strain 109-F29, IGS, and 874 were considered to be low, medium, and high, respectively, in titratable acidity. Their relative positions were maintained when the selections were grown in replicated plots at two locations (Table III). Titratable acidity comprised a greater percentage of total acidity in fruit samples from replicated plots than for varieties listed in Table I with similar acid ranges.

Citric acid exceeded all other acids in concentration and increased as the acidity level of the variety or strain increased (Table III). For example, from Field 1, citric acid levels for Ace, IGS, and 874 were 61.5, 75.6, and 89.4 meq. per liter, respectively, or 46.8, 52.7, and 57.1 per cent of the total acidity. Samples from Field 2 displayed essentially the same pattern.

The percentage that malic acid contributed to the total acidity decreased significantly in Field 1 as the acidity level of the strain or variety increased (Field 1, Table IV). No significant differences in malic acid content were found in samples from Field 2.

The combined concentration of acetic, lactic, and fumaric acids manifested no trends due to changes in acidity levels of varieties or strains. This was also the case with pyrrolidone carboxylic, sulfuric, and galacturonic acids. Phosphoric acid displayed an erratic pattern. The hydrochloric acid concentration was significantly greater in 874 than in Ace or 109-F29.

Analytical data for Ca, Na, K, and P in the tomato vines of Field 1 are summarized in Table V. Ace was significantly lower in K than 874. Differences in both K content and fruit acidity between Ace and 874 were significant. There were no differences between varieties in the content of Ca, Na, or P.

Data for Ca, Na, K, and P in tomato puree are summarized in Table V. The K content in the puree of 874 was significantly higher than for 109-F29.

**Root Cation Exchange Capacity.** No significant differences were noted in the cation exchange capacities of roots from three varieties of tomato seedlings with different acidity levels (Table VI).

**Grafted Plants.** Titratable acidities of tomato puree from control and grafted plants are shown in Table VII. Control 874 and Ace plants manifested their usual differences in titratable acidity. No significant differences in titratable acidity were noted between control Ace and Ace scions on 874 rootstocks. The titratable acidity of fruit from 874 scions on Ace rootstocks tended to be higher than the titratable acidity of control 874 plants.

### Literature Cited


(1) Anderson, R. E., Thompson, A. E., *Tomato Growers Cooperative Report* **6,** 5 (1956).
(2) Bradley, D. B., J. AGR. FOOD CHEM. **8,** 232 (1960).
(3) *Ibid.*, **10,** 450 (1962).
(4) Drake, M., Vengris, J., Colby, W. G., *Soil Sci.* **72,** 139 (1951).
(5) Lee, F. A., Sayre, C. B., *Food Res.* **5,** 69 (1940).
(6) Lee, F. A., Sayre, C. B., *N. Y. State Agr. Expt. Sta. Bull.* **278,** (1946).
(7) Manunta, C., Lafon, M., *Tomato Growers Cooperative Report* **10,** 25 (1960).
(8) *Ibid.*, p. 26.
(9) Patterson, H. J., *Maryland Univ. Agr. Expt. Sta. Bull.* **11,** 612, 663, 665 (1890).
(10) Reynard, G. B., Dept. Agr. Research, Campbell Soup Co., Riverton, N. J., unpublished data, 1951.
(11) Sherman, M. S., *Ind. Eng. Chem., Anal. Ed.* **14,** 182 (1942).



*Received for review April 10, 1962. Accepted November 14, 1963.*


**PLANT TISSUE ANALYSIS**

# Colorimetric Determination of Glucose, Fructose, and Sucrose in Plant Materials Using a Combination of Enzymatic and Chemical Methods


**GESTUR JOHNSON, CLYDE LAMBERT,[1] D. K. JOHNSON, and S. G. SUNDERWIRTH**

**Chemistry Section, Colorado Agricultural Experiment Station, Fort Collins, Colo.**



A combination of enzymatic and chemical colorimetric procedures for more specific determination of glucose, fructose, and sucrose in plant and food materials has been developed. These methods are simple and have a high degree of specificity. The usual clarification procedures often required for the removal of nonsugar-reducing substances are not necessary.


THE NEED for simpler and more specific methods for the quantitative determination of glucose, fructose, and sucrose in plant and food materials is well recognized. A specific enzymatic determination of glucose in serum, plasma, and urine was first reported in 1956 (*5, 13*). This method is based on the coupling of the two following enzymatic reactions:

$$\beta\text{-D-glucose} + \text{glucose oxidase} \rightarrow \text{D-glucono-}\delta\text{-lactone} + \text{hydrogen peroxide} \quad (1)$$

[1] National Science Foundation Research Participant.

$$\text{hydrogen peroxide} + \text{peroxidase} + o\text{-dianisidine} \rightarrow \text{color} \quad (2)$$

The color formed is measured colorimetrically.

Potter *et al.* (*8, 9*) used this method to determine the amount of fructose present in plant material. The difference between the amount of reducing sugar present before and after destruction of glucose by glucose oxidase was taken as a measure of fructose.

In the investigation reported in this paper, glucose, total fructose, and sucrose are determined directly. Free glucose is determined by the glucose oxidase method. After hydrolysis of the sucrose by invertase, the total glucose (free glucose + glucose in sucrose) is determined by the glucose oxidase method. The difference between total glucose and free glucose permits the calculation of the amount of sucrose. Then total fructose is determined by a modification of the method used by Wise *et al.* (*14*). Since the amount of sucrose is known from the glucose oxidase analysis, the amount of free fructose is readily obtained. For convenience, this method of analysis is called Method I.

An alternate procedure for the analysis of sugar in plant materials which does

not require the use of invertase is also described in this paper. In this method, free glucose and total fructose are determined as in Method I. Total sugar is determined by the well known anthrone technique (*1*, *3*, *7*, *12*, *15*). Total sugar less total fructose gives total glucose. Since free glucose is known, the amount of glucose in sucrose and therefore sucrose itself may be calculated. This gives a value for fructose in sucrose from which free fructose may be determined by subtracting the value for combined fructose from total fructose. This method is called Method II.

Glucose oxidase is specific for β-D-glucose (*4*), and the anthrone reagent at 50° C. is highly specific for ketohexoses. At 95° C., anthrone gives total sugars. Therefore, either of the two methods described in this paper should give reliable results provided that certain precautions are observed.

## Apparatus and Reagents

**Anthrone Reagent.** Place 0.5 gram of anthrone (Matheson Coleman & Bell) and 10 grams of thiourea (Baker Analyzed) in a flask and add 340 ml. of distilled water. Slowly add 660 ml. of sulfuric acid (specific gravity 1.84). As the temperature of mixing reaches 90° C., the anthrone will dissolve. The cooled solution may be stored in the refrigerator for 2 or 3 weeks without serious decomposition since thiourea stabilizes the reagent (*15*).

**Glucose Oxidase Reagent.** Dissolve 250 mg. of glucose oxidase (Worthington Biochemical Co.) and 10 mg. of horseradish peroxidase (Worthington Biochemical Co.) in 40 ml. of phosphate buffer (pH = 7). Bring the volume to about 175 ml. with distilled water. Add 2 ml. of a 1% solution of *o*-dianisidine (Eastman) in methanol (100 mg. in 10 ml.). After vigorous stirring, bring the volume to 200 ml. and filter. Store in a dark bottle in the refrigerator. Best results are obtained by using freshly prepared reagents.

**Invertase Solution.** Dissolve 10 mg. of melibiase-free invertase (Nutritional Biochemicals Co.) in 10 ml. of distilled water. Use only the freshly prepared solution.

**Ascorbic Acid Oxidase.** Prepare crude ascorbic acid oxidase from summer crook-neck squash using a procedure based on a method described by Powers *et al.* (*10*). Remove $Ba^{+2}$ ions and extraneous protein from the filtered barium acetate–clarified juice by means of ammonium sulfate (0.3 saturated). Precipitate the crude oxidase by increasing the ammonium sulfate concentration to 0.6 saturated. Dissolve approximately 1.0 gram of the precipitate in 10 ml. of 0.1*M* phosphate buffer, pH 7.

**Activated Carbon.** Baker and Adamson Code No. 1551, General Chemical Co., New York, N. Y. This carbon does not adsorb sugars. The amount used was 50 mg. per 100 ml.

## Procedure

**Preparation of Sample.** The sample of plant material need not be dried before extraction. Carry out the extraction with 80% ethanol. If the material contains considerable moisture, use enough 95% ethanol to maintain an extract concentration of about 80% ethanol. After blending at high speed in a Waring Blendor for 3 minutes, filter the mixture and store the filtrate in the refrigerator. Dilutions may be made from the extract to give suitable concentrations for analysis (50 to 150 μg. per ml. for glucose and 25 to 100 μg. per ml. for fructose).

**Determination of Ascorbic Acid in the Extract.** Determine ascorbic acid

**Table I. A Comparison of Method I and Method II**

| Each Sugar Present, μg. | μg. Recovered by: Method I Glucose | Fructose | Sucrose | Total | Method II Glucose | Fructose | Sucrose | Total |
|---|---|---|---|---|---|---|---|---|
| 25 | 20.7 | 24.3 | 26.9 | 71.9 | 20.7 | 25.0 | 27.0 | 72.7 |
| 50 | 48.3 | 47.6 | 53.4 | 149.3 | 48.3 | 51.3 | 48.8 | 148.4 |
| 100 | 95.0 | 107.0 | 99.0 | 301.0 | 95.0 | 112.2 | 93.4 | 300.8 |

**Table II. Comparison of Method I and Alkali Ferricyanide Methods for Sugar Determination in Plant Materials**

| % | Burbank Potato I[a] | Burbank Potato III[b] | Pontiac Potato I | Pontiac Potato III | Peaches I | Peaches III | Peas I | Peas III |
|---|---|---|---|---|---|---|---|---|
| Glucose | 0.23 | 0.31 | 0.21 | 0.21 | 0.63 | 0.69 | 0.14 | ... |
| Fructose | 0.47 | 0.48 | 0.18 | 0.21 | 1.41 | 1.19 | 0.24 | 0.27 |
| Sucrose | 0.27 | 0.26 | 0.23 | 0.19 | 3.90 | 4.31 | 3.98 | 3.91 |
| Total | 0.97 | 1.05 | 0.62 | 0.61 | 5.94 | 6.19 | 4.36 | ... |

[a] I = Method I.
[b] III = Hassid ferricyanide method combined with the anthrone total fructose determination.

**Table III. Comparison of the Hassid Method, Method I, and Method II Using Corn Stems and Leaves**

| Stems[a] | Method I, % Fructose | Glucose | Sucrose | Total | Method II, % Fructose | Glucose | Sucrose | Total | Hassid Method,[b] % Reducing | Nonreducing | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8.2 | 3.7 | 20.2 | 32.1 | 9.3 | 3.7 | 19.2 | 32.2 | 13.7 | 20.0 | 33.7 |
| 2 | 6.5 | 2.5 | 24.7 | 33.7 | 8.1 | 2.5 | 22.8 | 33.4 | 9.8 | 24.9 | 34.7 |
| 3 | 3.1 | 1.0 | 19.7 | 23.8 | 3.4 | 1.0 | 22.2 | 26.6 | 7.0 | 23.3 | 30.2 |
| 4 | 4.7 | 0.7 | 23.7 | 29.1 | 6.4 | 0.7 | 21.6 | 28.7 | 7.5 | 26.0 | 33.5 |
| 5 | 6.8 | 4.6 | 22.8 | 34.2 | 9.0 | 4.6 | 19.6 | 33.2 | 15.0 | 19.9 | 34.9 |
| 6 | 7.6 | 4.9 | 22.9 | 35.4 | 11.6 | 4.9 | 16.2 | 32.7 | 16.7 | 18.1 | 34.8 |
| 7 | 5.2 | 1.5 | 15.6 | 22.3 | 7.2 | 1.5 | 12.4 | 21.1 | 9.7 | 15.5 | 25.2 |
| Leaves | | | | | | | | | | | |
| 1 | 1.9 | 2.4 | 3.1 | 7.4 | 2.1 | 2.4 | 4.8 | 9.3 | 5.8 | 4.9 | 10.7 |
| 2 | 5.9 | 2.3 | 3.4 | 11.6 | 6.3 | 2.3 | 2.8 | 11.4 | 6.8 | 8.4 | 15.1 |
| 3 | 4.3 | 3.0 | 3.7 | 11.0 | 3.8 | 3.0 | 5.0 | 11.8 | 6.8 | 6.9 | 13.7 |
| 4 | 7.4 | 2.7 | 5.5 | 15.6 | 6.4 | 2.7 | 6.8 | 15.9 | 7.6 | 9.6 | 17.2 |
| 5 | 6.2 | 2.5 | 6.6 | 15.3 | 6.1 | 2.5 | 7.2 | 15.8 | 9.0 | 7.7 | 16.7 |
| 6 | 2.8 | 2.7 | 1.7 | 7.2 | 1.5 | 2.7 | 4.4 | 8.6 | 5.8 | 4.2 | 10.0 |
| 7 | 7.2 | 2.3 | 3.8 | 13.3 | 5.2 | 2.3 | 8.2 | 15.7 | 7.0 | 7.7 | 14.7 |

[a] Seven different varieties were used in the analyses. [b] These analyses were determined in the Regional Phosphorus Laboratory, Soil and Water Conservation Research Division, Agricultural Research Service, U.S.D.A., Fort Collins, Colo.

by the indophenol colorimetric method (*6*). Dilute all extracts with an oxalic acid solution; the final concentration of the oxalic acid in the test solution should be 0.25%.

**Method I.** Determination of Free Glucose. If an interfering amount of ascorbic acid is present, the determination is carried out as follows. Place 50 ml. of the properly diluted extract in a 100-ml. volumetric flask and add 0.1 ml. of ascorbic acid oxidase reagent. After allowing to stand for several hours (overnight is convenient, although 2 to 3 hours is sufficient), make up the volume to 100 ml. with distilled water, and rapidly add 1 ml. of this solution to an Evelyn colorimeter tube containing a mixture of 5 ml. of glucose oxidase solution and 3.9 ml. of distilled water. Shake immediately and allow to react exactly 10 minutes. Stop the reaction by the addition of 0.1 ml. of 4*N* HCl. Make up a blank using 1 ml. of distilled water, and a standard containing 1 ml. of glucose solution (100 μg. per ml.). Read the color at 400 mμ in the Evelyn colorimeter. A standard containing 100 μg. of glucose per ml. is always included in each run since the amount of color produced depends upon temperature and reaction time. If no significant ascorbic acid is present, the diluted extract may be analyzed without ascorbic acid oxidase treatment.

Determination of Total Glucose. Place 50 ml. of the properly diluted extract in a 100-ml. volumetric flask and add 1 ml. of invertase solution, 10 ml. of phosphate buffer, and 0.1 ml. of ascorbic acid oxidase solution. Allow to stand for several hours (overnight). A shorter time is sufficient if the alcohol content is less than 4%. Make up to 100 ml. and analyze as described above.

Determination of Total Fructose. Add 1 ml. of properly diluted extract to 10 ml. of anthrone reagent and agitate. Place the tubes in cold water until all tubes are prepared. Cover the tubes with rubber caps and place in a water bath maintained at 50° C. After 20 minutes, remove the tubes and place them in ice water for 1 minute. Transfer to colorimeter tubes and read at 620 mμ against a reagent blank containing 1 ml. of water. A standard containing 50 μg. of fructose in 1 ml. of solution must also be run along with the sample tubes.

**Method II.** Free Glucose. Determine free glucose in the same manner as in Method I.

Total Fructose. Determine total fructose in the same manner as in Method I.

Total Sugar. Use the same procedure to determine total sugars by the anthrone method as used for total fructose with the exception that the reaction is carried out in boiling water (95° C.) for 15 minutes. Include a 50-μg. standard in each run. In this method and also in Method I, duplicate analyses should always be run.

### Results and Discussion

The determination of glucose by the glucose-oxidase method followed Beer's law over the 50 to 150 μg. range. Analysis of fructose using anthrone reagent at 50° C. followed Beer's law over the 25 to 100 μg. range. The reproducibility of the glucose oxidase method and the anthrone method for total fructose was determined using 20 analyses from a standard solution. The mean absorbance was 0.2757 for 100 μg. of glucose (26° C.) and 0.2379 for 50 μg. of fructose, the standard deviations being ±0.0089 and ±0.0087, respectively.

The two methods, I and II, gave reliable and reproducible results when a known mixture of glucose, fructose, and sucrose was analyzed. A comparison of the two methods is shown in Table I. The values shown in this and subsequent tables are the averages of duplicate determinations. The glucose values were the same since glucose oxidase was used in both methods for the determination of free glucose. The comparisons were favorable over the concentration ranges used.

In early work, Method I was compared with a modified Hassid (*2*) determination using neutral lead acetate for clarification with four different plant materials. The usual Hassid method for total reducing sugars was used in combination with the anthrone determination for total fructose. Thus it was possible to determine glucose, fructose, and sucrose. The sucrose was hydrolyzed with invertase. As shown in Table II, close agreement between the two methods was found. In the case of peas, glucose was very low and a negative value was actually found using the Hassid method. Method I was superior in that it enabled one to determine this low glucose value.

Either method may be readily applied to the analysis of sugars in plant material. A comparison of Methods I and II and the Hassid method of analysis is shown in Table III. The plant materials used were seven samples of corn seedling stems and seven samples of corn seedling leaves. Ion exchange resins were used to remove nonsugar-reducing materials prior to analysis by the Hassid method. This was necessary because of the presence of significant amounts of these interfering substances. The total sugar values for

**Table IV. Comparison of Method I and Method II Using Various Plant Materials**

| Sample | Method I, % | | | | Method II, % | | | |
|---|---|---|---|---|---|---|---|---|
| | Fructose | Glucose | Sucrose | Total | Fructose | Glucose | Sucrose | Total |
| Elberta peaches | 1.5 | 0.9 | 6.6 | 9.0 | 1.8 | 0.9 | 6.4 | 9.1 |
| Watermelon—heart | 3.4 | 1.4 | 3.0 | 7.8 | 3.2 | 1.4 | 3.5 | 8.1 |
| Watermelon—flesh near rind | 2.9 | 1.1 | 0.8 | 4.8 | 2.8 | 1.1 | 1.2 | 5.1 |
| Strawberries | 2.6 | 1.4 | 0.9 | 4.9 | 2.3 | 1.4 | 1.5 | 5.2 |
| Russett Burbank potatoes | 0.4 | 0.4 | 0.3 | 1.1 | 0.4 | 0.4 | 0.2 | 1.0 |
| Garden peas | 0.8 | 0.3 | 2.6 | 3.7 | 0.6 | 0.3 | 3.2 | 4.1 |

**Table V. Influence of Ascorbic Acid (AA) on Enzymatic Determination of Glucose[a]**

| No. | Analyzed Immediately | | | | Analyzed after Standing 24 Hours | | | | Treated with Ascorbic Acid Oxidase | |
|---|---|---|---|---|---|---|---|---|---|---|
| | μg. AA/ml. added | Absorbance | μg. Glucose/ml. | μg. AA/ml. remaining | Absorbance | μg. Glucose/ml. | μg. AA/ml. remaining | Absorbance[b] | Absorbance | μg. AA/ml. remaining |
| 1 | 0 | 0.260 | 100 | 0 | 0.251 | 100 | 0 | 0.000 | 0.253 | 0 |
| 2 | 10 | 0.187 | 72 | 5.92 | 0.277 | 110 | 0 | 0.002 | Not analyzed | |
| 3 | 20 | 0.092 | 35.4 | 14.0 | 0.291 | 116 | 0 | 0.035 | 0.249 | 0 |
| 4 | 40 | 0.006 | 2.3 | High—not measured | 0.301 | 120 | 0 | 0.063 | 0.254 | 0 |
| 5 | 80 | 0.004 | 1.5 | High—not measured | 0.125 | 50 | 14.3 | 0.000 | 0.254 | 0 |

[a] Glucose concn. = 100 μg. per ml. [b] No glucose oxidase was added, thus absorbance is a measure of $H_2O_2$ produced upon autoxidation of AA.

Methods I and II were very close. The total sugar values from the Hassid method also compared favorably with those obtained by either of the new techniques. It can be seen that the Hassid values for total sugar were slightly higher. In the case of the stems, the reducing sugar values from the Hassid method were closer to glucose plus fructose values in Method II than the corresponding values in Method I. However, with the leaves, there was no regular deviation in any of the three methods.

Several fruits and vegetables were analyzed using both Methods I and II. A comparison of the results from the two methods of testing is shown in Table IV. In general, the agreement between the two analyses was good. The total sugars were somewhat higher in Method II. This is to be expected since anthrone will react with any minor sugars or glycosides present in the extract. However, ascorbic acid and other non-sugar-reducing substances such as the plant polyphenols do not interfere with the anthrone analysis.

Both methods are rapid and gave reproducible results in the absence of interfering substances. Especially important was the complete removal of ascorbic acid before the glucose oxidase method was used. The effect of ascorbic acid on the analysis for glucose is shown in Table V. The samples were analyzed immediately and also after standing for 24 hours at 21° C. The last two columns show how the serious effects of ascorbic acid may be eliminated by using ascorbic acid oxidase. Even at 80 μg. of ascorbic acid per ml. the analyses were good when ascorbic acid oxidase was used. It is interesting to note the change of absorbance by using glucose oxidase when the samples were allowed to stand for 24 hours. All the ascorbic acid had disappeared by this time (except in the sample in which 80 μg. per ml. were added) and the absorbance increased. In all cases (except No. 5), the addition of ascorbic acid caused an increase in the absorbance without the addition of glucose oxidase. For these reasons, it appears that if ascorbic acid is present, the dilutions from the ethanolic plant extract should not be made until immediately before the analysis.

**Table VI. Elimination of Ascorbic Acid (AA) with Activated Carbon Solution Analyzed after Standing 24 Hours at 21° C.**

| Sample | Treatment | Absorbance |
|---|---|---|
| 1 | 100 μg. per ml. glucose | 0.258 |
| 2 | 100 μg. per ml. glucose + 10 μg. AA | 0.299 |
| 3 | 100 μg. per ml. glucose + 40 μg. AA | 0.398 |
| 4 | 100 μg. per ml. glucose + carbon (50 mg. per 100 ml. soln.) | 0.260 |
| 5 | 100 μg. per ml. glucose + 10 μg. AA + carbon | 0.268 |
| 6 | 100 μg. per ml. glucose + 40 μg. AA + carbon | 0.295 |

Activated carbon has also been found suitable for removal of ascorbic acid. Table VI gives the results of treatment with carbon to remove ascorbic acid before glucose analysis. Samples 2 and 3 had a high absorbance when analyzed after standing 24 hours. Treatment with carbon removed most of the ascorbic acid from sample 5 (10 μg. of ascorbic acid) but failed to remove the ascorbic acid from sample 6 (40 μg. of ascorbic acid), which contained a larger amount of ascorbic acid. Carbon itself had no effect on the glucose content (sample 4). Apparently carbon is not as suitable as ascorbic acid oxidase for removing ascorbic acid, particularly at the higher concentrations, at which residual hydrogen peroxide interferes.

In samples containing small amounts of sugars, the dilutions from the alcoholic extracts may be so small that the alcohol concentration is high enough to interfere with the invertase reaction. In this case, Method II may be preferred over Method I. In general, Method I gives greater specificity for free fructose and sucrose because of the possible error in total sugar determination using anthrone. If this higher degree of specificity is not required, then Method II is satisfactory.

### Literature Cited


(1) Dreywood, R., *Ind. Eng. Chem., Anal. Ed.* **18,** 499 (1946).
(2) Hassid, W. F., *Ibid.*, **9,** 228 (1937).
(3) Johnson, R., *Anal. Chem.* **26,** 1331 (1954).
(4) Keilin, D., Hartree, E. F., *Biochem. J.* **50,** 331 (1952).
(5) Keston, A. S., Abstracts, p. 31C, 129th Meeting, ACS, April 1956.
(6) Loeffler, H. J., Ponting, J. D., *Ind. Eng. Chem., Anal. Ed.* **14,** 846 (1942).
(7) Morris, D. L., *Science* **107,** 254 (1948).
(8) Potter, E. F., Williams, K. T., *J. Assoc. Offic. Agr. Chemists* **41,** 681 (1958).
(9) Potter, E. F., Wilson, J. R., Williams, K. T., *Ibid.*, **42,** 650 (1959).
(10) Powers, W. H., Lewis, S., Dawson, C. R., *J. Gen. Physiol.* **27,** 107 (1944).
(11) Roe, J. H., *J. Biol. Chem.* **212,** 335 (1955).
(12) Sattler, L., *Science* **108,** 207 (1948).
(13) Teller, J. D., Abstracts, p. 69C, 130th Meeting, ACS, September 1956.
(14) Wise, C. S., Dimler, R. J., Davis, H. A., Rist, C. E., *Anal. Chem.* **27,** 33 (1955).
(15) Zill, L. P., *Ibid.*, **28,** 1577 (1956).



*Received for review May 13, 1963. Accepted October 21, 1963. Approved by the Director, Colorado Agr. Expt. Station, as Scientific Series Publication No. 858.*


**ANIMAL METABOLISM OF PLANT REGULATORS**

# Metabolism of 3-Amino-1,2,4-triazole-5-C$^{14}$ by Rats


**S. C. FANG, MARILYN GEORGE, and TE CHANG YU**

**Department of Agricultural Chemistry, Oregon State University, Corvallis, Ore.**


THE GROWTH regulating effects exerted by 3 - amino - 1,2,4 - triazole (ATA) on plants have attracted considerable interest in recent years. This chemical was first reported to cause leaf abscission, chlorosis, and growth inhibition in cotton (*2, 3*), and similar effects on many other plant species have subsequently been reported. Pyfrom *et al.* (*9*) reported the inhibitive effect of ATA on plant catalase activity and the depression of chlorophyll synthesis. Heim, Appleman, and Pyfrom (*4*) found that catalase activity in rat liver and kidney was reduced in animals injected with ATA. The blood catalase and hemoglobin of the treated animals remained normal. The purpose of the present investigation was to characterize excretory patterns and to study tissue residues following oral administration of 3-amino-1,2,4-triazole-5-C$^{14}$ to the rat.

### Materials and Methods

Several trials were conducted with adult rats of the Wistar strain (4 to 6