in those who had not (Table 2). This result supported the idea that a rise in the A/L ratio reflected hepatocellular

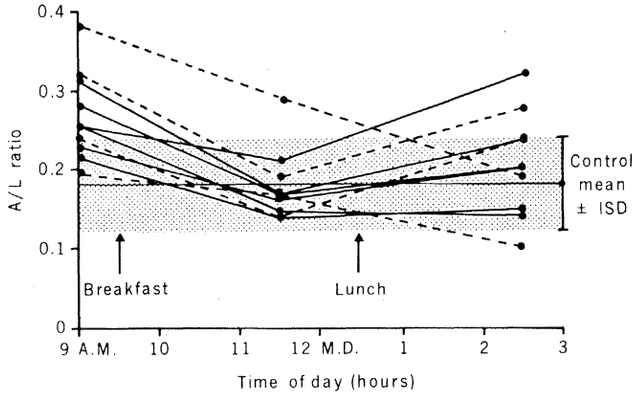
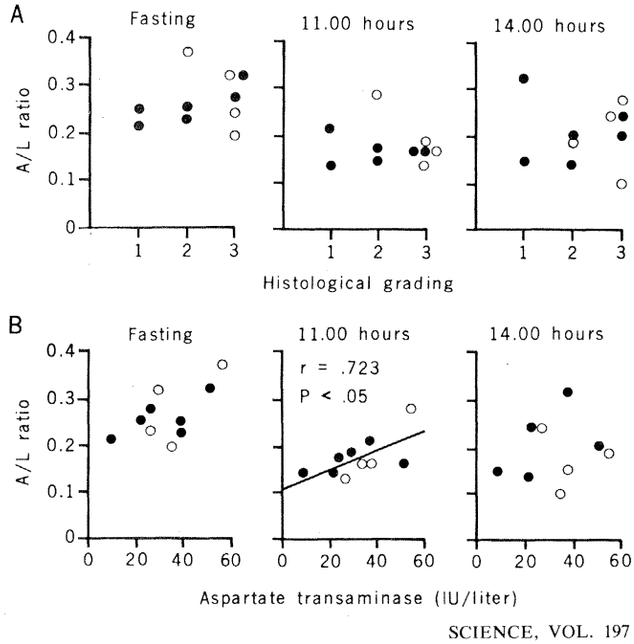


Fig. 3 (left). Fasting and 2-hour postprandial variation in the plasma A/L ratio in patients with alcohol-related and nonalcohol-related liver and biliary tract disease. (----) Alcoholic; (——) nonalcoholic. Fig. 4 (right). (A) Relation between plasma A/L ratio and liver histology in patients with liver disease. Histological grading: 1, mild; 2, moderate; and 3, severe liver damage. (B) Relation between the plasma A/L ratio and the aspartate transaminase in patients with liver disease. (○) Alcoholic; (●) nonalcoholic.



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.

MARSHA Y. MORGAN
JUDITH P. MILSOM
SHEILA SHERLOCK
Royal Free Hospital School of Medicine, University of London, Hampstead, London NW3 2QG, England

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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-