Bulletin 442 November, 1940

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VIII. THE EFFECT UPON THE COMPOSITION OF THE TOBACCO PLANT OF THE FORM IN WHICH NITROGEN IS SUPPLIED

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INTRODUCTION

ALTHOUGH nitrogen is supplied to plants in agricultural practice in many forms, it is generally held today that absorption by the roots takes place largely if not entirely as nitrate and ammonium ions. The biological mechanisms whereby nitrogen derived from the air, or from the decomposition of plant or animal matter in the soil, is converted into either or both of these ions have received a great deal of attention, and studies of plants in culture solution have shown that organisms, normal in every way so far as can be perceived, can be produced when the only nitrogen available to them is furnished under proper conditions in the form of nitrates or of ammonium salts. It is possible that other nitrogenous substances can and do enter the plant system through the roots. Recent observations on the stimulation of plant growth produced by traces of thiamine (vitamin B_1) (3, 20) suggest that this substance may be absorbed, but the evidence with respect to most substances that have been studied (e.g. urea, aspartic acid, etc.) is inconclusive since the entry into the plant system in unchanged form has seldom if ever been demonstrated.

The relative value or availability to the plant of ammonium and of nitrate ions has been much debated. In fairly recent times it has been commonly held that only the nitrate ion is really utilized, it being assumed that all forms of nitrogen in the soil are ultimately converted into nitrate by microorganisms before being absorbed by the plant. On the other hand, many early investigators (17) followed Liebig in maintaining that plants could utilize ammonium salts equally well or even better. The results of many of the older studies are of doubtful significance, however, through a lack of appreciation of certain factors that influence the absorption of the respective ions. A thorough review of the entire problem has been given by Pardo (16).

The work of Shive and his associates at the New Jersey Experiment Station (8, 11, 15, 24, 25) has, within the past few years, made it clear that differences that have been noted between the availability of these two ions, as expressed by the development of the plant, may frequently be accounted for in terms of the reaction of the culture solution that surrounds the roots. Absorption of ammonium ions is favored by maintenance of a reaction close to neutrality or even on the alkaline side, whereas absorption and assimilation of nitrate ions takes place best from culture solutions that are slightly acid (pH 4 to 5).

The removal of the ammonium ion from the culture solution by the plant leads to an increase of acidity which, unless corrected by renewal with fresh solution or by neutralization, may become sufficiently acute to interfere with the proper development of the plant. The impression may thus arise that ammonium salts are "toxic" (13, 18). Modern technics of experimentation to a large extent avoid this difficulty, however, and the conclusion has been drawn by Tiedjens and Robbins (25) that ammonium ions are no more toxic than nitrate ions under proper conditions.

The agricultural problems presented by the use of nitrogen in one or other of these forms are very complex. Choice is dictated by the conditions in the soil which must be controlled, by the tolerance of the species to the soil conditions that are achieved, and also by the susceptibility of the species to diseases which may arise or which may be controlled by the soil conditions that are established. If these factors are successfully dealt with, an organism that is regarded as a "normal" plant results and the agricultural problem is, to this extent at least, solved.

To the biochemist, however, the problem is presented in somewhat different terms. Absorption of ions by the roots is not merely a matter of accepting what chances to be present in the soil solution; the tissues exercise a very definite discrimination with respect to the nature and quantity of the ions they take in, and the mechanisms that control the selection are still imperfectly understood. However, granting that a plant, in order to reach a certain size within a certain time, must acquire a certain quantity of nitrogen, it seems obvious that there must be differences in the details of the mechanisms called into play if, on the one hand, the nitrogen is presented entirely in the form of cations, and, on the other hand, entirely in the form of anions. The exercise of these diverse mechanisms may well be expected to result in differences in the composition of the tissues ultimately produced.

A priori it would seem that ammonium salts are more readily available for use in the synthesis of tissue components than nitrates since there is little doubt that nitrates must be reduced to ammonia, or to substances of an allied nature, before the nitrogen can be assimilated. Such differences may find their expression in many ways, but one obvious difference would be in the disposition of the plants grown with nitrogen supplied in one or the other form to store the excess of absorbed nitrogen without chemical change. It is well known that many plants store nitrate nitrogen, sometimes in surprising quantities. Storage of substantial amounts of ammonium nitrogen is, however, a much less commonly encountered, although by no means rare, phenomenon.

The literature of plant composition, as influenced by the form in which nitrogen is supplied, is very limited. Most investigators have worked from the agricultural point of view and have recorded their results in terms of size, crop yield, luxuriance of foliage, etc., or at best in terms of dry weight of the plants. Tiedjens and Robbins

(25), however, have given fairly detailed analyses of tomato and soy bean plants grown with careful control of the cultural conditions. Their largest tomato plants, grown for 53 days with nitrogen supplied as ammonium sulfate, were obtained with a solution administered at pH 7.9. The stems of these plants weighed on the average 55 gm. (fresh) and contained 0.251 gm. of nitrogen exclusive of ammonia nitrogen. There was no nitrate nitrogen present and only a trace (<1 percent of the total nitrogen) of ammonia.[1] The largest plants grown on nitrate were obtained at pH 4.0. The stems of these weighed 77 gm. and contained 0.142 gm. of nitrogen. There was no detectable amount of ammonia, but storage of nitrate nitrogen was phenomenally high; no less than 30 percent of the total nitrogen present consisted of nitrate nitrogen. Somewhat similar results were secured with soy beans, although storage of nitrate was far less striking with this species.

These experiments suggest that important differences in the composition of plants grown under the two conditions mentioned may occur, and this conclusion is supported by the work of Clark (6). Clark grew tomato plants in culture solution administered continuously at pH 6.7[2] for 49 days and subjected the tissues to chemical analysis. The ash in the leaves and stalks of the nitrate plants was respectively 17.2 and 20.7 percent of the dry weight, that of the ammonium plants was 12.7 and 14.0 percent. There were wide differences in the nitrogenous components; the protein nitrogen and the soluble "organic" nitrogen were much higher in the ammonium plants than in the nitrate plants, and the amide nitrogen was enormously higher. Both glutamine and asparagine were present in stalks and leaves in approximately 10 times greater concentration in the ammonium plants than in the nitrate plants — in fact more than 5 percent of the dry weight of the stalk tissues of the former consisted of glutamine. Ammonia was stored in the tissues of the ammonia plants in only moderate amounts; for example, 2.4 percent of the total nitrogen of the leaves and 4 percent of that of the stalks was ammonia nitrogen. Nitrates were stored in far larger quantities in the nitrate plants; 7.3 percent of the leaf nitrogen and no less than 46.4 percent of that of the stalks was nitrate nitrogen.

The outstanding difference between the ammonia and the nitrate plants was, however, in the relative amounts of organic acids in the tissues. The leaves and stalks of plants grown on ammonium nitrogen contained respectively 71 and 65 milliequivalents of organic acids per 100 gm. of dry tissue, those of the plants grown on nitrate nitrogen contained 153 and 147 milliequivalents. Furthermore, the distribution of this organic acidity with respect to the relative pro-

[1]Smaller plants that resulted from growth at lower pH levels contained appreciable proportions of ammonia nitrogen.

[2]The tomato plant absorbs and assimilates nitrate nitrogen over a wider range of pH than it does ammonium nitrogen. The reaction selected was nearly optimal for ammonium absorption and only slightly higher than optimal for nitrate (7, 8, 25).

portions of oxalic, malic, citric, and unknown organic acids was widely different in the two cases.

The difficulty with these results of Clark is that the plants grown under the two sets of conditions were quite different in size. Although both groups of plants were the same age and both were beginning to set fruit, the ammonia plants were smaller and slimmer than the nitrate plants and both fresh and dry weight were much less.[1] The great differences in composition might, therefore, in part at least, be accounted for in terms of degree of tissue development.

Consideration of factors that may affect the growth of plants suggested that the comparison of greatest value, from the physiological point of view, is not that between plants that have been grown exclusively on one or the other source of nitrogen, but is the comparison of the relative effects of the two ions on the composition of the plant. The production of two plants of the same size, age, and degree of development, respectively, on each of the two different nitrogenous ions would probably be difficult if not impossible, and could almost certainly not be achieved if the nutrients were administered under identical conditions in each case. On the other hand, if plants were grown on a series of otherwise similar culture solutions of the same nitrogen content, in which the relative proportions of nitrate and ammonium ions were varied in steps between the limits 100 percent of nitrate to 100 percent of ammonium nitrogen, the effects of these ions on the composition of the plant should become apparent when expressed in terms of the nitrogenous composition of the culture solution. A description of a series of tobacco plants grown in this way forms the subject of the present Bulletin.

CULTURE SOLUTIONS

The culture solutions employed were patterned after Livingston and Tottingham's solution R_3S_4 (12), but were prepared at an osmotic pressure of approximately 0.5 atmos. This solution has a fundamental molecular relationship of KH_2PO_4:$Ca(NO_3)_2$:$MgSO_4$ of 3:4:1 and was selected so as to keep the proportion of sulfate ion in the initial solution as low as possible. In order to supply nitrogen as ammonium ion, calcium nitrate was replaced in a series of steps with ammonium sulfate, and to maintain the calcium content constant, calcium chloride in amount equivalent to the ammonium sulfate was also added. The composition of the solutions as administered is shown in Table 1.

The salts were made up in stock solutions at 0.5 M concentration and were diluted as needed in 8-liter lots with tap water. To each lot, sufficient of standard solutions of manganese, boron, and iron were added to provide 0.5 p.p.m. of each of these and of Hoagland's "AZ" (10) solution to provide 0.01 to 0.02 p.p.m. of each

[1]Nitrate plants: fresh weight per plant, leaves and stalks respectively, 94.4 and 175 gm.; dry weight 10.1 and 12.5 gm. Ammonia plants: fresh weight, leaves and stalks respectively, 38.7 and 53.7 gm.; dry weight, 4.1 and 4.1 gm.

minor element. The phosphate mixture was prepared from 0.5 M solutions of the mono-basic and dibasic salts and sufficient was taken of each to give the final reaction desired. For the first month of growth, the reactions varied from pH 4.9 for the nitrate solution to 6.3 for the 90 percent ammonia. For the balance of the 52-day period of growth, the reactions of all save the nitrate solution were adjusted to pH 6.4 to 6.6. To achieve this, 45 percent of the total amount of phosphate solution added consisted of K_2HPO_4. The solutions were found to be stable in this range but deposited a precipitate if the reaction was carried to pH 6.7.

It will be noted that the alteration of the composition with respect to the form of nitrogen involved a step-wise increase in the chloride and in the sulfate ion content of the solutions. Supplementary experiments designed to throw light upon the effects of these and of other negative ions were, therefore, carried out and the results are given in an appendix to the present Bulletin. It was found that the effects of changes in the proportion and nature of the negative ions seem to be far outweighed by the effects of the change in the form of nitrogen.

TABLE 1. MOLAR CONCENTRATION OF CULTURE SOLUTIONS THAT SUPPLY NITROGEN AS NITRATE AND AMMONIUM ION AT DIFFERENT RATIOS BUT WITH CONSTANT NITROGEN, POTASSIUM, AND CALCIUM

Percentage of N as ammonium ion	0	20	40	60	80	90
KH_2PO_4-K_2HPO_4 mixture	0.00325	0.00325	0.00325	0.00325	0.00325	0.00325
$Ca(NO_3)_2$	0.0043	0.00344	0.00258	0.00172	0.00086	0.00043
$CaCl_2$		0.00086	0.00172	0.00258	0.00344	0.00387
$(NH_4)_2SO_4$		0.00086	0.00172	0.00258	0.00344	0.00387
$MgSO_4$	0.00105	0.00105	0.00105	0.00105	0.00105	0.00105

CULTURE OF PLANTS

Tobacco seeds of the Rosenberg strain grown in 1936 were sprouted on sand moistened with culture solution at the 20 percent ammonia level and, on June 18, 1937, 31 days from germination, were transplanted to sand in individual crocks. Groups of five plants of uniform development were selected and each group was supplied with one of the culture solutions. The continuous drip method of Shive was started after four days and continued for 14 days when it became more convenient to resort to administration by flushing. Subsequently each plant received about 2 liters of solution daily, two-thirds of which was applied early in the morning and the balance late in the afternoon.

The plants grew well; those on the two highest proportions of ammonia were visibly retarded even at the end of one week, but

the plants on 20 percent of ammonia grew better than the nitrate controls or than any of the others. At the end of 52 days, a few flower buds were beginning to form and the plants were accordingly harvested. The stalks were cut at the base, the leaves were removed and weighed, and the stalks were measured and weighed. The buds were discarded. The roots were carefully washed free from sand, the short length of transitional tissue was discarded, and the remainder was weighed. All tissues were then dried for analysis, the stalks after being cut into short lengths, in a ventilated oven at 80°. The dried roots were found to have retained considerable sand which was separated from the dry material with a sieve and weighed. A correction of 22 percent, based on experiments with the sand, was added to the weight of the dry sand separated from the root tissue to allow for its water holding capacity, and this corrected value was subtracted from the weight of the roots to provide an estimate of their fresh weight. Neither fresh nor dry weight of the root tissue was exact since sand was still retained in considerable amounts. A simple method to remove most of this was, however, found: a portion of the dried fibrous material was stirred with a considerable volume of chloroform, whereupon the sand promptly settled to the bottom and the root particles floated. These were removed and dried quickly. They were exposed to the action of chloroform for only a few minutes, and the loss by extraction of chloroform-soluble components could not have been great. The dry weights given in Table 2 were obtained by this procedure.

Table 2 shows the fundamental data on this series of plants. One plant from the 60 percent and one from the 90 percent series were discarded because they failed to grow well. An index of the degree of variability of the individual plants is furnished by the standard error of the mean fresh weight of the leaves and the stalks. It is interesting and probably significant that the 20 percent ammonia plants were larger than any of the others and were less variable. With the exception of the 80 percent ammonia plants, the variation was of the order $\pm$ 10 percent.

ANALYTICAL METHODS

The analytical methods employed in this laboratory have for the most part been described in previous publications. An outline of the chief methods may be found in the sixth of this series of bulletins on the tobacco plant (29) and references to the original papers are there given. Minor modifications in these methods have been introduced from time to time in the interest of greater convenience and accuracy, but no fundamental changes have been made.

The residual moisture content of the crude dry tissue was obtained by heating for four hours at 105° in weighing bottles. The weighings were made without exposure of the dry materials to the air, and strict adherence to a time routine of heating and of cooling in the desiccator was found necessary.

TABLE 2. FRESH AND DRY WEIGHTS OF TOBACCO PLANTS GROWN IN SAND CULTURE WITH CONSTANT NITROGEN SUPPLY BUT WITH VARIATION IN THE RATIO OF AMMONIUM ION TO NITRATE ION

		LEAVES				STALKS				ROOTS
Percentage of N of culture solution as ammonium ion	Number of plants	Number of leaves	Fresh wt.	Fresh wt. per plant	Crude dry wt. per plant	Average length	Fresh wt.	Fresh wt. per plant	Crude dry wt. per plant	Crude dry wt. per plant
			gm.	gm.	gm.	cm.	gm.	gm.	gm.	gm.
0	5	214	2550	510± 44	41.40	186±11	1018	417±47	41.12	12.24
20	5	244	3384	677± 29	51.52	199± 4	2607	521±19	49.32	17.0
40	5	238	2894	579± 49	38.86	192± 7	2159	432±43	35.22	6.32
60	4	183	2583	646± 66	40.25	191± 5	1696	424±19	35.52	8.75
80	5	220	2787	557±106	30.46	164±10	1431	286±20	19.84	3.7
90	4	176	2008	502± 23	27.78	148±12	961	240±32	17.89	

Ash was determined by heating samples of tissue in porcelain capsules for 16 hours in an electric muffle at 600°, this technic having been found to yield closely reproducible results. Duplicate determinations were invariably made and if these failed to agree were repeated.

The composition of the ash was of interest in the present study and determinations were accordingly made of the alkalinity of the ash and its sulfate, chloride, calcium, and in two cases of its phosphorus content.

In order to determine the alkalinity of the ash, 0.500 gm. of tissue was ashed as usual and 10 ml. of accurately standardized 0.25 N hydrochloric acid were added. The capsule was heated on the steam bath for 10 minutes, with careful stirring, and the contents were transferred to a beaker and titrated with 0.1 N sodium hydroxide using methyl red as indicator. Twelve determinations on a single sample of a tobacco leaf tissue gave the results in Table 3.

TABLE 3. DETERMINATIONS OF ALKALINITY OF ASH IN A SINGLE SAMPLE OF TOBACCO LEAF TISSUE

Ash	0.1 N NaOH	0.1 N HCl used[1]	Alkalinity m. e. per 100 gm. dry tissue
%	ml.	ml.	
16.20	10.05	13.75	275
15.76	10.10	13.70	274
16.16	10.05	13.75	275
15.98	10.10	13.70	274
16.24	10.10	13.70	274
15.60	10.00	13.80	276
15.10	10.05	13.75	275
15.78	9.95	13.85	277
15.26	10.10	13.70	274
15.58	10.05	13.75	275
15.64	10.05	13.75	275
15.42	10.00	13.80	276
15.80	10.05	13.75	275
Av. 15.73 ± 0.35			275 ± 0.8

[1]Actual hydrochloric acid used was 0.238 N.

The data represent several groups of experiments in which such factors as the period in the muffle and the time of heating the ash with acid were varied between wide limits. No obvious effect of these variations was noted. The ash weight was reproducible within about 2 percent and the alkalinity determination within 0.3 percent.

The solutions from duplicate determinations of alkalinity were combined, acidified, and treated with barium chloride in order to determine sulfate ion. The filtrate from this precipitate was treated with ammonium molybdate in the usual way and phosphorus was determined as magnesium pyrophosphate. The chloride content of the ash was determined in a separate sample of leaf tissue gently

ignited with sodium carbonate. Calcium determinations were made in the usual way.

CALCULATION OF DATA

The analytical data obtained as percentages of the dry tissue (crude dry weight) were converted, by the use of factors computed for each set of plants, into grams of each constituent per single plant. In certain cases the data were then placed upon a concentration basis by calculation in terms of grams per kilo of fresh weight of each tissue. Factors to effect this conversion were calculated from the fresh weight of the respective samples. Since no reliable values for either fresh or dry weight of the root tissue were available, the concentration data were arbitrarily expressed in terms of grams per 50 gm. of organic solids of the roots. The organic solids could be determined accurately and the assumption involved in the use of 50 gm. is that fresh root tissue contains 5 percent of organic solids. This was approximately true and the root data on this basis, therefore, conform reasonably well with the leaf and stalk data expressed on a kilo of fresh weight basis.

WATER, SOLIDS, AND ASH

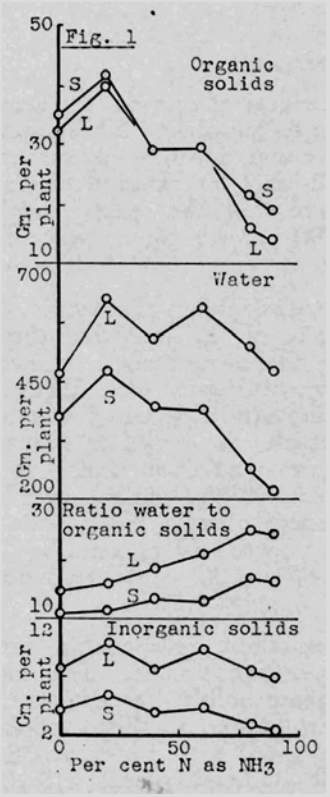
The curves in Figure 1 show the general effects on the size of plants of the change in the form of nitrogen supplied. The quantity of organic solids in the 20 percent ammonia plants was greater than that in the nitrate controls, but the 40 and 60 percent ammonia plants showed no striking difference save that the stalks weighed slightly less. The solids of the 80 and 90 percent plants were considerably less than those of the controls.

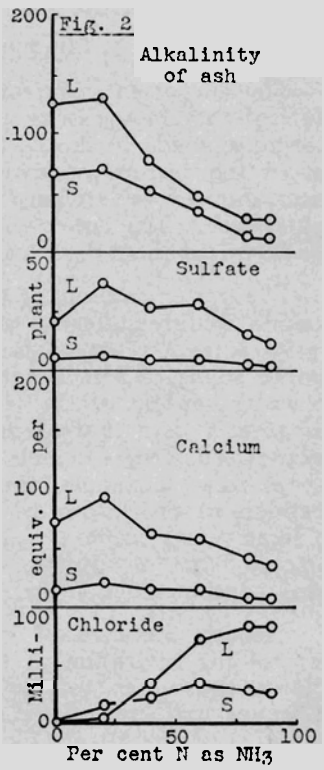
The water content of the plants, particularly in the leaves, followed a widely different course. In spite of the fact that the 80 and 90 percent plants were smaller than the nitrate controls, i.e. in organic solids and in height and number of leaves (see Table 2), the water content of the leaves of all plants that received ammonia was greater than that of the nitrate controls; the curve in Figure 1 is somewhat irregular but obviously represents a function that increases to a maximum and then decreases. The stalks, with the exception of the larger 20 percent ammonia plants, had practically the same water content as the controls up to the 60 percent level of ammonia nutrition, but the stalks of the 80 and 90 percent ammonia plants contained much less.

Perhaps the clearest way to express these relationships is in terms of the hydration of the solids in the two tissues. The ratios between the water content and the organic solids, respectively, of the leaves and stalks, are also plotted in Figure 1. The solids of the leaves of the nitrate plants were associated with 14.5 times their weight of water, those of the 90 percent ammonia plants with 24.2 times their weight of water; the ratios for the intermediate plants fall along a nearly straight line between these extremes. In the stalks, the solids of the nitrate plants were associated with 10.8 times

their weight of water, those of the 90 percent ammonia plants with 16.1 times, and again the intermediate plants lie along a very nearly straight line between these limits. The substitution of ammonium for nitrate ion in the culture solution, therefore, gave rise to plants characterized by progressively increasing degrees of hydration. This implies, in turn, a fundamental difference in chemical composition which extended to both leaf and stalk.

As a consequence of the increase in hydration of the tissues, it is clear that the organic solids, in terms of grams per kilo of fresh weight, decreased as the proportion of ammonia in the culture solution was increased. Curves for these relationships are not plotted, but the data are given in Table 4; for the leaves the concentration dropped from 63.3 gm. of organic solids per kilo of fresh tissue in the nitrate plants, along a nearly straight line, to 39.0 gm. for the 90 percent ammonia plants. The data for the stalks give a less satisfactorily straight line, but there was a more or less steady drop from 83.9 to 57.8 gm. per kilo.





The inorganic solids in the leaves (Figure 1) show very little consistent change over the series and only a minor diminution in the stalks of the plants at the highest level of ammonia nutrition.

However, as is clear from the data of Figure 2, although the quantity of ash per plant was not greatly affected, there was a marked change in the composition of the ash as a result of the change in composition of the culture solution. It will be recalled that, in order to maintain the calcium content the same in each culture solution, calcium chloride was added as calcium nitrate was removed. Thus the concentration of chloride ion (and also of sulfate ion) supplied to the plants was increased in step with the ammonium ion. These changes in the solutions, in addition to the change in the form of nitrogen, had their effect upon the composition of the ash. The behavior of the alkalinity of the ash is particularly striking. The nitrate plants yielded a highly alkaline ash, the 90 percent ammonia plants a moderately alkaline one and the intermediate data follow relatively smooth curves for both leaf and stalk. This at once suggests that there were wide differences with respect to the organic acid content of the tissues and, as will be shown below, such differences were, in fact, found.

TABLE 4. ORGANIC SOLIDS, WATER, AND ASH OF TOBACCO PLANTS, AT TIME OF FIRST FLOWER BUD FORMATION, AS INFLUENCED BY THE PROPORTION OF NITRATE AND AMMONIUM IONS IN THE CULTURE SOLUTION

Percentage of N of culture solution as ammonium ion	0	20	40	60	80	90
			LEAVES			
Organic solids, gm./plant	32.3	39.7	29.2	29.2	21.4	19.6
Organic solids, gm./kilo fresh wt.	63.3	58.7	50.4	45.2	38.4	39.0
Water, gm./plant	469	627	541	607	529	475
Ratio of water to organic solids	14.5	15.8	18.5	20.8	24.7	24.2
Inorganic solids, gm./plant	7.79	10.0	7.82	9.29	7.45	7.06
Alkalinity of ash, m.e./plant	125	130	77.7	47.5	28.8	27.8
Sulfate in ash, m.e./plant	21.1	37.4	27.5	28.4	15.6	11.2
Chloride in ash, m.e./plant	0.98	5.09	35.5	73.1	84.3	84.3
Calcium in ash, m.e./plant	72.0	93.3	61.7	58.4	41.9	36.6
Phosphate in ash, m.e./plant	6.45			7.24		
			STALKS			
Organic solids, gm./plant	35.0	41.4	29.5	29.5	16.0	13.9
Organic solids, gm./kilo fresh wt.	83.9	79.5	68.3	69.4	55.8	57.8
Water, gm./plant	378	474	398	390	267	224
Ratio of water to organic solids	10.8	11.5	13.5	13.2	16.7	16.1
Inorganic solids, gm./plant	4.29	5.60	3.99	4.30	2.92	2.56
Alkalinity of ash, m.e./plant	66.6	69.7	51.4	34.9	13.6	11.6
Sulfate in ash, m.e./plant	5.53	6.85	5.53	5.30	3.10	2.28
Chloride in ash, m.e./plant	0.69	17.3	21.3	33.9	28.9	26.9
Calcium in ash, m.e./plant	13.2	20.0	15.3	14.3	8.4	7.0
			ROOTS			
Organic solids, gm./plant	9.98	11.2	5.08	7.51	3.21	3.52
Inorganic solids, gm./plant	1.93	5.02	0.95	0.96	0.30	0.66
Water, gm./plant	131	136	103	99.4	52.8	54.3

The sulfate content of the ash of the leaves follows a curve similar in outline to the curve for organic solids, that is, the sulfate content was a function of the size of the plants rather than of the

composition of the culture solution in spite of the regular increase in the sulfate ion content of the solutions. It will be noted that the 90 percent ammonia plants were grown in a solution that contained nearly five times the molar concentration of sulfate ion as the nitrate plants but, nevertheless, contained less sulfate in the ash. The sulfate content of the ash of the stalks also followed the size of the stalks as measured by organic solids.

The chloride content of the ash, however, bore very little relation to the size of the plants. The curves in Figure 2 show that the chloride in the leaf ash increased nearly proportionally to the chloride content of the culture solution. The chloride content of the initial nitrate culture solution was very low, being made up chiefly from the traces of chloride ion added with the heavy metals in the "AZ" solution. The 20 percent ammonia plants, however, were grown in a solution 0.00086 M in calcium chloride and successive increments of chloride ion were added to prepare each of the solutions at the higher ammonia ratios. Accordingly, if the chloride in the leaf ash followed the composition of the culture solution precisely, the plot of the data would be a straight line inclined to the axis of abscissae and originating from a point just above the origin of the diagram as plotted. The data as a whole conform to this assumption but minor influences of the size of the plants are apparent at the higher levels of ammonia and chloride content of the culture solution.

The chloride in the ash of the stalks increased less rapidly than that in the leaves and was proportionately considerably lower in the stalks of the 80 and 90 percent ammonia plants than would be expected if a simple concentration rule was followed. The most important point is, however, the marked contrast in the behavior of the chloride and sulfate ions. The plants absorbed chloride ion more or less in proportion to the concentration provided in the culture solution, but they absorbed sulfate ion more nearly in proportion to the size of the plants regardless of change in the culture solution.

The calcium in the ash of the leaves (Figure 2) follows a curve that closely resembles that for the organic solids. At a constant calcium level in the culture solution, the calcium absorbed in the leaf was a function of the size and the variation was not nearly so striking as was the variation in the alkalinity of the ash, nor, as will later appear, as the variation in the organic acids. In the stalks there was, in fact, very little change in calcium content (with the exception of the 20 percent ammonia plants) up to the 60 percent ammonia level. The smaller stalks of the 80 and 90 percent ammonia plants contained somewhat less calcium. Thus calcium absorption at a constant level of calcium supply was little affected by the change in the form of nitrogen in the solution; such differences as were noted can be interpreted only as secondary results of the change in the nitrogen nutrition.

A complete series of analyses for phosphorus was not made. The few points obtained show, however, that the phosphate absorbed

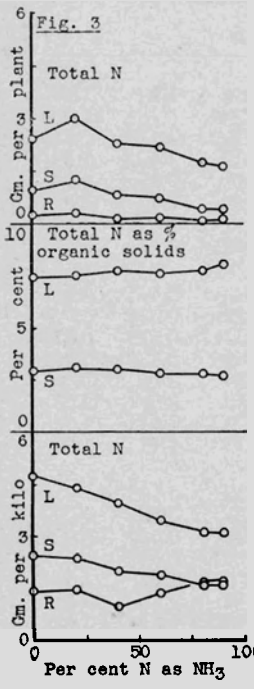
was little influenced by the change in the form of nitrogen. That is to say, phosphate absorption, like that of calcium, was not greatly affected by an increase in the proportion of ammonium ion available to the plants when the calcium and phosphate were provided at a constant concentration in the several culture solutions.

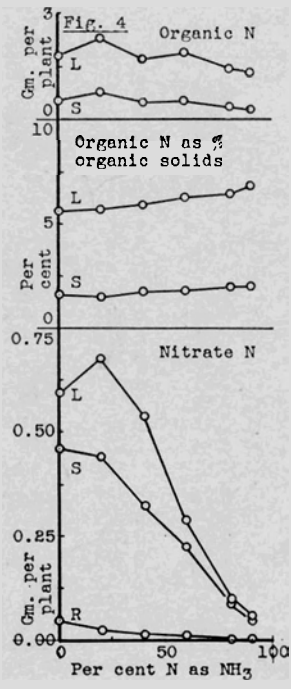
NITROGENOUS CONSTITUENTS

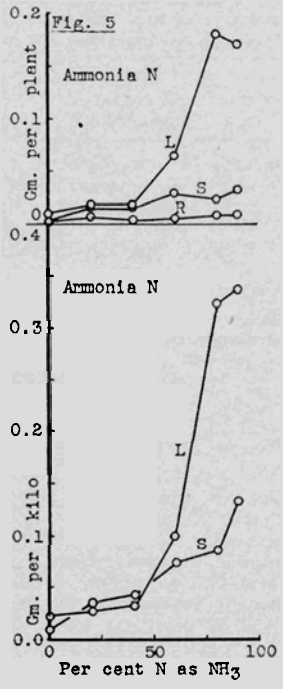
Total Nitrogen

The total nitrogen of leaves and of stalks, plotted in terms of grams per plant, is shown in Figure 3. The curves, as might be expected, are similar in appearance to those for the organic solids but they do not show whether there were differences in the concentration of nitrogen in the several plants. Small differences are, however, revealed by the plots of the nitrogen calculated as a percentage of the organic solids. The curve for the leaves shows a small but regular increase, that for the stalks a very small decrease.

The organic nitrogen, obtained by subtracting the sum of the nitrate and ammonia nitrogen from the total nitrogen, is plotted in Figure 4, and this same quantity, calculated as a percentage of the organic solids, is likewise given. The percentage of organic nitrogen in the organic solids shows clear evidence of progressive differences in the nitrogenous composition of the plants. In the leaves, the proportion of organic nitrogen in the organic solids increased







regularly from 5.6 percent to 6.8 percent with increase in the proportion of ammonia in the culture solution. This may be due either to the presence of progressively smaller proportions of non-nitrogenous solids in the plants grown at the higher ammonia levels, or to actual differences in average nitrogenous composition, or to both. The effect is less evident in the stalks, the small differences found probably being not substantially greater than the error of the determinations.

Still another method of expression of the data gives results that also imply definite differences in the average nitrogenous composition of these plants. In Figure 3 are plotted the results of calculations of the grams of nitrogen per kilo, respectively, of fresh leaf and fresh stalk tissue. The curves in both cases are smoother than the curves for grams per plant, and furnish a comparison on a concentration basis in terms of equal quantities of the tissue. Per unit of fresh weight, the concentration of nitrogen diminished rapidly as the relative proportion of ammonia in the culture solution was increased. This was largely an effect of the increased hydration at the higher levels of ammonia nutrition. Data for grams of organic nitrogen per kilo of fresh tissue (Table 5) give curves (not plotted) that resemble those for total nitrogen, but are placed lower on the scale of ordinates. They are also somewhat less smooth.

TABLE 5. TOTAL NITROGEN AND ORGANIC NITROGEN OF TOBACCO PLANTS, AT TIME OF FIRST FLOWER BUD FORMATION, AS INFLUENCED BY THE PROPORTION OF NITRATE AND AMMONIUM IONS IN THE CULTURE SOLUTION

Percentage of N of culture solution as ammonium ion	0	20	40	60	80	90
			LEAVES			
Total N, gm./plant	2.42	2.98	2.30	2.23	1.74	1.58
Nitrate N, gm./plant	0.592	0.675	0.536	0.290	0.101	0.063
Ammonia N, gm./plant	0.012	0.019	0.019	0.065	0.180	0.169
Organic N[1], gm./plant	1.81	2.29	1.74	1.87	1.46	1.34
Total N, % organic solids	7.48	7.51	7.87	7.63	7.78	8.03
Organic N, % organic solids	5.61	5.77	5.96	6.42	6.53	6.85
Total N, gm./kilo fresh wt.	4.74	4.41	3.97	3.45	3.13	3.14
Organic N, gm./kilo fresh wt.	3.56	3.38	3.01	2.90	2.62	2.68
			STALKS			
Total N, gm./plant	1.02	1.25	0.866	0.813	0.441	0.369
Nitrate N, gm./plant	0.461	0.444	0.324	0.229	0.086	0.047
Ammonia N, gm./plant	0.005	0.019	0.018	0.031	0.025	0.033
Organic N[1], gm./plant	0.558	0.790	0.525	0.553	0.330	0.289
Total N, % organic solids	2.91	3.02	2.94	2.76	2.76	2.66
Organic N, % organic solids	1.59	1.51	1.78	1.88	2.06	2.08
Total N, gm./kilo fresh weight	2.45	2.40	2.01	1.92	1.54	1.53
Organic N, gm./kilo fresh wt.	1.34	1.52	1.22	1.30	1.15	1.20
			ROOTS			
Total N, gm./plant	0.282	0.331	0.135	0.203	0.105	0.118
Nitrate N, gm./plant	0.048	0.029	0.017	0.013	0.003	0.002
Ammonia N, gm./plant	0.002	0.008	0.004	0.007	0.010	0.009
Organic N[1], gm./plant	0.231	0.295	0.113	0.183	0.091	0.107
Total N, % organic solids	2.82	2.95	2.66	2.70	3.27	3.35
Organic N, % organic solids	2.31	2.63	2.22	2.44	2.83	3.04
Total N, gm./kilo fresh wt.	1.41	1.48	1.05	1.35	1.63	1.67

[1]Organic N calculated by deducting sum of nitrate and ammonia nitrogen from the total nitrogen.

Data for the total nitrogen of the root tissue, calculated on a per plant basis and also in grams per 50 gm. of organic solids (i.e. the equivalent of grams per kilo of fresh weight), are also shown in Figure 3. No interpretation of these values is attempted owing to the probability of losses in collecting the root tissue from the sand. In general, however, the figures are of an order of magnitude that might be anticipated from the more reliable data for the leaves and stalks.

The effect on the nitrogen content of the tobacco plant of increase in the relative proportion of ammonia to nitrate in the culture solution is in general to increase the ratio of organic nitrogen to organic solids but to decrease the ratio of organic nitrogen to fresh weight. These effects are pronounced in the leaf tissue and are clear but less marked in the stalk tissue. The decrease in the ratio of organic nitrogen to fresh weight is probably largely due to the marked increase in hydration of the tissues at the higher ammonia levels.

Nitrate and Ammonia Nitrogen

It is to be expected that tobacco plants grown with nitrate ions as their entire source of nitrogen would contain an appreciable amount of nitrate nitrogen stored in the tissues, provided that the total nitrogen supplied is adequate for good growth. As the relative proportion of nitrate to ammonia in the culture solution is decreased, the storage of nitrates in the tissues might be expected to diminish. The curves in Figure 4 (data in Table 5) show that this is the case. Data for the concentration of the nitrate in terms of 1000 gm. of fresh tissue are given in Table 6, but are not plotted since they yield curves that depart but little from straight lines extending, for the leaf, from 1.16 to 0.12 gm. per kilo, and for the stalk, from 1.10 to 0.19 gm. per kilo over the entire series. The concentration of the nitrate in these plants was not widely different, respectively, in the leaf and the stalk in each case, and to a large extent is obviously a function of the concentration of nitrate ion in the culture solution.

The data for the roots are less satisfactory for technical reasons already mentioned, but show that the amount of nitrate nitrogen in the roots was small, and that the concentration, expressed in units that correspond to those used for the leaves and stalks (i.e. grams per 50 gm. organic solids), was also much less than that in the upper parts of the plant. One obtains the impression that nitrate nitrogen absorbed by the root system is transported into the tops in a manner that is disproportionate to the concentration in the roots when expressed in these units. It does not pass from a region of high to a region of low concentration but rather the reverse. In order to confirm this conclusion, calculations were made of the concentration of nitrate nitrogen in the water of each of the tissues. For this purpose the water content of the roots was estimated from the loss in weight when the fresh tissue was dried at 80°. The fresh tissue weight was corrected on the assumption that the sand,

which separated from the dried roots, retained 22 percent of water when attached to the moist roots. The weight of the dry roots was corrected for the weight of the dry sand that was removed. These data are given in Table 6 but are at best only rough approximations. It could be concluded, however, that the concentration of nitrate nitrogen in the water of the leaf and stalk tissue respectively was essentially the same for the plants grown in each culture solution, whereas the concentration in the water of the root tissue was in each case much lower, being from one-third to one-fifth as great as that in the corresponding leaf and stalk tissues.

TABLE 6. NITRATE NITROGEN AND AMMONIA NITROGEN OF TOBACCO PLANTS, AT TIME OF FIRST FLOWER BUD FORMATION, AS INFLUENCED BY THE PROPORTION OF NITRATE AND AMMONIUM IONS IN THE CULTURE SOLUTION

Percentage of N of culture solution as ammonium ion	0	20	40	60	80	90
			LEAVES			
Nitrate N, gm./kilo fresh wt.	1.16	0.997	0.927	0.449	0.180	0.125
Nitrate N, gm./kilo water	1.26	1.08	0.991	0.477	0.190	0.132
Ammonia N, gm./kilo fresh wt.	0.0235	0.0289	0.0335	0.101	0.323	0.338
			STALKS			
Nitrate N, gm./kilo fresh wt.	1.10	0.851	0.749	0.591	0.299	0.196
Nitrate N, gm./kilo water	1.22	0.936	0.813	0.586	0.320	0.210
Ammonia N, gm./kilo fresh wt.	0.012	0.036	0.0408	0.0737	0.0867	0.133
			ROOTS			
Nitrate N, gm./50 gm. organic solids	0.241	0.128	0.168	0.084	0.051	0.033
Nitrate N, gm./kilo water	0.368	0.211	0.166	0.126	0.062	0.043
Ammonia N, gm./50 gm. organic solids	0.011	0.034	0.043	0.048	0.159	0.125

The ammonia content of the plants varied in a manner the opposite to that of the nitrate being very low in the nitrate plants and high in the 90 percent ammonia plants. The demonstration of storage of excess quantities of these two ions at the extremes of the series is evidence for absorption of the ions in unchanged form. There is no suggestion of transformation of the one into the other during the absorption process. Storage of ammonia, however, took place upon a much smaller scale than storage of nitrate. Thus the 60 percent ammonia plants contained 0.065 gm. of ammonia nitrogen each while the 40 percent ammonia plants (60 percent nitrate) contained 0.536 gm. of nitrate nitrogen. These were plants of practically identical size and total nitrogen content. The curves in Figure 5 suggest, further, that ammonia nitrogen was absorbed materially in excess of actual needs only when the 60 percent level in the culture solution was reached, whereas nitrate was still being accumulated to an appreciable extent when nitrate nitrogen made up only 10 percent of the total supply of nitrogen available.

When the data for ammonia nitrogen are expressed on a concentration basis in terms of grams per kilo of fresh tissue, fairly smooth curves are obtained (Figure 5) which show that the con-

centration was substantially the same in both leaf and stalk of the nitrate plants and also in the plants grown at the 20 and at the 40 percent level of ammonia in the culture solution. Reference to Table 6 shows that this is true also for the roots. At higher levels, however, the ammonia concentration in the leaves mounted rapidly, while that in the stalks increased more slowly. The data in Table 6 also indicate high concentrations of ammonia in the root tissue in the 80 and 90 percent ammonia plants.

The constancy of the ammonia in all parts of the plants grown at low levels of ammonia nutrition suggests that we have here to do with an effect controlled by purely physiological processes. When ammonia is made available it is utilized and so is maintained at a low concentration that seems to prevail throughout the plant. Only when the supply is greater than the immediate needs of the plant does it accumulate as such. Accumulation of ammonia first became notable in the 60 percent ammonia plants, whereas storage of nitrate was significant even in the plants on the lowest proportion of nitrate nitrogen (90 percent ammonia). The inference seems warranted that ammonia supplied by the culture solution was more readily utilized by the plant than was nitrate.

An interesting point arises in connection with the relative rates of absorption of the two nitrogenous ions from the intermediate culture solutions. Measurements of the rate of removal of the two ions from the culture solution were not made in the present case, but in an analogous culture experiment in which a tobacco plant was grown in a solution that provided equal molecular proportions of nitrate and ammonium ions, the rates of disappearance of the two ions were far from equal. A plant 41 days old, that is, one that was somewhat less fully developed than the plants considered here but which was growing at approximately its maximum rate, removed 0.15 gm. of ammonia nitrogen and 0.075 gm. of nitrate nitrogen from the culture solution during a three-day test period. This result suggests that, at the reaction of the culture solution employed in this experiment, ammonia is preferentially absorbed when provided at a concentration equal to that of the nitrate ion. If, on further experimentation, this is found to apply under conditions analogous to those considered here, support would be lent to the idea that ammonia is both absorbed and assimilated more readily than nitrate when both ions are available. On this view, high accumulation of nitrate and low accumulation of ammonia under the conditions of the present experiment are to be expected.[1] A complete solution of such prob-

[1]The concentration of total nitrogen in the culture solution supplied these plants was only a little greater than the minimum necessary to provide for good growth. In other experiments in which nitrogen was supplied as nitrate at about two-thirds the present concentration, storage of nitrate was markedly reduced and growth was somewhat restricted. However, storage, particularly of nitrate, is affected by the growth; if growth is restricted by some factor other than the supply of nitrate, storage of nitrate may occur since it is supplied faster than it can be utilized. This may have been a factor in the surprisingly high nitrate content of the somewhat small plants of the present series grown at the 80 and 90 percent levels of ammonia.

lems as these can be obtained only when both ammonium and nitrate salts that contain nitrogen of atomic weight 15 become available for study. Comparative tests of both absorption and assimilation would then become relatively easy.

Amide Nitrogen

It has been a common experience that the introduction of ammonia into a plant system, either by way of the roots, or as a result of protein decomposition during culture of detached leaves, gives rise to a more or less prompt and often pronounced synthesis of amides (for literature see (29)). The experiments of Clark (6) showed that the leaves, and especially the stalks, of tomato plants become enormously enriched in glutamine when ammonia is provided as the sole source of nitrogen to the growing plant. Experiments in which beet plants growing in soil were furnished with a liberal supply of ammonium sulfate (28) also showed a marked enrichment with glutamine, particularly in the root tissue. Tobacco leaves cultured in darkness or in light (29) contain greatly augmented quantities of amides after a few days and similar observations have been made with rhubarb leaves (39). It had been expected, therefore, that tobacco plants grown with high proportions of ammonia in the culture solution would respond in a similar manner. The data plotted in Figure 6 show that, on the contrary, only a minor increase in asparagine took place in the leaves while the glutamine remained substantially unchanged. The curve for the stalks shows that asparagine increased up to the 60 percent ammonia group but the stalks of the plants at the 80 and 90 percent levels contained barely detectable traces of asparagine amide nitrogen. The glutamine in these last two groups of plants was scarcely different from that of the nitrate controls. Even the root tissue failed to show any significant effect. The total amount of either amide in the plants was very small at any point; the asparagine increased a little with increase in ammonia supply, the glutamine decreased.

Figure 7 shows the data plotted in another manner. The lower part of the figure shows the amide nitrogen (both glutamine and asparagine) as a percentage of the soluble nitrogen corrected for nitrate nitrogen. The curve for the leaves indicates a slight rise as the proportion of ammonia in the culture solution was increased; that for the stalks shows a significant increase for the 40 and 60 percent ammonia plants but a subsequent fall, the proportion of amide nitrogen in the 90 percent ammonia plants being in fact lower than that in the nitrate controls. For convenience in comparison, the ammonia nitrogen in the plants, calculated as percentage of the soluble nitrogen corrected for the nitrate, is plotted in the upper part of the figure. It is obvious that the marked increase in the proportion of ammonia nitrogen in these plants had very little effect on the proportion of amide nitrogen. In the leaves, there was a small increase over a part of the range while, in the stalks of the plants at high ammonia levels, there was a marked decrease in amide concentration coupled with an increase in ammonia content. It is nec-

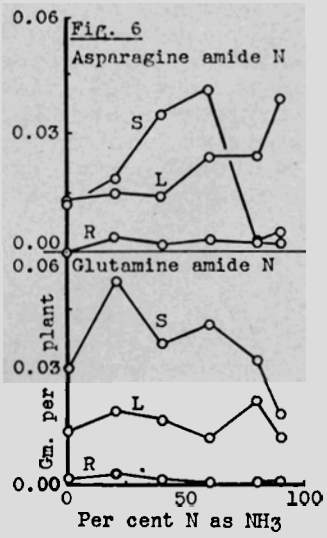


Fig. 6
Asparagine amide N
Glutamine amide N
Gm. per plant
0.06
0.03
0.00
S
L
R
0
50
100
Per cent N as NH3

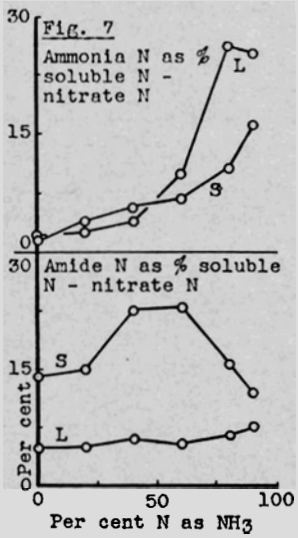


Fig. 7
Ammonia N as % soluble N - nitrate N
Amide N as % soluble N - nitrate N
Per cent
30
15
0
S
L
0
50
100
Per cent N as NH3

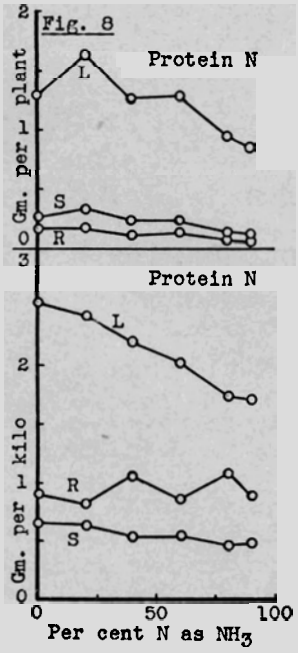


Fig. 8
Protein N
Gm. per plant
Gm. per kilo
2
1
0
3
L
S
R
0
50
100
Per cent N as NH3

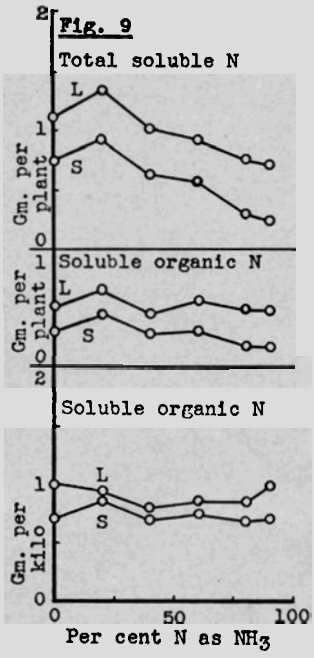


Fig. 9
Total soluble N
Soluble organic N
Gm. per plant
Gm. per kilo
2
1
0
L
S
0
50
100
Per cent N as NH3

essary to point out, however, that these plants were supplied with nitrogen at a total concentration only slightly in excess of what was needed for good growth. The failure of the plants up to the 60 percent level of ammonia nutrition to accumulate significant amounts of ammonia may merely mean that most of the ammonia supplied was being utilized in the synthesis of complex nitrogenous substances; very little remained in the presumably intermediate form of amide nitrogen. Had nitrogen been supplied in a bountiful excess over the amount the plants could readily use, it is probable that amide accumulation would have been far more pronounced.

TABLE 7. AMIDE NITROGEN OF TOBACCO PLANTS, AT TIME OF FIRST FLOWER BUD FORMATION, AS INFLUENCED BY THE PROPORTION OF NITRATE AND AMMONIUM IONS IN THE CULTURE SOLUTION

Percentage of N of culture solution as ammonium ion	0	20	40	60	80	90
			LEAVES			
Total amide N, gm./plant	0.0265	0.034	0.0303	0.035	0.0457	0.0509
Glutamine amide N, gm./plant	0.0137	0.0186	0.0159	0.0105	0.0213	0.0120
Asparagine amide N, gm./plant	0.0128	0.0154	0.0144	0.0245	0.0244	0.0389
Ammonia N, % soluble N minus nitrate N	2.25	2.84	3.92	10.3	26.6	25.5
Amide N, % soluble N minus nitrate N	5.06	5.08	6.19	5.51	6.79	7.70
			STALKS			
Total amide N, gm./plant	0.0415	0.0710	0.0715	0.0820	0.0347	0.0236
Glutamine amide N, gm./plant	0.0300	0.0523	0.0362	0.0408	0.0318	0.0183
Asparagine amide N, gm./plant	0.0115	0.0187	0.0352	0.0412	0.0030	0.0054
Ammonia N, % soluble N minus nitrate N	1.69	4.03	5.78	7.01	11.3	16.2
Amide N, % soluble N minus nitrate N	14.2	15.1	22.8	23.3	15.8	11.8
			ROOTS			
Total amide N, gm./plant	0.0018	0.0066	0.0035	0.0040	0.0032	0.0035
Glutamine amide N, gm./plant	0.0018	0.0029	0.0018	0.0006	0.0006	0.0010
Asparagine amide N, gm./plant	0.0000	0.0037	0.0017	0.0034	0.0026	0.0025

The failure of a large increase in the relative proportion of ammonia in the tissues of the growing tobacco plant to bring about an increase in that of the amide nitrogen raises a serious question with respect to the general validity of the "ammonia detoxication" view of amide metabolism expressed many years ago by Prianischnikow. This view is clearly much too simplified for general application. Although it serves more or less adequately to account for the behavior observed in many tissues, it fails entirely in the present case and also in the case of the behavior of rhubarb leaves cultured in water (30).

A re-examination of all the pertinent data, including the present observations, has led to the suggestion (27) that the synthesis of amides in plants represents the effect of at least two conditions, both of which must be satisfied: the availability of ammonia on the

one hand and the availability of suitable non-nitrogenous precursors of asparagine or glutamine on the other. These precursors are assumed, on theoretical grounds, to be oxaloacetic and α-ketoglutaric acids respectively, and they are presumably made available through interconversions of the organic acids involved in the respiratory mechanism. This view represents a return to early ideas of amide metabolism probably first suggested nearly a century ago in vague and purely hypothetical terms by Boussingault and advocated by many workers since, particularly in recent years by Chibnall (5).

On this view the present results become somewhat more easily understood. It seems to be true for many mature plant tissues under ordinary conditions that the amides are not present in high proportions. They are presumably synthesized, transported, and utilized at such a rate that no conspicuous accumulation occurs. The present experiment illustrates the fact that, even when the nitrogen supplied contains unusually high proportions of ammonia, a stimulation of amide synthesis is not a necessary outcome. With other species, such as the tomato and beet, synthesis of amides may ensue under these conditions, and, from what is known of seedling metabolism, it would appear that amide synthesis is a usual phenomenon in the early stages of growth when the protein of the cotyledon is furnishing a large proportion of the nitrogenous nutriment, in the form of soluble decomposition products, to the growing points. Under these circumstances, nitrogen is made available in what is really luxury concentration. Amide synthesis is, however, obviously the result of a mechanism that demands something in addition to a supply of ammonia, however ample, before it can operate. The nitrogen-free precursors must also be present, and this is probably as true for the mature plant as for the seedlings studied by Prianischnikow (see 29, p. 762 ff.). In the present case, only one of the necessary conditions was apparently satisfied, namely the provision of ammonia.

Protein Nitrogen

The determinations of protein nitrogen were made by the method in current use in this laboratory. The value obtained represents nitrogen that remains insoluble when the dry powdered tissue is exhaustively extracted with 70 percent alcohol and subsequently with boiling water. The method yields easily duplicated results and is the most satisfactory of any that have been studied. It is recognized, however, that it gives only an approximation, although probably a close one, to the true protein nitrogen content.

In Figure 8 the results for protein nitrogen are plotted in two ways. Curves that show the quantities per single plant, and also the quantities per kilo of fresh tissue are included. The chief variation brought about by the alteration in the form of nitrogen supplied to the plants is in the protein of the leaves. The quantity per plant was increased in the 20 percent ammonia plants, but was not significantly different from the nitrate controls in the 40 and 60 percent ammonia plants. The smaller plants at the two highest am-

monia levels, however, contained considerably less leaf protein. The irregularities in the curve are similar to those for the organic solids (Figure 1), and the amount of leaf protein is evidently to a large extent a function of the size of the plant.

TABLE 8. PROTEIN NITROGEN AND SOLUBLE NITROGEN OF TOBACCO PLANTS, AT TIME OF FIRST FLOWER BUD FORMATION, AS INFLUENCED BY THE PROPORTION OF NITRATE AND AMMONIUM IONS IN THE CULTURE SOLUTION

Percentage of N of culture solution as ammonium ion	0	20	40	60	80	90
			LEAVES			
Protein N, gm./plant	1.29	1.64	1.28	1.30	0.966	0.850
Protein N, gm./kilo fresh wt.	2.53	2.42	2.20	2.02	1.73	1.69
Protein N, % organic solids	3.99	4.13	4.38	4.45	4.51	4.34
Protein N × 6.25, % organic solids	25.0	25.8	27.4	27.8	28.3	21.1
Soluble N, gm./plant	1.12	1.35	1.02	0.925	0.777	0.725
Soluble organic N, gm./plant	0.521	0.650	0.466	0.570	0.497	0.493
Soluble organic N, gm./kilo fresh wt.	1.02	0.961	0.805	0.883	0.871	0.982
			STALKS			
Protein N, gm./plant	0.267	0.329	0.232	0.233	0.134	0.117
Protein N, gm./kilo fresh wt.	0.640	0.631	0.538	0.549	0.468	0.487
Protein N, % organic solids	0.76	0.79	0.79	0.79	0.84	0.84
Protein N × 6.25, % organic solids	4.77	4.97	4.92	4.94	5.24	5.26
Soluble N, gm./plant	0.756	0.923	0.634	0.580	0.307	0.252
Soluble organic N, gm./plant	0.291	0.461	0.293	0.320	0.196	0.171
Soluble organic N, gm./kilo fresh wt.	0.697	0.884	0.678	0.755	0.685	0.712
			ROOTS			
Protein N, gm./plant	0.182	0.183	0.107	0.130	0.070	0.063
Soluble N, gm./plant	0.100	0.149	0.028	0.073	0.035	0.055
Soluble organic N, gm./plant	0.050	0.112	0.006	0.053	0.021	0.044

The stalk protein diminished in quantity with increase in ammonia in the culture solution, but through only a small range. Again the irregularities followed those of the organic solids.

The curves which show the protein nitrogen as a function of the fresh weight of the tissues lead to a somewhat different conclusion in the case of the leaf protein. Although the effect is chiefly due to the increased hydration of the tissues at high ammonia levels, the proportion present decreased rapidly as the proportion of ammonia in the culture solution was increased. The results can be expressed by a relatively smooth straight line. Plants grown at the higher proportions of ammonia in the culture solution are thus definitely poorer in leaf protein in terms of fresh weight.

The stalk protein showed this effect also, but the total amount of protein was smaller. A calculation of the difference between the protein nitrogen of the stalk at the 80 percent ammonia level and that of the nitrate controls as a percentage of the protein nitrogen in the nitrate controls show that the stalk protein diminished by 28 percent over this range. The leaf protein diminished 31 percent of the amount in the nitrate controls, so that the two results agree in

showing a similar proportionate decrease in protein concentration with increase in ammonia in the culture solution.

The data for the roots are probably too uncertain to permit of definite conclusions and, in any case, the amounts of protein in these tissues were small in comparison with those in the leaf. The change with increase of ammonia in the culture solution, if any, was small. The irregularities in the curve for the concentration of protein nitrogen in the root furnish an example of the analytical difficulty encountered when the plants are grown in sand cultures. However, the curve suggests that increase in the proportion of ammonia in the culture solution had only a minor effect on the concentration of protein in the roots.

Water Soluble Nitrogen

Figure 9 shows the quantities of soluble nitrogen and of soluble organic nitrogen, the latter being obtained by deducting the sum of the nitrate nitrogen and the ammonia nitrogen from the former. The curve for the soluble nitrogen of the leaf tissue follows in general the shape of that for the organic solids (Figure 1) and soluble nitrogen is thus a function of the size of the plants. The curve for soluble organic nitrogen, on the other hand, is nearly a horizontal straight line suggesting that the soluble nitrogenous substances of these plants, exclusive of nitrate and ammonia, were but little affected by the change in composition of the culture solution. In order to afford a comparison independent of plant size, the soluble organic nitrogen expressed in grams per kilo of fresh leaf is also given. This curve indicates that the concentration of soluble organic nitrogen changed very little as the proportion of ammonia in the culture solution was increased. The soluble organic nitrogen provides an approximate measure of the simpler nitrogenous metabolites of the tissue, and it is clear that the amount of this group of substances was not greatly affected by the composition of the culture solution.

In the stalk, similar relationships also hold. The curve for the total soluble nitrogen resembles that for the organic solids, but that for the soluble organic nitrogen fluctuates much less; that for the soluble organic nitrogen expressed in grams per kilo of fresh tissue is, save for one point, very nearly a horizontal straight line.

Thus the greater part of the variation in the composition of these plants, with respect to soluble nitrogen, is due to the different amounts of nitrate and ammonium ions they contained. As has been pointed out above, the variation in nitrate storage is the largest single factor that influences this.

NITROGEN METABOLISM

The effects of the step-wise substitution of ammonia for nitrate in the culture solution upon the nitrogenous composition of tobacco plants may be briefly summarized at this point. At the 20 percent ammonia level, the plants were stimulated, being larger and more

uniform than the nitrate controls, but at the 40 and 60 percent level, they were essentially of the same size as the controls. Growth at higher levels was somewhat retarded, although no demonstration was provided that this was a necessary outcome. It is possible that, had the reaction of the culture solutions been maintained at a higher pH by more rapid renewal, or by adjustment of the original reaction, the plants at the 80 and 90 percent ammonia levels would have grown as well as the others. This was not done since the interest was chiefly in the effect on the plants of change in the nitrogenous composition of the culture solution furnished under otherwise comparable conditions. Whether or not tobacco plants of normal size can be produced on culture solutions that provide most of the nitrogen as ammonium ions remains for further study. The work of Shive's laboratory suggests, however, that with proper conditions this might be achieved.

In the present experiment, the total nitrogen content of the plants, whether calculated on a per plant basis or in terms of a kilogram of fresh weight, respectively, of leaf, stalk, or root tissue, diminished with increase in the relative proportion of ammonia in the culture solution. On the other hand, both the total nitrogen and the organic nitrogen of leaves and stalks either remained essentially constant or increased slightly, when expressed as a percentage of the organic solids. The overall nitrogen content of the organic solids was thus much less sensitive to the change in the composition of the culture solution than was the whole plant.

The most striking difference in the plants was in their storage of nitrate nitrogen. The nitrate controls contained no less than 29.6 percent of their total nitrogen (leaf, stalk, and root) in the form of nitrate. Over the entire series this diminished regularly to 5.4 percent in the 90 percent ammonia plants. On the other hand, the storage of ammonia was relatively smaller. The nitrate plants contained 0.51 percent of their total nitrogen in the form of ammonia, the 90 percent ammonia plants contained 10.2 percent. Furthermore, the increase in ammonia in the plants was not in proportion to the increase furnished in the culture solution; the plants on 40 percent ammonia contained only 1.24 percent of their total nitrogen as ammonia and those on the 60 percent contained only 3.1 percent. The lag in the rate at which ammonia accumulated as compared with the behavior of the nitrate suggests that, when both ions are available, the plant makes more rapid use of the ammonia than of the nitrate. Since the plants, up to the 60 percent level, were of about the same size, the total amount of nitrogen assimilated was nearly the same for each. Furthermore, the total organic nitrogen in all tissues of these plants changed but little save for the 20 percent plants in which it was substantially increased.[1] The failure of the plants to store ammonia obviously means that it was utilized

[1]The total organic nitrogen of the whole plants was respectively 2.60, 3.38, 2.38, and 2.61 gm. for the first four groups.

almost as rapidly as it was absorbed, while much of the nitrate nitrogen accumulated as such.

The stimulation of the plants grown at the 20 percent ammonia level is a further indication of the more ready availability of ammonia for assimilation than nitrate. Under these conditions nitrogen as ammonia was supplied at what appears to have been an especially favorable rate.

No data were secured from which a precise estimate of the relative rates of assimilation of nitrate and of ammonia by the plants could be obtained. A study of plants that had stored appreciable quantities of both ions and were then subjected to a brief period of nitrogen starvation would be desirable for this. Measurements of the rate at which the stored ions disappear from the tissues of such plants should furnish some indication of the relative assimilation rates.

The extraordinarily small effect of ammonia nutrition on the amide nitrogen of these tobacco plants has been already emphasized. Aside from the fact that storage of amides may be a function of the liberality of the supply of total nitrogen, it is clear that the production of amides is contingent on factors in addition to the mere presence of ammonia, and it has been postulated that whatever these factors are, they affect the course of the respiration of the tissues in such a way that oxaloacetic acid and α-ketoglutaric acid become available in sufficient amounts to permit of the synthesis of one or both of the amides asparagine and glutamine. It is obvious that the amides may and, in certain species and under the correct conditions, do serve as storage substances for nitrogen. The important inference from the present experiments is that this is not obligatory; ammonia itself or nitrate may also serve, and the great flexibility of the nitrogen metabolism of plants is illustrated by this fact. The precise course that will be followed is apparently determined by the complex of conditions in which the organism finds itself, including the rate at which nitrogen is supplied to the plant. A change in these conditions may be expected to result in a change in the course of the metabolism, but it seems clear that several, and perhaps many courses are open.

It is probably significant that what little response in amide synthesis these tobacco plants showed to increase in ammonia was largely confined to the stalks of the plants on the 40 and 60 percent levels (see Figure 7). The stalk tissue of the tomato plants in Clark's experiments was also the chief locus of amide enrichment and this seems to have been true for seedlings studied by Burkhart (4). The blade tissue of rhubarb leaves, however, was the chief locus of amide enrichment during culture of detached leaves in water (30), but the blade tissue was also richest in protein decomposition products as the culture progressed. A distinction should be made, however, between the behavior of the whole plant on the one hand and the behavior of a detached leaf, that is of a plant organ in an entirely abnormal environment, on the other hand.

It is of considerable interest, in its relationship to the course of the respiration in these tobacco plants, that the asparagine concentration was alone affected by the increase of ammonium in the culture solution. The glutamine amide nitrogen showed no regular change in the leaf, whether expressed on a grams per plant or on a concentration basis, and showed only minor changes in the stalk. The asparagine amide nitrogen, however, increased regularly in the leaf up to the highest ammonia level studied. In the stalk, it increased up to the 60 percent level but decreased again in the 80 and 90 percent ammonia plants. This observation is of interest in its contrast to the behavior of excised tobacco leaves subjected to culture in water. In these, asparagine, but not glutamine, rapidly accumulated when the leaves were kept in darkness, but both amides were formed when the leaves were exposed to light. The leaves of the whole plant, however, when analyzed immediately after being harvested, showed no response in glutamine concentration to a change in conditions of nutrition that might be expected to affect the amide metabolism.

It will be recalled that the data for organic solids, expressed in terms of grams per kilo of fresh tissue, showed a steady decrease for both leaf and stalk over the entire series, and that the data for water showed a steady increase; that is, the tissues of the plants grown in solutions with higher proportions of ammonia were more highly hydrated than those grown in solutions with lower proportions. The protein nitrogen, calculated as percentage of the organic solids, increased over the series from 4.0 to 4.5 percent (Table 8). Thus the greater hydration of the leaves of the plants on the higher proportions of ammonia is associated with a greater proportion of protein in the organic solids. This change is, however, a small one and it seems improbable that the far greater hydration of the plants on high proportions of ammonia can be accounted for in terms of an increase in protein alone.

Unlike the protein, the soluble organic nitrogen of the plants, particularly in the leaf, changed but little with the increase in the proportion of ammonia in the culture solution. The actual quantity as grams per plant varied hardly at all, certainly not significantly, while its concentration in grams per kilo of fresh weight decreased a little and then increased. Thus the composition with respect to simpler soluble organic nitrogenous substances was not greatly affected by the composition of the culture solution.

HYDROGEN ION ACTIVITY OF TISSUES

During the last month of growth of the plants, the culture solutions were supplied at a reaction of pH 6.4 with the exception of the nitrate control which was at pH 4.9. Frequent observations were made of the reaction of the solution that dripped from the crocks in which the plants were growing. The reactions varied more or less from day to day, and the change in reaction was greatest with the cultures on the higher proportions of ammonia. Aver-

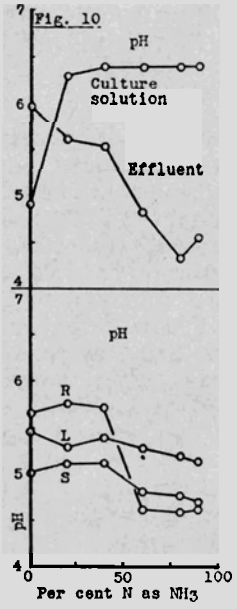


Fig. 10
pH
Culture solution
Effluent
pH
R
L
S
Per cent N as NH3

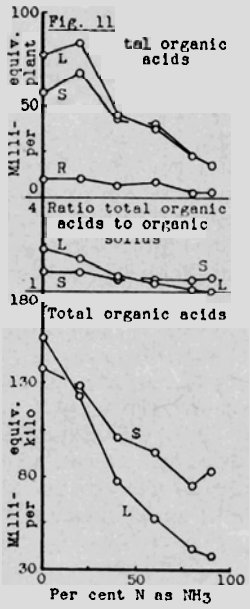


Fig. 11
tal organic acids
Milli-equiv. per plant
L
S
R
Ratio total organic acids to organic solids
Total organic acids
Milli-equiv. per kilo
Per cent N as NH3

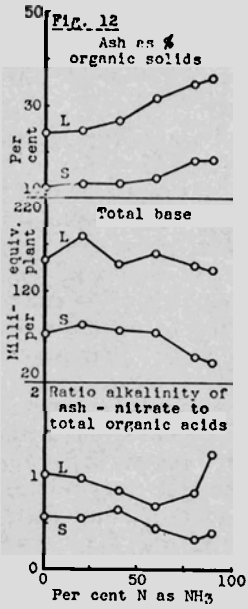


Fig. 12
Ash as % organic solids
Per cent
Total base
Milli-equiv. per plant
Ratio alkalinity of ash - nitrate to total organic acids
Per cent N as NH3

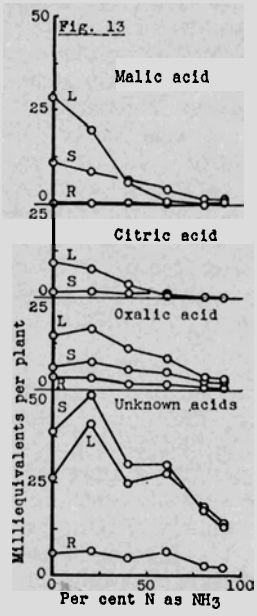


Fig. 13
Malic acid
Citric acid
Oxalic acid
Unknown acids
Milliequivalents per plant
Per cent N as NH3

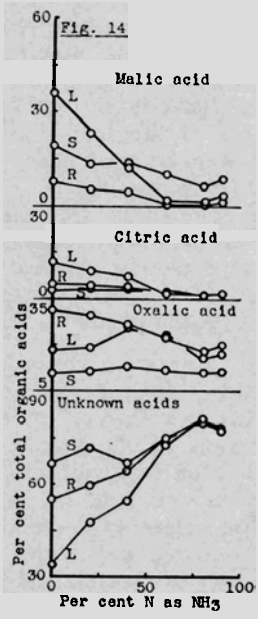


Fig. 14
Malic acid
Citric acid
Oxalic acid
Unknown acids
Per cent total organic acids
Per cent N as NH3

age values for the pH of the effluent on a few arbitrarily selected dates are plotted in Figure 10. The data serve to show the increasing effect on the production of acidity in the culture solution as the relative proportion of ammonium ion in it was increased, and also show the decrease in the acidity of the nitrate solution after use by the plant.

Figure 10, in addition, shows the reaction of extracts made from the dry tissues of the plants. The roots varied most widely over the entire range of culture solutions. The stalks showed little change until the 60 percent ammonia level was reached, the leaves showed even less variation. In no cases are the observations outside the limits of what might be regarded as normal variations in the acidity of tobacco plant tissues, although the trend of the observations as a whole is distinctly in the direction of a more acid reaction as the ammonia in the culture solution, and also the ammonia stored in the tissues, was increased. Nevertheless, even the plants grown with 90 percent of their nitrogen supplied as ammonia were by no means "acid plants" in the Ruhland sense, and, as will appear, they were actually very deficient indeed in organic acids. The trend of the pH does not at all conform to the trend in the organic acidity nor to the ammonia content and manifestly depends on quite different factors.

ORGANIC ACIDS

The experiments of Clark with tomato plants suggested that plants grown with nitrate as the sole source of nitrogen might be expected to contain a much larger quantity of organic acids than similar plants grown with ammonia alone. That this is also true for the tobacco plant is shown by the curves in Figure 11. The total organic acids of the leaf decreased very rapidly indeed as the proportion of ammonia in the culture solution was increased. The slightly larger plants at the 20 percent ammonia level contained a little more acid than the nitrate controls, but the 60 percent ammonia plants contained only about one-half as much although they were of very nearly the same size. The plants grown with the higher proportions of ammonia in the culture solution contained very little organic acid.

It is of interest to consider the ratio of the organic acids to the organic solids. If it be assumed that the total organic acidity represents acids that have, on the average, the composition of malic acid, the leaves of the nitrate plants contained 16.4 percent of their organic solids as organic acid. The 90 percent ammonia plants contained only 6.3 percent on the same assumption and the curve that expresses the intermediate points is very nearly a straight line. The actual ratio of organic acids, expressed in milliequivalents per plant, to the number of grams of organic solids per plant is plotted in Figure 11.

TABLE 9. ACIDITY AND ORGANIC ACIDS OF TOBACCO PLANTS, AT TIME OF FIRST FLOWER BUD FORMATION, AS INFLUENCED BY THE PROPORTION OF NITRATE AND AMMONIUM ION IN THE CULTURE SOLUTION

Percentage of N of culture solution as ammonium ion	0	20	40	60	80	90
pH culture solution	4.9	6.3	6.4	6.4	6.4	6.4
pH effluent (average)	5.98	5.62	5.53	4.83	4.34	4.55
			LEAVES			
pH extract	5.47	5.30	5.39	5.29	5.21	5.15
Total organic acids, m.e./plant	78.8	84.1	45.4	38.0	23.3	18.5
Oxalic acid, m.e./plant	14.5	16.4	11.3	8.69	3.66	3.11
Citric acid, m.e./plant	9.56	7.67	3.40	0.55	0.29	0.25
Malic acid, m.e./plant	28.2	19.7	5.62	0.96	0.50	0.75
Unknown acids, m.e./plant	26.6	40.5	25.0	27.8	18.8	14.4
Ratio of total organic acids to organic solids	2.44	2.12	1.56	1.30	1.09	0.94
Total organic acids, m.e./kilo fresh wt.	155	124	78.4	58.6	41.7	36.9
			STALKS			
pH extract	5.03	5.13	5.13	4.83	4.78	4.72
Total organic acids, m.e./plant	57.4	67.4	43.4	39.5	21.6	20.1
Oxalic acid, m.e./plant	6.27	7.77	5.72	4.79	2.38	2.33
Citric acid, m.e./plant	1.48	1.96	1.36	0.75	0.19	0.20
Malic acid, m.e./plant	11.2	8.91	6.31	4.24	1.53	1.84
Unknown acids, m.e./plant	38.4	48.7	30.0	29.7	17.5	15.7
Ratio of total organic acids to organic solids	1.64	1.63	1.47	1.34	1.35	1.45
Total organic acids, m.e./kilo fresh wt.	138	129	101	93	75.6	83.0
			ROOTS			
pH extract	5.67	5.78	5.73	4.63	4.61	4.64
Total organic acids, m.e./plant	11.1	11.6	7.8	9.2	3.7	2.9
Oxalic acid, m.e./plant	3.47	3.42	2.1	2.0	0.7	0.6
Citric acid, m.e./plant	0.57	0.53	0.31	0.17	0.05	0.05
Malic acid, m.e./plant	0.92	0.72	0.38	0.09	0.02	0.04
Unknown acids, m.e./plant	6.1	6.9	5.0	6.9	2.9	2.3
Ratio of total organic acids to organic solids	1.11	1.04	1.54	1.23	1.15	0.74
Total organic acids, m.e./kilo fresh wt.	55.5	51.6	76.5	62.9	56.9	41.7

It is evident from these curves that increase in the proportion of ammonia in the culture solution gave rise to plants that contained successively smaller actual amounts and smaller relative proportions of organic acids in the leaves.

The situation in the stalks was similar in one respect. Save for the nitrate and the 20 percent ammonia plants, the amounts of organic acids in the stalks were almost exactly the same as those found in the leaves in each case and, accordingly, the same rapid diminution in organic acids with increase in ammonia in the culture solution took place. When considered from the standpoint of concentration of organic acids in the organic solids, however, the behavior of the stalks was different from that in the leaves. The ratio of total organic acids (in milliequivalents) to organic solids (in grams) changed very little over the entire series. It dropped only from 1.6 to 1.4 while the ratio in the leaves dropped from 2.4 to 1.0.

Evidently, therefore, the relative proportion of organic acids in the stalks was less sensitive to the change in the culture solution than was that in the leaves.

The data for the roots show that in this tissue also the quantity of organic acids per plant diminished materially as the proportion of ammonia in the culture solution increased. The ratio of the organic acids to the organic solids in the roots was, however, like that in the stalks, nearly constant over the entire series. The data (Table 9) are not plotted in Figure 11 but only two points (40 percent and 90 percent plants) depart substantially from a horizontal straight line.

In Figure 11 are also plotted the quantities of organic acids per kilo of fresh tissue for both leaves and stalks of these plants. These curves illustrate the effect of the substitution of ammonia for nitrate in the culture solution perhaps more strikingly than the others. The concentration of organic acids in the leaves diminished to less than one-quarter of the value in the nitrate plants over the range studied, that in the stalks to slightly more than one-half. The data for the roots are not plotted. The values are given in Table 9 and lead to an irregular curve which suggests that the effect in the root tissue within the limits of accuracy of the data is much smaller than that in the stalks.

In general, therefore, the effect of increasing proportions of ammonia in the culture solution is to bring about a profound decrease in the amounts and concentration of the organic acids in all parts of the tobacco plant. The curves that represent concentration functions do not depart widely from straight lines over most of the range studied and suggest that the relationship is a relatively simple one, at least in the manner in which the ultimate metabolic effects upon the organic acids are expressed.

Alteration in the acid composition of the tissues involves alteration also in the base combined with these acids. Since a considerable part of the base must be inorganic, a correlation of the organic acidity with the inorganic constituents of the tissues is to be anticipated. The organic acids exist in the tissues in part as the anions of inorganic salts. Decrease in the quantity present might be expected, therefore, to be accompanied by a decrease in ash constituents. Clark's data on the tomato plant showed an effect that could be interpreted in this way, but, as has already been pointed out, his plants grown in ammonia culture solution were so much smaller than those in nitrate solution that a definite conclusion could not be drawn.

In the present case, unlike the results with the tomato, the data plotted in Figure 1 indicate that the change in the composition of the culture solution had remarkably little effect upon the total amount of ash per plant. Furthermore, when calculated as a percentage of the organic solids, the ash increased in the leaves over the range from 24 to 36 percent and in the stalks from 12.3 to 18.4 percent

(Figure 12). The inorganic substances absorbed by these plants were, however, derived from solutions that were varied in two ways; not only was the form of nitrogen changed but the proportions of chloride ion and sulfate ion present were increased. As a result, the plants absorbed a rapidly increasing amount of chloride ion and, as has already been pointed out in connection with the discussion of Figure 2, the chloride content of the leaf ash was roughly proportional to the concentration of chloride ion in the culture solutions. The increase in sulfate ion, on the other hand, had little influence on the sulfate content of the leaf ash. The higher relative proportions of ash, with respect to organic solids, in the plants grown at the higher levels of ammonia nutrition are thus to a considerable extent a result of the absorption of inorganic chlorides. This is an effect superimposed upon the effect of the alteration in the relative quantities of the nutrient ions from which the plants derived their nitrogen. Experimentally, it is not possible to replace nitrate ion by ammonium ion in culture solutions without at the same time altering the concentration of one or more of the anions made available to the plant.

The reaction of the leaf tissue of these plants did not vary widely over the series (see Table 9). Accordingly it may be assumed that there was little change in the proportion of each organic acid present as ions of salts. It happens that, in tobacco leaf tissue, the reaction (about pH 5.4) is one at which malic, citric, and oxalic acids are almost entirely present as the mono-basic and dibasic ions. Accordingly an equal quantity of base must be present to neutralize this acid.

An estimation of the total base present in the leaves can be made from the analysis of the ash. The alkalinity of the ash represents inorganic base in combination with acids that are decomposed during the ignition of the tissues. Most of the acid represented is, of course, organic but the nitrate also contributed and is represented by an equivalent of inorganic base. Table 10 shows the data for the correction of the alkalinity due to the nitrate present and it will be noted that, for the leaves, the residual alkalinity runs closely parallel with the observed total organic acidity and is very nearly identical with it. At the 40 and 60 percent points there is a significant excess of organic acids. This suggests that a part of the unknown acids, which are present in dominating proportions in these plants (see below), must represent acids definitely weaker than malic and citric acids. That is to say, the evidence points to the presence in these particular lots of plants of a significant amount of weak free acid groups.

In the stalks, the reaction of which was slightly more acid than the leaves (about pH 5.0), the excess of free acid was in all cases considerable. The stalks obviously contain appreciable quantities of weak acids in the unknown group.

An estimate of the inorganic base present in the tissues can be made by adding the alkalinity of the ash to the sulfate, chloride,

and phosphate contents. The necessary data are shown in Table 4 and it is assumed that the phosphate content of the leaf ash is 7.0 milliequivalents per plant in the cases where data were not available. The phosphate is omitted in the calculation for the stalks. In order to compute the total base of the tissues, the free ammonia must also be included. The data, expressed in milliequivalents, are given in Table 10. So little ammonia was present in the plants, up to and including the 60 percent ammonia plants, that its omission has little effect. The ammonia in the leaves of the 80 and 90 percent plants was, however, large enough to require consideration.

These estimates of the total base of the plants are plotted in Figure 12. It is most significant that the total base present both in the leaves and in the stalks was substantially constant up to the 60 percent level. The base in the 20 percent plants was significantly greater but it will be recalled that these plants were definitely larger than the controls. Even the leaves of the smaller 80 and 90 percent ammonia plants, when the correction for free ammonia is applied, contained nearly as much base as the nitrate controls.

TABLE 10. RELATIONSHIPS OF ORGANIC ACIDS AND ASH CONSTITUENTS OF TOBACCO PLANTS, AT TIME OF FIRST FLOWER BUD FORMATION, AS INFLUENCED BY THE PROPORTION OF NITRATE AND AMMONIUM ION IN THE CULTURE SOLUTION

Percentage of N of culture solution as ammonium ion	0	20	40	60	80	90
			LEAVES			
Ash, % organic solids	24.1	25.2	26.8	31.8	34.8	36.0
Alkalinity of ash, m.e./plant	125	130	77.7	47.5	28.8	27.8
Nitrate N, m.e./plant	42.3	48.2	38.3	20.7	7.2	4.5
Ammonia N, m.e./plant	0.8	1.4	1.4	4.6	12.8	12.1
Alkalinity of ash corrected for nitrate, m.e./plant	82.7	81.8	39.4	26.8	21.6	23.3
Total organic acids, m.e./plant	78.8	84.1	45.4	38.0	23.3	18.5
Excess "free" acid, m.e./plant	–3.9	2.3	6.0	11.2	1.7	–4.8
Total inorganic base, m.e./plant	153	179	148	156	136	130
Total base including ammonia, m.e./plant	154	180	149	161	149	142
Ratio of alkalinity of ash minus nitrate to total organic acids	1.05	0.99	0.87	0.71	0.84	1.26
			STALKS			
Ash, % organic solids	12.3	13.5	13.5	14.6	18.3	18.4
Alkalinity of ash, m.e./plant	66.6	69.7	51.4	34.9	13.6	11.6
Nitrate N, m.e./plant	32.9	31.7	23.1	16.4	6.1	3.4
Ammonia N, m.e./plant	0.4	1.4	1.3	2.2	1.8	2.3
Alkalinity of ash corrected for nitrate, m.e./plant	33.7	38.0	28.3	18.5	7.5	8.3
Total organic acids, m.e./plant	57.4	67.4	43.4	39.5	21.6	20.1
Excess "free" acid, m.e./plant	23.7	29.4	15.1	21.0	14.1	11.8
Total inorganic base, m.e./plant	73	84	78	74	47	41
Total base including ammonia, m.e./plant	73	85	79	76	49	43
Ratio of alkalinity of ash minus nitrate to total organic acids	0.587	0.564	0.652	0.468	0.347	0.413

The chief interest attaches to the comparison between the nitrate controls and the 60 percent ammonia plants. These were plants of essentially the same size and nitrogen content, yet they differed very widely indeed in organic acidity and in chloride and nitrate. The sum of the chloride and nitrate content of the leaves of the nitrate controls was 43.3 milliequivalents, that of the 60 percent ammonia plants was 93.8. Thus 50.5 more milliequivalents of base were required in these plants to neutralize these two acids alone. The 60 percent ammonia plants contained less organic acids by 40.8 milliequivalents and, accordingly, the balance between the positive and negative ions was preserved only in part by a diminution in the organic acids present. The compensating change in the organic acids, although it accounts for most of the difference, was not quantitatively equivalent and it seems clear that an important redistribution of basic and acidic ions must have occurred. The 60 percent ammonia plants were thus chemically widely different from the nitrate controls in spite of close similarities in many of the details of their composition.

Another method of presentation which illustrates the failure of the inorganic base associated with organic acids to vary in exact proportion with organic acids is also shown in Figure 12. The alkalinity of the ash, when corrected for the nitrate, furnishes a measure of the inorganic base in combination with the organic acids present in the tissues. If synthesis of organic acids takes place in response to the quantity of base available, the ratio between the alkalinity of the ash corrected for nitrate and the total organic acidity should be substantially constant. This ratio is plotted in Figure 12. In the leaves, the ratio is constant and is nearly unity up to the 40 percent level of ammonia. In these plants malic, oxalic, and citric acids made up a substantial part of the total acidity; these acids are present almost wholly as the ions of salts at the leaf tissue reaction and equivalence between the base and acid was maintained. With further increase in the proportion of ammonia in the nutrient, however, the ratio diminished, that is, more acid was present than was equivalent to the inorganic base. As is clear from the data in Table 9, the unknown acids in these plants assumed the dominating position and other factors must obviously have entered into the equilibrium relations. The most important of these is doubtless the increasing relative importance of acids weaker than malic and citric.

In the stalks, the ratio was also substantially constant up to the 40 percent ammonia plants but then diminished. Here, however, the ratio was always much less than unity and this conforms to the fact that a large part of the acids of the stalk belong to the unknown group and it would seem that a substantial part of these must also be very weak acids.

The quantities of malic, citric, oxalic, and unknown acids in the tissues of the plants are shown in Figure 13 (data in Table 9). Malic acid is the dominant acid in the nitrate plants but the amount

per plant present in the leaves diminished very rapidly indeed as the nitrogenous composition of the culture was changed so that even in the 40 percent ammonia plants the quantity of oxalic acid was greater than that of malic acid. The stalks showed a similar although less rapid fall in the malic acid content. Inspection of the curves for all three acids shows that malic acid is by far the most extensively affected by the composition of the culture solution. The citric and oxalic acids of both leaves and stalks also diminished, but these were present initially in smaller amounts and accordingly the changes took place over a narrower range. The unknown acids increased significantly in both leaves and stalks of 20 percent ammonia plants but were not greatly different from the nitrate controls at the 40 and 60 percent ammonia levels, although they fell again at the higher levels.

The mutual effects of these changes are perhaps more readily appreciated from the curves in Figure 14 which show the relative quantities of organic acids expressed in percentage of the total organic acidity. The change in the culture solution not only reduced the amount of each acid present, but this occurred in such a manner that from 70 to 80 percent of the organic acids present in all tissues of plants grown at or above the 60 percent ammonia level consisted of acids of the unknown group. Of the known acids, malic acid was the most profoundly affected by the change in the culture solution. Even the plants grown with 20 percent of their nitrogen supplied as ammonia showed a diminution in malic acid in spite of the fact that they were larger than the nitrate controls. At higher levels of ammonia nutrition, negligible absolute and relative amounts of malic acid were present in the leaves and only small absolute and relative amounts in the stalks. Citric acid behaved similarly, little more than traces being present either in leaves or stalks at the higher ammonia levels. Oxalic acid, however, underwent much less change in absolute amount and, save for small increases at the 40 and 60 percent levels of ammonia, preserved an interesting approach to constancy in relative proportion throughout.

The plants grown with 60 percent of their nitrogen supplied as ammonia deserve especial attention since these were practically the same size as the nitrate controls. The leaves contained more than half as much organic acid as the nitrate controls but only about 2 percent as much malic acid. The citric acid content was only about 4 percent of that in the nitrate controls. The oxalic acid content was, however, only reduced by one-third and the unknown organic acid content was practically the same. The entire picture of the organic acid composition of the leaves of these two groups of plants was thus utterly different and the stalks were only somewhat less widely different. From the organic acid analysis of the 60 percent ammonia plant leaves, it would not have been possible to recognize the tissue as belonging to the tobacco plant if comparisons were made with plants grown under normal field conditions (29). This, in turn, is evidence that normal field plants acquire a large proportion of their nitrogen in the form of nitrates regardless of the

TABLE 11. RELATIVE QUANTITIES OF ORGANIC ACIDS IN PERCENTAGE OF THE TOTAL ORGANIC ACIDS

Percentage of N of culture solution as ammonium ion	0	20	40	60	80	90
			LEAVES			
Malic acid	35.8	23.4	12.4	2.5	2.2	4.1
Citric acid	12.1	9.1	7.5	1.5	1.3	1.4
Oxalic acid	18.4	19.5	25.0	22.9	15.7	17.1
Unknown acids	33.7	48.0	55.1	73.1	80.9	77.5
			STALKS			
Malic acid	19.6	13.2	14.5	10.7	7.1	9.2
Citric acid	2.6	2.9	3.1	1.9	0.89	0.97
Oxalic acid	10.9	11.5	13.2	12.2	11.0	11.6
Unknown acids	66.9	72.3	67.1	75.2	81.0	78.3
			ROOTS			
Malic acid	8.3	6.2	4.9	0.94	0.38	1.4
Citric acid	5.1	4.6	4.0	1.8	1.3	1.7
Oxalic acid	31.3	29.5	26.4	22.1	18.4	19.7
Unknown acids	55.3	59.7	64.7	75.1	80.0	77.2

fertilizer source employed and, in confirmation of this view, is the fact that such plants usually contain large quantities of stored nitrate nitrogen.

From the present data, however, it is clear that the organic acid composition of the tobacco plant can be controlled within rather wide limits by attention to the relative proportion of nitrate and ammonia in the culture solution with which they are supplied. Certainly up to the 60 percent level of ammonia nutrition and probably somewhat beyond, plants that appear to be normal in size and general behavior can be produced without difficulty.

At the present time, detailed interpretation of such results as these is clearly impossible. Attention has been drawn to the constancy of the total base present in the leaves and to an approximate proportionality, valid over part of the range, between the quantities of organic acids and the alkalinity of the ash corrected for nitrate. Until more information is available on the qualitative composition of the unknown organic acid fraction, the assumption that an appreciable proportion of acids weaker than malic is present can be regarded only as an *ad hoc* hypothesis.

Perhaps the most important conclusion that can be drawn is that malic and citric acids are by far the most sensitive of the organic acids to the change in the cultural conditions. There is an obvious interlinkage between the metabolism of these two acids which recalls the interesting effects upon them of culture of tobacco leaves in darkness (29). Malic acid was apparently largely converted into citric acid under these conditions.

Previous work on the culture of tobacco and of rhubarb leaves has suggested that the organic acids, particularly malic and citric acids, are intimately concerned in the chemical mechanisms of respi-

ration. Present-day views of this function consider the quantities of the individual acids that accumulate to be a consequence of differences of rates of reaction at each of the several steps in the cycle of interconversions. According to this, malic acid is present in large amounts in normal tobacco leaves because the next step in its transformation is assumed to be ordinarily slower than its rate of production. It, therefore, accumulates until a mass action effect brings it into equilibrium with the next metabolic product. In the light of the present experiments, this view would seem to be oversimplified. There is no distinction possible, with respect to "normality" between the leaves of the nitrate plants, which contained 28 milliequivalents of malic acid per plant and those of the 60 percent ammonia plants which contained only 0.96 milliequivalent. The plants were of the same size and appearance although they were widely different in chemical composition. Yet one must assume that metabolic functions in the two were essentially similar; both were tobacco plants and were, therefore, presumably equipped with identical sets of enzymes and were capable of carrying out fundamentally similar kinds of chemical reactions. Yet in the one case malic acid and citric acid accumulated and in the other they did not. Does this imply that only a small part of the malic and citric acid in tobacco plants of the usual type is actually involved in the respiration cycle, the rest being primarily concerned with other cell functions? Is the concept of bars or inhibitions in the respiration cycle (5, Chapter X) or the notion of delayed or accelerated rates of reaction at one step or another really valid as an explanation of the relative quantities of organic acids present in plants of different nutritive history?

It is obvious that our present knowledge of plant composition and behavior is quite inadequate to deal in detail with the various situations that arise in experimental material. In general the most active metabolites among the organic acids appear to be malic and citric acids. The acids of the unknown group in the present case changed remarkably little in absolute quantity over a range of nitrogen nutrition (0 to 60 percent ammonia) in which the malic and citric acids changed very extensively indeed. Oxalic acid was also only moderately affected. Explanations of the behavior observed are thus more likely to be found in a consideration of the metabolism of other components of the tissues than the unknown acids.

CARBOHYDRATES

Figure 15 shows the effect of the substitution of ammonia for nitrate in the culture solution upon the soluble carbohydrate of the tobacco plant. The soluble carbohydrate, calculated as glucose from the reducing power after inversion of 75 percent alcohol extracts of the dried tissues, diminished in both leaves and stalks, the values in the 60 percent ammonia plants being about half those in the nitrate controls. The carbohydrates diminished still further in the plants on higher ammonia ratios. With respect to distribution within the plant, there was somewhat more than four times as much soluble carbohydrate in the stalks as in the leaves of the nitrate plants and

this proportion was maintained up to the 40 percent ammonia level; subsequently it dropped slightly, but even the 90 percent ammonia plants contained three times as much soluble carbohydrate in the stalks as in the leaves. The effect on the carbohydrates of change in the form of nitrogen in the culture solution thus extended throughout the plant.

TABLE 12. CARBOHYDRATES OF TOBACCO PLANTS, AT TIME OF FIRST FLOWER BUD FORMATION, AS INFLUENCED BY THE PROPORTION OF NITRATE AND AMMONIUM IONS IN THE CULTURE SOLUTION

Percentage of N of culture solution as ammonium ion	0	20	40	60	80	90
			LEAVES			
Soluble carbohydrate, gm./plant	0.617	0.701	0.414	0.393	0.299	0.319
Fermentable carbohydrate, gm./plant	0.348	0.396	0.229	0.211	0.132	0.157
Unfermentable carbohydrate, gm./plant	0.268	0.305	0.185	0.182	0.167	0.162
Sucrose, gm./plant	0.180	0.202	0.140	0.107	0.072	0.053
Soluble carbohydrates, % organic solids	1.91	1.76	1.42	1.35	1.40	1.63
Ratio of fermentable sugar to soluble carbohydrate	0.564	0.565	0.553	0.537	0.442	0.492
Ratio of sucrose to soluble carbohydrate	0.292	0.288	0.338	0.272	0.241	0.166
Soluble carbohydrate, gm./kilo fresh wt.	1.21	1.04	0.715	0.608	0.536	0.635
			STALKS			
Soluble carbohydrate, gm./plant	2.66	2.79	1.68	1.36	0.933	0.952
Fermentable carbohydrate, gm./plant	1.32	1.42	0.887	0.681	0.492	0.469
Unfermentable carbohydrate, gm./plant	1.34	1.37	0.796	0.682	0.441	0.483
Sucrose, gm./plant	0.579	0.773	0.275	0.182	0.117	0.116
Soluble carbohydrate, % organic solids	7.59	6.74	5.70	4.61	5.83	6.84
Ratio of fermentable sugar to soluble carbohydrate	0.496	0.509	0.528	0.501	0.527	0.493
Ratio of sucrose to soluble carbohydrate	0.218	0.277	0.164	0.133	0.125	0.122
Soluble carbohydrate, gm./kilo fresh wt.	6.36	5.36	3.90	3.22	3.26	3.96
			ROOTS			
Soluble carbohydrate, gm./plant	0.144	0.089	0.042	0.037	0.017	0.027
Fermentable carbohydrate, gm./plant	0.090	0.059	0.025	0.025	0.006	0.018
Unfermentable carbohydrate, gm./plant	0.054	0.031	0.017	0.015	0.011	0.009
Sucrose, gm./plant	0.021	0.049	0.020	0.015	0.005	0.012

The proportion of soluble carbohydrate in the leaf tissue dropped from 1.9 percent of the organic solids to a minimum in the 60 percent ammonia plants of 1.35 percent, and rose again to 1.6 percent in the 90 percent ammonia plants. In the stalks, the carbohydrate dropped from 7.6 percent to a minimum of 4.6 percent of the organic solids in the 60 percent ammonia plants and then increased again to 6.8 percent. The fact that the two groups of plants at the 80 and 90 percent levels of ammonia were materially smaller than the others accounts in part for the increase in concentration of soluble carbohydrate; the most important point for the present discussion is that the plants, up to and including those grown at the 60 percent ammonia level, that is in plants of substantially the same size, showed a regularly decreasing content of soluble carbohydrate whether calculated as absolute quantities per plant or in terms of the concentration in the organic solids of the leaf or stalk tissue

respectively. This is a result which recalls the observations of Sideris, Krauss and Young (22) who noted a lower concentration of sugars in pineapple plants grown with ammonium salts than in those grown on nitrates.

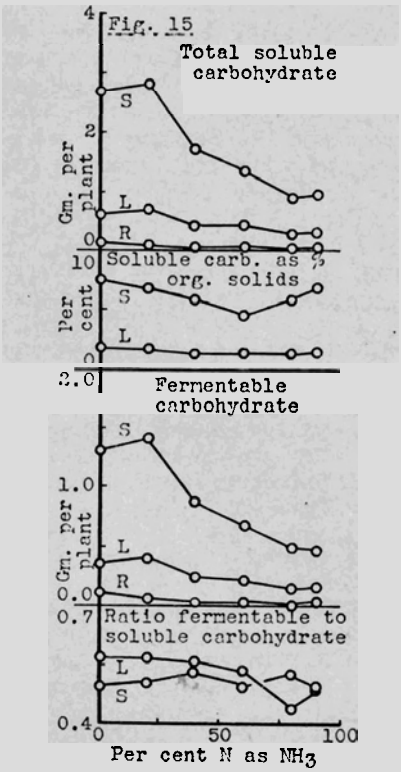
This observation has an important bearing on the carbohydrate metabolism under the various culture conditions. In view of the fact that the decrease of carbohydrates ran roughly parallel with the decrease of organic acids, particularly with the decrease of malic acid, it seems reasonable to infer that the two phenomena are in some way connected. Calculations of the ratios between soluble carbohydrates on the one hand and the total organic acids or the malic acid of the leaves on the other showed, however, that the relationship between these quantities is far from simple. With one exception, the ratios increased rapidly. That is to say, the organic acids decreased more extensively than the carbohydrates as the composition of the culture solution was changed. In the stalks, the ratio declined slightly up to the 60 percent level of ammonia in the culture solution and then rose again. Thus there is no indication of a simple direct proportion between the changes in the quantities of soluble carbohydrates and of those of the total organic acids or of malic acid.

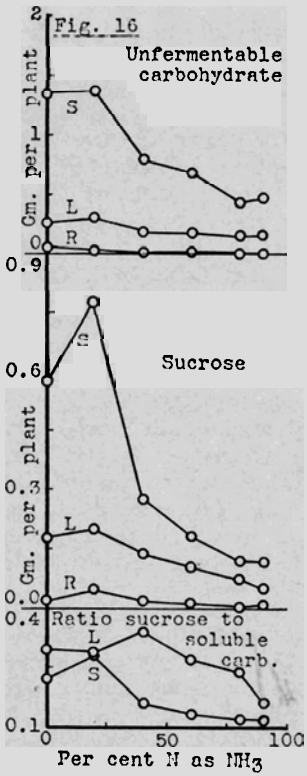
The total soluble carbohydrate of the tobacco plant, as determined by the customary method, consists of substances of two types of which one is fermentable by yeast. Roughly half of the fermentable sugar is sucrose, the rest being probably glucose. The curves in Figure 15, which show the fermentable sugar, resemble those for the total soluble carbohydrate in form. The ratio between the fermentable sugar and the total soluble carbohydrate, which is also plotted, approaches very closely indeed to a horizontal straight line for both leaf and stalk tissues; one point only, that for the 80 percent ammonia leaves, is appreciably discrepant. This implies that, in spite of the extensive change in the quantity of carbohydrates in these tissues, the effects of the change of the culture solution were exerted equally upon both the fermentable sugar (sucrose and glucose) and upon the unfermentable carbohydrate. Over the entire series, the fermentable sugar changed only from 56 to 49 percent of the total soluble carbohydrate in the leaves (average 52.5 percent) and from 49.6 to 49.3 percent in the stalks (average 50.9 percent). Both forms of carbohydrates are clearly concerned in the general metabolism as it is affected by the change in the culture solution.

The curves which show the behavior of that part of the soluble carbohydrate that is not fermented by yeast, the so-called unfermentable carbohydrate, are also closely like those for the fermentable sugar. The ratio between the amounts present in each group of plants and the corresponding amounts of total soluble carbohydrate or of fermentable carbohydrate is very nearly constant. This is a consequence of the fact that the ratio between fermentable carbohydrate and the total soluble carbohydrate is practically constant over the entire range of the experiment.

The readiness with which unfermentable carbohydrate enters into metabolism in the leaf of the tobacco plant has been pointed out in connection with studies of the behavior of the leaf during culture in both light and darkness (29). The present case is a further example of the significance of this analytical component as one of the more reactive metabolites of the tissues. Very little information with regard to its chemical nature has been obtained. In fact its classification as a "carbohydrate" is a matter of assumption since all that is really known is that it reduces sugar reagents and possesses the solubility relationships of the simple sugars. Substances of a similar nature are widely distributed (21) but have received little attention from the chemical point of view.

The sucrose, which forms a part of the soluble fermentable carbohydrate, is also plotted in Figure 16. The curves follow in general those for the other carbohydrate components, but sucrose does not make up a fixed proportion of the soluble carbohydrate fraction. The curve for the ratio of sucrose to soluble carbohydrate in the leaf does indeed show that this ratio changed very little over the range 0 to 60 percent ammonia in the culture solution but, in the plants on high proportions, it dropped significantly. The ratio for





the sucrose in the stalks, although a little higher in the plants grown at the 20 percent level, also diminished regularly as the ammonia in the culture solution increased. In general about one-third of the soluble carbohydrate in the leaves of the nitrate plants, and about one-fifth of that in the stalks, was sucrose; in the plants grown with 90 percent of the nitrogen as ammonia, these fractions became respectively one-sixth and one-eighth.

The soluble carbohydrate content of the leaves of the type of tobacco plant employed for this study is normally somewhat low. The nitrate plants, which may be regarded as fairly typical in their chemical composition, contained less than 2 percent of their organic solids as simple carbohydrates at the time of collection. This proportion was reduced still further by the substitution of ammonia for nitrate in the culture solution. Although the actual carbohydrate content of any individual plant is strongly affected by the conditions, with respect to exposure to light, previous to the time at which the samples are taken, variations between individuals on this account were not an important factor in the present case. All samples were harvested at substantially the same time — about 9 A.M. — on a cloudy morning, and the leaves were dried as promptly as possible, being kept on ice if necessary for a few hours until space was available in the drying apparatus.

The most significant result is that all of the several forms of carbohydrates were similarly affected by the conditions under which the plants were grown. Sucrose, glucose, and even the unfermentable carbohydrates were equally involved and, accordingly, are closely interrelated with each other. In exactly what way the quantity of carbohydrates present is connected with the quantities of other components of the tissues such as the organic acids or the proteins does not appear from the present data.

COMPOSITION OF TOBACCO PLANTS AS AFFECTED BY NITROGEN NUTRITION

In order to recapitulate the data which show the general effects of the substitution of ammonia for nitrate in the culture solution in which tobacco plants are grown, the composition of the leaf and stalk tissue has been calculated in terms of the chemical composition, so far as this is possible, for the plants grown on nitrate and for those grown with 60 percent of their nitrogen supplied as ammonia. These calculations are shown in Table 13. The values for constituents in terms of grams per single plant and also in grams per kilo, respectively, of leaf and stalk tissue, are both given.

In making these calculations, a number of assumptions, some of which are highly speculative, have been made. The 60 percent ammonia plants were selected for the comparison because they were of about the same size as the nitrate plants. They show the effects of ammonia nutrition on composition without the complication that would be introduced by corrections for size. Although the entire series of plants yielded many data that fall upon smooth curves

TABLE 13. THE CHEMICAL COMPOSITION OF TOBACCO PLANTS AS INFLUENCED BY THE FORM OF NITROGEN SUPPLIED

Percentage of N of culture solution as ammonium ion	LEAVES				STALKS			
	0	60	0	60	0	60	0	60
	gm. per plant		gm. per kilo of fresh tissue		gm. per plant		gm. per kilo of fresh tissue	
	1	2	3	4	5	6	7	8
Protein (N × 6.25)	8.06	8.13	15.8	10.0	1.67	1.46	4.00	3.43
Ammonia (N × 1.21)	0.015	0.079	0.029	0.123	0.006	0.037	0.015	0.089
Nitrate (N × 4.43)	2.62	1.28	5.14	1.99	2.04	1.01	4.89	2.62
Glutamine (amide N × 10.4)	**0.142**	**0.109**	**0.28**	**0.168**	**0.31**	**0.43**	**0.75**	**0.99**
Asparagine (amide N × 9.43)	0.121	0.231	0.236	0.358	0.11	0.39	0.25	0.91
Other soluble N compounds (corrected soluble N × 10)	4.68	5.0	9.16	7.75	2.08	1.56	4.97	3.69
Malic acid	1.89	0.064	3.70	0.099	0.751	0.284	1.80	0.67
Citric acid	0.612	0.035	1.20	0.054	0.095	0.048	0.227	0.113
Oxalic acid	0.653	0.391	1.28	0.606	0.282	0.215	0.676	0.508
Unknown acids (as malic)	1.78	1.86	3.49	3.65	2.57	1.99	6.17	4.69
Soluble carbohydrate	0.617	0.393	1.21	0.608	2.66	1.36	6.36	3.22
Undetermined solids soluble in 75% alcohol	0.26	5.32	0.53	7.45	0.43	1.42	1.02	3.12
Undetermined solids insoluble in 75% alcohol	19.2	16.0	37.6	27.3	26.9	23.8	64.3	56.0
Inorganic solids	7.79	9.29	15.3	14.4	4.29	4.30	10.3	10.1
Undetermined solids exclusive of inorganic solids	11.7	12.0	22.9	20.4	23.0	20.9	44.9	44.0
Total solids	40.1	38.5	78.6	59.6	39.3	33.8	94.1	79.7
Water	470	607	921	940	378	390	906	920

showing that the effects observed were truly functions of the change in the nutrient solutions, the failure of the plants on 80 and 90 percent ammonia in the culture solution to attain the same degree of development as the others renders detailed comparison of these individuals with the nitrate controls somewhat hazardous.

The calculations of the composition were made as follows: protein nitrogen was converted to weight of protein by the conventional factor 6.25 which assumes that the average true nitrogen content of the proteins is 16 percent. This is doubtless a fairly close approximation. Ammonia is given as weight of NH_3, regardless of the fact that it is present in solution as ammonium salts. Nitrate is calculated as NO_3 ion; an equivalalent quantity of base must, therefore, be present and is assumed to form a part of the inorganic solids. The amides are calculated from the respective forms of amide nitrogen. The balance of soluble nitrogenous substances is calculated on the assumption that the soluble organic nitrogen, exclusive of the amides, represents substances with an average nitrogen content of 10 percent. This is probably an underestimate and, accordingly, leads to an overestimate of the amount of substances of this type. Fortunately the difference in the quantity present in the two groups of plants is not great so that no serious error is introduced into the comparison. The organic acids are calculated from direct analyses on the assumption that the unknown acids may be calculated as malic acid from the titration data. The soluble carbohydrate is the glucose equivalent of the total reducing power after inversion of sucrose.

The estimation of the quantities of undetermined substances is subject to considerable error. The data are derived from the weights of the residues that remain when the tissues are exhaustively extracted with 75 percent alcohol. Under these circumstances, it is assumed that the malic, citric, and unknown acids are extracted while the oxalic acid is not. This is certainly not entirely correct. Although very little oxalic acid is soluble and much malic and citric acid are removed in this operation, some of these two acids always remains unless the tissue is acidified so as to liberate them from their salts. The proteins, of course, remain insoluble. The values given for undetermined soluble material are thus a compromise. They are computed by subtracting the sum of the items that appear above the estimate of undetermined soluble material (except protein and oxalic acid) from the analytically determined soluble material. Some of the inorganic material is included.

The undetermined insoluble substances represent the weights of the insoluble residues corrected for the proteins they contain. The values also include a part of the inorganic substances. The inorganic solids, or ash, are from the direct determinations on the whole tissue. The value for the ash in the leaves of the 60 percent ammonia plants chances to be somewhat out of line with the ash of the 40 and 80 percent plants, being apparently a little high.

What is probably a more trustworthy estimate of the quantity of undetermined substances is furnished by deducting the ash from

the sum of the undetermined soluble and insoluble substances. This value gives the order of magnitude of organic material the chemical nature of which is unknown save that it includes such components as the cellulose, pectins, and substances of allied nature, the chlorophyll, and the fat-solvent soluble material.

Leaves

The outstanding effects of the substitution of ammonia for nitrate on the chemical composition of the leaf of the tobacco plant (Table 13, Columns 1 and 2) are to decrease the quantities per plant of the nitrate, malic, citric, and oxalic acids, and soluble carbohydrate. The change in the amides was small, the increase in asparagine alone being significant. There was only a minor increase in ammonia content, when considered in terms of absolute quantity, although it represented actually a five-fold increase over the trace present in the nitrate plants. There was no significant change in the protein content and there seems little reason to suppose that any material change occurred in the amount of the undetermined group of substances that includes the complex carbohydrates, glucuronides and ether-soluble material. There was a remarkable increase in water content.

The data for concentration in terms of fresh weight (Columns 3 and 4) indicate a marked fall in protein, and a probably significant fall in the soluble organic nitrogenous substances other than the amides. In addition, extensive losses occurred of malic, citric, and oxalic acids and of soluble carbohydrate. The change in concentration of the unknown acids, the inorganic substances and the undetermined group was probably insignificant. The increase in water, although apparently not great, represents a change from leaves that contained 8 percent of solids to leaves that contained only 6 percent, that is, a change of 25 percent in the solids content of the fresh tissue.

Stalks

As in the leaves, the chief changes in terms of grams per plant (Columns 5 and 6) involved decreases in the quantities of nitrate and of malic acid and soluble carbohydrate. There was a small decrease in protein and in soluble organic nitrogenous substances (exclusive of the amides) and of citric, oxalic, and unknown acids. Only a minor increase in ammonia and amides occurred and the increase in water content was small compared to that in the leaves. It is possible that the decrease suggested by the figure for undetermined organic substances is significant but the change was small.

The data for stalk composition on a concentration basis (Columns 7 and 8) reflect the major changes already mentioned. It is interesting to note, however, that no change in the concentration of the group of undetermined organic substances is indicated.

DISCUSSION

The present results lead to the conclusion that the tobacco plant is extremely responsive to the nature of the nitrogenous nutriment with which it is supplied. Certain of the components, particularly the nitrate, the organic acids, and the carbohydrates undergo relatively enormous changes in the quantity present. When the extreme of ammonia nutrition is approached there is a marked accumulation of free ammonia in the tissues. The quantity of protein and, surprisingly enough, of the amides is not necessarily extensively altered by the change in nitrogenous nutriment. It must be emphasized, however, that the total nitrogen available to these plants was just sufficient to provide good growth; it was by no means a luxury supply. In later experiments (unpublished) it has been found that, if the nitrogen is furnished at a substantially greater total concentration, there is a definite effect upon amide concentration of the substitution of part of the nitrate by ammonia.

As perhaps might be anticipated, the group of substances that includes cellulose, pectins, and ether-soluble material appeared to be relatively insensitive. The results strongly suggest, however, that the groups of substances ordinarily regarded as the more active metabolites of plant tissues, the carbohydrates, organic acids, and certain simple nitrogenous substances, are chiefly concerned.

A clear inference may be drawn from these data with respect to the necessity of exercising control over the conditions under which plants to be employed for experimental purposes are grown. It is obvious that, by judicious selection of the salts employed in the culture solution, plant material of widely diversified composition within certain limits may be secured. Conversely when no control is exercised, regularities in composition are scarcely to be anticipated.

The use of the tobacco plant as an indicator of fertility in studies of soils (14) is clearly justified by the present results and it is possible that more detailed analysis of the tissues than is usually attempted in such tests might lead to even finer discriminations than can now be made.

No consideration has been given, in connection with the discussion of the present data, of possible application of the results to agricultural practice. The use of ammonium salts as the source of nitrogen for the growing of tobacco has been extensively studied by Anderson and his collaborators (1, 23). Their observations indicate that complications are introduced by the progressive acidification of the soil, if ammonium sulfate is used year after year. If this condition is controlled correctly, the crop can be produced in good yield but the quality of the cured and fermented tobacco is low. They particularly mention the poor fire holding capacity. The early work of Ridgway (19) and the more recent studies of Haley, Nasset and Olson (9) suggest a correlation between good fire holding capacity and high organic acid content. Although the organic acids

of manufactured tobacco are doubtless widely different in detail from those of the fresh leaf, there is reason to suppose, from our own work on the culture of fresh tobacco leaves in water (29), that high total organic acidity of the fresh leaf would result in high total organic acidity in the final product. It is doubtless also true that fresh leaves of low organic acidity would yield a manufactured tobacco of low organic acidity. If the present data obtained with culture solutions may be applied even in the broadest manner to the results of field experiments, they would lead one to anticipate that field tobacco grown with ammonium salts as the source of nitrogen would contain smaller proportions of organic acids than usual. To this extent, therefore, the low quality of the crops grown under these conditions receives a logical explanation.

It must be emphasized, however, that the ready conversion of ammonium salts to nitrates in the soil renders any such explanation as this somewhat uncertain. Records of the analysis of cured tobacco grown in 1927 at the Windsor Tobacco Substation (26) showed that a sample prepared from plants fertilized with sodium nitrate contained 3.5 percent of nitrate nitrogen while one from plants fertilized with ammonium sulfate contained 19.5 percent. Samples from plants that had received various organic nitrogenous fertilizers lay between these extremes. The explanation of the low nitrate nitrogen content of the sodium nitrate plants is doubtless the leaching of this soluble salt from the soil by the unusually heavy rains of that particular growing season. Ammonium salts, on the contrary, are not readily removed in this way and it is obvious that an extensive transformation to nitrates in the soil must have occurred.

The old problem of the form in which nitrogen is most readily available to plants, usually expressed in terms of the simple alternative between the supply of nitrate ions and ammonium ions, is clearly more complex than is often assumed. Although the present results may have little to do with the conditions encountered in agricultural practice, where the effects of organisms on the soil nitrogen play a dominating role with respect to the nitrogen compound actually absorbed by the roots of the plant, they do shed a little light upon the question. Both forms of nitrogen are available, and are utilized by the plant organism when conditions are correct. When both are furnished, there is considerable evidence that ammonium is more rapidly metabolized by the tobacco plant than nitrate. At this point, however, the complications begin, and the most fundamental of these appears to be related to the balance between the positive and negative ions introduced into the cells. The chemical responses evoked under different conditions are far-reaching indeed and satisfactory explanations will be obtained only when more detailed examination with more fully developed analytical technics has been made.

SUMMARY

Tobacco plants (Rosenberg strain) were grown in sand culture with nutrient solutions in which the relative proportion of nitrate nitrogen and ammonium nitrogen was varied by steps from all ni-

trate to a mixture of 10 parts of nitrate nitrogen with 90 parts of ammonium nitrogen. The total nitrogen content of all solutions was the same, and all known nutrient ions were provided in adequate and constant proportion with the exception of chloride ion and sulfate ion, both of which were increased in parallel with the increase in the proportion of ammonium ion. The nitrogen was supplied at a level high enough to provide for good growth but was not in excess of this. The plants were harvested at the time of first flower-bud formation and the quantities per plant of the more important chemical constituents of leaf, stalk, and root tissues were determined.

Wide variations in chemical composition of the plants were observed. The general effects of the substitution of ammonium nitrogen for nitrate nitrogen were briefly as follows: at the 20 percent level of ammonium nitrogen, a definite stimulation in size of the plants as compared with the nitrate controls was noted. The 40 and 60 percent ammonia plants were not greatly different in size from the controls but the 80 and 90 percent plants were smaller. There was a marked diminution in nitrate nitrogen stored in the tissues as the relative proportion of ammonium ion in the culture solution was increased, but there was a relatively much smaller increase in stored ammonia nitrogen. Possibly because of the failure to supply total nitrogen at a luxury level, the effects upon the amides of the tissues were very small; glutamine was not significantly changed although asparagine was slightly increased. Very little change in the amount of soluble organic nitrogen occurred. The protein content of the tissues in terms of grams per plant varied with the size but increased slightly when calculated in terms of organic solids.

The most striking effects of the change in the form of nitrogen supplied were shown by the water content of the leaves and by the non-nitrogenous components of the tissues. Hydration, as measured either by actual amount of water per plant or by concentration of total solids in unit weight of fresh tissue, increased phenomenally with increase in ammonia.

The organic acids, especially malic and citric acids, decreased remarkably both in quantity per plant and in concentration in the tissues, and evidence was found for a profound alteration in the distribution of acidic and basic components.

The soluble carbohydrates likewise diminished in quantity and in concentration and evidence for a close metabolic relationship between the several forms of carbohydrate was obtained.

The responses of the nitrogenous components, aside from the effects on the storage of nitrate and of ammonia nitrogen, were subtle and difficult to interpret from the present data. Far more detailed and extensive analysis of the tissues will be required before these can be fully understood.

The results of the experiments give the impression that the composition of the tobacco plant is profoundly influenced by the form in

which nitrogen is absorbed. Alterations in many aspects of the metabolism were noted and many of these may be provisionally attributed to the fundamental effects on the quantities of organic acids and to concomitant effects on the absorption of inorganic ions. A considerable sensitivity on the part of the mechanisms involved in the balance between positive and negative ions in the cells is suggested. Furthermore, the failure of the high ammonia plants to synthesize carbohydrates as extensively as those furnished with a high proportion of nitrate suggests that the photosynthetic mechanism is also intimately concerned.

Analytical studies such as these throw little light on the finer details of the enzymatic mechanisms that are involved but they emphasize the complexities of these systems. The plant is clearly a creature of its own environment; its chemical composition is by no means fixed and characteristic of the species but may vary within surprisingly wide limits in response to what may seem somewhat minor influences.

APPENDIX

Supplementary Experiments on the Effect of the Nature of the Negative Ion in the Culture Solution on the Composition of Tobacco Plants Grown With Both Nitrate Nitrogen and Ammonium Nitrogen Available

THE preparation of a series of culture solutions in which the relative proportions of nitrate and ammonium ions are varied involves variation also in one or more of the other ions. In the experiments already described, the chloride ion and the sulfate ion were both increased in concentration in the series of culture solutions as the composition with respect to nitrogen was changed from 100 percent nitrate nitrogen by steps to a mixture of 10 percent nitrate and 90 percent ammonium ion. Although little evidence was obtained that the increase in sulfate ion concentration had any material effect on the composition of the plants, the increase in chloride ion brought about a large relative change in the amount of chloride in the ash of the tissues. The chloride content of the leaf ash increased nearly proportionally with the chloride ion concentration in the culture solutions, and the effect on the chloride of the stalk ash was only moderately less pronounced. Although the change in the form of nitrogen seemed to be by far the most significant in its influence on the tissue composition, it appeared to be desirable to carry out tests to see if the chloride or sulfate ion could have contributed in any direct manner to the wide differences that were noted. The chief point at issue was whether or not the organic acids would respond to the alteration in the form of nitrogen, regardless of the nature of the negative ion that was also changed.

The comparison desired was one between nitrate control plants, and plants grown with half of their nitrogen supplied as nitrate, half as ammonium ion, the culture solutions of the latter type being prepared with increases respectively in sulfate, chloride, and phosphate parallel with the increases in ammonia. In addition a test with a solution prepared from ammonium nitrate was desired.

The details of the composition of these culture solutions are shown in Table 14. Each was obtained by dilution of mixtures of stock 0.5 M solutions in 10-liter lots using tap water. Boron, manganese, and iron (0.5 p.p.m. of each) and Hoagland's AZ solution were added so as to provide sufficient of the minor elements. The hydrogen ion activity was adjusted by the addition of 1.0 N sodium hydroxide or sulfuric acid as required. The nitrate control solution was furnished to the plants at or near pH 5.0, the other solutions at or near pH 6.2. Records of the reaction of the solution that dripped from the sand cultures were made at intervals. The drip from the nitrate controls ranged from pH 7.1 to 7.5, that from the other solutions was maintained for the most part in the range 6.0 to 6.6.

The solutions were based upon the system of solutions described by Beckenbach, Wadleigh and Shive (2) and represent a modification of their treatment 3, in which Ca, Mg, and K are present in the

TABLE 14. CULTURE SOLUTIONS DESIGNED TO PROVIDE EQUAL AMOUNTS OF NITRATE AND AMMONIUM NITROGEN WITH VARIATION IN THE NATURE OF ONE NEGATIVE ION

Figures give molarity of each salt in final solution; total concentration of ions to give approximately 0.5 atmos. osmotic pressure.

	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KNO_3	$Mg(NO_3)_2$	$CaSO_4$	$(NH_4)_2SO_4$	NH_4Cl	$NH_4H_2PO_4$	NH_4NO_3
Nitrate control	0.001	0.00225	0.0014	0.0021	0.0043					
Ammonium sulfate	0.001	0.00225	0.0014	0.0021	0.0005		0.0038			
Ammonium chloride	0.001	0.00225	0.0014	0.0021	0.0005			0.0076		
Ammonium phosphate	0.001	0.00225	0.0014	0.0021	0.0005				0.0076	
Ammonium nitrate	0.0031		0.0014			0.00225				0.0076

ratio 3:4:3 and NO_3, SO_4 and PO_4 in the ratio 8:1:1. The nitrate control solution was identical with their treatment 3, and other solutions were prepared by substituting the respective ammonium salts for magnesium nitrate in such a way as to preserve equality between nitrate ion and ammonium ion. The ammonium nitrate solution was a simple four-salt solution. The calcium was added as solid calcium sulfate after final dilution.

The concentration of magnesium was reduced in these ammonia-containing solutions as compared with the nitrate controls but not so as to become a limiting factor on the growth of the plants.

The general technic and methods of analysis of the tissues were identical with those already described. The plants were grown in the season of 1938 and were harvested 50 days after being transplanted to the sand. There were four plants on each treatment. It was necessary to eliminate one plant from each of three of the groups (nitrate control, chloride, and phosphate) because of failure to grow as well as the others or because of accidental damage.

In Table 15 are given data to permit a comparison between certain of these plants, grown in the season 1938, and the plants already described that were grown under similar conditions of nutrition in 1937. The nitrate controls were not grown with the same culture solution in the two instances, as will be seen on comparison of the data in Table 14 with those in Table 1, but in neither case were inorganic ions present in limiting proportion and in both the entire nitrogen supply was provided by nitrate ions.

TABLE 15. COMPARISON BETWEEN THE COMPOSITION OF TOBACCO PLANT LEAVES GROWN UNDER SIMILAR CONDITIONS IN TWO SUCCESSIVE SEASONS

Figures are grams per plant unless otherwise designated.

	Nitrogen supplied as nitrate ion		50% of nitrogen supplied as ammonium ion	
	1938	1937	1938	1937
Fresh weight	489	510	405	612
Organic solids	28.4	32.3	28.8	29.2
Inorganic solids	5.56	7.79	5.90	8.50
Nitrate nitrogen	0.561	0.592	0.440	0.413
Total nitrogen	2.26	2.42	2.60	2.27
Protein nitrogen	1.02	1.29	1.21	1.29
Total organic acids, m.e./plant	77.2	78.8	37.9	41.7
Malic acid, m.e./plant	31.3	28.2	2.70	3.29
Citric acid, m.e./plant	16.4	9.56	2.20	1.97
Oxalic acid, m.e./plant	11.5	14.5	8.35	10.0
Unknown acid, m.e./plant	18.0	26.6	24.7	26.4
Soluble carbohydrate	0.470	0.617	0.466	0.403
Sucrose	0.131	0.180	0.109	0.223
Fermentable sugar	0.186	0.348	0.180	0.220
Unfermentable carbohydrate	0.146	0.268	0.171	0.184

The growth period was slightly different in the two experiments, being 50 days in 1938 and 52 in 1937. Nevertheless, with respect to the points that are of significance for the present discussion the two lots of leaves from the nitrate plants were closely alike. Both stored a large amount of excess nitrate, both were rich in organic acids, especially malic acid, and both were poor in soluble carbohydrates. In Column 3 of the table is given the composition of the plants in which half of the nitrogen was supplied as ammonium ions and in which chlorides made up a large part of the negative ions. For comparison, the average between the 40 and 60 percent ammonia plants grown in 1937 is given in Column 4. As in the nitrate plants, the culture solutions differed somewhat widely in detail but were the same to the extent that equal amounts of nitrate and ammonium nitrogen were provided and that chloride made up a large part of the negative ions.[1]

Comparison of the data in Columns 3 and 4 shows an appreciable difference in fresh weight but the organic solids were almost identical. The nitrate nitrogen was the same within the limits of error and the organic acids were very similar in detail. Both groups of plants show the profound diminution in malic and citric acids as compared with the nitrate plants and, in both cases, there was little effect on the oxalic acid and unknown acids. A close resemblance in the quantities of the carbohydrates is perhaps not to be anticipated since these substances respond so readily to the conditions of illumination immediately preceding collection of the samples.

Accordingly, the plants grown in 1938 may be regarded as essentially similar in composition to those grown in 1937 in the cases where the nitrogen composition of the respective culture solutions was alike, and it may be further concluded that the effect upon the organic acids of the substitution of half of the nitrate nitrogen by ammonium nitrogen can be duplicated in successive years. It remains to see if this effect is conditioned by the presence of chloride.

The essential data are given in Table 16. The organic acid composition of the four groups of plants grown with half of their nitrogen supplied as ammonium ion was quite similar in detail. The citric acid and oxalic acid values for both leaves and stalks do not differ significantly from each other in any case. The total organic acids of the leaves of the chloride and nitrate plants were a little higher than those for the sulfate and phosphate plants and the unknown acids of these plants were also slightly higher than the others. The parallelism extends to the organic acids of the stalks. The tissues in each case, with only one or two minor exceptions, had a fundamentally similar organic acid composition.

Comparison with the nitrate controls shows in each case a sig-

[1]The culture solution used in 1938 was 0.0076 M in chloride ion. The average of the molarity of the chloride ion in the 40 and 60 percent ammonia plants grown in 1937 was 0.0037. In both cases the trace of chloride ion provided by the minor element solutions is ignored.

nificant and in many cases a wide difference with respect to the malic and citric acid. Regardless of the nature of the negative ion, the substitution of half of the nitrate by ammonium in the culture solution greatly reduced the quantity of malic and citric acid in the tissues. The effects upon the oxalic acid were in each case in the same direction although the relative magnitudes of the changes were much smaller. There was, however, only a moderate effect on the unknown acids; those in the leaves were a little higher and those in the stalks a little lower.

TABLE 16. THE EFFECT OF DIFFERENT NEGATIVE IONS IN THE CULTURE SOLUTION ON THE ORGANIC ACID CONTENT OF TOBACCO PLANTS: A COMPARISON BETWEEN NITRATE CONTROLS AND PLANTS GROWN WITH HALF OF THEIR NITROGEN SUPPLIED AS AMMONIUM SALTS

Figures are milliequivalents per plant.

	Nitrate control	Ammonium sulfate	Ammonium chloride	Ammonium phosphate	Ammonium nitrate
			LEAVES		
Total organic acids	77.2	34.3	37.9	33.6	40.8
Malic acid	31.3	4.85	2.69	3.24	4.68
Citric acid	16.4	2.02	2.20	2.10	2.56
Oxalic acid	11.5	7.43	8.35	7.12	8.37
Unknown acids	18.0	20.0	24.7	21.2	25.2
			STALKS		
Total organic acids	35.4	27.3	22.6	25.6	34.2
Malic acid	7.27	4.35	2.84	3.83	7.27
Oxalic acid	3.78	3.36	3.43	3.00	4.07
Unknown acids	22.6	19.5	15.3	17.8	21.7

It may be concluded that the alteration in the organic acid composition brought about by the substitution of ammonia for nitrate in the culture solution is a specific effect of this change and is not to any significant degree a function of the nature of the inorganic anion.

The data in Table 16 show several secondary effects, some of which may be significant. For example, the total organic acids and the unknown acids of the leaves of the chloride and ammonium nitrate plants were slightly higher than the others. This is possibly an effect of a difference between the rate of absorption of a monovalent and of a divalent anion. The effect is not apparent in the stalks of the same plants, however; on the contrary the total organic acids and the unknown acids were lower in the stalks of the chloride plants than in the others. To what extent such minor differences may be attributed to specific ions can be decided only by further investigation.

A few additional data for these groups of plants are shown in Table 17. Attention may be directed to the relative constancy of the organic solids throughout and accordingly to the greater hydration (as evidenced by the fresh weight) of the sulfate, chloride, and ammonium nitrate plants as compared with that of the nitrate controls. The somewhat smaller phosphate plants did not show this effect, however.

TABLE 17. THE EFFECT OF DIFFERENT NEGATIVE IONS IN THE CULTURE SOLUTION ON THE COMPOSITION OF TOBACCO PLANTS: A COMPARISON BETWEEN NITRATE CONTROLS AND PLANTS GROWN WITH HALF OF THEIR NITROGEN SUPPLIED AS AMMONIUM SALTS

Figures are grams per plant unless otherwise designated.

	Nitrate control	Ammonium sulfate	Ammonium chloride	Ammonium phosphate	Ammonium nitrate
			LEAVES		
Fresh weight	489	527	574	472	561
Organic solids	28.4	27.3	28.8	25.7	31.7
Inorganic solids	5.56	5.89	5.90	5.10	6.59
Total N	2.26	2.53	2.60	2.39	2.91
Nitrate N	0.561	0.517	0.440	0.609	0.631
Protein N	1.02	1.17	1.21	1.11	1.10
Soluble N exclusive of nitrate	0.684	0.845	0.950	0.675	1.20
Soluble carbohydrate	0.470	0.435	0.466	0.494	0.606
Sucrose	0.131	0.081	0.109	0.115	0.187
Fermentable sugar	0.186	0.177	0.180	0.234	0.179
Unfermentable carbohydrate	0.146	0.173	0.171	0.137	0.231
Alkalinity of ash, m.e./plant	111	64.0	60.9	65.8	73.3
Sulfate in ash, m.e./plant	10.7	29.1	15.0	19.4	33.4
Phosphate in ash, m.e./plant	5.72	8.11	7.63	8.75	8.96
Chloride in ash, m.e./plant	1.54	2.05	32.0	1.65	2.04
			STALKS		
Fresh weight	394	402	405	356	399
Organic solids	26.6	25.5	25.4	23.1	28.0
Inorganic solids	3.56	3.17	3.35	2.93	3.31
Total N	0.938	1.03	0.979	0.979	1.12
Nitrate N	0.510	0.626	0.648	0.585	0.715
Soluble carbohydrate	2.26	1.71	0.99	1.39	1.51
Sucrose	0.402	0.294	0.655	0.291	0.390
Fermentable sugar	1.38	0.814	0.493	0.605	0.586
Unfermentable carbohydrate	0.487	0.582	0.430	0.479	0.509
Alkalinity of ash, m.e./plant	58.2	49.1	40.2	45.4	47.1

The leaf protein was remarkably constant in all cases, but the soluble nitrogenous substances exclusive of nitrate were significantly greater (except for the phosphate plants) than in the nitrate controls.

It is interesting to note that the soluble carbohydrates of the leaves were not diminished by the alteration of the form of nitrogen in the culture solution; in fact, the soluble carbohydrate in the leaves of the ammonium nitrate plants was appreciably higher than that of the nitrate controls. Thus these plants did not show a diminution

in leaf carbohydrate with change in the form of nitrogen in the culture solution as did the plants grown in 1937.

The stalk carbohydrates, however, show a marked decrease in **the soluble carbohydrate and fermentable sugar.** Two of the four examples also show a decrease in sucrose. However, none of them shows an effect upon the unfermentable carbohydrate. Accordingly, with respect to the carbohydrates, the 1938 plants confirm the results with the 1937 plants only in part. It may be pointed out, however, that such labile components as the leaf and, probably, the stalk carbohydrates as well would be subject to precise control only if the conditions of illumination, temperature, and humidity were maintained exactly the same in two different experiments. This can be accomplished only when the plants are grown in an entirely artificial environment.

Specific effects of the several ions are best shown by the data **for the ash analysis.** Although the alkalinity of the ash varied, as between nitrate controls and the others, in a manner parallel to the total organic acids (Table 16), the detailed composition of the ash reflects the anion composition of the culture solutions, notably in the chloride and sulfate plants. The phosphate plants, on the contrary, showed no clear evidence of an increase in phosphate in the ash of the leaves. The true significance of the slightly greater organic solids and total nitrogen content of the ammonium nitrate plants, as compared with that of the other plants grown with half of their nitrogen supply as ammonium ion, is uncertain. It is a common experience, however, that larger and more vigorous plants are secured when this salt is used as nitrogen source than when the nitrogen is supplied entirely as nitrate (16). The present results illustrate this also, but show that the increased nitrogen content does not necessarily imply an increase in the tissue protein.

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