ica? It is the crowning distinction of the American university that it has built a worthy part of the national temple. While there may be confusion in confused minds concerning university purpose, could anything be clearer than that free inquiry and report is itself one of the greatest and most highly moral educational purposes in a democracy? The university is not designed to be authoritarian and say the last word in morals, ideas, research or teaching. We can avoid the authoritarian road only by choosing the free road. A body of prescribed doctrine, approved books, dialectical smartness and *a priori* opinion masquerading as "wisdom" can give "unity" also, as authoritarian governments have amply demonstrated. The strength of our American unity is in our free way of uniting. Our purpose is to train that freedom into responsibility. The growth of our educational program is due to the demonstrated need for the trained and responsible men and women we help to produce, with all faults of training and learning fully and freely admitted. In part our directive principles and powers grow out of what we find, in part out of a high court of moral judgment called informed opinion. No earthly device can make all our people wise or infallible in judgment. Wisdom that serves unity and fair social living is not an affair of graven tablets to be memorized, nor yet a delusion based on "ragged notions and babblements," but experience of the individual with the world after some thought about human experiences as recorded in the books by minds that are trained to be critical. "Scientific method" is one of several ways by which such training may be developed. We are not far enough advanced biologically to get assured wisdom at 21, as a result of some final scheme of formal education that has so far eluded us according to the critics.

SPECIAL ARTICLES

PYRIDOXINE AS A GROWTH FACTOR FOR GRAPHIUM

PYRIDOXINE (vitamin B_6) is known to be essential for the growth of excised plant roots,[1,2] for certain mutant strains of *Neurospora*,[3] and for the G.M. strain of yeast.[4] In connection with our studies on the vitamin requirements of fungi, an interesting example of pyridoxine deficiency has been found in cultures of *Graphium ulmi*, the organism which causes Dutch elm disease. This preliminary report on *Graphium* presents an example of the requirement of an accessory growth factor necessary for the life of a parasitic fungus. In view of recent work on chemotherapy by use of analogs which compete with natural growth factors in certain microorganisms causing diseases, it is hoped that this information may be of some use to phytopathologists who are attempting to control the Dutch elm disease.

The basal solution used for cultivating *Graphium* contained the following in one liter of triple distilled water: recrystallized asparagine, 4.0 gm; dextrose, 40 gm; KH_2PO_4, 1.5 gm; $MgSO_4 \cdot 7H_2O$, 0.5 gm; $CaCl_2 \cdot 2H_2O$, 0.1 gm. The elements, B, Mn, Zn, Cu, Mo and Fe were supplied in appropriately small quantities. Vitamins were added to this medium in the following amounts per liter: thiamin, 300γ; lactoflavin, 100γ; nicotinic acid, 500γ; pyridoxine, 200γ; inositol, 500γ; pantothen, 200γ; biotin, 0.4γ; and ascorbic acid, 500γ. The medium was dispensed into pyrex flasks, 25 ml per flask, and autoclaved at 15 pounds for 20 minutes. Inoculation was accomplished with bacteriological precautions by pipetting a drop of spore suspension into each flask. Growth was determined at the end of two weeks by weighing the washed and dried mycelium on filter paper.

A typical experiment was set up with basal medium to which no vitamins were added, basal medium containing the 8 vitamins, and a series of media in which one vitamin was omitted at a time to give 8 kinds of single deficiencies. Five replications were employed for each kind of medium. Growth was obvious only in the media containing pyridoxine. The presence or absence of the other accessory substances made little difference in growth. In another experiment each of the 8 vitamins was added singly to the basal medium in order to determine the effectiveness of each in the absence of the other 7 accessory factors. Growth occurred only in the medium which contained pyridoxine. It appears that *Graphium* is able to grow in the basal medium supplemented with only pyridoxine. Either these other vitamins are not essential, or what is more likely, the fungus synthesizes out of elementary compounds other factors which are necessary for growth.

TABLE 1

Pyridoxine gamma/liter	Dry weight of *Graphium* mg/flask
0.005	0.2
0.01	0.3
0.1	1.5
1.0	4.5
50.0	14.2
500.0	13.5

1 W. J. Robbins and M. B. Schmidt, *Proc. Nat. Acad. Sci., U. S. A.*, 25: 1, 1938.

2 J. Bonner, *Amer. Jour. Bot.*, 27: 692, 1940.

3 G. W. Beadle and E. L. Tatum, *Proc. Nat. Acad. Sci., U. S. A.*, 27: 499, 1941.

4 R. J. Williams, R. E. Eakin and J. R. McMahan, University of Texas Publication 4137: 24, 1941.

Further studies on the extent of growth in basal medium supplemented with increasing amounts of pyridoxine gave the results shown in Table 1 expressed as the average dry weight of five flasks for each treatment.

The fungus is sensitive to increments of pyridoxine at least in the range from 0.01 to 50.0 gamma per liter. No claim is made for a complete medium for growth of *Graphium*. Additions of yeast or of dark molasses to the medium containing pyridoxine permits still greater growth of the fungus. It seems probable that other unidentified essential factors may be made by the fungus in less than optimum amounts for growth in this synthetic medium.

PAUL R. BURKHOLDER
ILDA MCVEIGH
OSBORN BOTANICAL LABORATORY,
YALE UNIVERSITY

ELECTROPHORESIS OF THE CHLOROPHYLL-PROTEIN COMPLEX

A NUMBER of reports concerning the isolation and purification of a chlorophyll-protein complex from leaves have recently appeared. Although the protein nature of the material composing the complex has been supported by analytical evidence, it seemed desirable to investigate the problem by means of electrophoresis in an attempt to demonstrate the existence of an isoelectric point in the range of pH characteristic of proteins.

In 1912 it was observed by Herlitzka,[1] on subjecting press juice of leaves to electrophoresis, that the chlorophyll migrated toward the anode. More recently, Neisch[2] has reported that suspensions of chloroplast granules isolated from clover leaves are electrophoretically negative in distilled water and positive in N/5 HCl. Aside from these reports, there seems to be no information in the literature concerning the electrophoresis of the purified chlorophyll-protein complex.

We have prepared suspensions of the complex by a modification of the methods of Menke[3] and of Stoll and Wiedemann.[4] Young leaves of beans (*Phaseolus vulgaris* L., var. white navy) were ground in M/100 phosphate buffers at pH 7.2 and the deep green suspension was centrifuged to remove cellular debris. Neutralized ammonium sulfate was added to the supernatant solution until the molarity reached 1.3. After a wait of thirty minutes the green material was sedimented by centrifugation; this left the brown impurities dissolved in the supernatant. The operation of resuspension in the buffer and subsequent salting out was repeated several times in the course of several hours. The green material was finally resuspended in the buffer and dialyzed against the same buffer until free from sulfate. All preparative operations were carried out at 0° C. The purified green suspensions exhibited the physical characteristics described by other workers,[4, 5, 6] *e.g.*, a strong maximum absorption band at about 678 mμ, photostability, red fluorescence, sensitivity to protein coagulants. When the pH was reduced below 5, flocculation ensued.

Using a microelectrophoresis technique which has been described elsewhere,[7] we have found that the green microscopic particles, which make up the suspensions, are isoelectric at pH 4.7 in M/50 acetate buffers at 25° C. The particles migrate independent of size, shape or degree of clumping. Particles at the limits of microscopic visibility (at a magnification of 1,100) migrated at the same rate as larger ones. In fact, the particles behaved as though their surfaces were exceedingly uniform. By centrifugation at about 4,000 r.p.m. it was possible to remove all visible particles. Quartz or collodion particles placed in the resultant clear green solutions exhibited an electrophoretic behavior identical, within the limits of error, with the natural chlorophyll-containing particles of the original suspensions. No significant differences could be noticed between the electric mobilities of various preparations. Under the above ionic conditions the electric mobility curve tends to flatten out on the negative side at about pH 6.5 and on the positive side at about pH 3.6.

Smith[6] reports that suspensions flocculated at pH 4.5 or below are altered by the acid and more readily salted out after resuspension. We have found that particles from suspensions exposed to a pH of 3.1 (M/25 acetic acid) for more than thirty minutes exhibit an altered electric mobility when resuspended in more basic buffers. The electric mobility curve is shifted so that the isoelectric point lies at pH 5.0. The curves are nearly parallel on the negative side but converge on the positive side. Investigations of the rate of this denaturation process show that it is virtually complete within five to ten minutes at pH 3.1 (at 25°). At values of pH more basic than 3.1, the process is less rapid. Since at least five minutes are needed to make a measurement of electric mobility, it is apparent that the electric mobility of the undenatured material can not readily be determined below pH 4.5. In this respect the particles are similar to thyroglobulin in their behavior.[8]

1 A. Herlitzka, *Biochem. Z.*, 38: 321, 1912.
2 A. C. Neisch, *Biochem. Jour.*, 33: 293, 1939.
3 W. Menke, *Z. Bot.*, 32: 273, 1938.
4 A. Stoll and E. Wiedemann, *Fortschr. Chem. organ. Naturstoffe*, 1: 159, 1938.
5 J. Shafer, SCIENCE, 91: 580, 1940.
6 E. L. Smith, *Jour. Gen. Physiol.*, 24: 565, 1941.
7 L. S. Moyer, *Jour. Bact.*, 31: 531, 1936.
8 M. Heidelberger and K. O. Pedersen, *Jour. Gen. Physiol.*, 19: 95, 1935.