Table 1. Phosphorus analyses of anemone fractions.

Fraction	Phosphorus (%)	
	Total	Hydrolyzable
70% ethanol soluble	0.68	0.47
Chloroform soluble	2.28	1.53
Insoluble residue	0.95	0.66

total and hydrolyzable phosphorus were 9.39 and 9.45 percent, respectively (calculated for $C_{18}H_{15}O_4P$: P, 9.49 percent for both). For 2-aminoethylphosphonic acid, total and hydrolyzable phosphorus were 25.1 and 0.13 percent, respectively (calculated for $C_2H_8NO_3P$: P, 24.8 percent; no hydrolyzable phosphorus).

A portion (10 g) of the insoluble proteinaceous residue was hydrolyzed by refluxing for 24 hours in 4*N* hydrochloric acid. After filtration and evaporation, the hydrolysate was placed on a Dowex-50 (H^+) column. Compounds eluted with water had no carbon-phosphorus bonds detectable by analysis (total phosphorus, 14.3 percent; phosphate P, 14.0 percent). Elution with 1:9 concentrated ammonia-water gave 8.1 g of amino acids (total P, 0.66 percent). A 3.0-g portion was fractionated on a 2.8-cm by 28-cm column of Dowex 1 (×8, 200 to 400 mesh, acetate form) with 0.5*N* acetic acid (*3*). There was obtained from the 50- to 110-ml fraction, 2.4 g of solid (0.63 percent P), and from the 110- to 260-ml fraction, 0.3 g (0.085 percent P). Elution of the column with 3*N* acetic acid gave an additional 0.5 g (0.094 percent P). The first of these fractions was chromatographed again (in two portions) on the Dowex-1 column with water. The bulk of the solids (2.25 g) was eluted in the 50- to 130-ml fraction, but contained little phosphorus (0.088 percent). However, the 170- to 570-ml eluate contained a solid rich in phosphorous. Some amino acid impurities were removed from this solid by a second passage with water through the same column, and color was then removed with a pinch of Norite. The white solid (40 mg) obtained on evaporation gave a single spot (R_F, 0.24) on paper chromatography in a fresh mixture of butanol, acetic acid, and water (4 : 1 : 2) (*1*). Material accumulated in this fashion was twice recrystallized by adding ethanol to a concentrated water solution. The product melted at 282° to 283°C (with decomposition), contained 25.1 percent phosphorus, and had R_F of 0.54 in a pyridine and water mixture (65 : 55). Synthetic (*5*) 2-aminoethylphosphonic acid (calculated for $C_2H_8NO_3$ P: P, 24.8 percent) melted at 282° to 283°C (with decomposition) and gave R_F of 0.23 and 0.54 in the butanol and pyridine systems, respectively. The natural and synthetic compounds had identical proton magnetic resonance spectra (D_2O solution) in accord with the assigned structure. The amount of isolated phosphonic acid, scaled to the entire insoluble protein fraction, was 0.440 g. Additional small amounts were indicated by paper chromatography in various fractions from the ion-exchange chromatographic separation.

Application of the foregoing hydrolysis and separation procedures to 5.4 g of the 70-percent ethanol-soluble fraction gave 35 mg of 2-aminoethylphosphonic acid (107 mg for the entire fraction), and to 2.5 g of the chloroform-soluble fraction gave 37 mg (66 mg for the entire fraction). The total yield of 2-aminoethylphosphonic acid isolated from 467 g of *Metridium dianthus* was 0.613 g.

That 2-aminoethylphosphonic acid may be incorporated in the protein structure is suggested by its inclusion in material precipitated from partial hydrolysates by trichloroacetic acid. The free amino acid is not precipitated under similar conditions. Exposure of 2.0 g of insoluble proteinaceous material to 25 ml of 1*N* sodium hydroxide for 30 minutes at room temperature resulted in almost complete solution. The mixture was centrifuged; the supernatant liquid was adjusted to *p*H 7, and again centrifuged to remove a slight precipitate. The addition of 30-percent trichloroacetic acid caused immediate precipitation of 0.39 g (total P, 0.69 percent; hydrolyzable P, 0.53 percent). This precipitate was hydrolyzed, and the ion-exchange procedures applied. The presence of 2-aminoethylphosphonic acid in the proper fraction was demonstrated by two-dimensional paper chromatography in the butanol and pyridine systems.

Partial basic hydrolysates were also passed through a column of Dowex-50 (H^+). Material eluted with water, as well as that retained on the column and eluted with a mixture of ammonia and water (1 : 9), contained the carbon-phosphorus bond. These and enzymatic partial hydrolysates should be useful in studies to confirm and define a role for 2-aminoethylphosphonic acid in protein structures (*6*).

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Chlorophyll Content and Growth of Soybean Plants: Possible Interaction of Iron Availability and Day Length

Abstract. *Data indicate a relationship between the availability of iron and the response of "Hawkeye" soybeans to day length. The more readily available the iron, the less influence an increase in day length on dry weight. When iron was present but not chelated the plants remained green in a 16-hour day but became chlorotic in a 9-hour day.*

In some greenhouse studies on the responses of soybean plants to different iron chelates (*1*), it was noted that as the summer season approached, more and more days in iron-deficient solutions were required to induce the degree of chlorosis desired in the experimental plants. This suggested that day length might be a factor in iron availability or iron utilization by soybeans or, in turn, that the availability of iron might influence the response of the plants to day length.

Soybean plants *Soya max* var. Hawkeye were grown from seed in a complete nutrient solution, at *p*H 6, con-

Table 1. Milligrams of chlorophyll per gram of leaf dry weight of soybean plants grown in water cultures in indicated treatments. Each value is the mean of three replications.

Treatments	Days in treatments			
	7	14	21	28
No iron				
16-hour day	4.0	2.4	2.1	2.0
9-hour day	4.3	3.4	2.1	2.0
Iron as $FeNH_4(SO_4)$*				
16-hour day	4.6	4.6	5.0	4.7
9-hour day	4.6	4.0	3.1	2.7
Iron as FeNTA*				
16-hour day	5.2	5.0	5.3	5.3
9-hour day	5.0	5.3	5.5	5.6

* Iron supplied at concentrations of 0.075×10^{-5} mole/lit.

taining a minimum amount of iron (0.1 ppm $FeSO_4$) to keep the plants a healthy green color. After 3 weeks they were transferred to water cultures containing (i) no iron, (ii) 0.075×10^{-5} mole of $FeNH_4(SO_4)_2$ per liter, and (iii) the same concentration of ferric nitrilotriacetate (FeNTA). The plants in all three iron treatments were grown in the natural daylight existing in Pasadena, California, during October, in greenhouses from 8:00 a.m. to 5:00 p.m. At 5:00 p.m. each afternoon, one-half of the plants were transferred to aerated, light-sealed cabinets in an inside room. The other half were transferred to the same room and exposed from 5:00 p.m. to midnight to radiation of 10 ft-ca (110 lu/m^2) intensity from a 100-watt incandescent bulb. In this way the plants in the various treatments were exposed to the same conditions of temperature and humidity. The nutrient solutions were changed once during the 4-week period of the study. The temperature in the greenhouse ranged from 21° to 29°C (70° to 85°F) during the normal daylight hours, and was held at 22°C. during the "night" period.

The plants were held in the treatments from 4 October to 1 November 1961. During this time the chlorophyll content of the leaves was measured at weekly intervals by a reflectance method (*2*). The results of these determinations are shown in Table 1.

All the leaves on plants grown in solutions of the chelated iron, FeNTA, maintained or increased their chlorophyll content regardless of day length. The leaves on all the plants receiving no iron lost their chlorophyll and at about the same rate, regardless of the day length. However, the leaves of the plants given $FeNH_4(SO_4)_2$ maintained their chlorophyll content in the long day, but in the short days their chlorophyll content decreased almost as rapidly as in the leaves of the plants receiving no iron. An analysis of variance showed a highly significant interaction between day length and iron compounds. In other words, exposing the plants to low light intensities during normal night-time hours delayed or prevented the development of iron-deficiency symptoms (chlorosis) where iron was not readily available.

The dry weights of these plants are shown in Table 2. The results show that increasing the availability of the iron or adding supplemental light at night increased the growth of the plants. The results also show that the more readily available the iron, the smaller the proportionate increase in growth produced by lengthening the day.

The results of this preliminary study indicate that the response of plants to iron may be influenced by day length, or the response to day length may be influenced by the iron supply. It may be that iron is absorbed only during daylight hours and that the nonchelated iron was partially precipitated out as the result of reaction with the phosphate in the nutrient solution. Thus, during the short days the plants could not acquire sufficient iron to keep the leaves green. However, if such a precipitate was formed, it was quite small, since no noticeable amount was detected when the solutions were changed or at the close of the experiment. It would be of interest to repeat these studies with a long-day variety of soybean. The iron content of the leaves was not determined, so it is not possible to conclude whether there was actually less iron in the chlorotic leaves of the plants receiving $FeNH_4(SO_4)_2$ in the short days, or whether the iron was rendered nonavailable for chlorophyll formation.

Table 2. Dry weight of soybean plants grown under short and long days in water culture containing different iron compounds. Each value is the mean of three replications of four plants each.

Plant part and iron compound	Dry weight (g)		Ratio, 16-hr day wt ÷ 9-hr day wt
	9-hr day	16-hr day	
Leaf			
None (control)	1.33	2.53	1.90
$FeNH_4(SO_4)_2$*	2.40	3.43	1.43
FeNTA*	3.77	5.00	1.33
Stem			
None (control)	0.87	1.97	2.26
$FeNH_4(SO_4)_2$	2.30	2.57	1.12
FeNTA	3.10	3.13	1.01
Root			
None (control)	0.70	0.87	1.24
$FeNH_4(SO_4)_2$	0.77	0.93	1.21
FeNTA	1.23	1.43	1.16

* Iron concentration 0.075×10^{-5} mole/lit.

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Serotonin Binding to Nerve-Ending Particles of the Rat Brain and Its Inhibition by Lysergic Acid Diethylamide

Abstract. *The binding of serotonin to nerve-ending particles and other preparations from rat brain has been examined. By investigating the amount bound as a function of serotonin concentration from 10^{-7}M to 10^{-2}M, it was possible to identify three major components having K_{assoc} (association constant) values of 2×10^6, 5×10^4, and 5×10^2. The component having the highest binding constant was not present in liver and appeared to be confined to the cortex and midbrain regions. This component is inhibited by* d*-lysergic acid diethylamide at low concentrations. Solubilization of this binding component has been achieved.*

As part of a general investigation into the biochemistry of events at the synapse, the binding of serotonin to preparations of nerve-ending particles from whole rat brain has been examined. Previous studies have implicated serotonin in neural events (*1*). Many studies have been made on its binding to tissues; in this report we distinguish between a low-affinity nonspecific binding and a binding of very high affinity which is specific to certain brain components. Any tissue preparation may contain one or more binding compo-