Part I. THE DETERMINATION OF AMINO NITROGEN IN PLANT EXTRACTS

Part II. NITROGENOUS METABOLISM IN TUBERS OF SOLANUM TUBEROSUM L.

By

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PART I

THE DETERMINATION OF AMINO NITROGEN IN PLANT EXTRACTS

Introduction

In nitrogen partition studies it is customary to estimate the amount of nitrogen contained in the alpha-amino groups of amino acids by means of the Van Slyke gasometric or the Sorensen formol titration method. As is generally known, these methods were developed for the analysis of pure amino acids or products of protein hydrolysis as applied particularly to animal tissues. Since plant extracts usually contain water-soluble pigments and buffering substances which interfere with the formol titration, the Van Slyke method is more frequently employed.

While engaged in some nitrogen partition studies with alcoholic extracts of apple leaves (9) the writer observed that the yield of amino nitrogen by the standard Van Slyke procedure was in excess of the total soluble, non-protein nitrogen. This finding has been repeatedly confirmed and the same error noted in varying degrees in several other plant tissues extracted with water or 80% alcohol. The present investigation was undertaken to study the factors responsible for these anomalous results and to develop, if possible, corrective measures.

Materials and Methods

Plant tissues used in this study included: apple leaves, rhubarb petioles and leaves, clover roots and tops, young cabbage, soybean, and sunflower plants, begonia petioles and leaves, tomato plants and potato tubers.

Alcoholic Extraction of Soluble Nitrogenous Substances -

Representative 100 gram samples of tissue were preserved in sufficient hot 95% alcohol to give a final concentration of 80%. The alcohol was filtered off and the residue extracted eight times with 80% alcohol by the decantation method (9).

Water Extraction of Soluble Nitrogenous Substances - Similar samples of fresh tissue were passed through a Nixtamal mill and weighed portions of the pulp were ground in a mortar with nitrogen-free sand and water at a temperature of 25°C. The soluble material was expressed by hand through finely woven cloth. The residue was again ground in the mortar and the process repeated until complete extraction of the soluble, non-protein nitrogenous substances was obtained.

Analytical Procedure

Total Non-Protein Nitrogen - Total nitrogen of the protein-free extracts was determined by the Kjeldahl method. When it was possible to demonstrate the presence of nitrates

by the diphenylamine test the modified method of Pucher, et al. (6) was used.

Alpha-Amino Nitrogen - All amino determinations were made with the Van Slyke micro-apparatus using two or four ml. aliquots. Blank determinations and the proper corrections for temperature and pressure were made regularly. Deamination was allowed to proceed for a period of five minutes and the usual precautions to insure accurate, comparable results were consistently observed. Values reported for amino nitrogen are averages of duplicate determinations which agreed closely.

Difficulty was encountered in obtaining a satisfactory anti-frothing reagent for use during deaminization. Several samples of caprylic alcohol proved unsatisfactory due to the formation of a large blank. A number of other materials were tested in blank determinations and with plant extracts. Of these, toluol proved the best. When used in 0.25 ml. portions the troublesome frothing was largely suppressed.

Experimental Results

Substances in Plant Extracts Causing Errors in the Van Slyke Method.

Ammonia - It is well known that ammonia reacts with nitrous acid in the amino determination liberating free nitrogen. Van Slyke reported (13) that 36.3% of the ammonia contained in an M/5 solution of ammonium sulphate was liberated

in five minutes by nitrous acid in the macro-apparatus. It seemed desirable to repeat this experiment under the conditions encountered in this study. Solutions containing 1.0, 0.5, 0.25 and 0.125 mg. of ammonia per ml. were prepared from ammonium sulphate and four ml. aliquots analyzed in the micro-apparatus. It was established that the yield of nitrogen was a linear function of the concentration of ammonia and approximately constant under a given set of conditions. Thus, in one set of experiments conducted at a temperature of 30°C. and barometric pressure of 765 mm. an average of 35.2% of the ammonia was obtained as amino nitrogen following a five minute deaminization period. This percentage recovery was found to vary from time to time being influenced, apparently, by temperature, time and rate of deaminization, etc. Accordingly it is doubtful if attempts to apply a corrective factor for the ammonia present in solutions analyzed will prove uniformily satisfactory. Hence the removal of free ammonia, if present in appreciable quantities, is necessary for accurate amino determination.

It is generally considered that only small amounts of ammonia are found in plant tissues. However, in certain acid plants, as the begonia and rhubarb, ammonia may constitute a large proportion of the total soluble, non-protein nitrogen. Obviously any amino determinations made on these extracts would be greatly in error if the ammonia were not previously removed. It is of interest to note that Culpepper and Caldwell (3) who have recently studied the nitrogen metabolism of rhubarb in some detail, encountered difficulty in interpreting the results

of their amino determinations. These investigators attributed the irregularities and fluctuations of their data to the effect of preservation and storage of the tissue in alcohol. While this is undoubtedly a factor the ammonia content of the tissues, which apparently was not considered, would vitiate the results of the amino determinations if the rhubarb used in the present study was at all comparable.

Frequently plants grown under disturbed nutritional conditions are used for analytical studies. Under such conditions ammonia accumulation often occurs as a result of a breakdown in the nitrogen metabolism. Ammonia may also increase in stored alcoholic extracts of plant tissues, according to Webster (15). For these reasons some investigators as Thomas (11), Chibnall (2), Nightingale (4), and others, have employed methods whereby the ammonia is removed prior to the amino determination. This may be accomplished by a preliminary distillation to remove the free ammonia. In some instances the amide linkages are hydrolyzed and the total ammonia is then removed prior to making the amino determinations. This procedure measures all of the free alpha-amino groups but some hydrolysis of peptide linkages may have occurred during the amide hydrolysis. Frequently the filtrate from the phosphotungstic acid precipitate of basic nitrogen is analyzed for amino nitrogen. This filtrate contains only the mono-amino acids and simple peptides.

Derivatives of the Phenols - Investigation disclosed that the di- and tri-hydric phenols and their acid derivatives

as the tannins, were readily oxidized in the Van Slyke determination with the formation of gases difficully soluble in the alkaline permanganate. The amount of this gas decreased somewhat upon long continued contact with the permanganate. The amount of gas formed seemed to be correlated with the number and position of the hydroxyl groups present in the compound. Thus, the tri-hydric phenol, phloroglucinol, yields considerably more gas than the di-hydric resorcinol. When the hydroxyl groups are present in the ortho position as in catechol or pyrogallol much less of the insoluble gas is produced. Intermediate values were obtained for hydroquinone which has the para grouping of the hydroxyls. Ordinary phenol has little effect. It would therefore be expected that the compounds which occur in plants as esters and glucosides would react with nitrous acid according to the type of linkage, number of free hydroxyl groups, orientation of these groups, and probably other factors which are still obscure.

The tannins which may be regarded as phenol acids, or glucosides of these acids, are widely distributed in plant tissues. Pure tannic acid (Mercks) reacts readily with nitrous acid, forming a gas which would be measured as nitrogen in the amino determination. Extracts of oak galls known to contain large quantities of tannin were also found to react in the same manner. Many other so-called secondary plant substances are known to contain phenolic groups. Such a compound, for instance, is the glucoside phlorhizin in which

phloroglucinol occurs as an ester. This material is known to be present in apple tissue in large quantities. Pure phlorhizin was found to react in the amino determination with the formation of small quantities of insoluble gas.

Miscellaneous Substances - A further source of error lies in the compounds extracted with 80% alcohol but insoluble in water. When these compounds, consisting of pigments, lipides and other material of indefinite composition, are suspended in water and introduced into the Van Slyke apparatus, a variable amount of gas insoluble in the alkaline permanganate is obtained. It is, however, a simple matter to remove these materials by the addition of a small amount of toluol or chloroform to the concentrated water suspension remaining after the removal of the alcohol. The writer prefers chloroform since it sinks to the bottom of the flask, carrying the lipides with it and permitting the clear supernatant extract to be withdrawn. The possibility must be kept in mind that other chemical groups may react with nitrous acid reducing it to nitrogen gas or liberating other insoluble gases. Tangible evidence for this suggestion is afforded by the well known fact that ethyl alcohol and acetone form large quantities of gas when subjected to the conditions of the amino determination.

Procedures to Reduce Errors in the Van Slyke Method When Applied to Plant Extracts

In order to discover the most satisfactory method to reduce errors in the Van Slyke method when applied to plant extracts,

several plant tissues were extracted with water and with 80% alcohol and the extracts subjected to different treatments prior to the determination of amino nitrogen in the extracts. The soluble proteins were removed from the water extracts with colloidal ferric hydroxide as described by Thomas (10). The pigments, lipides, etc. were removed from the alcoholic extracts by adding chloroform to the alcohol-free filtrates. The emulsion was flocculated with magnesium sulphate as previously described by the author (9)[1]. Suitable aliquots of these partially cleared water and alcoholic extracts were then subjected to further treatments.

Precipitation with Neutral Lead Acetate Solution - One lot of aliquots was further cleared with saturated neutral lead acetate. The excess lead was removed with anhydrous sodium oxalate.

Adsorption with Decolorizing Carbon - A second lot of aliquots was stirred at intervals with decolorizing carbon for one hour, filtered, washed, and the filtrates concentrated. The degree of adsorption is admittedly indefinite and probably depends upon the concentration of adsorbent and extract and the time. As far as possible these factors were kept constant.

Adsorption with Calcium Oxide - To a third lot of aliquots sufficient calcium oxide was added to make the extract slightly alkaline. The mixture was stirred at intervals for one hour, filtered, washed, and the filtrate concentrated after making faintly acidic with acetic acid.

1. This method was originally suggested by Dr. T. G. Phillips of the University of New Hampshire.

Distillation with Solid Calcium Oxide - Distillation of a fourth lot of aliquots with solid calcium oxide under reduced pressure for one hour at 40-45°C. was carried out in the usual Van Slyke ammonia apparatus (12). A slight excess of calcium oxide was used as determined by preliminary titration of a small portion of the extract. The extract was filtered from the calcium, washed, acidified with acetic acid and concentrated. The ammonia was collected in 0.02 N. acid and titrated to a methylene blue-methyl red indicator with 0.02 N. base. This method was developed by Plimmer and Rosedale (5) to permit of controlled alkalinity in the determination of ammonia by the Van Slyke method. The same method was later applied to extracts of applewood and leaves by Thomas (11) who noted that this method, besides removing the ammonia, adsorbed the materials which cause frothing during deaminization. The results of the present study have fully confirmed the findings of these investigators and in addition have shown that this distillation results in a decrease in the amount of gas measured as amino nitrogen. The results of these preliminary analyses are shown in Tables 1 and 2.

Table 1 -- Amino nitrogen in water extracts of plant tissues after different treatments of the extracts

Treatment of extracts	Water extracts of 100 gm. of fresh tissue					
	Apple leaves		Tomato plants		Potato tubers	
	Amino N		Amino N		Amino N	
	mg.		mg.		mg.	
Colloidal ferric hydroxide to remove soluble proteins	80.3		38.5		148.0	
Further treatments of protein free extracts						
Neutral lead acetate	34.1		33.1		143.3	
Carbon	30.7		33.3		140.7	
Calcium oxide	33.5		33.4		137.6	
Distillation with calcium oxide	22.4		30.5		123.8	

Table 2 -- Amino nitrogen in 80 per cent alcoholic extracts of plant tissues after different treatments of the alcohol freed extracts

Treatment of extracts	Alcoholic extracts of 100 gm. of fresh tissue					
	Apple leaves		Tomato plants		Potato tubers	
	Amino N		Amino N		Amino N	
	mg.		mg.		mg.	
Chloroform to remove pigments, lipides	44.0		27.7		83.5	
Further treatments of pigments and lipide-free extracts						
Neutral lead acetate	19.5		23.2		78.2	
Carbon	18.5		21.8		76.0	
Calcium oxide	20.7		21.6		78.2	
Distillation with calcium oxide	14.8		20.4		70.2	

The data in Tables 1 and 2 indicate that all treatments resulted in a decrease in amino nitrogen as compared with the direct determination on the extracts which were only partially clarified in removing the alcohol, lipides, protein, etc. This decrease was fairly uniform for a given tissue when treated with the precipitating and absorbing agents but in no instance was it as great as that obtained by distillation. The latter method was accordingly selected for further study. The pronounced difference between the amount of nitrogen extracted by water and alcohol is typical of many analyses and will be discussed in a later section of this paper.

The possibility must be kept in mind that the distillation procedure as previously described may result in a loss of amino acids through occlusion or other means, thereby accounting for a portion of the reduction in yield. Since there are no methods for determining the absolute amount of any plant constituent this question cannot be definitely answered. However, numerous tests with the pure amino acids, leucine and arginine, and the half-amide of aspartic acid, asparagine, resulted in quantitative recovery after the distillation. Pure amino acids could also be quantitatively recovered when added to plant extracts which had been previously distilled with calcium oxide and analyzed for amino content. Furthermore, when the amino acid solutions were added to the partially clarified but undistilled plant extracts, the theoretical value could be obtained by analysis after distillation. Their value was determined by distilling other aliquots of the same extract without addition of amino

acids and noting the decrease in amino value.

Quadruplicate determinations on individual extracts were found to agree closely. Certain extracts were distilled and it was established that no further decrease occurred after the first treatment. On the basis of these studies it is concluded that treatment with calcium oxide has no effect on the amino acids contained in plant extracts. The decrease in yield must then be due to other factors.

The calcium oxide residue remaining after distillation was subjected to study. It was found possible to remove the adsorbed material with acetic acid and after neutralization it could be analyzed directly. Small amounts of gas measured as nitrogen in the Van Slyke determination were obtained which varied with different extracts. The amounts, however, could not account for the magnitude of the reduction in amino nitrogen brought about by distillation. The distillation must have exercised some denaturing effect on the interfering substances. Aside from precipitating or occluding certain materials on the surface of the calcium oxide, an oxidizing effect should be obtained as well since a stream of air is drawn through the suspension. Under such conditions it was observed that tannic acid as well as the tannin from oak galls could be quantitatively precipitated or denatured so that no gas not soluble in alkaline permanganate was liberated in the amino determination. Distillation with calcium oxide was therefore considered to be the best method since its use removes the free ammonia, adsorbs the materials which cause frothing

during deaminization, and precipitates or denatures the tannins.

After this investigation had been completed the work of Rahn (7) came to the writer's attention. This worker noted that when plant extracts were treated with tannic acid to precipitate the soluble protein difficulty was encountered in making the amino determination by the Van Slyke method. The difficulty was attributed to a reduction of nitrogen trioxide, $N_2 O_3$, said to arise in the determination, to elemental nitrogen. It is generally considered that nitric oxide, NO, rather than N_2O_3 arises in the determination. The latter compound is formed only at low temperatures from NO and NO_2 and at room temperature would not exist. Rahn reported that the tannic acid could be satisfactorily removed by allowing the extract to stand one day with a concentrated solution of potassium bichromate. Tannic acid is seldom used as a protein precipitant in this country. However, the widespread occurrence of tannins in plant tissue makes it imperative that their effect be removed before estimation of amino nitrogen. This is particularly important when tissues containing but a small amount of amino nitrogen are analyzed.

Limits of Error in Amino Nitrogen Determination in Different Tissues

In order to gain some information concerning the limits of error in amino nitrogen that may normally occur in tissues frequently analyzed, a number of such tissues were extracted with water and 80% alcohol and the amino content estimated before and after distillation with calcium oxide. The total soluble, non-protein nitrogen was also determined. The results appear in Table 3.

Table 3 -- Total Soluble, Non-Protein, Nitrogen and Amino Nitrogen in various Plant Extracts before and after Distillation with Calcium Oxide. The results are expressed as Milligrams per 100 grams of Tissue, Fresh Weight Basis

Tissue	Method of Extraction	Soluble Non-Protein Nitrogen	Amino nitrogen in plant extracts			
			Before distillation	After distillation	Decrease	Percent decrease
		mgm.	mgm.	mgm.	mgm.	mgm.
Cabbage plants	80% alcohol	23.8	14.3	10.8	3.5	24.5
	Water	51.5	23.6	19.3	4.3	18.2
Clover tops	80% alcohol	75.6	32.8	29.2	3.6	11.0
	Water	133.3	47.9	44.6	3.3	6.9
Clover roots	80% alcohol	117.2	48.7	41.7	7.0	14.4
	Water	170.2	60.7	56.2	4.5	7.4
Soybean plants	80%alcohol	105.7	43.0	39.0	4.0	9.3
	Water	163.0	55.4	52.0	3.4	6.1
Sun-flower plants	80% alcohol.	26.9	6.1	4.6	1.5	24.6
	Water	34.7	10.9	9.5	1.4	12.8
Begonia petioles	80% alcohol	24.3	9.3	1.4	7.9	84.9
	Water	42.9	11.3	3.9	7.4	65.5
Begonia leaves	80% alcohol	15.4	8.6	3.9	4,7	54.7
	Water	32.2	10.6	5.5	5.1	48.1
Rhubarb petioles	80% alcohol	65.0	17.4	3.2	14.2	81.6
	Water	77.8	19.1	6.7	12.4	64.9
Rhubarb leaves	80% alcohol	59.0	24.6	10.6	14.0	56.9
	Water	99.3	30.3	18.1	12.2	40.3

Without exception distillation with calcium oxide reduced the amount of "amino" nitrogen ranging from 6.1% to 84.9% of the values determined before distillation of the extracts with calcium oxide. Distinct tissue differences are evident which must depend upon the amount of non-amino substances free to react in the determination. In most cases good agreement is evident between the decrease in amino value of both water and alcoholic extracts

of the same tissue in spite of the fact that greater amounts of amino nitrogen were invariably removed by the water. Since the size of the aliquot distilled represented a much greater proportion of the total extract in the case of the alcoholic extracts than with the water extracts, the possibility of the decrease being due to occlusion or destruction of the amino acids through errors in the determination seems precluded. The percentage decrease of amino nitrogen was always greater in the case of the alcoholic extracts due to the smaller original amino content.

The analyses reported in Tables 1, 2 and 3 were carried out with freshly extracted tissues. Accordingly it seemed desirable to investigate the behavior of plant extracts which had been subjected to typical laboratory conditions. Tomato plants were grown in a complete nutrient solution and in a solution deficient in phosphorus, during the spring of 1932, the continuous renewal type of water culture being used. The plants were sampled for analysis in June, 1932, using the alcohol preservation method which has been described. Each series of plants was divided into upper and lower stems, upper and lower leaves, and roots. The primary branches were included with the stems, secondary branches and petioles with the leaves. The samples were extracted eight times by the decantation method during October, 1932. The extracts were made to definite volumes, sealed and allowed to stand six months. At the end of this period suitable aliquots were withdrawn and analyzed for amino nitrogen before and after

distillation with calcium oxide. The amount of ammonia present in the extracts was determined by the Sessions and Shive aeration method (8). The results are shown in Table 4.

Table 4 -- Ammonia and Amino Nitrogen in extracts of normal and phosphorus deficient tomato plants. The results are expressed as milligrams per 100 grams of tissue, fresh weight basis.

Tissue	Growth conditions of plants	Ammonia in Extracts	Amino nitrogen in extracts			
			Before distillation with CaO	After distillation with CaO	Decrease	Percent decrease
		mgm.	mgm.	mgm.	mgm.	Percent
Upper leaves	Normal	11.7	20.3	14.1	6.2	30.5
	Phosphorus deficiency	22.6	25.9	16.1	9.8	37.8
Lower leaves	Normal	13.5	20.8	14.2	6.6	31.7
	Phosphorus deficiency	19.3	27.2	19.2	8.0	29.4
Upper stems	Normal	19.4	25.4	18.0	7.4	29.1
	Phosphorus deficiency	29.3	31.4	21.2	10.2	32.5
Lower stems	Normal	29.9	40.9	30.1	10.8	26.4
	Phosphorus deficiency	83.3	65.2	37.2	28.0	42.9
Roots	Normal	13.5	20.7	13.7	7.0	33.8
	Phosphorus deficiency	26.4	26.3	17.1	9.2	35.0

A considerable difference exists between the normal and phosphorus deficient plants in ammonia content. Indeed, the ammonia content of the deficient plants is greater than their amino nitrogen content, which in turn is greater than the amino content of the normal plants. Such a large amount of ammonia would be expected to give erroneous amino values and the data indicate that this is the case, the amount of error ranging from 26.4% to 42.9% of the direct determination. The amino values after distillation are not greatly different in the normal and in the phosphorus deficient plants, whereas the direct determination exhibited much wider differences, particularly in the lower stems. It is obvious that determining the amino nitrogen in the presence of appreciable amounts of ammonia results in an overlapping of the nitrogen fractions. As a result the residual or "other" nitrogen is too low. At the present time investigators assign considerable importance to this fraction in the interpretation of metabolism studies. In the stems and roots the concentration of ammonia is sufficient to account for the decrease in "amino" nitrogen after distillation. In the leaves, however, other substances must have been present which added to the ammonia error.

Effect of Alcoholic Storage on Amino Nitrogen

Recently Webster (14,15) has reported that stored alcoholic extracts of plant tissue usually increase in ammonia and decrease in amino nitrogen. In Webster's work the free ammonia was

apparently not removed from the extract before making the amino determination. Since the ammonia content of the extracts increased, the direct determination of amino nitrogen should show an increase as well, unless the ammonia arose through deaminization of the alpha-amino groups. If the ammonia does increase in this manner then extracts should show even greater decreases after the removal of this ammonia than when the determinations are made without such treatment. Furthermore, the question naturally arises whether or not the observed decrease in amino nitrogen may be due to transformations of the non-amino reacting substances other than ammonia.

Preliminary experiments designed to throw some light on these questions have been carried out with alcoholic extracts of tomato leaves and stems stored in light and darkness. The results of periodic analyses clearly confirm the conclusions of Webster. In one instance with extracts of tomato stems the amino content decreased 35.8% in the dark and 47.3% in the light during a storage period of five months. The ammonia content, estimated before making the amino determinations, steadily increased. The rate of increase in ammonia and decrease of amino nitrogen was most rapid during the first four weeks of storage. The loss of amino nitrogen was far greater than could be accounted for by the increase in ammonia. This decrease must represent an actual change in the amino groups so that they are not free to react with nitrous acid. The mechanism of the change is not clear. It is quite probable that considerable variation

might exist in other tissue extracts. Also the rates might be somewhat different during storage in alcohol before complete extraction. However, the common practice of preserving large numbers of samples which cannot be analyzed for long periods of time must be questioned.

Comparison of Water and 80 per cent Alcohol as Extractives of Soluble Nitrogen

The data contained in Tables 1, 2 and 3 permit a direct comparison to be made between water and 80 per cent alcohol as extractives of soluble nitrogen and amino nitrogen. Without exception extraction with water removed larger amounts of nitrogen than did alcohol. In fact, the magnitude of the differences raises some question as to the value of 80 per cent alcohol as an extractive of the nitrogenous substances. In some instances 50 per cent alcohol proved to be about as effective as water for extraction of the soluble nitrogen. In nitrogen partition experiments with potato tubers it was established that 97 per cent of the non-protein nitrogen removed with water could be extracted from similar samples with 50 per cent alcohol. In such cases the tissue is best preserved in 95 per cent alcohol, by volume. It would seem that this method is deserving of further study and use where large numbers of samples must be handled in a limited period of time.(1)

The writer has frequently found that the excess of total

soluble non-protein nitrogen removed with water over that removed with 80 per cent alcohol is reflected in higher concentrations of all the soluble fractions with the exception of ammonia or other volatile bases measured as ammonia. This difference is usually most pronounced in the basic fraction. The increase is not due to incomplete removal of the soluble protein from the water extracts as preliminary experiments with other protein precipitants, acetic acid, trichloracetic, etc., gave entirely similar results. The possibility of proteolytic enzyme activity was restricted to a minimum since in this work duplicate samples of but a single tissue were taken for extraction at a time, thus permitting prompt treatment of the extracts. It is realized that not all tissues in their fresh state lend themselves to extraction with water. However, it seems desirable whenever possible in nitrogen partition studies to employ fresh tissue and water extraction.

Discussion

In this paper evidence has been presented indicating that the conventional methods for the determination of amino nitrogen in plant extracts may give erroneous results. It was shown that the preliminary distillation with calcium oxide as outlined made possible the determination of amino nitrogen values which more closely approached the true values.

The importance of this treatment depends upon the tissue involved. A comparatively wide range of plant tissues were found to show a decrease in amino nitrogen when distilled with calcium oxide but these data must be regarded as relative rather than absolute. While the composition of any species is doubtless fairly well defined, wide fluctuations in the proportions of the various fractions may occur through differences in physiological age, mineral and organic nutrition, etc. That the amount of amino nitrogen as well as the non-amino substances concerned will be influenced is certain. Aside from the errors involved in the actual amino determination, it is evident that the preservation and extraction of the tissue may greatly influence the results. The necessity of a careful preliminary study of the properties of a tissue before undertaking extensive analytical work must be emphasized.

Summary and Conclusions

Treatment of plant extracts with neutral lead acetate, decolorizing carbon, and solid calcium oxide, invariably resulted in a decrease of gas measured as amino nitrogen by the Van Slyke method. Evidence is presented supporting the validity of the method of low-temperature distillation with solid calcium oxide under reduced pressure which resulted in maximum decrease in all cases.

Acid derivatives of the phenols, as the tannins, were found to react with nitrous acid in the amino determination producing gases measured as nitrogen. This error is eliminated by distillation with calcium oxide. Limits of error caused by the presence of ammonia are discussed.

Reductions in gas measured as amino nitrogen ranging from 6.1 per cent to 84.9 per cent were found in 12 plant tissues extracted with water and 80 per cent alcohol after distillation with calcium oxide. Five plant fractions of normal and phosphorus-deficient tomato plants showed similar decreases of 26.4 per cent to 42.9 per cent after ten months storage in alcohol.

Alcoholic storage of tomato plant extracts was found to result in marked increases in ammonia nitrogen and decreases in alpha-amino nitrogen.

With 9 plant tissues 80 per cent alcohol extracted an average of only 63.7 per cent as much soluble non-protein nitrogen and 66.9 per cent as much alpha amino nitrogen as was removed with distilled water at a temperature of 25°C.

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PART II

Nitrogenous Metabolism in Tubers of Solanum Tuberosum L.

It is well known that potato tubers which have been exposed to temperatures approaching 0° C. become sweet to the taste. Müller-Thurgau (23) reported in 1882 that sugar accumulation occurs in potatoes stored at low temperature. The work of Appleman (2,3,4) extended this observation and showed conclusively that the equilibrium between starch and sugar in the potato tuber is extremely reversible and easily shifted by changes in storage temperature.

The effect of temperature on the nitrogenous metabolism of potato tubers seems to have received little attention. Grüntuch (15) and Rahn (33) have called attention to the fact that tubers, roots, bulbs, etc., contain a large proportion of their total nitrogen in the non-protein form. This condition is well illustrated in the potato tuber where approximately one-half of the total nitrogen is present in the non-protein or crystalloidal form. The situation with respect to nitrogen is, therefore, quite unlike that found in the other food storage organs, the seeds, where maturity is characterized by desiccation and synthesis of protein at the expense of the soluble nitrogen fractions. In the potato tuber a high moisture content coupled with an abundance of non-protein nitrogen and carbohydrate would seem to furnish the essential materials for further biochemical reactions which might involve the nitrogen. The present investigation was undertaken to determine the effect

of temperature on the nitrogenous metabolism of mature potato tubers. It was also a purpose of this study to note the changes in nitrogen distribution within potatoes as they emerged from the rest period and formed sprouts. Studies have also been made of the comparative composition of various areas within potatoes.

Review of Literature

No attempt will be made to review completely the extensive literature dealing with potato composition and storage or with the effect of temperature on plants. Only papers directly related to the present study will be cited.

In connection with the effect of temperature on plant metabolism it should be pointed out that low temperature appears to exert considerable influence on the nitrogen metabolism of growing plants. Subjecting certain plants, as the cabbage, to low temperature in the so-called hardening process has been observed to result in greater cold resistance and better ability to withstand the shock associated with transplanting. Thus Harvey (16) noted that hardening cabbage plants by cold treatment resulted in an increase in amino nitrogen. This was thought to have resulted from hydrolysis of the protein. Thompson (37) reported that exposure of celery plants to cold-frame temperature for 30 days resulted in the same increase in amino nitrogen as was observed by Harvey. The soluble nitrogen, however, decr ased with a corresponding increase in the insoluble fraction. This work suggested that synthesis rather than hydrolysis of protein had taken place. Platenius (30) confirmed Thompson's work with celery but failed to note any effect of temperature on synthesis of amino acids.

An inverse relation between amino and nitrate nitrogen was found.

Walster (41) reported that soluble nitrogen in barley plants increased with a rise in temperature. Tottingham et al.(38) observed the reverse condition in leaves of the sugar beet.

Nightingale and Schermerhorn (27) demonstrated that nitrate assimilation in the asparagus plant occurred rapidly at temper- of 20-30° C., very slowly at 10°C., and probably not at all much below that temperature. Nightingale and Robbins (26) also showed that the roots of the paper-white narcissus appeared to be the chief organs concerned with the transformation of nitrates to organic nitrogen. Recently Nightingale (25) has shown that tomato plants grown in humidity controlled chambers at 55o F., 70° F., and 95° F. absorbed nitrate rapidly but assimilation occurred satisfactorily only at the 70° F. temperature.

It is concluded, therefore, that temperature exerts a pronounced effect on the nitrogen metabolism of growing plants. The exact effect apparently depends upnn the plant, stage of growth, carbohydrate reserve, and probably environmental factors.

Several investigators have made nitrogen analyses of pota to tubers. Osborne and Campbell (28) reported that the proteins of the potato consist of a globulin which they named tuberin and a very small amount of a proteose. Pearsall and Ewing (29) reported the iso-electric range of potato tuberin to lie between pH 4.4 and 4.5.

Appleman (4) concluded that after-ripening in the potato tuber does not involve protein hydrolysis. Increases in the soluble nitrogenous fractions were detected after sprouting

occurred. Appleman and Miller (6) observed that immature potato tubers large enough for seed had practically the same percentage composition at the end of the rest period as similarly stored mature tubers. These writers also point out that hydrolysis of protein is one of the important ripening or maturing processes in potatoes.

Andre (1), working in France, studied the nitrogen distribution in two varieties of potatoes from October until May. The juice was expressed by pressure and analyses made for the total nitrogen, nitrogen which passed a collodion membrane, and nitrogen not coagulated by heat. Each nitrogen fraction was found to increase with the age of the potato.

Cook (9) analyzed skins, sprouts, and tubers of three varieties of Bordeaux sprayed potatoes held at a temperature of 70° F. It was concluded that the age of the sprout influenced its composition more than the variety. Spraying had no effect on the composition or growth of the sprouts. The sprouts showed a selective action in withdrawing from the tubers nitrogen, ash, phosphoric acid, and water in larger proportion than was originally present in the tubers. As the sprouts of Irish Cobbler potatoes constituted 17 per cent of the total weight of the sprouts and tubers while Green Mountain sprouts comprised only 5.5 per cent "..an increased concentration or activity of the growth-promoting agent or agents in the Irish Cobbler tubers is suggested."

Newton (24) found that the absorption of nitrates by potato tubers abbreviated the dormant period while absorption of ammonium salts had no effect. Amino and amide nitrogen were greater in non-dormant than in dormant tubers. Proteolytic enzyme activity was more intense in the expressed juice of the non-dormant tubers.

The rate of accumulation of amino acids from added casein was a straight-line function of the time of incubation. When potato juice was incubated amino nitrogen was converted into amide nitrogen.

Willaman and West (42) analyzed several varieties of potatoes grown under different soil conditions in Minnesota. The early varieties were characterized by lower dry matter content and higher protein. These investigators conclude (43) that the composition of American potato tubers is more affected by environment than by variety. Large and small tubers of the same stage of maturity have the same composition. In a later paper Willaman and Child (44) reported that smaller tubers have a higher nitrogen content than larger ones.

Headden (17) found that the application of 800 pounds of nitrate of soda per acre increased all of the forms of nitrogen compounds in the tubers for which analysis was made. An excess of nitrates radically modified the composition of potato tubers. Goldthwaite (14) made a thorough investigation of the variation in composition of Colorado potatoes. More than 400 potatoes of 11 varieties were analyzed separately for total nitrogen. This investigation disclosed that "no two potatoes having identical composition were found in the same variety, or in the same group or even in the same hill". The range in "nitrogenous matter" was from 1.462 per cent to 2.648 per cent of the fresh weight of the tubers. Nitrogenous matter was obtained by multiplying the total nitrogen by the usual factor 6.25.It was also concluded that the size of a potato was no criterion of its maturity, the

the ones having the longest growing season being most mature.

Coudon and Bussard (10) separated potato tubers into cortical, outer and inner medullary areas. Percentage of water increased from the cortex to the inner medullary area. The total nitrogen varied in the same manner while the starch exhibited a gradient in the reverse direction. Protein nitrogen was, however, richer in the cortex.

Frisbie and Bryant (12),working in the United States, also found the most protein in the cortex of potato tubers of the White Star variety. Otherwise their results were opposed to those of Coudon and Bussard. East (11) separated and analyzed the cortex and medullary area of Rural and Carman potatoes. His data confirmed the findings of Coudon and Bussard. Goldthwait (14) has also obtained similar data.

Glynne and Jackson (13), working in England, determined that in the King Edward variety the dry matter and total nitrogen were inversely correlated from zone to zone, the dry matter being lowest in the medullary area. Both dry weight and total nitrogen of the tissue increased from the terminal to the umbilical end of the tubers.

Chemical Methods

Considerable preliminary investigation was carried out in order to be able to choose the most satisfactory method of analysis for potato tissue.

It was established that the common method of preservation of plant tissue in 80 per cent alcohol is open to serious criti-

cism. The writer has pointed out in Part I of this paper that extraction with 80 per cent alcohol does not result in complete removal of the non-protein nitrogen. An added disadvantage of the alcohol method is that the alpha-amino content of the extract decreases upon storage. Virtually the same amount of non-protein nitrogen could be extracted from potato tubers by the method used by Appleman and Miller (6) as could be removed with water. This method makes use of 50 per cent alcohol as the extractive. However, in the present investigation it was unnecessary to store the samples taken for analysis. Accordingly the method of extracting the fresh tissue with water was used exclusively.

Sampling. The standard sample used for analysis consisted of eight carefully selected tubers weighing approximately 1000 grams. The tubers were washed, dried, passed through a Nixtamal mill, and the pulp thoroughly mixed. Triplicate portions of approximately five grams each were placed between tared watch glasses, weighed, and dried to constant weight in a vacuum equivalent to 30 inches of mercury, at a temperature of 80° C. Duplicate five gram portions of the pulp were also placed in weighing bottles, carefully weighed and transferred to Kjeldahl flasks for determination of total nitrogen. A 100 gram portion of the pulp was taken for extraction of the soluble nitrogenous fractions.

Extraction. The sample of potato pulp contained in a 400 ml. beaker was covered with a 200 ml. of distilled water at a temperature of 25° C., stirred a few minutes and decanted to a square of Huck toweling suspended over a two-liter beaker. After

draining the residue was transferred to a mortar. The pulp was thoroughly triturated with fused quartz sand with the gradual addition of about 200 ml. of distilled water. The material was again transferred to the extraction cloth, allowed to drain and then expressed by hand. The process of extraction was repeated two additional times resulting in complete removal of the non-protein nitrogenous constituents. Most of the extract was decanted to a second beaker. The remaining portion and the washings from the first beaker, containing most of the starch, were centrifuged and added to the extract. The combined extract was heated just to boiling, colloidal ferric hydroxide added, and boiling continued two minutes. The solution was filtered in a Buchner funnel, the beaker and iron gel being thoroughly washed with hot water. The non-protein filtrate was cooled, made to a volume of 1000 ml. and preserved with toluol. No proteins were present in the straw-colored filtrate.

Fractionation of the Non-Protein Nitrogen: Careful semi-quantitative tests with acidified diphenylamine reagent revealed the presence of only traces of nitrate nitrogen in the non-protein filtrate. Determination of total nitrogen was accordingly not modified to include nitrates. No attempt was made to measure the amount of extracted protein. As Chibnall (8) has pointed out the magnitude of the coagulable nitrogen varies with the method of extraction. There is, furthermore, no reason for believing that it differs from the unextracted cytoplasmic proteins.

Total nitrogen. The usual observation that the residue remaining from the moisture determination or a sample of the

fresh pulp could be used with equal accuracy for the determination of total nitrogen was confirmed. Determinations were therefore made on samples of the fresh pulp by the usual Kjeldahl method. Distillation was made into 0.1 N. sulfuric acid. The excess acid was titrated with 0.1 N sodium hydroxide to the combination Methylene Blue-Methyl Red indicator (19). Blank determinations were made regularly.

Total Non-protein Nitrogen: Duplicate 100 ml. aliquots of the non-protein filtrate were analyzed as described for total nitrogen.

Total Alpha-Amino nitrogen: As a result of numerous studies it was established that the determination of alpha-amino nitrogen in the protein-free extracts of potato tubers which had received no treatment other than that with colloidal ferric hydroxide yielded results by the Van Slyke method which were probably not a true measure of alpha-amino nitrogen. Any naturally-occurring, readily-oxidized compound could conceivably affect the determination with the production of gases measured as nitrogen. As a result the observed amino values are too high. A special preliminary procedure to correct this error was adopted. This consisted of removal of the effect of the interfering compounds by low-temperature distillation with calcium oxide under reduced pressure. A 200 ml. aliquot of the extract was transferred to a Claisen flask, sufficient solid calcium oxide added to make the solution slightly alkaline, 50 ml. of 95 per cent alcohol added, the flask evacuated and then immersed in a water-bath maintained at 45° C. Distillation was continued for one hour. This treatment removed the ammonia, adsorbed the materials which cause frothing during deaminization, and removed the effect of compounds like the tannins whose presence

increases the yield of gas measured as nitrogen. The calcium oxide residue was removed by filtration, the filtrate faintly acidified with acetic acid and concentrated in vacuo to a volume of 50 ml. Two ml. aliquots were used for the determination of total alpha-amino nitrogen in the Van Slyke micro-apparatus. Blank determinations and the proper corrections for temperature and pressure were made at each analytical period.

Basic Nitrogen. A 200 ml. aliquot of the non-protein extract was acidified with 2.5 ml. of concentrated sulfuric acid. An excess of freshly prepared phosphotungstic acid solution was added and the precipitate redissolved by heating the solution for two hours on the steam bath. The solution was then cooled and placed in a refrigerator for 24 hours. The phosphotungstate bases were removed by filtration and washed with a cold, dilute, sulfuric acid-phosphotungstic acid solution. The paper and precipitate were transferred to a Kjeldahl flask and thoroughly digested. Distillation was made into 0.02 N standard acid. The distillate was boiled, cooled, and the excess acid titrated with standard 0.02 N base.

Mono-Amino Nitrogen. The filtrate from the basic nitrogen was neutralized with sodium hydroxide and then treated as for the total alpha-amino nitrogen.

Ammonia Nitrogen. Free ammonia was always found to be low in potato tuber extracts regardless of the storage treatment of the tubers. Since this fraction is probably formed in part during heat coagulation of the protein, its metabolic significance is questionable. Its determination does, however, serve as a blank for the amide determination. The estimation of ammonia was made by the aeration method of Sessions and

Shive (36).

Amide Nitrogen. Duplicate 50 ml. aliquots of the non-protein solution were hydrolyzed with three ml. of concentrated sulfuric acid under reflux condensers for 2.5 hours. The solutions were nearly neutralized with sodium hydroxide and the ammonia determined by the method of Sessions and Shive(36).

Peptide Nitrogen. Aliquots of 100 ml. of the non-protein filtrate were hydrolyzed with 20 per cent hydrochloric acid for 12 hours. The acid was removed in vacuo, the ammonia expelled by distillation in vacuo with calcium oxide, the solution acidified with acetic acid and concentrated in vacuo to a volume of 50 ml. Total alpha-amino nitrogen was determined in two ml. aliquots. The increase in amino nitrogen due to hydrolysis was considered as combined amino or peptide nitrogen. Only small amounts of this fraction were found in mature potato tubers.

Residual Nitrogen. Determined by difference and consists of the difference between total soluble, non-protein nitrogen and the sum of the mono-amino, basic and amide nitrogen.

Experimental Results

Effect of Storage Temperature.

Irish Cobbler Potatoes

General Procedure. The potatoes used in the 1933 experiments were Maryland certified potatoes of the Irish Cobbler variety grown near Pocomoke, Maryland. The tubers were planted July 27, 1932, and harvested when fully mature on November 15. The potatoes were brought to College Park on November 17 and

stored in a laboratory behind a black sateen cloth which transmitted light of very low intensity. A number of samples consisting of eight tubers each, matched for size and uniformity, were weighed, placed in small wire baskets and stored on November 19 in an electric refrigerator maintaining a temperature of 2-3° C. One sample of tubers was analyzed on November 19 and the remaining ones returned to the storage at room temperature behind the black cloth. The room temperature fluctuated somewhat between day and night and from day to day, as shown by thermograph records, the mean temperature being about 20° C. Since the purpose of this first study was to determine the effect of rather large differences in storage temperature on the nitrogenous metabolism of mature potato tubers, typical storage conditions rather than rigidly maintained exact temperatures were considered satisfactory.

On January 2, 1933, a few of the potatoes stored at room temperature showed slight sprouting of the apical bud cluster, evidence that the tubers were emerging from the rest period. Accordingly the tubers were divided into samples of eight each, weighed, and an equal number of samples placed in the refrigerator or returned to the storage at room temperature. One sample was analyzed on January 2. At intervals of four weeks samples of the potatoes stored at the two temperatures were withdrawn, weighed, and analyzed separately. Additional experiments involving shifting of potatoes from one temperature to another, storage in pure nitrogen, etc., will be discussed separately in later sections of this paper.

Experiment 1--Irish Cobbler potatoes stored at 2-3° C. and at room temperature were analyzed at monthly intervals beginning at the end of the rest period, January 2, and ending April 24. The results are shown in Tables 1 to 8.

Table 1--Percentage of moisture and loss of weight in Irish Cobbler potatoes stored for varying periods at room temperature and at 2-3° C.

Date of analysis	Loss of weight of tubers		Moisture content of potato tubers	
	Stored at room. temp.	Stored at 2-3° C.	Stored at room temp.	Stored at 2-3° C.
	Per cent.	Per cent.	Per cent.	Per cent.
Jan. 2	--	--	82.98	(82.98)
Jan. 30	1. 18	0.44	82.55	82.36
Feb. 27	2. 54	.75	82.49	82.15
March. 27	4. 79	1.14	81.56	82.27
April 24	9. 57	2.08	81.38	82.29

With increasing senescence the potatoes stored at room temperature lose weight more rapidly than similar potatoes stored at 2-3° C. This decrease in total amount of water and dry substance per unit mass of tissue results in a fictitious apparent increase in nitrogen content of the tissue when the analytical data are expressed as percentages of the fresh weight at time of analysis. This fact is well illustrated in Table 2 where it appears that with increasing length of storage period the total nitrogen of potato tubers tends to increase.

Table 2--Distribution of nitrogen in potatoes stored at room temperature. The results are expressed as percentages of the fresh weight of the tissue at time of analysis.

Date of analysis	Distribution of nitrogen as percentages of the fresh weight of the tissue when analyzed						
	Total N	Non-Protein N	Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
Jan. 2	0.410	0.217	0.122	0.092	0.060	0.052	0.013
Jan. 30	.403	.239	..127	..087	.065	.052	.035
Feb. 27	.405	.249	.143	.104	.082	.062	.001
March 27	.452	.259	.144	.094	.078	.056	.031
April 24	.449	.267	.156	.111	.083	.064	.009

In order to correct the error caused by loss of weight of the tubers the analytical data have been recalculated to the original weight of the potatoes when the experiment was started on January 2.

Table 3--Distribution of nitrogen in potatoes stored at room temperature. The results are expressed as percentages of the fresh weight of the tissue on January 2.

Date of analysis	Distribution of nitrogen as percentages of the fresh weight of the tissue on January 2.						
	Total N	Non-Protein N	Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
Jan. 2	0.410	0.217	0.122	0.092	0.060	0.052	0.013
Jan. 30	.398	.236	.126	.086	.064	.051	.035
Feb. 27	.395	.243	.139	.101	.080	.060	.002
March 27	.430	.247	.137	.089	.074	.053	.031
April 24	.406	.241	.141	.100	.075	.058	.008

Study of the data presented in Table 3 leads to the conclusion that storage of Irish Cobbler potatoes at room temperature for a period of four months after the end of the rest period has resulted in an increase of the soluble nitrogenous fractions, particularly amino and basic nitrogen. Protein and residual nitrogen have been hydrolyzed in the process. The results of analyses of similar samples of potatoes stored at 2-3° C. are presented in Table 4.

Table 4--Distribution of nitrogen in potatoes stored at 2-3° C. The results are expressed as percentages of the fresh weight of the tissue on January 2.

Date of analysis	Distribution of nitrogen as percentages of the fresh weight of the tissue on January 2.						
	Total N	Non-Protein N	Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
Jan. 30	0.404	0.230	0.118	0.084	0.057	0.051	0.038
Feb. 27	.394	.225	.132	.085	.075	.052	.013
March 27	.416	.234	.131	.098	.075	.053	.008
April 24	.398	.222	.139	.092	.072	.053	.005

From the data in Table 4 it appears that low temperature has prevented the hydrolysis of protein, values for the non-protein nitrogen remaining approximately constant during the four-month period. However, as in the case of potatoes stored at the higher temperature, amino and basic nitrogen have definitely increased with senescence. The increase in concentration of these fractions has occurred at the expense of the residual nitrogen which has correspondingly decreased. It must be concluded that low temperature does not inhibit the hydrol-

ysis of this residual nitrogen although the protein seems to be unaffected. There is no evidence of any predominantly synthetic activity unless the appearance of amino and basic nitrogen and the disappearance of the residual nitrogen is a process involving synthesis.

The same relationship between the effect of temperature and nitrogen metabolism in potato tubers is apparent when the data are calculated on the basis of the oven-dry pulp. The results are shown in Tables 5 and 6. Clearly there is hydrolysis of protein and residual nitrogen with increases of basic and amino nitrogen in the potatoes stored at room temperature while in the potatoes stored at 2-3° C. only hydrolysis of the residual nitrogen has occurred.

Table 5--Distribution of nitrogen in potatoes stored at room temperature. The results are expressed as percentages of the dry weight of the tissue.

Date of analysis	Distribution of nitrogen as percentages of the dry weight of the tissue						
	Total N	Non-Protein N	Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
Jan. 2	2.409	1.275	0.717	0.541	0.353	0.306	0.075
Jan. 30	2.309	1.370	.728	.499	.372	.298	.201
Feb. 27	2.313	1.422	.817	.594	.468	.354	.006
March 27	2.451	1.405	.781	.510	.423	.304	.168
April 24	2.411	1.434	.838	.596	.446	.344	.048

Table 6--Distribution of nitrogen in potatoes stored at 2-3°C. The results are expressed as percentages of the dry weight of the tissue.

Date of Analysis	Distribution of nitrogen as percentages of the dry weight of the tissue						
	Total N	Non-Protein N	Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
Jan 30	2.302	1.310	0.669	0.476	0.323	0.289	0.222
Feb. 27	2.224	1.272	.745	.482	.426	.291	.073
March 27	2.374	1.337	.750	.558	.429	..305	.045
April 24	2.292	1.282	.802	..531	.418	.305	.028

The relative balance between the various nitrogen fractions is best portrayed by calculating them as percentages of the total nitrogen. This is particularly true in the case of tissues like the potato which contain a high proportion of soluble, non-protein nitrogen. In this calculation fluctuation in the weight of the potatoes due to changes in moisture do notaffect the accuracy of the results. These data for the potatoes stored at room temperature and at 2-3° C. are presented in Tables 7 and 8. The same conclusions previously pointed out are very evident. Low temperature has exercised a restraining effect on the normal drift of senescence in potato tubers.

Table 7--Distribution of non-protein nitrogen in potatoes stored at room temperature. Results are expressed as percentages of the total nitrogen.

Date of analysis	Non-Protein N	Partition of non-protein nitrogen as percentages of the total nitrogen				
		Alpha Amino N	Mono-Amino N	Basic N	Amide N	Residual N
	Per cent					
Jan. 2	52.93	29.76	22.44	14.63	12.68	3.18
Jan. 30	59.31	31.51	21.59	16.13	12.90	8.69
Feb. 27	61.48	35.31	25.68	20.25	15.31	0.24
March 27	57.30	31.86	20.80	17.26	12.39	6.85
April 24	59.47	34.74	24.72	18.49	14.25	2.01

Table 8--Distribution of non-protein nitrogen in potatoes stored at 2-3o C.Results are expressed as percentages of the total nitrogen.

Date of analysis	Non-Protein N	Partition of non-protein nitrogen as percentages of the total nitrogen				
		Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
	Per cent					
Jan. 30	56.90	29.06	20.69	14.04	12.56	9.61
Feb. 27	57.18	33.50	21.66	19.14	13.10	3.28
March 27	56.29	31.59	23.52	18.05	12.83	1.89
April 24	55.91	34.98	23.15	18.23	13.30	1.23

Experiment 2. Irish Cobbler potatoes were stored at room temperature and at 2-3° C. on November 19 immediately after harvesting. Samples of the potatoes were analyzed on November 19, at the end of the rest period on January 2, and on May 15. The results are shown in Table 9.

Table 9--Distribution of non-protein nitrogen in potatoes stored at room temperature and at 2-3° C. from November 19 until May 15. The results are expressed as percentages of the total nitrogen.

Date of analysis	Storage temp.	Weeks in Storage	Non-Protein N	Partition of non-protein nitrogen as percentages of the total N				
				Alpha Amino N	Mono-Amino N	Basic N	Amide N	Residual N
			PER CT					
Nov. 19	--	0	52.34	29.44	18.69	13.55	11.68	8.42
Jan. 2	Room temp.	6	52.93	29.76	22.44	14.63	12.68	3.18
May 15	Room temp.	25	57.99	33.70	23.41	18.38	13.32	2.88
May 15	2-3° C.	25	54.63	31.95	21.95	18.29	11.95	2.44

The data contained in Table 9 show that during the rest period of potato tubers hydrolysis of the residual nitrogen to amino and basic nitrogen took place. From January 2 until May 15 the potatoes stored at room temperature exhibited further hydrolysis of the residual nitrogen as well as a depletion of the protein reserve. More than twice as much protein was hydrolyzed in the potatoes stored at room temperature as in similar samples maintained at 2-3o C. As a result all of the nitrogen fractions are greater in the potatoes stored at the higher temperature. It is of particular interest to note that on May 15 the residual nitrogen is of equal magnitude in the potatoes stored at either temperature.

This suggests that residual nitrogen is composed of more than one type of compound, at least one of which is fairly readily hydrolyzed at either high or low temperature. The accelerating action of the higher storage temperature in hydrolytic activities is very striking.

McCormick Potatoes

General Procedure. The experiments conducted during 1932-1933 showed that the nitrogen metabolism of Irish Cobbler potatoes during storage was characterized by a consistent dominance of protein hydrolysis over synthesis. The effect of low temperature was to inhibit this response without changing its qualitative aspects. It was considered desirable to repeat this study with another variety of potatoes stored under carefully controlled conditions.

Potatoes used in the 1933-1934 experiments were of the McCormick variety grown under the direction of Dr. R.A. Jehle, specialist in Plant Pathology at the University of Maryland. The potatoes were harvested about the 6th of November and stored in a cellar at a temperature of 40-50°F. until November 17 when they were brought to the laboratory.

The tubers were divided into uniform lots and stored in cheese cloth sacks at constant temperatures of 2-3° C., 6-7° C. and 22° C. Other lots were stored in damp sawdust at laboratory temperatures and check lots were left in air at the same temperature. The methods of analysis were the same as have been described under Chemical Methods and found to be very satisfactory for this tissue. One sample of the potatoes was analyzed and the remaining lots stored at the various temperatures on November 21, 1933.

Experiment 1. McCormick potatoes stored at 2-3° C. were analyzed at intervals beginning shortly after harvesting, November 21 and ending April 24. The results are shown in Tables 10-14.

Table 10--Percentage of moisture and loss of weight in McCormick potatoes stored for varying periods at 2-3°C? and at 22° C.

Date of analysis	Storage at 22° C		Storage at 2-3° C	
	Moisture	Loss of Weight	Moisture	Loss of Weight
	Per cent	Per cent	Per cent	Per cent
Nov. 21	77.03	---	(77.03)	--
Dec. 11	75.75	4.28	79.02	2.10
Jan. 8	75.72	6.07	76.37	4.31
Jan. 22	74.73	6.70	76.35	4.68
Feb. 5	75.33	7.33	75.64	5.06
March 12	78.23	7.97	77.36	7.59
April 16	74.12	15.48	74.56	9.05

The moisture content of the McCormick potatoes was lower on November 21, when the storage experiments were started, than the mositure content of the Irish Cobbler potatoes at any time during the preceding year. Greater loss of weight occurred at the 22° C. storage than at 2-3° C. During the early storage period the mdsture percentage remained higher in the potatoes stored at the lower temperature but this difference gradually disappeared.

It was previously pointed out that the loss of moisture and dry substance by stored potatoes results in an apparent increase of total nitrogen with increasing length of storage period. The increase in nitrogen content of potato tubers upon storage which has been reported by various workers is entirely fictitious and caused by the manner of calculation of the analytical data. In order to avoid this error the data have been recalculated to the original fresh weight of the tubers at the time the storage experiments were started. The data appear in Tables 11 and 12.

Table 11--Distribution of nitrogen in potatoes stored at 22° C. The results are expressed as percentages of the fresh weight of the tissue on November 21.

Date of analysis	Distribution of nitrogen as percentages of the fresh weight of the tissue on Nov.21						
	Total N	Non-Protein N	Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
November 21	0.326	0.147	0.076	0.068	0.010	0.035	0.034
December 11	.352	.148	.080	.073	.013	.034	.028
January 8	.328	.157	.082	.074	.015	.034	.034
January 22	.321	.145	.071	.067	.017	.036	.025
February 5	.332	.152	.079	.063	.039	.041	.009
March 12	.331	.158	.087	.073	.010	.037	.038
April 16	.315	.151	.074	.066	.018	.035	.032

Table 12--Distribution of nitrogen in potatoes stored at 2-3°C. The results are expressed as percentages of the fresh weight of the tissue on November 21.

Date of analysis	Distribution of nitrogen as percentages of the fresh weight of the tissue on November 21.						
	Total N	Non-Protein N	Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
Dec. 11	0.322	0.143	0.074	0.061	0.014	0.031	0.037
Jan. 8	.329	.143	.076	.069	.012	.033	.029
Jan. 22	.305	.127	.064	.048	.013	.031	.035
Feb. 5	.360	.169	.089	.085	.024	.044	.016
March 12	.311	.123	.063	.057	.015	.023	.028
April 16	.347	.142	.071	.065	.018	.033	.026

The data in Table 11 indicates that with increasing length of storage period at 22° C. the non-protein nitrogen tends to increase slightly. There is a well-defined inverse relation between residual and basic nitrogen. Just as with the Irish Cobbler potatoes early senescence is characterized by hydrolysis of residual nitrogen and increase in basic nitrogen. When growth starts the basic nitrogen decreased accompanied by an increase in residual nitrogen.

The results obtained with potatoes stored at 2-3° C. (Table 12) lead to the conclusion that low temperature has had no effect on their nitrogen metabolism. The tubers analyzed January 22, February 5, and March 12 unquestionably differed from the average in nitrogen distribution. The data for the April 16 analysis differed from the December 11 analysis only in showing a slightly higher content of basic nitrogen and correspondingly lower content of residual nitrogen, confirming the results obtained each year with Irish Cobbler potatoes.

The fluctuation in total nitrogen shown by the various samples is somewhat greater than might be desired. That these fluctuations are due to sample heterogeneity and not to analytical errors is shown by the fact that the various fractions, whether determined by Kjeldahlization, gasometrically, or by aeration, also fluctuate in the same direction as does the total nitrogen. This fact is clearly demonstrated in the data for the February 5 analysis of potatoes stored at 2-3C. (Table 12).

Nitrogen distribution data are also presented in Tables 13 and 14 as percentages of the total nitrogen.

Table 13--Distribution of non-protein nitrogen in potatoes stored at 22° C. Results are expressed in percentages of the total nitrogen.

Date of analysis	Non Protein N	Partition of non-protein nitrogen as percentages of the total nitrogen				
		Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
	Per ct.					
Nov. 21	45.09	23.31	20.86	3.07	10.74	10.42
Dec. 11	42.12	22.83	20.65	3.80	9.78	7.89
Jan 8.	47.85	24.93	22.64	4.58	10.32	10.31
Jan. 22	45.06	22.09	20.93	5.23	11.34	7.56
Feb. 5	45.81	23.74	18.99	11.73	12.29	2.80
March 12	47.78	26.11	21.94	3.06	11.11	11.67
April 16	47.99	23.59	20.91	5.63	10.99	10.46

Table 14--Distribution of non-protein nitrogen in potatoes stored at 2-3° C. Results are expressed as percentages of the total nitrogen.

Date of analysis	Non-Protein N	Partition of non-protein nitrogen as percentages of the total nitrogen				
		Alpha-Amino N	Mono Amino N	Basic N	Amide N	Residual N
	Per cent					
Dec. 11	44.38	23.10	18.84	4.26	9.73	11.55
Jan. 8	43.31	22.97	20.93	3.78	9.88	8.72
Jan. 22	41.56	20.94	15.62	4.38	10.00	11.56
Feb. 5	46.97	24.80	23.75	6.60	12.14	4.48
March 12	39.47	20.18	18.40	4.75	7.42	8.90
April 16	40.99	20.37	18.80	5.22	9.40	7.57

The increase in non-protein nitrogen, constancy of amino and amide content, and the inverse relationship between basic and residual nitrogen are outstanding in the data for the potatoes stored at 22° C. and presented in Table 13. The variatibility in the analytical data for the potatoes stored at 2-3° C. make it difficult to accurately interpret the results. As Goldthwaite (14) observed, the total nitrogen content of individual tubers is not constant. Furthermore, the ratio between total and non-protein nitrogen as well as the balance among the several soluble fractions is not constant. The data in Table 14 afford good evidence that low temperature inhibits the normal senescence processes in potato tubers. The relation between residual and basic nitrogen has been mentioned. There is no change in content

of amide or mono-amino nitrogen. The slightly lower content of total alpha-amino nitrogen and total non-protein nitrogen with increasing length of storage period cannot be definitely interpreted on the basis of the information at hand.

It should be pointed out that the emergence of the tubers from the rest period was not signalized by any noticeable effect on the nitrogen metabolism aside from an increased rate of accumulation of basic nitrogen at the expense of the residual nitrogen. It seems quite evident that after-ripening in the potato tuber is characterized by a hydrolysis of the unanalyzable residual nitrogen to amino acids and bases. Rosa (34) observed that the primordia of the vegetative sprouts develop during the latter stages of tuber growth and during the dormant period and that the emergence from the rest period is a gradual and not a sudden change. Rosa states that "no significant difference in the chemical composition of dormant and non-dormant potato tubers has yet been established". Loomis (20) thought that dormancy was probably related to a greater degree to cytoplasmic structure than to chemical composition or enzyme formation and suggested that "...the rest period is broken by some such change as thereversal of lipoidal proteinaceous phases in the cytoplasm whereby the permeability of the cell is increased and the enzymes associated with the protein phases are liberated". Such a hypothesis would be extremely difficult of proof. The present study has shown that a gradual hydrolysis of protein occurs with the drift in senescence. The rate of hydrolysis is inhibited by low temperature storage. After the termination of the rest period the rate of hydrolysis tends

to increase but it must be concluded that the ability to produce sprouts cannot be correlated with a marked shift in the nitrogen metabolism of the tubers.

Experiment 2. McCormick potatoes stored at 2-3° C., 6-7° C., and 22° C. on November 21, were analyzed March 12 and April 16. On March 12 one sample of potatoes from the 6-7 C. storage was shifted to 22° C. and analyzed April 16. The results are shown in Table 15.

Table 15.--Distribution of non-protein nitrogen in potatoes stored at 2-3° C., 6-7° C., and 22 C. The results are expressed as per cent of the total nitrogen.

Date of analysis	Storage Temp.	Weeks in Storage	Non-Protein N	Partition of non-protein nitrogen as percentages of the total nitrogen				
				Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
			Per ct.					
March 12	2-3C	16	39.47	20.18	18.40	4.75	7.42	8.90
" 12	6-7C	16	37.36	19.25	17.82	4.60	7.47	7.47
Apr. 16	2-3C	21	40.99	20.37	18.80	5.22	9.40	7.57
" 16	6-7C	21	39.14	17.69	16.89	4.02	8.85	9.38
" 16	2-3C 6-7C	16 5 21	42.38	19.64	18.60	3.88	10.08	9.82
" 16	22 C	21	47.99	23.59	20.91	5.63	10.99	10.46

It is evident from the data in Table 15 that storage of the potatoes at 22° C. results in a greater accumulation of non-protein nitrogen than that occurring in similar potatoes stored at either 6-7° C. or 2-3° C. When potatoes which had been stored at 6-7°C. were transferred to 22° C. the concentration of amino

and amide nitrogen increased significantly.

It is not clear whether the smaller amount of non-protein nitrogen in the potatoes stored at 2-3° C. or 6-7o C. as compared with those stored at 22o C. is due to protein synthesis or simply sample heterogeneity. The fact that this observation was made in both analyses made March 12 and April 16 support the hypothesis that protein synthesis occurred at low temperature storage. The experiments conducted in 1932-1933 failed to reveal any evidence of protein synthesis dominating protein hydrolysis at low temperature storage. The present experiments with McCormick potatoes show definitely that storage at 22° C results in protein hydrolysis with increasing length of storage period. They show further that little difference exists between temperature of 2-3° C. or 6-7° C with respect to their effect on the nitrogen metabolism in potato tubers. It should be pointed out that this is unlike the carbohydrate metabolism of potatoes where temperatures approaching 0°C. are necessary for any considerable accumulation of sugar to occur.

Comparative distribution of Nitrogenous Compounds in Irish Cobbler and McCormick Potatoes

Irish Cobbler potatoes were analyzed November 19, 1932, and McCormick potatoes were analyzed November 21, 1933. The results are shown in Table 16.

Table 16. Distribution of nitrogen in Irish Cobbler and McCormick potatoes. The results are expressed as percentages of the fresh weight of the tissue.

Variety	Distribution of nitrogen as percentages of the fresh weight of the tissue.						
	Total N	Non-Protein	Alpha Amino N	Mono-Amino N	Basic N	Amide N	Residual N
Irish Cobbler	0.428	0.224	0.126	0.080	0.058	0.050	0.036
McCormick	.326	.147	.076	.068	.010	.035	.034

It is clearly evident from the data in Table 16 that the nitrogen content of tubers of the two varieties of potatoes is quite different. Greater amounts of total nitrogen and of all of the nitrogen fractions were present in the Cobbler potatoes. This difference is particularly striking in the case of the basic nitrogen. The difference in basic nitrogen is largely due to a much higher content of diamino acids in the Cobbler potatoes. It is of considerable interest to note that the residual nitrogen content in tubers of the two varieties is nearly identical. When calculated on the basis of the total nitrogen, relatively higher percentages of non-protein nitrogen are present in the Cobbler potatoes, more protein being in the McCormick potatoes. It should be pointed out that this observation is in agreement with the findings of Willaman and West (43) who noted that early potatoes were higher in total nitrogen and lower in dry matter than late potatoes. Despite the fact that the Irish Cobbler potatoes, an early variety, were grown in the late summer the

original difference in the tubers persisted. It is, of course realized that soil type, fertilization, etc., profoundly affect the nitrogen content of potato tubers.

Effect of Storage in an Atmosphere of Pure Nitrogen

Irish Cobbler potatoes stored at room temperature and at 2-3° C. November 19 were transferred to an atmosphere of pure nitrogen gas on February 27. One sample was stored at 2-3° and one at 22° C. Incipient breakdown necessitated the analysis of the sample held at 22° C. at the end of 10 days on March 9. The potatoes stored in nitrogen at 2-3° kept in good condition for a period of 28 days and were analyzed on March 27. A check lot which had been held in air at room temperature was also analyzed on March 27. The results are shown in Table 17.

Table 17--Distribution of non-protein nitrogen in Irish Cobbler potatoes stored in air and in an atmosphere of pure nitrogen at temperatures of 2-3° and 22° C. Results are expressed as percentages of the total nitrogen.

Date of Analysis	Storage atmosphere	Storage period	Storage Temp.	Non-Protein N	Partition of the non-protein nitrogen as percentages of the total nitrogen				
					Alpha Amino N	Mono-Amino N	Basic N	Amide N	Residual N
				Per cent					
Mch.27	Air	28	Room Temp.	57.30	31.86	20.80	17.26	12.39	6.85
Mch. 9	Nitrogen	10	22o C	60.95	35.24	26.67	18.81	14.05	1.42
Mch.27	Nitrogen	28	2-3°C	61.17	37.86	26.94	17.23	12.38	4.62

Storage of the potatoes in nitrogen gas has accelerated the hydrolysis of protein to the soluble fractions. A period of 10 days in an atmorphere of pure nitrogen increased the concentrations of all of the analyzable nitrogen fractions, decreasing the protein and residual nitrogen. When the storage was carried on at low temperature the same response occurred with slightly more hydrolysis than took place at a temperature of 22° C. Although hydrolysis must have occurred at a slower rate at the lower temperature over a longer storage period the net effect was greater than in the potatoes stored at 22° C.

Comparative Distribution of Nitrogenous Compounds in Potato Tubers and Sprouts

After the initiation of growth processes the nitrogen metabolism in potato tubers is in part governed by the sprout. It was therefore considered desirable to compare the composition of tubers and the sprouts which grow from them. Irish Cobbler potatoes were used for this study. All of the sprouts were removed from a number of potatoes and analyzed as described for the tubers. The tubers had received only diffuse light and the sprouts were slightly etiolated, ranging from six to ten inches in length. The nitrogen distribution in the tubers from which these sprouts were obtained was also determined. The results are shown in Tables 18 and 19.

Table 18-- Distribution of nitrogen in potato tubers and sprouts. The results are expressed as percentages of the fresh weight of the tissue.

Tissue	Distribution of nitrogen as percentages of the fresh weight of the tissue.						
	Total N	Non-protein N	Alpha-Amino N	Mono Amino N	Basic N	Amide N	Residual N
Tubers	0.458	0.259	0.143	0.089	0.084	0.054	0.032
Sprouts	.658	.356	.161	.138	.041	.068	.109

Table 19 --Distribution of non-protein nitrogen in potato tuber and sprouts. The results are expressed as percentages of the total nitrogen.

Tissue	Non-Protein N	Partition of non-protein nitrogen as percentages of the total nitrogen.				
	Percent	Alpha-Amino N	Mono-amino N	Basic N	Amide N	Residual N
Tubers	56.55	31.22	19.43	18.34	11.79	6.99
Sprouts	54.10	24.47	20.97	6.23	10.33	16.57

The data in Table 18 reveal that the sprouts are considerably higher in nitrogen than the tubers from which they grew. This is true of the total nitrogen and of all of the nitrogen fractions with the exception of basic nitrogen which is strikingly lower in the sprouts than in the tubers. The true bases and the diamino acid bases are both lower in the sprouts than in the tubers. The sprouts are particularly rich in protein, mono-amino

acids, and residual nitrogen.

When the data are calculated as percent of the total nitrogen the sprouts are seen to be slightly higher than the tubers in protein and mono-amino nitrogen, and correspondingly lower in total alpha-amino nitrogen and amide nitrogen, much lower in basic nitrogen yet considerably higher in residual nitrogen. It would appear that this latter fraction is associated with growth and its decrease in stored potatoes precedes the initiation of growth processes.

The Comparative Distribution of Nitrogenous Compounds in Tissue-at-eyes, Cortex, and Medulla of Irish Cobbler Potatoes

Irish Cobbler potatoes stored November 19 at 2-3 C. were transferred to room temperature on June 12. One sample consisting of eight tubers was divided into three areas as follows. Each bud cluster and adjacent tissue was removed from the tubers with a No. 8 size cork borer. This sample then consisted of cylinders of tissue each 0.5 inch in length and 0.5 inch in diameter. The remaining portion of the tubers was divided into two areas, cortex and medulla, by separation at the vascular ring which lies about one centimeter beneath the surface of the tuber. All of the center of the tuber within this vascular ring was termed the medulla. Some investigators have attempted to divide the medulla into inner and outer areas but there is no definite line of demarcation between these areas as there is between cortex and medulla so

further fractionation was not attempted. Each sample of the three areas, tissue-at-eyes, cortex, and medulla, was extracted and analyzed separately as described under methods of analysis.

A second sample of potatoes was separated into areas and analyzed on July 5 when they had been exposed to room temperature since June 12. When potatoes are transferred from low temperature to room temperature the accumulated sugars rapidly disappear. Preliminary experiments revealed that nitrogen transformations were not brought about so rapidly. The present study was made to detect changes in the course of the nitrogen metabolism occurring within limited areas of potatoes. When the potatoes were removed from storage at 2-3 C. on June 12 the eyes on the seed end of the potatoes showed faint sprout growth being 2-3 mm. in length. Storage at room temperature increased the growth rate so that by July 5 the terminal sprouts were about one centimeter in length. The other sprouts on the seed end of the potatoes were slightly shorter while the eyes on the stem end had not sprouted. The sample of tissue-at-eyes, however, included all of the eyes on the eight potatoes. The data for the changes in moisture in the potatoes during the course of the experiment are shown in Table 20.

Table 20 -- Percentages of moisture in tissue-at-eyes, cortex, and medulla, of Irish Cobbler potatoes.

Date of analysis	Percentages of moisture		
	Tissue-at-eyes	Cortex	Medulla
June 12	81.88	80.78	83.50
July 5	80.36	80.85	82.28

There has been a marked decrease in moisture in the tissue-at-eyes during the storage period. This is probably due to the effect of the sprouts. The medulla also lost a considerable amount of moisture. The cortical tissue, however, showed no change in percentage of moisture. This is due without doubt to the steep gradient of moisture existing between the medulla and the cortex. The moisture lost from the medulla must inevitably have entered the cortex.

Data showing the distribution of nitrogen in the potatoes are presented as percentages of the total nitrogen in Table 21 and as percentages of the dry weight in Table 22.

Table 21 -- Distribution of non-protein nitrogen in tissue-at-eyes, cortex, and medulla of Irish Cobbler potatoes stored at 2-3 C. November 19 and shifted to room temperature on June 12. Results are expressed as percentages of the total nitrogen.

Date of Analysis	Tissue	Non-Protein N	Partition of non-protein nitrogen as percentages of the total nitrogen.				
			Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
		Percent					
June 12	Tissue-at-eyes	49.89	27.74	18.34	15.21	10.07	6.27
July 5	Tissue-at-eyes	48.31	28.69	17.30	14.98	9.92	6.11
June 12	Cortex	47.43	26.64	17.76	13.32	9.58	6.77
July 5	Cortex	49.76	28.77	18.40	15.57	11.32	4.47
June 12	Medulla	61.75	38.25	23.27	18.89	14.06	5.53
July 5	Medulla	59.42	34.53	22.20	17.26	13.45	6.51

Table 22 -- Distribution of nitrogen in tissue-at-eyes, cortex, and medulla of Irish Cobbler potatoes stored at 2-3 C. November 19 and shifted to room temperature on June 12. Results are expressed as percentages of the dry weight of the tissue.

Date of Analysis	Tissue	Distribution of nitrogen as percentages of the dry weight of the tissue.						
		Total N	Non-Prot N	Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
June 12	Tissue-at-eyes	2.467	1.231	0.684	0.453	0.375	0.248	0.155
July 5	Tissue-at-eyes	2.413	1.166	.692	.418	.362	.239	.147
June 12	Cortex	2.227	1.056	.593	.395	.297	.213	.151
July 5	Cortex	2.214	1.102	.637	.407	.345	.251	.099
June 12	Medulla	2.630	1.624	1.006	.612	.497	.370	.145
July 5	Medulla	2.517	1.495	.869	.559	.435	.339	.162

The data contained in Tables 21 and 22 show conclusively that percentage and absolute amounts of nitrogen per unit of tissue are considerably greater in the medulla than in the cortex. This is also true for the various nitrogen fractions which are far greater in the medulla of the potatoes. As would be expected intermediate values are characteristic of the tissue-at-eyes. This is due to the inclusion of both cortex and medulla in the analytical sample. A greater proportion of the sample was made up of a cortical tissue. This sample was taken to detect metabolic changes in a limited area of the potatoes.

The changes in nitrogen distribution within the areas of the potatoes are due in part to the difference in storage temperature and in part to the initiation of growth. It has been pointed out that storage of potato tubers at room temperature results in a hydrolysis of protein reserves. The effect of growth cannot be definitely measured. Differences in distribution of nitrogen therefore represent the mean effect of processes which may be in part synthetic and in part hydrolytic. It was of particular interest in the present experiment to note the differences in metabolic rate within the various areas.

The most outstanding differences in distribution of nitrogen were found in the medulla. In this tissue all of the non-protein analyzable nitrogen fractions decreased in concentration. The absolute amount of total nitrogen also decreased indicating that translocation of soluble nitrogen away from the medulla had occurred. In the cortex there has been a corresponding increase in non-protein nitrogen. Since steep gradients of water and nitrogen exist between the medulla and cortex it seems probable that the amino acids, amides, and bases present in the medulla were translocated into the cortex. In the tissue-at-eyes the non-protein nitrogen has decreased slightly indicative of protein synthesis. The differences are, however, very slight. The fact that certain fractions accumulate or are depleted more rapidly than

others is explained by the differential rate of their utilization in condensation of new protein, ease of translocation, etc. The three areas of potato tubers exhibit distinct specificity in their nitrogen metabolism.

The Effect of Storage in Damp Sawdust

Samples of McCormick potato tubers were placed in damp sawdust on November 21 and similar samples left in air subject to ordinary temperature fluctuations of a laboratory. Water was added to the sawdust at intervals in amounts sufficient to keep it damp. This increased humidity resulted in an abbreviation of the rest period. By February 19 the tubers stored in sawdust bore short sprouts while similar tubers stored in cheesecloth sacks at the same temperature exhibited only a trace of sprout development at the terminal bud cluster. It should be pointed out that other lots of McCormick potatoes stored at 22 C. emerged from the rest period about January 22 as evidenced by slight sprout growth of the terminal bud. Other lots held at 2-3 C. had not produced sprouts by April 16. Not only did storage in sawdust somewhat abbreviate the rest period but the subsequent growth of the sprouts was much more rapid than that of potatoes stored in air.

Typical samples of air-stored and sawdust-stored potatoes were analyzed on February 19 for distribution of nitrogen. The tubers in sawdust increased in total water necessitating the recalculation of the data to the original fresh weight basis.

The moisture content and loss of weight of the two lots of tubers are shown in Table 23.

Table 23 -- Moisture content and loss of dry weight of McCormick potatoes stored at room temperature from November 21 until February 19 in air and in damp sawdust.

Date of analysis	Storage medium	Moisture	Loss of dry weight
November 21	-	77.03	-
February 19	Air	76.62	4.93
February 19	Damp sawdust	81.06	9.20

Although the sawdust-stored potatoes increased in moisture they showed a greater loss of dry substance than did the air-stored potatoes. The effect on the distribution of nitrogen is shown in Table 24.

Table 24 -- Distribution of nitrogen in potato tubers stored in air and in damp sawdust. The results are expressed as percent of the original fresh weight of the tubers at the beginning of the experiment.

Storage medium	Distribution of nitrogen as percentages of the fresh weight of the tissue at the beginning of the storage.						
	Total N	Non-Protein N.	Alpha-Amino N	Mon-Amino N	Basic N	Amide N	Residual N
Air	0.318	0.149	0.077	0.072	0.013	0.032	0.032
Damp sawdust	.315	.144	.077	.068	.009	.032	.035

It must be concluded from the data presented in Table 24 that storage of potatoes in sawdust despite its effect in abbreviating the rest period and increasing the loss of dry substance, has had no effect on the course of the nitrogen metabolism of the tubers. There is no significant difference between the relative amounts of the various nitrogen fractions in the tubers stored at the same temperature but under otherwise different environmental conditions.

Nitrogen Metabolism of Wounded Potatoes

When a potato tuber is wounded the injury is soon healed in such a manner as to prevent bacterial or fungal invasion as well as excessive dehydration. The sequence of events in this process consists first of a rapid blocking of the exposed surface by a fatty suberin deposit. This material serves to check the rapid loss of water and protect the underlying cells. If favorable conditions obtain some of the parenchymatous cells below the wounded surface become transformed to cork initials. The new layer of cells formed when suberized is as effective as the original cork in protecting the interior of the potato.

This process of wound periderm formation has occasioned considerable research, histological, cytological, and biochemical in nature. It was observed a number of years ago that the starch grains disappeared from the cells which are transformed to the phellogen. The manner in which this process is brought about has never been explained. Priestley and Woffenden (32) maintained that fatty acids were produced anaerobically from the starch. Herklots (18) states that the fats released by

the potato are in the form of unsaturated fatty acids which are most easily immobilised at low hydrion concentrations. Hence suberization occurred only in an alkaline or neutral media. However, after a suberin block had been deposited phellogen activity was promoted by high hydrion concentration.

Lutman (21) has emphasized that the change from senescence to rejuvenescence in cells of the potato tuber is characterized by an accumulation of protein in the nuclei of the periderm initials. This protein was presumably obtained from the cells lying between the wounded surface and the cork initials. Artschwager (7) however, observed that while the starch disappeared from the cell layers next to the cut surface, protein crystals were not used up in the process. Both periderm formation and suberization were affected by temperature and humidity. Shapovalov and Edson (35) reported that sprouting of tubers did not affect their ability to form wound periderm. Dusting with sulphur was also without effect. Dehydration, however, closely correlated with failure to form a periderm. The central portion of the tuber did not heal as readily as did the periphery.

Study of the experimental work which has been conducted with the problem of periderm formation indicates that three conditions are necessary for the process to occur satisfactorily. There must be an abundance of atmospheric oxygen, a warm temperature, and moist atmosphere. Preliminary experiments were conducted in which the tubers were cut in halves and placed in moisture chambers lined with wet paper. Since it had previously been established that pronounced differences in

composition occur between the medulla and cortex of potato tubers, care was taken to sample equivalent areas for analysis. The tubers were divided longitudinally and a slice 2-3 mm. in thickness taken from one half of each tuber. Moisture and nitrogen distribution were determined in this sample. The remaining halves were placed in a moisture chamber and allowed to remain seven days when a sample exactly like the original check was taken for analysis. It was established that changes in the moisture and nitrogen distribution had occurred. The ether extract of the dry tissue increased from 0.06 percent to 0.12 percent of the dry weight of the tissue.

The loss of dry substance through accelerated respiratory activity as well as changes in the moisture content of the tissue make it exceedingly difficult to express analytical data on a satisfactory basis. The nitrogen fractions might be calculated as percentages of the total nitrogen if the latter fraction did not change appreciably. In case it increased as Lutman (21) states this basis of calculation might not show it. In order to overcome this difficulty the following procedure was adopted.

A ten-liter desiccator was emptied of calcium chloride and partially filled with water. A wire rack was constructed to fit inside the desiccator in such a manner that it would be suspended over the water surface. Potato tuber slices were skewered on pointed glass rods supported on the wire rack so that they did not touch one another. Gaseous exchange was

provided through a side opening in the desiccator which was closed with a rubber stopper bearing two glass rods, one of which was attached to a filter pump. The flow of water in the pump was adjusted to provide a slow but constant replacement of air in the vessel at such a rate that water was just prevented from condensing on the sides of the vessel.

In one experiment six carefully selected Green Mountain potatoes were divided into halves longitudinally and one set of halves analyzed at once. The other six halves were again divided longitudinally, skewered on the glass rods, weighed, and suspended in the vessel. The cover was adjusted, the filter pump started, and the apparatus covered with a dark cloth which transmitted only diffuse light. The experiment was allowed to continue 13 days at a temperature ranging from 70 to 80 F. At the end of this time the slices were weighed, removed and analyzed. The data appear in Table 11.

Table 25 -- Distribution of nitrogen in Green Mountain Potatoes after wounding. The data are expressed as percentages of the original fresh weight of the potatoes.

Condition of potatoes	Distribution of nitrogen as percentages of the fresh weight of the tissue before wounding.						
	Total N	Non-Prot. N	Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
Uninjured	0.516	0.262	0.153	0.133	0.032	0.067	0.030
Wounded	.518	.250	.125	.102	.061	.067	.020

Table 26 -- Distribution of non-protein nitrogen in Green Mountain potatoes after wounding. The data are expressed as percentages of the total nitrogen.

Condition of potatoes	Non-Protein N	Distribution of non-protein nitrogen as percentages of the total nitrogen.				
	Percent	Alpha-Amino N	Mono-Amino N	Basic N	Amide N	Residual N
Uninjured	50.78	29.65	25.78	6.20	12.98	5.82
Wounded	48.18	24.16	19.76	11.85	12.92	3.65

It may reasonably be assumed that if the analytical technique is satisfactory the total nitrogen of the wounded tuber slices should not differ from that of the original tubers. Inspection of the data in Table 25 shows that they are nearly identical. Changes in distribution of the non-protein nitrogen have occurred. Protein has increased, total amino, mono-amino, and residual nitrogen have decreased, while the basic nitrogen has sharply increased and the amide nitrogen remained constant. A portion of the amino nitrogen has undoubtedly been condensed to form new protein. The decrease is, however, greater than could be accounted for by that process. The basic nitrogen has nearly doubled in concentration and it appears certain that a portion of the amino nitrogen has been converted to substances measured in the basic fraction. Careful analyses revealed the presence of only very small amounts of peptide nitrogen in the wounded tubers. It is possible that the poisonous alkaloid, solanine, known to be present in potato tubers may have been formed. Votchal (40) (quoted by Palladin) reported an

accumulation of solanine in wounded potatoes. While the data, per se, do not prove that the increase in basic nitrogen is due to the formation of increased amounts of solanine, the possibility should be kept in mind. Vickery (39) has pointed out that the nitrogen precipitated from plant extracts by phosphotungstic acid is a complex fraction. The inverse relation existing between residual and basic nitrogen is once more clearly demonstrated.

The process of wound periderm formation did not involve the amide fraction. This observation is quite remarkable in view of the fact that potatoes are rich in amide nitrogen which is considered to play a prominent role in nitrogen metabolism. Obviously the results of this experiment support the theory that amino acids rather than amides are concerned in the primary synthesis of protein in this species. Wound periderm formation in potato tubers has diverted the course of the nitrogen metabolism from that found in the ordinary drift with senescence. Moreover, the effect was measurable throughout the tuber halves. Without doubt the processes occurring in the regenerating periderm area were more intense but the fact has been demonstrated that severe wounding completely disrupts and changes the course of the nitrogen metabolism within the tubers.

Discussion

It was pointed out in the review of literature on the subject of nitrogen metabolism in potato tubers that few nitrogen

partition experiments had been conducted with this species. Most nitrogen analyses of potato tubers have been confined to determinations of total nitrogen. When the soluble nitrogen was estimated separately it was found to increase with increasing storage life of the potato. No study seems to have previously been made of the effect of low temperature on the nitrogen metabolism of potato tubers.

The original purpose of the present investigation, to study the effect of storage temperature on the nitrogen metabolism in potatoes, has been accomplished. It may safely be concluded that there is no such effect of low temperature on the nitrogen metabolism of potatoes as that which has been observed in the carbohydrate transformations in the potato or the distribution of nitrogen in growing plants subjected to low temperatures. Coincident with the drift of senescence there is a gradual hydrolysis of the complex nitrogenous compounds to simpler products, largely amino acids and bases. This hydrolysis occurs stepwise in the sense that the unanalyzable residual nitrogen first diminishes being replaced by basic nitrogen. No further hydrolysis may occur over a period of several months in potatoes stored at low temperature but if they are held at room temperature the proteins are broken down to amino acids. The chief product of residual nitrogen hydrolysis appears to be basic nitrogen. It is of course obvious that in a system involving fluctuating variables, if all are held constant but two, one of which is determined by difference, an inverse

relationship inevitably results. This is much the situation obtained with basic and residual nitrogen. However, the results of a comparatively large number of analyses support the contention that residual nitrogen is composed at least in part of compounds which are directly or potentially basic in nature.

It is certain that in potatoes stored at room temperature the equilibrium, synthesis $\rightleftarrows$ hydrolysis of protein, is always displaced to the right. There is some evidence that prolonged storage at low temperature not only inhibits protein hydrolysis but also may result in a slight synthesis of new protein from the soluble fractions. When potatoes stored at 6-7 C. are shifted to room temperature, protein hydrolysis increases and they tend to resemble in nitrogen distribution potatoes stored continuously at room temperature.

Evidence that temperature is the most important factor governing nitrogen metabolism in potatoes was obtained from the experiment in which potatoes were stored in damp sawdust and in air at room temperature. Although the sawdust-storage abbreviated the rest period, increased the rate of sprout growth as well as loss of dry substance as compared with potatoes held in air at the same temperature, no distinctive effect was observable on the distribution of nitrogen in the potatoes.

During the rest period the unanalyzable residual nitrogen partially disappeared,being replaced by amino and basic nitrogen. With the initiation of growth as evidenced by sprout production, hydrolytic activities became somewhat more active. While it was not the primary purpose of this study to investigate the

chemical changes associated with the emergence from the rest period, certain pertinent observations may be made. From the standpoint of the nitrogen metabolism the ability to produce a sprout, signifying the end of the rest period, is inconsequential. That is the conclusion when the entire tuber is sampled for analysis. Probably the chemical changes accompanying sprout formation are localized in the tissue immediately adjacent to the bud cluster and when the entire tuber is sampled countless storage cells are analyzed whose composition bears little or no causal relation to the initiation of growth processes. Despite the abundance of carbohydrates and nitrogen in the dormant potato tuber there is no evidence that synthetic processes involving the nitrogen exceed hydrolysis until the initiation of growth. At that time the tissue adjacent to the bud cluster shows limited synthesis of protein while hydrolysis and translocation of nitrogen are the dominant activities throughout the remainder of the potato. It should be pointed out that there is no marked accumulation of any nitrogen fraction when potatoes are subjected to low storage temperature as there is in the case of the carbohydrates. It is evident, therefore, that neither the lability of the starch-sugar equilibrium nor the remarkable acceleration in respiration induced by low temperature storage of potatoes can be associated with their nitrogen metabolism.

Potato sprouts are able to withdraw nitrogen from the potatoes in greater concentrations than it is originally present in the tubers. The low concentration of basic nitrogen in the

sprouts is noteworthy. While this may be interpreted as meaning that the plant bases and diamino acids are translocated only with difficulty from tuber to sprout it seems more probable that they are utilized rapidly in synthetic reactions. Basic nitrogen appears in the potatoes as one of the first hydrolytic products before the termination of the rest period. When tubers are wounded it increases significantly in concentration.

Amide nitrogen, usually present in low concentrations in actively growing plants, constitutes an appreciable portion of the non-protein nitrogen of potato tubers. The analytidal data show little change in this fraction regardless of treatment of the tubers. The inference is that amino acids play a more important role in protein synthesis in this species than do the amides. The data for the total alpha-amino nitrogen and alpha-amino nitrogen of the mono-amino acids show that the diamino acids are not accumulated or depleted at the same rate as are the mono-amino acids.

As was previously pointed out the residual nitrogen appears to be made up of more than one constituent. This may consist in part of peptide units not precipitated by phosphotungstic acid but capable of hydrolysis to bases and amino acids. The rest period is characterized by hydrolysis of this fraction although the protein appears relatively stable during this period of early senescence. The high concentration of residual nitrogen in the tubers occurs prior to the initiation of growth.

The present study has confirmed the pioneer work of the French investigators Coudon and Bussard (10) who observed the total nitrogen in various areas of the potato tuber to be inversely correlated with the amount of dry substance. The present investigation has shown that the higher content of total nitrogen in the watery medulla is due to higher concentrations of the soluble non-protein fractions. The protein is slightly lower in the medulla than in the cortex. The possibility of translocation of these soluble nitrogen constituents within the potato tuber has been little emphasized. It is well recognized that a growing potato plant is able to extract the reserves of the original seed piece and it seems extremely probable that changes in nitrogen distribution within the tuber might be brought about by any marked shift in the course of the metabolism of the tuber. For example, shifting the potatoes to higher temperature with resulting production of sprouts appeared to result in definite withdrawal of soluble nitrogen from the medulla to the cortex while some elaboration of new protein occurred in the immediate vicinity of the growing sprout. While the data are admittedly meager there is good evidence of well defined specificity of nitrogen metabolism in various areas of potato tubers.

It is of interest to note that Appleman (5) after extensive studies with the growth of potato sprouts and plants from variously located seed pieces advised the partition of the mother tuber into pieces containing a portion of the center or

medulla. When the seed pieces were of suitable size much better growth of the sprouts was obtained if a portion of the medulla was included. The experiments conducted in the present investigation clearly demonstrate that the nitrogen distribution within the medulla is much different than that found in the cortex. The possibility that the growing sprout is better able to make use of the readily translocatable, soluble nitrogen occurring in the medulla must be considered. It is of course equally possible that substances were present in the medulla not detected in the nitrogen analyses which promote the growth of sprouts.

It is obvious that the differences in nitrogen distribution of potatoes due to treatment are very small. Hence it is necessary to make use of closely comparable material for analysis. The sampling error may be virtually eliminated by analyzing duplicate portions of the same tubers as was done in the experiment dealing with the effect of wounding on nitrogen metabolism. This procedure also made possible a study of the processes involved in nitrogen metabolism where synthesis rather than hydrolysis was the dominant activity. The results of the wounding experiment lead to the conclusion that the amino acids and residual nitrogen were involved in synthesis of new protein and in formation of peptides and other substances, possibly alkaloids, which were basic in nature.

Summary and Conclusions

Senescence in the potato tuber results in a loss of weight and decrease in percentage of moisture. These processes occur more rapidly at high than at low temperature of storage.

During the rest period of potatoes basic nitrogen increases in concentration at the expense of residual nitrogen. Storage for a period of five months at room temperature results in hydrolysis of the protein reserves with increases in all of the soluble nitrogen fractions. Hydrolysis of protein in potatoes occurs very slowly, if at all, at temperatures approaching 0 C.

A comparatively wide range of temperature is effective in preventing hydrolysis of protein in potatoes. When potatoes stored at low temperature are shifted to higher temperatures the rate of hydrolysis increases.

Storage in damp sawdust abbreviated the rest period, increased the rate of sprout growth and loss of dry substance but had no observable effect on the nitrogen metabolism.

Storage in an atmosphere of pure nitrogen accelerated the hydrolysis of protein at both high and low temperature. The storage life of the potatoes was longer at the lower temperature.

Potato sprouts are richer in nitrogen than the tubers from which they grow. The sprouts are much lower in basic nitrogen

and higher in residual nitrogen than the tubers.

In Irish Cobbler potatoes percentage of moisture, total and non-protein nitrogen were much higher in the medulla than in the cortex. Sprout production resulted in translocation of soluble nitrogen from the medulla to the cortex where an accumulation of non-protein nitrogen occurred. Protein synthesis slightly exceeded hydrolysis in the tissue-at-eyes.

Wounding Green Mountain potatoes resulted in an increase of protein and basic nitrogen at the expense of amino and residual nitrogen.

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