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.

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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.

It would seem premature to assert that these observations characterize the chlorophyll-protein itself. In any event, the electrophoretic properties are closely correlated with the general colloidal behavior of the complex. We intend to publish an extended account of these investigations in the near future.

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EFFECTS OF OXYGEN ON RESPIRATION, FERMENTATION AND GROWTH IN WHEAT AND RICE

RICE seeds are capable of germinating in the absence of oxygen and the seedlings grow well when submerged in water containing little oxygen, whereas wheat is unable to germinate or develop under such conditions.[1] In an attempt to ascertain the physiological basis for this difference, a study was made of the influence of variations in oxygen tension (pO_2), from 0 to 20.8 per cent., on respiration, fermentation and growth in seedlings of each of these cereal grains.

Manometric measurements of the gas exchanges occurring in seedlings of wheat (*Triticum vulgare* var. Leap's Prolific) and of rice (*Oryza sativa* var. Early Prolific) were conducted at 30° C., using a Barcroft-Warburg apparatus according to the method of Dickens and Simer.[2] The seedlings had roots 5 to 8 mm long when germinated in the dark at 30° C. on moist filter paper; for this development wheat required 24 to 26 hours, rice 30 to 33 hours. Groups of uniform seedlings (25 wheat or 30 rice) were soaked for one hour in a 0.013 molar phosphate buffer adjusted to pH 5.8, containing 4 per cent. sucrose and having a K/Ca balance of 7.5/1. Then the seedlings of each group were placed on a bed of glass beads in the manometer vessel with their roots submerged in the buffer solution to a depth of 1 to 2 mm. Prior to each test of 1 to 1.5 hours' duration, the vessels were thoroughly flushed with the required gas mixture, prepared by partially replacing normal air with nitrogen.

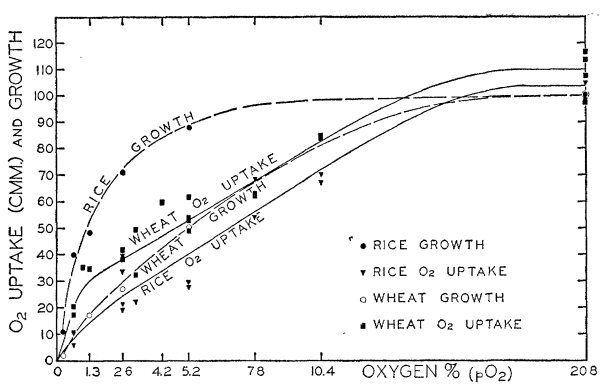

FIG. 1.

The results of the gas-exchange measurements are shown in Figs. 1 and 2, in which smoothed curves have been drawn in solid lines to show the trend of the experimentally determined points, each of which represents the average of 4 or 5 separate determinations. In 20.8 per cent. O_2 (air) the average activity of rice seedlings, as shown by CO_2 evolution, was found to be 87 per cent. of the average of wheat seedlings on the basis of dry weight of embryos. To facilitate comparison of the experimental data, the curves in all cases have been plotted for the activity of a number of seedlings (wheat, 6.4; rice, 13.3) calculated to produce 100 cmm of CO_2 per hour in 20.8 per cent. O_2.

In the growth experiments 225 twelve-hour-old seedlings of wheat and rice were allowed to develop for 96 hours at 30° C. on moist filter paper in gas streams containing from 0.25 to 20.8 per cent. O_2. Increment in dry weight of embryo (*i.e.*, of all the seedling except the endosperm) was used as a measure of growth. The curves depicting the results of the growth experiments are presented in Fig. 1, plotted on a scale of ordinates in which 100 represents the increment in embryo dry weight in 20.8 per cent. O_2.

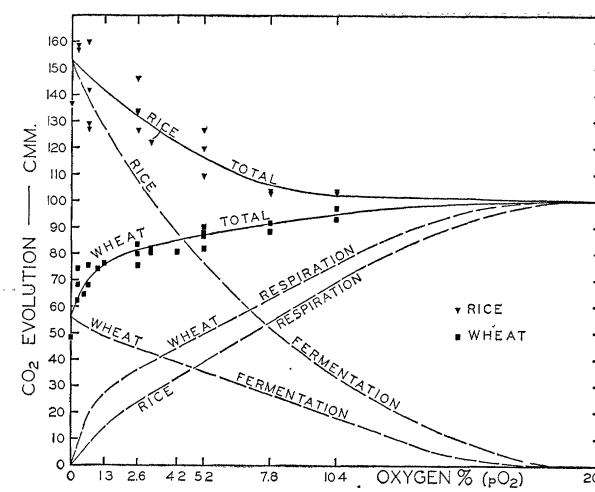

FIG. 2.

Inspection of the curves of Fig. 1 for O_2 consumption reveals the following relations: The O_2 intake by rice is less rapid than that by wheat over the range of O_2 tensions in which tests were conducted. With decrease in O_2 tension, the growth curve for wheat

[1] I. Nagai, *Tokyo Imp. Univ. Jour. Coll. Agr.*, 3: 109, 1916. C. Stich, *Flora*, 74: 1, 1891.

[2] M. Dixon, "Manometric Methods," pp. 61–67. London: Cambridge University Press. 1934.