side cup (cup 3), there is a lag of fully 120 minutes before this cell-suspension reaches a rate comparable to the others. A reasonable explanation for the existence of this lag is that time is required for the conversion of the vitamin B_1 into cocarboxylase in which form it acts as the catalyst. In any case, however, if no previous incubation with the bacterial cells is permitted, cocarboxylase is a more effective stimulant than is vitamin B_1.

Summary. Evidence is presented that cell-suspensions of *P. pentosaceum* are capable of converting vitamin B_1 into cocarboxylase.

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Occurrence and Significance of Oxalacetic Acid in Plant Tissues.*

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The investigations of Szent-Györgyi,[1] Stare and Baumann,[2] and Krebs[3] have established the importance of the 4 carbon dicarboxylic acids in the respiration of animal tissues and several species of bacteria, but extension of their findings to plant tissues has not been made. Such extension might be naturally assumed since succinic, fumaric and malic acids, as well as the corresponding amino acid, aspartic, and its amide, asparagine, are well-known constituents of most plant species. One line of evidence for demonstration of this assumption would be the detection of oxalacetic acid in respiring plant tissues. In connection with our studies on the respiratory enzymes of the root nodules of leguminous plants, examination of these was made for the presence of oxalacetic acid, but none was found.

Both the colorimetric method of Szent-Györgyi and Straub[4] and the manometric method of Ostern[5] were used for detection of the oxalacetic acid. Fresh plant tissue was cooled to 0°C, ground in a Nixtamal mill and the sap expressed in a Carver hydraulic press.

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1 Szent-Györgyi, A., *Studies on Biological Oxidations and Some of Its Catalysts*, Budapest, 1937.

2 Stare, F. J., and Baumann, C. A., *Proc. Roy. Soc.* (London), 1936, **121B**, 338.

3 Krebs, H. A., *Biochem. J.*, 1937, **31**, 2095.

4 Straub, F., *Z. Physiol. Chem.*, 1936, **244**, 117.

5 Ostern, P., *Z. Physiol. Chem.*, 1933, **218**, 160.

The proteins were precipitated with tungstic acid. The manipulations were made as rapidly as possible, and all instruments were chilled before use in order to reduce decomposition of any oxalacetic acid which might be present. In the recovery tests the oxalacetic was added immediately to the expressed plant sap.

In the colorimetric method hydrazine forms a pyrazalone ring compound with oxalacetic acid which gives a deep yellow color on the addition of sodium nitrite and potassium hydroxide. Although this method is quite satisfactory for animal tissues, it was found unsuitable for detection of small quantities of oxalacetic acid in plant tissues. The data in Table I are typical of results obtained with a large number of tissues. Consideration of these data shows that the sap was free of oxalacetic acid.

TABLE I.
Estimation of Oxalacetic Acid in Young Pea Roots.

Treatment	Oxalacetic added, micrograms/ml	Color computed as oxalacetic, micrograms/ml	Recovery, micrograms/ml
Nitrite only	0	34	
All reagents	0	15	
	6.5	16	1
	13.0	34	19
	26.0	40	25
	65.0	83	68
	130.0	145	130
	320.0	313	298

Color measured by light absorption with 4200 Å filter; all values corrected for blank on sap alone, which was almost colorless. Plants used were actively fixing nitrogen.

With the addition of nitrite alone a yellow color develops which corresponds to more oxalacetic acid than is found with all 3 reagents. This color can not be distinguished from that formed by oxalacetic in presence of all reagents even with the selective filters of the Evelyn colorimeter. The decrease in apparent oxalacetic content on addition of all reagents probably arises through decomposition of part of the nitrite by the hydrazine. It follows that the "blank" due to these interfering substances cannot be estimated with any great degree of accuracy, and hence estimation of oxalacetic acid by the colorimetric method is not possible unless the quantity present is relatively large—order of 50 micrograms per ml.

As all attempts to remove the interfering substances by various precipitation, extraction and adsorption procedures were unsuccessful, the manometric method[5] was tested. With proper temperature control, this method proved satisfactory with plant tissues. As little as 25 micrograms per ml of added oxalacetic acid could be quantitatively recovered, and 10 micrograms per ml could be detected.

A large number of saps from the tops and roots of leguminous plants in various stages of growth were tested for oxalacetic acid by the manometric method, but all results were negative. These data are in agreement with the experience of Szent-Györgyi and his associates, who report that oxalacetic acid is detectable in respiring muscle tissue only after addition of a suitable precursor, as fumarate, and/or inhibitors (arsenite, hydrazine) which "fix" the acid by preventing its reduction. Both fumarate and malate were added to the saps and minced tissues of young pea plants with and without arsenite (M/100); analyses were made after incubation periods of 15 minutes to 5 hours, but oxalacetic acid was not detected. These negative results do not necessarily mean that the cycle proposed by Szent-Györgyi is not concerned with the respiration of the plants examined, only that efforts to obtain a certain type of supporting evidence have been unsuccessful. This failure is not too surprising since even in animal tissues which respire at a much higher rate than do most plant tissues, oxalacetic acid occurs only as a fleeting transitory intermediate whose detection is quite difficult.

In light of the foregoing discussion the recent reports by Virtanen and Laine[6] of finding from 500 to 1000 micrograms of oxalacetic acid per gram of wet tissue in the tops and roots of the pea plant are quite unexpected. Initially, these workers used the colorimetric method, but in a recent note[7] they state that since this method is unsatisfactory for colored portions of the plant, they have discarded it in favor of the manometric procedure.

It is suggested that the high content of oxalacetic acid found in the pea plants grown in Finland may be correlated with excretion of nitrogen by these plants. Although Virtanen and associates[6] consistently have obtained excretion of nitrogen by leguminous plants, repetition of their experiments at other stations, in general, has been unsuccessful. Our recently published evidence[8] indicates that differences in the environment at the various stations may be an important factor in determining whether or not excretion is obtained. Such differences should be reflected in biochemical variations in the plant which would control the excretion process. The accumulation of a compound such as oxalacetic acid which is usually regarded as a transient intermediate in the respiration cycle might well represent such a biochemical variation as has been postulated. This compound

[6] Virtanen, A. I., *Cattle Fodder and Human Nutrition*, Cambridge University Press, 1938.

[7] Virtanen, A. I., Laine, T., and Roine, P., *Suomen Kemistilehti*, 1938, **11B**, 25.

[8] Wilson, P. W., and Wyss, O., *Soil Sci. Soc. Proc.*, 1937, **2**, 289.

could be readily converted into aspartic acid which is the sole compound excreted.[8]

A second difference which has been noted in the plants grown in Finland and those at the Wisconsin Experiment Station is the relation of excised nodules to oxalacetic acid. Virtanen[6] reports that excised pea nodules supplied with oxalacetic acid will fix free nitrogen as rapidly as does the intact plant, but if no oxalacetic acid is supplied, fixation does not take place. We have repeated these experiments, but have never succeeded in securing fixation. In a typical experiment 100 mg of oxalacetic acid were added to 5 g of ground nodules from Torsdag peas which had been inoculated with the Finnish culture, *Rhizobium leguminosarum* HX and which were actively fixing nitrogen. Initially, the nitrogen content of quadruplicate samples was: 23.45, 23.40, 23.25, and 23.35 mg; after 24 hours' incubation the following values were obtained: 23.45, 23.40, 23.60, and 23.45 mg. Other experiments already have been reported for peas,[9] and similar results have been obtained with nodules from the soybean and cowpea. It should be noted, moreover, that not only do the nodules from pea plants grown at this station contain no oxalacetic acid, but that they decompose this compound very rapidly. Under the conditions of the nitrogen fixation experiments, 30 to 40% of the added oxalacetic acid was decomposed in 30 minutes, about 75% in one hour and almost all in 3 hours. If the nodules are not minced, decomposition is not so rapid, but in this case it is doubtful if much of the added acid actually diffuses into the nodules.

Test of the hypothesis proposed in this paper can best be made by workers at the several experiment stations who are conducting experiments on the excretion of nitrogen. If the plants are regularly examined for oxalacetic acid, it should be readily established whether or not the occurrence of large quantities of this compound in leguminous plants is correlated with the excretion phenomenon.

Summary. Because a yellow color is produced on the addition of the nitrite reagent alone, the colorimetric method of Szent-Györgyi and Straub was found unsuitable for the determination of oxalacetic acid in plant tissue, unless relatively large quantities are present. The manometric method of Ostern proved satisfactory, and though as little as 10 micrograms of added oxalacetic acid per ml of plant sap could be detected by the method, the presence of oxalacetic acid in leguminous plants actively fixing nitrogen could not be demonstrated.

It is suggested that the presence of oxalacetic acid in leguminous plants may be correlated with the phenomenon of nitrogen excretion.

[9] Wilson, P. W., *Ergeb. Enzymforsch.*, 1939, **8**, 67.