THE BOTANICAL REVIEW

VOL. III MARCH, 1937 NO. 3

THE NITROGEN NUTRITION OF GREEN PLANTS*†

GORDON T. NIGHTINGALE
Pineapple Producers' Cooperative Association, Hawaii.

* Nitrogen-fixation by leguminous plants is a specialized phase of nitrogen nutrition which is not reviewed here. There is likewise omitted any discussion of the interrelation between disease and nitrogen nutrition, and of responses to mineral deficiencies. For an excellent review of the theories of protein metabolism, consult Robinson (208). Especial attention should also be called to the hypotheses of the structure of proteins by Vickery and Osborne (271).

† Published with the approval of the Director as Miscellaneous Paper No. 21 of the Pineapple Experiment Station, University of Hawaii.

Plants absorb and utilize inorganic salts of nitrogen including ammonium, nitrite and nitrate with various degrees of efficiency. The relative rate at which plants can absorb and elaborate nitrogenous nutrients is dependent upon such external factors as the pH of the soil or nutrient solution, its concentration of solutes, and the relative availability of calcium, potassium, phosphate, etc. External factors such as light, temperature, moisture and oxygen supply also play an important rôle.

But internal factors, frequently given no consideration, are at least of equal if not of greater importance. Seeds, storage organs and growing plants vary enormously in their content and quality of nitrogenous and carbohydrate, or related nitrogen-free, reserves. The pioneer work on nitrogen nutrition, initiated nearly 40 years ago by Prianischnikov and his students (181, etc.), shows clearly that the quantity and nature of reserve materials in the plant profoundly influence nitrogen nutrition: the absorption and new synthesis of amino acids and associated materials from inorganic sources of nitrogen.

The subject of nitrogen nutrition in its several aspects to be discussed in the following pages can best be considered following a brief review of the metabolic changes which have been found to take place in organic compounds of nitrogen already in storage or contained in the plant. This phase of nitrogen metabolism is in contrast to new synthesis from inorganic nitrogenous salts. It is mainly concerned with the hydrolysis and regeneration of proteinaceous materials as they have been found to occur under various conditions of environment and carbohydrate supply.

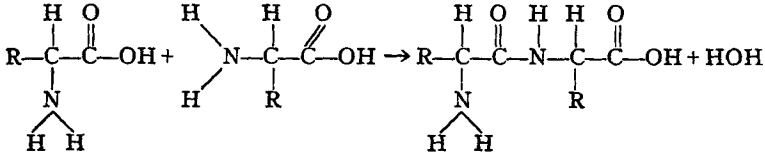
SYNTHESIS AND HYDROLYSIS OF STORAGE PROTEINS

There is available no satisfactory chemical basis for distinguishing between storage proteins and proteins of the protoplasm of active leaves or of growing or meristematic tissues such as cambium, root tip or stem tip. The former are found in tissues low in moisture and high in carbohydrates or their derivatives, in tubers and similar organs and in the cotyledons or endosperm of seeds. The latter are found in cells relatively high in moisture and lower in nitrogen-free materials.

Protoplasmic proteins have not been thoroughly investigated but it is well known that they do not give characteristic protein tests

except when preceded by denaturization. They occur apparently in a relatively latent condition and include complex nucleo-proteins which may in turn be cleavage products of more complex units. The cyclic structure hypothesis may perhaps lead to a better understanding of the nature of the protoplasmic proteins, for it indicates that amino acids can enter into combinations that are not included in the conventional peptide hypothesis (271).

Synthesis of Storage Proteins: The peptide hypothesis of Emil Fischer and others (271) is primarily one of synthesis of polypeptides by dehydration of amino acids. For every two molecules of amino acid that condense to form a more complex compound there is loss of a molecule of water thus:



This hypothesis is demonstrable in the laboratory and while it does not explain the synthesis of protoplasmic proteins of active plant tissues, it still remains one of the foundation stones of protein chemistry and, as will be seen in the following paragraphs, is in complete harmony with certain phases of protein metabolism in plants.

Schulze and his co-workers (230, 231) studied the chemical changes that occurred in the ripening of seeds of legumes. In the pea plant they found that the proteins of the pod were broken down to soluble organic compounds of nitrogen of which about one-half was asparagine and the remainder the amino acids tryptophane, histidine, leucine, and a small quantity of arginine. As the seeds ripened there was a decrease in the soluble nitrogen of the pods and a closely corresponding increase in the relatively complex amino acids and proteins of the seeds. In the completely ripe seeds there were found only extremely small quantities of asparagine, monamino acids, arginine and histidine. Wassilieff's (284) results indicate, however, that amino acids and asparagine do not always accumulate in the pod but may apparently be derived directly from stems and leaves. At least with accumulation of storage protein in the seed there was a marked decrease in the simpler soluble forms of organic nitrogen in all parts of the plant.

Zaleski (301) worked with the maturing seeds of pea which had been removed from the plant and he found that as the protein of the seeds increased with ripening there was a corresponding decrease in amino acids. Consistent results were not obtained in the case of all other species under similar treatment but this may well have been associated with removal of seeds prior to the deposition in the developing seed of an adequate carbohydrate reserve for respiration. Under such conditions, as will be shown presently, the dominant phase of protein metabolism is not of synthesis but of hydrolysis.

Many different kinds of seeds have been analyzed at various immature stages until completely ripe, and without exception the results have shown that as ripening progressed there was a rapid condensation of amino acids into polypeptides and storage proteins. (230, 303, 74, 17). In coffee and tobacco seeds there occurred with ripening not only dehydration of amino acids but also practically complete disappearance from the ripe seed of caffein and nicotine, respectively. (75, 84). A discussion of the relationship of protein metabolism to xanthine derivatives and alkaloids is given by Weevers (286, 287). It has been found also by Klein and Taubock (100) that various immature seeds and fruits may contain free urea but that with maturation of the seed it can no longer be detected.

The synthesis of protein in ripening grain has been frequently studied (17, 21, 118, 119, 254, 297), various methods have been employed and the results are in general accord with those of Eckerson (56) who found no storage protein in the endosperm of the full grown, still green wheat kernel containing about 90% moisture. The aleurone layer and the layer of cells immediately below it contained more protoplasm than the other endosperm cells and gave a protein reaction; this, however, as she points out, was not storage protein. No storage protein was formed in the endosperm until desiccation began. From her tests it appears that the proteins gliadin and glutenin are formed when drying of the grain causes the amino acids in the endosperm to condense into proteins. She found no gluten until desiccation of the wheat grain began.

Grain, which when brought into the laboratory gave no protein reaction but contained much asparagine, arginine, histidine and leucine, was dried for 12 hours. It then gave a strong protein

reaction and contained gluten but had much less asparagine than before. On further desiccation, arginine, histidine and leucine disappeared and there remained only a trace of asparagine. The rôle of asparagine will be considered elsewhere but these results, with those of Schulze and others cited, are clearly in harmony with the peptide hypothesis of the synthesis of storage proteins by dehydration of amino acids.

It should be mentioned also that the chemical aspects of dehydration are not confined to amino acids (252). Monosaccharides condense to form disaccharides with loss of a molecule of water thus: $C_6H_{12}O_6$ plus $C_6H_{12}O_6$ equals $C_{12}H_{22}O_{11}$ plus H_2O. In the formation of starch $(C_6H_{10}O_5)^n$ or any other polysaccharide there is further dehydration. Both processes, the synthesis of storage proteins and the synthesis of reserve carbohydrates, are in harmony with the well known fact that seeds, storage organs and the plant as a whole decrease in moisture content as ripening or maturation progresses.

Hydrolysis of Storage Proteins: Whereas the synthesis of storage proteins as exemplified in the ripening of seeds involves the loss of water and is associated with carbohydrate accumulation, the breaking down or hydrolysis of storage proteins is correlated with the taking on of water and decrease in concentration of carbohydrates. Germination of seeds is one of the most common examples of a plant response in which proteolysis is a dominant phase of metabolism although of course accompanied, at least during early stages, by a gain in complex nucleo-proteins owing to increase in amount of meristematic tissue (166). A few illustrations may be cited but first it should be pointed out that in the plant, in addition to urea, two acid amides containing the characteristic $-CONH_2$ group have been found: asparagine, a semi-amide of amino-succinic acid ($COOH . CHNH_2 . CH_2 . CONH_2$), and glutamine, a semi-amide of amino-glutaric acid ($HOOC . NH_2 . CH_2 . CH_2 . CH_2 . CONH_2$). Both are very common and one or the other or both are practically always present in higher plants (243, 135).

Schulze (220, etc.) permitted seeds of various legumes to germinate in darkness and made a remarkable series of isolations of many of the actual nitrogenous compounds concerned. Seedlings left in the dark for a week decreased greatly in storage protein owing to the formation by hydrolysis of the amino acids leucine, histidine,

lysine, tyrosine, and a small quantity of asparagine or glutamine. After the seedlings remained without light for two or three weeks and necessarily with accompanying decrease in carbohydrates, the amino acids mentioned decreased and there was a striking increase in amide. The form of amide varied with the kind of plant and possibly with the conditions of environment and the nature of the nitrogen-free reserves in the cotyledons (224). Both asparagine and glutamine were found in different proportions, one amide in some plants apparently almost completely replacing the other.

In the etiolated seedlings the decrease in amount of storage protein was relatively great if the seeds were low in their initial reserve of carbohydrates or fat. Likewise the decrease in amino acids and increase in amides was most pronounced under conditions of carbohydrate deficiency. For example, Schulze and Castoro (227) found that the amino acid content of etiolated seedlings one week old was much greater than that of comparable plants two weeks old growing in light, whereas amide nitrogen was about the same. It has been shown also that seedlings grown in the dark *with no external source of inorganic nitrogen* and supplied with sugar, form relatively little asparagine and ammonium (250, 251). Many additional experiments (87, 88, 136, 248), similar in nature, have since corroborated the earlier work of Schulze (220, etc.).

Results similar to those described were obtained by Prianischnikov (181). In fact, he found that asparagine accumulation continued even after protein hydrolysis had ceased and that this was accompanied by a diminution in concentration of amino acids and an increase in ammonium as carbohydrates became depleted in darkness. On the basis of their experimental evidence, Schulze and Prianischnikov eventually came to much the same conclusion. Their results show that it is essential to distinguish between the hydrolytic splitting of storage protein by proteolytic enzymes and a further oxidation change of the cleavage products. The former primary process yields amino acids, organic bases and small quantities of amides, but probably only insofar as they were preformed in the disintegrated protein.* Portions of these primary hydrolytic products, apparently especially the bases alanin and leucin (68, 228), undergo oxidation with the formation of ammonia.

* Of course, it may be possible, as suggested by Schulze and others, that some ammonia formed in plants may be split off directly from the protein molecule and with the simultaneous presence of malic acid in germinating seeds there could well occur the union of this acid with ammonia to form asparagine.

The investigations of Godlewski (68) furnish additional proof of the secondary origin of amides, in that he was able to show that germinating seeds in oxygen-free air formed amino acids, whereas asparagine and ammonium, which normally appeared, were not present in measurable quantity. Likewise Butkewitch (26), who successfully demonstrated the presence of proteolytic enzymes in seeds, was able to show that in anaesthetized seedlings no asparagine was formed even though ammonium was formed (*cf.* 117, 249, 284, 135, 300).

Prianischnikov especially has compared the rôle of ammonium and amides in plants with urea formation in animals (189). This immediate discussion, while concerned with the internal formation of ammonia, may be compared with Prianischnikov's work to be considered in greater detail later. He was concerned with the formation of asparagine from ammonium derived from external as well as internal sources. However, other conditions being equal, there seems not the slightest reason to think that the origin of ammonia would in any way modify its subsequent metabolism within the plant.

Prianischnikov's views, which have apparently become rather generally accepted, are summarized by Murneek (146). With a decrease in carbohydrates, amino acids are formed through hydrolysis of proteins by means of proteolytic enzymes. With considerable depletion of available carbohydrates it appears that amino acids are oxidized and amino groups released. Asparagine, containing two amino groups, is considered to be formed from two molecules of amino acids, one of these being oxidized to aspartic acid, the other much further, with splitting off of ammonia. There is presumed to be then a union of aspartic acid with ammonia to form ammonium aspartate, from which, through dehydration, asparagine would be produced in the same manner as, in the animal organism, urea is formed from carbamate of ammonia. When a plant containing an adequate carbohydrate reserve is supplied with an abundance or slight excess of ammonium, asparagine may presumably be formed from one molecule of an amino acid or even from an organic acid such as malic or succinic acid. With growth of the plant and development of new tissues, asparagine may apparently be broken down, ammonia released, and employed for amino acid synthesis and subsequent protein formation. Thus asparagine and

likewise glutamine are said to function in removal of injurious ammonia and in storage of nitrogen (*cf. Detoxication of Ammonia*). Studies by Klein and his coworkers (99, 100, 103, 104) show that urea is present in some green plants and seems to be in many respects similar to asparagine and glutamine in function. Urease (93, 94, 96, 175) has been found in plants as well as asparaginase (71), arginase (102) and other enzymes apparently associated with protein metabolism (26, 280, 296).

Storage Organs: It would not seem essential to discuss in detail metabolic changes occurring in the nitrogenous compounds of storage organs such as corms, bulbs, rhizomes and storage roots. In general, they follow the same course of metabolism as seeds. Associated with much higher percentages of moisture in storage structures, there is invariably found, as would be expected, a relatively high proportion of soluble organic nitrogen and a proportionately lower concentration of protein than in seeds where ripening has been shown to be characterized by desiccation and the synthesis of protein at the expense of amino acids. It should also be recalled that many storage organs contain a comparatively high proportion of meristematic tissue such as the supernumerary cambiums of the storage root of beet (7) or sweet potato (6) and the specialized dividing cells of the tubers of the white potato (5). The proteins of such cells can scarcely be considered storage proteins.

It is not surprising, therefore, that studies of metabolic changes of entire storage organs have sometimes yielded less precise results than investigations of seeds. Grüntuch (72) has reviewed much of the earlier work concerning storage organs and has conducted an extensive series of experiments with various underground reserve structures of many plants. Unfortunately his analyses do not differentiate between organic and inorganic nitrogen. Nitrate was not determined, yet some of the plants he worked with store nitrate in high concentrations. However, where nitrate has been determined and where it has been possible to separate storage tissues, as the older storage scales of bulbs, from the central relatively meristematic tissues, responses have been obtained which corresponded closely to those already recorded for seeds (153, 200); that is, sprouting of storage organs in darkness is associated with a decrease in carbohydrates in strictly storage cells, hydrolysis of proteins to amino acids and the later appearance of amides very much as in seeds

(220, etc., 299, 151, 247). In general, as in seeds, the higher the concentration of carbohydrates the less drastic the proteolytic changes. In fact, dormant onion bulbs notably high in sugars and amino acids but low in amides may during sprouting go so far as to exhibit protein regeneration from amino acids in darkness, although in part of a non-storage type, if the period of etiolation is not unduly prolonged (302). In accord with the results just cited and the previously described work of Schulze, Stuart and Appleman (247) make the significant observation that in the process of development of the meristematic cells involved in wound periderm formation in potatoes, the synthesis of the proteins concerned was associated with utilization of amino- rather than amide nitrogen. This response would seem particularly notable in view of the fact that amides are high in potato tubers. Obviously these results support the theory of protein regeneration from amino acids rather than from amides.

SYNTHESIS AND HYDROLYSIS OF LEAF PROTEINS

Assuming that both storage and protoplasmic proteins occur in leaves that are not of a specific storage type, there is no information making it possible to differentiate between them. In studies of progressive changes in leaf metabolism the proteins have in most cases been estimated on the basis of the total nitrogen content of a heterogeneous coagulum rather than as individual proteins. Various protein materials have been obtained, however, from leaves and compared as to their isoelectric point and hydrolytic products with similar preparations from other organs (49, 95, 165).

Chibnall (29, 31, 33) and Chibnall and Grover (34) indicate that there is a protein fraction in bean leaves considered to be cytoplasmic protein that, while varying in amount in young and old leaves and under different external conditions, nevertheless seems to remain constantly of about the same quality. Thomas (256) likewise found that the preparation which he designates as cytoplasmic protein of apple leaves varied in amount but was approximately the same in amino acid constitution regardless of the stage of development of the leaf. On the other hand, Vickery *et al.* (274) reported that somewhat more than half of the protein of the tobacco leaf was less stable to the enzymes of the cells than the remainder, which they suggest may be in the nature of a reserve protein. The

methods of extracting the leaf proteins are of course described in the several cases but it remains, so far, uncertain as to the exact part or parts of the leaf protoplasmic mass contributing to the protein preparations.

Ullrich's (269) results are of interest in this connection since on the basis of microchemical and cytological examinations he observed a close relationship between the size of the chloroplasts and the protein content of the leaf. Meyer (133) likewise noted that as leaves became older or etiolated there was the usual decrease in green color, relatively little change in cytoplasm or nucleus, but a marked decrease in size of the chloroplasts that was correlated with disappearance of protein from the leaf.

Metabolism of Attached Leaves: Schulze and Schutz (229) followed the diurnal changes in leaves from two box elder trees and found that there was a loss of nitrogen from the leaves at night. This was correlated with a decrease in carbohydrates and protein whereas the soluble organic fractions of nitrogen changed little. Bean leaves also lost in protein content at night, according to Chibnall (31). He ascribes this to the breaking down of cytoplasmic proteins. In these experiments, however, as in similar cases (10, 138), there was furnished little evidence as to the immediate products of protein hydrolysis because they were apparently translocated from the leaf. As leaves of trees mature or as the lower leaves of herbaceous plants approach senescence there is, before leaf-fall, a loss of protein and a gain in nitrogenous material in other organs (41, 52, 138, 145, 173, 229, 257). If nitrogen is deficient there is decrease in protein in the lower leaves and migration of nitrogen from these organs to younger tissues, as shown by Richards and Templeton (206), Mason and Maskel (120, etc.) and others (62, 135). This process, of course, involves protein cleavage and translocation of the hydrolytic products. Proteolysis in leaves, as in other organs, may be greatly accelerated in rate if nitrogen deficient plants, usually high in carbohydrates, are shifted to darkness, thereby decreasing their carbohydrate reserves (154). Here again detailed information concerning the nature of the metabolic changes is obviously made uncertain owing to translocation. Accordingly detached leaves have frequently been investigated.

Metabolism of Detached Leaves: The objection is often raised that in experiments with detached leaves abnormal conditions are

created. This is obvious and in fact the primary object in such investigations is to avoid normal translocation losses of nitrogenous bodies and thereby have such materials available for study. Although caution must necessarily be employed in interpretation it should be pointed out that work with excised leaves has supplied information of value that might not otherwise have been obtained. It may be pertinent to mention here that Mothès (135) found that when the petioles of detached leaves were immersed in water there was no significant loss of nitrogen from the cut ends. It should be said, however, that he took special precautions, supplying sterile water with frequent changes, and at least minimized the chances of contamination with microorganisms by washing the leaf surfaces with 1 to 2.5 per cent hydrogen peroxide, taking special care to employ leaf blades that were intact.

His work and that of others (150, 169, 170) indicate that there is a remarkable similarity between the nitrogenous metabolism of leaves and seedlings. This is especially evident in the so-called "protein-sparing" action of carbohydrates. For example, Deleano (50), working with detached shaded grape leaves with their petioles in water, found that respiration during the first three days went on only at the expense of carbohydrates. After that time, when a large part of the carbohydrate supply was exhausted and starches had practically disappeared, proteolysis began and after several days in darkness was accompanied by a considerable increase in ammonium. The total nitrogen content did not change appreciably. Spoehr and McGee (241) studied the responses of excised sunflower leaves in darkness and obtained a striking increase in amino acids when the petioles were in water only, whereas similar leaves with their petioles in a sugar solution exhibited practically no increase in amino nitrogen. The rate of hydrolysis of protein of grape leaves also was greatly reduced when the detached leaves were supplied with glucose (50).

Chibnall (29, 32) found that the attached leaves of the runner bean decreased materially in amount of protein at night although the quality, as already stated, apparently remained practically unchanged. In a parallel experiment using detached leaves with their petioles in water, he found that the decrease in protein occurring in darkness was associated with an increase in amino acids and asparagine.

The apparent secondary origin of asparagine and glutamine in seedlings has already been discussed and it seems reasonably clear that amide nitrogen in leaves also is formed through the oxidation of amino acids. Mothes (135) showed by several experiments with detached bean leaves, receiving no external supply of nitrogen, that amides began to appear in quantity only after 2 to 4 days in darkness, hydrolysis of proteins to amino acids apparently predominating during the early stages.

As further proof of the origin of amide nitrogen there are available the effects of anaesthesia. Mothes (135) placed the petioles of detached bean leaves in water in darkness under 8 liter flasks in which was evaporated .5 cc. of chloroform. This concentration was not apparently injurious to the leaves as some of them, returned to normal air conditions subsequent to treatment, followed the usual course of metabolism. These experiments and others with oxygen-free air indicated that oxygen was essential for amide formation. In anaesthetized high-carbohydrate leaves, not too old, neither amide nor ammonia appeared but apparently proteolysis to amino acids occurred unhindered in darkness. When similar leaves low in carbohydrates were employed, proteolytic activities were greater; ammonium accumulated but there was no formation of amide.

Mothes' (135, 137) results fully corroborated the earlier work of Schulze and Prianischnikov with seedlings. In addition, he found that old leaves, as the primary leaves of bean even before they showed any external signs of senesence, exhibited proteolysis and amide formation both on increase of carbohydrates in the light and on addition of glucose in the dark. This was not owing to exceptionally rapid catabolism but rather to limited synthesis. Leaves which had but recently become fully expanded were much more active proteolytically on decrease in carbohydrates in the dark. These results, in harmony with those of Everingham and Pearsall (64), emphasize the necessity of care in selecting plant material.

In brief, excepting "old leaves," Mothes (135) found that when there was opportunity for carbohydrate synthesis in sunlight or the leaves had an available supply of glucose in darkness, protein synthesis rather than hydrolysis predominated and there was a decrease rather than an increase in amides. The higher the temperature, however, the higher the concentration of glucose which had to be supplied to check proteolysis. On the other hand, when carbohy-

drate reserves became reduced through exposure to darkness there occurred first proteolysis and with further decrease in carbohydrates an increase in amides. Finally, ammonia accumulated with eventual injury to the leaf under conditions of extreme carbohydrate deficiency. In the presence of abundant carbohydrates in darkness or in light, ammonia was used in the formation as asparagine. Mothes (135) found that this was true whether the ammonia was derived externally from ammonium salts absorbed by the cut petiole or internally by proteolytic action. Whether or not there was direct synthesis of amino acids from ammonia was not made clear. However, amides entered directly or indirectly into the synthesis of proteins in darkness or in light if there was available an abundant carbohydrate supply. It made no difference whether the amide nitrogen was supplied from external sources, as asparagine, or was derived internally through the oxidation of amino acids.

In this connection Wood's (296) studies of the pH of leaf sap and accompanying proteolytic activity should be recorded even though the lack of pH data in Mothes' (135) experiments and the lack of carbohydrate determinations in those of Wood make suggestions of interrelations purely speculative. Wood noted that the pH values of the leaves of *Atriplex nummularium* were intimately associated with enzymatic activity. He obtained leaves of different degrees of acidity by employing old and young leaves, by allowing plants to wilt and by supplying the petioles of excised leaves with acidulated water. When the sap was below pH 5.5 amino acids predominated; when less acid, there was an increase in amide plus ammonia. Extracts of leaves adjusted with a phosphate buffer to various pH values and subjected to autolysis, gave comparable results.

This matter will be considered again in connection with the ammonium and nitrate nutrition of plants of different degrees of acidity. Fife and Frampton's (65) results may, however, be cited. They decreased the acidity of the sap of beet plants and detached leaves by placing their plant material in an atmosphere high in carbon dioxide. In an hour or less there was a decrease in amide and a comparable increase in ammonium. A return of the plants or detached leaves to normal atmosphere resulted in a prompt reversal of the reaction, the amide increasing with a decrease in ammonium.

Vickery and Pucher (274) obtained results which are in part similar to those of Mothes (135). They followed the chemical changes that occurred in mature detached leaves of tobacco that were placed with their petioles in distilled water. Some of the leaves were analyzed at the beginning of the experiment and others at intervals up to 303 hours. Decrease in carbohydrates in diffuse light was accompanied by a slow decrease in nicotine and by protein hydrolysis. It is significant that a gain in amides was correlated with a decrease in amino acids.

They also record a result that seems to be without precedent and for which no explanation is available and for which they offer none. There apparently occurred during the early period of the experiments, while the leaves were still turgid, an increase in nitrate in the detached leaves which, it will be recalled, had no external nitrogen supply. This was soon followed by an apparent reduction of nitrate to ammonia. Mothes (135), peculiarly enough, in all his extensive work with excised leaves, for the most part obtained from soil-grown plants, gives no records as to the presence or absence of nitrate. Spoehr and McGee (241), in comparable experiments, state that special tests were made to determine whether there was an accumulation of nitrate in the leaves of sunflower which had been kept in the dark. However, no indication could be found that this was the case; in fact, no tests for nitrate could be obtained in the water extracts. The writer has frequently placed in darkness tomato plants that had no external supply of nitrogen and no nitrate in their tissues; in no case, regardless of the period of proteolysis in darkness, was a positive test for nitrate obtained. As Vickery *et al.* point out, further investigation will be required before an explanation can be offered as to the apparent synthesis of nitrate from organic nitrogenous materials already in storage in the plant.

Vickery and Pucher (272), in following chemical changes in slowly drying (curing) tobacco leaves, noted that during the period of wilting proteolysis was much more rapid than during a comparable period of time in the case of turgid detached leaves supplied with distilled water (277). Mothes (138) and Wood (296) also found that wilting materially increased the rate of protein hydrolysis.

METABOLISM OF STEMS

Aerial stems vary greatly in structure and function and in their rôle in nitrogen metabolism. Some types of stems are concerned

primarily with translocation, some with translocation and storage and others with both these functions and new synthesis of organic nitrogen as well (57, 60, 153, 154). Stems being less specialized in function than seeds, storage organs and leaves, have lent themselves less readily to studies of protein metabolism. Nevertheless, there is considerable evidence to show that in nitrogen economy they follow trends similar to those already recorded for other organs.

An accumulation of carbohydrates usually occurs under favorable light conditions in plants lacking an abundant external supply of nitrogen. With the onset of senescence there is undoubtedly a breakdown of stem protein (154), regardless of carbohydrate reserves, probably much as Mothes (135) reports for "old leaves". At high temperature in plant stems, as in leaves (135), proteolytic action takes place even in the presence of a high concentration of carbohydrates, although much less rapidly than in a low-carbohydrate plant (158, 160, 161). However, a high carbohydrate reserve is very frequently associated with condensation of amino acids to polypeptides and complex proteins, probably in part of a non-meristematic or storage type (153, etc.). At least under such conditions in tomato few meristematic cells are present except at the apex of the stem and roots .

Richards and Templeton (206) take exception to this view on the basis that under conditions of shortage of nitrogen the plants should not be expected to store the limiting element. Although this is a convenient hypothesis it means little. Their work, on the other hand, and that of Mason and Maskel (124, 125) are convincing. It indicates clearly that under conditions of nitrogen deficiency there is a definite migration of nitrogen from relatively mature or senescent tissues to meristematic zones. It should be emphasized, however, that this migration of nitrogen to meristematic tissues can be greatly accelerated and increased in amount by shifting high-carbohydrate, nitrogen-deficient plants to darkness (153, 154, 155, 157). Under these conditions there is a very rapid increase in volume of new tissue of stem and leaves that may very well be initiated by growth-substances or hormones, the gross chemical changes being possibly secondary in nature (*cf.* 289, 198). Nevertheless, this phenomenon is accompanied by a decrease in carbohydrates and there does occur protein hydrolysis much as in seeds, storage organs

and leaves. As long as some carbohydrates are available there is very rapid regeneration of the proteolytic products to form new proteins of the meristem (152, 154, 155). Concerning these proteins there is little known except that, as already pointed out, they are in contrast to storage proteins which are apparently most readily synthesized under conditions of high dry matter content; that is, a high concentration of carbohydrates or other nitrogen-free reserves. Further evidence in this direction is furnished by the chemical composition and growth responses of tomato plants and apple trees grown with no external nitrogen supply at high and low relative humidity. Low humidity clearly favored condensation of amino acids to proteins and little development of meristematic tissue; high humidity, the reverse (162) (*cf.* 128).

There has recently been accomplished some excellent work on the seasonal changes in the nitrogenous materials of trees (90, 141, 142, 143, 145, 173, 239, 257, 258, 260). Reference should also be made to earlier work cited by Chandler (28) and Gardner, Bradford and Hooker (67). This type of work has been carried out under different circumstances of environment and soil conditions, and different kinds and varieties of trees have been employed; therefore, a coherent adequate account of this work showing the proper interrelations cannot be readily included in the space allotted to this review.

The autumnal migration of nitrogen from leaves has already been mentioned in connection with the metabolism of these organs. This phenomenon seems well established and in general it also appears from the work cited above that in the late winter and early spring soluble organic compounds of nitrogen accumulate and are translocated to the developing buds and shoots. The probable origin of these materials will be considered later, especially in their relationship to the metabolism of roots. In some cases it appears that amino acids or polypeptides have been primarily concerned in transport; in other cases, amides.

It is generally considered that the soluble or crystalloid forms of nitrogen are concerned in translocation. Many references might be cited favoring a particular form of nitrogen but there is no data available at present which makes it possible to point out one form of soluble nitrogen found in plants as being of more importance than another in translocation (31, 32, 40, 51, 62, 114, 172, 208, 257).

For a consideration of the possible channels of nitrogen transport the reader is referred to the work of Maskel, Mason and their collaborators (120, etc.) and to a review by Curtis (44). It would seem reasonable to think, however, that the metabolic activities of the plant and the seat of initial organic nitrogen synthesis to be considered later, must in a considerable degree determine the form of nitrogen available for translocation. A material must certainly be present before it can be translocated and there is abundant evidence, to be considered in the following pages, indicating that with shift in environment or nutrient supply one or another form of nitrogen may predominate. For example, asparagine may be almost completely replaced by glutamine and the ratio of amide to amino nitrogen may be greatly changed depending simply upon whether the external nitrogen source is from an ammonium or nitrate salt (39).

THE NEW SYNTHESIS OF ORGANIC NITROGEN FROM NITROGENOUS NUTRIENTS

The metabolic changes that take place in organic compounds of nitrogen stored or contained in the plant have been considered in the preceding pages. The quantity and quality of these materials and carbohydrate reserves present and their relation to new synthesis of organic nitrogen from inorganic nitrogenous salts will next be discussed along with external factors influencing this process.

Absorption is one of the first stages in any phase of plant nutrition and, although closely associated with the general topic under consideration, will not be discussed in detail (*cf.* 79, 180, 261, 262). It may be well to recall, however, that living root cells can absorb solutes from very dilute nutrient or soil solutions. They can with extraordinary rapidity accumulate much higher concentrations of salts in their cells than in the surrounding nutrient solution. Granted an adequate growing root system in the presence of some free oxygen and a complete nutrient solution that permits salt dissociation, absorption or permeability of non-injured root cells seems seldom if ever to be a serious limiting factor in nitrogen nutrition. Failure of a plant to remove nitrate continually, for example, from a nutrient solution is, of course, failure to absorb but it seems, at least in the cases where plant analyses have been made, to be associated with capacity accumulation of nitrate within the plant rather than with impermeability of the absorbing cells of the root system.

If internal and external conditions are so modified as to permit reduction of the contained nitrate and new synthesis of amino acids, absorption is again renewed (37, 38, 47, 48, 233, 242).

As will be brought out in the following discussions, there is obviously no one best nutrient solution (110). The nutrient solution will necessarily vary with the experimental objectives, with the quality of plant growth desired, with the opportunity for carbon dioxide assimilation, with the initial reserves in the seed or other organ of propagation and with the stage of growth of the plant. Certain precautions are, however, absolutely essential. The pH of the culture or soil solution is of major importance, as has been pointed out repeatedly by Prianischnikov (181, etc.), Shive and his students (37, 38, 47, 48, 233, 242) and by many others. Methods of pH control of culture solutions have been given by Shive and Stahl (234), Trelease (267, 268), and Zinzadze (305). Many workers, however, have given no consideration to, or at least no report of, the pH of the soil or nutrient solution employed in their studies of nitrogen nutrition.

A commonly ignored factor in water culture experiments is the oxygen supply available to the immersed root system. For most non-aquatic plants aeration is absolutely essential. Shive (235) reports that when tomato plants were grown in culture solutions that were continuously aerated the average total yields of plants were more than 50 per cent higher than those of plants grown in non-aerated cultures. Determinations of the oxygen content of the culture solutions, both before and after the plants had grown in them for six hours, showed that even with continuous aeration the roots of the plants used up in a short time the greater portion of the oxygen which was presented in the culture solution before the plants were placed in them. The aerated solutions contained four to five times as much oxygen as did the non-aerated solutions. Plants grown in aerated cultures removed up to 60 per cent more nitrate and ammonium nitrogen from the cultures than did those grown in non-aerated cultures; the influence of aeration on ammonium nitrogen absorption rates was much more pronounced than on the nitrate absorption rates. Low nitrogen absorption rates and retarded growth rates in non-aerated cultures were caused by a deficiency of oxygen and not by an accumulation of carbon dioxide through excretion from the plant roots.

From these responses it is obvious that the metabolism of the plant is greatly modified when the nutrient lacks oxygen. Loehwing (111) grew sunflower and soy bean plants in aerated and non-aerated soil. His plants lacking aeration were soft, succulent and poorly developed mechanically in that lignification was limited. This was associated with carbohydrate deficiency and late flowering. Additional references might be cited (*cf.* 111, 171) but it will be sufficient to recall that, in addition to effects on carbohydrate reserves, lack of oxygen materially modifies protein metabolism, the dominant phase being hydrolysis rather than synthesis, not only in aerial organs (26, 135, 139) but in roots as well (14, 79).

Unfortunately, many experiments on nitrogen nutrition have been conducted in water cultures with no provision for aeration and in sand, soil and water culture with no record or no control of the pH value of the nutrient medium. There are cases where methods of plant analysis do not permit conclusions, where attempts have been made to estimate amide nitrogen by hydrolysis with hydrochloric acid in the presence of nitrate, where nitrate has been recorded as the difference between the Kjeldahl and the modified Kjeldahl procedure, where ammonium in plant tissues has been recorded as the amount of ammonia yielded after boiling plant extracts with sodium hydroxide, etc. (*cf.* 30, 35, 246, 273, 285).

Because the results of such work seem to the writer to be impossible of accurate interpretation, they are in general omitted in whole or in part from the following discussions without further reference. A useful and comparatively complete list of experiments on ammonium and nitrate nutrition has been recently made available by Pardo (168). As intended, it is primarily an annotated bibliography in which experimental results have been catalogued according to the plant family concerned.

STORAGE AND ASSIMILATION OF NITRATE

Nitrate Storage: Nitrate is apparently freely absorbed by uninjured roots (*cf. The pH of the Nutrient Solution*) over a wide range of pH values of a complete nutrient or soil solution and it may accumulate in the plant in enormous quantities without injury. Woo (295) reported that in the stems of *Amaranthus* 56 per cent of the total nitrogen was nitrate and Campbell (27) obtained similar results. Even in storage organs, as the roots of mangold, nitrate

has been found in high concentrations. In celery plants Platenius (179) recovered as high as 80 per cent of the soluble nitrogen as nitrate; in the vegetative organs of wheat McCalla (118) obtained, as the plants were maturing, 50 per cent of the total nitrogen was nitrate and in the oat plant Sessions and Shive (233) found that one-third of the total nitrogen was in this form. Chibnall and Miller (35) obtained high yields of nitrate from the leaves of rye grass, Vickery *et al.* (274) and Eisenmenger (61) from the tobacco plant, and many more references might be cited showing that nitrate accumulation commonly occurs in a wide variety of plants (43, 149).

Nitrate Reduction: The amount of nitrate stored and the organs concerned vary greatly with the kind of plant (55). In some plants, as tomato, if the sole external source of nitrogen is nitrate, a fairly high concentration must continually be maintained in the vegetative tissues to insure a synthesis of organic nitrogen from nitrate that is sufficiently rapid to maintain vigorous vegetative growth (105, 154, 265, 39). On the other hand, with ammonium as the sole external source of nitrogen, vigorous growth of tomato and many other plants may be maintained even though the plants concerned are entirely or practically free of nitrate (47, 48, 194, 197, 263, 265). Nitrate may, however, accumulate in tomato and other plants during a period of little growth with no apparent external effect on the plant (55, 58, 60, 152, 158). Any cell constituent may well be considered as exerting some effect on the development of the plant but it is obvious that nitrate is not a form of nitrogen essential for plant growth; it rather represents nutrient material not yet metabolized, a potential source of organic nitrogen.

There are many theories, for the most part purely speculative, concerning the processes involved in the transformation of nitrate to organic nitrogen. Robinson has contributed an excellent review partly on this subject (208). Knowledge of organic nitrogen synthesis from nitrate is far from complete but there is abundant evidence that the initial phases of the process include the reduction of nitrate to nitrite and ammonia. It has been reported that hydroxylamine is present in the leaves of certain plants in minute quantities, supposedly being formed following the appearance of nitrite, but at present any discussion concerning its possible significance would be purely speculative (108). In would be of interest, however, to know whether or not nitrate-free ammonium-supplied plants ever contain hydroxylamine.

Nitrate reduction will take place in plant cells in the dark (*cf. Nitrate Reduction in Roots*), although light is of course necessary in carbohydrate synthesis. The reduction of nitrate is a definitely endothermic reaction, however, and there cannot be said to be unequivocal proof that there may not be in nitrate reduction in some plants direct utilization of the energy of light through transformation to chemical energy. Nevertheless, in cases where carbohydrate analyses have been made, the reduction of nitrate in light as well as in darkness has been found to be accompanied by oxidation of sugars or their derivatives and a decrease of reserve carbohydrates in the organs concerned (57, etc., 257, 258, 259, 153, etc., 264, 265, 195, 47, 48, 55, 118, 203).

It is most important to appreciate the significance of this process in relation to carbohydrates in any consideration of nitrate nutrition. Eckerson (57) has followed the transformation of nitrate in tomato plants and has also recorded the associated changes in carbohydrate reserves. Her plants, after an initial period in soil, were grown for several weeks in quartz sand with no external supply of nitrogen. At the end of that time they exhibited typical symptoms of lack of nitrogen. They contained an abundance of glucose, some fructose, a little sucrose and a high concentration of starch in stems and leaves. The plants were free of nitrate, nitrite and ammonium and practically no amino acids could be detected. Some of the plants in this condition were supplied with calcium nitrate; in 24 hours all parts of the plant contained abundant nitrate and the tops of some of the plants gave a slight reaction for nitrite but no ammonium was found. Twelve hours later all the plants had considerable nitrite localized in the cortical cells of the stem tips and especially in the phloem region of the stem. After 48 hours there was slightly less nitrite but more ammonium. It is notable that a decrease of starch occurred in the tissues in which nitrite was observed. These responses were followed by the appearance of amino acids. However, in vigorously growing plants continually supplied with nitrate there seldom may be detected more than traces of nitrite and usually none is found. This is in accord with the results of many workers who have reported the presence of traces of nitrite in different kinds of plants (155, etc., 9, 55, 73, 86, 217, 259, 285, 108).

In connection with student instruction during the past few years the writer has frequently followed in tomato plants reactions essen-

tially the same as those recorded by Eckerson. Apparently the same series of chemical reactions have been obtained in varied experiments with other plants, some of which are to be described later. The reduction of nitrate to nitrite may be easily and consistently demonstrated in tomato by employing plants that are low in nitrogen but high in carbohydrate reserves and supplying them with abundant nitrate under favorable growing conditions. As a rule, the lower the nitrogen content of the plants, when coupled with high carbohydrate reserves, the more rapid the absorption and reduction of nitrate. Often two or three hours after application, nitrate can be found in abundance in all parts of a tomato plant and frequently nitrite will appear two or three hours later. In plants considerably higher in organic nitrogen and lower in carbohydrates, but likewise lacking nitrate, 12 hours or more are required for a comparable absorption response and two days may elapse before the appearance of nitrite. Dittrich's (55) results showed similar correlations between nitrogen concentration, carbohydrate content and reducase activity.

Hamner (73) worked with tomato plants in sand culture which were very similar in quality of growth to those employed by Eckerson. In a typical experiment his high-carbohydrate plants lacking nitrate were shifted from a greenhouse to a chamber maintained at a practically constant temperature of 22° C. and a relative humidity of 75 per cent. In alternate periods the plants were in darkness for 14 hours, and for 10 hours were exposed to light from tungsten lamps averaging about 850 foot candles at the surface of leaves which were enclosed in a chamber employed for measuring carbon dioxide exchange. The concentration of carbon dioxide in the air entering the leaf chamber was the same as in outside air. There was no evidence in this work that addition of nitrate to the tomato plants increased the rate of photosynthesis. As Hamner points out, this may have been owing to the light source, which compared unfavorably with that of sunlight in its effect on tomato, and to the fact that carbon dioxide may have been a limiting factor.

His results on respiration are of remarkable interest. Some of his plants, lacking an external supply of nitrate and containing none in their tissues, were supplied with nitrate *at the beginning of the period of darkness.* Nitrate, as part of a complete nutrient solution containing no ammonium, was added to the quartz sand of the

culture jars in the form of calcium nitrate. In four to six hours after adding this salt, tests for nitrate were positive well up in the plants. Nitrite became apparent two hours after nitrate was detected in the tops and shortly thereafter a striking increase in rate of respiration of carbon dioxide was observed as compared to comparable plants lacking nitrate.

His several different series of experimental plants were different in degree of nitrogen deficiency and carbohydrate content and, therefore, as would be anticipated, differences in the rate of nitrate absorption and reduction occurred. To be emphasized, however, are the facts that (1) he obtained reduction of nitrate to nitrite in darkness and (2) that in all cases the increase in rate of respiration occurred not with the initial appearance of nitrate in the plant but accompanying or following the reduction of nitrate to nitrite. Undoubtedly this was accompanied by the appearance of amino acids, as described by Eckerson, for his plants were definitely darker green 24 hours after receiving nitrate. In his series of plants which were comparatively high in carbohydrates this increase in respiration rate was of the order of 300 per cent; in plants lower in carbohydrates it was about 100 per cent. In wheat Hamner found that the responses in respiration under comparable conditions were very much the same as in tomato. This was true not only for the tops but for the roots of the wheat plant (*cf. Nitrate Reduction in Roots*). Further, Gregory and Richards (70) report that barley lacking abundant nitrogen was at all stages of growth much lower in respiration than plants abundantly supplied.

There are many complicating factors in studies of photosynthesis and it would be premature to draw conclusions, as Hamner points out. Nevertheless, it is of great interest that under conditions of light, nutrition and carbon dioxide supply that were apparently fairly satisfactory for wheat, his relatively dark green nitrate-supplied plants at first markedly increased and then later actually decreased in photosynthetic rate per unit of leaf area. Gregory and Richards (70) report for barley a similar response that was thought to be associated with advanced age of the plants. Hamner's low-nitrogen plants, high in carbohydrates and light green in color, exhibited during the same period of time no such decrease; they continued to increase in dry weight, the greatest gain in volume and dry weight being of the root system rather than of the tops. It

appears, according to Lundegardh (116), that the leaves of nitrogen-deficient oat plants were much more active photosynthetically than comparable plants which were supplied with nitrate.

Some of the various factors affecting the rate of nitrate assimilation will be considered later but it is clear that under conditions which favor rapid nitrate reduction and amino acid synthesis there may also be expected rapid utilization of carbohydrates or their derivatives, often resulting in very low carbohydrate reserves in the plant (57, 105, 153, etc., 215).

The carbon skeleton of the protein or amino acid molecule is necessarily derived directly or indirectly from carbohydrates. About 85 per cent of the more complex protein bodies are of carbohydrate origin although the nitrogen content of proteins is commonly emphasized. In addition to this, the fact of greatly increased respiration under conditions of nitrate reduction and amino acid synthesis explains, at least in part, why excessively heavy applications of nitrogenous fertilizers have frequently resulted in plants high in organic nitrogen but deficient in carbohydrates. Just what phase or phases of nitrate assimilation are most intimately associated with increased respiration is not apparent. Nevertheless, there is considerable evidence showing that an increase in the amino acid content of plants is associated with increased respiration (23, 232). Spoehr and McGee's (241) results are especially pertinent. They worked first with entire sunflower plants which were placed in darkness for 71 hours. During this period there was decrease in carbohydrates and, as usual, a material increase in concentration of amino acids undoubtedly owing mainly to proteolysis. Associated with the increase in amino acid content of their plants there was an enormous increase in respiration rate until the carbohydrate reserves became greatly depleted. Many of their experiments with detached leaves in darkness included the addition of amino acids to the water in which the cut ends of the petioles were immersed. For example, when glycine was supplied there was a gain in amino acid content and the leaves respired and decreased in carbohydrates much faster than other leaves which lacked glycine.

Discussion as to interrelations between these results and the increase in respiration that occurred following nitrate reduction would be premature. Still, the increase in respiration was undoubtedly associated with the appearance of amino acids, whether

supplied externally, formed through protein cleavage, or formed through new synthesis from reduction of nitrate and the oxidation of carbohydrates or their derivatives. Further, the comparatively high plane of respiration was maintained in tomato after nitrite could no longer be detected following the first flush of nitrate reduction (73).

Nitrate Reduction in Roots: The extracts of the fresh tissues of various plant organs have frequently been employed to measure the nitrate reducing ability of the structure concerned. Work of this type has been especially valuable in studies of roots (55, 59, 60, 155, 163, 259). Briefly, the technique for determining nitrate reducase, activity, as developed by Eckerson (57, 58), consists of taking an aqueous extract of fresh plant tissue and measuring the amount of nitrite reduced from nitrate, in the presence of nitrate and glucose and some free oxygen, by a given sample under specific conditions of time, pH and temperature. It is, of course, essential to maintain a constant pH as well as a constant temperature, to avoid a deficiency of nitrate, glucose or oxygen and to eliminate microorganisms through the use of toluene. The amount of nitrite formed from nitrate gives a measure of the reducase activity of the particular plant or organ sampled. Analyses have repeatedly shown (57, 60, 155, etc., 259, 260, 262) that reducase activity closely parallels the synthesis in the plant of amino acids and other forms of elaborated nitrogen. Reducase activity, therefore, furnishes an index of the relative rate of nitrate assimilation.*

Although in the older literature (259) occasional statements may be found indicating that nitrate can be reduced to nitrite in the roots of plants, Thomas (259) seems to have been among the first to appreciate the significance of roots in nitrate assimilation. He conducted quantitative studies of the nitrogenous constituents of

* Erratic results in the attempted use of this method have recently been reported by Sommer (240), probably owing, in the opinion of the writer, to the fact that although she adjusted the initial pH of her plant extracts the pH was not controlled during the period of incubation (private communication). As recently emphasized by Hibbard (76), it is essential to bring the pH of the plant extract to pH 7.2 to 7.4, as recommended by Eckerson (58), and to so maintain it for the entire incubation period. Some plant extracts contain natural buffers which are adequate for this purpose; to others a phosphate buffer must be added. Dittrich (55) favors a pH value of 7.6 for nitrate reduction and it may be pertinent to mention, with further discussion later, that Dikussar (54), who grew maize with nitrite as the sole external source of nitrogen, employed a pH value of 7 in his culture medium.

apple trees through a year's cycle of growth. The material included mature and seedling trees receiving heavy applications of sodium nitrate at regular intervals throughout the vegetative period. In the aerial organs positive tests were obtained for nitrate, or nitrite, in one structure only and this at just one period of the year, in the leaf buds just as they were opening. On the other hand, the fine roots gave nitrate reactions throughout the season, although there was little in the main roots. It is highly significant that quantitative tests for amino acids were always higher in the roots than in the aerial parts.

Eckerson (59) has followed the reducase activity of apple trees during a year's cycle of growth and, in complete harmony with Thomas' chemical analyses, found that high reducase during the fall and winter was localized in the fine roots. The maximum reducase in early spring was localized in both fine roots and buds. There was very little reducase in the leaves at any time. Later work by Thomas (257, 258) is in harmony with his earlier observations and he further determined that, accompanying assimilation of nitrate, there was a marked increase in utilization of starch (260, 261). As already indicated, this would be anticipated since reduction of nitrate and synthesis of amino acids in roots can obviously not occur without oxidation of carbohydrates or their derivatives. Other work with apple trees, including many carbohydrate analyses, has corroborated the results of Thomas and Eckerson (159, 160, 163, 264, 265).

Stuart (245) reports that if nitrate is applied in extremely high amounts to small apple trees it may appear in the aerial organs. Accompanying the appearance of nitrate in the leaves, his trees exhibited severe scorching of the foliage; there was, however, no indication that this was directly caused by the presence of nitrate. His results do not minimize the value of the observations discussed above but furnish additional information. There can no longer be any doubt that in this species the reduction of nitrate and synthesis of amino acids takes place mainly in the roots.

In the asparagus plant Nightingale and Schermerhorn (155) found that active reduction of nitrate, both in darkness and in light, took place mainly in the fine rootlets. When the plant was in a condition of active vegetative growth of tops, nitrate was found only in the fine roots. In plants lacking nitrate in their tissues and

nutrient medium but containing reserve carbohydrates in their rhizomes and roots, an external supply of nitrate resulted in the appearance of nitrate, nitrite and ammonium in the fine rootlets only and not in the storage roots. At the same time, amino acids and asparagine appeared in these organs in considerable quantities and the amount of reserve carbohydrates was reduced. The results were later reflected in all parts of the plant. Computations on an absolute amount as well as on a percentage basis showed a striking decrease in carbohydrates and increase in organic nitrogen, as compared to plants that lacked nitrate.

The storage roots and actively growing tops of the asparagus plant apparently assimilated nitrate, but seemingly seldom had an opportunity to do so because nitrate was reduced in the fine rootlets before reaching other organs of the plant. If, however, the temperature was 10° C. or lower, nitrate was translocated to other parts of the plant, owing apparently to cessation of reduction at that temperature. Later with a rise in temperature, nitrate was assimilated by and disappeared from both the storage roots and the actively growing tops and was then found again only in the fibrous roots. In harmony with the preceding results, Eckerson (60) found that the tops or succulent shoots of young vigorously growing asparagus plants contained only traces of reducase, the new storage roots contained very little, and the older storage roots practically none, whereas the fine rootlets were in all cases high in reducase.

A similar transformation of nitrate to organic nitrogen occurred in the rootlets of narcissus bulbs both with plants continually in darkness and others under seasonal light. Reduction of nitrate resulted in a definite increase in organic forms of nitrogen and a decrease in carbohydrates as computed on a percentage and absolute amount basis (153). These results were in contrast to control plants that received no external supply of nitrogen.

Some underground storage organs, however, may reduce nitrate as, for instance, the roots of mangold (49). Dittrich (55) also found that the storage roots of plants of the family Chenopodiaceae reduced nitrate and he reported nitrate reduction by the roots of plants of several different families including the Graminaceae. Many other references might be cited but it will be sufficient to point out that in the Graminaceae Sani (217) found very active nitrate reduction by extracts of roots. It is notable that he found

the rate of nitrate reduction was doubled by extracts of maize roots when citric acid was introduced. Apparently organic acids or sugars may be oxidized in nitrate reduction. The assimilation of nitrate by the roots of the Graminaceae is in apparent accord with Hamner's (273) work (already discussed), wherein he reports a greatly increased respiration rate of the roots of wheat plants that received nitrate as compared to others lacking an external supply of nitrogen.

The importance of the roots of some plants in assimilation of inorganic nitrogen has been emphasized but the fact should be kept in mind that in many plants, such as tomato (57, 158), peas (164), soy beans, etc. (152, 60), the tops, rather than the roots, play the dominant rôle in reduction of nitrate and synthesis of amino acids. Before taking up in detail the matter of ammonium and nitrate nutrition it is of interest to note that Dittrich (55) found that beets when supplied with ammonium sulphate gave a negative test for reducase. Tiedjens (264) reports a similar situation for the extracts of apple roots when the trees in sand culture were supplied with the same salt. Nevertheless, he found, as did Davidson and Shive for peach trees (48), that both nitrate and ammonium assimilation took place mainly in the fine rootlets.

EXTERNAL FACTORS INFLUENCING AMMONIUM AND NITRATE NUTRITION

If vigorously growing plants in a complete nutrient medium are supplied with ammonium sulphate, the culture solution usually tends to increase in acidity, owing to the fact that the anions are absorbed by the plant in a smaller proportion than the cations. On the other hand, calcium nitrate under similar circumstances commonly causes an increase in alkalinity as the nitrate anion is usually absorbed with relative rapidity. Therefore, a mixture of ammonium sulphate and calcium nitrate in proper proportions for the internal and external conditions concerned, or under some circumstances the use of ammonium nitrate, may minimize pH changes of the residual nutrient solutions (267, 268, 303, 234, 191, etc., 130, 131, 63, 174, 176, 178). Because of the residual effect on the nutrient substrate, salts such as ammonium sulphate are commonly called "physiologically acid" and those such as calcium nitrate "physiologically alkaline." On the same basis ammonium nitrate is "physiologically amphoteric."

Nitrate Absorption: In the case of nitrate salts (as calcium nitrate), the differential intake of the two elements is probably wholly an expression of the ionic absorption (167). A calcium nitrate solution sufficiently acid to permit seemingly significant formation of molecular nitric acid (HNO_3) would probably kill the root hairs or other absorbing cells of most plants. Many investigations, some of which will be considered presently, show that a rather acid medium is more favorable for absorption and assimilation of nitrate than a neutral or slightly acid culture solution. Hoagland and Davis (78), working with the large-celled alga *Nitella,* found that penetration of nitrate was much more rapid from a slightly acid solution than from an alkaline one. But, directly or indirectly, it is probably assimilation rather than absorption of nitrate which limits growth. This is indicated by the responses of peach trees maintained by Davidson and Shive (47, 48) in sand culture with nitrogen supplied only as calcium nitrate at pH 6 (plus or minus .5). The trees removed relatively less nitrate from the nutrient medium and made somewhat less growth than comparable cultures at pH 4 (plus or minus .5) but lack of a nitrate supply within the trees of the less acid cultures was not a limiting factor. On the contrary, the peach tree, which ordinarily contains and assimilates nitrate mainly in the fine rootlets, had in this case nitrate not only in these organs but in the tops of the trees as well. Nitrate in the cultures at pH 4 was limited strictly to the roots (*cf. Nitrate Reduction in Roots*).

Their results and others to be discussed show that plants may absorb, assimilate nitrate and grow luxuriantly over a much greater pH range of the nutrient solution than is possible with the use of ammonium salts in solutions lacking nitrate. This would seem to indicate, along with accompanying records of differential absorption or shift in pH of solution, that adequate ionic dissociation of the nitrate salts occurs at the several pH levels concerned. Even at low temperature there seems to be sufficient dissociation for unhindered absorption of nitrate, as emphasized by Mevius and Engle (131) and frequently corroborated by the writer, the limiting factor with low temperature being assimilation rather than absorption of nitrate (155, 158, 160, 161, 163).

Ammonium Absorption: Mevius and Engle (63, 130, 131) also conducted extensive investigations designed to determine conditions

governing the absorption of nitrogen from solutions of ammonium salts at different pH values. The hydrogen ion concentration of nutrient solutions, one of the most critical environmental factors, was studied by observations of the responses of corn plants at different seasons of the year when supplied with ammonium sulphate in a complete nutrient solution at various concentrations and pH values. They conclude that when carbohydrate reserves in the plant are not a limiting factor, the effect of ammonium sulphate is determined in large part by the pH of the nutrient medium. This in turn determines the amount of cleavage of ammonium sulphate to free or molecular ammonia (NH_3, or NH_4OH). They believe that the amount of free ammonia formed determines the amount of nitrogen which is available for absorption from a nutrient solution containing nitrogen only as the ammonium salt of a strong acid.

It is known that free ammonia is toxic to plants. This has been demonstrated by Willis and his collaborators (292, 293, 294). Their results indicate that where ammonium hydroxide has been successfully employed in significant concentrations as a source of nitrogen, its introduction into a nutrient solution or soil is associated with interaction of materials of the soil or nutrient substrate to form relatively insoluble compounds or non-toxic salts.

Mevius and Engle (63, 130, 131) record in support of their ideas the fact that the more alkaline the nutrient solution was the more rapid the absorption of nitrogen from ammonium salts. They further report that under the same conditions with high concentrations of the solution there was an increase in amount of absorption of free ammonia and death of the root tips. The surrounding residual nutrient solution increased in acidity owing to the usual differential absorption. Nevertheless, the death of the root tissues was preceded by an increase in alkalinity of the cells concerned that was apparently correlated with the presence within the cells of free ammonia. At pH values slightly below 6, large concentrations of ammonium sulphate were supplied without accumulation of free ammonia within the cells and without injury to the roots. Low temperature was also said to permit the addition of high levels of ammonium sulphate to the cultures without injury to the roots. The explanation given was that both low temperature and low pH limited the hydrolytic cleavage of ammonium sulphate to free

ammonia and, according to their hypothesis, must necessarily, therefore, have limited absorption of the nitrogen of this compound. On the other hand, Prianischnikov (196) claims that although nitrate absorption increased with rise in temperature that of the nitrogen of ammonium remained unaffected. Undoubtedly, the matter of assimilation was a dominant factor indirectly controlling absorption but the point to be emphasized here is that Prianischnikov, in accord with others, records absorption of nitrogen from ammonium sulphate under conditions that apparently eliminated the possibility of the presence of free ammonia in the culture medium.

In brief, Mevius and Engle (63, 130, 131) place the emphasis upon the absorption of molecular ammonia (NH_3 or NH_4OH) rather than upon absorption of the ammonium ion (NH_4^+), the product of electrolytic dissociation. They ascribe any injurious effects of "physiologically acid" ammonium salts to the accumulation of free ammonia in the plant cells rather than to the direct or indirect effect of residual acidity that may develop in the nutrient medium or at the absorbing surfaces of the roots. Their plant responses to the nutrient conditions employed are in excellent agreement with the work of many others if due allowance is made for the cultural technique followed by the various workers and the resulting degree of control of the pH of the respective nutrient media. This will be further discussed.

Unquestionably, Mevius and Engle have experimental evidence that supports their contentions but they probably have unduly minimized the importance of electrolytic dissociation of ammonium salts and the direct or indirect effects of residual acidity (*cf. The pH of the Nutrient Solution*). When the pH of the nutrient medium is nearly neutral or slightly alkaline, molecular ammonia may perhaps play the dominant rôle but there are many experiments indicating that ionic ammonium (NH_4^+) may be removed by plants from a nutrient solution at a pH value much too low for the hydrolytic cleavage from ammonium sulphate to ammonia (NH_3 or NH_4OH) (*cf. The pH of the Nutrient Solution*).

It is true with usual cultural conditions, as will be shown presently, that the absorption of the nitrogen of ammonium when coupled with assimilation is much more rapid at neutral or slightly acid pH than under more acid conditions of the nutrient medium. But it does not necessarily follow that *absorption* of the nitrogen of ammonium is necessarily a limiting factor in an acid nutrient solution.

Shive and his students (3, 38, 47, 233, 242), by analyses of residual nutrient solutions, have repeatedly determined that, at least under some circumstances the nitrogen of ammonium salts may be absorbed in measurable quantity by several different kinds of plants even though the pH values of the nutrient solutions were well below neutral. Very recently, Davidson and Shive (47, 48) grew peach trees in sand culture with excellent control of the pH of the nutrient solution. The entire external nitrogen supply for the trees under consideration was ammonium sulphate. The nutrient solutions were applied at pH 4 for some of the trees and the remainder received the same solution adjusted to pH 6 (plus or minus .5 in both cases). Absorption tests were conducted to determine the rates in milligrams per gram of dry plant material per hour at which nitrogen was absorbed by plants in the two treatments. These figures for the more acid series averaged for the growing season .052 as compared to .114 at pH 6. Further, the tendency of the culture solutions was to increase in acidity at both low and high pH values and, at least in the more acid culture, there could have been no free ammonia. The shift in pH must, therefore, have been correlated with differential ionic absorption of ammonium (NH_4^+). The trees at pH 4 exhibited a typical response in that they made less growth than those supplied with the less acid solution but the limiting factor was clearly not absorption for the percentage concentration of ammonium nitrogen (plus glutamine?) in the fresh absorbing roots was .011 and .013, respectively. The roots were not noticeably injured in either case (1932 series) although the volume of growth was greatest in the solution at pH 6. It is notable that Davidson and Shive say that the rootlets were relatively short in the pH 4 series. This is not a characteristic of trees deficient in organic nitrogen. Mevius and Engle, on the other hand, state that where the acidity of the medium was not low enough to cause root injury, plants at low pH supplied with nitrogen only as ammonium sulphate exhibited the same long slender type of roots that were characteristic of their cultures lacking nitrogen. Indubitably, this response is typical of nitrogen deficiency (*cf. Growth in Relation to Available Nitrate*) and supports their contention that at low pH values of the nutrient medium the nitrogen from ammonium sulphate was largely unavailable to the corn plant under the conditions of their experiments, but it is apparent that this does not hold true

for all plants under some environmental conditions. As will be shown presently, internal factors and other external factors in addition to pH, very greatly modify the utilization of ammonium nutrients, as for example, the relative amounts of calcium and other non-nitrogenous ions. It may also be mentioned, with further discussion in the following pages, that some plants in an acid medium may absorb ammonium without there occurring any noticeable root injury, whereas others not only fail to absorb but actually excrete ammonia, thereby tending to neutralize external acidity. The difference in plant response to the acid environment of the roots seems to be determined by the quantity and proportions of protein and carbohydrate reserves (188, 191, 192).

The same general principles that have been discussed apply to ammonium nitrate. However, consideration to it can be most conveniently given elsewhere as the responses of a plant to nutrition with this "physiologically amphoteric salt" are closely correlated with metabolic activities. Likewise a discussion of Prianischnikov's views and evidence concerning the effects on plant cells of residual nutrient acidity and free ammonia can be presented to better advantage a little later.

The pH of the Nutrient Solution: The importance of the hydrogen ion concentration of the nutrient solution has been emphasized but a catalogued list of the experimental results of various workers recording the pH which gave best growth for each particular set of experimental conditions would avail little. It must already be apparent to the reader that there is no one best pH value for a given nutrient solution for all plants nor for the same kind of plant under different environmental conditions. Mevius and Engle (63, 130, 131), whose work has already been discussed, found that ammonium salts could be supplied to corn plants without injury during the summer months at a pH value considerably less acid than could be employed in the winter months when light conditions were less favorable for carbohydrate synthesis. Although they strongly emphasize the matter of pH of the nutrient solution they are not less emphatic in pointing out the importance of other factors. In a general way, under excellent control of pH, it may be said that at pH 5.3 to 5.6 they obtained approximately equal growth with both nitrate and ammonium salts.

When nitrate was applied at much less acid pH, corn was detrimentally affected, apparently owing to iron deficiency. This ele-

ment is notably difficult to keep in solution as the culture medium shifts towards alkalinity (210) when there are present salts such as calcium nitrate. As will be shown presently, this is apparently owing in part to differential absorption and the resulting increase in alkalinity at the surface of the absorbing root where it causes iron precipitation. This may occur even though the bulk of the nutrient solution is sufficiently acid to maintain iron in solution (159). Internal factors may, however, be of equal or greater importance (210). Pirschle (177, 178) worked with different plants and noted that, while certain kinds made excellent growth with nitrate supplied at an intial pH well above the neutral point, several species were limited in growth at least in part by inability to absorb iron under these conditions. In general, he found, as many others have, that nitrate gave excellent growth responses over a much greater pH range than ammonium. Nitrate utilization and growth was distinctly favored, however, by a pH value in the vicinity of 5, varying somewhat with other external and internal conditions to be discussed. In the case of some plants he obtained two pH optima; although the reasons for this are not entirely clear in every case, he indicates that in general it was associated with indirect effects of the hydrogen ion concentration resulting in the harmful effect of iron deficiency or in interference with the absorption of other non-nitrogenous ions. He states very pertinently that there is no absolute pH optimum and that his statements about it are only approximate for the immediate conditions of his experiments. With these reservations he concluded that under a system of constant renewal of nutrient solutions most plants had their optima between pH 5.5 to 6.5. Within this range there was usually very little difference in the growth responses of ammonium- and nitrate-supplied plants. Loo's (112) extensive experiments are in harmony with those just cited. He reports different pH optima but, in general, nitrate was most available at a weakly acid reaction and ammonium at a neutral or slightly alkaline pH value.

The work of Shive and his students and of Prianischnikov and his collaborators carried on over a number of years is in complete accord with the preceding observations but as their work is of additional significance in its bearing upon metabolic responses it will be discussed later in that connection along with the results of Tiedjens and others. It should be mentioned here, however, that in earlier

experiments Tiedjens and Robbins (263) reported that tomato plants supplied with ammonium nitrogen at an initial pH of 8 absorbed nitrogen and grew luxuriantly with uninjured roots. This observation, in apparent contrast to others already cited, is not actually in conflict in principle for the initial pH was subject to rapid change in their sand culture medium, especially as the root systems increased in volume.

It may well be asked what the real pH of a nutrient solution is, if subject to shift in value as a result of more or less continual differential absorption of anions and cations. The answer must be that only a range of pH values can be determined. Apple trees supplied with a complete nutrient solution were employed in a test involving the determination of the pH ranges occurring in cultures supplied with ammonium sulphate at pH 6 or with calcium nitrate at pH 4.5 (159). The pH values were not chosen arbitrarily but were those found by Tiedjens (265) to be favorable for the growth of apple trees in sand culture under conditions of constant solution renewal. The trees were grown at a practically constant temperature of 10° C. in both sand and water cultures. In both media the solutions were constantly renewed according to the method of Shive and Stahl (234) at the rate of 36 liters per culture every 24 hours. This rate of renewal was such that the solution after bathing the roots and passing out of the culture vessel did not change more than plus or minus .1 pH. As usual, the ammonium sulphate cultures tended to become more acid and the calcium nitrate cultures more alkaline. Absorption and assimilation of both ammonium and nitrate was verified by plant analysis. The results were very definite as there were available for comparison similarly treated trees that were grown with no external nitrogen supply at pH 5.

Associated with absorption of nitrate and ammonium, respectively, and with nearly perfect control of the pH of the bulk of the solution, it was found by the range indicator method of Small (236) that the trees in sand culture receiving ammonium sulphate at pH 6 had, at the absorbing surface and tips of the fine fibrous roots, a pH of 4 to 4.5. The sand immediately adjacent to the roots also had about the same H-ion concentration, with a gradient reaching pH 6 at about 2 cm. from the absorbing surface of the root. The calcium nitrate cultures at pH 4.5 had, at the absorbing surface and tips of the fine roots, a pH of 5.6, as did also the sand in contact with the

roots; although less than 1 cm. from the roots the solution on the sand particles was about pH 4.5. On the other hand, the minus-nitrogen cultures at pH 5 did not noticeably affect the H-ion concentration of the solution as a whole, and the surface of the fine roots appeared to have a pH value of approximately 4.8 to 5. Yet the roots, although somewhat more slender, were growing, at least in length, at about the same rate as in the case of the trees receiving nitrogen in the nutrient solution.

The ammonium and the nitrate water culture series were vigorously stirred with a motor-driven agitator. Even under these conditions the ammonium sulphate (pH 6) cultures had at the surface of the fine absorbing roots a capillary film of pH 5.4 while the trees supplied with calcium nitrate nutrient solution at pH 4.5 had at the surface of the absorbing roots a film of pH 5.2. It is obviously impossible, in sand culture, absolutely to control the pH of the solution bathing the roots, if the plant is absorbing from the nitrogen-containing salt of the nutrient solution in largest part ammonium from ammonium sulphate or nitrate from calcium nitrate. The preceding results would not indicate that a widely different pH of the nutrient medium is required for ammonium from that required for nitrate nutrition. Actually, the trees of the ammonium series assimilated nitrogen rapidly when the solution bathing the roots was pH 4.5, and those of the nitrate series when the absorbing surface of the roots was pH 5.6. Practically, under conditions of sand culture it is essential that the initial solution containing ammonium sulphate be approximately neutral (pH 6) and that of a calcium nitrate culture be relatively more acid, in order that the absorbing root surfaces may not become extremely acid in the former case or excessively alkaline in the latter case.

Non-Nitrogenous Ions: Prianischnikov and his students over a considerable period of years have been studying in part the effects of the pH of the nutrient medium on ammonium and nitrate nutrition (191, 194, 196, 53, 54, 85, 86). Their results are very similar to those already cited. In addition, they determined the ash content of a considerable number of species of plants and found almost without exception that their ammonium-supplied plants contained much less calcium than similar series receiving nitrate (.18 against .32 per cent CaO in sugar beet). Upon the addition of more calcium to the nutrient solution there was greater intake of this element and

good growth of sugar beet was obtained when ammonium sulphate was supplied at a practically constant pH of 4 (constant renewal culture). The recorded yield in this particular instance was slightly greater than that of comparable cultures with the same or less calcium at pH 6 (194). Other tests were conducted in which the pH was controlled by either flowing cultures or frequent additions of increments of acid or alkali as required to solutions containing ammonium sulphate. The results were similar to those described. Diminishing the amount of calcium was unfavorable to the growth of the plants in the ammonium cultures. Potassium was much less marked in effect although an abundance seemed more essential in ammonium than in nitrate nutrition. High concentrations of calcium were distinctly detrimental to plants of the nitrate-supplied series at both high and low pH values of the culture solutions.

Holly *et al.* (80, 81, 82), working with ammonium and nitrate-supplied cotton plants in sand culture, report that the use of the ammonium ion as a source of nitrogen reduced the absorption of bases, the greatest effect being on calcium and magnesium. Although the use of ammonium was associated with reduced calcium absorption there was no evidence that the presence of calcium was correlated with a reduced absorption of ammonium. They state in addition that the differences in calcium content between the nitrate and ammonium-supplied plants was due principally to leaf calcium content. The differences in magnesium were evident, however, in roots, stems and leaves. Differences in absorption of sulphate and phosphate were small and varied at different stages of development.

Ivanova (85), likewise using cotton, reports that in sand culture best growth was obtained at pH 7 but that through the introduction of additional calcium to Naftel's (147) solution as calcium chloride or sulphate, cotton was able to utilize ammonium at pH 3 throughout the vegetative period. Additions of potassium had no apparent effect and increased magnesium as the sulphate or chloride was detrimental. In comparable studies with sugar beets, cabbage and flax, Dikussar (53) found that ammonium at pH 7 gave as good yields as nitrate at pH 5 and he obtained responses similar to those described through the use of different proportions of calcium and magnesium. Various other ions have been investigated (45, 46, 177, 178, 281) to determine their relationship to ammonium and nitrate utilization but the results permit no conclusions, probably

partly because of precipitation of materials in the nutrient solution at the more alkaline ranges or of variations in experimental conditions.

Considerable additional work on calcium has been reported by Prianischnikov and will be considered later in connection with metabolic responses (189, 196). It is significant that he strongly emphasizes the fact that there is no single coefficient through which the optimum pH value of a nutrient medium can be determined. It depends not alone upon such external conditions as the concentration, temperature and calcium content of the nutrient solution, but upon internal factors presently to be discussed.

Stage of Plant Development: Shive and his students have made extensive quantitative studies of the absorption of nitrogen by oat and buckwheat plants which received equal proportions of nitrogen as ammonium and as nitrate at various stages in the life cycle of the plants (233, 242). They found that the rate of absorption of nitrogen as nitrate by oats was lowest in early growth, reached a maximum at the blossom stage and then declined. Ammonium, on the other hand, was absorbed most rapidly during early stages and declined with increasing age of the plant. The absorption of total nitrogen also reached a maximum at the flowering stage. Quantitative determinations of the nitrogenous materials of the oat plants served further to verify the conclusions arrived at through analysis of the residual nutrient solutions. With buckwheat, on the contrary, ammonium absorption predominated over nitrate absorption during the greater part of the life cycle of the plant. The nitrate absorption rate exceeded that of ammonium only in the very late stages of growth when the rate of intake of both forms of nitrogen was extremely low.

Later work with tomato in which ammonium and nitrate nitrogen was supplied in equal proportions, indicated that ammonium absorption tended to predominate over that of nitrate in young plants, but the pH value of the nutrient media produced a very striking response (3, 37, 38).This was decisively demonstrated by shifting plants from solutions of one pH to those of another pH. Twelve days later the plants were tested as to their rates of absorption. The effect was to retard the rate of nitrate intake regardless of whether the transfers were made from solutions of relatively high to those of low pH values, or *vice versa.* On the other hand, plants transferred

from pH 7 solutions to cultures at pH 4 or *vice versa* for immediate absorption tests showed that the effects on the rates of absorption of ammonia were immediate. Although the reaction change exerted an immediate influence upon nitrate intake the effect was not so pronounced as in case of ammonium absorption, possibly because of the high nitrate content in the plants of the several series. Ammonium, in contrast, was present as usual only in small concentrations.

The results with oats are in harmony with the responses of young cotton plants as observed by Naftel (147). This species first absorbed more ammonium but later more nitrate. Prianischnikov (197) repeated Naftel's experiments and came to the conclusion that the character of the culture solution employed by Naftel, rather than the stages of development of the plant, was the determining factor. In Prianischnikov's opinion the plants had received a great excess of nitrogen (80 mg. N per L. every two days). He considers that the comparatively high proportion of magnesium sulphate employed must on account of the abundant sulphate have favored the absorption of nitrate (*cf.* Loo, 112) which in conjunction with less calcium than magnesium indicated to him a solution particularly adapted to nitrate rather than ammonium nutrition.

Prianischnikov, in repeating Naftel's experiments, took as a starting point his solution "C" which contained equivalent amounts of ammonium and nitrate. The unmodified solution gave results similar to those of Naftel but with a doubled amount of calcium introduced as the chloride or sulphate, the plants absorbed in every stage of development more ammonium than nitrate and made more growth. The following data indicate the results obtained:

Age of plants in days		Mg. nitrogen absorbed per 24 hours per culture				Dry wt. of plants per culture grams
		30	60	80	100	
Nutrient solution "C" of Naftel at pH 4.8	NH_4-N	9.3	8.7	12.2	6.9	30.7
	NO_3-N	15.6	11.9	13.4	6.0	
Same solution "C" with double the calcium, pH*	NH_4-N	19.9	37.5	29.6	19.8	67.1
	NO_3-N	9.8	19.4	23.6	11.3	
Same solution "C" at pH 7	NH_4-N	13.8	20.6	24.0	10.8	58.6
	NO_3-N	7.1	17.9	15.1	6.8	

* Presumably pH 4.8.

Prianischnikov concluded, therefore, that the pH and calcium content of the solution exerted more influence upon the relative absorption of ammonium and nitrate than the stage of plant development.

He suggests that the results obtained by Stahl and Shive (242) with oats were associated with the fact that they employed a nutrient mixture high in nitrogen (240 mg. per liter) and supplied it as a flowing culture. [This does not, however, account for the responses with buckwheat nor for the fact that equivalent amounts, or greater, of ammonium or nitrate supplied separately will carry tomato plants through their entire life cycle (37, 38, 39, 265).] When Prianischnikov furnished oat plants with the same solution containing only 24 milligrams of nitrogen per liter, he recorded in all developmental stages greater absorption of ammonium than of nitrate. His results agreed with those of Stahl and Shive when the same amount of nitrogen was used, *i.e.,* in later stages of growth there occurred mainly nitrate rather than ammonium absorption. He obtained the greatest dry weight yield of plant material when the nitrogen supplied was 48 mg. per liter. (*cf.* GROWTH IN RELATION TO AVAILABLE NITRATE). His plants began to excrete ammonia into the external medium after 20 to 30 days when receiving 480 mg. per liter of nitrogen; at least, his solutions gained in amount of ammonium and became less acid whereas nitrate in the solution decreased. He ascribes the excretion of ammonia to continued absorption and reduction of nitrate by the plants without further assimilation to organic nitrogen.

These results are not of minor importance but, as will be indicated presently, Prianischnikov's work and that of many others emphasizes the significance in nitrogen nutrition of the chemical constitution of the plant. Detailed descriptions and carbohydrate analyses of the plants of the several experiments cited would have been of value. Obviously, the change in appearance of plants with different stages of development is an external expression of a continually changing internal status. The pH and calcium content of the nutrient solution and other external factors are clearly important but not to the exclusion of the internal condition of the plant which is often the chief limiting factor in nitrogen nutrition. A discussion of such relationships follows.

INTERNAL FACTORS INFLUENCING AMMONIUM AND NITRATE NUTRITION

The pH of Root Cells: Conrad (42) grew maize seedlings in a complete nutrient solution in which nitrogen was supplied as ammonium nitrate. After some growth had been made the plants were shifted to single salt solutions of ammonium sulphate and potassium nitrate as well as to solutions containing, respectively, only the corresponding acid. The initial pH of the salt-containing cultures was about 5.8; that of the acid-containing media, approximately 2.4. The length of time the plants remained in these cultures is not stated but it was apparently for a sufficient length of time to affect more or less complete removal of nitrogen from the solutions containing it. At least the ammonium sulphate cultures showed a shift in pH value to 2.7 and the potassium nitrate and nitric acid series to 6.5 and 6, respectively. There was a decrease of .2 only in pH value of the sulphuric acid solution. Comparable cultures of sodium nitrate and carbonate gave residual pH values of about 7.2.

It is not made clear whether the pH values of cultures ranging from 2.7 to 7.2 in any way affected the external or internal appearance of the roots, but the composite tissues of roots, stems and leaves from single cultures were dried in an oven at 70° and ground. Aqueous suspensions of this material from the several series gave pH values ranging from 5.1 to 5.7 which corresponded, respectively, to nutrient media with the residual values of 2.7 and 7.2.

Keyssner (92), using flowing complete nutrient solutions maintained at practically constant pH, supplied oat plants and various other species with combinations of ammonium and nitrate to which had been added dilute acid or alkali as required. The pH values of his solutions ranged from 4 to 9. The plants were grown in the respective solutions for about two weeks, after which the roots were washed in distilled water and ground to a pulp. The pH_H of this freshly ground pulp was determined with the quinhydrone electrode. In typical experiments the root sap of nitrate-supplied cultures varied from 5.6 to 8 and that of corresponding ammonium series from 6.7 to 7.7; the external pH of the cultures, going in the same direction, ranged from 4 to 9. It is significant that in cases where the extreme pH values of root sap occurred the plants were

badly stunted. For example, the dry weight yield of plant substance for the ammonium series was 38 grams when the culture pH was 9 as compared to a maximum yield of 481 grams at a nutrient solution pH of 6 and a sap pH of 6.3. Various other experiments might be described in which composite extracts of all parts of the plant or single organs consisting of various tissues have given one or another result (46, 236).

Such results are difficult of interpretation as in a single cross-section of a root tissues will usually be found having pH values ranging from less than 4 to about 7 (236). It is obvious that if the nutrient treatment is associated with change in relative proportions of the respective tissues there will be a change in pH of extracted sap, yet during the same period cells of a given tissue at a given stage of development may not have exhibited any change in hydrogen ion concentration. Juices expressed from complex plant tissues have a hydrogen ion concentration which represents the algebraic sum of that of the sap of phloem, xylem, parenchymatous tissue, any specialized cells present, dead tissue, etc.

Hoagland and Davis (78) have eliminated this objection by employing the expressed sap of single cells of *Nitella*. They found that the hydrogen ion concentration of healthy cells of this plant was approximately constant at pH 5.2. The nutrient media employed by them varied from pH 3.8 to 9.4, yet no appreciable change of pH occurred in the cell sap, except below 5, and at the lower values they state the cells were unquestionably injured. In connection with studies of penetration of ammonium and nitrate ions which have already been mentioned, they noted that the ammonium ion penetrated rapidly and caused a change of reaction in the cell sap. With .005 molecular ammonium salts, the reaction was, in most cases, changed from pH 5.2 to pH 5.6–6.2 in the course of 24 hours or less but the cells were injured.

Cells of a kind at a given stage of maturity do not apparently have an absolutely constant pH value. Young respiring cells seem to fluctuate more than mature elements (236, 163, 159), presumably owing at least in part to the relative rate of respiration and effects of carbon dioxide under different conditions (65). But aside from fluctuations within rather narrow limits, comparable cells of a given tissue probably maintain a fairly constant hydrogen ion concentration. Observations of the tissues of the roots of apple trees

grown at a practically constant temperature of 10° C. with ammonium and nitrate, respectively, at various pH values of the nutrient media were in accord with this view (159). Associated with a high degree of residual acidity in cultures supplied with a high concentration of ammonium sulphate, the cortical cells became abnormally acid but this was associated with severe injury. The roots were short, stubby and bulbous in appearance. This was due mainly to the development of the primordia of lateral roots, most of which never developed sufficiently to emerge through the cortex, probably because of the extremely acid condition of the root surface and of the outer cortical cells which were about pH 2.8 to 3. There was no evidence of increased alkalinity owing to the absorption of ammonium, which certainly occurred as indicated by the increased ammonium and organic nitrogen content of the root system as well as by the shift in pH from 6 to the values mentioned. This shift in pH occurred only at or near the root surfaces, not throughout the nutrient medium.

It should be emphasized, however, that these trees were grown at low temperature preventing thereby, according to Mevius and Engle (130, 131), the hydrolytic cleavage of ammonium sulphate to molecular ammonia. The trees also had an unusually high carbohydrate reserve, an important factor in detoxication of ammonia (see this heading). There would seem no question but that in this case injury to the roots was caused directly or indirectly by the residual acidity of the nutrient medium. This is not necessarily in conflict with the observations of Mevius and Engle, Ribbert and others (204), who have reported that although accompanied by residual acidity the major factor bringing about injury to plant tissues was probably the presence of free ammonia in the cells. In addition to tests with culture solutions, Ribbert injected leaf cells with ammonium sulphate and obtained an increase in alkalinity of the cells which he ascribes to the probable presence of free ammonia. It would seem obvious that, if extreme, either external acidity or internal alkalinity could well result in injury to plant tissues. It should be emphasized again that the gross pH value of the nutrient medium as a whole gives no measure of the hydrogen ion concentration at the absorbing surfaces of the root.

Regardless of the origin of the free ammonia, the degree of pH change of the protoplasm and the extent of injury from it will be

dependent very largely upon the capacity of the plant for disposal of any ammonia present in the tissues. Much of the following discussion is concerned with this matter.

Sources of Ammonia: (1) To recapitulate, one source of ammonia is that formed from the cleavage of proteins, polypeptides, etc. The resultant amino acids are deaminized, leaving the carbon skeleton of the original amino acid plus free ammonia. If carbohydrate reserves are adequate or there is opportunity for carbon dioxide assimilation, detoxication of ammonia takes place through synthesis of one or both of the amides, asparagine and glutamine, some free oxygen being required for amide synthesis, however, even though carbohydrates may be present.

(2) There is also the possibility, emphasized by Mevius and Engle, of direct absorption of free ammonia (NH_3 or NH_4OH) from a neutral or slightly acid nutrient solution containing ammonium sulphate or a similar salt. However, with absorption of the nitrogen of ammonium sulphate there is rapidly developed considerable residual acidity, especially at the absorbing surfaces of the roots. The resultant pH value is much too low for hydrolytic cleavage and formation of free ammonia. At least in a nutrient medium much below neutral, plants probably absorb the nitrogen of ammonium sulphate as the ammonium ion (NH_4^+) (167).

(3) The reduction of nitrate with accompanying oxidation of sugars or their derivatives may take place in darkness or in light, the first product to appear being nitrite and then ammonia. Regardless of the initial source of free ammonia, other conditions being equal, its subsequent disposal by the plant will be the same whether it was derived through protein cleavage, by direct absorption or through reduction of nitrate.

Disposal of Free Ammonia by the Plant: The preceding observations are clearly in harmony with Prianischnikov's now generally accepted theory that ammonia is the "alpha and omega" of nitrogen metabolism in the plant (188, etc.). Much of his work and that of Mothes (135), already discussed, has emphasized the fact that ammonia was not stored in the plant as such but was metabolized to amides in the presence of carbohydrates and thus rendered inocuous.

Storage as Ammonium Salts: In a series of papers published a few years ago, Ruhland and Wetzel (212, 213, 214, 291) have

shown that in plants with a very acid sap there may be storage of ammonia as the ammonium salts of organic acids, not free or molecular ammonia. For this there is required not only a low pH value but actual acidity as well. Some of their first observations were made with the leaves of *Begonia semperflorens* which had a pH value of about 1.5 and contained 20 per cent of their dry weight as oxalic acid. In a typical experiment some of these leaves were placed in darkness at 28–35° to bring about carbohydrate deficiency and proteolysis (cf. *Metabolism of Leaves*). At the end of 106 hours the ammonium nitrogen amounted to 30 per cent of the total nitrogen whereas the small amount of initially present amide completely disappeared. Accompanying protein cleavage and loss of amide nitrogen they record an increase in acidity to pH 1.3, which they believe was directly correlated with deamination. The metabolized acid, whatever the origin, was apparently adequate to react with the ammonia formed proteolytically so that no free ammonia injury was possible.

Ruhland and Wetzel worked also with the usual garden rhubarb, *Rheum hybridum* Hort, the rhizome of which had only a slightly acid reaction and contained about the usual distribution of amino and amide nitrogen and no more than traces of ammonium nitrogen. As the young leaves developed there was rapid growth and protein synthesis with accompanying decrease in the amide and amino nitrogen of the rhizome. As the leaves approached maturity there was apparently deamination to the extent that the petioles of fully developed leaves contained about 60 per cent of their total nitrogen as ammonium. Correlated with deamination—or at least it is so considered by Ruhland and Wetzel—there appeared first malic and succinic acids chiefly, followed, as the petiole matured, by an increase in oxalic acid and a decrease in the acids mentioned. There was present at all times an adequate amount of organic acid to prevent the appearance of injurious free ammonia.

Ruhland and Wetzel's interpretation of the organic acid metabolism of rhubarb has been criticized by Bennet-Clark and Woodruff (13) who have reinvestigated the matter, conducting their experiments in a manner to permit computations on an absolute amount basis. They concluded there is little justification for the view that malic acid is derived from the carbon residues of deaminated amino acids. They did not question the ability of the rhubarb petioles to

store ammonia as the ammonium salt of organic acids but emphasized that their results showed that the increase in organic acid content of the petioles is associated with translocation in the spring and that malic acid is apparently a product of carbohydrate metabolism.

Recent work by Kultzscher (106) and Rahn (200) gives further evidence to show that highly acid plants store ammonia as the ammonium salts of organic acids. Kultzscher, in addition, supplied acid plants, *Begonia semperflorens* pH 1.4 and *Oxalis deppei* pH 1.3, with calcium nitrate and found that there was a striking increase in ammonium stored as the salts of organic acids, presumably indicating nitrate reduction to nitrite and ammonia with delayed or limited assimilation of the ammonia. Other plants, the expressed sap of which gave a pH value of about 5, followed the conventional course of metabolism, amides being metabolized with increase in ammonia from nitrate reduction or deamination. They describe also plants which are intermediate between the so-called "ammonium plants" of Ruhland and Wetzel and the "amide plants," the type upon which all the earlier work was based. In general, the intermediate plants had an intermediate pH value of expressed sap but the ratio of amide to ammonium bore no constant relationship to the hydrogen ion concentration of the heterogeneous sap extract. It will be recalled that Wood (296), in his studies of *Atriplex nummularium* leaves, found marked variations in the proportions of ammonium plus amide and amino nitrogen depending upon the pH values of the tissue. Unfortunately, he did not determine ammonium and amide nitrogen separately.

Both Kultzscher and Rahn follow Ruhland and Wetzel's hypothesis and concluded, on the basis of percentage determinations, that the storage organs of acid plants typically are high in amino nitrogen and that with expansion of new shoots there is rapid deamination, the carbon skeleton of the amino acids concerned forming organic acids which react with the ammonia produced from the amino group to form ammonium salts. In the storage structures of the "amide plants" there was nearly as much amide as amino nitrogen.

Detoxication of Ammonia: The matter of detoxication of free ammonia has already been discussed in connection with formation of ammonia from proteinaceous reserves contained in various organs

of the plant. A few experiments will be cited sufficient to indicate the apparent course of metabolism in detoxication when the ammonia is derived from external sources. The investigations of Prianischnikov and his collaborators with seedlings in darkness and in light furnish excellent illustrations (183, 184, 185, 186, 187, 189, 190). Summing up briefly the work of many years of research, it may be said that seedlings such as oats, barley and maize, relatively high in carbohydrate reserves, and pumpkin, containing reserve fats, absorbed ammonium rapidly in darkness or in light from solutions containing ammonium sulphate or chloride. This was associated with no appreciable accumulation of ammonium but with a marked increase in asparagine or in glutamine, the latter amide occurring especially in the Cucurbitaceae. If seedlings containing abundant nitrogen-free reserves were grown in darkness until the carbohydrate or fat content became practically exhausted and were then supplied with ammonium, there was practically no elaboration of amide nitrogen; ammonium accumulated probably in part as free ammonia and the plants died. Others received an external supply of glucose, synthesized asparagine, accumulated little ammonium and were not injured.

Seedlings such as *Vicia sativa, Vicia faba* and *Pisum sativum* were unable to elaborate asparagine from absorbed ammonium except when there was present in the external medium an abundance of calcium, as the chloride, sulphate or carbonate. Work was done also with low-carbohydrate seedlings such as *Lupinus* that store mainly hemicelluloses. *Lupinus,* even with addition of calcium, was unable to synthesize asparagine from absorbed ammonium in darkness. The seedlings were accordingly injured. But it is notable that, with an external supply of glucose in darkness or when the seedlings were grown in light, formation of asparagine occurred, there was little increase in concentration of ammonium in the plants and they were uninjured. Burkhart (24) very recently studied the metabolism of etiolated seedlings, some of which received no external nitrogen supply and others a typical ammonium sulphate nutrient solution. He found an increase in absolute amount of organic nitrogen, owing to assimilation of ammonium in darkness, in case of all seedlings employed except *Lupinus.* In other seedlings containing abundant carbohydrate reserves, he obtained striking increases in organic nitrogen as a result of assimilation of am-

monium in darkness. He obtained the usual formation of asparagine during the early period; then, following or accompanying carbohydrate depletion, there was proteolysis and increase in ammonium. In cases where sugars became extremely low there was injury and presumably the formation of free ammonia.

Smirnov (237) studied in some detail the metabolism of etiolated seedlings of *Hordeum sativum*. During the early period in darkness there was an increase in amino acids owing to assimilation of ammonium derived from a nutrient solution containing ammonium chloride. As carbohydrates were consumed, amide nitrogen increased and with extreme depletion asparagine was no longer elaborated, ammonium or free ammonia accumulated and the plants were injured. With no attempt at explanation, it is of interest to note that calcium appeared to accelerate the several metabolic steps in both synthesis and hydrolysis of proteins. It was associated with more rapid decrease in dry matter.

Smirnov supplied some of his plants in darkness with ammonium malate and succinate, respectively, for comparison with ammonium sulphate cultures. The salts of these organic acids were associated with an apparently significant increase in synthesis of amino acids and asparagine but for further synthesis to proteins the presence of abundant gl ose was required. In this connection it may be said that the technique of infiltration indicated synthesis of asparagine from ammonium malate. This synthesis recorded by Mothes (140) occurred in leaves which were deficient in carbohydrates (*cf.* 18, 19).

Many more examples might easily be given but sufficient evidence has been presented to show that detoxication of ammonia in plants of the "non-acid" class is often dependent in large part upon the ability of the plant to elaborate asparagine or glutamine. For this purpose the presence of reserve carbohydrates in darkness is essential, or sunlight and the opportunity for CO_2 assimilation. This varies with seasonal conditions and location and although seldom considered is very frequently a serious limiting factor in studies of nitrogen nutrition.

*Excretion of Ammonia**: The phenomenon of ammonia excretion was observed by Prianischnikov when pea seedlings were tested as to their response to a nutrient solution containing ammonium nitrate (188, 191, 192). It was noted that in comparatively acid solutions

* Ammonia and volatile amines have been reported in the exhalate of certain flowers (97).

there was excretion of ammonia by the roots instead of absorption which occurred in neutral or slightly acid media. Other seedlings were selected for trials so as to obtain plant material containing different proportions of protein and carbohydrate reserves. For example, in oats the proportion of protein to carbohydrates is about 1:6; in peas, 1:2. Results were obtained as follows:

	Concentration of HCl			
	0.00075 N		0.001 N	
	Oats	Peas	Oats	Peas
First occurrence of NH_3 after ..	19 days	4 days	12 days	3 days
Death of the plants after	20 days	7 days	15 days	5 days
Initial pH of media.....	3.1	3.1	2.9	2.9
Final pH of media	5.1	5.4	5.1	5.6

The results are in accord with the previous discussion in showing that the appearance of ammonium and ammonia is dependent in part upon the ability of the plant to synthesize asparagine which in turn is dependent upon the presence of carbohydrates. They indicate, in addition, that with increase in acidity of the nutrient medium the appearance of ammonia was greatly accelerated and was accompanied by injury to the plant.

Following these and similar tests with seedlings a large number of trials were made employing ammonium nitrate and calcium or sodium nitrate as the nitrogen source. The object was to obtain further information concerning possible excretion of ammonia from plants high and low in carbohydrates when grown in culture solutions of different degrees of acidity (191, 193, 195, 196, 86, 115). Their results were consistent in showing that when plants were supplied with a nutrient solution containing ammonium nitrate, ammonium was invariably absorbed more rapidly than nitrate as long as the carbohydrate reserves in the plant were abundant and the pH of the nutrient media as a whole did not drop below pH 5. With decrease in carbohydrate reserves, whether it was an inherent condition of the plant or the result of etiolation, nitrate was absorbed rather than ammonium. However, regardless of how high the carbohydrate content, nitrate absorption predominated when the nutrient media *en masse* was less than pH 5. By greatly increasing the relative proportions of nitrate in an ammonium nitrate medium

through the addition of calcium nitrate, there was, even at neutral or slightly acid values of the solutions, some increase in nitrate utilization; but the dominant factors in determining the relative importance of the respective nitrogenous ions was, as indicated, the available carbohydrate supply and the pH of the nutrient solution.

When for any reason carbohydrates approached depletion in plants supplied with ammonium nitrate, then, as usual, the formation of asparagine or glutamine was inadequate for detoxication of ammonia. Eventually death occurred but before carbohydrates became depleted to the point of practical exhaustion of supply there occurred a striking increase in ammonia in the nutrient medium as already stated. This was greatly increased when the cultures were relatively acid.

Perhaps the most surprising phenomenon was the continued reduction of nitrate by plants that were rapidly decreasing in carbohydrate reserves. However, there was apparently no new synthesis of amino acids, the reaction stopping with formation of ammonia. This was most pronounced in acid media. Anaylses of residual solutions of cultures supplied with ammonium nitrate, in which had been placed low-carbohydrate plants, showed continued decrease in nitrate in the nutrient medium but very definite increase in ammonium. Many tests with ammonium nitrate indicated this to be true. In addition, plants were supplied with a complete solution containing nitrogen only as nitrate and there occurred absorption of nitrate and increase in ammonia in the residual solution when carbohydrates in the plant were too low to permit formation of asparagine or glutamine with resultant detoxication of the ammonia originating from nitrate reduction.

There are in the literature the results of a considerable number of experiments involving the relative absorption rates of nitrate and ammonium from solutions containing these two ions in various proportions. The results of many of these determinations have already been cited in connection with absorption rates at different stages of development of the plant. It will be recalled that Prianischnikov (197) reported that mature plants (carbohydrate content unknown), when supplied with high concentrations of ammonium and nitrate, absorbed the latter but that there occurred, instead of synthesis of organic nitrogen, considerable storage of nitrate and reduction to ammonia which was excreted into the nutrient media.

COMPARATIVE METABOLISM OF AMMONIUM- AND NITRATE-SUPPLIED PLANTS

It has been shown that plants absorb ammonium and nitrate with varying ability depending in part upon the concentrations employed, the pH value of the nutrient solution, the presence of some free oxygen in the medium, and the carbohydrate content of the plant or its opportunity for new synthesis of sugars. Unless otherwise stated, the experiments chosen for the immediate discussion are concerned with the growth and metabolic responses exhibited by plants grown under reasonably favorable nutrient treatment for the form of nitrogen supplied. Unfortunately few, if any, experiments are available for discussion, concerning which it can be said that carbohydrates or opportunity for their synthesis may not have been a limiting factor in the utilization of ammonium nitrogen and in the growth of the plants. However, carbohydrate analyses which are in some cases available help materially in an understanding of the results obtained. It may be well to remind the reader that the experiments to be considered were conducted in glasshouses. These structures, even when in excellent condition, shut out 20 per cent of natural sunlight and often very much more (244).

It has been shown that, in the process of protein synthesis from nitrate, nitrite and ammonium are found in the plant in successive stages. Obviously on a theoretical basis, ammonium should be more rapidly assimilated by the plant, therefore, than nitrate. Under conditions of a culture medium favorable, respectively, for ammonium and nitrate, this has been found to be invariably true if carbohydrates have been present in sufficient quantity for synthesis of amino acids or other forms of elaborated nitrogen, and for detoxication of absorbed ammonium through the elaboration of asparagine or glutamine.

Tiedjens *et al.* (263, 264, 265) worked with tomato and several other species and supplied them with nitrate and ammonium, respectively, from the seedling stage to maturation of fruit. Young apple trees also were carried through several months of vegetative growth, receiving in some cases nitrogen only as ammonium and in other instances only as nitrate. Some of this work was in a degree duplicated in studies of the metabolic responses of apple trees at low temperature (159). When plant material was employed that contained an abundant carbohydrate reserve there was very much more rapid

synthesis of organic nitrogen from ammonium and much more rapid increase in volume of the plant than in the case of comparable cultures supplied with nitrate. Invariably this was accompanied by rapid depletion of sugars and starch. The organ first becoming depleted of carbohydrate reserves varied with the kind of plant, depending of course upon the seat of initial amino acid synthesis. In fruit trees this is mainly in the rootlets and it was in the root system that there occurred first the most striking decrease in nitrogen-free reserves. This was apparent earliest in the ammonium-supplied trees although the cultures receiving nitrate decreased in carbohydrates materially as compared to similar trees lacking an external nitrogen supply. The latter steadily increased in concentration of starch (*cf.* 73). Also the total organic nitrogen, amino acid and determined amide was very much higher in the roots of the trees receiving ammonium as compared to those receiving nitrate. The trees of the cultures lacking an external source of nitrogen were extremely low in concentration of nitrogen and contained little more than traces of the simple soluble forms mentioned.

In tomato plants and in the other herbaceous species under investigation there was, with continuous ammonium nutrition, a comparatively low carbohydrate content in all parts of the plants as compared to others receiving nitrate. The carbon skeleton of a carbohydrate or derivative is clearly essential for the synthesis of any organic form of nitrogen and it is, therefore, not surprising that with relatively rapid synthesis of organic nitrogen rapid consumption of carbohydrates occurred. The assimilation of organic nitrogen involves not only utilization of carbon in the amino acid or protein molecule but involves also marked increase in rate of loss through respiration (*cf. Nitrate Reduction*).

Except in midsummer under nearly ideal light conditions, the carbohydrate reserves of plants continually supplied with ammonium tended to become very low (263, 265, 154). This was associated with a soft, dark green, succulent growth and relatively unfruitful plants. These responses can scarcely be considered to be peculiar to ammonium nutrition for identical responses occurred during winter months in the case of nitrate-supplied plants. They were clearly associated with a condition of carbohydrate deficiency; at least carbohydrates were deficient in the sense that their concentration was inadequate for formation in abundance of the carbo-

hydrate derivatives, lignin and cellulose, the major constituents of the mechanical tissues of plants. Blossoming was seldom entirely eliminated but many of the flowers or developing fruits abscissed, as is commonly the case when plants are deficient in carbohydrates. It may, of course, also occur if plants are deficient in organic nitrogen (*cf.* GROWTH IN RELATION TO AVAILABLE NITRATE).

The cultures concerned were in all cases supplied with a flowing nutrient solution or were given daily or twice daily applications. Under these conditions even a very dilute solution containing ammonium resulted in extremely rapid organic nitrogen synthesis. A very low ammonium content in the plants was apparently adequate for new synthesis of amino acids. This was in contrast to nitrate-supplied tomato plants which apparently required, at least under the experimental conditions obtaining, a very high concentration of nitrate in their tissues. Otherwise, reduction of nitrate to ammonium was seemingly inadequate in amount or rate to maintain sufficient synthesis of organic nitrogen for vigorous vegetative growth (cf. 105).

That these results in general are not peculiar to the location of the experiment or type of solution employed is obvious. Mevius and Engle (63, 130, 131) stress the importance of opportunity for carbohydrate manufacture and note the fact that available ammonium must be less in the winter under unfavorable light conditions than during the summer months. They feel that their analytical data, especially in its relation to seasonal conditions, substantiates the views of Prianischnikov in showing that carbohydrates, or opportunity for their synthesis, is essential for amino acid formation from ammonium and detoxication of ammonia through amide formation.

In connection with depletion of carbohydrates in the tissues of ammonium-supplied plants, the results of Holly *et al.* (82) are interesting. Owing to circumstances which were unavoidable, cotton plants during the later stages of vegetative development received less ammonium than they were capable of metabolizing so that in effect his plants were changed periodically from plus-ammonium to minus-nitrogen culture, to plus-ammonium, etc. Unfortunately, his carbohydrate analyses include only sugars which fluctuate materially with minor changes in environment but they showed a tendency to increase under the conditions of intermittent

ammonium nutrition and there occurred abundant boll development. He emphasizes the fact that with conditions favorable for carbohydrate formation, ammonium was much more rapidly converted into organic nitrogen than was nitrate.

Prianischnikov has repeatedly stressed the importance of carbohydrates in nitrogen nutrition in his work with seedlings and, in a recent paper (197) concerning the ammonium and nitrate nutrition of plants carried to maturity, he suggests that ammonium-supplied plants be occasionally deprived of all external nitrogen supply not because of any factor directly inherent in the nutrient material itself but rather to permit carbohydrates to accumulate.

The writer has frequently used ammonium nutrition of plants in sand culture in the greenhouse in preparing them for other experimental work, solely because more rapid responses can usually be obtained in this manner under conditions of adequate light; but for best results, it is absolutely essential to observe the quality of growth of the plants and when the current growth becomes unduly succulent, which invariably means carbohydrate deficiency, to omit all external sources of nitrogen and thereby permit carbohydrate reserves to become replenished, after which ammonium may be added again. It may be recalled that ammonium, excepting in very acid plants (cf. *Ammonium Storage*), is not stored in quantity, as nitrate often is (cf. *Nitrate Storage*). Accordingly, with ammonium nutrition it is possible to maintain, by observation of the quality of plant growth, a nice adjustment of protein and carbohydrate synthesis, the objective being to avoid a deficiency of either. With nitrate nutrition plants often accumulate such an enormous nitrate reserve in their tissues that under unfavorable light conditions a month or more may be required, with no external nitrogen supply, for disappearance of nitrate through reduction and assimilation.

From another viewpoint the desirability of avoiding carbohydrate deficiency is indicated by an interesting result reported by Beaumont *et al.* (12). They supplied various grasses and clovers with ammonium sulphate under sterile conditions in a complete water culture with no aeration. The tops of the plants were enclosed in clear glass lamp chimneys, the non-sterile controls presumably being likewise enclosed. These conditions are cited not in point of adverse criticism, for ideal environmental conditions could

not easily have been maintained and at the same time have kept the cultures sterile, but because the conditions are highly significant in that the opportunity for carbohydrate synthesis was limited by limited light, and the detoxication of ammonia was presumably seriously impaired through lack of aeration and consequent limited synthesis of amide (*cf.* 26, 111, 135, 235).

Although in addition to nutrient supply the environmental factors mentioned played an important rôle, it is significant that in the unsterilized solutions the roots decayed whereas roots of plants in the sterilized cultures did not decay. They suggest that the decay of roots was due to carbohydrate depletion coupled with accumulation in the roots of unassimilated nitrogen from ammonium sulphate, thus making the root tissue susceptible to the attack of organisms causing decay.

Clark and Shive's (37, 38) work on tomato, although already considered in connection with absorption, is of unusual significance in that the plants were grown in flowing cultures with adequate aeration and careful control of pH values. This technique was also followed even during the periods of the actual absorption tests. These results would indicate that, directly or indirectly, the pH of the nutrient medium limits assimilation rather than absorption, at least within the pH ranges employed (4, 5, 6 and 7). At these several hydrogen ion concentrations there was found in the roots, on a percentage of fresh weight basis, almost exactly the same concentration of nitrate. The ammonium, as recorded, allowing for glutamine hydrolysis that undoubtedly occurred with the analytical technique employed (39, 199, 276), indicates no deficiency of this ion in the roots at any pH value. They conclude that each ion was most rapidly absorbed when most rapidly assimilated. Their further results on analysis are difficult of interpretation as the plants were supplied with both ammonium and nitrate. Later work by Clark (39) on the composition of the tomato plant will be discussed in detail.

Davidson and Shive (47, 48) grew young peach trees in sand culture in two series of treatments, in one of which the cultures received nitrogen only as ammonium, in the other, only as nitrate. The nutrient solutions in both series were applied at pH 4 and 6. Constant renewal of cultures was employed and excellent control of pH obtained. Nitrate was limited exclusively to the roots except

in the pH 6 cultures. The trees of this group absorbed nitrate relatively slowly but it was found in both roots and tops indicating that the pH value of the culture medium limited assimilation rather than ability of the plant to take in the nitrate ion. The ammonium situation was essentially the same as that found by Clark and Shive for tomato (37, 38). The pH value of the solution seemed directly or indirectly to limit assimilation rather than intake of ammonium. Between the two better lots, the trees of the ammonium cultures at pH 6 and the nitrate group at 4, there was practically no difference in volume or quality of growth; it being excellent in both cases. It is of interest that the stems of both groups were very closely similar in percentage of total organic nitrogen and the fractions determined, including cyanogenetic material (cf. 209). The function of the cyanogenetic fraction is not clear and the concentration was low.

On the other hand, the roots, the principal seat of initial organic nitrogen synthesis in peach, were very much higher in elaborated nitrogen in the ammonium supplied trees at pH 6 than in the nitrate group at 4. This was owing mainly to a higher protein content. The pH 4 cultures which received ammonium characteristically made little growth, but they were high, rather than low, in total organic nitrogen. The detailed fractions furnish no explanation. The trees which received nitrate at pH 6 exhibited root and top growth that was not greatly less than that of the ammonium and nitrate series at pH 6 and 4, respectively. The most striking feature was the comparative deficiency of soluble organic nitrogen in the roots of the tree receiving nitrate from the less acid solution which, coupled with the appearance of nitrate in the tops, would seem, as already mentioned, to indicate limited ability to reduce nitrate.

In so far as possible, a technical discussion of methods of chemical analysis of plant tissue has been avoided in this review. However, it seems essential to point out here that the several experiments cited, including those with which the writer has been associated, although they have yielded certain proximate information on the amounts of total organic and protein nitrogen, include serious inaccuracies with respect to the determinations of ammonium and amide (cf. 39, 199, 276, 270). This is owing to the fact that boiling plant extracts at ordinary pressure hydrolyses the amide group of glutamine, resulting in a greatly increased ammonia value that

is, of course, spurious. Moreover, the amide-free residue of glutamine forms pyrrolidone carboxylic acid. This compound, which includes the original amino group of glutamine, yields no amino nitrogen by the usual Van Slyke procedure (36, 276). Nitrate-supplied plants, at least of tomato, contain relatively little glutamine or ammonia and while the fractionation of nitrogenous groups in the boiled extracts of such plants is probably approximately correct, the so-called "combined ammonia," referred to by Tiedjens (265) in the case of his ammonium-supplied plants, was presumably the product of hydrolysis in whole or in part of the amide group of glutamine.

There are unfortunately a considerable number of papers concerning the metabolism of ammonium and nitrate that are based upon analyses of plant tissue dried gradually in ovens at low temperatures such as 45° or 65°. This results in autolysis, as Chibnall has shown (30). Such analytical results do not represent or even remotely approach the conditions actually occurring in the plant. Drying finely minced tissue rapidly, brittle and practically to constant weight in the first two hours, at a temperature of about 80° in a forced draft very largely eliminates autolytic changes in the simpler nitrogenous constituents (272). However, results recently obtained in the writer's laboratory indicate that the amount of insoluble or "protein" nitrogen, at least in some plant material, varies with fresh tissue as compared to tissue dried rapidly at 80°. This is not an argument for or against the use of fresh tissue in determination of the heterogeneous fraction called protein; but the method chosen and the coagulent employed should be considered in comparing the yield of "protein" obtained by the two procedures.

The careful study of analytical technique carried on cooperatively in the laboratories of Vickery and Chibnall (276) has resulted in methods of analysis which, when applied to experimental plant material, have furnished the most complete information yet obtained on the nitrogenous and related organic acid metabolism of ammonium- and nitrate-supplied plants. Clark (39, 275) grew tomato plants in sand culture with continuously renewed complete solutions at pH 6.7. One solution contained only calcium nitrate as a source of nitrogen; another, an equivalent amount of ammonium sulphate; a third contained one-third as much ammonium sulphate. These plants, one month old at the initiation of his ex-

periments, were grown for 49 days with a relatively limited opportunity for carbohydrate synthesis as the glasshouse employed was severely shaded by surrounding trees. This is clearly reflected in the extremely low percentage dry matter in the stems of all the plants of the several cultures. The differences in percentage dry matter are small and it should be borne in mind that soft tomato plants of the type described fluctuate materially in water content (154). The plants were of a soft succulent vegetative type in all cases. The undoubtedly low carbohydrate content of his plants was an influencing factor which should be considered in evaluating the growth and metabolic responses obtained.

However, the fresh weight of the nitrate-supplied cultures was over twice that of the ammonium groups. There was in this respect no significant difference between the cultures receiving the high and low concentrations of ammonium. Probably carbohydrates were less a limiting factor in the nitrate- than in the ammonium-supplied plants (*cf.* 263, 265, 159). There certainly was the usual indication of relatively rapid assimilation of the ammonium ion in that the stems of the plants of the series receiving the high level of ammonium were over twice as high in concentration of soluble organic nitrogen and proportionately low in protein as compared to the nitrate series. With a higher carbohydrate reserve a greater degree of condensation of amino acids to protein would be anticipated. Tiedjens (265) obtained a similar response with tomato. This is not peculiar to ammonium nutrition but is intimately correlated with the concentration of carbohydrates (*cf. Synthesis of Storage Proteins*).

It is probably significant that the roots of the several series were practically the same in percentage content of soluble organic nitrogen. In tomato the initial stages of ammonium and nitrate assimilation take place mainly in the aerial organs of the plant. It will be recalled that peach trees (48) exhibited similar differences in organic nitrogen concentration but it was in the roots, the organs of initial synthesis of amino acids, rather than in the tops where there was little or no difference in quantity or quality of nitrogenous materials.

Clark's determinations of ammonium contained in the plants are probably the most accurate available for tomato and they indicate that this plant contains little ammonium even when abundantly

supplied. Moreover, the concentration is greater in the aerial organs than in the roots. As usual, the plants supplied with nitrate accumulated it in enormous concentrations especially in the stems (*cf. Nitrate Storage*).

As already mentioned, the soluble organic nitrogen was extremely high in the stems of the ammonium series. Within this fraction much larger percentages of glutamine and asparagine nitrogen were found than in the nitrate group. As a result, total amino nitrogen and known soluble organic nitrogen composed large percentages of the soluble organic nitrogen in the plants receiving ammonium. In contrast, the major part of the soluble organic nitrogen in the plants supplied with nitrate was composed of unknown soluble organic nitrogen. It may have included in large part polypeptides or related compounds as none of the determinations made included materials of this general group.

One of the most pronounced contrasts between plants of the ammonium and those of the nitrate series was in the concentration of organic acids in their tissues. The concentrations of the individual acids, oxalic, malic, and citric, were all higher, respectively, in the plants receiving nitrate than in those supplied with ammonium. His data show that both the known and the total acids were much more abundant in the plants of the nitrate than in those of the ammonium cultures. Moreover, the percentage of the total acids composed of the three known acids was much greater in the former than in the latter case in corresponding organs. Unknown acids not only formed a large fraction of the total acids of the ammonium-supplied plants, but also were present actually in relatively large amounts per unit of tissue, except in the roots of the plants that received the higher level of ammonium.

Ruhland and Wetzel (212), it will be recalled, found that in *Begonia* oxalic acid was relatively high when nitrate was supplied, relatively low when ammonium was the external source of nitrogen. Clark suggests that these results, in harmony with his, and the relatively low concentration of total acids may have been correlated with the available carbohydrate reserves which, under comparable conditions, seem to be invariably lower with abundant available ammonium than with nitrate freely supplied. It would be of interest to know the organic acid situation in tomato plants high in carbohydrates that had been grown for some time with no exter-

nal supply of nitrogen. The stems of such plants as frequently observed by the writer commonly exhibit an abundance of calcium oxalate crystals in the mature relatively acid parenchymatous tissues. Schneider (218) also found that calcium oxalate accumulated in nitrogen-deficient plants. Clark suggests the remarkably high asparagine and glutamine content of the plants of the ammonium groups may have been formed at the expense of organic acids, or their precursors, with consequent reduction in the amounts of organic acids in tissues rich in amides.

It is further suggested that the data do not warrant an assertion that the unfavorable effect of a high concentration of ammonium nitrogen in the tissue was due to any "toxic" effect of this ion. The relatively low ash content found to obtain in the ammonium-supplied plants (*cf. Non-Nitrogenous Ions*), coupled with the low concentration of organic acids, is indicated as being a possible causal factor contributing to the lesser growth of the plants of the ammonium groups. The suggestion concerning ash is in harmony with Prianischnikov's (197) work on beneficial effects of added calcium in ammonium nutrition, and the apparent importance of organic acids is not in conflict with the view that carbohydrates are essential for detoxication of ammonia through amide synthesis. Rather, the function of carbohydrates in this connection is seemingly made more specific, suggesting very strongly that the apparent rôle of carbohydrates in amide synthesis is indirect. However, carbohydrates are directly or indirectly necessary for organic acid formation and, as has been frequently shown by many experiments, formation of amide and detoxication of ammonia fail when the carbohydrate reserves of the plant become depleted.

In this connection the importance of asparagine has been especially emphasized in the literature but the work of Greenhill and Chibnall (69) and Vickery *et al.* (278) make it apparent that glutamine should not be assigned a minor rôle. The former workers found that, when perennial rye grass was supplied with ammonium sulphate in conjunction with abundant calcium, a white exudation appeared on the upper half of the blades. The exudate consisted almost entirely of glutamine.

The latter group of workers supplied beets in the open field with frequent applications of ammonium sulphate. Under these circumstances the glutamine content of the root tissue rapidly increased

although the asparagine content was not affected. At the final stage, before definite injury to the plants occurred, a glutamine concentration of 5.4 per cent of the dry weight of the root tissue was attained. There was little effect upon the composition of the tops. Only after severe damage had occurred did the concentration of glutamine in the tops increase (*cf. Nitrate Reduction in Roots*) (49).

These experiments and Clark's (39) show that the quantity and kind of amide present in the plants concerned was directly or indirectly dependent upon the external source of nitrogen. Mothes (135: *472*), in discussing the two amides, remarks that "in Russian sugar beets asparagine occurred in large quantities while in German sugar beets glutamine was found." It is not clear whether he implies a difference owing to variety or that induced by unlike environment. Both tomato and beet synthesized large amounts of glutamine when ammonium was supplied whereas with nitrate nutrition (tomato) amide nitrogen was low. Many examples have been cited of cases where asparagine fulfilled the function of ammonia detoxication (*cf. Detoxication of Ammonia*). Glutamine apparently performed this rôle in tomato, and asparagine in beet appeared in quantity only as the plants approached the stage of definite tissue injury.

In considering their experimental results, Vickery *et al.* (*278*) emphasize the fact that the increase in soluble nitrogen of beet root tissue, on treatment of the soil with ammonium sulphate, was equivalent to the glutamine nitrogen rather than to the glutamine amide nitrogen. Although they feel that the close quantitative agreement in their experiments may have been fortuitous, they emphasize the fact that it was apparent that both nitrogen atoms of glutamine functioned in detoxication of ammonia. Simple dehydration of the ammonium salt of glutamic acid might account, as they point out, for the amide group. It would seem obvious that the precursor of glutamine is a nitrogen-free carbohydrate derivative but the manner of function of the amino group is obscure. An explanation will necessarily await additional experimental evidence.

NITRITE NUTRITION

It has been pointed out that nitrate under usual nutritional conditions is not found in the plant at all, or only in very minute quan-

tity. Certain experiments have been described, however, by means of which the presence of nitrite can be detected in plants supplied with nitrate during a period of rapid reduction, nitrite being a transitory intermediate product in the formation of the end product ammonia (*cf. Nitrate Reduction*). It would seem reasonable, therefore, to expect that nitrite under suitable conditions would be readily absorbed and assimilated by plants. In the earlier literature there are records of occasional tests of sodium nitrite as a source of nitrogen. The quite contradictory results are of little more than historical interest, there being no record of the pH value of the nutrient medium and no provision for maintaining a constant concentration of solutes in the substrate.

One of the first extensive investigations on nitrite nutrition in which reasonably favorable cultural technique was employed was reported by Mevius and Dikussar (54, 132). Without describing all of their earlier work in detail it will be sufficient to summarize the more recently reported results.

Corn was grown in flowing water cultures, all of the cultures being supplied with the usual essential elements. It was found that with sodium nitrite as the sole external source of nitrogen, concentrations of nitrite as high as 200 mg. of nitrogen per liter of nutrient solution, at a pH of 7, could be employed without injury to the plants if light conditions were favorable for rapid CO_2 assimilation. During most of the period of their experiments, however, they found that 50 mg. of nitrite nitrogen per liter gave the most desirable growth responses.

With excessive applications of nitrite under unfavorable light conditions there was a very rapid increase in soluble organic compounds of nitrogen but little condensation of amino acids to protein, and amide nitrogen accumulated. It is apparent that these responses are strikingly similar to those already recorded for low-carbohydrate plants supplied with high concentrations of ammonium. Much as in the case of ammonium-supplied plants, the more acid the nutrient medium the lower the concentration of nitrite had to be to avoid injury to the plants.

In comparison of cultures supplied with nitrate, nitrite and ammonium, respectively, under pH conditions of the culture solution reasonably favorable for each, they observed the usual tendency for nitrate to accumulate in the plants, whereas nitrite, like ammonium,

occurred only in very low concentrations, presumably being rapidly reduced to ammonium with subsequent assimilation at the expense of carbohydrate reserves. This is suggested by the distribution of organic compounds of nitrogen appearing in the plants receiving nitrite. In both roots and tops the highest percentage of amide nitrogen as well as total soluble organic nitrogen was found in the plants of the ammonium series. The nitrite group was only slightly lower and in striking contrast to the values recorded for the nitrate-supplied plants. A relatively high proportion of the elaborated nitrogen of the plants furnished with nitrate was present as protein, and amino acids rather than amide nitrogen predominated.

Fraps and Sterges (66) grew cotton seedlings for 18 days in water culture with a complete nutrient solution containing nitrogen only as nitrate. They used Shive and Stahl's (234) technique of continuous renewal and aeration of the water cultures. At the end of that time, after the roots were washed, some of the plants were shifted for 24 hours to a similar solution containing nitrogen only as sodium nitrite. The initial pH of the solutions was approximately 6 and at the end of the absorption test was slightly less acid, about pH 6.2. There was slightly greater absorption of nitrite than of nitrate nitrogen. It is probable that somewhat more rapid absorption of nitrate would have occurred from a solution at about pH 5 (*cf. The pH of the Nutrient Solution*). They also made the observation that with both sources of nitrogen more than six times as much was absorbed in the 24 hour period as in the first six hour interval, though the time was only four times as long.

Effects of the pH of the culture solution on the utilization of nitrate have already been discussed but the results of Fraps and Sterges (66) are of additional interest in that they afford a basis for comparison with the pH values found most favorable for nitrite nutrition. They found that corn made the greatest growth with nitrate when the nutrient solution had a pH of 3.9 to 4.1, the least growth at pH 6.4 to 7.7, whereas with nitrite the lowest growth was at pH 3.9 to 4.1 and the highest at pH 6.6 to 7.7. A similar response was exhibited by cotton and oats. However, the best growth of the nitrite-supplied plants under the most favorable pH conditions employed, being much the same as those considered optimum by Mevius and Dikussar (54, 132), was much less than that of the most vigorous plants which received nitrate. Judging from their

plant descriptions this may have been associated partly with iron deficiency. The high pH values essential for nitrite nutrition necessarily make it difficult to maintain in the culture medium a source of soluble iron that is adequate for some plants. (210).

Their nitrification work with soils, which may be briefly mentioned, indicates that nitrite is more likely to be produced in alkaline soils than in soils with a lower degree of acidity. Apparently the soils in which nitrites are likely to be produced are those in which they are not likely to be toxic in small amounts.

ABSORPTION OF ORGANIC COMPOUNDS OF NITROGEN

The proteins and related compounds are obviously essential for plant growth. It has been shown that plants can synthesize proteins from inorganic salts of nitrate, nitrite and ammonium. The synthesis to proteins is inadequately understood but, nevertheless, it is clear that the chemical transformation does not take place in one step. Some of the intermediate products in this synthesis are known, however, and have been indicated in the preceding discussions. They include semi-amides, asparagine and glutamine along with a large number of amino acids. The latter especially are formed both in the synthesis of new proteins and in the breaking down of storage proteins through hydrolysis as, for example, in the germination of seeds. It is well known that many of these compounds are found in soils where they are formed through the cleavage of the proteins of decaying organic materials (282, 107, 22).

It seems reasonable to think, therefore, that if the plant absorbs such compounds as amino acids from a soil or nutrient solution, its utilization of them, if in equivalent amounts, must occur just as if they had been synthesized in the plant from nitrate, nitrite or ammonium. The nitrogen of amino acids and related compounds represents a greatly reduced form of nitrogen. As already pointed out, it necessarily follows that their synthesis from inorganic nitrogenous nutrients can occur only following expenditure of energy apparently obtained in large part if not entirely through oxidation of reserve carbohydrates or their derivatives (*cf. Nitrate Reduction*). It would seem, therefore, that the amino acids of the soil, or related compounds, should be a singularly efficient source of nitrogen.

That plants can utilize various compounds of organic nitrogen when supplied externally through the cut ends of stems or petioles has already been indicated. Klein and Linser (104), for example, placed the ends of cut stems of tobacco plants in a nutrient solution containing proline and greatly increased the nicotine content of the plants as compared to the controls. By injection into the hollow stems of growing plants they obtained an increase in the betaines on supplying glutamic acid, ornithine, proline or hexamethylenetetramine (101). Many other results of this type might be cited (*cf.* 18, 19, 140). These results, while showing that plants can metabolize certain compounds of organic nitrogen when supplied externally, can scarcely be considered in the same category as absorption by intact roots.

Some of the earliest work on the absorption of organic compounds of nitrogen was reported by Hutchinson and Miller (83). They grew pea plants in sterile cultures usually with two plants per culture and determined by analyses of the plants whether or not an increase in absolute amount of total nitrogen occurred. Proceeding on this basis, they listed certain organic compounds that were good sources of nitrogen, others that were doubtful or even toxic. Schreiner and Skinner (219), at about the same time, tested the effects of a very large number of compounds of organic nitrogen upon the growth of wheat seedlings, employing compounds they had found to be present in soils. They changed the solutions of their water cultures every three days and although the plants were not grown under strictly sterile conditions great care was taken in that the solutions were changed every three days and the plants shifted to sterilized receptacles at that time. They obtained increased growth of their plants with additions, respectively, of creatinine, creatine, nucleic acid, xanthine, guanine, histidine, arginine and asparagine. In the case of nutrient solutions containing nitrate, added compounds of organic nitrogen increased growth from 11 to 23 per cent as compared to the cultures receiving nitrogen only as nitrate. Analyses of residual solutions showed definite removal of organic compounds of nitrogen from the cultures. In certain cases removal was faster in the presence than in the absence of nitrate.

Unfortunately, the matter of aeration and of the pH of the culture medium was not at that time appreciated but the work of Schreiner and Skinner definitely established the fact that many

organic compounds of nitrogen may be absorbed by intact roots. Whether or not increased growth occurs with additions of amino acids will of course depend in part upon the degree of nitrogen deficiency of the plants being supplied with the material in question; more specifically, probably, with the content of the compound in the plant that is being furnished to it externally. For example, a carbohydrate-deficient plant containing a high concentration of proteolytic products and asparagine would scarcely be expected to absorb asparagine as freely as a plant of the same kind containing less organic nitrogen and an abundant carbohydrate reserve. It should also be remarked that seedlings which have been used for the most part in this type of work are notably high in proteolytic products. Also the use of sterile cultures, although clearly desirable, has tended undoubtedly to accentuate the already high amino acid content of seedlings, owing to the limited light available in the flasks often employed for enclosing the aerial organs. The lack of aeration of the culture medium, as already shown, greatly modifies the external responses and metabolism of most plants.

The intention is not to minimize the value of much careful work with sterile cultures wherein ideal conditions of environment are difficult if not impossible of attainment. It should, nevertheless, be emphasized that under usual field ecological conditions there may well occur much greater utilization of amino acids than the results of present cultural technique indicate.

Some of the work concerning utilization of nitrogenous compounds diffusing from the root nodules of legumes supplies considerable pertinent information. Lipman (109) demonstrated that oats, a non-leguminous plant, absorbed nitrogenous compounds derived from a host legume, in this case the pea plant. Sand culture was employed and the two kinds of plants were separated from each other by an impermeable partition and other similar groups by a permeable partition. The peas exerted a definite beneficial effect on the growth of oats where diffusion between the two plants was possible. The effects were so marked even when the plants were very young that decay of cortical root tissues could scarcely have been a significant contributing factor.

Recently, Virtanen (279, 280) has reported the results of extensive experimental work. In typical experiments his technique has been to grow one leguminous and one oat plant together in flasks

under sterile conditions except for the presence of specific legume nodule bacteria. In some cases the plants were entirely enclosed in glass receptacles; in others, bottles with two or more necks were employed out of which emerged through a cotton plug the tops of the plants which were grown in sand or agar culture. He found, as did Lipman, that legumes with nodules supplied nitrogenous material which permitted growth of oats. He found that the materials diffusing from the root nodules were principally amino acids. Neither ammonium nor nitrate was present. Barley, for example, made excellent growth when its only external source of nitrogen consisted of the organic compounds of nitrogen furnished by the host legume. It is of interest that in other experiments in which barley was grown under sterile conditions independently of legumes, asparagine proved to be one of the best of the organic compounds tested and was apparently absorbed, as was aspartic and glutamic acid, without any material shift in the pH of the nutrient media. Aspartic acid was associated with good growth of various leguminous plants but did not seem to be utilized by barley and certain other grains under the experimental conditions. Employing somewhat similar technique and sterile conditions, Klein apparently found considerable variation in the absorption of amino acids, glycine and alanine seeming in general to be less freely utilized than asparagin, aspartic and glutamic acid (98).

Certain experiments concerning the utilization of urea are of interest. Pirschle (175) found that the roots of plants which had been supplied with this compound in water culture exhibited greater urease activity than roots of similar plants supplied with the usual forms of inorganic nitrogen. Pirschle considers that the cleavage of urea to ammonia may take place in the plant independently of bacterial action. Yamaguchi (298), in studies of the responses of corn seedlings to urea supplied in sterile cultures, concluded that urea may be absorbed as such. He found no ammonium in his cultures but records an initial pH value of the nutrient medium of 4.6 which shifted to 5.2 to 5.5 and then again became more acid, 3.6 to 3.8. In Pirschle's experiments the change in pH was towards increased alkalinity. Yamaguchi's analytical technique (Xanthydrol method) would not, however, distinguish between urea and ureides. He reported that urea (ureide?) was present in the aerial organs and in the guttation water of the leaves.

Klein and Taubock (102, 103), in experiments with corn and beans in sterile culture supplied with arginine, indicate that this amino acid may be absorbed by plant roots but that owing to the presence of arginase in the plants there is rapid decomposition of it to urea and presumably to ammonia. The matter of metabolism of directly absorbed organic compounds of nitrogen remains, however, an almost uninvestigated field of research. It should be an extremely profitable one if considered in relation to any concentration and pH changes of the culture medium in their interrelation to the initial and subsequent nitrogenous and carbohydrate metabolism of the plant. Finally, attention may again be called to the earlier investigations of Schreiner and Skinner wherein a combination of nitrate and organic compounds of nitrogen gave better growth responses than either employed alone. Of course, this may have been a matter of a favorable change in pH of the media brought about by addition of the "physiologically alkaline" nitrate salt to the solution containing an amino acid. Nevertheless, it is of interest that they suggest that if a soil is liberally supplied with all the building units for proteins (amino acids etc.), it is conceivable that good plant growth might result without nitrate. If only a limited amount or assortment of units were present in the soil, then the plant might presumably require nitrate with which to synthesize the lacking units. However, they point out quite reasonably that plant enzymes may be able to transform one or more of these units into other or closely related units.

A few additional references are given that are of interest but merely listing compounds of organic nitrogen that have been reported to have given good, bad or indifferent results means little for reasons already discussed (1, 2, 11, 12, 253, 255).

GROWTH IN RELATION TO AVAILABLE NITRATE

Abundant evidence has been presented showing that ammonium may be absorbed and assimilated by plants with extraordinary rapidity. Although we know little about the direct absorption and utilization of organic compounds of nitrogen it may well be of considerable importance in many fertile agricultural soils. Under usual commercial field conditions the principal source of nitrogen is undoubtedly nitrate, nitrite being but a transitory product. Nitrogenous materials very quickly change to nitrate in most tillable

soils so that no matter what kind of nitrogen-containing fertilizer is applied the plants absorb mainly nitrate (205, 238). There are some exceptions where heavy applications of ammonium sulphate are applied in a localized area about the plant. But where ammonium is found as a more or less permanently present constituent of a given soil, it is usually owing to lack of nitrifying organisms and is correlated with poor aeration or a low pH value of the soil, or both; and these conditions, as already have been shown, practically eliminate the possibility of efficient utilization of ammonium except by certain specialized types of plants (129, 148, 215).

Studies of effects of a lack of available nitrogen have been in continual progress in fertilizer plots at the Rothamsted Experimental Station for nearly 100 years (215). Considerable interest must, therefore, be attached to the responses exhibited by the plants of the several levels of fertilization. The plants lacking adequate nitrogen are described as being stunted in growth and yellowish green. The red pigments of anthocyanins are often conspicuous on the foliage. Apples, when present, are few and limited in size but very highly colored with red for the variety. These characteristics are too well known to require further comment.

Also, the plants are typically stiff and woody when nitrate is low, owing to thick cell walls and the formation of mechanical fibers, sclerenchymatous tissue, etc. These observations have been corroborated by Kraus and Kraybill (105), by Welton (288) and recently by Schneider (218). The leaves of such plants usually have a thick cuticle and epidermis; in short, the effect of lack of nitrogen on mechanical structure is to produce a relatively xeromorphic plant.

The fibrous roots of low-nitrogen plants are notable in that, although of small diameter, they are usually very extensive and branch and rebranch with many fine sublateral rootlets. This again is an observation so frequently recorded in horticultural literature that little more need be said (*cf.* 201, etc.). In this connection, however, may be recalled the striking increase in dry matter yield and in volume of roots recorded by Hamner in the case of the series of wheat plants deprived of nitrate (73).

Additions of nitrate to cereal plants exhibiting such symptoms were said by Russell (215) to have caused a marked and rapid increase in green color and volume of growth. On the other hand,

greater quantities of nitrate led to the development of large, dark green leaves which were often "soft, sappy, and liable to insect and fungous pests". The cuticle and cell walls of the leaves were thin and the development of mechanical tissues was limited, a result which has very frequently been associated with heavy applications of nitrate (105, 218, 288, 155, etc.).

Further effects of a large supply of nitrogen are recorded (215) as having retarded ripening. Plants receiving different amounts of nitrate were thus at different stages of their development at any given time, even though they were all sown on the same day. Those supplied with large quantities of nitrate continued vegetative growth for a longer period. Russell remarks further that seed crops, like barley, that should be cut "dead ripe," should not be supplied with much nitrate, but oats which are cut before being ripe can receive larger quantities. It was said, however, of all the cereal crops, that they produced too much straw if the nitrate supply was excessive and that the straw did not stand up well but was beaten down or "lodged" by wind and rain. Crops such as mangolds and potatoes also produced abundant leaf growth but not proportionately more root or tuber, etc.

In one case it was reported that at the Cheshunt Station the omission of nitrogenous compounds from the fertilizer mixture caused an increase of 11 per cent in yield. Wallace and Sylvester (283) found that lowering the nitrogen content in apples with accompanying increase in sugars by cultural treatments, or ringing, raised the vitamin C content of the fruit as much as 1.5 to 2 times. Obviously, an excess of available nitrate as well as a deficiency is undesirable, deficiency being simply a relative term, for, as Russell (215) indicates, a deficiency of available nitrate for oats might well be an excess for a crop like barley. These or similar results obtained over a long period of years can scarcely be discarded and, furthermore, are in harmony with an enormous amount of horticultural literature.

Without repeating in detail certain interrelations of nitrate assimilation and carbohydrate metabolism, the reader may be reminded that nitrate will not materially effect the growth responses of plants—that there must first occur organic nitrogen synthesis. This involves an endothermic reaction with oxidation of carbohydrates or their derivatives and a greatly increased rate of respiration

(*cf. Nitrate Reduction*). Clearly, new synthesis of organic nitrogen, if *carried to excess*, will tend greatly to deplete the carbohydrate reserves of the plant. Correlated with such a deficiency there can scarecly occur strongly developed mechanical elements, for lignins and cellulose are carbohydrate derivatives in their most condensed form. Peaches from trees heavily fertilized with nitrate are usually relatively late in ripening, comparatively acid and low in sugars (156). In extreme cases of over-fertilization with nitrate, although blossoms may appear, they, or their partially developed fruits, absciss. This probably is not owing directly to excessive organic nitrogen synthesis but rather to the resulting deficiency of carbohydrates. Likewise failure of plants to fruit abundantly when no nitrate is available is not due directly to a high carbohydrate reserve, although it is true that at least prior to senescence the high carbohydrate content is frequently associated with immobilization or condensation of proteinaceous materials (*cf.* METABOLISM OF STEMS). In that sense, cleavage and reutilization of protein goes on slowly unless carbohydrates are decreased by shading, pruning, high night temperatures with increased respiration, etc. (105, 154, 158).

The dominant limiting factor in the latter case is unquestionably a lack of available nitrogen; in the former, a lack of carbohydrates. This, in brief and in the opinion of the writer, is the essence of the relationships discussed by Kraus and Kraybill (105) in their studies of vegetative and sexual reproduction in tomato. There are, of course, all degrees of carbohydrate and organic nitrogen deficiency and an optimal status wherein, for the growth response desired, there is no deficiency of either, as indicated in Kraus and Kraybill's four classes. However, if the varied functions of carbohydrates and the many forms of nitrogen found in the plant are considered, it seems highly improbable that a ratio of significance can ever be obtained by dividing the total carbohydrate content of a plant by its nitrogen content. It is certain that Kraus and Kraybill never intended to make this and similar fantastic claims. Yet in practical agriculture the nitrogen nutrition of plants must be considered in relation to opportunity for carbohydrate synthesis in order to avoid a deficiency of either plant constituent.

There is an enormous amount of literature on the carbohydrate and nitrogen content of plants in relation to growth responses, much

of which is very contradictory. Aside from translocation phenomena and the fact that specialized organs have often been indiscriminantly included with all other parts of the plant making an heterogeneous sample of dubious significance, the biggest source of error lies in the fact that it has not yet been possible to express results on the basis of "protoplasmic mass," as do the animal physiologists. This has recently been brought to the attention of the writer in working with the pineapple plant. Often in a plant in which all the potential starch-storing cells were filled to capacity, macroanalysis, as expressed on the basis of percentage of green or dry material, indicated little starch. The reason was obvious: the plant contained an enormously high proportion of inert mechanical fibers, and analyses of expressed juice, although of interest, naturally did not include starch, the major carbohydrate reserve. Another plant type, relatively soft and succulent and with limited fibers, was by microscopic examination very low in starch, yet on a percentage of dry or green weight basis relatively more starch was indicated than in the case of the plant filled to capacity with this material. Of course, the absolute amount of starch or other material could be easily computed but concentration or quality of protoplasmic materials, not absolute amounts, is associated with quality of growth. Absolute amounts are correlated with size, not quality of plant.

Very recently it has been discovered that unsaturated compounds such as acetylene properly employed can induce sexual differentiation of floral organs almost at will (91). The carbohydrate and organic nitrogen content of the plant can vary within very wide limits and yet on treatment with acetylene differentiation occurs. The writer has recently had an opportunity to participate in experimental work involving the use of acetylene. As might be anticipated, the nitrogen (and CO_2 nutrition) of the acetylene-treated plants still remains as critical a factor as in the case of plants which differentiated without the acetylene stimulus. Fruits of high quality, of desirable texture, containing abundant sugar were produced only on plants containing abundant carbohydrate and proteinaceous reserves in their vegetative organs. Although the nitrogen and CO_2 nutrition of plants is apparently only an attendant factor in relation to sexual reproduction, the fact remains that years of agricultural research show beyond doubt that a serious deficiency

of either organic nitrogen or carbohydrates in the plant is often associated with failure to produce flowers, or, if flowers appear, fruits are abortive and absciss prematurely (*cf.* 28, 67).

Nitrogen nutrition may also greatly modify the quality of fruit even though neither carbohydrates nor organic nitrogen are low enough to prevent their eventual maturation (156). Bishop and Russell (16, 17, 216), in summing up the results of investigations of barley, show that no matter where a given variety was grown nor what the fertilizer treatment—and there were included different sources and amounts of nitrogen—there was, over a period of 10 years, a consistent correlation between the concentration of the individual proteins and the total protein nitrogen and carbohydrate content of the grain. A relatively low percentage of total protein nitrogen (high percentage of carbohydrates) in the grain was associated with a high proportion of salt soluble nitrogen and low concentration of hordein nitrogen. Conversely, when total protein nitrogen was high (and carbohydrates were low) in the grain, there was proportionately less salt soluble nitrogen and a high percentage of hordein nitrogen. The proportion of glutenin was practically constant (*cf.* 89). Although we know little about the factors directly responsible for sexual reproduction, it is obvious that nitrogen nutrition plays a dominant rôle in determining the responses obtained. Certain ecological factors are discussed in the following pages in their relationship to nitrate nutrition.

EFFECTS OF TEMPERATURE ON NITRATE NUTRITION

The term nitrogen deficiency, so commonly employed, is not strictly applicable in many cases to plants which are low in content of assimilated or organic nitrogen. They may exhibit all the symptoms so characteristic of plants commonly included in this category yet may contain very high concentrations of nitrate. Nitrate, as already pointed out, is a potential source of organic nitrogen that may be stored in many plants but it is effective only following reduction and assimilation, not before. In recent work with pineapple plants at comparatively high elevations, where during the winter months the temperatures became relatively low, the plants exhibited all the typical symptoms of "nitrogen deficiency" yet contained in the massive stump or stem as much and sometimes more nitrate than organic nitrogen. Clearly, the external supply of nitrate was ade-

quate and it was equally apparent that absorption and translocation were not limiting factors.

Similarly in tomato plants grown in sand culture at a temperature of 13° C. nitrate was absorbed instantaneously, in about five hours was present in high concentration throughout the plant and remained high; but nitrate was reduced and synthesized to organic nitrogen very slowly. The plants accumulated carbohydrates, especially starch, in large quantities and in appearance were almost identical with other plants that were grown with no external nitrogen supply but a temperature of 21° C. Rosa (211) found a similar response in tomato, and Werner (290) in potato, the external expression of carbohydrate deposition being in greatly increased tuberization in the latter case. Platenius (179), during periods of low temperature, found that carbohydrates and nitrate accumulated in celery. Robbins *et al.* (207) report much the same situation in cauliflower and many more similar cases may be found in the literature.

Carbohydrate accumulation is undoubtedly the result of decreased utilization of carbohydrates in organic nitrogen synthesis but also, as was found in peach trees, with a greatly reduced respiration rate at low temperature yet a remarkably high plane of CO_2 assimilation (161). High temperatures (35° C.), on the contrary, were associated with nearly negative CO_2 exchange in peach under the same light conditions that resulted in carbohydrate increase at low temperature. Vigorous nitrate assimilation and rapid respiration continued until carbohydrates became depleted and protoplasm seriously injured. Associated with carbohydrate deficiency, the plants at high temperature, as might be anticipated, were weakly vegetative, soft and succulent, and exhibited all the symptoms so characteristic of plants lacking an adequate nitrogen-free reserve. The seat of nitrate reduction and initial amino acid synthesis in the fruit trees mentioned was limited as usual to the fine rootlets but metabolism was apparently similar to that already recorded.

EFFECTS OF DAY-LENGTH ON NITRATE NUTRITION

In conclusion, the much discussed problem of the effects of length of day and night may be mentioned in its relation to nitrate nutrition. The subject has been adequately reviewed by Burkholder (25) and the writer is heartily in accord with the view expressed. As he points out, the direct causal factors associated with floral

differentiation remain to be determined. The most extensive investigations under the most carefully controlled conditions of environment have been conducted by Arthur *et al.* (4). They list the responses of a large number of plants but in their chemical analyses unfortunately did not determine nitrate which under short day conditions may accumulate in very high concentrations and in some plants in amounts equal to or greater than their content of organic nitrogen (60, 77, 152, 157, 304). Nitrate accumulation is not directly associated with cessation of vegetative growth—that occurs in many plants with initiation of flowering—for in Biloxi soy bean plants nitrate accumulated even though the day-length was such (6 hours) as to entirely prevent any sexual response (157). The high nitrate content is the result of inability on the part of the plant to reduce and assimilate the contained nitrate. Accompanying this response, carbohydrates, especially starch, usually accumulate in very high concentration unless the long nights are warm, when a decrease in carbohydrates occurs presumably because of a relatively high rate of respiration (*cf.* 290).

It may also be remarked here that amino acids and amide nitrogen were made to accumulate in short-day Biloxi soy bean plants by shifting the plants to continual darkness for several days—a typical proteolytic response (*cf.* SYNTHESIS AND HYDROLYSIS OF STORAGE PROTEIN). Following the period of darkness, some of the plants were returned to short-day conditions and yet even with eventual increase in carbohydrates condensation of amino acids to proteins was limited and there was no significant change in concentration of amide nitrogen. Apparently the short days (long nights) directly or indirectly limited not only the initial reduction and assimilation of nitrate but the later stages of synthesis to protein. Under variable conditions of temperature, including especially high night temperature, amino acids might, therefore, accumulate in short-day plants as the result of cleavage of stored proteins rather than through new synthesis from nitrate (unpublished results by writer).

Arthur *et al.* (4), in considering the interpretation of their plant analyses, segregated plants into groups on the basis of whether or not any flowering response was exhibited. Fruiting of tomato plants was "taken to mean the setting and continued growth of three or four fruits per plant" and, of course, in the same class were included other plants with many fruits that must have been vastly

different in quality of growth. They divided the total carbohydrate content of their plants by the total nitrogen content and the quotient, or "C/N ratio," as might be anticipated, showed no correlation with anything.

Other factors than the supply of carbohydrates and organic nitrogen in the plant obviously influence sexual differentiation, as is shown by the effects of acetylene already mentioned, but, nevertheless, there seems to be under many circumstances an intimate association of the direct causal factors for flowering with the proteinaceous and carbohydrate reserves of the plant, even though many different combinations of ecological factors contribute to their synthesis. Some plants at least, as strains of *Salvia* that ordinarily blossom only during a short photoperiod, can be made to bloom profusely under long-day conditions simply by limiting the external nitrogen supply and thereby permitting carbohydrates to accumulate (152). Later experiments have shown also that *Salvia* blossomed little or not at all under short-day conditions if a state of carbohydrate deficiency was maintained by shading or effected by the use of high night temperatures, the latter result presumably being due to high respiration rate during the long night. Borodina (20) reported that the external nitrogen supply influenced seeding of barley, and by limiting nitrogenous nutrients she induced flowering at a day length usually associated with vegetative growth. Any number of references might be cited showing that short-day conditions favor the formation of carbohydrate storage organs. Zimmerman and Hitchcock (304) found that heavy root storage in 6 varieties of dahlia was correlated with a short day, and nitrate accumulated in the leaves and stems of the short-day plants but was absent or present in only small amounts in the long-day plants. Maximov (127) obtained tuber formation in *Solanum demissum* in a short day but not in a long day. Tincker (266) observed carbohydrate accumulation under short-day conditions as did Moshkov (134). The former author suggests that carbohydrates accumulate because the plants blossom. This is not always the case, however, for if the day length is extremely short no blossoms appear on Biloxi soy beans yet carbohydrates accumulate in high concentration (152, 157, 60). Werner (290) goes a step further, emphasizing that short days, although giving in general the influences recorded, can give the opposite response with respect to carbohydrates if

high temperatures are employed. The rate of nitrate reduction and assimilation may be greatly modified also according to the osmotic concentration of the nutrient medium. With a high salt concentration the sweet pea plant absorbed nitrate freely but under such conditions the intake of water was greatly limited (*cf.* 128). This was associated with early maturation of cells and a greatly decreased rate of nitrate assimilation owing to the fact that only comparatively young cells containing abundant protoplasm were capable of active nitrate assimilation (164). Thus, carbohydrates were made to accumulate and a deficiency of them avoided to a considerable extent, even with unfavorable light conditions and the presence of abundant nitrate in the plant.

The organic nitrogenous and carbohydrate constituents of the plants can scarcely be considered to be the immediate cause of flowering (25, 105, 113, 144, 15, 8), but it is clear that intelligent steps may be employed in nitrogen nutrition to decrease or increase vegetative growth, and to hasten, delay or entirely eliminate flowering responses under many different conditions of environment.

Literature Cited

1. Addoms, Ruth M., and Mounce, F. C. Notes on the nutrient requirements and the histology of the cranberry (*Vaccinium macrocarpon* Ait.) with special reference to mycorrhiza. Plant Physiol. **6**: 653–668. 1931.
2. ————, ————. Further notes on the nutrient requirements and the histology of the cranberry, with special reference to the sources of nitrogen. Plant Physiol. **7**: 643–656. 1932.
3. Arrington, L. B., and Shive, J. W. Rates of absorption of ammonium and nitrate nitrogen from culture solutions by ten-day-old tomato seedlings at two pH levels. Soil Sci. **39**: 431–435. 1935.
4. Arthur, J. M., Guthrie, J. D., and Newell, J. M. Some effects of artificial climates on the growth and chemical composition of plants. Amer. Jour. Bot. **17**: 416–482. 1930.
5. Artschwager, E. F. Anatomy of the potato plant, with special reference to the ontogeny of the vascular system. Jour. Agr. Res. **14**: 221–252. 1918.
6. ————. On the anatomy of the sweet potato root, with notes on internal breakdown. Jour. Agr. Res. **27**: 157–166. 1924.
7. ————. Anatomy of the vegetative organs of the sugar beet. Jour. Agr. Res. **33**: 143–176. 1926.
8. Austin, Stanley. Effects of exfloration on plant metabolism. Plant Physiol. **10**: 225–243. 1935.
9. Bambacioni, Valeria. Ulteriori osservazioni sul luogo in cui se compie l'organicazione dell'azoto nei vegetali superiori. Ann. Botanica **17**: 4–23. 1926.
10. Barton-Wright, E., and McBain, Alan. Studies in the physiology of the virus diseases of the potato. III. A comparison of the nitrogen metabolism of normal with that of leaf-roll potatoes. Ann. Appl. Biol. **20**: 549–589. 1933.

11. BEAUMONT, A. B., LARSINOS, G. J., PIEKENBROCK, P., AND NELSON, P. R. The assimilation of nitrogen by tobacco. Jour. Agr. Res. **43**: 559–567. 1931.
12. ————, EISENMENGER, W. S., AND MOORE, W. J., JR. Assimilation of fixed nitrogen by grasses and clovers. Jour. Agr. Res. **47**: 495–503. 1933.
13. BENNET-CLARK, T. A., AND WOODRUFF, W. M. Seasonal changes in acidity of rhubarb (*Rheum hybridum*). New Phyt. **34**: 77–91. 1935.
14. BERTEL, R. Ueber Tyrosinabbau in Keimpflanzen. Ber. Deut. Bot. Ges. **20**: 454–463. 1902.
15. BIDDULPH, O. Histological variations in *Cosmos* in relation to photoperiodism. Bot. Gaz. **97**: 139–155. 1935.
16. BISHOP, L. R. The nitrogen content and "quality" of barley. Jour. Inst. Brewing **36**: 352–369. 1930.
17. ————. The proteins of barley during development and storage and in the mature grain. Jour. Inst. Brewing **36**: 336–349. 1930.
18. BJÖRKSTEN, J. Zur Kenntnis der Synthese von Eiweissstoffen und ihrer Bausteine bei höheren Pflanzen. Biochem. Zeits. **225**: 1–80. 1930.
19. ———— und HIMBERG, I. Spielt Ammoniak eine direkte Rolle bei der Eiweisssynthese höherer Pflanzen? Biochem. Zeits. **225**: 441–446. 1930.
20. BORODINA, IRENE N. The influence of nitrogenous and mineral nutrition on the time of heading in barley and millet with different daylight durations (Russian with English summary). Bull. Appl. Bot., Genet., Plant Breed. (Leningrad) **27**: 171–193. 1931.
21. BRENCHLEY, W. E., AND HALL, A. D. The development of the grain of wheat. Jour. Agr. Sci. **3**: 195–217. 1908–1910.
22. BRIGHAM, R. O. Assimilation of organic nitrogen by *Zea mays* and the influence of *Bacillus subtilis* on such assimilation. Soil Sci. **3**: 155–195. 1917.
23. BURGE, W. E., WICKWIRE, G. C., ESTES, A. M., AND WILLIAMS, MAUDE. Stimulating effect of amino acids on sugar metabolism of plant and animal cells. Bot. Gaz. **85**: 344–347. 1928.
24. BURKHART, L. Metabolism of etiolated seedlings as affected by ammonium nutrition. Plant Physiol. **9**: 351–358. 1934.
25. BURKHOLDER, P. R. The rôle of light in the life of plants. Bot. Rev. **2**: 1–52; 97–172. 1936.
26. BUTKEWITSCH, W. Das Ammoniak als Umwandlungsprodukt stickstoffhaltiger Stoffe in höheren Pflanzen. Biochem. Zeits. **16**: 411–452. 1909.
27. CAMPBELL, E. G. Nitrogen content of weeds. Bot. Gaz. **78**: 103–115. 1924.
28. CHANDLER, W. H. Fruit growing. (Boston) 1925.
29. CHIBNALL, A. C. Investigations on the nitrogenous metabolism of the higher plants. II. The distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**: 344–362. 1922.
30. ————. Investigations on the nitrogenous metabolism of the higher plants. III. The effect of low-temperature drying on the distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**: 595–603. 1922.
31. ————. Investigations on the nitrogenous metabolism of the higher plants. V. Diurnal variations in the protein nitrogen of runner bean leaves. Biochem. Jour. **18**: 387–394. 1924.
32. ————. Investigations on the nitrogenous metabolism of the higher plants. VI. The rôle of asparagine in the metabolism of the mature plant. Biochem. Jour. **18**: 395–404. 1924.
33. ————. Leaf cytoplasmic proteins. Jour. Amer. Chem. Soc. **48**: 728–732. 1926.

34. ————, AND GROVER, C. E. A chemical study of leaf cell cytoplasm. I. The soluble proteins. Biochem. Jour. 20: 108–118. 1926.
35. ————, AND MILLER, E. J. Some observations on the distribution of nitrogen in plant extracts that contain a high proportion of nitrate nitrogen. Jour. Biol. Chem. 90: 189–196. 1931.
36. ————, AND WESTALL, R. G. The estimation of glutamine in the presence of asparagine. Biochem. Jour. 26: 122–132. 1932.
37. CLARK, H. E., AND SHIVE, J. W. The influence of the pH of a culture solution on the rates of absorption of ammonium and nitrate nitrogen by the tomato plant. Soil Sci. 37: 203–225. 1934.
38. ————, ————. The influence of the pH of a culture solution on the assimilation of ammonium and nitrate nitrogen by the tomato plant. Soil Sci. 37: 459–476. 1934.
39. ————. Effect of ammonium and of nitrate nitrogen on the composition of the tomato plant. Plant Physiol. 11: 5–24. 1936.
40. CLEMENTS, H. E. Translocation of solutes in plants. Northwest Sci. 8: 9–21. 1934.
41. COMBES, R. Absorption et migrations de l'azote chez les plantes ligneuses. Ann. Physiol. Physicochim. Biol. 3: 333–376. 1927.
42. CONRAD, J. P. Physiological acidity and alkalinity of inorganic nitrogenous compounds in solution cultures. Jour. Amer. Soc. Agron. 26: 364–372. 1934.
43. CULPEPPER, C. W., AND CALDWELL, J. S. Relation of age and of seasonal conditions to composition of root, petiole, and leaf blade in rhubarb. Plant Physiol. 7: 447–479. 1932.
44. CURTIS, O. F. The translocation of solutes in plants. (New York) 1935.
45. DASTUR, R. H., AND MALKANI, T. J. The intake of nitrogen by the rice plant (*Oryza sativa* L.). Indian Jour. Agr. Sci. 3: 157–206. 1933.
46. ————, AND KALYANI, V. V. Hydrogen-ion concentration and the intake of nitrogen by the rice plant. Indian Jour. Agr. Sci. 4: 803–831. 1934.
47. DAVIDSON, O. W., AND SHIVE, J. W. The influence of the hydrogen-ion concentration of the culture solution upon the absorption and assimilation of nitrate and ammonium nitrogen by peach trees grown in sand cultures. Soil Sci. 37: 357–385. 1934.
48. ————, ————. Determination of the nitrogenous fractions in vegetative tissue of the peach. Plant Physiol. 10: 73–92. 1935.
49. DAVIES, W. L. The proteins of green forage plants. II. The proteins of the mangold root. Comparison with the proteins of mangold seed. Jour. Agr. Sci. 16: 293–301. 1926.
50. DELEANO, N. T. Studien über den Atmungsstoffwechsel abgeschnittener Laubblätter. Zeits. Wiss. Bot. 51: 541–592. 1912.
51. DENNY, F. E. Rôle of mother tuber in growth of potato plant. Boyce Thompson Inst. Contr. 2: 77–114. 1929.
52. ————. Changes in leaves during the period preceding frost. Boyce Thompson Inst. Contr. 5: 297–312. 1933.
53. DIKUSSAR, I. G. The physiological significance of ammonium salts in relation to the composition changes of the nutrient solution. (Russian with German Summary.) Lenin. Acad. Agr. Sci., Gedroiz Inst. Fertilizers Agron-Soil Sci. 3: 67–76. 1934.
54. ————. Nitrite als Stickstoffquelle für höhere Pflanzen. Ber. Agr. Vers. Sta. (Moscow) 16: 76–86. 1935.
55. DITTRICH, WERNER. Zur Physiologie des Nitratumsatzes in höheren Pflanzen. (unter besonderer Berücksichtigung der Nitratspeicherung.) Planta (Abt. E. Z. Wiss. Biol.) 12: 69–119. 1931.

56. ECKERSON, SOPHIA H. Microchemical studies in the progressive development of the wheat plant. Wash. Agr. Exp. Sta. Bull. 139. 1917.
57. ————. Protein synthesis by plants. I. Nitrate reduction. Bot. Gaz. 77: 377–390. 1924.
58. ————. Influence of phosphorus deficiency on metabolism of the tomato. Boyce Thompson Inst. Contr. 3: 197–217. 1931.
59. ————. Seasonal distribution of reducase in the various organs of an apple tree. Boyce Thompson Inst. Contr. 3: 405–412. 1931.
60. ————. Conditions affecting nitrate reduction by plants. Boyce Thompson Inst. Contr. 4: 119–130. 1932.
61. EISENMENGER, W. S. The distribution of nitrogen in tobacco when the supplies of nitrogen and of light are varied during the growing period. Jour. Agr. Res. 46: 255–265. 1933.
62. ENGLE, H. Beiträge zur Kenntnis des Stickstoffumsatzes grüner Pflanzen. Planta (Abt. E. Z. Wiss. Biol.) 7: 133–163. 1929.
63. ————. Die Wirkung der Ammoniumsalze in Abhängigkeit von der Wasserstoffionenkonzentration. Zeits. Pflanzenernährung. Dung. u. Dodenk. 16A: 226–233. 1930.
64. EVERINGHAM, E. T., AND PEARSALL, W. H. The effects of tartaric acid and of glucose on the metabolism of vine leaves. Proc. Leeds Phil. Lit. Soc. Sci. Sect. 2: 303–308. 1932.
65. FIFE, J. M., AND FRAMPTON, V. L. The effect of carbon dioxide upon the pH and certain nitrogen fractions of the sugar-beet plant. Jour. Biol. Chem. 109: 643–655. 1935.
66. FRAPS, G. S., AND STERGES, A. J. Availability of nitrous nitrogen to plants. Texas Agr. Exp. Sta. Bull. 515. 1935.
67. GARDNER, N. R., BRADFORD, F. C., AND HOOKER, H. D. The fundamentals of fruit production. (New York.) 1922.
68. GODLEWSKI, E. Ein weiterer Beitrag zur Kenntnis der intramolekularen Atmung der Pflanzen. Bull. Acad. Cracovie, Math.-Naturw. Kl. 115. 1904.
69. GREENHILL, A. W., AND CHIBNALL, A. C. The exudation of glutamine from perennial rye-grass. Biochem. Jour. 28: 1422–1427. 1934.
70. GREGORY, F. G., AND RICHARDS, F. J. Physiological studies in plant nutrition. I. The effect of manurial deficiency on the respiration and assimilation rate in barley. Ann. Bot. 43: 119–161. 1929.
71. GROVER, C. E., AND CHIBNALL, A. C. The enzymic deamidation of asparagine in the higher plants. Biochem. Jour. 21: 857–868. 1927.
72. GRÜNTUCH, R. Untersuchungen über den N-Stoffwechsel unterirdischer Reservestoffbehälter. Planta (Abt. E. Z. Wiss. Biol.) 7: 388–421. 1929.
73. HAMNER, K. C. Effects of nitrogen supply on rates of photosynthesis and respiration in plants. Bot. Gaz. 97: 744–764. 1936.
74. HANSTEEN, B. Ueber Eiweisssynthese in grünen Phanerogamen. Jahrb. Wiss. Bot. 33: 417–486. 1899.
75. HERNDLHOFER, E. Menge und Verteilung von Protein, Cäffein, Mono- und Diamino-Sauren in der Kaffeipflanze und deren monatliche Schwankungen während einer Vegetationsperiode. Tropenpflanzer 36: 279–308. 1933.
76. HIBBARD, A. D. Modification of the Eckerson method for determining nitrate reducase. Plant Physiol. 11: 657–658. 1936.
77. HIBBARD, R. P., AND GRIGSBY, B. H. Relation of light, potassium, and calcium deficiencies to photosynthesis, protein synthesis, and translocation. Mich. Agr. Exp. Sta. Tech. Bull. 141: 1934.
78. HOAGLAND, D. R., AND DAVIS, A. R. The composition of the cell sap of the plant in relation to the absorption of ions. Jour. Gen. Physiol. 5: 629–646. 1923.

79. ————, AND BROYER, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.
80. HOLLEY, K. T., PICKETT, T. A., AND DULIN, T. G. A study of ammonia and nitrate nitrogen for cotton. I. Influence on absorption of other elements. Ga. Agr. Exp. Sta. Bull. 169: 3–14. 1931.
81. ————, DULIN, T. G., AND PICKETT, T. A. A study of ammonia and nitrate nitrogen for cotton. Ga. Agr. Exp. Sta. Bull. 273. 1932.
82. ————, ————, ————. A study of ammonia and nitrate nitrogen for cotton. II. Influence on fruiting and on some organic constituents. Ga. Agr. Exp. Sta. Bull. 182. 1934.
83. HUTCHINSON, H. B., AND MILLER, N. H. J. The direct assimilation of inorganic and organic forms of nitrogen by higher plants. Jour. Agr. Sci. **4**: 282–302. 1912.
84. I'LIN, G. Die Umwandlung des Nicotins beim Reifen der Tabaksamen. Biochem. Zeits. **268**: 253–259. 1933.
85. IVANOVA, V. S. Utilization of ammonia nitrogen by cotton. (Russian with English summary.) Lenin. Acad. Agr. Sci., Gedroiz Inst. Fertilizers Agron-Soil Sci. **3**: 77–103. 1934.
86. ————. Ueber die Bildung von Ammoniak bei Reduktion von Nitraten bei höheren Pflanzen. Ber. Agr. Vers. Sta. (Leningrad) **16**: 27–61. 1935.
87. JODIDI, S. L. Nitrogen metabolism in etiolated corn seedlings. Jour. Agr. Res. **31**: 1149–1164. 1925.
88. ————. Isolation and identification of some organic nitrogenous compounds occurring in etiolated corn seedlings. Jour. Agr. Res. **34**: 649–656. 1927.
89. ————, AND BOSWELL, V. R. Chemical composition and yield of the Alaska pea as influenced by certain fertilizers and by the stage of development. Jour. Agr. Res. **48**: 703–736. 1934.
90. KARMARKAR, D. V. The seasonal cycles of nitrogenous and carbohydrate materials in fruit trees. I. The seasonal cycles of total nitrogen and of soluble nitrogen compounds in the wood, bark, and leaves portions of terminal shoots of apple trees under two cultural systems—grass plus annual spring nitrate and arable without nitrogenous fertilizer. Jour. Pomol. Hort. Sci. **12**: 177–221. 1934.
91. KERNS, K., AND MEHRLICH, F. P. Unsaturated hydrocarbons force flowering in pineapple plants. Science. (In press.)
92. KEYSSNER, E. Der Einfluss der Wasserstoffionenkonzentration in der Nährlosung auf die Reaktion in der Pflanze. Planta **12**: 575–587. 1931.
93. KIESEL, A. The enzymic decomposition of arginine in plants. II. Zeits. Physiol. Chem. **118**: 267–276. 1922.
94. ————, AND TROITZKI. Formation of urease in plants. Zeits. Physiol. Chem. **118**: 247–253. 1922.
95. ————, BELOZERSKII, A., AGATOV, P., BIVSHICH, N., AND PAVLOVA, M. Comparative investigations on organ protein of plants. Zeits. Physiol. Chem. **266**: 73–86. 1934.
96. KLEIN, G., UND TAUBÖCK. Physiologie des Harnstoffs in der höheren Planze. II. Osterreich. Bot. Zeits. **76**: 195–221. 1927.
97. ————, AND STEINER, M. Stickstoffbasen im Eiweissabbau höherer Pflanzen. I. Ammoniak und flüchtlige Amine. Jahrb. Wiss. Bot. **68**: 602–710. 1928.
98. ————. Der Wandel des Stickstoffs in der grünen Pflanze. Ergebn. Agr. Jahrb. Landw. Chem. **2**: 143–158. 1930.
99. ————, TAUBÖCK, K., AND LINSER, H. Harnstoff und Ureide bei den höheren Pflanzen. I. Das Vorkommen von Harnstoff im Pflanzenreich und sein Wandel im Laufe der Vegetationsperiode. Jahrb. Wiss. Bot. **73**: 193–225. 1930.

100. ————, ————. Harnstoff und Ureide bei don höheren Pflanzen. III. Das Vorkommen von Ureiden. Quantitative Bestimmung von freiem und gebundenem Harnstoff. Biochem. Zeits. **241**: 413–459. 1931.
101. ————, AND LINSER, H. Zur Bildung der Betaine und der Alkaloide in der Pflanze. I. Die Bildung von Stachydrin und Trigonellin. Zeits. Physiol. Chem. **209**: 75–96. 1932.
102. ————, AND TAUBÖCK, K. Argininstoffwechsel und Harnstoffgenese bei höheren Pflanzen. Biochem. Zeits. **251**: 10–50. 1932.
103. ————, ————. Argininstoffwechsel und Harnstoffgenese bei höheren Pflanzen. Biochem. Zeits. **255**: 278–286. 1932.
104. ————, AND LINSER, H. Zur Bildung der Betaine und der Alkaloide in der Pflanze. III. Vorversuche zur Bildung von Nikotin. Planta (Abt. E. Z. Wiss. Biol.) **20**: 470–475. 1933.
105. KRAUS, E. J., AND KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
106. KULTZSCHER, M. Die biologische NH_3-Entgiftung in höheren Pflanzen in ihrer Abhängigkeit von der Wasserstoffionen-Konzentration des Zellsaftes. Planta (Abt. E. Z. Wiss. Biol.) **17**: 699–757. 1932.
107. LATHROP, E. C. The organic nitrogen compounds of soils and fertilizers. Jour. Franklin Inst. **183**: 169–206; 303–321; 465–498. 1917.
108. LEMOIGNE, M., MONGUILLON, P., AND DESVEAUX, R. Recherches sur le rôle biologique de l'hydroxylamine. III. Présence de l'hydroxylamine dans les feuilles des végétaux supérieurs. Bull. Soc. Chim. Biol. **18**: 868–876. 1936.
109. LIPMAN, J. G. The associative growth of legumes and non-legumes. N. J. Agr. Exp. Sta. Bull. 253. 1912.
110. LIVINGSTON, B. E. A plan for cooperative research on the salt requirements of representative agricultural plants, prepared for a special committee of the division of biology and agriculture of the Nat. Res. Council. 2nd. Ed. Baltimore. 1919.
111. LOEHWING, W. F. Physiological aspects of the effect of continuous soil aeration on plant growth. Plant Physiol. **9**: 567–583. 1934.
112. LOO, TSUNG-LE. Studies on the absorption of ammonia and nitrate by the roots of *Zea mays* seedlings, in relation to the concentration and the actual acidity of the culture solution. Jour. Fac. Agr. Hokkaido Imp. Univ. **30**: 1–118. 1931.
113. LOOMIS, W. E. Growth-differentiation balance *vs.* carbohydrate-nitrogen ratio. Proc. Amer. Soc. Hort. Sci. **29**: 240–245. 1932.
114. ————. Translocation and growth balance in woody plants. Ann. Bot. **49**: 247–272. 1935.
115. LUBARSKAYA, L. S. N-Stoffwechsel in Zuckerrübenkeimlingen und seine Abhängigkeit von der Ammoniak- und Nitraternährung. Zeits. Pflanzenernährung. u. Bodenk. **28A**: 340–368. 1933.
116. LUNDEGARDH, H. Die Nährstoffaufnahme der Pflanze. Jena. 1932.
117. ————, UND BURSTROM, H. Untersuchungen über die Atmungsvorgänge in Pflanzenwurzeln. Biochem. Zeits. **277**: 223–249. 1935.
118. MCCALLA, A. G. The effect of nitrogen nutrition on the protein and non-protein nitrogen of wheat. Canad. Jour. Res. **9**: 542–570. 1933.
119. MCKIE, PHYLLIS. The nitrogen metabolism of the lupin seedling. Biochem. Jour. **25**: 2181–2188. 1931.
120. MASKELL, E. J., AND MASON, T. G. Studies on the transport of nitrogenous substances in the cotton plant. I. Preliminary observations on the downward transport of nitrogen in the stem. Ann. Bot. **43**: 205–231. 1929.
121. ————, ————. Studies on the transport of nitrogenous substances in the cotton plant. II. Observations on concentration Gradients. Ann. Bot. **43**: 615–652. 1929.

122. ————, ————. Studies on the transport of nitrogenous substances in the cotton plant. IV. The interpretation of the effects of ringing, with special references to the lability of the nitrogen compounds of the bark. Ann. Bot. **44**: 233–267. 1930.
123. ————, ————. Studies on the transport of nitrogenous substances in the cotton plant. V. Movement to the boll. Ann. Bot. **44**: 657–688. 1930.
124. MASON, T. G., AND MASKELL, E. J. Further studies on transport in the cotton plants. II. An autogenetic study of concentrations and vertical gradients. Ann. Bot. **48**: 119–141. 1934.
125. ————, AND PHILLIS, E. Studies on the transport of nitrogenous substances in the cotton plant. VI. Concerning storage in the bark. Ann. Bot. **48**: 315–333. 1934.
126. ————, MASKELL, E. J., AND PHILLIS, E. IV. On the simultaneous movement of solutes in opposite directions through the phloem. Ann. Bot. **50**: 161–174. 1936.
127. MAXIMOV, N. A. Experimentelle Änderungen der Länge der Vegetationsperiode bei den Pflanzen. Biol. Zentrbl. **49**: 513–543. 1929.
128. ————. The plant in relation to water. (London) 1929.
129. METZGER, W. H., AND JANSSEN, G. The relation of sodium nitrate and certain other nitrogen carriers to the development of chlorosis in rice. Jour. Agr. Res. **37**: 589–602. 1929.
130. MEVIUS, W. Die Wirkung der Ammoniumsalze in ihrer Anhängigkeit von der Wasserstoffiionenkonzentration. Planta (Agr. E. Z. Wiss. Biol.) **6**: 379–455. 1928.
131. ————, AND ENGLE, H. Die Wirkung der Ammoniumsalze in ihrer Abhängigkeit von der Wasserstoffionenkonzentration. Planta **9**: 1–83. 1929.
132. ————, AND DIKUSSAR, I. Nitrite als Stickstoffquellen für höhere Pflanzen. Jahrb. Wiss. Bot. **73**: 633–703. 1930.
133. MEYER, A. Eiweissstoffwechsel und Vergilbung der Laubblätter von *Tropaeolum majus*. Flora **11**: 85–127. 1918.
134. MOSHKOV, B. S. Photoperiodicity of certain woody species. (English summary.) Bull. Appl. Bot. Genet. Plant Breed. **23**: 479–510. 1930.
135. MOTHES, K. Ein Beitrag zur Kenntnis des N-Stoffwechsels höherer Pflanzen. Planta **1**: 472–552. 1926.
136. ————. Über den N-Stoffwechsel der Coniferen. Ber. Deut. Bot. Ges. **45**: 472–480. 1927.
137. ————. Physiologische Untersuchungen über das Asparagin und das Arginin in Coniferen. Ein Beitrag zur Theorie der Ammoniakentgiftung im Pflanzlichen Organismus. Planta **7**: 585–649. 1928.
138. ————. Zur Kenntnis des N-Stoffwechsels höherer Pflanzen. Planta **12**: 686–731. 1931.
139. ————. Die natürliche Regulation des pflanzenlichen Eiweissstoffwechsels. Ber. Deut. Bot. Ges. **51** Gen.-Heft. 1: 31–46. 1933.
140. ————. Die Vakuuminfiltration im Ernährungsversuch. (Dargestellt an Untersuchungen über die Assimilation des Ammoniaks). Planta (Arch. Wiss. Bot.) **19**: 117–138. 1933.
141. MULAY, A. S. Seasonal changes in total, soluble, soluble-protein, nonprotein, and insoluble nitrogen in current year's shoots of Bartlett pear. Plant Physiol. **6**: 519–529. 1931.
142. ————. Seasonal changes in the composition of the non-protein nitrogen in the current year's shoots of Bartlett pear. Plant Physiol. **7**: 107–118. 1932.
143. ————. Seasonal changes in the composition of the insoluble nitrogen fraction in the current year's shoots of Bartlett pear. Plant Physiol. **7**: 323–327. 1932.

144. MURNEEK, A. E. Physiology of reproduction in horticultural plants. I. Reproducton and metabolic efficiency in the tomato. Mo. Agr. Exp. Sta. Res. Bull. 90: 1–19. 1926.
145. ————, AND LOGAN, J. C. Autumnal migration of nitrogen and carbohydrates in the apple tree with special reference to leaves. Mo. Agr. Exp. Sta. Res. Bull. 171. 1932.
146. ————. Physiological rôle of asparagine and related substances in nitrogen metabolism of plants. Plant Physiol. 10: 447–464. 1935.
147. NAFTEL, J. A. The absorption of ammonium and nitrate nitrogen by various plants at different stages of growth. Jour. Amer. Soc. Agron. 23: 142–158. 1931.
148. ————. The nitrification of ammonium sulfate as influenced by soil reaction and degree of base saturation. Jour. Amer. Soc. Agron. 23: 175–185. 1931.
149. NEDOKUTSCHAEFF, N. Über die Speicherung der Nitrate in den Pflanzen. Ber. Deut. Bot. Ges. 21: 431–435. 1903.
150. NEWBY, H. S., AND PEARSALL, W. H. Observations on nitrogen metabolism in the leaves of *Vitis* and *Rheum*. Proc. Leeds Phil. Lit. Soc. Sect. 2: 81–85. 1930.
151. NEWTON, W. Metabolism of nitrogen compounds in dormant and non-dormant potato tubers. Jour. Agr. Res. 35: 141–146. 1927.
152. NIGHTINGALE, G. T. The chemical compostion of plants in relation to photo-periodic changes. Wis. Agr. Exp. Sta. Res. Bull. 74. 1927.
153. ————, AND ROBBINS, W. R. Some phases of nitrogen metabolism in *Polyanthus narcissus*. N. J. Agr. Exp. Sta. Bull. 472. 1928.
154. ————, SCHERMERHORN, L. G., AND ROBBINS, W. R. The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. N. J. Agr. Expt. Sta. Bull. 461. 1928.
155. ————, ————. Nitrate assimilation by asparagus in the absence of light. N. J. Agr. Exp. Sta. Bull. 476. 1928.
156. ————, ADDOMS, RUTH M., AND BLAKE, M. A. Development and ripening of peaches as correlated with physical characteristics, chemical composition, and histological structure of the fruit flesh. III. Macrochemistry. N. J. Agr. Exp. Sta. Bull. 494. 1930.
157. ————, SCHERMERHORN, L. G., AND ROBBINS, W. R. Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. N. J. Agr. Exp. Sta. Bull. 499. 1930.
158. ————. Effect of temperature on metabolism in tomato. Bot. Gaz. 95: 35–58. 1933.
159. ————. Ammonium and nitrate nutrition of dormant delicious apple trees at 48° F. Bot. Gaz. 95: 437–452. 1934.
160. ————, AND BLAKE, M. A. Effect of temperature on the growth and composition of Stayman and Baldwin apple trees. N. J. Agr. Exp. Sta. Bull. 566. 1934.
161. ————, ————. Effect of temperature on the growth and metabolism of Elberta peach trees with notes on the growth responses of other varieties. N. J. Agr. Exp. Sta. Bull. 567. 1934.
162. ————, AND MITCHELL, J. W. Effects of humidity on metabolism in tomato and apple. Plant Physiol. 9: 217–236. 1934.
163. ————. Effects of temperature on the growth, anatomy, and metabolism of apple and peach roots. Bot. Gaz. 96: 581–639. 1935.
164. ————, AND FARNHAM, R. B. Effects of nutrient concentration on anatomy, metabolism, and bud abscission of sweet pea. Bot. Gaz. 97: 477–517. 1936.

165. OSBORNE, T. B. The vegetable proteins. 2nd Ed. New York. 1924.
166. PALLADIN, M. W. Recherches sur la correlation entre la respiration des plantes et les substances azotées actives. Rev. Gen. Bot. **8**: 225–248. 1896.
167. PANTANELLI, E. Über Ionenaufnahme. Jahrb. Wiss. Bot. **56**: 689–733. 1915.
168. PARDO, J. H. Ammonium in the nutrition of higher green plants. Quart. Rev. Biol. **10**: 1–31. 1935.
169. PEARSALL, W. H., AND EWING, J. The relation of nitrogen metabolism to plant succulence. Ann. Bot. **43**: 27–34. 1929.
170. ———. The distribution of the insoluble nitrogen in *Beta* leaves of different ages. Jour. Exp. Biol. **8**: 279–285. 1931.
171. PETRIE, A. H. K. The intake of ions by the plant and its relation to the respiration of the roots. Austral. Jour. Exp. Biol. Med. Sci. **11**: 25–34. 1933.
172. PETTIBONE, C. J. V., AND KENNEDY, CORNELIA. Translocation of seed protein reserves in the growing corn seedling. Jour. Biol. Chem. **26**: 519–525. 1916.
173. PINEY, M. Variations qualitatives et quantitatives des substances azotées chez une plante ligneuse au début de la période de végétation. Rev. Gen. Bot. **41**: 65–94. 1929.
174. PIRSCHLE, K. Nitrate und Ammonsalze als Stickstoffquellen fur höhere Pflanzen bei konstanter Wasserstoffionenkonzentration. Planta (Abt. E. Z. Wiss. Biol.) **9**: 84–104. 1929.
175. ———. Zur Assimilation des Harnstoffs durch die höhere Pflanze. Biochem. Zeits. **212**: 466–474. 1929.
176. ———. Nitrate und Ammonsalze als Stickstoffquellen für höhere Pflanzen bei konstanter Wasserstoffionenkonzentration. Ber. Deut. Bot. Ges. **47**: 86–92. 1929.
177. ———. Nitrate und Ammonsalze als Stickstoffquellen für höhere Pflanzen bei konstanter Wasserstoffionenkonzentration. III. Planta **14**: 583–676. 1931.
178. ———. Nitrate und Ammonsalze als Stickstoffquellen für höhere Pflanzen bei konstanter Wasserstoffionenkonzentration. IV. Zeits. Pflanzenernährung. Dung. u. Bodenk. A Wiss. **22**: 51–86. 1931.
179. PLATENIUS, H. Carbohydrate and nitrogen metabolism in the celery plant as related to premature seedling. Cornell Univ. Agr. Exp. Sta. Mem. 140. 1931.
180. PREVOT, P., AND STEWARD, F. C. Salient features of the root system relative to the problem of salt absorption. Plant Physiol. **11**: 509–534. 1936.
181. PRIANISCHNIKOV, D. Eiweisszerfall und Eiweissrückbildung in den Pflanzen. Ber. Deut. Bot. Ges. **17**: 151–155. 1899.
182. ———. Die Ruckbildung der Eiweisstoffe aus deren Zerfallsprodukten. Landw. Vers. Sta. **52**: 347–381. 1899.
183. ———. Zur Frage der Asparaginbildung. Ber. Deut. Bot. Ges. **22**: 35–43. 1904.
184. ———, AND SCHULOV, J. Über die synthetische Asparaginbildung in den Pflanzen. Ber. Deut. Bot. Ges. **28**: 253–264. 1910.
185. ———. La synthèse des corps amidés aux dépens de l'ammoniaque absorbée par les racines. Rev. Gén. Bot. **25**: 5–13. 1913.
186. ———. Das Ammoniak als Anfangs- und Endprodukt des Stickstoffumsatzes in den Pflanzen. Landw. Vers. Sta. **99**: 267–286. 1922.
187. ———. Über den Aufbau and Abbau des Asparagins in den Pflanzen. Ber. Deut. Bot. Ges. **40**: 242–248. 1922.
188. ———. Ammoniak als Alpha und Omega des Stickstoffumsatzes in den Pflanzen. Landw. Vers. Sta. **99**: 267–286. 1922.

189. ————. Asparagin and Harnstoff. Biochem. Zeits. **150**: 407–423. 1924.
190. ————. Sur le rôle de l'asparagine dans les transformations des matières azotées chez les plantes. Rev. Gén. Bot. **36**: 108–122; 159–181. 1924.
191. ————. Über physiologische Aciditat von Ammoniumnitrat. Biochem. Zeits. **182**: 204–214. 1927.
192. ————. Über die Ausscheidung von Ammoniak durch die Pflanzenwurzeln bei Säurevergiftung. Biochem. Zeits. **193**: 211–215. 1928.
193. ————, AND IVANOV, V. Absorption and excretion of ammonia by roots. (Russian with English summary.) Compt. Rend. Acad. Sci. U. S. S. R. 327–331. 1929.
194. ————. Zur Frage nach der Ammoniakernährung von höheren Pflanzen. Biochem. Zeits. **207**: 341–349. 1929.
195. ————, AND IVANOVA, V. S. Formation of ammonia during the reduction of nitrates in higher plants. (Russian with English summary.) Compt. Rend. Acad. Sci. U. S. S. R. 205–209. 1931.
196. ————. Über die äusseren und inneren Bedingungen der Ausnutzung des Ammoniakstickstoffs durch die Pflanzen. Zeits. Pflanzenernährung. Dung. u. Bodenk. A **30**: 38–82. 1933.
197. ————. Über den Einfluss des Entwickelungsstadiums auf die Ausnutzung des Ammoniak und Nitratstickstoffs durch die Pflanzen. Trans. 3rd. Int. Cong. Soil Sci. **1**: 207–209. 1935.
198. PRIESTLY, J. H. Light and growth. I. The effect of brief light exposure upon etiolated plants. New Phyt. **24**: 271–283. 1925.
199. PUCHER, G. W., VICKERY, H. B., AND LEAVENWORTH, C. E. Determination of ammonia and of amide nitrogen in plant tissue. Indus. and Engin. Chem. Anal. Ed. **7**: 152–156. 1935.
200. RAHN, H. Untersuchungen über den N-Stoffwechsel pflanzlicher vegetativer Speicherorgane. Planta (Abt. E. Z. Wiss. Biol.) **18**: 1–51. 1932.
201. REED, MARY E. Growth of tomato cuttings in relation to stored carbohydrate and nitrogenous compounds. Amer. Jour. Bot. **13**: 548–574. 1926.
202. ————. Relation of composition of seed and the effects of light to the growth of seedlings. Amer. Jour. Bot. **16**: 747–769. 1929.
203. ————. Growth of seedlings in light and in darkness in relation to available nitrogen and carbon. Bot. Gaz. **87**: 81–118. 1929.
204. RIBBERT, A. Beiträge zur Frage nach der Wirkung der Ammonsalze in Abhängigkeit von der Wasserstoffionenkonzentration. Planta **12**: 603–634. 1931.
205. RICHARD, H. L., AND CROWTHER, E. M. Studies on calcium cyanamide. V. The utilization of calcium cyanamide in pot culture experiments. Jour. Agr. Sci. **25**: 132–150. 1935.
206. RICHARDS, F. J., AND TEMPLEMAN, W. G. Physiological studies in plant nutrition. IV. Nitrogen metabolism in relation to nutrient deficiency and age in leaves of barley. Ann. Bot. **50**: 367–402. 1936.
207. ROBBINS, W. R., NIGHTINGALE, G. T., AND SCHERMERHORN, L. G. Premature heading of cauliflower as associated with the chemical composition of the plant. N. J. Agr. Exp. Sta. Bull. 509. 1931.
208. ROBINSON, MURIEL E. The protein metabolism of the green plant. New Phyt. **28**: 117–149. 1929.
209. ————. Cyanogenesis in plants. Biol. Rev. & Biol. Proc. Cambridge Phil. Soc. **5**: 126–141. 1930.
210. ROGERS, C. H., AND SHIVE, J. W. Factors affecting the distribution of iron in plants. Plant Physiol. **7**: 227–252. 1932.
211. ROSA, J. T. Investigations on the hardening process in vegetable plants. Mo. Agr. Exp. Sta. Res. Bull. 48: 1–97. 1921.

212. RUHLAND, W., AND WETZEL, K. Zur Physiologie der organischen Säuren in grünen Pflanzen. I. Begonia semperflorens. Planta 1: 558–564. 1926.
213. ———, ———. Zur Physiologie der organischen Säuren in grünen Pflanzen. III. *Rheum Hybridum* Hort. Planta 3: 765–769. 1927.
214. ———, ———. Zur Physiologie der organischen Säuren in grünen Pflanzen. V. Weitere Untersuchungen an *Rheum Hybridum* Hort. Planta (Abt. E. Z. Wiss. Biol.) 7: 503–507. 1929.
215. RUSSELL, SIR E. JOHN. Soil conditions and plant growth. 6th Ed. London. 1932.
216. ———, AND BISHOP, L. R. Investigations on barley. Jour. Inst. Brewing 39: 287–343. 1933.
217. SANI, G. Intorno all'attivita riduttrice delle graminacee; la riduzione del nitrato di calcio per le radici delle graminacee. Atti Accad. Lincei 10: 197–201. 1929.
218. SCHNEIDER, K. Beeinflussung von N-Stoffwechsel und Stengelanatomie durch Ernährung. Zeits. Bot. 29: 545–569. 1936.
219. SCHREINER, O., AND SKINNER, J. J. Nitrogenous soil constituents and their bearing on soil fertility. U. S. Dept. Agr. Bur. Soils Bull. 87. 1917.
220. SCHULZE, E. Ueber Zersetzung und Neubildung von Eiweissstoffen in Lupinenkeimlingen. Landw. Jahrb. 7: 411–444. 1878.
221. ———. Ueber die Bildungsweise des Asparagins und über die Beziehungen der stickstoff freien Stoffe zum Eiweissumsatz in Pflanzenorganismus. Landw. Jahrb. 17: 683–711. 1888.
222. ———. Über das Vorkommen von Arginin in den Wurzeln und Knollen einiger Pflanzen. Landw. Vers. Sta. 46: 451–458. 1896.
223. ———. Ueber die beim Umsatz der Proteïnsstoffe in den Keimpflanzen einiger Coniferen-Arten enstehenden Stickstoffverbindungen. Zeits. Physiol. Chem. 22: 435–448. 1896.
224. ———. Über die Verbreitung des Glutamins in den Pflanzen. Landw. Vers. Sta. 48: 33–55. 1897.
225. ———. Ueber den Umsatz der Eiweissstoffe in der lebenden Pflanze. Zeits. Physiol. Chem. 24: 18–114. 1898; 30: 241–312. 1900.
226. ———. Ueber die Bildungsweise des Asparagins in den Pflanzen. II. Landw. Jahrb. 30: 287–297. 1901.
227. ———, AND CASTORO, N. Beiträge zur Kenntnis der Zusammensetzung und des Stoffwechsels der Keimpflanzen. Zeits. Physiol. Chem. 38: 199–258. 1903.
228. ———. Über den Abbau und den Aufbau organischer Sticksstoffverbindungen in den Pflanzen. Landw. Jahrb. 35: 621–666. 1906.
229. ———, AND SCHUTZ, J. Landw. Vers. Sta. 71: 229– . 1909.
230. ———, AND WINTERSTEIN, E. Studien über die Proteinbildung in reifenden Pflanzensamen. Zeits. Physiol. Chem. 65: 431–476. 1910.
231. ———. Studien über die Proteinbildung in reifenden Pflanzen. Zeits. Physiol. Chem. 71: 31–48. 1911.
232. SCHWABE, G. Über die wirkung der Aminosäuren auf den Säuerstoffverbrauch submerser Gewächse. Protoplasma 16: 397–451. 1932.
233. SESSIONS, A. C., AND SHIVE, J. W. The effect of culture solution on growth and nitrogen fractions of oat plants at different stages of their development. Soil Sci. 35: 355–374. 1933.
234. SHIVE, J. W., AND STAHL, A. L. Constant rates of continuous solution renewal for plants in water cultures. Bot. Gaz. 84: 317–323. 1927.
235. ———. Nitrogen absorption and aeration. N. J. Agr. 16: 2–3. 1934.
236. SMALL, J. Hydrogen-ion concentration in plant cells and tissues. Protoplasma Monographien. Berlin. 1929.

237. SMIRNOV, A. I. Über die Synthese der Säureamide in den Pflanzen bei Ernährung mit Ammoniaksalzen. Biochem. Zeits. **137**: 1–34. 1923.
238. SMOCK, R. M. Some physiological studies with calcium cyanamide and certain of its decomposition products. Ohio Agr. Exp. Sta. Bull. 555. 1935.
239. SMYTH, ELSIE S. The seasonal cycles of nitrogenous and carbohydrate materials in fruit trees. II. The seasonal cycles of alcohol soluble materials and of carbohydrate fractions and lignin in the wood, bark, and leaves portions of terminal shoots of apple trees under two cultural systems—grass plus annual spring nitrate and arable without nitrogenous fertilizer. Jour. Pomol. Hort. Sci. **12**: 249–292. 1934.
240. SOMMER, ANNA L. Reduction of nitrates to nitrites by the expressed juice of higher green plants. Plant Physiol. **11**: 429–436. 1936.
241. SPOEHR, H. A., AND MCGEE, J. M. Studies in plant respiration and photosynthesis. Carnegie Inst. Wash. Pub. 325: 1–98. 1923.
242. STAHL, A. L., AND SHIVE, J. W. Studies on nitrogen absorption from culture solutions. I. Oats. II. Buckwheat. Soil Sci. **35**: 469–483; 375–399. 1933.
243. STIEGER, A. Untersuchungen über die Verbreitung des Asparagins, des Arginines, und der Allantoins in den Pflanzen. Zeits. Physiol. Chem. **86**: 245–269. 1913.
244. STONE, G. E. The relation of light to greenhouse culture. Mass. Agr. Exp. Sta. Bull. 144. 1913.
245. STUART, N. W. Nitrogen and carbohydrate metabolism of young apple trees as affected by excessive applications of sodium nitrate. N. H. Agr. Exp. Sta. Tech. Bull. 50. 1932.
246. ————. Determination of amino nitrogen in plant extracts. Plant Physiol. **10**: 135–148. 1935.
247. ————, AND APPLEMAN, C. O. Nitrogenous metabolism in Irish potatoes during storage. Md. Agr. Exp. Sta. Bull. 372. 1935.
248. SURE, B., AND TOTTINGHAM, W. E. The relation of amide nitrogen to the nitrogen metabolism of the pea plant. Jour. Biol. Chem. **26**: 535–548. 1916.
249. SUSUKI, U. On the formation of asparagine in plants under different conditions. Japanese Imp. Univ. Coll. Agr. Bull. 2: 409–457. 1897.
250. ————. On the formation of asparagin in the metabolism of shoots. Japanese Imp. Univ. Coll. Agr. Bull. 4: 351–356. 1902.
251. SUSUKI, S. A study of the proteolytic changes occurring in the lima bean during germination. Jour. Biol. Chem. **3**: 265–277. 1907.
252. TADOKORO, T., AND ABE, M. Studies on the ripening of rice grains. II. Jour. Fac. Agr. Hokkaido Imp. Univ. **27**: 349–387. 1930.
253. TANAKA, I. Studien über die Ernährung der höheren Pflanzen mit den organischen Verbindungen. Japan. Jour. Bot. **5**: 323–350. 1931.
254. TELLER, G. L. Changes in nitrogen compounds in the wheat grain at different stages of development. Plant Physiol. 10: 499–509. 1935.
255. THELIN, G., AND BEAUMONT, A. B. The effect of some forms of nitrogen on the growth and nitrogen content of wheat and rice plants. Jour. Amer. Soc. Agron. **26**: 1012–1017. 1934.
256. THOMAS, W. The nitrogenous metabolism of *Pyrus malus* L. II. The distribution of nitrogen in the insoluble cytoplasmic proteins. Plant Physiol. **2**: 67–70. 1927.
257. ————. The nitrogenous metabolism of *Pyrus malus* L. III. The partition of nitrogen in the leaves, one- and two-year branch growth and nonbearing spurs throughout a year's cycle. Plant Physiol. **2**: 109–137. 1927.
258. ————. The nitrogenous metabolism of *Pyrus malus* L. IV. The effect of sodium nitrate applications on the total nitrogen and its partition products in the leaves, new and one year branch growth throughout a year's cycle. Plant Physiol. **2**: 245–271. 1927.

259. ————. The seat of formation of amino acids in *Pyrus malus* L. Science **66**: 115–116. 1927.
260. ————. Composition of current and previous season's branch growth in relation to vegetative and reproductive responses in *Pyrus malus* L. Plant Physiol. **7**: 391–445. 1932.
261. ————. The reciprocal effects of nitrogen, phosphorus, and potassium as related to the absorption of these elements by plants. Soil Sci. **33**: 1–20. 1932.
262. ————. Absorption, utilization, and recovery of nitrogen, phosphorus and potassium by apple trees grown in cylinders and subjected to differential treatment with nutrient salts. Jour. Agr. Res. **47**: 565–581. 1933.
263. TIEDJENS, V. A., AND ROBBINS, W. R. The use of ammonia and nitrate nitrogen by certain crop plants. N. J. Agr. Exp. Sta. Bull. 526. 1931.
264. ————, AND BLAKE, M. A. Factors affecting the use of nitrate and ammonium nitrogen by apple trees. N. J. Agr. Exp. Sta. Bull. 547. 1932.
265. ————. Factors affecting assimilation of ammonium and nitrate nitrogen, particularly in tomato and apple. Plant Physiol. **9**: 31–57. 1934.
266. TINCKER, M. A. H. The effect of length of day upon the growth and chemical composition of the tissues of certain economic plants. Ann. Bot. **42**: 101–140. 1928.
267. TRELEASE, S. F., AND TRELEASE, HELEN M. Physiologically balanced culture solutions with stable hydrogen-ion concentration. Science **78**: 438–439. 1933.
268. ————, ————. Changes in hydrogen-ion concentration of culture solutions containing nitrate and ammonium nitrogen. Amer. Jour. Bot. **22**: 520–542. 1935.
269. ULLRICH, H. Die Rolle der Chloroplasten bei der Eiweissbildung in den grünen Pflanzen. Zeits. Bot. **16**: 513–562. 1924.
270. VICKERY, H. B. Some nitrogenous constituents of the alfalfa plant. IV. The betaine fraction. Jour. Biol. Chem. **65**: 81–89. 1925.
271. ————, AND OSBORNE, T. B. A review of hypotheses of the structure of proteins. Physiol. Rev. **8**: 393–446. 1928.
272. ————, AND PUCHER, G. W. Chemical investigations of the tobacco plant. II. The chemical changes that occur during the curing of Connecticut shade-grown tobacco. Conn. Agr. Sta. Bull. 324. 1931.
273. ————, ————. A source of error in the determination of amide nitrogen in plant extracts. Jour. Biol. Chem. **90**: 179–188. 1931.
274. ————, ————, WAKEMAN, A. J., AND LEAVENWORTH, C. S. Chemical investigations of the tobacco plant. Carnegie Inst. Pub. 445. 1933.
275. ————, ————, AND CLARK, H. E. Glutamine in the tomato plant. Science **80**: 459–461. 1934.
276. ————, ————, ————, AND CHIBNALL, A. C., AND WESTALL, R. G. The determination of glutamine in the presence of asparagine. Biochem. Jour. **29**: 2710–2720. 1935.
277. ————, ————, LEAVENWORTH, C. S., WAKEMAN, A. J., AND NOLAN, L. S. Chemical investigations of the tobacco plant. V. Chemical changes that occur during growth. Conn. Agr. Exp. Sta. Bull. 374. 1935.
278. ————, ————, AND CLARK, H. E. Glutamine metabolism of the beet. Plant Physiol. **11**: 413–420. 1936.
279. VIRTANEN, A. I., HAUSEN, S., AND KARSTRÖM, H. Untersuchungen über die Leguminose-Bakterien und Pflanzen. XII. Die Ausnutzung der aus den Wurzelknollchen der Leguminosen herausdiffundierten Stickstoffverbindungen durch Nichtleguminosen. Biochem. Zeits. **258**: 106–117. 1933.

280. ————. Zur Kenntnis der Aminosäuresynthese in der Pflanze. Proc. Int. Bot. Congr. **2**: 275–276. 1935.
281. VLADIMIROW, A. W. Über den Einfluss von Stickstoffhaltigen Düngern auf die Eigenschaften der Zuckerrube. Ber. Agr. Vers. Sta. (Leningrad) **16**: 381–398. 1935.
282. WAKSMAN, S. A. Humus. The Williams & Wilkins Co. Baltimore. 1936.
283. WALLACE, T., AND SYLVESTER, S. Z. The antiscorbutic potency of apples. VI. Biochem. Jour. **27**: 693–698. 1933.
284. WASSILIEFF, N. Eiweissbildung in reifenden Samen. Ber. Deut. Bot. Ges. **26A**: 454–467. 1908.
285. WEBSTER, J. E. Nitrogen changes in stored alcoholic extracts of plant tissues. Plant Physiol. **8**: 166–168. 1933.
286. WEEVERS, TH. The function of xanthine derivatives and alkaloids in the metabolism of the higher plants. 5th Int. Congr. Rept. Proc. Cambridge. pp. 454–458. 1930.
287. ————. The relation between secondary plant products and protein metabolism. Proc. Int. Bot. Congr. **2**: 276–278. 1935.
288. WELTON, F. A. Lodging in oats and wheat. Bot. Gaz. **85**: 121–151. 1928.
289. WENT, F. W. Auxin, the plant growth-hormone. Bot. Rev. **1**: 162–182. 1935.
290. WERNER, H. O. The effect of a controlled nitrogen supply with different temperatures and photoperiods upon the development of the potato plant. Neb. Agr. Exp. Sta. Res. Bull. 75. 1934.
291. WETZEL, K. Zur Physiologie der organischen Säuren in grünen Pflanzen. IV. Zur Entstehung der oxalsaure. Planta **4**: 476–525. 1927.
292. WILLIS, L. G., AND DAVIS, E. A. The toxicity to cotton seedlings of high concentrations of soluble nitrogenous fertilizers. N. C. Agr. Exp. Sta. Tech. Bull. 30. 1928.
293. ————, AND RANKIN, W. H. Free-ammonia injury with concentrated fertilizers. Indus. Engin. Chem. **22**: 1405–1407. 1930.
294. ————, AND PILAND, J. R. Ammonium calcium balance: A concentrated fertilizer problem. Soil Sci. **31**: 5–23. 1931.
295. WOO, M. L. Chemical constituents of *Amaranthus retroflexus*. Bot. Gaz. **68**: 313–344. 1919.
296. WOOD, J. G. The nitrogen metabolism of the leaves of *Atriplex nummularium*. Austral. Jour. Exp. Biol. Med. Sci. **11**: 237–252. 1933.
297. WOODMAN, H. E., AND ENGLEDOW, F. L. A chemical study of the development of the wheat grain. Jour. Agr. Sci. **14**: 563–586. 1924.
298. YAMAGUCHI, S. Studies on the resorption of urea by roots of *Zea mays* seedlings in sterile culture. Jour. Fac. Sci. Hokkaido Imp. Univ. Set. 5. **1**: 37–55. 1930.
299. ZALESKI, W. Zur Keimung der Zwiebel von *Allium cepa* and Eiweissbildung. Ber. Deut. Bot. Ges. **16**: 146–151. 1898.
300. ————. Über die Rolle des Sauerstoffs bei der Eiweissbildung in den Pflanzen. Biochem. Zeits. **23**: 150–152. 1909.
301. ————. Zur Kenntnis der Stoffwechselprozesse in reifenden Samen. Beih. Bot. Centralbl. **27**: 63–82. 1911.
302. ————, AND SHATKIN, W. Untersuchungen über den Eiweissaufbau in den Pflanzen. I. Über den Eiweissaufbau in den Zwiebeln von *Allium cepa*. Biochem. Zeits. **55**: 72–78. 1913.
303. ZELENY, L. The distribution of nitrogen in the seed of *Zea mays* at different stages of maturity. Cereal Chem. **12**: 536–542. 1935.
304. ZIMMERMAN, P. W., AND HITCHCOCK, A. E. Root formation and flowering of dahlia cuttings when subjected to different day lengths. Bot. Gaz. **87**: 1–13. 1929.
305. ZINZADZE, CH. Nutrition artificielle des plantes cultivées. I. Mélanges nutritifs à pH stable. Ann. Agron. Nov.–Dec., 1932: 809–853; Jan.–Feb., 1933: 53–72.