probably does not contain a phenylalanine decarboxylase. Experiments in which the cat's kidney is perfused with blood containing phenylalanine confirm these results. These experiments demonstrate that the decarboxylating enzyme responsible for the transformation of phenylalanine into its corresponding amine is species specific.

Summary. The decarboxylating enzymes contained in the kidney are specific for certain amino acids and vary with the species.

We express our sincere appreciation to the Department of Urology of the Presbyterian Hospital, for help in providing us with the human material.

11989

Quantitative Spectroscopic Analysis of Stem "Tracheal" Fluids for Inorganic Constituents.*

PHILIP HAMM, R. B. HARVEY AND ELMER S. MILLER.

From the Departments of Botany and Plant Pathology, University of Minnesota.

Many workers have reported quantitative spectrographic methods for the determination of major and trace elements necessary for plant growth. The major plant nutrition studies by spectrographic analysis have concerned either leaf tissue analysis or plant ash analysis Heretofore, the general practice consisted of volatilizing a homogenized ash in a 220 volt d-c arc with a current of 9-12 amperes. Estimations of the amounts of the constituents present have usually been carried out either by employing standard powders according to Nitchie[1] for comparison purposes, or by using suitable internal standards and determining the ratios of certain emission lines of the unknown to the suitable emission spectra of an internal standard as outlined by Gerlach.[2] Studies of ashed leaf tissue have furnished data concerning the number and respective amounts of the soil

* Aided by research grants from the Graduate School, University of Minnesota, Minneapolis, Minnesota. Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official No. 65-1-71-140, Sub-project No. 331.

The authors wish to express their sincere thanks to Dr. R. E. Oltman for assistance in certain studies on electrode impurities.

1 Nitchie, C. C., *Ind. and Eng. Chem.* Analyt. Ed., 1929, **1**, 1.

2 Gerlach and Schweitzer, *Foundations and Methods of Chemical Analysis by the Emission Spectrum.*

elements that are taken up by the plant. However, because only a portion of each element present in a plant ash is available in a mobile form, it has been difficult to interpret the significance of these analyses. Recently, Duffendack and Wolffe[3] and Hamm, *et al.*,[4] have presented evidence that both accuracy and sensitivity may be increased by employing an a-c arc across the graphite electrodes. This additional sensitivity has extended the spectrographic method to such low concentrations (10 parts per billion) as found in certain tissue fluids.

The purpose of this paper is to present in detail a rapid and direct spectroscopic method for the quantitative analysis of the inorganic constituents of plant stem "tracheal" fluids, which permits the determination of what elements are absorbed and are available at any phase of plant growth.

Experimental. Four to 12 plant stems are collected and cut into pieces 4 inches long. Usually, it is necessary to store the stems

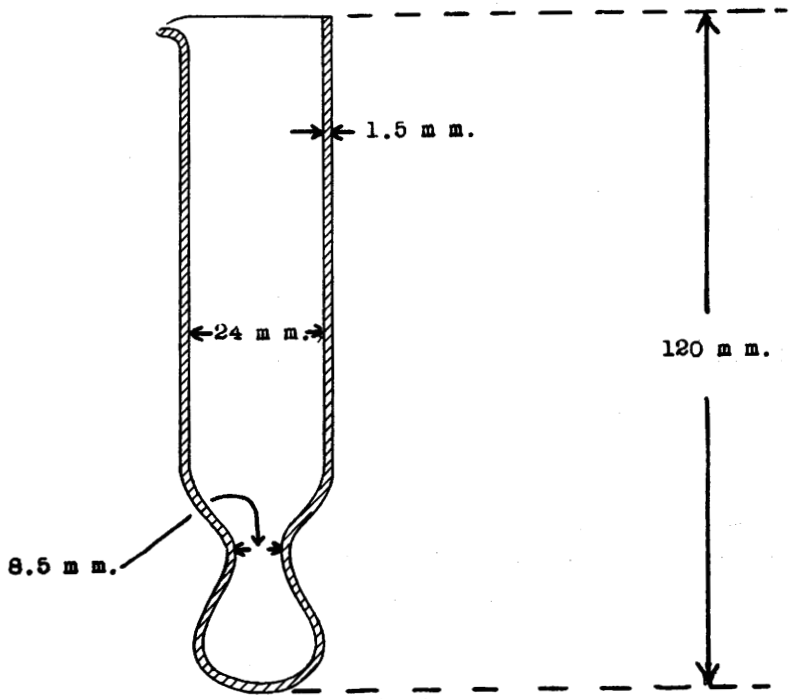

FIG. 1.
Centrifuge tube for collecting tracheal fluids.

[3] Duffendack, O., and Thompson, *Proc. Am. Soc. Testing Materials*, **1936**, **36**, 310.

[4] Hamm, Philip, *et al.* (In press.)

24 to 72 hours to permit transportation to the laboratory. Under such conditions, immediately upon collection in the field, the stems are dipped in a low melting paraffin to minimize desiccation while samples are in transit and storage. In the laboratory after the ends of the stem are cut off and the stems brushed free of dirt, the sample is placed in specially designed centrifuge tubes (Fig. 1). The stems are centrifuged at 1500 r.p.m. for 15 minutes. The exact centrifugal force required is determined somewhat by the species and the time of collection. Six 4-inch potato stems will yield 0.10 to 0.6 cc tracheal fluid. Should several centrifuge tubes be necessary for the collection of the sample, all portions are combined to give a composite example.

Preparation of Graphite Electrodes. For these studies graphite electrodes "Spectroscopic Carbons" (National Carbon Company, Cleveland) 0.25 inch in diameter are employed. The ends of the graphite electrodes are rendered flat by touching the end against a motor-driven grinder. The electrodes are pretreated by 2 drops of Skelly-solve containing 1% paraffin. Aliquot portions, 0.075 ml, of "tracheal" fluid are deposited on the end of the graphite electrodes in quadruplicates. After the fluid is evaporated to dryness under an infra-red lamp, the internal standard, in this case lead acetate, is deposited on the end of the electrode. It is preferable to add the internal standard separately to avoid precipitation of certain fluid constituents. The transit holder with graphite electrodes is placed under the infra-red lamp until the sample is *dry*. The entire holder is then placed in a desiccator until the spectrographic analyses are made. The piece of graphite containing the sample forms the lower electrode 1 mm below the upper piece of graphite. The sample is excited in an a-c arc at 3000 volts and 2.5 amperes and 60-second exposures are employed. Any fluctuation in the arc is reflected by the density of the emission lines of the internal standard. The plates, either Cramer contrast or Eastman 103-O, are developed to a gamma of about 0.6 for those elements in the lowest concentration. The density progression with respect to concentration is extended by including a step sector in the optical train. The absolute concentration of any constituent is determined by constructing a working curve, by plotting the log of the ratio of the density of a certain line to that of a suitable emission line of the internal standard against concentration of the element on the other axis. These working curves are constructed by obtaining a series of spectrograms of an artificial standard at different dilutions through

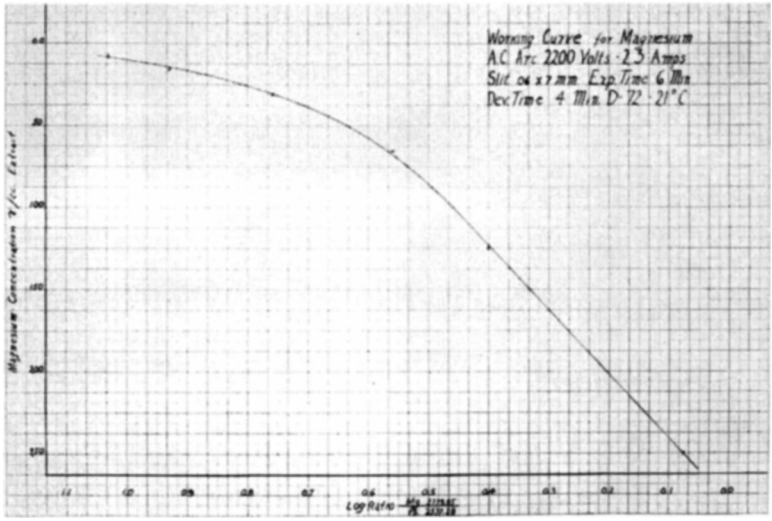

FIG. 2.
A working curve for quantitative analyses of magnesium by emission spectra.

a concentration range corresponding to the range found in the tracheal fluid investigated (Fig. 2).

Electrode Impurities. Because the concentrations of inorganic constituents in the tracheal fluid are so much lower than that in the leaf tissue, particular precaution must be observed insofar as electrode impurities are concerned. The inexpensive grade of "Spectroscopic Carbons" distributed by the National Carbon Co., Inc., revealed on spectroscopic examination the impurities listed in Table I.

The highest quality of graphite electrodes contain minute quantities (in concentrations less than 0.01 p.p.m.) of copper, calcium, iron and silicon. Because of these impurities a "blank" correction at equivalent exposures must be made in using the working curves.

Typical analyses of potato "tracheal" fluid are presented in Table II.

TABLE I.
Impurities in Graphite Electrodes as Determined by an A-C Arc.

Element	p.p.m.
Boron	0.01
Magnesium	0.10
Manganese	0.01
Calcium	0.01
Copper	0.02
Silicon	appreciable
Iron	"
Aluminum	trace

TABLE II.
Quantitative Analysis of Inorganic Constituents in Potato Stem "Tracheal" Fluid.

Element Treatment	B p.p.m.	P p.p.m.	Mn p.p.m.	Mg p.p.m.	Cu p.p.m.	Ca p.p.m.
B	0.80	27.6	.40	345	.65	110
Zn	1.80	18.0	.23	348	.90	136
Mn	1.20	18.0	.38	366	—	125
K	1.10	18.0	.44	363	.55	183
Mix	1.20	17.0	.52	399	—	183
Control	0.40	10.3	.22	294	—	62

Summary. (1) A rapid and reliable method for quantitatively analyzing "tracheal" fluid by emission spectroscopy has been described in detail. (2) Evidence has been presented showing that a correction should be made for electrode impurities. (3) Typical analyses of potato tracheal fluid for B, P, Mn, Mg, Cu, and Ca are presented.

11990

Etioallocholanol-3(β)-17-One (Isoandrosterone) as a Metabolite of Testosterone in the Human Male.*

RALPH I. DORFMAN.

From the Laboratory of Physiological Chemistry and the Adolescence Study Unit, Yale University School of Medicine, New Haven, Conn.

Continuing the study of the metabolic fate of testosterone in the human male, we have now been successful in demonstrating that etioallocholanol-3(β)-17-one (isoandrosterone) is a possible metabolite of testosterone. Previous communications have established androsterone and etiocholanol-3(α)-17-one as metabolic products of testosterone in the human male.[1, 2, 3] When testosterone was administered to the adult male guinea pig, etioallocholanol-3(β)-17-one was recovered in the urine.[4]

* This work was aided by grants from the Rockefeller Foundation, the Fluid Research Fund of Yale University School of Medicine, and the International Cancer Research Foundation. The latter grant was made to Dr. James B. Hamilton of the Department of Anatomy.

1 Callow, N. H., *Biochem. J.*, 1939, **33**, 559.

2 Dorfman, R. I., Cook, J. W., and Hamilton, J. B., *J. Biol. Chem.*, 1939, **130**, 285.

3 Dorfman, R. I., PROC. SOC. EXP. BIOL. AND MED., 1930, **45**, 739.

4 Dorfman, R. I., and Fish, W. R., *J. Biol. Chem.*, 1940, **135**, 349.