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CHEMICAL INVESTIGATIONS OF THE METABOLISM OF PLANTS

I. The Nitrogen Nutrition Of *Narcissus Poeticus*

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I. The Nitrogen Nutrition Of *Narcissus Poeticus*[1]

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The transformation of the nutritive material in the storage organs of a plant into the substances found in the cells of the growing tissues involves phenomena concerning which remarkably little has been learned in spite of the large volume of research that has been carried on since the pioneer investigations of Schulze in this field seventy years ago. Storage tissue, whether that of the dehydrated endosperm of a seed, the fleshy thickened scales of a bulb or that of a relatively highly hydrated tuber or specialized root-stock, is characterized by the presence of nutriment deposited in chemically stable forms. Proteins, carbohydrates and fats, together with a small proportion of inorganic substances, make up the greater part of the material upon which the development of the new plant depends. During the early stages of growth, these substances undergo transformation into soluble products that are translocated into the newly developing cells where they are converted into the specific components of the new tissue. The details of the innumerable reactions that take place are for the most part unknown although it is obvious that many, if not most, of the chemical changes are brought about by enzymes; nevertheless, the general nature of the reactions can be studied, at least to some extent, by the application of analytical chemical methods on the one hand to the storage tissue and, on the other hand, to the tissues that have been produced from it. In this way information can be obtained regarding the end products of the reactions and some light thrown upon the sequence of chemical events that must have occurred.

The narcissus plant was selected for the present study in part because of the convenience with which experiments could be arranged, and in part because of the preliminary investigation of Nightingale and Robbins (14) which had shown that the bulbs of a closely allied species are eminently suited for the examination of the chemical changes that take place during the early stages of growth. There is sufficient nutriment in the bulb of this plant to permit it to develop the leaf tissue fully and also to produce flowers even if nothing in addition to water is supplied. Accordingly, it is possible to isolate the system under study more effectively and for a far longer relative period of time than is the case with the seedlings even of such large-

[1] This Bulletin is a continuation, under a broader general title, of a series of nine Bulletins, eight of which (Numbers 323, 324, 339, 352, 374, 399, 407 and 442) dealt with various phases of the chemistry of the tobacco plant. These appeared under the general title "Chemical Investigations of the Tobacco Plant". The ninth Bulletin (Number 424) was entitled "Chemical Investigations of the Rhubarb Plant".

[2] The technical assistance of Mr. Laurence S. Nolan is gratefully acknowledged.

seeded species as the legumes. Few if any seedlings can be grown successfully beyond the point at which the first leaves have opened unless an outside source of nutriment is provided.

DESIGN OF THE EXPERIMENT

It is customary, in biological experimentation, so to design the work that groups of replicated data will be obtained which can be examined by statistical methods. Estimates of accuracy and precision can then be derived and statements made which are true within ascertained and expressed quantitative limits. The comprehensive analytical investigation that was contemplated in the present case made it necessary, however, to restrict the number of samples as much as possible, if the work was to be kept within feasible limits. A choice accordingly had to be made between an experiment in which replication in all details of a single treatment was carried out, an experiment with a single treatment in which samples were to be taken at intervals in order to show the serial progress of the chemical changes, or an experiment without replication in which several treatments were to be applied. The last alternative was selected as it promised to yield the maximum amount of information without unduly increasing the analytical work.

Bulbs of paper-white narcissus (*Narcissus poeticus*) were sorted into seven lots of approximately equal weight, each lot containing 30 bulbs. One lot was retained for analysis to establish the composition of the system at the start, and the other six lots were planted in clean glazed crocks, 10 bulbs per crock, in washed sand, the level being so adjusted that 1 liter of water or culture solution could be added and allowed to drain through.

The treatments selected were: (a) distilled water alone to give a basis for comparison with the effect of a culture solution, (b) a solution that provided nitrogen as nitrate ion, and (c) a solution that provided nitrogen as ammonium ion, each to be applied in continuous darkness and continuous light. The plants obtained by treatment (a) are referred to in the text, for convenience, as the starved plants, the plants obtained by treatment (b) are similarly referred to as the nitrate plants, and those by treatment (c) as the ammonia plants.

The culture solutions were prepared by the dilution of the 0.5 M stock solutions of reagents selected to conform with the Livingston solution R_3 S_4 (12). The nitrate solution was prepared by diluting with distilled water to 8 liters a mixture of 104 ml. of stock solution of potassium dihydrogen phosphate, 137.6 ml. of calcium nitrate, and 33.6 ml. of magnesium sulfate, to which was added 8 ml. of a solution containing 2.032 gm. of manganese sulfate and 2.86 gm. of boric acid per liter and 8 ml. of a solution containing 2.6 gm. of ferric tartrate per liter. Other trace elements were supplied by the addition of 10 ml. of Hoagland's A-Z solution (9) per 8 liters of culture solution. The ammonium salt solution was prepared by similarly diluting a mix-

ture of 78 ml. of potassium dihydrogen phosphate, 26 ml. of dipotassium hydrogen phosphate, 33.6 ml. of magnesium sulfate, 137.6 ml. of calcium chloride, and 137.6 ml. of ammonium sulfate; additions of manganese, boron, iron and trace elements were made as before. The nitrate solution was at pH 4.4, the ammonium salt solution at pH 5.8 as prepared.

The cultures were started on February 3, 1938, three groups of three crocks each being placed in a completely dark room in which the temperature remained within 2° of 23° C, for the entire period of the experiment. The other three groups of crocks were placed in a greenhouse where the temperature varied, according to the weather, from 17° to as high as 30° during the early afternoon of sunny days. Each crock was flushed with 1 liter of water or of the appropriate culture solution each alternate day. In six days, all of the bulbs had sprouted well and at this point the cultures in the dark used up from 200 to 300 ml. of solution per 48 hours. By the ninth day, about 400 ml. were being used and the quantity gradually increased thereafter until the experiment was terminated on the 28th day. At this time the plants in the dark were tall (for details of length of leaf see Table 1) and the tissues were yellow, no trace of green being detected. The plants in light were somewhat shorter and were fully green. During the course of the experiment, the flower stalks with buds were cut off as soon as they had become separated from the sheath of leaves. The number of buds removed during the course of the experiment and the time of removal are shown in detail in the following table. For weights and other pertinent data, see Table 1.

Elapsed time days	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
11		2		12		3
13	13	8	15	6	9	8
15		3		2		7
18	5		6		7	
19		8		2		1
20	4	1			2	1
21				2	1	
23						1
25				1		
Total	22	22	21	25	19	21

The tissue was weighed, dried and again weighed, being later added to the appropriate sample of leaf tissue after this had been harvested and dried. Because the quantities of bud tissue were so small, no attempt was made to analyze them separately.

The removal of the buds rendered the plants so treated entirely vegetative and was necessary inasmuch as only about three-quarters of the bulbs developed flower stalks. All were thus placed upon a similar basis and the study was restricted to a single aspect of the metabolism of this species, the complications that would arise from the initiation of the reproductive cycle being avoided.

At the termination of the experiment, the leaves were cut from the plants, separated, counted, the length of each leaf was measured and the weight of the crop from each culture was obtained. The leaves were then dried at 80° C. in a ventilated oven, equilibrated with air for a few hours, weighed to give what is designated the crude dry weight, and the material was then ground for analysis. The sand was washed from the crocks and the roots were separated from the bulbs and freed from sand as far as possible. Any shoots that emerged from the side of the bulb were removed and added to the leaf sample. After being weighed, the bulbs were sliced and dried. The root tissue was weighed and dried with care to preserve any adhering sand. Without being broken up, the dry root tissue was then floated on the surface of about 200 ml. of chloroform contained in a 600 ml. beaker and stirred in order to loosen the adhering sand. This settled at once and the tissue, substantially free from sand, was then removed and dried. The time of exposure to the solvent was of the order of two minutes. After being dried, the tissue was again weighed. The sand that separated was also recovered from the chloroform and weighed to provide a double check on the quantity present. The sum of the weight of the treated tissue and the sand that had separated from it agreed with the weight of the tissue before treatment within 0.3 to 0.4 gm., showing that mechanical losses or losses of soluble components were negligible, inasmuch as the samples weighed from 20 to 36 gm.

All of the dried samples were carefully preserved in tightly closed containers after being weighed, ground and thoroughly mixed. The analytical data were subsequently obtained in terms of percentage of the weight of these samples and the quantity of the component in the whole of the crude dry weight was ascertained. Table 1 shows the data for the weights and measurements of the fresh and the dry tissues as obtained for the samples of 30 bulbs. These figures are presented so as to show the order of magnitude of the quantities observed. The analytical data were subsequently calculated to a basis of 50 bulbs so as to permit direct comparison of the present results with those of Nightingale and Robbins. The calculations were carried out by the use of factors based upon the crude dry weight of each sample of tissue and the factor 1.667 to convert from 30 to 50 bulbs. The concentrations of the constituents expressed as percentage of the dry weight or of the fresh weight or any desired ratios between the quantities could then be readily obtained.

ANALYTICAL METHODS

The analytical methods used in this laboratory for the examination of plant tissues have been briefly described in previous bulletins (44, 47) and in detail in journal papers, but, as experience has broadened, many modifications of the techniques have been introduced. Evolution of this kind is essential and is still continuing. Furthermore, few methods can be applied without minor modification to tis-

TABLE 1. FUNDAMENTAL DATA ON FRESH AND DRY WEIGHTS OF THE PLANTS GROWN FROM 30[1] NARCISSUS BULBS

Figures not otherwise designated are grams.

	CB	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
Fresh weight, leaves ..		1302	883.5	2154	1549	1169	791.6
Number of buds		22	22	21	25	19	21
Fresh weight, buds		13.6	19.4	26.8	20.9	17.4	16.4
Crude dry weight, leaves		104.3	86.5	138.5	126.1	88.8	76.8
Crude dry weight, buds		3.2	3.1	3.2	3.1	2.4	2.7
Fresh weight, leaf fraction + buds		1316	902.9	2181	1570	1186	808
Dry weight, leaf fraction + buds		107.5	89.6	141.7	129.6	91.2	79.5
Number of shoots		80	94	105	91	95	86
Number of leaves		285	309	336	300	295	283
Total length of leaves (cm.)		12671	9497	20887	12939	12396	7800
Average length per leaf (cm.)		44.8	30.7	62.1	43.2	42.2	27.6
Fresh weight, bulbs, at start	1122	1121	1122	1122	1123	1121	1123
Fresh weight, bulbs, at end		1186	1258	1136	1181	1169	1179
Crude dry weight, bulbs, at end	436	284.8	292.7	190.1	214	264.7	307.8
Fresh weight, roots[2] ...		392.2	399.5	633.6	731.4	413	507.3
Crude dry weight, roots		21.2	22.5	30.3	37.0	21.2	24.6

[1] All subsequent analytical data are arbitrarily expressed in terms of 50 original bulbs.

[2] The fresh weight is corrected on the assumption that the sand separated by the use of chloroform retained 20 per cent of moisture under the conditions at the time the fresh tissue was weighed. The dry weights are those obtained after the sand had been separated.

sues other than the ones for which they were developed, and occasionally cases are encountered that require complete revision of a method to make it applicable to the material in hand.

In the following paragraphs, brief descriptions of the techniques employed in the present investigation are given, reference being made to earlier publications for those details that have already been fully described. It must be emphasized, however, that no method can be applied to a given plant tissue without preliminary study to demonstrate that it is suitable for the particular case. Even such apparently simple determinations as the total nitrogen by the Kjeldahl method or the estimation of the dry weight and the ash require study if true accuracy is to be attained. The precision must always be controlled by replication of determinations until the limits actually secured under the working conditions have been established.

Preparation of Dry Tissue

A Proctor and Schmidt tray drier provided with a fan for the circulation of the air and heated by a gas burner is employed. The temperature is controlled by a thermostat within a few degrees of 80° C. This temperature has been found adequate to stop enzyme action promptly by heat denaturation of the proteins of the tissues, but brings about little decomposition of such unstable components as glutamine (43). Fleshy tissues such as bulbs are sliced by means of a vegetable slicing machine before being dried. After the tissues have become crisp they are allowed to equilibrate with air for a few hours in order to establish a reasonably stable moisture content before being weighed. The weight obtained at this point is designated the "crude dry weight", and is taken before the tissues are broken up so as to avoid error due to the loss of fragments in the necessary transfers. After being weighed, the tissues are ground in a Wiley mill and are preserved in tightly stoppered glass containers. Tissues prepared in this way in general maintain their moisture content with little change if care is taken that the containers are opened for as short a time as possible. However, the moisture content should be established without undue delay and must be checked if the analytical studies are delayed or postponed. If significant change occurs, a correction factor can be established to be used for computation of the analytical results.

All analytical results are obtained in terms of percentage of the crude dry weight of each sample. The factor necessary to calculate from this percentage the quantity of substance determined in the whole of the crude dry weight is established for each sample and enters into the calculation of all results for it. Thus, the accuracy of the final data depends upon the care with which the crude dry weight is obtained in the first place and upon the maintenance of the moisture content of the sample.

Moisture Content

Pyrex glass weighing bottles 25 x 40 mm. in size with flat standard taper ground glass covers are used. Exactly 0.5 gm. of tissue is weighed into the bottle and heated at 110° for four hours. The bottle is closed in the oven and is cooled in a desiccator and weighed. Preliminary tests of the time required for reproducible loss of weight must be made with all tissues and this time must be strictly adhered to thereafter.

Ash

The determination of the ash is made on the same sample. The Pyrex bottles are gently heated on a wire gauze until the sample is completely charred and are then placed in a muffle furnace at 580 ± 20° for two hours. If traces of carbon still remain, heating is continued for another two hours. The bottles are removed from the muffle, allowed to cool for a few minutes, the covers are put in place and the bottles are further cooled in a desiccator and weighed. Pro-

longed heating is undesirable owing to the possible conversion of calcium carbonate into calcium oxide; the conditions described bring about minimal decomposition, but reproducible results cannot be obtained if liberties are taken with the temperature or the time.

Total Nitrogen

The Kjeldahl method is unreliable for the determination of nitrogen if nitrates are present. A qualitative test with diphenylamine upon a water extract of a plant tissue must therefore be made to establish the absence of nitrate before the unmodified Kjeldahl method is employed. In the presence of nitrate nitrogen, the procedure is as follows: to 0.100 gm. of dry tissue in a 300 ml. Kjeldahl flask are added 12 ml. of water and 1.5 ml. of concentrated sulfuric acid (special nitrogen-free). After being mixed, 0.3 gm. of reduced iron powder is added from a calibrated scoop and the mixture is boiled gently for five minutes to reduce the nitrate to ammonia. The digestion reagents, 5 ml. of 20 per cent sodium sulfate, 5 ml. of concentrated sulfuric acid (special), and 1 drop (0.6 gm.) of mercury are added and the mixture is digested in the usual way for two hours after becoming colorless. The flask is cooled, 50 ml. of water are added and 15 ml. of concentrated sodium hydroxide-sodium thiosulfate reagent are added so as to underlay the aqueous solution. After the addition of a little granulated zinc, the ammonia is distilled into standard acid. Any suitable distillation technique may be used provided that reproducible and standardized conditions are established. In this laboratory, a Folin and Wright (6) distillation tube modified by the addition of a bulb trap at the top is used without any condenser and the distillate is collected in 25 ml. of 0.02 N hydrochloric acid contained in a 300 ml. Erlenmeyer flask. The distillation time is six minutes from the appearance of the first drop of condensate in the tube. The titration is carried out with 0.02 N sodium hydroxide after the distillate has been cooled to room temperature. Methyl red-methylene blue indicator solution is used in a standard quantity and the titrations for the standarization of the solutions are carried out at a final volume similar to that obtained in the course of routine determinations.

If no nitrate is present, the preliminary reduction with iron powder is omitted. A blank on the reagents is determined frequently.

Protein Nitrogen

Protein nitrogen is determined in the tissue that has been extracted with alcohol for the removal of the soluble carbohydrates as described later. Of this material, 0.100 gm. is treated in a centrifuge tube with 25 ml. of boiling water, the mixture being continually stirred with a rod in a steam bath for five minutes. The suspension is centrifuged and the wash fluid is poured off through a small paper filter. The residue is extracted twice more in the same way. The final residue, together with any particles that have been collected on the filter paper, is washed into a Kjeldahl flask with cold water and total nitrogen is determined. The result, as a percentage of the

weight of the dry alcohol-insoluble residue, is converted to a percentage of the weight of the original tissue by the use of a factor obtained from the loss of weight during the alcohol extraction.

Ammonia and Amide Nitrogen

The several forms of soluble nitrogen are determined in an extract of the dry tissue obtained as follows. Exactly 1.0 gm. of the powder is treated in a beaker with 80 ml. of hot water, being stirred for 10 minutes in a water bath at 80 to 85° and then cooled rapidly to room temperature. The suspension is transferred to a centrifuge tube graduated at 100 ml., made to volume, mixed thoroughly and centrifuged, and the clear fluid is poured off through a small plug of dry glass wool in a funnel into a stoppered flask. If necessary, the extract is preserved with a little toluene and stored in the refrigerator. Aliquots are taken for the subsequent determinations.

Ammonia nitrogen is determined, usually in a 10 ml. aliquot, as described by Pucher, Vickery and Leavenworth (27). Glutamine and asparagine amide nitrogen are determined in 5 ml. aliquots as described by Vickery, Pucher, Clark, Chibnall and Westall (43), except that the intensity of the color of the Nesslerized solution is determined by means of a Beckman spectrophotometer at wave length 430 mμ.

Amino Nitrogen

The residue from the determination of ammonia nitrogen is acidified with 2 ml. of glacial acetic acid and diluted to 50 ml. Of this solution, 5 ml. aliquots are used for the determination of amino nitrogen in the Van Slyke manometric apparatus. For the determination of the total amino nitrogen after hydrolysis, a 5 ml. aliquot of the extract is mixed with 5 ml. of 12 N sulfuric acid and is heated with a capillary air condenser, as used for the hydrolysis of the glutamine amide nitrogen, in a boiling water-bath for six hours. The solution is transferred to the ammonia distillation apparatus and made alkaline with 8 ml. of 10 N sodium hydroxide. The ammonia is then distilled, the residue is acidified with acetic acid, diluted to 50 ml. and amino nitrogen is determined as before.

The "Organic Acid Fraction"

The preparation of an extract that contains the organic acids and the nitrate nitrogen of the tissue is carried out as described by Pucher, Vickery and Wakeman (28) [see also Pucher, Wakeman and Vickery (31)] save that the technique of the ether extraction has been simplified. A 0.500 gm. sample of the dry tissue, after being acidified and mixed with asbestos as described, is packed directly into the glass siphon cup of the ether extraction apparatus as follows: A sufficient quantity of angular quartz pebble fragments (approximately 5 mm. size) is placed in the cup to form a layer 1.5 cm. deep. Over this is placed a thin plug of glass wool,[1] care being taken not to compress

[1] Cheesecloth has been recommended instead of glass wool in an earlier publication. This material has certain advantages but extraction with 0.1 N sulfuric acid in the cold and then with ether is necessary before it can be used without giving rise to error.

the fibers so snugly as to interfere with the ready flow of ether through them. The sample is then added and is covered with a second pad of glass wool that has been used to wipe the last particles of tissue from the beaker. This pad is pressed down gently so as to cover all particles of the sample. The quantities of pebbles and sample should be such that the sample occupies the middle third of the siphon cup.

The siphon cup is held in a special glass frame (described below) that rests on the bottom of the extraction flask and holds it directly under the condenser. Specially prepared ether is employed, and extraction is continued overnight (16 hours) at the rate of from 40 to 60 siphoning cycles per hour. If apparatus that siphons less frequently is used, extraction must be continued until an equivalent number of cycles has been completed. The ether in the extraction flask is treated with 7 ml. of water and 5 ml. of 1 N sodium hydroxide, the ether is evaporated and the solution is made to 25 ml. in a volumetric flask. Aliquots of this solution are used for the determination of the organic acids and of the nitrate nitrogen.

Organic Acids

The details of the methods used for the determination of oxalic, malic, citric and succinic acids and the calculation of the residual so-called unknown organic acids have been described in journal papers as follows: oxalic acid (28, 31), malic acid (29, 31), citric acid (20, 25, 26), succinic acid (22, 23).

Nitrate Nitrogen

Nitrate nitrogen is also determined in the organic acid fraction inasmuch as nitric acid is quantitatively extracted from plant tissue along with the organic acids under the conditions used. A 5 ml. aliquot is treated, in a 300 ml. Kjeldahl flask, with 2.5 ml. of 18 N sulfuric acid and 300 mg. of reduced iron powder. The mixture is boiled gently over a micro burner for five minutes, cooled and diluted with 50 ml. of water; 10 ml. of concentrated sodium hydroxide are added. The solution is then distilled as in the usual determination of nitrogen. The distillate may be titrated with the aid of 0.02 N reagents, or diluted to standard volume and aliquots removed for colorimetric determination of the nitrogen by the Nessler method using the Beckman spectrophotometer. Blanks on the apparatus and reagents must be carried out at frequent intervals. Furthermore, the apparatus must be steamed out at the beginning of a series of determinations by distilling water through it for the usual six minutes.

The method makes no distinction between nitrate and nitrite nitrogen both of which are extracted under the conditions and reduced to ammonia. Nitrite nitrogen is of importance only in fresh tissue. Little or no nitrite could be expected to survive after tissues

have been dried unless special precautions are taken to maintain an alkaline reaction.

Starch

The determination of starch from the intensity of the color of the starch-iodine complex formed under specified conditions, as described by Pucher and Vickery (21), has been simplified by the adoption of perchloric acid as the solvent as recommended by Nielsen (13). The extract is prepared from 0.100 gm. of dry tissue to which are added 4 ml. of water and 0.2 gm. of sharp sand in a heavy wall 200 x 25 mm. test tube calibrated at 50 ml. The mixture is heated in a boiling water bath for 15 minutes with occasional shaking, is cooled, and 3 ml. of 72 per cent perchloric acid are added. The tissue is then ground in the test tube with a stout glass rod for 15 minutes after which the suspension is diluted to 50 ml., mixed for two minutes and centrifuged. Five ml. aliquots are transferred to 200 x 25 mm. test tubes, calibrated at 20 ml. and at 50 ml., and diluted to 10 ml.; phenolphthalein indicator is introduced and 10 N sodium hydroxide is added dropwise until the solution is alkaline, and then 5 N acetic acid until the solution is colorless and 1 ml. of excess is present. To each tube, 0.5 ml. of fresh 10 per cent potassium iodide and 2 ml. of exactly 0.025 N potassium iodate are added and the mixture, after being allowed to stand for five minutes, is diluted either to 20 ml. or to 50 ml. depending on the intensity of the color. After being allowed to stand for another five minutes, the tubes are centrifuged for a short time and the extinction coefficient is determined with a Beckman spectrophotometer set at 610 mμ against a blank prepared from 10 ml. of water to which the reagents (except the alkali) are added as described. A standard curve is prepared from high grade potato starch that has been extracted with ether, alcohol and cold water, dried and equilibrated with air. Moisture and ash determinations are made and the starch content of the preparation is calculated. A solution of a known quantity, approximately 0.1 gm., is prepared as described and suitable aliquots are treated with the reagents and used to establish the curve between the limits 0.1 mg. to 4 mg. It is important to note that this colorimetric method gives results in terms of the color value of potato starch used as standard and, accordingly, is a relative, not an absolute method.

Soluble Carbohydrates

The determinations of the soluble carbohydrates involve the estimation of the reducing power with sugar reagents directly and after inversion with invertase; also, after treatment of the solution with yeast to remove glucose and sucrose. A further determination after inversion and subsequent hydrolysis with dilute hydrochloric acid is sometimes made. The Shaffer-Somogyi method (36, 37) is employed with modifications that are to be described in another publication. The extract of the tissue is prepared from 0.5 gm. which is treated with 15 ml. of water and warmed to 50° with stirring for 15 minutes. The suspension is transferred to a centrifuge tube calibrated at 20 ml.,

diluted to the mark, mixed and centrifuged and the clear solution is filtered through a plug of dry glass wool in a funnel. Aliquots of this extract are clarified with lead acetate and potassium phosphate and used for the determinations. Alternatively, the alcohol extract of the tissue prepared as described below is used.

Carbon

Carbon is determined by the wet combustion method with reagents and apparatus modified slightly from those of Friedemann and Kendall (7). The combustion is carried out in a 300 ml. Kjeldahl flask by means of chromic acid in a mixture of sulfuric and phosphoric acids, the rate of heating being adjusted so that the sulfuric acid vapors are condensed and returned while the carbon dioxide passes on to be absorbed in a carbon dioxide-free sodium hydroxide solution in a bead tower which drains into a 200 x 32 mm. test tube. This is attached to the tower by means of a standard taper ground glass joint. The combustion is carried out in a current of carbon dioxide-free air. At the end of the operation, the bead tower is washed with a liberal amount of recently boiled water, the contents of the test tube are made to standard volume and aliquots are analyzed for carbon dioxide in the manometric apparatus of Van Slyke. The result is corrected for blanks obtained by analyzing a standard quantity (0.100 gm.) of pure succinic acid.

Carbon determinations are made on 0.100 gm. of the tissue and also upon samples of tissue that have been extracted with alcohol and with ether after acidification to pH 1 so as to remove organic acids. The alcohol extraction is carried out by the technique already described for the preparation of the organic acid fraction save that 70 per cent alcohol is used as solvent and the sample of tissue is not acidified and mixed with asbestos. Furthermore, the sample (2 to 5 gm.) is weighed directly in the siphon cup already provided with quartz pebbles and gauze pads, the upper pad being put in place after the sample has been introduced. During this operation, it is convenient to support the cup in a large weighing bottle, using a second one of the same size as a tare. After being extracted overnight, the siphon cup is freed from as much alcohol as possible by suction, is dried at 105° and is then allowed to equilibrate with air at room temperature before being weighed again. The loss of weight is employed to compute the factor for calculating results of the analysis of the samples of the extracted residue back to the original tissue. The alcohol extract obtained can be used in alternative procedures for the determination of the sugars while the extracted residue is used for the determination of the protein nitrogen and the insoluble carbon.

Although extraction with alcohol at the reaction of the tissue can be depended upon to remove a part of the organic acids, namely, that part that is not in ionic form in equilibrium with inorganic bases, acidification is necessary to ensure complete extraction. Accordingly, 0.100 gm. of the alcohol extracted material is mixed to a paste with

0.3 ml. of 4 N sulfuric acid, and 0.3 gm. of ignited fine asbestos fibers is mixed thoroughly with the paste a little at a time until a dry friable mass is secured. This mixture is packed into a micro Gooch crucible small enough to be inserted into the neck of the Kjeldahl flask of the carbon apparatus. The bottom of the crucible is covered with a thin layer of glass wool. The balance of the asbestos is used to transfer all particles from beaker to crucible and is then placed on top; a small pad of glass wool is finally added.

The crucible is placed in the ether extraction apparatus and the organic acids are extracted overnight. Carbon is then determined in the residue by transferring the crucible and its contents to the Kjeldahl flask of the carbon apparatus and proceeding in the usual way. The carbon, calculated as a percentage of the alcohol extracted tissue, is corrected by the factor to the percentage of the original tissue.

Ether Extraction Apparatus

The apparatus (Eimer and Amend catalogue No. 30754) consists of a small coil of block tin tubing attached to a plate that serves as a cover for a special wide mouth Erlenmeyer flask. From this a siphon cup is usually suspended. This apparatus has been modified by designing[1] a special support for the siphon cup. A piece of Pyrex tubing of internal diameter slightly larger than the external diameter of the siphon cup and long enough to stand inside the flask and reach nearly to the lowest portion of the tin condenser is ground on its side on a flat glass-worker's wheel until a longitudinal slot has been cut away the entire length of the tube. The width of this slot should be just sufficient to admit one of the micro Gooch crucibles. By means of a small sharp flame, the glass is softened and small indentations are made in the walls of the tube so placed that they will support the siphon cup at the correct height when the frame is placed inside the Erlenmeyer flask. The frame is then turned end for end and another set of indentations made to accommodate and support one of the Gooch crucibles. It has also been found to be possible to arrange indentations so that two or three crucibles can be supported over each other in the frame; if this is done a separate frame is made for use with the siphon cups. This device adapts the extraction apparatus for both crucibles and cups and eliminates the necessity for making wire supports.

EXPERIMENTAL RESULTS

The analytical data are shown in the form of block diagrams as well as in tables. In many of these diagrams, the quantity of each constituent derived from the roots of 50 plants is shown in solid black at the bottom of the block, that from the bulb as a diagonally hatched block placed above the root, and that from the leaves as an open block at the top of the diagram. The blocks that describe the several cul-

[1] For the design of this support, we are indebted to Mr. Laurence S. Nolan of this laboratory.

tures are arranged with the quantity from the original bulbs at the left, the quantities from the starved cultures, i. e., from the bulbs grown in water alone in darkness and in light respectively, immediately at the right of the original bulbs, and the blocks for the cultures upon the solutions that provided nitrogen as nitrate and as ammonium salt successively in order to the right of this. Comparison of the effect of darkness and of light as well as the effects of the addition of the two respective forms of nitrogen can thus readily be made. The designations at the bottom of the diagrams indicate the nature of the culture solution. The symbol CB refers to the control bulbs. The symbol DS refers to the starved culture in darkness, LS refers to the starved culture in light, the other symbols are self-explanatory.

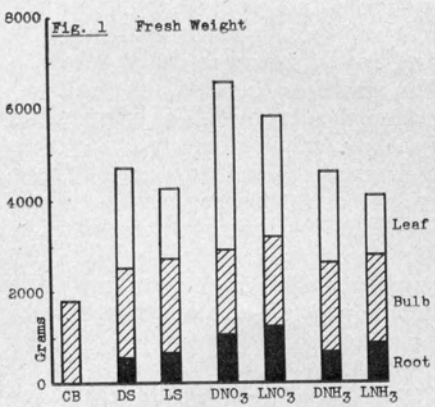
Fresh Weight

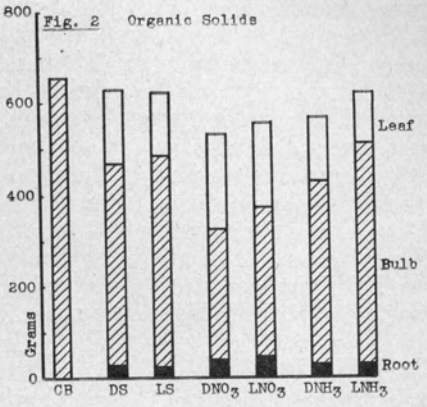
A rough, although somewhat misleading, idea of the size of the narcissus plants[1] grown under the culture conditions that have been described is obtained from the data shown in Figure 1 in which the fresh weight of the tissues is plotted. It is clear that culture in darkness leads to slightly larger fresh weight of the whole plant in all three cases but that the difference is chiefly due to the weight of the leaves. The fresh weight of the bulbs changed remarkably little in spite of the fact that the organic solids of the plants grown in darkness were entirely derived from them. Obviously, the loss in solids was compensated by the addition of water, and the details of this are shown in subsequent diagrams. The root tissues of the plants grown in light were in each case somewhat heavier than those of the plants grown in darkness. An impression of greater succulence on the part of the leaves of the plants grown in darkness is evident.

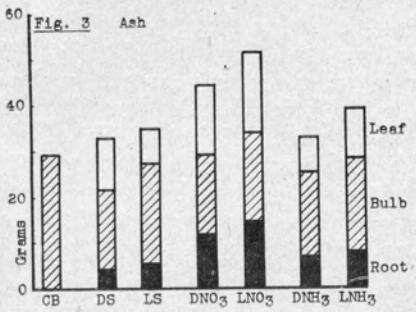
Organic Solids

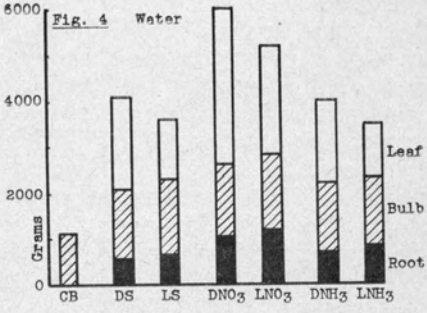
Figure 2 shows the organic solids of the tissues. In no case did the weight of the organic material in the plants grown in light, as shown by the height of the entire block diagram, exceed the weight of the organic material in the original bulbs, and it is apparent that the losses from the plants grown in light due to respiration were not entirely compensated by photosynthesis. Respiration losses were indeed small in the DS culture and amounted to only about 30 gm. of organic solids from the whole plants, a quantity that is only 4.5 per cent of the organic solids of the original bulbs. The net loss from the plants of the LS culture was only 36 gm. or 5.5 per cent. The higher metabolic rate of the plants furnished with nitrate nitrogen is illustrated by the apparent respiration loss of 127 gm. of organic solids from the plants of the DNO_3 culture, which is 19.3 per cent of the organic solids of the original bulbs. The ammonium salt culture occupied an intermediate position since the DNH_3 samples lost only 91 gm. or 13.8 per cent of the original solids. The effect of light was to diminish the losses of organic solids slightly in the case of the

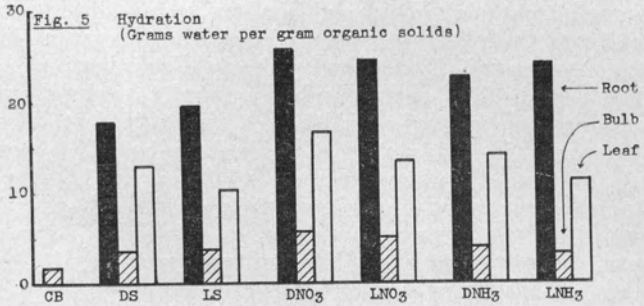
[1] The relative "size" of the plants as judged by various criteria is discussed in a later section (see p. 75).











plants to which nitrogen was supplied but this was not clearly apparent in the starved plants.

The details of the changes in the separate tissues can be most readily appreciated from the data shown in Table 2. The organic

TABLE 2. FRESH WEIGHT, ORGANIC SOLIDS, ASH, WATER AND HYDRATION OF NARCISSUS PLANTS

Figures not otherwise designated are grams calculated on the basis of the tissues from 50 bulbs.

	CB[1]	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
Fresh weight, leaf		2194	1505	3636	2617	1977	1347
bulb	1810	1977	2097	1893	1969	1949	1965
root		577	666	1056	1219	689	846
total		4748	4268	6585	5805	4615	4158
Organic solids, leaf ...		157	134	206	181	137	110
bulb ...	658	441	456	286	327	401	478
root ...		31	32	39	47	29	33
total ...		628	622	531	555	567	621
Ash, leaf		11.4	8.1	15.2	17.3	8.2	11.1
bulb	29.6	17.6	21.5	17.7	19.7	18.5	20.5
root		4.1	5.4	11.3	14.3	6.3	7.7
total		33.1	35.0	44.2	51.3	33.0	39.3
Water, leaf		2026	1363	3414	2418	1832	1226
bulb	1125	1518	1620	1589	1622	1529	1466
root		542	628	1005	1157	654	805
total		4086	3611	6008	5197	4015	3497
Hydration, leaf ..... (gm. water		12.9	10.2	16.5	13.3	13.9	11.2
per gm. bulb ..... organic	1.71	3.4	3.6	5.6	4.9	3.8	3.1
solids) root		17.4	19.6	25.6	24.4	22.5	24.2

[1] The figures in this column refer to the control bulbs at the start of the culture.

solids of the leaf tissue of the plants of the DS culture amounted to 157 gm. while the leaves of the LS culture contained 134 gm. There is no clear-cut evidence for photosynthesis in these figures and the data for the starch and soluble carbohydrates to be presented later likewise suggest none since both of these components were lower in the leaves grown in light than in the leaves grown in darkness.

The residual bulbs of the DS culture contained 441 gm. of organic solids while those of the LS culture contained 456 gm. Thus a slightly smaller net quantity of organic solids was withdrawn from the bulbs grown in light than from the bulbs grown in darkness. The sums of the weight of the leaf and bulb tissues in the two cases were 597 and 590 gm., quantities that are probably not significantly different. Moreover, both sets of plants produced the same quantity of root tissue as measured by the organic solids. Accordingly, the effect of light in the absence of inorganic nutriment appears to have been a stimulation of metabolic activity of the leaves. This is expressed chiefly by a smaller net loss of organic material from the bulb tissues. If photosynthesis did indeed occur, and there is no *a priori* reason to doubt that it did, the stimulation was greater than is indicated by

the data for the organic solids since the equivalent of any new organic matter acquired was also consumed.

The effect of the addition of nitrate was apparently to increase the rate of the metabolism as measured by the respiration loss. Evidence of this is the fact that the weight of the organic material remaining in the bulbs of the DNO_3 plants was by far the smallest. Considerably more than half of the organic solids of the bulbs were mobilized in these plants, and almost exactly half was mobilized from the bulbs grown in nitrate solution in the light. The material that disappeared from the bulbs was utilized for the production of a large mass of highly succulent leaf and root tissue and gave rise to the largest plants as measured by the fresh weight and length of the leaves. The plants in light show moderately clear evidence of an increase in organic solids as a result of photosynthesis since their total weight was greater than those grown in darkness by about 24 gm., but there was no increase in the organic solids of the leaf tissue. On the contrary, the quantity was actually less than that in the leaves of the plants grown in darkness. Detailed interpretation is, of course, speculative at the present stage of our knowledge, but it would appear that in light a smaller demand was placed upon the stores of nutriment in the bulb tissue because of the availability of carbohydrates newly synthesized in the leaves. It should be noted that the roots of the plants grown in light were heavier by some 8 gm. in spite of the smaller quantity of solids withdrawn from the bulbs.

The plants grown upon the ammonium salt culture solution either in light or darkness differed only moderately in fresh weight from the corresponding starved plants (see Figure 1) but the total amount of organic solids present in the ammonia plants grown in darkness was markedly less than that in the DS plants. The actual loss in organic solids from the whole plant, as compared with the starved plants, was 61 gm. which may be compared with the actual loss from the nitrate plants in darkness of 97 gm. Measured in these terms, therefore, culture on ammonium salts produced plants in which the total metabolism was materially greater than that in plants cultured on water alone, although it did not attain the level observed with the nitrate plants. There was certainly no serious disadvantage to the plants nor evidence of "toxic" effects. In light, on the other hand, the total weight of organic solids in the ammonium plants was practically the same as that in the starved plants and no advantage for the supply of nitrogen can be claimed with respect to the total metabolism as measured by the net loss of organic solids. Furthermore, the solids of the leaf tissue were less than those in the starved plants and the solids remaining in the bulbs were greater. One obtains the impression that less extensive chemical transformations took place in the ammonium plants in light than in the starved plants in light. However, this impression of a retardation of the general metabolism with ammonium salt nutrition, as expressed by the weight of the or-

ganic solids, is not borne out by other items of the data. As will appear, the protein metabolism was materially stimulated in the ammonia plants.

Ash

The quantities of ash in the tissues are shown in Figure 3. The original bulbs contained 29.6 gm. of inorganic solids, but additional inorganic material was acquired by all of the plants during growth. Since the cultures were grown in sand, the origin of a part of this is obvious. Although the sand was thoroughly washed before being used, significant amounts of soluble inorganic material remained so that the plants grown with water alone were able to secure 3.5 gm. of additional inorganic substances in the culture in darkness and 5.4 gm. in the culture in light. The nitrate plants were provided with a culture solution that contained inorganic salts and their more vigorous growth, especially in light, is illustrated by the marked increase in ash content. The ammonium plants in darkness, on the other hand, in spite of the availability of inorganic salts in the culture solution, acquired no more inorganic material than the starved plants. It would appear that light promoted an increase in ash constituents, a behavior which reflects that of the plants with respect to the losses of organic solids already pointed out in connection with the discussion of the data plotted in Figure 2.

Because of the availability of inorganic substances to all of the plants, the details of the transport of the substances originally present in the bulbs cannot be followed with accuracy. However, it is clear that migration to the growing tissues of a substantial part of the inorganic substances in the bulb took place. In each of the three treatments, a larger part of the original inorganic material in the bulb was apparently transported to the other tissues in the culture in darkness than in that in light.

The leaf tissue of the starved cultures contained more ash in the plants grown in darkness than those grown in light but the reverse was the case for the two cultures in which nitrogen was supplied. In the root tissue the ash was higher in all cases in the plants grown in light. Attempts to correlate the distribution of the ash with that of the organic solids, the fresh weight or with the water content of the tissues were not successful; what was true in one case did not follow in another and it was clear that far more detailed chemical examination than was attempted would be necessary before generalization upon the distribution of the inorganic components and the relation of this to the distribution of the organic components would become possible.

Water Content

The water relations of the tissues are shown in Figures 4 and 5. Figure 4 shows the quantities of water present and, as might be anticipated, resembles the diagram for fresh weight very closely in detail, confirming the impression already given by that figure regard-

ing the greater succulence of the leaves of the plants grown in darkness. This is shown even more strikingly by the data for the hydration of the tissues given in Figure 5. These quantities are the ratios of the water content to the organic solids, that is, the number of grams of water associated with 1 gm. of organic solids in each case, and are plotted side by side to illustrate the differences more clearly. The root tissue is outstanding in its capacity to hold water; a hydration of 25 gm. of water per gram of organic solids means that the tissues, aside from ash components, contain 96.1 per cent water. It is clear that the cells of this tissue are provided with components of highly specialized properties. The hydration of the roots does not run parallel to that of the leaves save in the nitrate plants; in both starved plants and ammonia plants, the roots were more highly hydrated and the leaves less highly hydrated in the plants grown in light than in those grown in darkness.

Comparison of the data in Figure 5 with those in Figure 2 for the organic solids furnishes a striking illustration of the wide differences in the properties of the substances in the tissues formed under the different conditions of culture and emphasizes the futility of attempting to interpret the effect of these conditions upon growth when only data on the fresh or dry weights are collected.

NITROGEN METABOLISM

Total Nitrogen

The total nitrogen content of the separate tissues of the plants is shown in Figure 6. When grown upon nitrate or ammonium salt culture solution, the plants assimilated substantial quantities of nitrogen, but, when grown with no external source of nitrogen, a significant loss of this element took place. The original bulbs contained 10.8 gm. of nitrogen, but the plants when harvested at 28 days contained only 9.18 and 9.56 gm. after growth, respectively, in darkness and in light. The losses thus amounted to 1.62 and 1.24 gm. or 15.0 and 11.5 per cent, respectively, of the quantity of nitrogen originally present in the bulb tissue, and were therefore much larger than could reasonably be accounted for by mechanical loss of fragments of the root tissue in the course of the preparation of the material for analysis. The evidence points to a loss of nitrogen from the system through the operation of some obscure metabolic change.

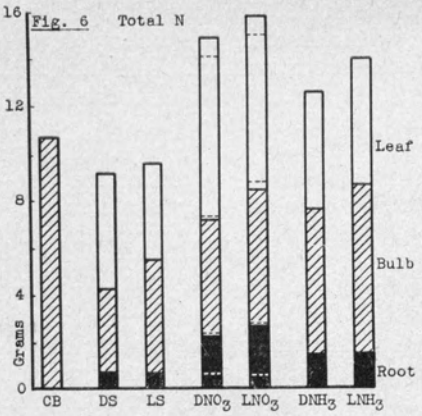
There are few if any records in the literature of loss of nitrogen from growing plants. Experimental conditions under which such a behavior could be detected are only rarely established since the interest of investigators is usually centered upon the absorption of nitrogen rather than the reverse. Furthermore, only in those species that are provided with a large quantity of storage tissue for the support of the young plant could the phenomenon be readily observed, if it is indeed a common one, and in practice this limits the demonstration in general to plants that are reproduced from bulbs or from fleshy tubers.

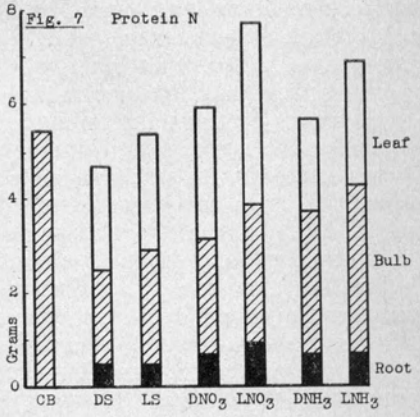
Plants as a rule are extremely economical in their use of the stores of nitrogen available to them.

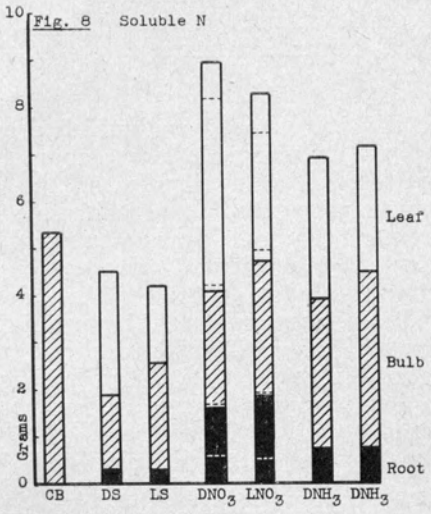
Nevertheless, the leaves of the daffodil, a closely related species, have been shown by Pearsall and Billimoria (15) to lose nitrogen under certain experimental conditions, and interest in this behavior was one of the factors that led to the present investigation. The English workers were concerned with the metabolic behavior of detached daffodil leaves cultured in solutions that provided nitrogen in different forms. The leaves from either young plants or plants in full flower were treated with calcium hypochlorite solution to minimize bacterial infection and were divided laterally into four segments to permit a study of the effect of the age of the tissue. Each segment was then divided longitudinally in half to provide a sample for analysis before the experiment was begun. Samples of the half segments, in sufficient numbers to make upwards of 15 gm. of fresh tissue, were floated upon measured quantities of sterile culture solution buffered at pH 5.4 in a closed vessel. After being exposed to the experimental conditions (light or darkness at controlled temperature) for 60 hours, the samples were withdrawn and analyzed. The remaining culture solution was also analyzed to obtain data on the nitrogen absorbed from it and to permit a balance sheet of the nitrogen exchange to be set up. Bacterial infection was rare and could always be detected when present in significant amount, such infected samples being discarded. The general limits of accuracy were established by suitable control experiments.

A wide range of data was obtained which showed that a significant, and frequently a surprisingly large, quantity of nitrogen was lost from the isolated culture system when the solution furnished nitrogen as ammonium nitrate, potassium nitrate, ammonium chloride, or ammonium tartrate. These salts were supplied at the level of 0.2 per cent (together with 3 per cent of glucose) in the solution which was buffered with phosphates. There was no detectable loss when the solution provided nitrogen as urea, asparagine or alanine. Adequate summarization of their extensive results in a brief statement is scarcely possible but, in general, the English workers noted losses that ranged from 3 to 27 mg. of nitrogen from the system when the data were calculated upon a uniform basis of 10 gm. of fresh leaf tissue. The higher losses affected the apical or older segments of the leaves and were in general greater in light than in darkness. Detailed examination of the composition of the tissues after treatment brought out the fact that about one-half of the nitrogen that disappeared was derived from the culture solution, the other half originating from the organic nitrogen of the leaves. Since there was appreciable hydrolysis of the leaf protein, it was a fair assumption that a part, at least, of the nitrogen lost from the leaves was nitrogen originally combined in the protein. Pearsall and Billimoria accordingly set up the hypothesis that, under the experimental conditions, an equilibrium was established between the ammonium and the nitrate

ions, the intermediate being nitrite ion. Inasmuch as the nitrite was produced in cells abundantly provided with amino acids derived from the hydrolysis of the protein, and the reaction of the cell solution was







definitely acid, interaction of nitrite and amino nitrogen became possible with the production of free nitrogen that was lost from the system. Their assumption that nitrite ion may be formed as an intermediate in the assimilation of nitrate ion is supported by considerable evidence as, for example, by the experiments of Eggleton (5) who has observed the presence of nitrite in tissues of grass leaves which were actively assimilating nitrate. Confirmation of this hypothesis must necessarily await the application of the tracer element technique.

The application of these results of the English workers to the present investigation is by no means straightforward. The conditions under which they established loss of nitrogen to occur were entirely unlike those that prevailed in plants growing in normal fashion upon water in the absence of other nutriment. Nevertheless, certain analogies can be pointed out.

In the first place, the quantities of nitrogen lost from their leaf samples were relatively large. If a rough average figure of 15 mg. per 10 gm. of leaf tissue be taken to represent the magnitude of these losses, then a quantity equal to the 2194 gm. of leaf tissue produced by the bulbs in the DS experiments would be expected to lose 3.3 gm., and a quantity equal to the 1503 gm. of leaf tissue in the LS experiment would lose 2.25 gm. Half of these quantities would be derived from the nitrogen of the leaf tissue itself so that the losses from this tissue would be respectively 1.7 and 1.1 gm. The actual losses observed in the present experiment were 1.6 and 1.2 gm. Thus the *order of magnitude* of the losses in 60 hours from leaf tissue under the conditions established by the English workers was the same as that observed in the present experiment during the entire growth period of the plants. Substantial quantities of leaf tissue were present, however, only during the last 14 days of the experiment. This comparison may be entirely misleading, and the agreement of the figures is of course fortuitous, since *all* of the nitrogen lost by the present leaves was derived from the tissues themselves; but the similarity of the quantities suggests that one is dealing with a phenomenon that may be characteristic and general for plants of this genus.

If the mechanism proposed is indeed the correct explanation, one must assume that the nitrogen of the bulbs in the present experiment underwent transformations such that 0.8 and 0.6 gm. of nitrogen ultimately passed through the stage of nitrite ion. These quantities are respectively 7.4 and 5.5 per cent of the total quantity of nitrogen present. That such large amounts were converted via nitrate ion is at least surprising. Nitrate ion was, in fact, detected in the roots of the DS culture to the extent of 0.003 gm. and in the roots and leaves of the LS culture to the extent of 0.010 gm., but these quantities are far too small to make it likely that so substantial a part of the nitrogen lost from the plants passed through the stage of nitrate and nitrite ion during the course of normal growth.

The alternative is to suppose that a part of the nitrogen in the tissues was converted into nitrite via ammonium ion. This assumption involves a mechanism whereby amino acids or possibly other components are deaminized with the production of ammonium ion which is then oxidized to nitrite. The possibility of this reaction is envisaged in the hypothesis of Pearsall and Billimoria although, in their case, the ammonium ion was presumably mostly derived from the culture solution in which the leaves were immersed. There is no information available by means of which the hypothesis can be either

denied or verified. Significant quantities of ammonium ion were present in the tissues of these plants (see Figure 11) and there is reason to suppose that even larger amounts were produced and transformed into other substances during the experimental period. Thus, the substance that is assumed to serve as a starting point in the series of reactions was in fact present. The difficulty is to secure evidence for the existence of an oxidation mechanism which might give rise to the nitrite ion under the present circumstances.

Such observations as have been recorded upon the presence of nitrites in plant tissues have dealt almost exclusively with the phenomenon of nitrate reduction. There are many cases in the literature, one of the most striking, although rarely quoted, examples being the study of Rimington and Quin (32). These authors were concerned with the toxicity of various species of *Tribulus* to sheep, fatal poisoning having been observed when certain areas upon which the plant was growing were grazed. The toxic plants were found to be extraordinarily rich in nitrates and the tissue was shown to contain an enzyme system capable of reducing nitrate rapidly to nitrite. Accordingly, extracts of the plants, prepared by procedures that did not destroy enzymes, soon became enriched in nitrites, sufficient being formed presumably in the rumen of the animals after ingestion of the plant to provide a fatal dose under certain circumstances. The facts were established by unusually able chemical analysis, while the toxic symptoms could be reproduced in all details by the administration of potassium nitrite. Thus, it seems quite possible that substantial amounts of nitrites can be formed in certain plant tissues when these are grown under conditions that give rise to the presence of a high concentration of nitrates. No analogous case has, however, come to the attention of the writers in which evidence for the production of nitrites from ammonium ion has been established by direct analysis. Although Pearsall and Billimoria demonstrated appreciable loss of nitrogen from daffodil leaves cultured in solutions of ammonium salts, they did not provide chemical evidence for the formation of nitrite, either from nitrate or from ammonium ion, in their experimental material.

The interpretation of the loss of nitrogen from the narcissus plants at present under consideration must therefore remain in abeyance until further evidence can be offered. The observations of Pearsall and Billimoria suggest that the phenomena are similar in mechanism, but application of the hypothetical explanation offered by these investigators offers serious difficulties in the present case. It it perhaps pertinent to remark that a survey of the behavior of the leaves of several plant species by Mothes (11) under conditions essentially similar to those employed by Pearsall and Billimoria failed to reveal losses of nitrogen from the system unless nitrites were added to the culture solution. Accordingly, it would seem that loss of nitrogen from excised leaves in culture is not a usual phenomenon.

It would be surprising indeed if it were common in the case of plants grown under more or less normal conditions.

Turning now from the consideration of the starved plants grown in water to those grown in the presence of culture solutions that provided nitrogen, it is clear from Figure 6 that assimilation of substantial net quantities of nitrogen occurred whether or not losses similar to those observed in the starved plants took place. The nitrate plants acquired 4.1 and 5.1 gm. of nitrogen respectively in darkness and light, the ammonia plants 1.8 and 3.2 gm. The diagrams for the nitrate plants show the quantities of nitrate nitrogen in the root, bulb and leaf tissue indicated by dotted lines, while the total nitrogen of the plants exclusive of nitrate nitrogen is indicated by a dotted line near the top. The quantities of free ammonium nitrogen in the ammonia plants were too small to represent on the scale of the figures in a similar way. It is clear that the nitrate plants were grown with a luxury supply of nitrogen[1] since accumulation of a moderate excess of this component was possible, and the large fresh weight of these plants (See Figure 1) illustrates their response to this condition. It has already been pointed out that the ammonia plants were unable to grow as luxuriantly as the nitrate plants, and this is shown also by the data in Figure 6. Even when corrected for the unutilized nitrate nitrogen stored in the tissues, the nitrate plants assimilated more nitrogen than the ammonia plants. In other words, the ammonia plants were grown under conditions that were somewhat less favorable than were the nitrate plants.

The behavior of the nitrogen of the bulb tissue is instructive. The data are summarized in Table 3 where the quantities that remained in the bulbs are calculated as percentage of the nitrogen of the whole plant in the first line and as percentage of the original

TABLE 3. PROPORTION OF THE NITROGEN IN THE BULB TISSUE OF NARCISSUS PLANTS

	CB	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
Per cent of total N of whole plant	100	39.5	50.0	32.8	36.3	49.5	51.7
Per cent of total N of control bulbs	100	33.6	44.3	45.2	53.5	57.9	66.9
Per cent of original N transported from bulbs into other tissues	0	66.4	55.7	54.8	46.5	42.1	33.1

[1] It is important to note that the presence of nitrate nitrogen in a plant tissue is, in general, an expression of the fact that, just previous to the time of observation, nitrates were being absorbed by the roots at a rate greater than they could be assimilated, that is, converted into other nitrogen compounds. The situation at any point of time is a complex one in which such factors as the concentration of nitrates in the solution that bathes the roots, the total mass of metabolizing tissue and its nature, and the availability of other essential nutrients all play their part. A given concentration of nitrate in the culture solution may give rise to storage of nitrate and thus be a luxury supply at one stage of growth but may prove to be an inadequate supply at another.

nitrogen content of the bulbs in the second line. The third line shows the percentage of the original nitrogen of the bulb that was transported into the other tissues during the growth period. The starved plants, especially those grown in darkness, drew heavily upon the nitrogen of the bulb, this being the only source available. As a consequence, about 66 per cent of the original nitrogen of the bulb was transported to the other tissues during growth in darkness and 56 per cent was similarly transported during growth in light. Reference to Figure 3 shows that the behavior of the ash content of the bulb tissue followed a similar pattern, more being lost from the bulbs in darkness than in light.

The nitrate plants and the ammonia plants show a similar effect in that growth in light gave rise to the transport of a somewhat smaller proportion of the original nitrogen than growth in darkness. The important point is that the proportion of the original bulb nitrogen that was translocated to the other tissues in the nitrate plants was only about 10 per cent less than that in the starved plants, whereas the proportion transported in the ammonia plants was about 24 per cent less, being only 42 and 33 per cent in the two cases. When compared on the basis of equal *periods of growth*, the ammonia plants therefore appear to have made more efficient use of the nitrogen they acquired from the culture solution than did the nitrate plants. This impression is confirmed by the following calculation. The net quantity of nitrogen absorbed from the culture solution can be calculated from the total nitrogen of the plants by deducting 10.8 gm., the nitrogen present in the original bulbs. The quantities found are respectively 4.1, 5.1, 1.8, and 3.2 gm. in the four sets of plants grown on culture solutions. The nitrogen transported from the bulbs can similarly be calculated from the quantities in the bulbs at the end (see Table 4) by deducting these from 10.8 gm., the nitrogen present in the bulb at the start. The figures are 5.9, 5.0, 4.5, and 3.6 gm., respectively. The sums of these respective quantities are the amounts of nitrogen available in each set of plants for the synthesis of new tissue and are 10.0, 10.1, 6.3, and 6.8 gm. If the ratios of these last quantities to the total nitrogen in the leaf tissue of each set are calculated, it is found that for each gram of nitrogen available for synthesis, 0.76 and 0.70 gm. of leaf tissue nitrogen, corrected for the nitrate present in it, were produced in the plants grown respectively in darkness and in light on nitrate solution, while 0.79 and 0.78 gm. of leaf tissue nitrogen were produced in the plants grown on ammonium salt solution. With respect to the production of root tissue (as measured by the nitrogen it contained), there was a less marked difference in the dark experiments and none in the light; the figures are 0.17 and 0.22 gm. for the nitrate plants and 0.21 and 0.21 gm. for the ammonium plants. This result does not mean that growth upon ammonium salts was superior to that on nitrates; quite the reverse was the case if such usual criteria as the fresh weights are used as the basis of comparison. All that is implied is that the utilization of ammonium ion from the culture solution in the synthetic processes of

the plant as expressed by the quantity of what is often called "organic nitrogen" in the leaf tissue was not inferior to the utilization of nitrate; on the contrary, there was a slight advantage in favor of the ammonium salt.

A somewhat analogous case was encountered in the study of the effect upon the growth of tobacco plants (50) of culture solutions that provided a series of mixtures of nitrate and ammonium nitrogen varying from one the nitrogen of which was all nitrate to one in which nitrate made up only 10 per cent of the whole. In these experiments it was found that, although the plants grew poorly upon the solutions that furnished most of the nitrogen as ammonium ions, the inclusion of 20 per cent of the nitrogen in the form of ammonium ion with the balance as nitrate furnished the plants with an advantage over those grown upon nitrate alone. This was expressed by the size of the plants whether measured by fresh or dry weight or by total nitrogen. All of the evidence secured pointed to the more rapid utilization by the plants of the ammonium ions than of the nitrate ions derived from the culture solutions up to and including one that contained 60 per cent of its nitrogen in the form of ammonium ion.

Examination of the details of the data in Figure 6 shows that growth as measured by the nitrogen content of the respective tissues was more luxuriant upon the nitrate solution than upon the ammonium salt solution. Unfortunately, there is no evidence upon which to base a judgment of the relative physiological age of these plants. The experiment was arbitrarily terminated at the expiration of a definite period of time without regard to degree of development of the plants. This procedure was dictated by the practical consideration that comparison upon a basis of equal time of treatment was far easier to establish than a comparison upon a basis of equal physiological age. The considerable variation between the growth rates of the individual bulbs and the plurality of shoots from single bulbs in each set precluded any attempt to harvest the plants at some point such, for example, as the time when the first bud separated from the sheath of leaves, or when the flower stalk reached an arbitrarily selected length. Presumably, such a comparison as has been attempted in the present experiment could successfully be made only with plants of a strain that had been inbred so that the growth characters had become fixed and reproducible.

In spite of the technical deficiencies of the experiment, it is of interest to note that the leaves of the water culture and of the nitrate plants grown in darkness contained appreciably more nitrogen than those of the corresponding plants grown in light. The reverse was, however, the case for the ammonia plants and this is a circumstance that is difficult to understand, especially as the total nitrogen content of the whole plants was in each case greater in light, and assimilation might be assumed to be favored in these circumstances.

No evidence was obtained, by analysis of the culture solutions, on the matter of losses of nitrogen from these plants. Culture in sand is unsuitable for this type of study, and even if the plants had been grown directly in the culture solutions, clear-cut evidence would be difficult to obtain owing to the probability that bacterial infection would obscure the issue. The most conclusive method to establish such a behavior would be the use of heavy nitrogen in the culture solution with subsequent isotope analysis of the air surrounding the leaves. It should be pointed out, however, that if nitrogen loss did in fact occur, the quantities of nitrogen absorbed by the plants were to that extent greater than those observed, and the present figures can, therefore, at best, be regarded as net results. The conditions in the leaf tissues grown upon nitrate or ammonium salt were, at least to some extent, the duplicate of those present in the experiments of Pearsall and Billimoria, since ample amounts of nitrate ion or ammonium ion were transported from the culture solutions into the leaves. It is possible, accordingly, that a reaction similar to that observed in the present water cultured starved plants and in the excised leaves studied by the English investigators did occur.

Protein Nitrogen

From many points of view, the determination of the protein nitrogen of the tissues is the most important index of growth available since the success of the plants as organisms is largely dependent upon their capacity to synthesize protein. Accordingly, it is necessary to specify the nature of the data obtained in the present investigation with some care. The figures that are given for protein nitrogen were obtained by determining the total nitrogen of samples of the dried tissues that had been extracted thoroughly first with alcohol, to remove carbohydrates and simple nitrogenous substances soluble in this solvent together with the chlorophyll, and subsequently with hot water, to remove a small residual quantity of soluble nitrogenous material. It is assumed that this procedure extracts all nitrogenous substances other than protein, and that all of the protein is converted during the drying operation at 80° and by the treatment with hot alcohol into the insoluble denatured condition. Although the prolamins of the cereal grains provide an exception, proteins in general, and especially the proteins of living tissues, are known to to be rendered insoluble under these conditions.

The data are plotted in Figure 7 and are given in detail in Table 4, together with calculations of the proportions of the total nitrogen of the individual tissues present in the form of protein, and of protein nitrogen as a percentage of the organic solids. The original bulbs contained 50.6 per cent of their nitrogen in the form of protein, the balance being combined in substances that were soluble in hot alcohol or hot water. Both protein nitrogen and soluble nitrogenous components evidently contributed to the growth of the plants since both decreased in the tissues of the bulbs that remained after the plants had developed. In order for transport to take place, protein

must have been decomposed by the proteolytic enzymes of the cells into soluble products, these being in turn conveyed to the meristematic tissues where they were utilized for the synthesis of the components of the developing cells.

The proportions of the protein of the original bulbs that were so digested and utilized for the growth of the plants are shown in Table 4 and amounted to approximately 62 and 55 per cent for the DS and LS cultures, 56 and 46 per cent for the nitrate cultures, and 44 and 35 per cent for the ammonia cultures respectively in darkness and in light. In all cases, therefore, less of the original bulb protein was metabolized in the plants grown in light than in those grown in darkness, and culture upon solutions that provided extraneous

TABLE 4. TOTAL NITROGEN AND PROTEIN OF NARCISSUS PLANTS

Figures not otherwise designated are grams calculated on the basis of the tissues from 50 bulbs.

		CB	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
Total nitrogen,	leaf ..		4.84	4.09	7.72	7.41	5.02	5.30
	bulb ..	10.8	3.63	4.78	4.88	5.78	6.26	7.23
	root ..		0.71	0.69	2.30	2.75	1.36	1.45
	total ..		9.18	9.56	14.90	15.94	12.64	13.98
Protein nitrogen,	leaf ..		2.17	2.46	2.81	3.83	1.98	2.58
	bulb ..	5.46	2.06	2.48	2.41	2.95	3.08	3.56
	root ..		0.45	0.45	0.72	0.90	0.65	0.71
	total ..		4.68	5.39	5.94	7.68	5.71	6.85
Per cent of protein of original bulbs utilized..			62.2	54.6	55.9	46.0	43.6	34.8
Protein nitrogen as per cent of total nitrogen of tissues	leaf ..		44.8	60.1	36.4	51.7	39.4	48.7
	bulb ..	50.6	56.7	51.9	49.3	51.0	49.2	49.2
	root ..		63.4	62.5	31.3[1]	35.7[1]	47.8	48.9
	total ..		51.0	56.3	39.9	48.2	45.2	49.0
Protein nitrogen[2] as per cent of organic solids	leaf ..		1.39	1.83	1.36	2.11	1.44	2.34
	bulb ..	0.83	0.47	0.54	0.84	0.90	0.77	0.75
	root ..		1.45	1.41	1.84	1.91	2.24	2.15

[1] These percentages are calculated without correction for nitrate nitrogen. If this correction is made, the percentages in leaf and bulb tissue are from 1 to 2 per cent higher; the values for the root tissue become, however, 42.3 and 40.7 per cent, respectively.

[2] These figures can be converted to percentage of protein in the organic solids by multiplication by the conventional factor 6.25.

nitrogen diminished the drain upon the bulb protein. Appreciably less protein was called for from the bulbs in the ammonia culture than in the nitrate culture experiments. Clearly, the rate of digestion of the bulb protein during the growth period is under the control of mechanisms that are remarkably sensitive to the conditions that surround the plant. This conclusion is valid even if protein

synthesis actually did take place in the bulb tissue of the growing plants; for if the situation was really one in which both hydrolytic and synthetic reactions were going on continuously—and this is today a fundamental concept in the theory of protein metabolism in living cells—the extent to which the hydrolysis predominated over the synthesis is what is really involved in the changes revealed by the data. The net result was hydrolysis and the extent of this was controlled by the external conditions under which the plants were grown.

The protein nitrogen of the leaf tissue was in all cases higher, both in absolute amount and in relation to the total nitrogen or to the organic solids of the leaf (i. e. as percentage of the total nitrogen or of the organic solids), in the plants grown in light as compared with the similar plants grown in darkness. Thus, although the plants grown in light drew less heavily upon the protein of the bulbs than the plants grown in darkness, they synthesized more protein both absolutely and relatively in their leaves. It seems likely that this is an effect of the larger quantities of energy presumably available in plants grown in light. A parallel behavior is less apparent in the root tissue, the differences being too small, save possibly in the nitrate plants, to be significant.

The quantity of protein in the leaves was greatly influenced by the availability of nitrate nitrogen in the culture solution, especially in light. Owing to the larger relative quantity of soluble nitrogen in the leaves of the nitrate plants, the proportion of protein nitrogen, in terms of total nitrogen, was much smaller than was the case in the starved plants and the same is even more strikingly shown in the root tissue. In terms of organic solids, the leaves of the nitrate plants contained a slightly higher proportion of protein than the starved plants, the roots a definitely higher proportion. It may thus be assumed that culture upon a nitrate solution brought about a stimulation of protein synthesis, especially pronounced in the leaves, and, since the nitrate plants drew less heavily upon the original stores of protein in the bulbs than the starved plants, the total protein in the nitrate plants was materially greater at the end of the experiment than it was either in the starved plants or in the ammonia plants.

Culture upon the ammonium salt solution did not give rise to any striking difference in the protein content of the leaves as compared with those of the plants supplied only with water. The DNH_3 plants contained a little less protein in the leaves than the DS plants and the LNH_3 plants a little more than the LS plants, but in neither case is the difference striking. The roots of the ammonia plants were a little richer in protein than were those of the respective starved plants but the actual quantities were small in both cases. Only in the bulb tissue was there a large difference, and this is clearly an expression of the fact that there was a smaller drain upon the protein of the bulbs in the plants grown on ammonium salt solution than

there was in the plants grown on water. Accordingly, although the total protein of the whole plants was appreciably greater in the case of the plants on ammonia culture than in the plants on water culture, this does not necessarily imply a stimulation of protein synthesis in the ammonia plants. It appears to mean that the plants to which ammonium ions became available were able to utilize this form of nitrogen for the synthesis of new tissue protein about as effectively but no better than the starved plants were able to utilize the nitrogen derived exclusively from the bulb tissue. There is evidence neither for significant stimulation nor for repression of synthetic activity with respect to protein. As far as it goes, the data suggest that the leaf tissues were able to do as well upon one source of nitrogenous nutriment as upon the other.

It should perhaps be pointed out that a similar inference could not have been drawn from data calculated in terms of percentage of the total nitrogen or as percentage of the organic solids. Such figures give measures of concentration rather than amount, and it is clear that the concentration of the leaf protein in the ammonia plants was lower than that in the starved plants, when the comparison is made in terms of percentage of the total nitrogen but was higher when it is made in terms of the organic solids. The same is true for the root tissues. These differences are due to the effects upon other components of the tissues than the protein of the conditions under which the respective plants were grown. The ammonium plants were much higher in soluble nitrogen than the starved plants and were lower in organic solids. Thus, the percentage method of expression presents a different picture of the final results than does the calculation of the absolute quantities.

Soluble Nitrogen

The soluble nitrogen of a plant tissue is a purely conventional analytical quantity and, moreover, is one the magnitude of which depends to a considerable extent upon the method employed for its determination. The kind of solvent, the conditions under which it is applied, the previous treatment, as well as the nature of the tissue and the cultural conditions that prevailed immediately before the plants were harvested, are all factors that influence the result. The nitrogenous substances that are included in determinations of the soluble nitrogen differ widely from each other both in chemical properties and in significance in the metabolic scheme. It would be misleading indeed to consider this analytical quantity as a measure of merely the amino acids, amides, and simple peptides of the cell solution; these substances are included, but many other products that are not nearly so obviously related to the protein metabolism are likewise involved, and among these the purines, choline, the betaines, and, in the present experiments, the chlorophyll, should be specifically mentioned. As will become clear as the details of the analytical data are presented, only from one-third to three-quarters of the soluble nitrogen in the present experimental material is demonstrably re-

lated to the protein metabolism although the actual proportion is probably somewhat higher; the balance is made up of what is doubtless a large number of other nitrogenous substances, the metabolic relationships of few of which are at all well understood.

In the present case, the soluble nitrogen represents those nitrogenous substances that could be extracted from the dried tissue by means of hot diluted alcohol followed by treatment with hot water. Little nitrogenous material other than protein can be expected to remain undissolved under these conditions provided that the dried tissue is well comminuted and the extraction procedure is sufficiently thorough. The preliminary treatment with alcohol is especially advantageous since it removes the chlorophyll as well as much, if not all, of the nitrogenous lipids in addition to nitrates, ammonia, amides, amino acids, and simpler peptides, and the purines and methylated basic substances. With respect to the chemical nature of the small quantity of nitrogenous material that is subsequently removed by the hot water, nothing has been learned.

The data (see Table 5) plotted in Figure 8 show the quantities of soluble nitrogen in the tissues under discussion. On the diagrams for the nitrate plants, the quantities of nitrate nitrogen have also been indicated according to the conventions that were used in Figure 6 in order to illustrate the relative order of magnitude; the quantities of free ammonia nitrogen in the plants grown on ammonium salt culture were, however, too small to represent on the scale chosen.

The soluble nitrogen of the leaf tissue of the plants grown in light was in all cases lower than that of the plants grown in darkness; this is also true of the root tissue of the starved plants, but the reverse is the case for the roots of the nitrate plants and of the ammonia plants. The bulb tissues of all three sets grown in light contained more soluble nitrogen than the corresponding sets grown in darkness. This observation can perhaps be correlated with the data for protein nitrogen in the leaf tissue. Protein nitrogen was greater in the leaves grown in light and the soluble nitrogen was correspondingly smaller, a result that may be due to the more intense protein synthetic activity in the illuminated leaves. If this were supposed to call forth a greater supply of soluble nitrogenous substances from the bulbs, the conditions would be similar to those observed.

The effect of the availability of nitrogen in the form of nitrate is strikingly shown by the data for soluble nitrogen in the nitrate plants as compared with the starved plants. Even when allowance is made for the appreciable quantities of nitrate nitrogen that these plants contained in both root and leaf tissues, it is clear that the nitrogen metabolism, as expressed by the quantity of soluble substances synthesized, was markedly stimulated. This is true, although to a smaller extent, of the ammonia plants also, and, as will become clear, is in part an expression of the increased amino acid and amide synthesis in these plants. Both leaf and root tissues of the nitrate plants

show an enrichment in soluble nitrogen, the effects upon the roots being especially pronounced.

The extracts from the present samples were subjected to somewhat detailed chemical analysis in order to discriminate as far as possible among the different forms of nitrogen that are grouped together under the designation soluble nitrogen. Briefly, these analyses involved the determination of the ammonia and nitrate nitrogen, the glutamine and asparagine amide nitrogen, and the amino nitrogen both before and after severe hydrolysis, this last quantity being determined in solutions that had been freed from ammonia by distillation at alkaline reaction. Consideration of the data so obtained permits the allocation of a large part of the soluble nitrogen to groups of substances of similar chemical properties. The diagrams shown in Figures 9, 10 and 11 are plotted from the data given in Table 5. In addition to the fundamental assumptions that the extracts from the tissues contained the whole of the material being studied and that the analytical methods are trustworthy, it has also been assumed that peptide nitrogen is adequately estimated from the difference between the values for amino nitrogen before and after hydrolysis with 6 N sulfuric acid, and that the interference of phenolic substances with the determinations of amino nitrogen could be neglected. Aside from this, the only complication that arises is that due to the peculiar behavior of glutamine when treated with nitrous acid under the conditions of the Van Slyke amino nitrogen determination. In these circumstances, not only the whole of the α-amino group reacts but 80 per cent of the amide nitrogen group is likewise decomposed; accordingly, the determinations of the free α-amino nitrogen of an extract are too high by a quantity equal to 80 per cent of the separately determined glutamine amide nitrogen. The observed quantities are given in Table 5. On the other hand, the total amino nitrogen, that is the amino nitrogen present after the complete hydrolysis of the peptide groups, is accurately determined. To estimate the peptide nitrogen, it is necessary to subtract the true free amino nitrogen from the total amino nitrogen. In plotting the results in the lower part of Figures 9, 10 and 11, the sum of the total amino nitrogen and 80 per cent of the glutamine amide nitrogen is represented on the graph and the observed free amino nitrogen plus 20 per cent of the glutamine amide nitrogen is also plotted. The difference shown graphically thus gives the closest possible approximation to the true peptide nitrogen. When the whole of the glutamine amide nitrogen is set off downward from the plotted level of the free amino nitrogen, the quantity below this point represents the free amino nitrogen due to the amino groups of both asparagine and glutamine as well as of any other amino acids in the extracts. The amino nitrogen of amino acids other than asparagine and glutamine is then obtained by setting off the sum of the asparagine and the glutamine amide nitrogen (which would be equal to the sum of the amino nitrogen in these substances) below the glutamine amide nitrogen. This quantity is designated "corrected amino acid nitrogen" in Table 5.

TABLE 5. DISTRIBUTION OF THE SOLUBLE NITROGEN OF NARCISSUS PLANT TISSUES
Figures not otherwise designated are grams calculated on the basis of the tissues from 50 bulbs.

	CB	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
			Bulb Tissue				
Total soluble N	5.36	1.57	2.30	2.47	2.83	3.19	3.67
Soluble N as per cent total N	49.6	43.2	48.1	50.6	48.9	50.9	50.7
Free amino N (observed)	1.18	0.48	0.45	0.83	0.70	1.10	0.81
Total amino N	2.91	1.12	1.28	1.20	1.63	1.91	2.10
Asparagine amide N	0.42	0.12	0.13	0.31	0.17	0.38	0.26
Glutamine amide N	0.15	0.07	0.10	0.08	0.13	0.22	0.17
Ammonia N	0.03	0.03	0.04	0.04	0.04	0.08	0.07
Nitrate N				0.06	0.05		
Corrected amino acid N	0.50	0.23	0.15	0.37	0.29	0.33	0.24
Peptide N	1.85	0.70	0.90	0.44	1.04	0.98	1.43
Other soluble N	1.88	0.24	0.77	0.80	0.84	0.64	1.10
			Root Tissue				
Total soluble N		0.27	0.24	1.58	1.85	0.72	0.75
Soluble N as per cent total N		38.0	34.8	68.7	67.3	52.9	51.7
Free amino N (observed)		0.04	0.02	0.33	0.29	0.26	0.19
Total amino N		0.12	0.09	0.42	0.41	0.29	0.28
Asparagine amide N		0.01	0.007	0.15	0.13	0.06	0.04
Glutamine amide N		0.008	0.008	0.04	0.04	0.06	0.07
Ammonia N		0.003	0.004	0.013	0.015	0.061	0.065
Nitrate N		0.003	0.003	0.596	0.527	0.016	0.016
Corrected amino acid N		0.01	0.00	0.11	0.09	0.09	0.03
Peptide N		0.09	0.08	0.12	0.15	0.08	0.13
Other soluble N		0.12	0.13	0.37	0.74	0.24	0.30
			Leaf Tissue				
Total soluble N		2.67	1.63	4.91	3.58	3.04	2.72
Soluble N as per cent total N		55.2	39.8	63.7	48.3	60.6	51.3
Free amino N (observed)		0.54	0.19	0.73	0.65	0.39	0.60
Total amino N		1.25	0.55	2.06	1.52	1.13	0.83
Asparagine amide N		0.24	0.08	0.58	0.44	0.29	0.25
Glutamine amide N		0.09	0.06	0.13	0.19	0.16	0.24
Ammonia N		0.02	0.02	0.04	0.05	0.03	0.07
Nitrate N		0.00	0.01	0.14	0.27	0.00	0.00
Corrected amino acid N		0.12	0.00	–0.08	–0.12	–0.19	–0.09
Peptide N		0.79	0.41	1.43	1.02	0.87	0.42
Other soluble N		1.09	0.92	1.99	1.15	1.46	1.38

The analytical data for the bulb tissue are given in Figure 9, the upper part of the diagram showing the individual quantities plotted each on the same level to permit ready comparison of the relative magnitudes and, accordingly, of the effects of the culture conditions. In the lower part of the diagram, the separate quantities are divided off from each other in a block the height of which shows the total soluble nitrogen. This part of the diagram thus represents the detailed composition of the soluble nitrogen fraction from the bulb tissue. Similar diagrams for the root tissue are given in Figure 10,

and for the leaf tissue in Figure 11. In Figure 9, the composition of the original bulb tissue is shown at the left to serve as a basis of comparison with the composition of the bulb tissue that remained after the plants had been grown for 28 days. The quantities represent soluble material which was doubtless for the most part transported into the growing parts of the plant in the early stages of the process of development. As growth proceeded, digestion of the storage proteins in the bulb tissue took place with the production of soluble nitrogenous substances that were utilized in the development of the leaves and roots. The quantities of protein that survived this process have already been shown in Figure 7 while the quantities of soluble substances that were presumably largely produced from the protein in the starved plants but which were still present in the bulbs at the time of harvest are shown in Figure 9 together with the quantities in the bulbs of the plants that were supplied with nitrogen from the culture solution. The soluble nitrogen in the bulbs of these plants was in part derived from the protein, in part from the extraneous nitrogen of the culture solution. In comparing the composition of the original bulbs with that of the bulbs of the plants at the time of harvest, it must be held in mind that although there was, for example, 1.85 gm. of peptide nitrogen in the original bulbs and only 0.70 gm. in the bulbs of the DS plants, it cannot be inferred that the difference, 1.15 gm., represents the quantity of peptide nitrogen that was utilized during the growth of the plants. The actual amount must have been a great deal larger than this since the DS plants grew at the expense of 3.4 gm. of protein nitrogen derived from the bulbs and a considerable part of this must have passed through the stage of peptide and amino nitrogen during the growth period.

The effect of light upon the soluble nitrogenous components of the bulbs of the starved plants is best seen from the upper part of Figure 9. The most conspicuous differences were in the peptide nitrogen and the "other" nitrogen, both being increased in light. There was a small similar effect upon the two amides, but the quantity of amino acids other than glutamine and asparagine was a little less in light than in darkness although the difference is perhaps hardly significant.

The effects upon the two amides in the starved plants differed from those in the plants supplied with nitrogen in the culture solution. The asparagine in the bulbs of both the nitrate plants and the ammonia plants was less in light than in darkness; the glutamine was greater in light in the nitrate plants but less in the ammonia plants. The effect of the supply of ammonium ions upon the total quantity of the two amides in the bulbs was surprisingly small although this is a condition that, in other plant species, notably the tomato (41) and the beet (42), leads to a marked stimulation of the production of glutamine. As will be seen, none of the tissues of the ammonia plants could be regarded as being unusually enriched in amides, and in fact the leaves of the nitrate plants contained appreci-

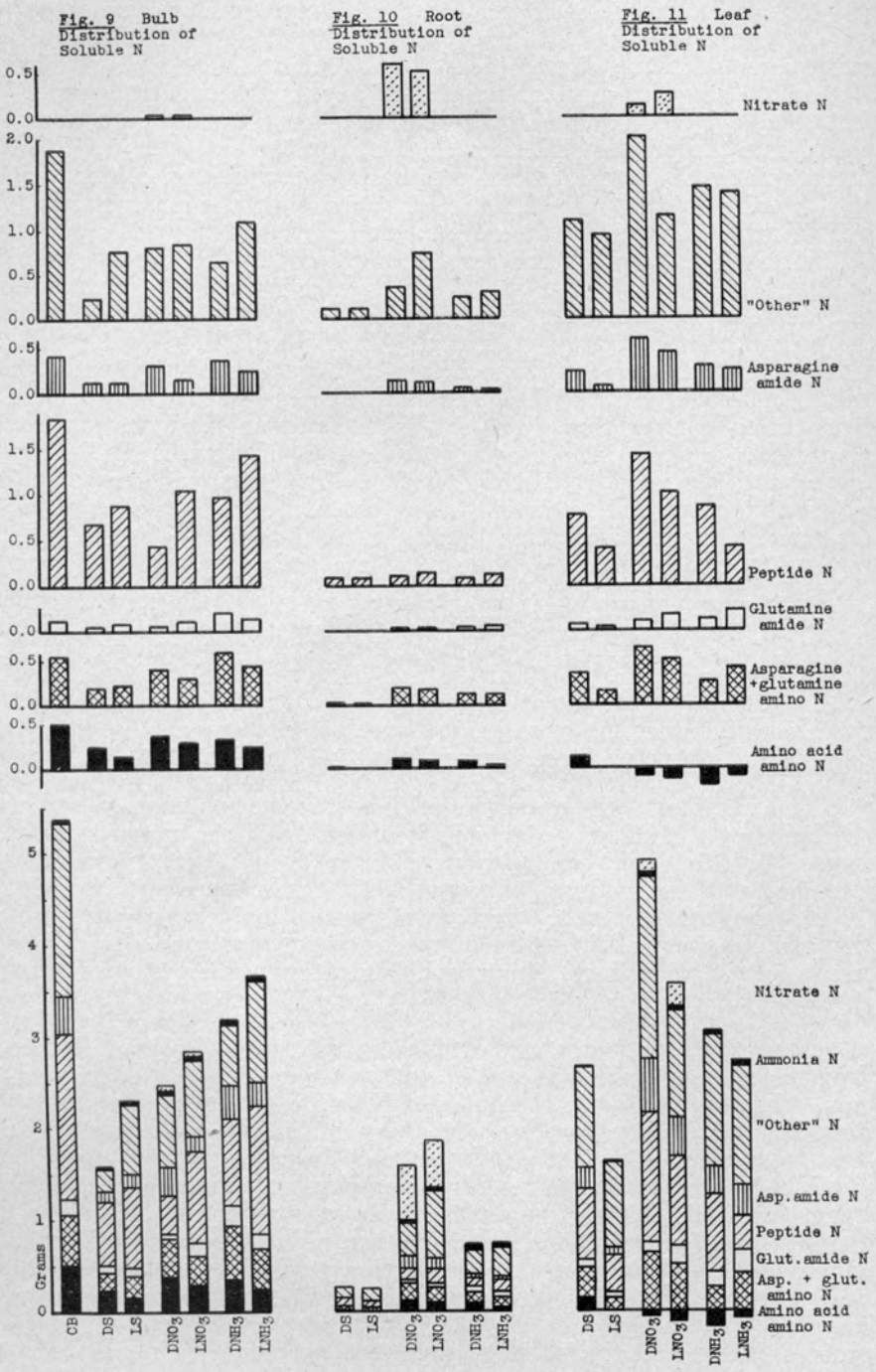


Fig. 9 Bulb Distribution of Soluble N
Fig. 10 Root Distribution of Soluble N
Fig. 11 Leaf Distribution of Soluble N
Nitrate N
"Other" N
Asparagine amide N
Peptide N
Glutamine amide N
Asparagine +glutamine amino N
Amino acid amino N
Grams
CB
DS
LS
DNO3
LNO3
DNH3
LNH3
Nitrate N
Ammonia N
"Other" N
Asp. amide N
Peptide N
Glut. amide N
Asp. + glut. amino N
Amino acid amino N

ably more asparagine than the leaves of the ammonia plants. Thus, ammonia nutrition, contrary to what might have been expected, did not result in a significant increase of the amide metabolism as expressed by the composition of the bulb tissue and, as will be seen, this is true also for the leaf tissue.

Figure 10 shows the distribution of the soluble nitrogen in the root tissue, the details being given in the upper part, the combined data with some simplification owing to the small quantities of certain components in the lower. It will be noted that in general the relative magnitudes in the tissues of the plants grown in darkness and in light follow those for the bulbs in close detail. Of special significance are the facts that neither the asparagine nor the glutamine amide nitrogen was affected by the availability of ammonia in the culture solution; on the contrary, the general stimulation of nitrogen metabolism under nitrate nutrition gave rise to a greater total quantity of amides in the roots of the nitrate plants than in those of the ammonia plants.

The outstanding result is the unusually large quantity of "other" nitrogen in the roots of the LNO_3 plants and to a somewhat lesser extent in the DNO_3 plants. The relative importance of this group of substances is particularly well shown in the combined diagram in the lower part of the figure. The total soluble nitrogen in the roots was greatly increased in the nitrate culture, a considerable part of it being unassimilated nitrate nitrogen. The process of assimilation was also greatly increased, although only about one-half of the soluble nitrogen other than nitrate of the plants grown in light had been converted into substances for which analytical methods are available. We have here, then, evidence for the presence of nitrogenous substances, in substantial proportions, that do not yield amino nitrogen on hydrolysis, that is, for substances that are apparently not associated in a direct way with the protein metabolism. It may be well to digress for a moment to inquire into such chemical possibilities as can be suggested to account for this group of components.

All of the amino nitrogen, whether present in amino acids or amides, or potentially present in peptide linkage, is accounted for by the part of the diagram that includes the asparagine amide nitrogen and the forms plotted below this. However, the nitrogen of the basic amino acids histidine and arginine other than the α-amino groups would be reckoned in the "other" nitrogen. The same is true for the nitrogen of proline and hydroxyproline, and for the indole nitrogen atom of tryptophane, as well as for a part of the nitrogen of the end-standing amino group of lysine, since the analytical procedure employed (i. e., a short reaction time with nitrous acid in the Van Slyke apparatus) would probably not suffice for the complete decomposition of all of this form of nitrogen present. The "other" nitrogen likewise includes the whole of the nitrogen of the purines of which adenine is probably the chief representative, and the nitrogen of the

traces of vitamins, e. g., thiamine, nicotinic acid, riboflavin, etc., that are doubtless present.

The data for the leaf tissues are plotted in Figure 11. The soluble nitrogen of the leaves of the DS and LS plants was less than that of the leaves cultured on nitrate or ammonium salt solution but, nevertheless, it formed a substantial part of the total nitrogen being, respectively, approximately 55 and 40 per cent in the two cases. The analogous figures for the nitrate plants were 64 and 48 per cent while those for the ammonia plants were 61 and 51 per cent. In each case, therefore, the effect of light was to diminish the relative proportion of soluble nitrogen in the leaf tissue as well as to diminish the actual quantity present. With a few exceptions, this was also true of the several forms of nitrogen that are plotted in the upper part of the figure, and the impression is given of a stimulated protein metabolism in light. The exceptions are the ammonia nitrogen, where the total amount present was very small in any case and the difference due to growth in light was scarcely more than significant, and the glutamine amide nitrogen; this last in turn affected the free amino nitrogen. Thus, in the leaf tissue there is evidence for a small stimulation of the synthesis of glutamine as a result both of nitrate and of ammonia nutrition in light. The quantity present is both actually and relatively to the soluble nitrogen greater in light than in darkness although the differences are small. Reference to Figures 9 and 10 shows that this is not so in the bulb and root tissues.

The asparagine amide nitrogen was small in the starved plants grown in darkness and was minute in those grown in light. In the nitrate plants, however, it was greatly increased under both conditions of culture but was small and was little affected by light in the ammonia plants. The evidence with respect to asparagine in narcissus, then, points to a function associated with general nitrogen metabolism rather than specifically with ammonia metabolism inasmuch as more was present in the larger and more highly nitrogenous leaves of the nitrate plants than in those grown on ammonia culture. Although by no means so clearly expressed as to be convincing, there is thus a suggestion that the two amides play somewhat different metabolic roles in the leaves of this species, a conclusion that conforms with results obtained with the tobacco plant (47).

In the leaves of the plants supplied with nitrate or with ammonia, the amino nitrogen present in free form can be accounted for in its entirety by the amino groups of the two amides, asparagine and glutamine. In fact, when the amino nitrogen from this source is subtracted from the corrected free amino nitrogen, small negative values are obtained that are represented on the diagram by blocks plotted below the axis. These values may arise from cumulative analytical error. The evidence thus points to an extraordinarily active metabolic process in these leaves since free amino acids did not accumulate in quantities that could be demonstrated by the analytical

methods applied. The inference is clear that the synthesis of protein was proceeding with striking vigor and called upon the entire available supply of free amino acids. This result in turn correlates with the fact that these leaves, particularly those grown in light, were higher in protein nitrogen than the leaves of the starved plants.

The observations on the peptide nitrogen likewise agree with this general conclusion. In each case the peptide nitrogen was depressed in light, a result that would suggest a more rapid utilization of the peptides for protein synthesis in light than in darkness. The somewhat higher level of protein metabolism in the nitrate plants is reflected in the higher peptide nitrogen they contained. On the other hand, the peptide nitrogen of the leaves of the ammonia plants scarcely differed from that of the starved plants.

It should perhaps be emphasized that the present evidence is altogether in favor of a theory of protein metabolism that assumes that proteins arise in plant tissues from the condensation of amino acids and of peptides. This is the simplest and most straightforward view of the problem and is one that is widely, although not universally, held. The more recent investigations of Petrie and his collaborators in Australia [for a full review of the problem, see Petrie (17)], as well as of many others, point all but conclusively to the validity of this conception of the general mechanism of protein synthesis in plant tissues.

As in the case of the root tissue, the leaves of the plants grown on nitrate culture solution were greatly enriched in "other" nitrogen. The leaves of the plants grown in darkness contained slightly over 40 per cent of the soluble nitrogen in this group of substances, those grown in light 32 per cent. Although smaller in absolute amount in the starved plants and ammonia plants, the "other" nitrogen formed a higher proportion of the soluble nitrogen, being 41 and 56 per cent in the starved plants and 48 and 51 per cent, respectively, in the ammonia plants. These high proportions are evidence of the inadequacy of the analytical methods that are available for the examination of the soluble nitrogen of plant tissues; conclusions that rest upon the detailed composition of only somewhat over one-half of the soluble nitrogen are necessarily largely speculative. Nevertheless, it is clear from the discussion already given for the composition of the root tissue that an appreciable part of the "other" nitrogen is to some extent at least involved directly in the protein metabolism, inasmuch as it includes a part of the nitrogen of the basic amino acids and tryptophane as well as all of that of proline and hydroxyproline. Thus, the "other" nitrogen should be decreased under conditions which bring about a more active protein metabolism and this is seen to be the case.

One of the surprising results of the present study of the effect of nutrition with ammonium salts is the failure of the plants to accumulate more than traces of ammonium ion in their tissues. The contrast

with the behavior of the nitrate ion, which accumulated in substantial quantities especially in the roots, is marked. Narcissus does not behave at all like the tobacco plant. When grown upon culture solutions that provide a high proportion of the nitrogen in the form of ammonium ion, the tobacco plant becomes greatly enriched in ammonia nitrogen (50), and the storage of substantial amounts of ammonia nitrogen in such plants as the rhubarb (*Rheum*) (49), *Begonia* and *Oxalis* has long been recognized. Plant species thus differ widely in their capacity to accumulate ammonia nitrogen and attempts have been made, for example, to classify together such highly acid plants as those of the last three mentioned genera on the basis of their behavior with respect to the storage of ammonium ions (34). More recent investigation of the rhubarb plant has shown, however, (45) that, under certain not yet fully defined conditions, the leaves are able to accumulate relatively high concentrations of glutamine. Accordingly, although the capacity to store ammonia is undoubtedly high, this is not because of the absence of the enzyme systems required for the synthesis of an amide. The tobacco plant, in contrast, is rarely found to store substantial amounts of ammonia under normal cultural conditions. Enrichment of the leaves with the two amides is the more common result of the presence of ammonia in the tissues, especially when this substance arises from the decomposition of the protein during water culture of the detached leaves (47). Nevertheless, there is no essential incompatibility between the presence of ammonia and the reasonable welfare of the plant; ammonium ion is not of itself toxic to this species.

The narcissus plants in the present experiment, on the other hand, showed neither a marked tendency to accumulate either of the amides nor to store ammonium ion. They utilized the ammonium ion derived from the culture solution with moderate efficiency for the promotion of growth and the synthesis of protein. Ammonia nutrition was somewhat less effective than nitrate nutrition, but the behavior of the plants on ammonia was definitely superior to that of plants that obtained no extraneous nitrogen. The flexibility of the plant organism in its response to different conditions of nutrition is thus well illustrated, but far more detailed study than was attempted in the present case would be necessary before it could be demonstrated that the one or the other nutritive course possesses special advantages.

A word should perhaps be added on the behavior of the nitrate nitrogen in these plants. Traces of nitrate were detected in the roots of the starved plants and also in those of the ammonia plants; a trace was likewise found in the leaves of the LS plants. This nitrate could have arisen in either or both of two ways. Inasmuch as the plants were grown in sand, it is possible that infection of the sand with nitrifying bacteria took place so that towards the end of the growth period a little nitrate nitrogen was available to these plants in all cases. The procedure of flushing the pots with a large volume of water or of culture solution would minimize the effect of such an

event with the starved plants and the ammonia plants, and the observation that a trace of nitrate was present is not regarded as an evidence that any substantial error occurred in the technique, or that a blunder was made in the treatment of the plants. The sand employed was washed but was not bacteriologically sterile; accordingly, traces of nitrogen were present even in that used for the water controls. The water also, like most laboratory supplies of distilled water, likewise contained a chemically demonstrable trace of nitrogen so that even the starved plants must have been able to acquire a minute quantity. Under the circumstances, therefore, the presence of a trace of nitrate in all of the plants is perhaps not to be wondered at.

The other possible explanation is that the small quantity of nitrate nitrogen in the starved plants and the ammonia plants represents the existence of an oxidative mechanism in the tissues that gave rise to the formation of a detectable quantity of nitrate. On this view, the trace of nitrate in the starved plants was intrinsic nitrogen produced from some precursor originally present in the bulbs. The original bulbs gave no test whatever for nitrate so that the nitrate must have been produced during the culture period. This view is suggested because of the results of experiments with tobacco and with rhubarb leaves in culture in water in darkness (47, 49) which demonstrated that during the early course of the treatment a significant increase in the quantity of nitrate nitrogen took place. The observation has been confirmed upon several other species of plants by McKee and Lobb (10). More apropos to the present experiment is the observation of Pearsall and Billimoria (15) who obtained evidence for the production of detectable quantities of nitrate in narcissus leaves of another species when floated for 60 hours on an ammonium salt culture solution in darkness. Their experiment was carried out under conditions entirely different from those of the present one, the effect being obtained only in darkness. It demonstrated, however, the existence in the tissues of this genus of a mechanism capable of producing nitrate and, accordingly, the possibility of a similar origin for the trace of nitrate found chiefly in the roots of the starved plants and of the ammonia plants should not be overlooked.

The storage of nitrate in the tissues of the nitrate plants was substantial especially in the roots. A moderate quantity was also found in the bulbs and an appreciable amount in the leaves especially of the plants in light. Its presence is evidence that these plants were subsisting upon a luxury supply of nitrogen at the time of harvest since assimilation even in the leaves could not keep pace with absorption.

ORGANIC ACID METABOLISM

So far as the writers are aware, the present data are the first that have been obtained upon the composition, with respect to organic acids, of the tissues of a plant that grows from a bulb. Accordingly, it may be desirable to consider the order of magnitude of the results

in relation to the composition of better known plant tissues as a preliminary to the description of the details of the data. The analytical values in Table 6 are expressed in terms of milliequivalents of each acid in the original bulb tissue and in the roots, leaves and residual bulb tissue of the grown plants, the figures being calculated on the basis of 50 plants. The choice of this method of expression is dictated by the fact that a large proportion—more than half in most cases—of the organic acidity belongs to substances that have not yet been identified. The measurements of the total acidity are obtained in terms of a titration and, accordingly, can be expressed with accuracy only in terms of chemical equivalents since the ratio between the titration value and the weight of the organic acid is not known unless the identity of the acid has been ascertained. Calculation of the actual weight present of the individual known organic acids can be made, but there is no way to calculate that of the unknown acids unless an assumption is made as to the magnitude of the factor. It seems best, therefore, for the present, to express all of the results upon a similar basis. In order to obtain an approximate idea of the percentage composition, however, the weights of the individual acids in the original bulb tissue and in the leaves of the LNO_3 plants have been computed by the use of the appropriate equivalence factors, it being assumed that the unknown organic acids have substantially the same composition as citric acid. The original bulbs were thus found to contain, per 50 bulbs, 3.32 gm. of oxalic acid, 4.45 gm. of citric acid, 1.33 gm. of malic acid, 0.21 gm. of succinic acid, and 11.2 gm. of unknown acids making in all 20.5 gm. of total organic acids. The sum is 0.11 per cent of the fresh weight of the bulb tissue and 3.1 per cent of the organic solids. Accordingly, this tissue is definitely low in organic acids. The leaves of the LNO_3 plants, which were the richest in organic acids of any of the tissues examined, contained 0.46 gm. of oxalic acid, 0.70 gm. of citric acid, 3.54 gm. of malic acid, 0.41 gm. of succinic acid, and 5.44 gm. of unknown organic acids, in all 10.5 gm. This is 0.40 per cent of the fresh weight and 5.8 per cent of the organic solids. Contrasted with the leaves of the tobacco and the rhubarb plants, for example, which may contain from 20 to 30 per cent of the organic solids in the form of organic acids, it is clear that the leaf of the narcissus plant is, like the bulb, definitely low in its content of organic acids.

Unfortunately, sufficient detailed data are not yet available to provide for adequate comparison among different species of plants. Tobacco leaves (47) and rhubarb leaves (49) have been investigated somewhat thoroughly in this laboratory, and a briefer study has been made of the buckwheat (30) and of *Bryophyllum* leaves (19, 24). Pierce and Appleman (18) have furnished data for the organic acids of twelve common agricultural species and Wood (51) has recently described the composition of a number of samples of Sudan grass with respect to oxalic, citric and malic acids. Consideration of all of this information suggests that, with few exceptions, the leaves of mono-

TABLE 6. ORGANIC ACIDS OF NARCISSUS PLANTS

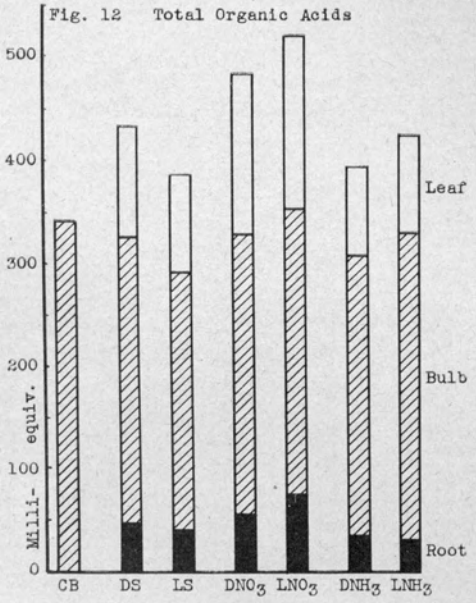
Figures not otherwise designated are milliequivalents calculated on the basis of the tissues from 50 bulbs

		CB	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
Total organic acids,	leaf		107	97	154	166	88	94
	bulb	342	280	252	273	280	274	301
	root		46	38	55	73	33	29
	total		433	387	482	519	395	424
Total organic acids, (m. e. per gm. of organic solids)	leaf		0.686	0.724	0.747	0.917	0.642	0.854
	bulb	0.520	0.635	0.553	0.954	0.856	0.683	0.630
	root		1.48	1.19	1.41	1.55	1.14	0.763
	whole plant		0.689	0.622	0.907	0.935	0.697	0.677
Oxalic acid,	leaf		6.6	9.2	6.5	10.2	6.3	7.1
	bulb	73.8	55.1	67.3	71.6	77.1	66.2	61.6
	root		6.2	10.5	16.9	19.8	10.8	6.7
	total		68	87	95	107	83	75
Citric acid,	leaf		4.7	3.5	7.2	10.9	2.7	3.1
	bulb	69.6	24.9	19.9	16.9	17.2	23.7	37.4
	root		1.6	1.3	2.9	3.4	0.8	0.8
	total		31	25	27	32	27	41
Malic acid,	leaf		22.9	10.6	40.2	52.8	11.8	7.4
	bulb	19.9	34.0	25.0	29.3	25.0	19.7	24.7
	root		14.5	7.9	6.4	11.3	0.6	0.9
	total		71	44	76	89	32	33
Succinic acid,	leaf		3.3	1.3	3.6	6.9	3.3	7.4
	bulb	3.6	2.4	5.0	3.2	3.0	3.0	9.5
	root		0.5	0.5	0.9	1.4	2.3	2.1
	total		6.2	6.8	7.7	11.3	8.6	19.0
Unknown organic acids,	leaf		69	72	96	85	64	69
	bulb	175.5	164	135	152	158	162	167
	root		24	18	28	37	19	18
	total		257	225	276	280	245	254
Unknown organic acids as per cent of total organic acids,	leaf		64.5	74.2	62.3	51.7	72.7	73.4
	bulb	51.1	58.6	53.6	55.7	56.4	59.1	55.5
	root		52.1	47.3	50.9	50.7	57.5	62.0

cotyledonous plants are characterized by a low content of organic acids as compared with dicotyledons. This conclusion is, however, by no means certain at the present time in view of the paucity of available information.

The data for the total organic acids are shown in Table 6 and plotted in Figure 12, the quantities in the three main tissues of the

plants being indicated. Synthesis of organic acids took place, this being especially marked in the plants grown upon nitrate culture solution. The decrease in total organic acids in the bulbs suggests that transport of moderate quantities occurred during the growth process, although this is by no means certain; the observations could be equally well accounted for on the assumption that a small proportion of the organic acids of the bulbs was metabolized to non-acidic substances and that the organic acids present in the leaves and roots



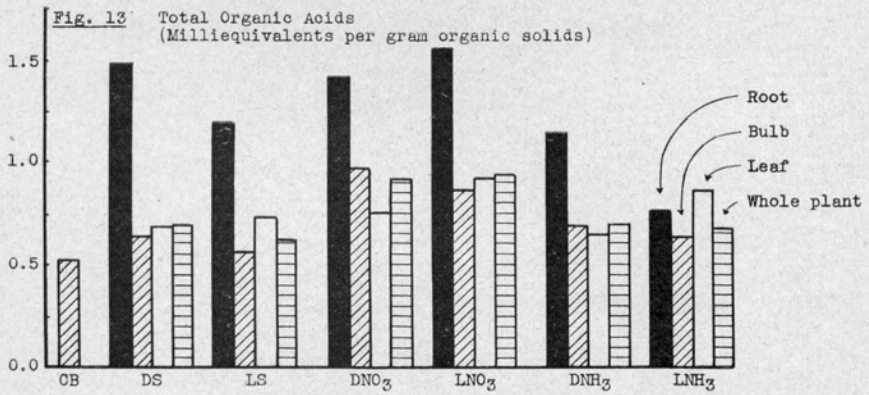
represent newly synthesized material. In the absence of experiments with tracer isotopes, the relative importance of transport and of new synthesis cannot be demonstrated.

The effect of growth in light as compared with growth in darkness differed in the three sets of plants. The starved plants in light contained less organic acid than those in darkness, but, in both the nitrate plants and the ammonia plants, growth in light increased the quantity of organic acids. The effect of the availability of nitrate nitrogen was to increase the quantity of organic acids whether in light or in darkness as compared with the starved plants, but the effect of ammonium salt nutrition was small. The DNH_3 plants contained slightly less acid than the DS plants while the LNH_3 plants contained slightly more than the LS plants.

In order to obtain figures that would indicate the concentration, as distinct from the actual quantity, of the organic acids in the tissues, a calculation was made of the number of milliequivalents of acid per gram of organic solids. The results are shown in Table 6 and are

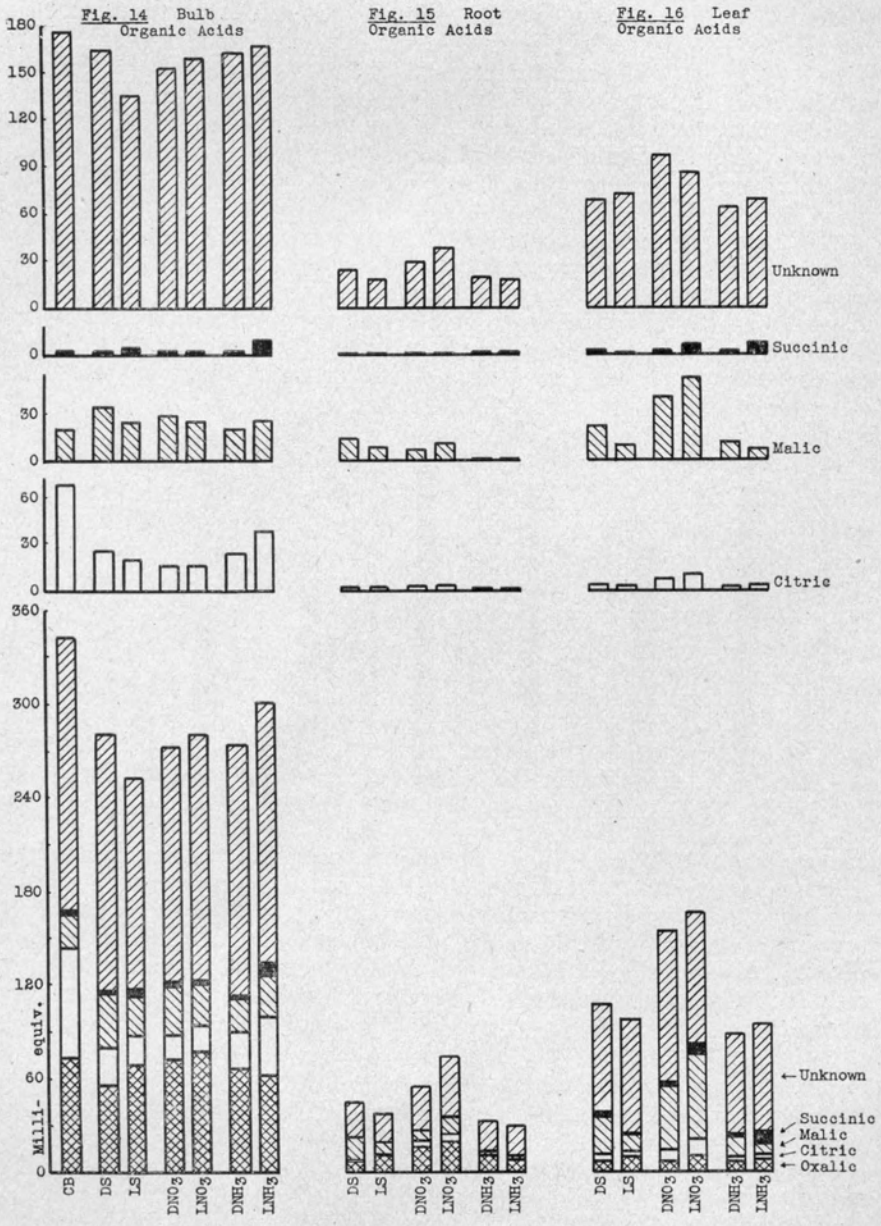
plotted in Figure 13. On this basis, the root tissue stands out as the richest in organic acids in all cases save the LNH_3 plants. The roots of the starved plants contained about twice as great a concentration of acid as the bulb or leaf tissue while in the nitrate plants the difference was almost as great. The ammonia plants grown in darkness also show a similar effect although to a less pronounced degree, but those grown in light do not show it at all.

Figure 13 indicates that the other tissues of the plants contained rather closely similar concentrations of organic acids; all were somewhat richer than were the original bulbs. The effect of culture upon nitrate solution is well shown by the increased concentration of organic acids in the tissues of these plants. Detailed examination of the diagram shows that growth in light brought about an increased



concentration of organic acids in the leaves in all cases although the difference was small. On the other hand, the concentration in the residual bulb tissue was slightly decreased by growth in light. Culture upon ammonium salt had a much smaller effect than culture on nitrate; in fact the plants grown in darkness on ammonia differed only a little from the starved plants in darkness save in the root tissue.

The detailed data for the composition of the plants with respect to individual organic acids have been plotted in Figures 14, 15 and 16, which show respectively the data for the bulb, root and leaf tissues. In the lower part of the diagrams, the block that shows the quantity of total organic acids is sub-divided so as to show the relative quantities of oxalic, citric, malic, succinic, and unknown organic acids present. In the upper part, separate blocks are plotted to facilitate comparison of the quantities of citric, malic, succinic, and unknown acids. Oxalic acid is shown at the bottom of the block in the lower part of the diagram where comparison of the relative quantities can be made.



The most striking features of the diagram are the large proportion of the total organic acids of the plants in the residual bulb tissue and the high proportion of unknown organic acids in all parts of the plant. It is clear from Figure 14 that only a relatively small part of

the acids in the original bulb disappeared during the process of growth, and whether the diminution represents transport from the bulb to the growing tissues or utilization within the bulb, possibly due to respiration, or both, is by no means obvious. The greatest loss from the bulb occurred during the development of the starved plants in light, the least during the growth of the ammonia plants in light, but the differences were not great, and the other four treatments resulted in no clearly significant difference in the effects upon the total acids of the bulb tissues. Reference to Figure 3 shows that there is no obvious correlation between the ash content and the behavior of the total organic acids of the bulbs as might be anticipated if the diminution of acid in the bulb represented migration in the form of salts of inorganic bases into the growing tissue. On the other hand it is unlikely that the magnitude of the changes in organic acid is sufficiently great to be clearly evident in the ash data in view of the errors that affect these latter figures owing to the absorption of inorganic material from the sand in which the plants were grown.

The quantity of oxalic acid in the original bulbs was diminished after the growth of the starved plants, somewhat more in darkness than in light; there was no significant effect upon this component in the bulbs of the nitrate plants and only a small diminution in the bulbs of the ammonia plants, the change in this case being somewhat greater in light than in darkness. The impression is given that oxalic acid is a sluggish metabolite under any conditions. Figures 15 and 16 show that small amounts of oxalic acid were formed in both root and leaf tissue so that, in general, there was more oxalic acid present after growth of the plants than there was at the start. No interpretation of this result can be suggested in view of our ignorance of the metabolic role of oxalic acid. There is an indication, however, that the oxidation reactions which presumably produced oxalic acid were especially vigorous in the roots of the plants grown on nitrates.

Citric acid, unlike oxalic acid, is known to be an active metabolite in plant tissues and the present case is no exception. There were approximately 70 milliequivalents of citric acid in the original bulbs but the plants that were produced from them contained only from 25 to 31 milliequivalents save in the single case of the LNH_3 plants which contained 41 milliequivalents. Thus, about one-half of the original citric acid disappeared from the system during the growth process. This is, however, a superficial view of the results. There is little doubt that citric acid is both utilized and synthesized so that the observed effects are net results and by no means necessarily represent the total metabolism of citric acid in the plants. Figure 14 shows that a considerable part of the total citric acid in the plants at the time of harvest was located in the bulb tissue and that the effect of growth upon the citric acid in the bulb was greatest in the nitrate plants and least in the ammonia plants, particularly in the LNH_3 culture. The total amount of citric acid in the root tissue (Figure 15) was very small in all cases, although appreciable in the roots of

the nitrate plants. In the leaves (Figure 16), the citric acid was also highest in the nitrate plants while there was only a trace present in the leaves of the ammonia plants.

Malic acid, like citric acid, is also known to be an active metabolite in plant tissues and the data in Figures 14, 15 and 16 show that this substance underwent marked changes during the development of the plants. It was far more responsive to the conditions of the culture than any of the other acid components. The malic acid content of the bulb tissue increased during the growth of the plants in every instance. This observation furnishes direct evidence for synthesis and proof that the bulb is something more than merely a storehouse from which the growing tissues abstract the substances needed for their development. The increase was greatest in the DS bulbs and was only slightly less in the DNO_3 bulbs. In the ammonia plants, the change in the bulbs of the plants in darkness was too small to be significant but there was a definite increase in the bulbs of the LNH_3 plants. Thus, the effect of light on the plants cultured on ammonium salts was the reverse of the effect upon those grown on nitrates.

The quantities of malic acid in the root tissue were small but differed according to the treatment. The roots of the starved plants contained the largest quantity of malic acid, there being more in the DS roots than in the LS roots. On the other hand, the LNO_3 roots contained more malic acid than the DNO_3 roots. Only traces of malic acid were present in the roots of the ammonia plants.

The leaves showed the greatest effect of the different treatments of the plants. The leaves of the starved plants grown in darkness contained somewhat more than twice as much malic acid as the corresponding leaves grown in light. Culture on nitrate led to a marked enrichment of the leaves in malic acid, and this was more pronounced in light than in darkness. The LNO_3 leaves in fact contained almost one-third (32 per cent) of the organic acidity in the form of malic acid, a higher relative proportion than was the case for any other of the tissues. This result recalls the observations of Clark (4) on the tomato plant as well as the effects of nitrate and ammonium salt nutrition upon tobacco (50). In both of these species, culture on nitrate led to enrichment of the tissues, especially the leaves, in organic acids as compared with similar plants cultured on ammonium salts, and malic acid showed the effect to a more pronounced extent than any of the other organic acid components. The suggestion that malic acid is in some way concerned with the metabolism of the nitrate ion, possibly with its reduction, is difficult to escape, and the comparative data in the present case are especially striking inasmuch as the leaves of the ammonia plants were lower in malic acid even than the starved plants.

The present data on the succinic acid content of the tissues is, so far as the writers are aware, the first that have been accumulated

which show the effect of different cultural treatments on the metabolism of succinic acid. This substance is probably widely, if not universally, distributed in plant tissues but is rarely present in substantial quantities. It has been shown to be an active metabolite in the tobacco plant (23), being synthesized in all of the tissues examined during the course of the development of the plant, but, in general, it is a minor organic acid component; a survey of a number of species showed that it makes up as a rule from 0.2 to 0.5 per cent of the dry weight of the tissues examined. The present case is similar. The largest quantity of succinic acid was found in the bulb tissue of the LNH_3 plants; these contained 0.56 gm. in a total weight of 478 gm. of organic solids, or 0.11 per cent. The richest tissue was that of the leaves of the nitrate plants grown in light; these contained 0.40 gm. in 181 gm. of organic solids or 0.22 per cent.

The significance of the small differences in the quantities of succinic acid that were observed in the several tissues is somewhat problematical in view of the presumed high reactivity of this substance in the general metabolic scheme of the plant. However, the data suggest that the bulb tissues of the starved plants and of the ammonia plants grown in light contained a slightly larger amount than the bulbs of the plants grown in darkness. There was no difference in the bulbs of the nitrate plants. The quantities found in the roots were too small to draw any conclusions from save that growth in ammonium salt solution appears to have promoted the synthesis of a little more succinic acid than growth in nitrates. In the leaves, however, there is a clear effect of growth in light on both nitrate and ammonium salt solutions which led to an increase in succinic acid, although the reverse was the case in the leaves of the starved plants. Perhaps the most important conclusion from the data is that the behavior of the succinic acid does not follow in detail that of any of the other organic acids, a conclusion that emphasizes the desirability of the collection of as complete information as possible on the organic acid composition of plant tissues under investigation.

The problem presented by the large proportion of unknown organic acids in this plant is a difficult one. There is no information available upon which to establish a reasonable hypothesis regarding the identity of the unknown organic acid or acids of these tissues, but the fact that unknown components make up a fairly constant proportion of the acids of the bulb tissue, the figures ranging from 51 per cent of the total organic acids in the original bulbs to 59 per cent in the residual bulb tissue of the DNH_3 plants, suggests that a large part of the unknown acidity may consist of some single acid component. If this is so, the hypothetical component may well be the predominant organic acid of the bulb tissue. Examination of the data in Table 6 on the percentage of organic acids in terms of the total acidity indicates that the same is true both for the root and especially for the leaf tissues. We are thus faced in the present case with a situation in which the identity of what may be quantitatively

the most important organic acid of the plant is unknown. Speculation at the present time is doubtless premature, but recent experience in this laboratory with the organic acids of the *Bryophyllum* plant (19, 24), in which a similar situation was found to obtain, raises the interesting question whether or not isocitric acid may be present in substantial relative proportions in the tissues of the narcissus plant as well. No evidence one way or the other has been secured and none can be had at the present stage of the development of the analytical chemistry of these substances until an examination of the organic acids of this plant has been made by means of the classical ester distillation method, this being the only technique available whereby malic, citric and isocitric acid can be separated for identification. Should this speculation turn out to be justified, however, the leaves of the narcissus plant may well become an important and convenient source of optically active isocitric acid, since this plant can be more readily and quickly grown in substantial quantities than the *Bryophyllum* plant. Even the bulbs should prove interesting in this connection.

With respect to the metabolism of the unknown organic acids, it is evident from the data in Table 6, for the total organic acids and for the total amount of unknown organic acids, that the greater part of the net increase in organic acids in the plants as they grew from the bulbs was due to the synthesis of unknown acids. Thus, for the starved plants, of the net increase, respectively, of 91 and 45 milliequivalents of total acidity, 90 per cent in each case arose from the increase in the unknown acids. For the nitrate plants, the analogous figures were 72 and 59 per cent while, for the ammonia plants, the figures were 132 and 96 per cent. The high figure in the DNH_3 plants is a result of the sharp drop in citric acid which was not compensated by a corresponding rise in malic acid. The point is that, considered as a whole, the increase in the quantity of the unknown acid or acids as the plants grew was by far the greatest net change that occurred. Thus, the unknown acid or acids must be regarded as quantitatively the most active of the organic acid metabolites.

The details of the changes are shown at the top of Figures 14, 15 and 16. With the possible exception of the starved plants, the differences in the quantities that resulted from the effect of light are relatively small even in the leaf tissue. Culture upon nitrates stimulated the production of unknown organic acid in both root and leaf tissue as compared either with the starved plants or with the ammonia plants, although in the leaves the effect was proportionately less striking than it was upon the malic acid. Culture upon ammonium salts slightly depressed the quantity of unknown organic acid in the leaves as compared with the starved plants, the effect being greater in light than in darkness. There was only a small and probably insignificant similar effect upon the unknown acids of the root tissue. Thus, the unknown organic acids, although quantitatively the most important acid components and although they underwent greater changes in absolute amount during growth of the plants under the

different conditions of treatment than any of the known acid components, were relatively less affected by these differences of treatment than was malic acid. It must be remembered, however, that the unknown organic acids were determined by difference in each case, and, accordingly, the actual quantity in each tissue is in doubt by the sum of the analytical errors that affect the determination of each of the four known acid components. For this reason no emphasis has been placed upon small differences in the amounts recorded. For example, it seems probable that the leaves of the LS plants actually contained more unknown acid than those of the plants grown in darkness. The difference in the case of the leaves of the ammonia plants is doubtless too small to be significant. It seems clear, however, that the unknown organic acid components play a large and important part in the general organic acid metabolism of this species of plant.

CARBOHYDRATES

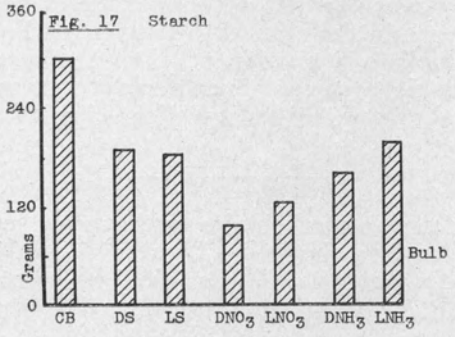
The several forms of carbohydrates that were analytically determined in the tissues are shown in Figures 17 to 23, the individual data being given in Table 7. The original bulbs contained 301 gm.

TABLE 7. CARBOHYDRATES OF NARCISSUS PLANTS

Figures not otherwise designated are grams calculated on the basis of the tissues from 50 bulbs.

		CB	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
Starch,	leaf		0.8	0.4	0.8	0.4	1.6	0.5
	bulb	301.3	190	184	96.7	127	162	201
	root		0.05	0.07	0.04	0.11	0.08	0.09
	total		191	184.4	97.5	127.5	164	201.6
Total soluble carbohydrate,	leaf		44.9	35.0	56.2	32.2	35.7	18.9
	bulb	65.8	69.1	65.5	51.7	49.2	54.7	54.8
	root		4.8	6.1	1.3	2.9	1.6	2.8
	total		118.8	106.6	109.2	84.3	92.0	76.5
Total soluble carbohydrate as per cent of organic solids,	leaf		28.6	26.2	27.2	17.8	26.1	17.3
	bulb	10.0	15.7	14.4	18.0	15.0	13.6	11.4
	root		15.4	18.9	3.3	6.1	5.5	7.4
	total		18.9	17.1	20.5	15.2	16.2	12.2
Sucrose,	leaf		12.5	10.5	16.3	11.2	12.8	7.8
	bulb	58.2	50.2	46.2	33.6	33.1	38.2	40.7
	root		1.8	2.1	0.8	1.4	1.0	1.7
	total		64.5	58.8	50.7	45.7	52.0	50.2
Glucose,	leaf		28.3	20.2	29.8	14.9	15.6	3.5
	bulb	3.1	15.8	14.6	15.8	14.0	12.9	11.3
	root		2.5	3.4	0.3	1.0	0.4	0.8
	total		46.6	38.2	45.9	29.9	28.9	15.6
Unfermentable carbohydrate,	leaf		4.1	4.3	10.1	6.1	7.3	7.8
	bulb	4.4	3.1	4.8	2.3	2.1	3.6	2.8
	root		0.4	0.5	0.2	0.5	0.2	0.3
	total		7.6	9.6	12.6	8.7	11.1	10.9

of starch as determined colorimetrically with potato starch as standard. This is 45.8 per cent of the total organic solids of the bulb tissue, and, together with the 65.8 gm. of soluble carbohydrates accounts for 55.7 per cent of these solids. The bulbs are therefore richly supplied with carbohydrates both in the form of soluble sugars and as starch. The way in which the stores of starch in the bulbs were drawn upon during the growth of the plants is shown in Figure 17. The data plotted refer only to the bulb since the quantities of



starch in either the root or the leaf tissues were too small to be represented on the scale of the diagram (see Table 7). The starved plants supplied only with water utilized about one-third of the starch stored in the bulbs, there being no great difference attributable to the effect of light. The nitrate plants, on the other hand, required more than two-thirds of the starch of the bulbs for development in darkness but used appreciably less in light. The ammonia plants grown in darkness utilized a little less than one-half of the starch in the original bulbs but those grown in light drew upon even less than did the starved plants.

It is of interest to compare the utilization of the starch in the bulbs of these plants with that of the protein, which is one of the other important storage substances. Table 8 shows calculations of the percentages of the protein and starch that disappeared from the bulb during the growth of the plants. The starved plants made far greater relative demands upon the protein than upon the starch, but the nitrate plants utilized a higher proportion of the starch than of the protein. The ammonia plants, however, required approximately equal

TABLE 8. RELATIVE PROPORTIONS, EXPRESSED AS PERCENTAGES, OF THE PROTEIN AND OF THE STARCH OF THE ORIGINAL BULB TISSUE UTILIZED DURING THE GROWTH OF THE PLANT

	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
Protein	62.2	54.5	55.9	45.9	43.6	34.8
Starch	36.9	38.9	67.9	57.8	46.2	33.2

proportions of the protein and of the starch of the bulbs during their development, these proportions being greater in darkness than in light. The nitrate and the ammonia plants had an outside source of nitrogen available, and reference to Figure 7 shows that, under these circumstances, the drain on the protein of the bulbs was diminished. Furthermore, less nitrogen was withdrawn from the bulb by the plants grown in light than by the plants grown in darkness. Similarly, the plants grown in light had an outside source of carbohydrate inasmuch as photosynthesis was possible, and this may account to some extent for the difference shown in Figure 17 in the quantities of bulb starch utilized by the nitrate and the ammonia plants. These comparisons are suggestive, but it must be remembered that there was a large difference in the actual quantities of protein and of starch utilized. For example, the starved plants used 111 and 117 gm., respectively, of starch derived from the bulbs; they likewise used 3.40 and 2.98 gm. of protein nitrogen as computed from the loss of protein nitrogen from the bulb. This is the equivalent of 21.3 and 18.6 gm. of protein if the conventional factor 6.25 is used to convert protein nitrogen to weight of protein. Thus, the utilization of starch was from five to six times as great as the utilization of protein in these two sets of plants. The nitrate plants grown in darkness, which drew remarkably heavily upon the starch in the bulbs, required about ten times as much starch as protein during their development.

A comparison of the losses of starch from the bulbs with the losses of organic solids during the growth period is also instructive. The pertinent data are shown in Table 9, and suggest that a nearly constant proportion of the organic solids that disappeared from the bulbs consisted of starch, this being true regardless of the conditions under which the plants were grown. The average is 54.5 ± 2.4 per

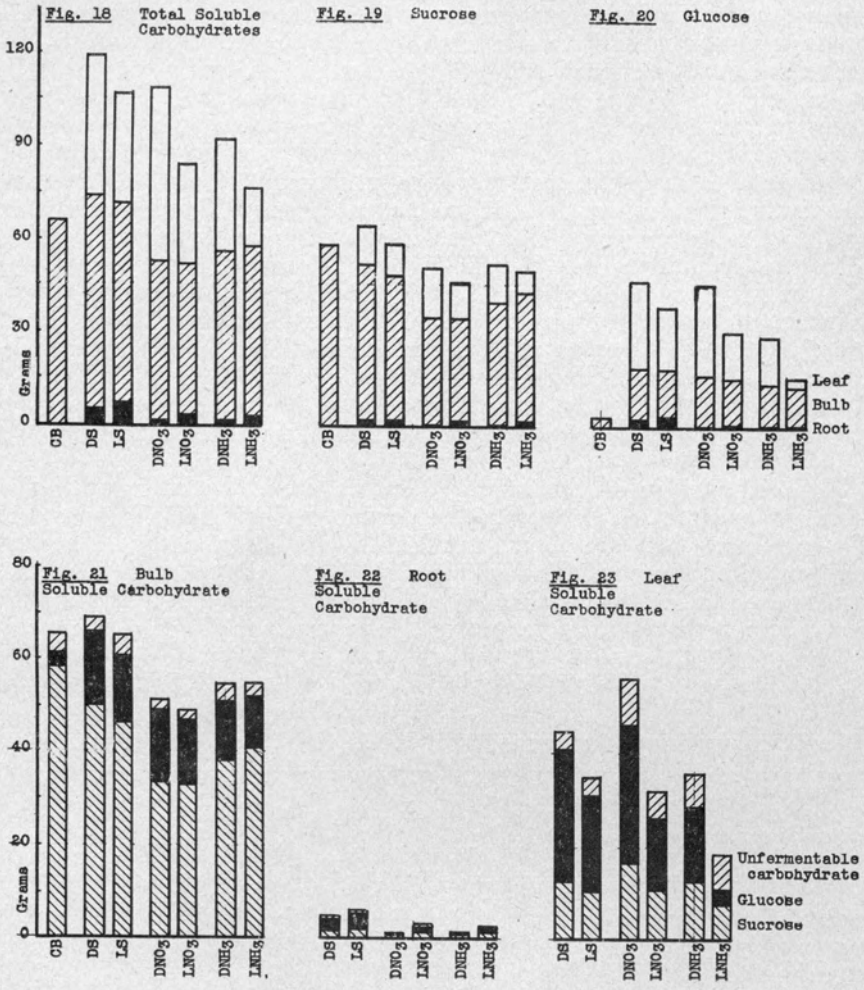
TABLE 9. LOSSES OF ORGANIC SOLIDS AND OF STARCH FROM 50 BULBS DURING GROWTH OF THE PLANTS

	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
Organic solids (gm.)	217	202	372	331	257	180
Starch (gm.)	111	117	205	175	139	100
Starch as per cent of solids	51	58	55	53	54	56

cent of the organic solids and is valid within the limits shown over a range of starch utilization of from 100 to 205 gm. in the different experiments.

Clearly, the metabolism of the starch of the bulb is one of the major events, in terms of actual quantities involved, that took place during the growth of the plants. It may be assumed that the transfer of starch from the bulb took place after its conversion to a soluble sugar, presumably glucose, and that this was used in part in the respiration and in part for the synthesis of new tissue components.

At all events, the presence of appreciable quantities of soluble carbohydrates in the tissues of the plants, whether grown in light or in darkness, is to be expected and the data in Table 7, which are plotted in Figures 18, 19 and 20, show this to be the case. It is a striking fact that the plants grown in darkness under any of the three conditions of culture contained more soluble carbohydrates than the correspond-



ing plants grown in light, and this is evidence that photosynthesis played a minor role in comparison with the results of the starch metabolism. The generalization is true not only for the total soluble carbohydrates but also for the individual components sucrose and glucose, and, within the limits of error and with one possible exception, also for the small quantities of unfermentable carbohydrate

that were present. This is shown by the details plotted in Figures 21, 22 and 23.

Figure 18, which shows the total soluble carbohydrates in the separate tissues as well as in the whole plants, indicates that the starved plants were richer in sugars than the nitrate plants and these, in turn, than the ammonia plants. In view of the large quantities of sugars that must have been produced in the bulbs owing to the digestion of the starch, it is perhaps surprising that the amounts of soluble carbohydrate in the residual bulb tissue were as small as they were and were so little affected by the conditions of the culture. The original bulbs contained nearly 66 gm. of sugar, mostly sucrose, while the residual bulb tissue after the growth of the plants contained practically the same amount in the starved plants and the bulbs of the nitrate and ammonia plants contained only from 10 to 15 gm. less, the quantities varying from 49 to 55 gm. The demands of the growing tissues upon the soluble sugar present originally in the bulb and that produced by the digestion of the starch did not result in any great depletion of the soluble sugars in the residual bulb tissue. On the contrary, the concentration of the sugars in terms of organic solids of the bulbs increased somewhat as a result of the growth process as is shown by the percentage data in Table 7, the increase being the greatest in the DNO_3 bulbs which, it will be recalled, were the ones in which the greatest quantity of starch was digested.

It is natural to assume that a part of the carbohydrate that disappeared from the plants during the growth process was utilized for respiration. No experimental evidence can be offered on this point; the respiration of the plants was not measured directly, and the information that was obtained regarding it is chiefly that shown in Figure 2 on the losses of organic solids from the tissues. Data on actual carbon losses from the plants are presented in the next section. The losses of organic solids from the whole plants grown in darkness, where no complication from possible photosynthesis arises, amounted to 30 gm. in the DS plants, 127 gm. from the DNO_3 plants and 91 gm. from the DNH_3 plants. These quantities may be compared with the sums of the losses of starch and soluble carbohydrate from the same plants which were, respectively, 58, 162 and 106 gm. Thus, the quantities involved are such that it is possible that the whole of the supposed respiration loss from the plants grown in darkness may have resulted from the metabolism of the sugars and starch;[1] there is, moreover, a moderate degree of parallelism in the quantities involved in the three separate cases. This is scarcely evidence, however, that the respiration in these plants drew exclusively upon the sugars and starch throughout the period of growth, although such a conclusion is not in conflict with the observations on the carbohydrates.

[1] Notwithstanding this observation, it will be shown in the next section that the average carbon content of the organic solids that disappeared from the plants grown in darkness was appreciably lower than would be the case if the main metabolite were starch or sugar.

The fact that the quantities of soluble carbohydrates in the leaves of the plants grown in light were notably less than those in the corresponding plants grown in darkness suggests that, if the experiments were indeed comparable, more rapid use was made of the sugars in the illuminated leaves. Inquiry into the details of the fate of this sugar is hardly justified, when it is recalled that what is measured is in effect a net quantity which represents the difference between the carbohydrate supplied by the bulb and the quantity of reducing sugar that arose from photosynthesis. The total carbohydrate metabolism in the leaves of the plants grown in light was thus doubtless considerably greater than is represented by the difference between the amounts found respectively in the light and the dark plants since the equivalent of any newly formed sugar disappeared along with a part of that transported from the bulb. One interesting parallelism may, however, be pointed out. Among the substances that can be recognized analytically in the leaves and which were conspicuously increased by growth in light were the proteins (see Figure 7). The relative enrichment of the leaves in protein thus runs in the same direction as the total carbohydrate metabolism and suggests that the two phenomena are an expression of related biological events. From the chemical point of view, the determination of protein furnishes at least a rough measure of the quantity of protoplasm, together with its inclusions, since it represents essentially the sum of the chloroplastic and the cytoplastic proteins of the leaf cells. It measures, therefore, something which is related to the success of the organism as an individual, the more successful plant being assumed to be the one that has become the more enriched in living protoplasm. That the synthesis of protein took place at the expense of sugar is not implied; the point is that those leaves which synthesized more protein had available more energy derived from the metabolism of the carbohydrates.

The effects of the conditions of culture upon the total soluble sugars present in the tissues are perhaps not those that would be anticipated. The starved plants were conspicuously enriched as compared with the nitrate and the ammonia plants, and the nitrate plants were notably higher in sugars than the ammonia plants. The presence of soluble sugar *per se* in large quantities is, therefore, not necessarily a driving force that serves directly to stimulate protein synthesis, other factors being necessary as well.

In Figure 19 are plotted the quantities of sucrose in the tissues, and attention is immediately attracted by the fact that the quantity in the bulbs of the starved plants is only slightly less than that in the original bulb tissue. Whether this means that sucrose is only sluggishly metabolized in this species is by no means clear. It is difficult to imagine a mechanism in the growing bulbs that would allow more than 110 gm. of carbohydrate derived from the hydrolysis of the starch to be transported out of the bulbs into the growing leaves and

roots and at the same time prevent any substantial movement of sucrose. Yet the residual bulbs of the starved plants contained respectively only 8 and 12 gm. less sucrose than the original bulbs. Equally puzzling is the behavior of the bulbs of the nitrate plants. These lost about 25 gm. of sucrose each in the two experiments but at the same time lost 204 and 174 gm. of starch respectively in darkness and in light, this being presumably for the most part transported out of the bulbs as glucose. Figure 20 shows that the glucose content of the bulb tissue did indeed increase during the growth of the plants. The original bulbs contained only about 3 gm. whereas the residual bulbs after growth had occurred contained from 11 to 16 gm. in the different experiments. Thus, although the glucose content of the bulb tissues increased proportionately very much indeed, that is by a factor of from 4 to 5, the increase in terms of quantity was almost negligible in comparison with the large amounts of starch that were metabolized and removed from the bulbs. The glucose data, in combination with that for the starch, suggest an extremely rapid and effective mechanism for the transport of glucose from the bulb into the growing parts; the sucrose data, on the other hand, imply a behavior that was slow and relatively ineffective.

A suggestion that may account in part for these observations is that the sucrose found in the residual bulb tissue is not identical with that originally present but represents material brought down from the leaves for storage. This implies that the bulb, even at a fairly early period of the development of the plant, is already in a position to resume its function as a storage organ, a view which involves the assumption of a downward stream of metabolites at a time when the main function of the bulbs is undoubtedly to supply an upward stream. Neither view of the situation is entirely satisfactory, and the dilemma furnishes an excellent example of the difficulty of interpretation in the absence of serial experiments.

More detailed information on the behavior of sucrose during the growth of the narcissus plant is fortunately available in the work of Nightingale and Robbins (14). Their observations refer to a different species (*Polyanthus narcissus*) but include determinations of the sucrose at several stages of growth. Their sample of original bulbs weighed, per 50 bulbs, 980 gm. and contained 687 gm. of starch and dextrin and 63 gm. of sucrose. Their bulbs were, therefore, considerably heavier than the bulbs used in the present experiment. During the first 13 days of growth of the plants supplied with nitrate in the dark, the storage tissue lost all but 8.5 gm. of sucrose while the centers and tops had acquired 4.4 gm. At 20 days, the storage tissue contained 19 gm. of sucrose, the tops, 8.8 gm., while at 27 days the storage tissue contained 14 gm., the tops, 9 gm. The behavior of the plants grown in darkness without nitrate was closely similar and it is clear that, with their material, sucrose in the storage tissue was drawn upon early in the growth process and was extensively utilized. A little later, there was a suggestion that a moderate quantity of sucrose

was restored to the bulb tissue and the subsequent changes were not great. Parallel with these changes, however, there was a utilization of starch fully as extensive as that observed in the present experiments. Accordingly, the observation that sucrose was present in the bulbs of the DS and LS plants at the end of 28 days in an amount nearly equal to that in the original bulbs may well be the result of a storage phenomenon if these plants behaved in a manner similar to those of Nightingale and Robbins.

Still another explanation of the behavior of the sucrose may be offered. The maintenance of a relatively constant quantity of sucrose in the bulbs in spite of the rapid mobilization of the starch in the form of glucose may represent direct synthesis of sucrose in the bulb tissue at a rate commensurate with the rate of withdrawal. Choice among these alternatives must await more complete and detailed studies.

Inasmuch as glucose is a metabolite that undergoes rapid and extensive changes in plants, too much significance cannot be attached to the moderate differences in the quantities of glucose in the plants at the end of the experimental period. The unusually small amount in the ammonia plants grown in light, particularly in the leaves, is, however, striking and suggests that the metabolic processes in these plants were drawing heavily upon the supply of sugar immediately preceding the time of harvest. Comparison of the LNH_3 plants with the LS plants which had metabolized substantially the same amount of starch in the bulbs is interesting since the LS plants contained six times as much glucose in the leaves as the LNH_3 plants.

In Figures 21, 22 and 23, the same data are plotted so as to show the distribution of the soluble carbohydrates in the separate tissues. Sucrose was the predominant sugar in the bulbs in all cases and glucose in the leaves in all save the LNH_3 plants. The roots contained very little soluble carbohydrate, the starved plants, being, however, the richest in these components. There is an exception to the rule that the plants grown in darkness contained more of each sugar component than the plants grown in light since the bulbs of the LNH_3 plants contained somewhat more sucrose than the bulbs of the DNH_3 plants. The rule does not apply to the root tissue either since here the plants grown in light contained a little more than those grown in the dark.

The component designated unfermentable carbohydrate represents the analytical fact that the reducing power is not completely abolished after treatment of the solutions with yeast. There is little information available in the literature on the chemical nature of this type of component; dilute acid hydrolysis after enzymatic inversion gave essentially similar results for the quantity of unfermentable carbohydrate, so that the inference could be drawn that the substance involved was not sedoheptose, nor were fructosans present in significant amounts. Nevertheless, the component was found in appreciable

amounts in all of the tissues, traces being detected even in the roots. In the leaves of the LNH_3 plants, this material exceeded glucose in quantity and even more was found in the leaves of the DNO_3 plants.

Substances of analogous chemical behavior are present in tobacco leaves (47, 50) and in rhubarb leaves (49), and in these tissues have been found to play an important role in the general carbohydrate metabolism. Schlenker has detected material of similar properties in leaves of a number of other species (35). The only indication that this component is to any great extent influenced by the conditions under which the plants were grown is the fact that the leaves of the DNO_3 plants were markedly enriched in it. However, these leaves were the highest in both sucrose and glucose, and their general carbohydrate metabolism was obviously stimulated as compared with the other plants.

CARBON CONTENT OF NARCISSUS PLANT TISSUES

In order to throw additional light upon the transformations that occurred during the development of the plants, the carbon content of the dried tissues was determined as well as that of the tissue residues that remained insoluble after thorough extraction with alcohol and ether. The weight of carbon in soluble components could then be obtained by difference. The data are shown in Table 10 and are plotted in Figures 24, 25 and 26.

In each case, there was a net loss of carbon from the plants when the carbon of the original bulb is compared with the sum of the carbon contents of the root, residual bulb and leaf tissues. The loss from the

TABLE 10. CARBON OF NARCISSUS PLANT TISSUES

Figures not otherwise designated are grams calculated on the basis of the tissue from 50 bulbs.

		CB	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
Total carbon,	leaf		72.8	62.9	101.0	91.1	63.1	54.7
	bulb	286	189	197	127	142	177	209
	root		14.0	15.2	16.7	20.4	13.0	14.2
	total		275.8	275.1	244.7	253.5	253.1	277.9
Insoluble carbon,	leaf		38.3	30.3	43.3	45.3	30.7	27.8
	bulb	233	138	151	86.5	105	126	169
	root		7.9	7.9	10.6	12.7	7.8	9.4
	total		184.2	189.2	140.4	163.0	164.5	206.2
Soluble carbon,	leaf		34.5	32.6	57.7	45.8	32.4	26.9
	bulb	52.9	51.0	46.3	40.3	36.4	51.0	39.5
	root		6.1	7.3	6.1	7.6	5.2	4.8
	total		91.6	86.2	104	89.8	88.6	71.2
Carbon content of organic solids (per cent)	leaf		46.7	46.9	49.0	50.3	46.1	49.7
	bulb	43.5	42.8	43.2	44.4	43.4	44.1	43.7
	root		45.2	47.5	42.8	43.5	44.8	43.0

plants grown in darkness can be assumed to represent the total loss due to respiration that occurred during the growth of the plants. The quantities amounted to 10.2 gm. from the starved plants, 41.3 gm. from the nitrate plants, and 32.9 from the ammonium salt plants. These figures are probably the most accurate measures available of the respiration loss.

Reference to Table 2 shows that these three groups of plants lost, respectively, 30, 127 and 91 gm. of organic solids during the growth period of 28 days in darkness. Accordingly, the carbon content of the organic solids consumed during this period was, respectively, 34.0, 32.5 and 36.2 per cent. These are interesting figures. Elaborate experimentation and numerous repetitions would be required in order to establish the limits of their validity; nevertheless, it is obvious that the three experiments agree among themselves fairly closely and there seems no reason to doubt that the magnitude is approximately correct. If this is so, the inference is clear that the average carbon content of the substances lost from the plant tissues during the growth period was definitely lower than the carbon content of glucose which is 40.0 per cent. Thus, the respiration must have made use of a greater or smaller proportion of substances other than glucose and the greater the proportion of glucose that was actually used, the lower the average carbon content of these other substances must have been in order that the final average carbon content of the substances utilized should have been as low as 34 per cent. It may perhaps be pointed out that the figures would have to be in error by as much as 20 per cent of themselves, if the foregoing inference is incorrect and if the entire substrate of respiration were in fact glucose or a carbohydrate of similar composition. These data have, accordingly, raised a puzzling and difficult question which can be answered only by further research.

It should perhaps be emphasized that the figures refer to the total respiration loss over the entire growth period and thus differ fundamentally from the respiration data usually collected for plant tissues which refer to a single tissue observed for a relatively short time. Attention may also be directed to the fact that among substances of carbon content lower than that of glucose which are concerned in respiration are the common organic acids. Nevertheless, as will be shown, there were only 7.3 gm. of carbon belonging to organic acids present in the original bulbs, a quantity entirely inadequate to account for the losses actually observed even if all of it had disappeared, which was not the case. The difficulty of the dilemma is increased when it is recalled that data already presented have shown that the actual losses of starch and soluble sugar are sufficient to account for the separately determined losses of organic solids.

The net loss of carbon from the plants grown in light was, respectively, 10.9, 32.3 and 8.1 gm. from the starved, the nitrate and the ammonia plants. With the exception of the starved plants where the loss was the same, these figures are much smaller than those observed

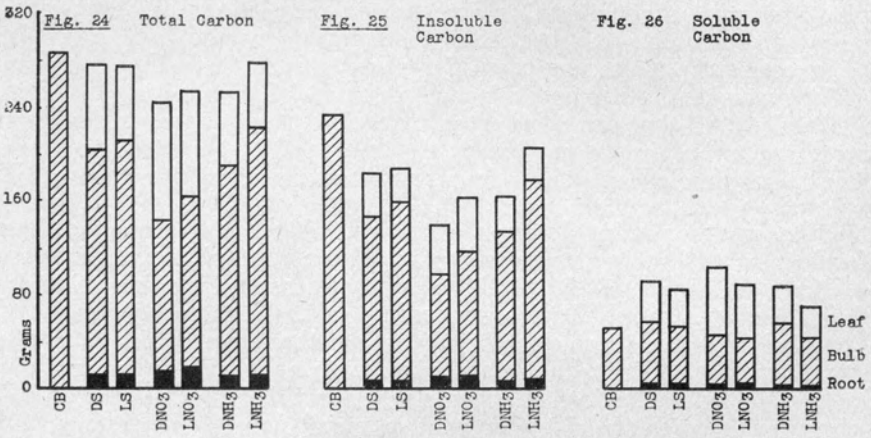
for the plants grown in darkness, and it can thus be inferred with some assurance that the respiration loss was to a considerable extent compensated by a gain of carbon through photosynthesis. It is extremely unlikely that the plants grown in darkness can be assumed to be controls on the parallel plants grown in light with respect to the quantity of carbon consumed by respiration. Respiration is a function of the vigor of the metabolism, and there is little doubt that the plants grown under more or less normal conditions in light were more vigorous in this respect than those in darkness. Thus, it would be inaccurate to look upon the differences between the carbon losses in darkness and in light respectively as measures of the amount of photosynthesis. All that can be asserted regarding these figures is that they represent approximate net losses, that is, the difference between the loss by respiration and the gain by photosynthesis in the three cases.

One further point deserves attention. It is clear from the data for loss of carbon in darkness that the plants supplied with nitrate existed at a higher level of metabolism as measured by this criterion than the plants supplied with ammonium salt. Both conditions, however, led to a far greater total metabolism than was observed in the starved plants. The carbon data agree with and confirm the entirely independent data for loss of organic solids in this respect and are likewise parallel with the data for protein metabolism as represented by the quantities of protein synthesized in the tissues.

The details of the carbon content of the several tissues in Table 10, plotted in Figure 24, show many analogies with the data for the total nitrogen plotted in Figure 6. Transport of carbon from the bulbs into the growing tissues frequently parallels that of the nitrogen, although the access of nitrogen from the culture solutions renders exact comparison difficult. In the starved plants, the relative proportion of the carbon that was transported from the bulb into the growing tissues was much smaller than that of the nitrogen. Thus, under these conditions of culture, the leaves and roots made far higher relative demands upon the nitrogen of the bulb than they did on its carbon. The leaves and roots of the nitrate plants, on the other hand, drew upon the bulb for about one-half of its nitrogen and about equally upon the carbon it contained; the demands of the ammonia plant tissues were proportionately somewhat less but were similar as respects both nitrogen and carbon. Calculations are shown in Table 11 and serve to illustrate the behavior of the nitrogen and carbon of the bulb tissue during growth under the experimental conditions studied.

The data for the insoluble carbon, plotted in Figure 25, show that more insoluble carbon remained in the bulbs of the plants grown in light than in those of the plants grown in darkness under all conditions of culture. The leaves of the starved plants grown in darkness contained somewhat more insoluble carbon than those grown in light and this was also true of the ammonia plants, although in this

case the difference was small. The leaves of the nitrate plants, however, contained slightly more insoluble carbon in the plants grown in light than in those grown in darkness. The roots of both nitrate plants and ammonia plants grown in light contained a little more insoluble carbon than those grown in darkness. The roots of the starved plants showed no difference. In all cases, there was somewhat more



insoluble carbon in the whole plants grown in light than in those grown in darkness.

TABLE 11. RELATIVE PROPORTIONS OF THE NITROGEN AND CARBON OF THE BULB TISSUES UTILIZED FOR THE DEVELOPMENT OF THE LEAVES AND ROOTS

Figures are the per cent of the original bulb nitrogen or carbon that disappeared from the bulb tissue during growth of the plants.

	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
Nitrogen	66	56	55	47	42	33
Carbon	33	31	56	50	38	27

The data for soluble carbon (Figure 26) in general show the reverse effect; there was more in the plants grown in darkness than in those in light, the difference being especially pronounced in the nitrate plants and being evident in both bulb and leaf tissue.

Table 10 also shows calculations of the carbon content of the organic solids of the separate tissues. As might be anticipated, the carbon content of the bulb tissue differed only slightly from that of starch, its major component. The average of all samples was 43.6 per cent whereas starch contains 44.4 per cent of carbon. The root tissue had a similar composition with respect to carbon, on the average 44.5 per cent, thus also closely resembling starch and cellulose. The leaf tissue, however, was significantly higher in carbon content, averaging 48.1 per cent, in part because of the presence of a substantial pro-

portion of protein (carbon content, approximately 52 per cent) and also of a moderate proportion of ether extractable components which would be expected to contain 70 per cent of carbon or more.

COMPOSITION OF NARCISSUS PLANT TISSUES

If certain assumptions are made, the analytical data that have been presented can be used for the calculation of the chemical composition of a substantial fraction of the tissues. The results of such a calculation are shown in Table 12. The assumptions that have been made are discussed in the following paragraphs.

It is assumed that the nitrogen content of the proteins of the tissues is 16 per cent, that is, that the quantity of protein present can be computed from the protein nitrogen by the conventional factor 6.25. In the absence of information upon the actual average nitrogen content of the tissue proteins, this is the closest approach that can be made at present. Chibnall's work upon leaf proteins (2) indicates that the nitrogen content of most of the grass-leaf proteins that he and his associates prepared contained from 14 to 15 per cent of nitrogen which would suggest that the conventional factor is somewhat

TABLE 12. COMPOSITION OF THE NARCISSUS PLANT

Figures not otherwise designated are grams calculated on the basis of the tissues from 50 bulbs.

	CB	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
		Leaf Tissue					
Protein (N x 6.25)		13.6	15.4	17.6	23.9	12.4	16.1
Starch		0.8	0.4	0.8	0.4	1.6	0.5
Crude fiber		24.2	20.3	34.5	29.0	20.3	16.1
Ether extract		2.31	4.38	3.66	4.91	2.17	3.83
Soluble carbohydrates		44.89	35.02	56.20	32.21	35.73	18.95
Oxalic acid		0.30	0.41	0.29	0.46	0.28	0.32
Citric acid		0.30	0.22	0.46	0.70	0.17	0.20
Malic acid		1.53	0.71	2.69	3.54	0.79	0.50
Succinic acid		0.19	0.08	0.21	0.41	0.19	0.44
Unknown acid as citric		4.41	4.61	6.14	5.44	4.10	4.41
Asparagine		2.30	0.75	5.48	4.12	2.74	2.39
Glutamine		0.97	0.64	1.38	1.95	1.65	2.51
Amino acids (NH_2–N x 10)		1.20	0.00	0.00	0.00	0.00	0.00
Peptides (peptide N x 10)		7.9	4.1	14.3	10.2	8.7	4.2
Other soluble nitrogenous components (N x 5)		5.5	4.6	9.9	5.8	7.3	6.9
Total known organic solids (estimated)		110.4	91.6	154	123	98.1	77.4
Total organic solids (determined)		156	134	206	181	137	110
Unknown organic solids		45.6	42.4	52	58	38.9	32.6
Unknown organic solids as per cent of total organic solids		29.2	31.6	25.2	32.0	28.4	29.6
Inorganic solids		11.4	8.1	15.2	17.3	8.2	11.1

TABLE 12 (*Continued*)

	CB	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
			Bulb Tissue				
Protein (N x 6.25)	34.1	12.9	15.5	15.1	18.4	19.3	22.3
Starch	301.3	190	184	96.7	127	162	201
Crude fiber	21.8	19.0	20.4	19.9	20.0	18.7	18.8
Ether extract	3.80	2.37	3.4	2.0	2.3	2.8	3.5
Soluble carbohydrates	65.75	69.1	65.5	51.6	49.2	54.7	54.8
Oxalic acid	3.32	2.48	3.03	3.22	3.47	2.98	2.77
Citric acid	4.45	1.59	1.27	1.08	1.10	1.52	2.39
Malic acid	1.33	2.28	1.68	1.96	1.68	1.32	1.65
Succinic acid	0.21	0.14	0.30	0.19	0.18	0.18	0.56
Unknown acid as citric	11.2	10.5	8.64	9.73	10.1	10.4	10.7
Asparagine	3.92	1.12	1.20	2.96	1.61	3.58	2.42
Glutamine	1.54	0.74	1.02	0.86	1.34	2.25	1.81
Amino acids (NH_2–N x 10)	5.00	2.30	1.50	3.70	2.90	3.30	2.40
Peptides (peptide N x 10)	18.5	7.0	9.0	4.4	10.4	9.8	14.3
Other soluble nitrogenous components (N x 5)	9.40	1.20	3.85	4.00	4.20	3.20	5.50
Total known organic solids (estimated)	485.6	322.7	320.3	217.4	253.9	296.0	344.9
Total organic solids (determined)	658	441	456	286	327	401	478
Unknown organic solids	172.4	118.3	135.7	68.6	73.1	105.0	133.1
Unknown organic solids as per cent of total organic solids	26.2	26.8	29.8	24.0	22.4	26.2	27.8
Inorganic solids	29.6	17.6	21.5	17.7	19.7	18.5	20.5
			Root Tissue				
Protein (N x 6.25)		2.81	2.81	4.50	5.62	4.06	4.44
Starch		0.046	0.068	0.040	0.110	0.081	0.094
Soluble carbohydrates		4.80	6.06	1.31	2.88	1.59	2.78
Oxalic acid		0.276	0.473	0.758	0.891	0.484	0.301
Citric acid		0.103	0.084	0.182	0.217	0.050	0.051
Malic acid		0.968	0.529	0.427	0.758	0.040	0.060
Succinic acid		0.030	0.030	0.055	0.080	0.137	0.126
Unknown acid as citric		1.54	1.15	1.79	2.37	1.21	1.15
Asparagine		0.10	0.07	1.38	1.24	0.54	0.37
Glutamine		0.08	0.08	0.42	0.44	0.65	0.69
Amino acids (NH_2–N x 10)		0.10	0.00	1.10	0.90	0.90	0.30
Peptides (peptide N x 10)		0.90	0.80	1.20	1.50	0.90	1.40
Other soluble nitrogenous components (N x 5)		0.60	0.65	1.85	3.70	1.20	1.50
Total known organic solids (estimated)		12.3	12.8	15.1	20.7	11.8	13.3
Total organic solids (determined)		31	32	39	47	29	38
Unknown organic solids		18.7	19.2	23.9	26.3	17.2	24.7
Unknown organic solids as per cent of total organic solids		60.3	60.0	61.2	55.9	59.3	65.0
Inorganic solids		4.1	5.4	11.3	14.3	6.3	3.5

too low. They noted, however, that these preparations usually contained non-protein material that was difficult, if not impossible, to separate from the protein itself, and they encountered a few cases, notably the protein of spinach leaves (1) and that of the leaf of *Vicia faba* (3), preparations of which were found to contain well in excess of 16 per cent of nitrogen. Thus the assumption that, on the average, these tissue proteins contain 16 per cent of nitrogen is probably not seriously in error.

The situation with respect to the proteins of the bulb and root tissues is different. No records of the composition of proteins of analogous tissues have been found in the literature and the use of the conventional factor is therefore entirely arbitrary.

The "crude fiber" represents material that remains insoluble when a sample of the tissue is treated with a succession of solvents including ether, hot dilute aqueous acid and hot dilute alkali. Roughly speaking, this residue consists of the cellulose and cellulose-like materials of the tissue, although there is no certainty that moderate quantities of other components may not also be present. The "crude fiber" is thus a purely conventional component.

The ether extract, notably that from the leaf tissues, undoubtedly contains substances other than fat. Chlorophyll was present in the ether extracts from the leaf samples grown in light, and all of the leaf extracts contained lipid pigments. In addition, such substances as sterols, hydrocarbons, phosphatides and waxes would be included if present.

The data for the organic acids can be computed with accuracy in the cases where the acid has been identified, but approximately one-half of the total organic acidity consisted of unknown acids. It has been assumed that no great error would result if the factor for citric acid were used for calculating the quantity present. It should perhaps be pointed out that the value for the unknown acids is obtained by difference and, accordingly, is subject to the combined analytical errors of all of the individual determinations.

The quantity of amino acids other than the asparagine and glutamine has also been arrived at by difference, the soluble amino nitrogen of the extract being corrected for the amino nitrogen from the asparagine and that from the glutamine. This last quantity is computed with allowance for the unusual behavior of the amide nitrogen of glutamine when this substance is treated with nitrous acid in the Van Slyke apparatus. The factor 10 has been adopted to compute the amount of amino acids from the estimated amino nitrogen, a procedure that assumes that the average mixture of unknown amino acid contains 10 per cent of amino nitrogen. Actual amino acids range from 18.6 per cent in the case of glycine to 7.7 per cent in the case of

tyrosine, but most of them fall within the limits of from 9 to 11 per cent; accordingly, the factor 10 cannot lead to grave error.[1]

A similar argument applies to the calculation of the quantity of peptides. The peptide nitrogen is obtained by difference from the amino nitrogen before and after severe hydrolysis, with correction for the glutamine, and it is assumed that the average peptide will contain 10 per cent of peptide nitrogen. The actual value will depend upon the complexity of the peptides. The dipeptide of leucine, for example, contains 5.7 per cent of peptide nitrogen, while the hexapeptide of leucine contains 10.0 per cent, the intermediate members of the series falling between these limits. If the actual peptides present in the mixture of substances derived from the tissues are tetrapeptides or larger, the factor 10 would give only a moderate overestimate of the quantity present.

For the estimation of the quantities of other unknown nitrogenous components, no logically derived factor is possible. The factor 5 has been used on the assumption that the substances involved are on the average twice as rich in nitrogen as amino acids. A relatively small portion of adenine, which contains nearly 52 per cent of nitrogen, would compensate for a considerably larger proportion of choline that contains 11.6 per cent; the factor used merely assumes that the average nitrogen content of these substances is 20 per cent.

It is obvious from these considerations that the calculation of the composition of the plant tissues from the available analytical data is, at best, an extremely rough approximation and serves merely to give an estimate of the proportion of the organic solids present concerning which nothing is known. Nevertheless, as will be seen from the data in Table 12, from two-thirds to three-quarters of the organic solids have been accounted for on this basis in the bulb and leaf tissues although a little less than one-half could be allocated in the case of the root tissue.

Although Table 12 shows results that are, for the most part, a recapitulation of data already presented, it contains two items not previously mentioned, the crude fiber and the ether extract.[2] The crude fiber of the bulb tissue is clearly little affected during the process of growth; the original bulb tissue contained 21.8 gm. and this diminished very little during culture of the plants. Certainly, there is no significant effect of culture in light in comparison with culture in darkness in the three cases. The crude fiber was not determined in the root tissue, but that of the leaf tissue shows evident effects not only of growth in light as compared with growth in darkness but also of the treatment of the plants.

These results would perhaps be anticipated. The bulbs contained a small proportion of cellulose originally, probably chiefly in the

[1] This assumption is equivalent to the assumption that the average protein containing 16 per cent of nitrogen yields a hydrolysate in which 62.5 per cent of the nitrogen is amino nitrogen.

[2] We are indebted to the Department of Analytical Chemistry of this Station for the data which were obtained by standard official methods.

structure of the cell walls and in the envelope of the bulb. Growth would be expected to draw upon the contents of the cells but not upon the components of their walls; thus the crude fiber in the residual bulb tissue after the plants had been harvested would be expected to be essentially unchanged, as is found to be the case. The leaf tissue, on the other hand, was produced mostly from components derived from the cells of the bulbs and transported to the developing tissue in soluble form. The expansion of the leaf took place with an increase in the quantity of material laid down in the cell walls and this is clearly illustrated in the data for the crude fiber; the quantities observed are, within the limits of error, in constant ratio to the total organic solids, being 15.5 and 15.1 per cent for the starved plants, 16.7 and 16.0 per cent for the nitrate plants and 14.8 and 14.6 per cent for the ammonia plants. The quantity of crude fiber is thus a rather accurate measure of the size and degree of development of the leaves of the plant if this is understood to mean their content of organic solids.

The ether extract, a measure of the entire lipid content of the tissue, forms only a small part of the organic solids of the original bulb tissue, the 3.8 gm. present being slightly under 0.6 per cent of these solids. During growth, the quantity of ether extract in the residual bulb tissue diminished by from 1 to 2 gm., presumably owing to the metabolism of a part of the components represented. The metabolism of lipids in this tissue is, however, minute in comparison with that of the fats in such a tissue as, for example, the nuts or the seeds of the peanut or soybean. These seeds contain from 20 to 40 per cent or even more of their organic solids in the form of ether soluble components and, during growth of the plant in the early stages, much of this fat is utilized. Narcissus bulbs, in contrast, are high in starch and the early stages of metabolism call chiefly upon this component, and only slightly upon the ether soluble material.

The ether extract in the leaves is likewise a relatively small component, although doubtless a very important one. The figures in Table 12 show a marked increase in the ether extract of the leaves grown in light owing in part at least to the formation of chlorophyll. There is evidence for an increase in lipid synthesis in the leaves of the nitrate plants as compared with the others, but the leaves of the ammonia plants contained less ether extract than the leaves of the starved plants.

To return to the compiled data of Table 12: The sum of the components, for which estimates of quantity could be provided, is shown in the rows marked total organic solids (estimated). Beneath this item is the quantity of organic solids as determined analytically, i. e., the total solids corrected for the ash and moisture. The difference between these two estimates thus provides a measure of the quantity of organic solids of unknown chemical nature, and the percentage of these unknown solids in terms of the determined organic solids is

also shown. For the residual bulb tissue, this proportion is 27 and 30 per cent, respectively, in the starved plants, 24 and 22 per cent in the nitrate plants and 26 and 28 per cent in the ammonia plants. It was 26 per cent in the original bulb tissue. Thus, nearly three-quarters of the solids of the bulb tissue can be reasonably accounted for in terms of known substances. The chemical nature of the unknown part can only be guessed at, but one would expect that a moderate proportion of such substances as the so-called hemicelluloses and glucuronides might be present, together with small amounts of, doubtless, a large number of other substances. The average composition of the unknown part with respect to carbon might be expected to approach that of the carbohydrates and evidence that this is the case will be presented in the following section.

The composition of the root tissue is in marked contrast to that of the bulbs. The analytical investigation was less extensive with this tissue and it happens that none of the components that were determined make up any substantial part of the whole. The soluble carbohydrates and the proteins were the most important from the point of view of quantity present but even these, together with the organic acids and the known simple nitrogenous substances, account for much less than half of the solids. The unknown solids in fact range from 56 to 65 per cent of the total organic solids, most of the values being close to 60 per cent. No information upon the nature of this material is available, although it might be hazarded that the composition would not be far from that of the carbohydrates. Whatever its nature, however, from data already presented, it obviously contains components of remarkably large water-holding capacity.

The leaves, in turn, differ in composition from the other tissues. The chief known components are the soluble carbohydrates, the crude fiber and the protein and peptides appear to make up a moderate proportion of the soluble nitrogenous substances. The amides were present in substantial amounts only in the leaves of the nitrate plants. The organic acids rank in order of quantitative importance with the peptides but the relative proportion present was small, ranging from 4.3 to 5.8 per cent of the organic solids. The composition of the leaves of the narcissus plant is thus in marked contrast to that of such more thoroughly investigated leaves as those of the tobacco plant which frequently contain from 20 to 30 per cent of the organic solids as organic acids.[1]

The unknown organic solids amounted to 29 and 32 per cent, respectively, of the organic solids of the leaves of the starved plants, 25 and 32 per cent of the leaves of the nitrate plants, and 28 and 30 per cent of those of the ammonia plants; thus, as with the bulbs, a considerable part of the organic solids can be reasonably accounted for. Again, however, nothing is known as to the composition of the balance. The presence in this tissue of hemicelluloses and glucuronides,

[1] Tobacco leaves studied in detail in 1937 (47) for example contained 26.9 per cent of organic acids.

and possibly of substances allied with the pectins, may be suspected and, as will appear, there is also evidence for the presence of substances appreciably richer in carbon. Far more elaborate analytical studies than those at present reported will be required before more exact information becomes available.

DISTRIBUTION OF CARBON IN NARCISSUS PLANT TISSUES

From the data for the composition of the tissues shown in Table 12 and the data for the total and the insoluble carbon in Table 10, it is possible to compute the quantities of carbon in the several tissues that presumably belong to identifiable components, if the admissibility of certain assumptions may be granted. The assumptions, in addition to those already discussed in connection with the data of Table 12, are as follows.

Pure proteins contain as a rule a remarkably constant proportion of carbon, the observed values being found in most cases to cluster closely around 52 per cent. This value is more nearly constant than the nitrogen which is conventionally assumed to be 16 per cent in the case of proteins that have not been studied in the pure state. In the absence of evidence in the present case, the carbon content of the proteins in the tissues is calculated from the protein nitrogen by multiplication by the factor 3.25. This merely assumes that the hypothetical average protein contains 52 per cent of carbon and 16 per cent of nitrogen.

Starch carbon is calculated from the theoretical carbon content of starch (44.4 per cent), soluble carbohydrate from that of glucose (40.0 per cent), while the carbon of crude fiber is calculated on the assumption that the material involved has the composition of cellulose, which is identical with that of starch. The carbon of the ether extract is calculated on the purely arbitrary assumption that it has the composition of an average true fat which is 76.7 per cent carbon. Chlorophyll, which is one of the components of this fraction in the case of the leaf tissue grown in light, contains 73.2 per cent of carbon, sterols such as cholesterol contain 83.9 per cent and carotene, 89.5 per cent. Thus, the carbon content of fat probably represents a fair approximation to the average composition of this fraction.

No comment on the calculation of the carbon of the organic acids or the amides is necessary. The peptide carbon and that of the amino acids is computed on the assumption that the hydrolysis of 100 gm. of an average protein yields 116 gm. of amino acids. If the original protein contained 52 per cent of carbon, the amino acids derived from it would contain 44.8 per cent; this is 2.8 times their average nitrogen content on the assumption that the original protein contained 16 per cent. Thus, the carbon attributable to the peptides and amino acids can be reasonably calculated from the nitrogen by multiplying by 2.8.

The carbon of the other soluble nitrogenous components is arbitrarily assumed to be twice their nitrogen content.

The carbon of the several soluble and insoluble components of the tissues has been calculated on the basis of these assumptions and is shown in Table 13. The proportions of the total carbon of the tissues that can thus be more or less reasonably accounted for differ with the different tissues. In the bulbs, the separate sets of figures agree fairly closely; on the average, only 27.1 per cent of the carbon present must be ascribed to unknown substances and this agrees substantially with the average of the figures from Table 12 for the relative proportion of unknown organic solids which is 26.2 per cent. The unknown organic solids of the bulb must, therefore, have about the same average carbon content as the known organic solids. In the root tissue, the unknown carbon amounts to 62.5 per cent of the total carbon on the average and, again, the agreement with the proportion of unknown organic solids (average 60.3 per cent) from Table 12 is close. In the leaf tissue, on the other hand, the unknown carbon makes up 37.0 per cent of the total carbon whereas the unknown organic solids account for only 29.3 per cent. Clearly, therefore, the carbon content of the unknown solids of the leaf must be substantially higher than that of the substances that have been recognized and accounted for, and this observation raises an interesting and important question regarding the chemical nature of these substances.

TABLE 13. DISTRIBUTION OF CARBON IN COMPONENTS OF THE TISSUES OF THE NARCISSUS PLANT

Figures not otherwise designated are grams calculated on the basis of the tissues from 50 bulbs.

	CB	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
		Leaf Tissue					
Protein C (N x 3.25)		7.05	8.00	9.13	12.4	6.44	8.39
Starch C		0.35	0.18	0.35	0.18	0.71	0.22
Crude fiber C		10.7	9.02	15.3	12.9	9.02	7.15
Ether extract C		1.77	3.36	2.81	3.76	1.67	2.93
Soluble carbohydrate C		18.0	14.0	22.5	12.9	14.3	7.58
Oxalic acid C		0.079	0.110	0.078	0.123	0.075	0.086
Citric acid C		0.113	0.083	0.173	0.263	0.064	0.074
Malic acid C		0.548	0.254	0.963	1.27	0.283	0.178
Succinic acid C		0.077	0.033	0.085	0.167	0.077	0.179
Unknown acid[1] C		1.654	1.728	2.303	2.040	1.537	1.654
Asparagine C		0.837	0.271	1.99	1.50	0.995	0.868
Glutamine C		0.399	0.261	0.566	0.801	0.677	1.03
Amino acid C(NH_2–N x 2.8)[2]		0.34	0.0	0.0	0.0	0.0	0.0
Peptide C(peptide N x 2.8)		2.21	1.15	4.00	2.86	2.44	1.18
C of other soluble nitrogenous components (N x 2)[3]		2.18	1.84	3.98	2.30	2.92	2.76
Total C of known components (estimated)		46.30	40.27	64.22	53.46	41.21	34.28
Total C (determined)		72.76	62.88	101.0	91.09	63.08	54.74
Unknown C		26.4	22.59	36.8	37.6	21.9	20.5
Unknown C as per cent of total C		36.3	35.9	36.4	41.3	34.7	37.4
C content of unknown solids (per cent)		57.9	53.3	70.8	64.8	56.3	62.9

TABLE 13 (*Continued*)

	CB	DS	LS	DNO_3	LNO_3	DNH_3	LNH_3
			Bulb Tissue				
Protein C (N x 3.25)	17.7	6.70	8.06	7.83	9.59	10.0	11.6
Starch C	134.0	84.4	81.8	43.0	56.4	72.0	89.3
Crude fiber C	9.7	8.4	9.1	8.8	8.9	8.3	8.4
Ether extract C	2.91	1.82	2.61	1.53	1.76	2.15	2.68
Soluble carbohydrate C	26.3	27.6	26.2	20.6	19.7	21.9	21.9
Oxalic acid C	0.885	0.661	0.808	0.859	0.925	0.794	0.739
Citric acid C	1.670	0.597	0.477	0.405	0.413	0.569	0.896
Malic acid C	0.478	0.816	0.600	0.701	0.600	0.473	0.591
Succinic acid C	0.086	0.057	0.120	0.077	0.072	0.072	0.228
Unknown acid C	4.200	3.936	3.240	3.648	3.792	3.888	4.008
Asparagine C	1.42	0.408	0.436	1.08	0.586	1.30	0.878
Glutamine C	0.634	0.304	0.420	0.351	0.549	0.926	0.746
Amino acid C(NH_2–N x 2.8)	1.40	0.64	0.42	1.04	0.81	0.92	0.67
Peptide C(peptide N x 2.8)	5.18	1.96	2.52	1.23	2.91	2.74	4.00
C of other soluble nitrogenous components (N x 2)	3.76	0.48	1.54	1.60	1.68	1.28	2.20
Total C of known components (estimated)	208.3	138.8	138.4	93.8	108.7	127.3	148.8
Total C (determined)	286.3	189.4	197.1	126.8	141.6	177.3	208.7
Unknown C	78.0	50.6	58.7	33.0	32.9	50.0	59.9
Unknown C as per cent of total C	27.2	26.7	29.8	26.0	23.2	28.2	28.7
C content of unknown solids (per cent)	45.2	42.8	43.3	48.1	45.0	47.6	45.0
			Root Tissue				
Protein C (N x 3.25)		1.46	1.46	2.34	2.92	2.11	2.31
Starch C		0.020	0.030	0.018	0.049	0.036	0.042
Soluble carbohydrate C		1.92	2.42	0.524	1.15	0.636	1.11
Oxalic acid C		0.074	0.126	0.202	0.238	0.129	0.080
Citric acid C		0.039	0.032	0.068	0.081	0.019	0.019
Malic acid C		0.346	0.189	0.153	0.271	0.014	0.021
Succinic acid C		0.012	0.013	0.022	0.032	0.056	0.051
Unknown acid C		0.577	0.431	0.671	0.888	0.454	0.431
Asparagine C		0.038	0.024	0.501	0.449	0.196	0.134
Glutamine C		0.034	0.034	0.171	0.18	0.266	0.28
Amino acid C (NH_2–N x 2.8)		0.03	0.00	0.31	0.25	0.252	0.084
Peptide C (peptide N x 2.8)		0.252	0.224	0.336	0.420	0.252	0.392
C of other soluble nitrogenous components (N x 2)		0.24	0.26	0.74	1.48	0.48	0.60
Total C of known components (estimated)		5.04	5.24	6.06	8.41	4.90	5.55
Total C (determined)		14.03	15.16	16.71	20.35	13.04	14.22
Unknown C		8.99	9.92	10.65	11.94	8.14	8.67
Unknown C as per cent of total C		64.1	65.4	63.7	58.7	62.4	61.0
C content of unknown solids (per cent)		48.1	51.7	44.6	45.4	47.3	35.1

[1] Unknown acid calculated as citric acid.

[2] The factor 2.8 is calculated as follows: an average protein contains 52 per cent of carbon and 16 per cent of nitrogen and yields 116 gm. of amino acids on hydrolysis. The average carbon content of these is therefore 44.8 per cent which is 2.80 times the average nitrogen content.

[3] The factor 2 is a compromise between adenine (0.86) and choline (4.3).

In order to obtain more detailed information, the carbon content of the unknown organic solids has been calculated by subtraction of the sum of the carbon of the known components from the total carbon as determined. The ratios of these quantities expressed as a percentage of the quantities of unknown organic substances taken from Table 12 are shown in Table 13. For the bulb tissue these are quite satisfactorily constant when the errors that affect the calculation are considered, the average value being 45.3 per cent, and the range from 42.8 (DS) to 48.1 per cent (DNO_3). This average is about what might be expected if the unknown organic solids of the residual bulb tissue consisted largely of substances allied to the complex carbohydrates. Inasmuch as the average carbon content of the total organic solids of the residual bulb tissue was 43.6 per cent, while that of the original bulb was 43.5 per cent (Table 10), it is clear that the growth and development of the leaf and root tissue of these plants took place with only minor change in the average composition of the bulb. The change was in such a direction that the composition of the residual unknown material at the time of harvest was slightly enriched in carbon.

In the roots, the average carbon content of the unknown organic solids was 45.4 per cent with a range from 35.1 (LNH_3) to 51.7 (LS). This is a far broader spread than with the bulb tissue, but it happens that four sets of samples agree rather closely. The omission of the highest and lowest values would alter the average only to 46.3 per cent. Comparison with the figures in Table 10, which show that the total organic solids of the roots contained on the average 44.5 per cent of carbon, indicates that the unknown portion of the solids of the root tissue are probably not significantly different in composition, with respect to carbon, from the whole tissue and that there is no suggestion of the presence of any great quantity of material of substantially higher carbon content.

The leaf tissue presents a different picture. Here, the average carbon content of the unknown solids is 61.0 per cent with a range from 53.3 to 70.8 per cent. The experimental error here is undoubtedly high and the agreement among the separate sets of data is poor; nevertheless, it seems certain that the carbon content of the unknown solids in this tissue is substantially greater than is the case in the other two tissues. Both bulb and root tissue yielded figures that are not too far from what might be expected if the unknown components were related to the carbohydrates. The leaf tissue, on the other hand, must contain an appreciable proportion, roughly one-quarter of the organic solids, of higher carbon content, so high in fact as to suggest that considerable material related to the aromatic organic substances must be present. This is not an unreasonable deduction; the presence of tannins and of lignins and related substances in plant tissues is a commonplace, although the tannins are usually recognized as components of bark and lignins are associated with the wood of stems. The present data merely suggest the possibility that sub-

stances of an aromatic nature may be present in significant amounts in the leaves of this plant. Clearly, far more extensive qualitative study is necessary before this observation can be explained.

THE RELATION OF CULTURAL CONDITIONS TO "SIZE" IN NARCISSUS PLANTS

Many studies of the conditions under which plants are produced raise the question which of the treatments selected is the "best", or which produces the "best" plants. In dealing with an agricultural crop, there are usually certain more or less obvious criteria by which judgments may be made. Since such crops are grown for the benefit of mankind, the criteria chosen will naturally be those which express the degree of this benefit. Thus, the weight or number of seed or fruit, the fresh or dry weight of the leaves of a fodder crop or of the roots of a root crop are natural grounds for a discrimination between various applied conditions of culture. More recondite methods are sometimes selected in special cases such, for example, as attempts to determine "quality" in the case of the leaf of the tobacco plant, although this is recognized to be a complex property depending upon many conditions and in practice is determined by the subjective judgment of a trained observer.

A somewhat different problem is presented by a purely ornamental plant such as the narcissus. Here, the decision between what is "best" and what is less so is also largely a matter of subjective judgment and would probably rest upon an opinion that was formed from such components as the number and size of the flowers, the height of the flower stalk and especially upon the beauty of the flowers. The producer of narcissus bulbs would also be concerned with the number and average weight and the perfection of the bulbs that were developed after the flowering period was over. Some of these components are matters of counting or measurement, others are definitely not. The present experiment raises still other questions. The plants were harvested before they bloomed and, in fact, blooming was prevented by the removal of the buds as soon as they began to be formed. Which, then, of the various measurements that were carried out can be relied upon to serve as a criterion as to the most advantageous cultural treatment?

It is perhaps instinctive in such a case to turn to some measurement that may lead to a judgment of "size" with the implicit assumption that the biggest will *ipso facto* be the best. Whether or not such an assumption is justifiable cannot be decided from the present evidence. Experiments in which the entire life cycle of the plant were studied would be required for this. Nevertheless, a clear question of the "size" is raised, and it is the purpose of the present discussion to point out the uncertainty of this concept in the case of a plant where factors other than the strictly utilitarian ones of agriculture may play a part.

A general consideration of the conditions under which the present plants were grown would probably lead to some such conclusion as the following. Culture in the dark is entirely abnormal and leads to plants that contain no chlorophyll and possess no esthetic appeal; the three sets of plants produced in this way can therefore be eliminated at once as representatives of a practical method of growth. Culture in the absence of nitrogenous nutriment would not be expected to give plants as large or as well developed as those that had received nitrogen; the starved plants grown in light can therefore be eliminated on these grounds. The two cultures that were supplied with nitrogen remain and general experience with fertilizers would suggest to many that the nitrate plants would probably be better developed than the ammonium salt plants. Thus, the rating of the sets of plants on the basis of what would be suggested by general experience would place the LNO_3 plants at the top of the list, the LNH_3 possibly second, and the LS plants third. One might assume that, of the plants grown in darkness, the DNO_3 would be best with respect to "size", the DNH_3 next, and the DS plants last, although some difference of opinion might well arise on this. How the light and dark plants would rate relative to each other would be difficult to anticipate.

The data that have been collected furnish a number of criteria of "size". The first and most obvious is the fresh weight of the leaves, and relative ratings of the bulb and root tissue as well as the sum of these, the fresh weight of the whole plant, might be attempted. The order in which the different cultures are placed by this criterion is shown by the first four lines of Table 14. The two nitrate cultures

TABLE 14. RELATIVE RATINGS OF THE NARCISSUS PLANTS ACCORDING TO VARIOUS CRITERIA OF "SIZE"

Fresh weight,	leaves	DNO_3,	LNO_3,	(DS,	DNH_3,)	LS,	LNH_3
	roots	LNO_3,	DNO_3,	LNH_3,	(DNH$_3$,	LS,)	DS
	bulbs	LS,	(DS,	LNO_3,	LNH_3,	DNH_3,)	DNO_3
	whole plant	DNO_3,	LNO_3,	(DS,	DNH_3,)	LS,	LNH_3
Total solids,	leaves	DNO_3,	LNO_3,	DS,	(DNH$_3$,	LS,)	LNH_3
	whole plant	(DS,	LNH_3,	LS,)	(LNH$_3$,	DNH_3,)	DNO_3
Leaf length,	total	DNO_3,	LNO_3,	DS,	DNH$_3$,	LS,	LNH_3
	average	DNO_3,	(DS,	LNO_3,	DNH_3,)	LS,	LNH_3
Total N,	leaf	DNO_3,	LNO_3,	LNH_3,	DNH$_3$,	DS,	LS
	whole plant	LNO_3,	DNO_3,	LNH_3,	DNH$_3$,	LS,	DS
Total N	leaf	LNH_3,	LNO_3,	(DNO$_3$,	DNH_3,)	(DS,	LS
Organic solids	whole plant	LNO_3,	DNO_3,	(LNH$_3$,	DNH_3,)	LS,	DS
Leaf protein		LNO_3,	DNO_3,	(LNH$_3$,	LS,)	DS,	DNH_3
Whole plant protein		LNO_3,	LNH_3,	DNO_3,	DNH$_3$,	LS,	DS
Protein N	leaf	LNH_3,	LNO_3,	LS,	DNH$_3$,	DS,	DNO_3
Organic solids	whole plant	LNO_3,	(DNO$_3$,	LNH_3,)	DNH$_3$,	LS,	DS

head the list in all save the rating on fresh weight of the bulb, but the positions of the other sets of plants do not follow in any regular order. The cases where the numerical differences are small and perhaps not entirely significant are enclosed in parentheses. The relative position of the plants as rated on the weight of the residual bulb tissue is not surprising; it would perhaps be expected that the bulbs of the starved plants would be the heaviest since presumably less material had been abstracted from them during growth; thus, the reversed order on this basis is logical. Nevertheless, the bulbs of the DNO_3 culture would probably not have been placed last in advance of information.

Ratings on the basis of total solids of the leaves and of the whole plants differ widely; the DNO_3 leaves are placed first by the first criterion but the DNO_3 whole plants come last by the second. There is close agreement, however, in the ratings on total leaf length and average leaf length, and the first of these agrees exactly with the rating on leaf fresh weight.

The order in which the plants are placed on the basis of total nitrogen content, either of the leaves or of the whole plant, puts the nitrate plants ahead of the ammonia plants and these in turn ahead of the starved plants. On whole plant nitrogen, the LNO_3 plants are first; on total leaf nitrogen, the DNO_3 plants come first. Both criteria place the LNH_3 plants ahead of the DNH_3 plants, but the position of the starved plants differs in the two cases.

Plant analysts usually express their results in terms of concentration rather than in terms of quantity as is done here. Accordingly, the concentration of nitrogen as a percentage of the organic solids both for the leaves and for the whole plants has been computed and used for rating. On this basis, the LNH_3 plants head the list in terms of leaf concentration, the LNO_3 plants in terms of whole plant concentration; in both, the starved plants fall at the end.

The protein nitrogen has been assumed in an earlier section to represent a measure of the quantity of metabolizing tissue since it is presumably proportional to the amount of protoplasm in the cells. This quantity may, therefore, be expected to furnish a rating that has some relation to the success of the individual plants as living organisms. In terms of leaf protein, the LNO_3 plants head the list, the DNO_3 plants follow them but the DNH_3 plants are at the end, being below the starved plants. Whole plant protein, however, corrects this; the LNO_3 plants again head the list, the DNH_3 occupy an intermediate position, and the starved plants come at the end. When expressed in terms of concentration of protein in the organic solids, the LNH_3 plants come first if the calculation is made for the leaves, and the DNO_3 plants come last; in terms of the whole plant, the LNO_3 plants come first and the starved plants last.

It is clear from the consideration of these sets of data that no single criterion can with logic be selected as the "best". Furthermore, there is little agreement in the details of the rating by the different methods. In general, the nitrate plants occupy the first place according to the greater number of methods of judgment, the starved plants the last, but there are many exceptions and some of these occur in cases that would not perhaps be expected. "Size" is obviously a vague concept which is difficult to define in terms of actual physical and chemical measurements upon plants, and the fundamental question as to which of the different treatments of the plants is "best", is not to be determined from the present data. A general impression only is furnished.

COMPARISON WITH THE EXPERIMENTS OF NIGHTINGALE AND ROBBINS

Direct comparison of the present results with those of Nightingale and Robbins (14) on the metabolism of the allied plant species, *Polyanthus narcissus*, is difficult because of the difference in the technique employed for the dissection of the plants previous to the preparation for analysis. Nightingale and Robbins took advantage of the fact that the center tissue of the bulbs, which comprises the base of the leaves, can be separated from the surrounding sheath of thickened bulb scales or storage tissue proper. Thus, their analytical determinations, instead of being made upon the leaves above the point of emergence, were made on the leaves, together with their basal regions located in the center of the bulb and the fraction is denoted by the term "centers and tops". Accordingly, a portion of the tissue that has been considered as residual bulb tissue in the present experiment is included in the leaf samples in theirs.

The bulbs of *Polyanthus* were considerably heavier than those of *Narcissus* and were far richer in starch. A comparison of some of the analytical results on the bulbs of the two species is shown in Table 15; the proportion of nitrogen in terms of dry solids was similar as well as the proportion of soluble carbohydrates, but there were wide differences in the composition with respect to soluble nitrogenous components as well as in the proportion of starch.

Nightingale and Robbins carried out a series of analyses during growth in darkness with and without the addition of a culture solution that provided nitrate nitrogen. Their last harvest was made at 27 days and, accordingly, corresponds closely with respect to time to the present experiment. It is difficult, if not impossible, however, to determine from the data the relative physiological age of the plants in the two experiments. They record that parallel sets of samples transferred to the greenhouse on the 20th day had come into full bloom at about the 38th day from planting of the bulbs in the case of the starved plants, but that blooming was delayed from 10 days to two weeks further in the plants supplied with a culture solution that con-

TABLE 15. COMPARISON OF THE COMPOSITION OF BULBS OF *Narcissus poeticus* WITH THE OBSERVATIONS OF NIGHTINGALE AND ROBBINS ON *Polyanthus narcissus*

Figures are grams calculated on the basis of 50 bulbs and as per cent of dry weight.

	Narcissus poeticus		*Polyanthus narcissus*	
	gm.	per cent	gm.	per cent
Fresh weight	1870		2680	
Dry weight	687.7		980.3	
Total nitrogen	10.82	1.57	16.18	1.65
Protein nitrogen	5.46	0.794	12.33	1.26
Soluble nitrogen	5.36	0.779	3.85	0.393
Amide nitrogen	0.563	0.0819	0.750	0.0765
Amino nitrogen	1.18	1.72	0.427	0.0436
Total soluble carbohydrates	65.75	9.56	100.4	10.2
Starch	301	43.8	687.4	70.1
Hemicellulose			71.1	7.25

tained nitrate nitrogen. Thus, it may be supposed that their plants on the 27th day of culture in the dark were still relatively undeveloped with respect to bud formation. The present plants were deprived of bud tissue in all cases and, unfortunately, a control set exposed to normal conditions was not allowed to develop blossoms in order to ascertain when this event might have occurred. However, the comparison of the fresh weight of the centers and tops as well as of the dry weights in Nightingale and Robbins' experiments at 27 days with the weight of the leaves at 28 days in the present experiment shown in Table 16 bears out the impression that their plants were considerably less fully developed than the present ones at about the same age. The relative length of the leaf tissue in the two experiments supports

TABLE 16. COMPARISON OF PORTIONS OF THE PRESENT DATA FOR *Narcissus poeticus* WITH ANALOGOUS DATA OF NIGHTINGALE AND ROBBINS FOR *Polyanthus narcissus*

In both sets of data, figures not otherwise designated are grams calculated on the basis of 50 bulbs grown in darkness.

	Present data		Nightingale and Robbins' data	
	28 days' growth		27 days' growth	
	DNO_3	DS	Plus NO_3	Minus NO_3
Fresh weight, leaves	3636	2194	1090	1600
Total length of leaves (cm.)	20887	12671	5241	3623
Number of shoots	105	80	191	157
Average length of leaf (cm.)	44.8	62.1	30.4	23.1
Dry weight, leaves	221.7	168.2	190.0	180.8
Total nitrogen, leaves	7.72	4.84	6.37	5.32
Protein nitrogen, leaves	2.81	2.17	4.22	3.51
Protein nitrogen, bulbs	2.41	2.06	9.06	7.05
Dry weight, roots	50.5	35.3	57.8	56.3
Total nitrogen, roots	2.30	0.71	2.84	1.73
Protein nitrogen, roots	0.72	0.45	1.59	1.27
Soluble carbohydrates (sucrose + glucose), leaves	56.2	44.9	35.5	33.2
Starch, leaves	0.80	0.82	35.6	36.6
Starch, bulbs	96.6	189.9	311.3	390.1

this conclusion. Accordingly, attention need be directed to only a few points of resemblance and differences in behavior and composition in the two experiments.

The quantity of nitrogen in the leaves of the plants supplied with nitrate nitrogen, as compared with the quantity in the leaves of the starved plants, was widely different in the present experiment but there was only a moderate difference in the Nightingale and Robbins' experiment. This again suggests that the analyses were made at a relatively earlier point in the life cycle of the plants in the latter case as it seems probable that a greater effect of nitrogen supply would subsequently have become established. The chemical composition of the two species of leaf was also different with respect to protein. The relatively small leaves of the *Polyanthus* plants at 27 days contained, respectively, 66.2 and 65.9 per cent of their total nitrogen in the form of protein nitrogen in the nitrate and in the starved plants, whereas the larger leaves of the plants of the present experiments contained, respectively, 36.4 and 44.8 per cent of the total nitrogen as protein nitrogen in the nitrate and the starved plants. Moreover, the wide difference brought about by the addition of nitrate to the culture solution was not at all a result of the presence of a large proportion of nitrate nitrogen in the leaves; rather, it was due to the presence of increased proportions of soluble metabolites of which peptides and amides were conspicuous representatives. Thus, although the *Polyanthus* leaves were relatively smaller and apparently less mature, they were proportionally much richer in protein nitrogen as well as being richer in the absolute quantities of nitrogen and of protein nitrogen.

The differences extended to the nitrogen of the bulbs. The *Polyanthus* bulbs originally contained more than twice as much protein nitrogen as the *Narcissus* bulbs whether reckoned as absolute quantity or as percentage of the solids. The residual bulb tissue after 27 days of growth had been depleted in protein nitrogen from an original quantity of 12 gm. to 9 gm. in the plus nitrate plants, and to 7 gm. in the minus nitrogen plants. The *Narcissus* bulbs, on the other hand, dropped from an initial 5.5 gm. to 2.4 and 2.1 gm. of protein nitrogen in the analogous respective cases, and, accordingly, a far larger proportion of the original protein nitrogen had been used for the development of the plants.

The root tissues of the two species behaved in much the same way. The *Polyanthus* plant roots contained about the same weight of organic solids in the two cases while the *Narcissus* plant roots differed moderately as a result of treatment; both species showed the influence of the availability of nitrate in that roots that were conspicuously richer in nitrogen were produced.

The soluble carbohydrates of the leaves (reducing sugars plus sucrose in the *Polyanthus* plants) were about the same in the two experiments and there was no great effect of the availability of nitrate. The starch in the leaves was, however, widely different in the two

cases. The *Polyanthus* plants contained a considerable quantity of starch in the leaf fraction, but this was presumably a result of the method of dissection used inasmuch as the tissue from the centers doubtless contained an appreciable quantity of residual starch. The leaves of the *Narcissus* plants developed in darkness were almost free from starch.

Both species drew heavily upon the starch of the original bulb during growth. About one-half of the original bulb starch was used during the growth of the *Polyanthus* plants in the two experiments; the *Narcissus* plants provided with nitrate utilized two-thirds while the starved plants utilized a little over one-third.

Further discussion of the differences and analogies in the observed behavior of these two allied species is perhaps scarcely worth while. The general picture of a plant that develops from bulbs that are richly provided with starch is similar in the two cases; species differences doubtless account for the differences in the rate of growth and in the composition of the tissues, and it is clear that agreement in details could only be anticipated if the tissues were examined at analogous stages in their physiological cycle of development. Even so, it is by no means certain that the similarities would be conspicuous. The two experiments thus furnish an excellent example of the far-reaching effects upon chemical composition and behavior of species difference.

DISCUSSION AND RECAPITULATION

Although a number of the points that have been raised by the present analytical studies of the narcissus plant have already been discussed, a brief recapitulation of the main results seems desirable. The loss of organic solids from the plants grown in darkness has been assumed throughout to represent the effect of respiration. This view is supported by the data for carbon which show losses of carbon that ran parallel with the losses of organic solids. The behavior was analogous to that encountered in many series of previous experiments carried out in this laboratory in which samples of detached leaves have been studied during culture in darkness in water and in various nutrient solutions (47, 48, 49). In the present case, as in that of the detached leaves, a moderately accurate estimate of the weight and composition of the tissues at the start of the experiment as well as that at the end was possible. The fundamental difference is that, in the narcissus plants, for the starting point the composition of the original bulb from which the entire plant grows was chosen, and the experiments were prolonged for a substantial portion of the early period of development of the plant. The detached leaf experiments, on the other hand, dealt with already matured leaves the samples of which were assumed to have a similar composition. Analysis of one of these samples gave the starting point while others were subjected to the experimental conditions and then analyzed. Losses of organic

solids were estimated over rather brief periods since the tissues could be maintained in apparent health only for a relatively short time. In all cases in which leaves were kept in darkness, loss of organic solids occurred, the magnitude of the loss depending on the conditions of the culture. Many of the details of the disappearance of organic solids could be followed by serial analyses during the entire period of study, but this was not attempted in the experiments with narcissus.

If the validity of the fundamental assumption is admitted, the problem of the nature of the substrate that was consumed during the experimental period is at once raised. The leaf culture experiments in general have suggested that the situation is seldom simple; the soluble sugars invariably diminished rapidly in the early stages of the experiments but were never found to account for the whole of the solids that were lost. The organic acids likewise appeared to play a part, but the most important conclusion, at least in the case of rhubarb leaves where the full data for carbon were obtained (40, 49), was that the residues, which remained after intracellular hydrolysis of the leaf protein and deamination of the resulting amino acids had occurred, were likewise drawn into the respiration and oxidized to carbon dioxide. Respiration of protein is not usually regarded as a normal event in plant leaves; at least it has been detected only in the case of detached leaves exposed to the entirely abnormal conditions of culture in darkness (46, 52). It is, however, an event which may take place under these circumstances and its existence furnishes an example of the resourcefulness, so to speak, of plant leaves, when they are placed in abnormal conditions, in maintaining the life of the cells even at the expense of an essential component of the protoplasm. Mention of this possibility is made in connection with the present experiments only to illustrate the wide difference from the conditions that exist in detached leaves and in the normal leaf still growing and attached to the plant. The solids that disappeared from the samples of rhubarb leaves that were examined after culture in water in darkness were, on the average, somewhat higher in carbon content than are carbohydrates. At the end of 72 hours, calculations from the loss of solids and from the loss of carbon showed that the solids that disappeared contained 44.7 per cent of carbon, at 96 hours 46.9 per cent, while at 165, 213 and 261 hours the figures were, respectively, 52.1, 50.3 and 50.4 per cent of carbon.[1] The picture conforms with the view that, early in the culture period, carbohydrates and substances of similarly low carbon content were being consumed, while at later stages substances of higher carbon content played an increasingly important part. The figures are calculated from the cumulative losses and thus represent the average composition of the entire substrate of respiration during the previous period of experimentation.

The results with rhubarb leaves differ widely from those of the present experiment. The narcissus plants were living and growing organisms although they were maintained in darkness and thus had

[1] These figures are calculated from data given in Table 13 of Bulletin 424 (49).

not developed chlorophyll. The experience of Nightingale and Robbins shows, however, that such plants, if removed to the greenhouse, rapidly become green and soon come into full bloom. Although abnormal from certain points of view, they were not suffering from such inevitably lethal factors as are introduced when leaves are detached from the plants and subjected to analogous experimental conditions. Accordingly, their behavior might well be expected to be different. One of the most striking of these differences was the nature of the tissue components that were consumed in the respiration. It has already been pointed out that the average carbon content of the substances that were utilized was low, lower in fact than the average composition of carbohydrates, the figures being 34.0, 32.5 and 36.2 per cent in the three sets of experiments. These results depend upon analytical operations that are relatively easy to carry out without error, namely, the determinations of dry weight, ash weight and total carbon. It seems certain that, if the substrate of respiration during the entire growth period of these plants had consisted on the average of substances of the carbon content of a sugar, figures closer to 40 per cent would have been obtained. It is equally clear that substances of as high carbon content as the average composition of the amino acids derived from protein could have entered into the respiration only to a small extent if at all.

The earlier experiments with detached leaves in darkness are all in agreement in showing that simple sugars are promptly drawn into the metabolism after the culture conditions are set up. In darkness, there is no possibility that these sugars can be replenished by photosynthesis and the life of the tissues can only be sustained if other substances are then utilized. Analytical techniques that make it possible to detect the nature of all of these other substances have not yet been developed, but sufficient has been learned to make it moderately certain that the organic acids soon become involved, although it seems evident that transformation of these substances, that is to say, the conversion of one organic acid into another, as well as more or less complete oxidation, both take place. During the growth of the narcissus plant, on the other hand, there was no lack of simple sugars even when the plants were grown in darkness since there was a bountiful store of starch in the bulbs. This underwent hydrolysis, presumably with the production of glucose, which was withdrawn from the bulb into the leaf and root tissue. Thus, there is no necessity for assuming that any other source of energy would be required than that derived from the oxidation of simple sugars. Nevertheless, the data clearly imply that other components were in fact drawn upon, these other components being substances of carbon content lower than that of the sugars.

The observation that a moderate proportion of the nitrogen may disappear from the plants in the normal course of their development is an unusual one and presents a theoretical problem of great difficulty. The speculation of Pearsall and Billimoria has already been discussed

as a possible explanation. According to this view, the loss of nitrogen involves the interaction of amino acids with nitrous acid, this substance being produced either by the reduction of nitrates or the oxidation of ammonia. The evidence for the presence of nitrates in the original bulbs was completely negative and, although traces were detected in the roots of the starved plants, the order of magnitude was so small as to make it difficult to believe that sufficient nitrate could have been formed in the tissues to account for the relatively much larger quantity of nitrogen that disappeared. Accordingly, if one is to accept the view of Pearsall and Billimoria as the explanation of what occurred it must be assumed that all or nearly all of the necessary nitrite was formed by the oxidation of ammonia present or produced within the tissues. This involves the assumption of the existence of a vigorous oxidation system in the cells of this plant. It is difficult to avoid this conclusion in the light of the experiments of the English workers in which they demonstrated loss of substantial quantities of nitrogen from leaf segments floated upon culture solutions of ammonium salts, but the question arises as to why no losses were observed by them in experiments in which the culture solution contained amino acids or urea. If the leaf cells contain a mechanism capable of oxidizing ammonia to nitrate,[1] it must be assumed from the present experiments that they also contain a mechanism capable of producing ammonia in considerable amounts from other intracellular substances. Thus, although the English workers found that the presence of ammonium salt (or nitrate) in the culture solution was an essential feature of their observations and that the salts participated directly in the reaction, it would seem from the present results that this condition is not obligatory. Accordingly, there is still much to be accounted for before the two widely different experiments can be reconciled.

The quantity of protein in the leaves of the narcissus plants was markedly affected on the one hand by light and on the other hand by the availability of nitrogen in the culture solution. Light stimulated protein synthesis in each of the three treatments, and both nitrate and ammonia nitrogen increased the quantity of protein in the leaves as compared with the starved plants. The quantity of protein that remained in the bulb was greater in the plants that were grown in light; thus, the effect of light was to bring about a more effective synthesis of protein from the nitrogen available to the plants.

There has been much interest in recent years in the problem of protein synthesis in plants. The extensive literature has been carefully reviewed by Petrie (17) and considerable evidence can now be marshalled in favor of the view that the protein concentration attained in the leaf is a function, among other things, of the amount of energy available to the organism. Petrie has summed up the situation in general terms as follows, "We may thus expect the amounts of

[1] Pearsall and Billimoria observed the formation of nitrate in narcissus leaf samples floated upon ammonium chloride solutions.

a metabolic product to be related to the concentration of the reactants and also to certain parameters relating directly or indirectly to the energy release. These parameters are the regulators of the metabolic processes concerned." He goes on to point out that the respiration rate is one of the parameters which affect the synthesis of protein and quotes the work of Steward and Preston (38), and of Steward, Stout and Preston (39), as well as that of Gregory and Sen (8) in support of this view. The present experiments are likewise in agreement, inasmuch as there is evidence from several groups of the data that the leaves grown in light did so at the expense of larger quantities of carbohydrates consumed in the tissues during the metabolic process than was the case in the leaves grown in darkness. The soluble carbohydrates in the leaves of the plants in light were lower than in the leaves grown in darkness in spite of the probable availability of the products of photosynthesis. One of the effects of this condition was an increase in the level of the protein, in the leaves grown in light, that was evident when expressed not only in terms of absolute quantity but also in terms of concentration.

That the concentration of the reactants is likewise a factor in the efficiency of the synthesis of protein is evident from a comparison of the nitrate plants with the ammonia plants. The nitrate plants absorbed more nitrogen from the culture solution than the ammonia plants and were correspondingly enriched in protein in the leaves. This was true for both light and dark plants. It is to be noted, however, that the ammonia plants show little, if any, stimulation of protein synthesis in the leaves as compared with the starved plants. The great difference in this case is in the residual protein in the bulb. The ammonia plants made a smaller demand upon the protein in the bulb for their development than did the starved plants and, accordingly, at the time of harvest, the whole plant contained more protein. But the leaves did not differ significantly in protein content and it could be concluded that these plants made as advantageous use of the nitrogen obtained in the form of ammonia from the culture solution as did the starved plants of the nitrogen furnished as products of protein decomposition derived from their own bulbs. It would doubtless be going too far to infer from this observation that the protein synthesized in the leaves of the starved plants arose from nitrogen supplied to the cells directly in the form of ammonia, inasmuch as no proof whatever was secured that the nitrogen supplied to the leaf cells in the plants grown upon ammonium salt culture reached them as this substance. On the contrary, there was no apparent tendency for the tissues of the ammonia plants to become enriched with ammonium ion.

The variation in the quantity of protein that was metabolized in the bulbs during culture under the several conditions presents a series of difficult problems. Conditions were entirely different in this case from those which obtain in detached leaves in culture. The protein of the detached leaf promptly begins to undergo hydrolysis and the products pass through a series of reactions of which oxidation with

the production of ammonia seems to be of primary importance. The net result of the changes is frequently the accumulation of substantial quantities of either asparagine or glutamine or both, but there appears to be a limit beyond which this process cannot be carried. Ammonia itself then usually begins to accumulate and not long after this event assumes importance, death of the cells supervenes. Chemical changes do not entirely cease even then, but evidences of respiration, i. e., loss of carbon, can no longer be detected, the pigments are destroyed, the leaves become flaccid from the loss of water and brown, presumably from the action of tyrosinase or of allied oxidative enzymatic processes. The exact sequence of chemical events is not the same in all species, but the general course of the reactions is probably similar and is in all cases inevitable; there is no evidence for truly effective counteraction of the catabolic process in such leaves even when culture conditions are established that may be reasonably assumed to supply the cells with glucose or nitrogen compounds. Thus, detached leaf culture experiments furnish a picture of the disintegration of the chemical systems of the cell when the controls upon these delicately adjusted relationships are removed. The inference is unavoidable that these controls play their roles effectively only when the organism is intact; accordingly, certain factors in the controlling mechanisms must have their origin in other tissues than the leaves.

The behavior of the protein in the bulbs of the narcissus plants during growth under various cultural conditions furnishes an illustration of a protein metabolism that is indeed fully under the control of the organism as a whole. The picture presented is one that implies a sensitive adjustment of the quantity of the bulb protein to be utilized according as the demands of the growing tissues are modified by the cultural conditions. If the quantity of protein synthesized in the leaves and roots is taken as a measure of the relative level of the intensity of the metabolism in the plants, and compared with the quantity of bulb protein that was decomposed as such and utilized in the metabolic processes, it is found that, in darkness, the newly synthesized protein amounts to 77 per cent of this utilized protein in the starved plants, 116 per cent in the nitrate plants and 111 per cent in the ammonia plants. In light, the corresponding figures are, respectively, 98 per cent, 188 per cent and 173 per cent. Clearly, the synthetic processes were greatly stimulated by light, and this stimulation was nearly twice as great when nitrogen was supplied to the plants. There is no reason to assume from these figures that nitrate nutrition was more efficient than ammonia: on the contrary, the agreement between the figures is close and would lead one to infer that the two forms of nitrogen were equally readily used by the plants. There was a definite *quantitative* difference, however, inasmuch as the nitrate plants contained appreciably more leaf and root protein than the ammonia plants and correspondingly used up more of the bulb protein. But, if the premises upon which the comparison is made can be accepted, the plants differed little if at all in the efficiency with which the bulb protein was metabolized.

The contrast between these observations and those made with detached leaves is sharp. The narcissus plants used that part of the storage protein of the bulb that was needed but retained the rest intact; furthermore, they were especially responsive and efficient in their utilization of the extraneous nitrogen from the culture solution. Detached leaves, on the other hand, immediately begin to suffer loss of protein and only in rare cases, and even then only for a relatively short time, can this loss be brought under control or reversed by the conditions of culture. In the experience of this laboratory, only one clear case of protein synthesis has been encountered in detached plant parts; this occurred in the petiole of rhubarb leaves (49) cultured in light during the second to fourth day of the experiment when large quantities of protein decomposition products from the leaf blade were apparently transported into the petiole. The effect was to bring about an increase in the protein nitrogen in the petiole tissue. Pearsall and Billimoria (16) have, however, observed a few cases that could be interpreted as protein synthesis in detached leaves of *Narcissus pseudo-narcissus*. Their experiments were carried out with segments of leaves floated for 70 hours upon a culture solution that provided 3 per cent of glucose and 0.2 per cent of ammonium nitrate buffered at pH 5.4 with phosphates. The lower, that is the younger, segments of the leaves in a number of experiments gave significant evidence of an increase in the protein, although decrease was found in all other segments. In marked contrast to the present experiments with whole plants, no significant effect of light could be demonstrated upon either the decomposition or the synthesis of the protein.

Together with the enrichment of the leaves in protein as a result of the supply of nitrates in the culture solution, there was also enrichment in soluble nitrogenous components. A similar but less pronounced effect was found in the ammonia plants. It had been anticipated that ammonia nutrition would increase the quantity of amide nitrogen in the leaves and roots, but this did not occur. The quantity of asparagine or of glutamine present was in no instance high but asparagine occurred in greater amounts in the nitrate plants than in the ammonia plants. The leaves of the ammonia plants, however, contained a slightly larger quantity of glutamine than those of the nitrate plants. The narcissus plant is evidently not a species that responds to ammonia in the culture solution by the accumulation of large amounts of amides.

The sharpest contrast was in the capacity of the plants to store excess of the nitrogenous nutrient ion in the tissues. The nitrate plants contained a substantial amount of nitrate nitrogen, the roots being especially enriched in this component. The ammonia plants, on the other hand, contained only a trace of "free" ammonia nitrogen, differing hardly at all in this respect from the nitrate plants. The presence of substantial quantities of nitrate in plant tissues is an evidence that the rate of supply through the roots has outstripped the

rate of assimilation, that is that the plant is existing, temporarily at least, at a luxury level of nitrogen nutrition. Storage of ammonia in plant tissues is far less common than is storage of nitrate, although it does occur in certain species, rhubarb being a well known example. Under specially contrived culture conditions, however, even the tobacco plant can be made to accumulate substantial amounts and it does this without evidence of serious harm. The narcissus plants showed none of this capacity. They responded to ammonia nutrition to the extent that nitrogen derived from the culture solution was readily used for metabolic processes. The plants grew in most respects at least as well as the starved plants and on many points of composition approached the nitrate plants somewhat closely. Thus, ammonia nutrition placed no drawback upon their development that could be clearly interpreted as a "toxicity". However, inasmuch as the plants were in many ways smaller than the nitrate plants, especially in leaf development, it is natural to infer that ammonium ion is a less suitable form in which to supply nitrogen to this species than nitrate.

The failure of the plants to show evidence for the capacity to store ammonium ion hints at some impediment to its effective absorption from the culture solution. Although the molar concentration of the nitrogen in the two culture solutions was the same, the ammonia plants were not existing at a luxury level of nutrition as seemed to be the case for the nitrate plants. Accordingly, another problem is presented. It will be necessary to discover at what level of ammonia nitrogen concentration in the culture solution the effects on general metabolism would simulate those observed with the present level of nitrate nitrogen. This raises a question regarding the other components of the culture solution, in particular those that serve to maintain the acidity at a suitable point for maximum availability of nitrogen under the two sets of conditions.

The low level of organic acids in the tissues of the narcissus plants places this species in a different category from most of the few other species that have been studied in detail. Organic acids are known to form a far larger proportion of the solids, particularly of the leaf tissues, in most of the known cases than was observed with this plant, and their synthesis and transformations accordingly occupy a more important place in the general metabolism of such plants. Furthermore, the fact that nearly one-half of the organic acidity of the narcissus plant tissues belongs to substances of unknown identity places a serious limitation upon the possibility of understanding their organic acid metabolism at the present stage of development of the analytical methods.

Only one of the known organic acids showed marked effects of the conditions of culture. Malic acid was proportionately and absolutely greatly increased in the leaves and to a lesser extent in the roots of the plants grown upon nitrates. Citric acid showed a similar vari-

ability but upon a much smaller scale. The observations bear a close analogy to those upon the tobacco plant (50) in this respect, although the scale of relative magnitudes was smaller. It seems clear, however, that in both of these widely different species nitrate nutrition calls forth an enrichment of the leaves in malic acid.

In the absence of a theoretical approach to the problem of nitrate metabolism in plant leaves, the explanation of this fact is impossible and speculation upon the problem is still scarcely justified owing to the limited nature of the information available. Nevertheless, the time is approaching when the details of the mechanisms that bring about the reduction of the nitrate ion in the plant must be ascertained. The correlation between an enhanced concentration of organic acids, in particular of malic acid, and the presence of an intense nitrate metabolism is too close to be the result of chance and provides a suggestion that, whatever the mechanism of nitrate reduction may be, it involves transformations of organic acids. Present day views of respiration in living tissues suggest that a series of complex and interrelated reactions of organic acids is concerned. Carbon fixation in the plant (33) also appears to be a reaction that occurs through the agency of the organic acids. Many of the oxidation-reduction reactions of plants thus depend in part at least upon these substances and it is no serious stretch of the imagination to assume that nitrate reduction, another of the fundamental reactions of plant tissues, may likewise involve the interconversion of certain of the organic acids. If this is the case, the enrichment with acids of the tissues of plants in which nitrate reduction is proceeding would have a reasonable explanation. Furthermore, it would seem from what evidence has been accumulated that malic acid is specifically concerned with this reaction.

With respect to the quantities of material involved, by far the most important reaction that occurred during the growth of the narcissus plants was the decomposition of the starch in the bulbs and the mobilization of the soluble products into the growing tissues. This decomposition has been assumed to represent conversion into glucose. The evidence points to the presence in the translocation streams to the leaves and roots of large quantities of glucose, whereas the failure of the tissues to become unusually enriched with this sugar indicated that it was rapidly and effectively metabolized. This is particularly true of the roots which contained only minute quantities. There was a marked difference between the leaves of the plants grown in light and those grown in darkness with respect to the quantity of glucose found; the leaves in light contained far less, in spite of the fact that there was every opportunity for photosynthesis and a corresponding accumulation of reducing sugars. The contrast with the behavior of detached tobacco leaves cultured in darkness and in light (47) is marked. In this case, the leaves in light rapidly increased in carbohydrate content as a result of photosynthesis, although those cultured in darkness soon became nearly sugar-free. The fundamental

difference in behavior may possibly be attributed to the wide difference in the protein and general nitrogen metabolism in the two cases. In the tobacco leaves, even in light, decomposition of the protein proceeded rapidly with accumulation of a complex mixture of soluble products. This seemed to have little effect upon the mechanisms provided for the synthesis of sugars and so the two systems, namely, the protein metabolism and the carbohydrate metabolism, pusured separate courses. In the narcissus leaves, on the ohter hand, the course of the metabolism was essentially normal; the protein metabolism was upgrade rather than downgrade, and the drain upon the carbohydrates for the support of this metabolism was not relaxed. Accumulation of a large excess of glucose under these circumstances would not be anticipated and it did not occur. The more vigorous metabolism in the leaves in light effectively disposed of the products of photosynthesis and also diminished the quantity of sugars that may have been presumed to have been derived from the decomposition of the starch in the bulbs.

The fact that the sucrose content of the original bulb was only moderately decreased at the time of harvest has already been mentioned as one of the more puzzling observations. It implies that sucrose was not transported from the bulb at a rate at all comparable to that of the starch decomposition products or else suggests that there was a tendency to store this substance. The data of Nightingale and Robbins indicate that sucrose at first decreases in the bulb and then shows a tendency to increase. Whether this is a result of a downward stream of metabolic products or is the effect of synthesis from precursors already present in the bulb is not clear. At all events, sucrose is the predominant soluble carbohydrate of the bulb tissue, even in the case of the plants that were metabolizing relatively very much larger quantities of starch.

SUMMARY

Groups of bulbs of *Narcissus poeticus* of equal weight were grown under conditions that supplied, respectively, water alone, a complete culture solution that contained nitrate and a complete culture solution that contained ammonium salts. Duplicate sets were maintained in complete darkness and in continuous light, the flower stalks being removed as they separated from the sheath of leaves so that the plants were entirely in a vegetative state. At the end of 28 days, the leaves and the roots were separated from the bulbs and all tissues were dried for chemical analysis, the small quantities of flower stalk tissue being added to the leaf fraction. Analytical data for all tissues, as complete as possible with existing methods, was collected and computed upon a basis of 50 original bulbs. A separate sample of the original bulbs was also dried and analyzed. Comparison of the composition of the plants with that of the bulbs thus permits inferences to be drawn with regard to the main types of chemical reactions that took place during the development of the plants from the storage tissue under

conditions of starvation with respect to the supply of inorganic nutrients, with supply of nitrates and with supply of ammonium salts, in each case both in light and in darkness.

The major component of the bulbs is starch, protein making up a relatively much smaller proportion of the storage material. Growth upon nitrates led to what appeared to be in most respects a superior development of the plants either in darkness or in light; growth upon ammonium salts yielded plants that with respect to size were not greatly different from the starved plants supplied only with water. There was, nevertheless, a marked stimulation of the protein metabolism in the ammonia plants as compared with the starved plants and considerable evidence was found that this species can make effective use of ammonium salts in the nitrogen metabolism. No marked effect upon the composition with respect to the amides asparagine and glutamine was, however, noted; the nitrate plants were in general richer in these components than the ammonia plants.

The plants grown with no external supply of nitrogen gave evidence for loss of nitrogen from the tissues during the course of their development. The loss amounted to 15 per cent of that present in the original bulbs in the plants grown in darkness and 11.5 per cent in the plants grown in light. This unusual observation has been discussed in terms of the hypothesis of Pearsall and Billimoria, according to which loss of nitrogen is due to interaction between amino acids and nitrous acid, this last substance being formed either by reduction of nitric acid or oxidation of ammonia.

Data for the organic acids of the tissues were obtained, being the first to become available for plants of this botanical group. The tissues were unusually low in organic acids and present a marked contrast in composition to such better known plants as tobacco and rhubarb. It was found that the common plant tissue components, malic, citric, oxalic and succinic acids account together for only about one-half of the total organic acids of the tissues and the impression was obtained that one or other of the acids for which no analytical methods are at present available is in fact the predominant organic acid component of the tissues of this plant.

The organic acids were not strikingly responsive to the variations in the conditions of culture with the exception that the tissues of the nitrate plants were especially enriched in malic acid. This observation is in line with data already recorded concerning the specific effect of nitrate nutrition upon the organic acids of plants.

The carbohydrate metabolism in this species is dominated by the presence of large quantities of starch in the bulbs. The starch was drawn upon rapidly for the development of the leaves and roots, the loss from the bulbs being greatest in the nitrate plants. The starved plants and the ammonia plants drew about equally upon the starch stores in the bulbs. Conclusive evidence for photosynthesis in the

plants grown in light was not secured inasmuch as the gain in organic solids from this cause did not compensate for the losses due to respiration, and the soluble carbohydrate content of the leaves of the plants grown in light was lower than that of the plants grown in darkness. Nevertheless, the net loss of carbon from the plants grown in light was much smaller than that from the plants grown in darkness. Accordingly, there is little doubt that photosynthesis did in fact occur.

Examination of the carbon content of the tissues gave evidence for the extent of the respiration of the plants grown in darkness. It was observed that the average carbon content of the substances that disappeared from the plant tissues was somewhat lower than that of glucose.

The composition of the several tissues was calculated from the analytical data with the aid of certain assumptions regarding the nitrogen content of the main known and unknown components. In this way, approximately three-quarters of the leaf and bulb tissue could be fairly reasonably accounted for, but only about 40 per cent of the solids of the root tissues could be allocated. The presence in the roots of a considerable proportion of substances characterized by an unusually high capacity to become hydrated was inferred.

A brief comparison has been made between the results of the present analytical study and those of Nightingale and Robbins who have investigated an allied species.

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