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THE BASIC AMINO ACID CONTENT OF SOME PLANT PROTEINS

William E. Brown

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University of Alberta

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THE BASIC AMINO ACID CONTENT OF
SOME PLANT PROTEINS

William E. Brown

Department of Plant Science

A THESIS

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THE BASIC AMINO ACID CONTENT OF SOME PLANT PROTEINS

William E. Brown

INTRODUCTION

It is now generally recognized that the biological values of different proteins are not equal. The majority of agriculturists still evaluate rations simply on the basis of their protein content, although it is known that feeding some animal protein supplement is advantageous with at least some farm animals. The amino acid content of plant proteins differs from species to species. Block and Bolling (4) even suggest that "just as the vitamin and mineral content of a plant can be varied by breeding and cultivation it is probable that the amino acid composition of its proteins can likewise vary".

To insure optimum health the amino acid requirements of the animal body must be met. Certain of these amino acids are essential and cannot be dispensed with in the diet (25). The three basic amino acids--arginine, histidine, and lysine--have been classified as being either indispensable or semi-indispensable. The main animal feeds in this country are the

THE BASIC AMINO ACID CONTENT OF

BOVINE MILK

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INTRODUCTION

It is now generally recognized that the biological values of different proteins are not equal. The majority of scientists still evaluate proteins simply on the basis of their protein content, although it is known that feeding some animal protein supplements is advantageous with at least some farm animals. The amino acid content of plant proteins differs from species to species. Direct and indirect methods suggest that "just as the vitamin and mineral content of a plant can be varied by breeding and cultivation so is possible that the amino acid composition of its proteins can likewise vary."

To insure optimum health and amino acid requirements of the animal body must be met. Certain of these amino acids are essential and cannot be synthesized within the body. The other basic amino acids--arginine, histidine, and lysine--have been classified as being either indispensable or semi-indispensable. The main clinical tests in this connection are the

cereal grains which have been shown by both biological and chemical assays to be deficient in lysine (18). On the other hand, animal proteins have a relatively high lysine content. Comparative figures for some proteins reported by Block and Bolling (4) are tabulated below.

TABLE I

Basic amino acids as percent of total protein

	Meat	Cow's milk	Whole beef blood	Wheat	Corn
Arginine	7.1	4.3	4.2	3.0	4.0
Histidine	2.2	2.5	5.9	1.2	2.4
Lysine	8.1	7.5	8.0	2.7	2.5

The need for protein supplements to make up the deficiencies in grain is obvious since grain feeding is a very common practice with all types of livestock. Tankage, fish meal, buttermilk, and other animal supplements are generally considered to be best. The demand, however, has at times far exceeded the supply. It is here that vegetable proteins enter the picture. The question of their nutritional value becomes of importance if they are to be used in quantity.

The main ^{immediate} purpose of this investigation was to determine the amount of each of the three basic amino acids in pea meal, linseed oil meal, and alfalfa meal, three feeds of relatively high protein content which can be produced on our

essential amino acids which have been shown by both biological and chemical assays to be deficient in lysine (13). On the other hand, animal proteins have a relatively high lysine content. Comparative figures for some proteins reported in block and boiling (1) are tabulated below.

TABLE I
Basic amino acids as percent of total protein

	Wheat	Wheat	Wheat	Wheat	Wheat
	Wheat	Wheat	Wheat	Wheat	Wheat
Arginine	4.1	4.8	4.8	4.8	4.8
Lysine	3.2	3.9	3.9	3.9	3.9
Isoleucine	3.1	3.6	3.6	3.6	3.6

The need for protein supplements to make up the deficiencies in grain is obvious since grain feeding is a very common practice with all types of livestock. Tankage, fish meal, buttermilk, and other animal supplements are generally considered to be best. The demand, however, for animal products has increased so rapidly. It is here that vegetable proteins enter the picture. The question of their nutritional value becomes of importance if they are to be used in place of animal products.

The main purpose of this investigation was to determine the amount of each of the three basic amino acids in soy meal, linseed oil meal, and alfalfa meal. These levels of relatively high protein content which can be produced on our

farms.

The problem of determining the amino acids present in natural feeds is even more difficult than when purified proteins are analyzed. It is generally recognized that amino acid determinations which may give reasonably accurate results on purified proteins fail miserably when applied to natural feeds. The acid hydrolysis of protein produces a nitrogen-containing substance called humin, an insoluble fraction important because of its bearing on the quantitative determination of certain of the amino acids. The presence of carbohydrates greatly increases this fraction. Roxas (26) found that small amounts of humin were formed when the bases, arginine, histidine, and lysine, were heated with carbohydrate solution. Tristram (33) in 1939 hydrolyzed the three bases individually in the presence of various furfuraldehyde-yielding carbohydrates. He reported that arginine suffers a loss roughly proportional to the amount of carbohydrate present, that histidine recoveries may be very irregular and that lysine recoveries are normal unless a large excess of carbohydrate is present. A preliminary study of the dispersion of the proteins of pea meal, linseed oil meal, and alfalfa meal was made. The aim was to extract a high percentage of the total protein in a relatively pure form and, at the same time, maintain its original chemical characteristics.

The need for a comparatively simple method of analysis was apparent. In 1940 Albanese (1) reported an

electrolytic method for the determination of the basic amino acids in protein. The author claims for this micro-method two desirable features, namely, simplicity of operation, and precision. An investigation of the possibility of using the method was undertaken.

The work involved in this research is consequently reported in three sections:

I. Dispersion studies with the proteins of pea meal, linseed oil meal, and alfalfa meal;

II. An investigation of the electrical transport method of basic amino acid analysis.

III. A presentation of the results obtained with some plant proteins using the electrical transport method of analysis.

electrostatic method for the determination of the basic amino acids in protein. The author claims for this micro-method two distinctive features, namely, simplicity of operation, and precision. An investigation of the possibility of using the method was undertaken.

The work involved in this research is summarized

as follows in three sections:

- I. Comparison studies with the method of use of methyl, lysine, and arginine.
- II. An investigation of the electrostatic transport method of basic amino acid analysis.
- III. A presentation of the results obtained with some plant proteins using the electrostatic transport method of analysis.

DISPERSION STUDIES WITH THE PROTEINS OF
PEA MEAL, LINSEED OIL MEAL, AND
ALFALFA MEAL

Literature Review

The investigations of Denis, Seyler, Ritthausen, and others (30) in the 19th century made it clear that plant proteins exist in many diverse forms. Owing to the differences in the properties of the protein constituents in the different meals to be investigated, each is discussed in turn.

Pea Meal

Legumin was referred to in the early literature as a substance extracted from seeds by caustic alkalis and more or less altered by the action of the solvent. Osborne and Campbell (30) reported that pea seeds contain two globulins--legumin and vicilin--and an albumin--legumelin. Legumin was thought to be the chief protein constituent. A water extraction of peas yielded 4% legumin and 2% legumelin, in terms of the weight of the seed.

In a later experiment Osborne and Campbell (30) extracted pea meal with 10% NaCl solution and obtained a ratio of one part albumin to 3.42 parts globulin. Unfortunately

DISCUSSION OF THE LITERATURE

AND THE RESULTS OF THE INVESTIGATION

BY

WILLIAM H. RAY

The investigation of the literature and the results of the investigation are presented in the following order: (1) the literature, (2) the results of the investigation, (3) the discussion of the literature, and (4) the conclusions. The literature is divided into two parts: (a) the literature on the general subject, and (b) the literature on the specific subject. The results of the investigation are presented in the following order: (1) the results of the investigation, (2) the discussion of the literature, and (3) the conclusions.

1921

The literature was reviewed in the early literature as a substance extracted from seeds of various kinds and more or less altered by the action of the solvent. Campbell (50) reported that the seeds contain two alkaloids: leucine and valine--and an amino-acid. Leucine was thought to be the chief alkaloid constituent. A water extract of seeds yielded a leucine and a valine, in cases of the weight of the seed.

In a paper on the leucine and valine (51) extracted from seeds of 100 seeds obtained and obtained a ratio of one part leucine to 5.42 parts valine. The leucine was

Osborne, interested primarily in the nature of the proteins, does not report yields in many of his papers.

Staker and Gortner (30) dispersed the protein of Andes pea meal in water and in M/2 neutral salt solutions. They reported water peptized 78.87% of the total nitrogen, potassium fluoride 85.23%, potassium sulphate 83.93%, potassium chloride 84.05%, potassium bromide 88.33%, and finally potassium iodide 95.18%. The anions formed the following lyotropic series on the basis of dispersing power,

By extracting the meal with ether the subsequent dispersion of protein in these solvents was increased by from 0.05% to 7.28%. The theory is advanced that the protein is imbedded in a layer of fat-like substance which prevents the salt solutions from exerting their maximum peptizing effect. The ratio of albumin to globulin was reported as 1:6.26.

Dermanskii, Antonovich, and Silaev (7) found two peptization maxima for pea protein, one in 2% NaCl corresponding to vicilin, the other in 10 - 20% NaCl corresponding to legumin. Using KCl as the dispersing agent one maximum was obtained in a 0.02 - 0.05% N solution at pH 1.4 - 1.8. Ba Cl₂ had a stronger peptizing action than KCl.

Smith and Circle (28) determined the dispersion of the protein of Alaska peas over a pH range of 2.0 - 10.5, using distilled water adjusted to these acidities. A maximum dispersion of 82.8% was obtained at pH 2.0, with a second

Osborne, interested primarily in the nature of the proteins, from our report yields in many of his papers.

Becker and Gortner (30) dispersed the protein of which was used in water and in 0.2 normal salt solutions. They reported water required 78.4% of the total nitrogen, potassium chloride 83.8%, potassium sulphate 83.7%, potassium nitrate 84.0%, potassium bromide 83.8%, and finally potassium iodide 90.1%. The values formed the following lyotropic series on the basis of dissolving power.

I Br T Cl NO₃

By extracting the meal with ether the subsequent dispersion of protein in these solvents was increased by from 0.05% to 0.23%. The theory is advanced that the protein is included in a layer of two-fold thickness which prevents the salt solutions from exerting their maximum peptizing effect. The ratio of albumin to globulin was reported as 1:0.75. Bernanelli, Antonovich, and Aliev (7) found two peptization maxima for pea protein, one in 0.2% normal solution, the other in 10 - 30% meal corresponding to 10% and 15% respectively. Using KCl as the dissolving agent one maximum was obtained in a 0.05% solution, at pH 1.4 - 1.8. The other was a stronger peptizing action than 10%.

Salin and Ullrich (28) determined the dispersion of the protein of Alaska (see over a pH range of 1.0 - 10.0, using distilled water adjusted to these acidities. A maximum dispersion of 83.8% was obtained at pH 2.0, with a second

maximum of 97.7% at pH 9 - 10.5. The minimum dispersion of 14.8% was at pH 3.9.

Linseed Oil Meal

The literature contains no references to studies on the dispersion of the proteins in commercial linseed oil meal. Considerable data have, however, been reported on oil-free flax seed.

In 1892 Osborne (30) found that the proteins of ether-extracted ground flax seed were largely soluble both in water and in sodium chloride solutions. He extracted with water and with saturated sodium chloride protein equal to 10.5% and 9.25% respectively of the original weight of the oil-free meal.

Staker and Gortner (30) attempted to disperse the nitrogen-containing substances of oil-free Redwing flax in water and M/2 neutral salt solutions. Water peptized 64.07% of the total nitrogen, potassium fluoride 53.53%, potassium sulphate 65.26%, potassium chloride 57.64%, potassium bromide 63.51% , and potassium iodide 60.94%. The lyotropic series differed from that reported for pea protein. The series for dispersion of flax seed protein was

but water was as effective as the best salt.

O'Hara and Saunders (19) investigated the solubility of the nitrogenous constituents of defatted flax seed meal in

maximum of 57.74 at 10.5. The minimum absorption of 14.84 was at 2.5.

Isolated Oil Seed

The literature contains no reference to studies on the absorption of the proteins in commercial isolated oil seed. Potentially these have, however, been reported on oil-seed. These are:

In 1933 Gossard (2) found that the proteins of water-extracted ground flax seed were largely soluble in water and in sodium chloride solutions. He extracted with water and with saturated sodium chloride solution equal to 10.5 and 0.25% respectively of the original weight of the oil-seed.

Stark and Gortner (3) attempted to determine the nitrogen-containing substances of oil-seed during this in water and 5% sodium salt solutions. Water solution of 0.25% of the total nitrogen, combined fraction 0.55%, combined soluble to 0.55%, potassium chloride 0.55%, combined soluble to 0.55%, and potassium iodide 0.55%. The nitrogen was different from that removed for the protein. The nitrogen for digestion of flax seed (4) was

1.1 I 1.1

but water was as effective as the salt.

Gossard and Gortner (1) investigated the solubility of the nitrogenous constituents of isolated flax seed in

sodium chloride solutions. Water dispersed 24% of these constituents. In concentrations of sodium chloride ranging from 0.125 N to saturation, the dispersion varied from approximately 42% to 65%. Maximum dispersion was obtained in 0.375 N to 2.0 N solutions.

Smith, Circle, and Brother (29) obtained a 58% extraction of Bison flaxseed protein with water. After the seed was defatted with petroleum ether the water extract contained 66% of the total protein. A 2N solution of NaCl dispersed 82% of the protein of the defatted meal. More concentrated solutions gave no higher extraction.

Alfalfa Leaf Meal

No figures were available in the literature on the peptization of alfalfa leaf meal protein. In fact, very little work has been done on dry leaves of any species. Plant physiologists have been quite interested in the extraction of the protein from the fresh leaves of plants. Miller (17) summarizes briefly the methods that have been used for extraction. The methods consist of macerating the leaves and plasmolizing the cells with "ether-water"; grinding and pressing the leaves; grinding the leaves, followed by high speed centrifuging. It is quite apparent that the methods for the extraction of the proteins and nitrogenous products from leaves vary greatly and the indications are that for best results special methods of

extraction must be applied to different types of leaves.

Osborne, Wakeman, and Leavenworth (21) ground and pressed fresh young alfalfa plants. They extracted from the expressed juice in succession: 43.8% of the total nitrogen with water; 2.0% with 93% alcohol; 7.0% with 0.3% aqueous NaOH; and 39.3% with 0.3% alcoholic NaOH. Total extraction was 92.1% of the nitrogen present.

The effect of drying fresh leaves on the subsequent extraction has been investigated. Chibnall (5) reported that the total water-soluble nitrogen of the fresh leaves of the runner bean was increased from 21.57% to 33.68% by drying the leaves in a current of warm air at 40°C. Conversely, Tottingham, Schulz, and Lepkovski (32) found that drying sugar beet leaves without aeration at 40°C. lowered the total amount of soluble nitrogen.

Materials

The peas used in this study were of three common field varieties--Idabel, Chancellor, and Early Blue--grown at Edmonton. The linseed oil meal was a commercial sample. The alfalfa meal consisted of the air-dried leaves and blossoms of Ferax alfalfa plants grown in 1944 on the University plots at Edmonton. The alfalfa was 60% in bloom at the time of picking.

All samples were coarsely ground in a Wiley mill, ground for six hours in a ball mill, and then stored in bottles.

Extraction must be applied to different layers of leaves.
Glycerine, benzene, and low-boiling (20) ether and
treated from white solids. The extracted from was
expressed in anhydrous: 17.8% of the total nitrogen
with water; 3.0% with 95% alcohol; 0.3% with 0.5% aqueous
sugar; and 20.2% with 0.5% alcoholic sugar. Total nitrogen
was 22.1% of the nitrogen present.

The effect of drying leaves on the nitrogen
extraction has been investigated. Williams (2) reported that
the total water-soluble nitrogen of the fresh leaves of the
tender herb was increased from 11.5% to 15.5% by drying the
leaves in a current of warm air at 40°C. Conversely, drying-
less, Schuler, and Japrovski (3) found that drying warm fresh
leaves without ventilation at 40°C. lowered the total amount of
soluble nitrogen.

Materials

The grass used in this study was of white common
this varieties--Lobel, Campbell, and Early Blue--grown at
Shenandoah. The leaves all had a somewhat similar
alfalfa meal consisted of the dried leaves and blossoms of
Tern's alfalfa plants grown in 1932 on the University farm at
Shenandoah. The alfalfa was cut in bloom at the end of May.
All samples were carefully ground in a mill mill,
ground for six hours in a mill mill, and then stored in bottles.

Analysis of the meals yielded the following data:

	<u>Protein, %</u>	<u>Moisture, %</u>
Idabel	29.6	6.76
Chancellor	25.4	8.37
Early Blue	27.1	8.58
Linseed Oil Meal	40.9	8.64
Alfalfa Leaf Meal	27.6	7.72

The term "protein" is used throughout this study to include all nitrogenous compounds present in the meals.

Methods

Protein Dispersion

Since the weights of meal, quantities of solvent, and the methods of extraction varied, details are given for each individual experiment. The flasks containing the meal-solvent mixtures were centrifuged for 10 minutes at 2000 r.p.m. and the supernatant liquid decanted. The nitrogen content of the extract was determined on an aliquot. All extractions were made at room temperature and in duplicate.

Oil Extraction

A 10-gram sample of meal was extracted with 50 ml. of ether, and shaken intermittently for a period of two hours. Two subsequent extractions, each with 50 ml. of ether and of one-half hour duration, gave complete extraction.

Analysis of the results of the following tests:

Protein	Protein
8.75	8.75
8.75	8.75
8.75	8.75
8.75	8.75
8.75	8.75
8.75	8.75

The term "protein" is used throughout this report to include all nitrogenous compounds present in the media.

Methods

Protein Determination

Since the content of protein in the media was known, the amount of protein in the media was determined by the method of Lowry (1956). The tissue homogenates were centrifuged for 10 minutes at 2000 r.p.m. and the supernatant liquid assayed. The nitrogen content of the extracts was determined on an aliquot. All determinations were made at room temperature and in duplicate.

Oil Extraction

A 10-gram sample of meat was extracted with 50 ml. of ether, and shaken thoroughly for a period of 24 hours. Two subsequent extractions, each with 50 ml. of ether, were made. The ether was removed by evaporation, and the residue was dried in a vacuum oven at 40°C. for 24 hours.

Nitrogen

The Kjeldahl method with mercuric oxide as catalyst was used. The protein was calculated as $N \times 6.25$.

Experimental Results

Pea Meal

The protein was extracted from 6-gram samples of pea meal with three successive treatments of one-half hour duration, using 50 ml. portions of the dispersing agent.

Experiment I

The effect of shaking during extraction on the dispersion of pea meal protein:

The protein of Idabel pea meal was extracted with distilled water using two methods of shaking:

- (a) Continuous shaking for one-half hour;
- (b) Intermittent shaking - 2 minutes shaking, 5 minutes at rest, for one-half hour.

The results as shown in Table II would appear to indicate that excessive agitation lowers the dispersion of pea meal protein. The difference was so slight, however, that not much significance may be attached to it.

All subsequent extractions of pea meal

10-97

126 301

protein were made using intermittent shaking.

TABLE II

The dispersion of pea meal protein as affected
by shaking during extraction

Method of shaking	Protein extracted, %			
	Extraction			Total
	I	II	III	
Intermittent	46.3	36.7	6.8	89.8
Continuous	45.0	33.3	8.0	86.3

Experiment II

The dispersion of the protein of Idabel pea meal in different concentrations of neutral salt solutions:

The protein was dispersed in concentrations of 1%, 2%, 3%, and 4%, of the three salts--sodium chloride, sodium salicylate, and sodium sulphate. The figures for dispersion in distilled water are included for comparison. The results are presented in Table III and Figure 1. The protein dispersed in 1% solutions was in every case less than that dispersed in distilled water. This decreased dispersion is similar to the effect of weak neutral salt solutions on the dispersion of soybean protein as reported by Smith and Circle (28).

protein were also being investigated.

TABLE II

The dispersion of the heat protein as observed
in shaking during experiments

Heat Protein				
Method of shaking	I	II	III	Total
Intermittent	46.5	50.7	50.5	50.5
Continuous	46.0	50.5	50.5	50.5

Experiment II

The dispersion of the protein of heat was
studied in different concentrations of sodium
chloride:

The protein was dispersed in concentrations of
1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%. The results are presented in Table III and Figure 1. The protein dispersed in 1% solution was in every case less than that dispersed in distilled water. This decrease in dispersion is similar to the effect of salt on the dispersion of the protein of sodium chloride as reported by Smith and Circle (35).

As one would expect, the decrease in dispersion varied with the salt used. Maximum dispersions were obtained in 3% and 4% concentrations. The results for the chloride and sulphate ions agree with the results of Staker and Gortner (30).

TABLE III

The dispersion of Idabel pea meal protein in neutral salt solutions

Solvent	Protein extracted, %			
	Extraction			Total
	I	II	III	
Distilled H ₂ O	46.3	36.7	6.8	89.8
NaCl 1%	49.3	9.6	4.3	63.1
2%	77.0	12.6	3.5	93.1
3%	80.5	11.6	2.5	94.6
4%	82.2	10.2	1.9	94.3
NaSal. 1%	62.3	12.1	4.0	78.4
2%	78.5	10.7	2.5	91.7
3%	83.0	9.6	1.5	94.1
4%	83.8	9.5	1.4	94.7
Na ₂ SO ₄ 1%	74.9	11.4	2.9	89.2
2%	78.5	10.7	2.2	91.4
3%	79.0	11.2	2.3	92.5
4%	80.4	10.0	2.1	92.5

Figure 1

The dispersion of Idabel pea meal protein
in neutral salt solutions of
various concentrations

Experiment III

A comparison of the dispersion of the protein of Idabel, Chancellor, and Early Blue pea meals in 3% sodium chloride:

The results are reported in Table IV. The dispersion of the protein in each variety was well over 90%. The results of this experiment indicate that the solubility of the nitrogen-containing constituents may vary from sample to sample.

TABLE IV

The dispersion of the protein of different varieties of peas in 3% NaCl

Variety	Protein extracted, %			
	Extraction			Total
	I	II	III	
Early Blue	83.3	11.5	2.5	97.3
Chancellor	78.6	11.5	2.7	92.9
Idabel	80.5	11.6	2.5	94.1

Linseed Oil Meal

The dispersion of linseed oil meal was studied in the following experiments, using three successive extractions of one-half hour duration on the mechanical shaker:

Experiment III

A comparison of the dispersion of the protein

of labeled, Chancellor, and Early Blue beans in 3%

sodium chloride:

The results are reported in Table IV. The dis-

tribution of the protein in each variety was well over

50%. The results of this experiment indicate that the

solubility of the nitrogen-containing compounds was

very from sample to sample.

TABLE IV

The dispersion of the protein of different
varieties of beans in 3% NaCl

Protein extract, %				
Variety				
Distribution				
Total	I	II	III	Total
37.3	33.8	11.8	4.5	37.3
38.8	38.3	11.3	8.7	38.8
34.1	30.5	11.8	2.5	34.1

Linseed Oil Seed

The dispersion of linseed oil seed was studied in

the following experiments, using three successive extractions

of one-half hour duration on the mechanical shaker:

Experiment I

The meal-solvent ratio giving maximum dispersion of linseed oil meal protein:

Samples of "natural" and "oil-free" linseed oil meal weighing 2 grams were extracted with 50 ml. and 100 ml. portions of 3% NaCl. The results are summarized in Table V.

TABLE V

The meal-solvent ratio giving maximum dispersion of linseed oil meal protein

Meal	Volume of solvent, ml.	Protein extracted, %
Oil-free	50	64.3
	100	65.8
Natural	50	61.4
	100	63.6

The greater solubility of the protein of the "oil-free" samples should be noted. The slight increase in dispersion obtained using 100 ml. of solvent can scarcely be considered significant. Despite this, in the following experiments 100 ml. of solvent were used in each extraction for every 2 grams of meal.

Experiment I

The meal-solvent ratio giving maximum disper-

sion of linseed oil meal protein

samples of "natural" and "oil-free" linseed

oil meal weighing 2 grams were extracted with 50 ml. and 100 ml. portions of 2% NaCl. The results are summarized

in Table V.

TABLE V

The meal-solvent ratio giving maximum dispersion of linseed oil meal protein

Meal	Volume of solvent, ml.	Protein extracted, g.
Oil-free	50	84.8
	100	83.8
Natural	50	81.4
	100	83.8

The greater solubility of the protein of the "oil-

free" samples should be noted. The slight increase in dis-

persions obtained using 100 ml. of solvent can scarcely be con-

sidered significant. Despite this, in the following experiments

100 ml. of solvent were used in each extraction for study 2

grams of meal.

Experiment II

The dispersion of linseed oil meal protein in various solvents:

A. As a preliminary test the dispersion of the protein of the "oil-free" and of the "natural" linseed oil meals was determined in 3%, 6%, and 10% sodium chloride. The results are reported in Table VI, with the dispersion in distilled water given for comparison.

TABLE VI

The dispersion of linseed oil meal protein in various concentrations of NaCl

Solvent	Meal	Protein extracted, %			
		Extraction			Total
		I	II	III	
Distilled water	Oil-free	25.9	11.1	5.8	42.8
NaCl 3%	Natural	50.4	10.4	2.8	63.6
	Oil-free	51.4	11.4	3.0	65.8
6%	Natural	67.7	6.1	1.0	74.8
	Oil-free	67.0	6.5	1.1	74.6
10%	Natural	66.9	6.2	1.2	74.3
	Oil-free	67.0	6.7	1.2	74.9

Maximum dispersion was obtained in the 6% and 10% solutions. From the practical point of view no significance can be attached to the differences

between the dispersion of the protein of the natural and oil-free meals, although the slight difference in favor of the oil-free meal is probably real.

B. The dispersion of the protein of linseed oil meal was determined in 4% sodium sulphate, 8% sodium salicylate, 6% sodium carbonate, 7% ammonium sulphate, and 0.5% NaOH. The pH of the ammonium sulphate solution was adjusted to 7.0. Dispersion values for distilled water and 6% NaCl are included for comparison. Table VII summarizes the data.

TABLE VII

The dispersion of the protein of linseed oil meal in various solvents

Solvent	Meal	Protein extracted, %			
		Extraction			Total
		I	II	III	
Distilled water	Oil-free	25.9	11.1	5.8	42.8
NaCl 6%	Natural	67.7	6.1	1.0	74.8
	Oil-free	67.0	6.5	1.1	74.6
Na ₂ SO ₄ 4%	Natural	63.6	6.7	0.8	71.1
(NH ₄) ₂ SO ₄ 7%	Natural	62.8	7.5	1.5	71.8
	Oil-free	63.1	8.2	2.4	73.7
Na Sal. 8%	Natural	65.8	8.4	1.1	75.4
	Oil-free	62.2	12.5	2.1	76.8
Na ₂ CO ₃ 6%	Oil-free	64.4	8.7	0.8	73.8
NaOH 0.5%	Natural	86.5	5.6	0.3	92.4

between the dispersion of the protein in the water and oil-free media, showing the slight difference in favor of the oil-free media is readily seen.

5. The dispersion of the protein in limited oil media was determined in 5% sodium sulfate, 5% sodium sulfate, 5% sodium carbonate, 7% ammonium sulfate, and 0.5% NaCl. The η_{sp}/c of the ammonium sulfate solution was adjusted to 7.0. Dispersion values for distilled water and 5% NaCl are included for comparison. Table VII summarizes the data.

TABLE VII

The dispersion of the protein in limited oil media in various solvents

Protein concentration, %					
Dispersion					
Solvent	NaCl	Oil-free	Oil	Water	Oil-free
Distilled water	42.0	3.8	11.1	35.8	Oil-free
NaCl 5%	74.8	1.0	8.1	57.7	Water
	74.8	1.1	8.5	57.0	Oil-free
Na ₂ SO ₄ 5%	41.1	0.8	8.7	55.8	Water
(NH ₄) ₂ SO ₄ 7%	71.8	1.5	7.5	55.8	Water
	73.7	2.4	8.5	55.1	Oil-free
Na ₂ SO ₄ 10%	73.8	1.1	8.4	55.3	Water
	73.8	2.1	12.5	55.2	Oil-free
Na ₂ CO ₃ 5%	73.8	0.8	5.7	54.4	Oil-free
Na ₂ CO ₃ 0.5%	52.4	0.5	5.5	55.5	Water

Although the dispersion of the protein of the "oil-free" meals tends to be higher, the differences are so slight that they are of no practical importance.

The dispersions in the neutral salt solutions all fell within the range of from 71% to 77%. It is open to question whether the dispersion in 8% sodium salicylate is significantly higher than that in 6% sodium chloride.

The 0.5% NaOH gave the highest extraction. This was expected as many compounds are soluble in alkaline solutions. This extract cannot, however, be used in amino acid analysis for two reasons: firstly, much interfering material is present in the extract; and secondly, alkaline solutions alter the chemical properties of proteins (18).

Experiment III

The dispersion of the protein of linseed oil meal in 6% NaCl solutions adjusted to various acidities:

The pH's of the solutions were adjusted with dilute HCl. Table VIII summarizes the results.

Although the dispersion of the particles of the
"colloidal" water seems to be higher, the dispersion is
so slight that it is not noticeable.
The dispersion in the water and solution
all fall within the range of from 10 to 100. It is only
in question whether the dispersion is so slight as to be
significantly higher than that in the water.
The 0.5% water gave the highest dispersion.
This was expected as many compounds are soluble in water.
This experiment. This experiment cannot, however, be used
in water and analysis for the reason: firstly, when
interfering material is present in the solution; and
secondly, alkaline solution gives the chemical reaction
of the solution (10).

Experiment III

The dispersion of the particles of the water is
only in 0.5% solution and is not very noticeable;
the 0.5% of the solution is not very noticeable.
The 0.5% water gives the highest dispersion.

TABLE VIII

The dispersion of the protein of linseed oil meal in 6% NaCl solutions adjusted to various acidities

pH	Protein extracted, %			
	Extraction			Total
	I	II	III	
2.3	54.0	2.1	0.8	56.9
3.1	61.8	6.7	1.0	69.5
4.1	62.9	7.1	1.3	71.3
5.3	63.4	7.1	1.1	71.5
6.4	64.9	6.9	1.1	72.9
7.0	64.7	6.2	1.3	72.2

No appreciable differences in dispersion were noted in the pH range 4 to 7. In solutions of increased acidity there was an indication that protein dispersion decreased markedly. Rich (23) reports similar results with wheat gluten. The dispersion of gluten in dilute magnesium sulphate solutions was very little influenced by the pH unless the solution was strongly acid or alkaline. Smith, Circle, and Brother (29), however, obtained maxima and minima dispersions of soybean proteins by altering the pH of salt solutions.

The decreased dispersion of the protein of linseed oil meal in 6% NaCl in this experiment, as compared with that reported in Experiment II, is attributed to the effects of three months' storage of the meal.

TABLE VIII

The dispersion of the emulsion of linseed oil
 made in 6% well solutions adjusted
 to various acidities

Protein extracted				
Dispersion				
%	I	II	III	Total
2.5	54.0	7.1	0.8	58.9
3.1	61.8	6.7	1.0	59.5
4.1	68.0	7.1	1.3	71.3
5.3	68.4	7.1	1.1	71.3
6.4	64.9	6.9	1.1	72.9
7.0	64.7	6.8	1.3	72.8

No appreciable differences in dispersion were
 noted in the pH range 4 to 5. In solutions of increased
 acidity there was an indication that protein dispersion
 decreased markedly. Also (23) the other similar results
 with wheat gluten. The dispersion of gluten is almost
 negligible in acidic solutions and very little influenced
 by the pH value the solution was strongly acid or
 alkaline. Salt, chloride, and protein (24), however,
 obtained maximum and minimum dispersions of systems pro-
 teins by altering the pH of salt solutions.
 The bestest dispersion of the protein of
 linseed oil was in 6% well in this experiment, as was
 noted also that reported in experiment II, as mentioned
 to the effects of these various 'stages' of the seed.

Smith and Circle (28) reported that the aging of soybean meal decreased the water-soluble nitrogen. This decrease was even greater with salt-soluble nitrogen.

Alfalfa Leaf Meal

The protein was extracted from 2-gram samples with three successive treatments of one-half hour duration on the mechanical shaker, using 100 ml. portions of the dispersing agent.

Experiment I

The dispersion of alfalfa leaf meal protein in various solvents:

The concentrations of the neutral salt solutions were selected because they had given the maximum dispersion obtainable with other proteins. Comparative results for distilled water, 6% NaCl, 8% sodium salicylate, 8% potassium iodide, 0.5% HCl, and 0.5% NaOH are given in Table IX.

Smith and Smith (1931) reported that the same

of which was collected and was collected in 1931.

It is believed that the same was collected in 1931.

Smith and Smith

The results were summarized in Table 1.

Three descriptive measurements of one-half inch diameter on the

measured number, being 100 in. diameter of one diameter.

Table 1.

Table 1

The distribution of the data is given in

Table 1.

The concentration of the data is given in

Table 1.

Table 1.

Table 1.

Table 1.

Table 1.

TABLE IX

The dispersion of alfalfa leaf meal protein
in various solvents

Solvent	Protein extracted, %			Total
	Extraction			
	I	II	III	
Distilled water	42.1	5.5	2.1	49.7
NaCl 6%	49.2	6.6	1.3	57.1
Na Sal. 8%*	41.3	13.1	3.8	58.2
KI 8%	54.3	5.8	2.3	62.4
HCl 0.5%	41.9	5.7	2.8	50.4
NaOH 0.5%	75.0	8.2	0.9	84.1

* Three 40-ml. extractions

The results obtained indicate that 8% KI has the strongest dispersing action. As might be expected, the 0.5% HCl dispersed no more protein than water. Higher extraction was again obtained with 0.5% NaOH. For reasons stated previously this protein extract cannot be used for accurate analytical work.

Experiment II

The dispersion of alfalfa leaf meal protein in 6% NaCl adjusted to various acidities:

The pH was adjusted using dilute HCl. The results are recorded in Table X. The dispersions obtained resemble those reported previously for the protein of linseed oil meal in similar solutions. Over a

TABLE IX

The dispersion of alfalfa leaf meal protein in various solvents

Protein extracted				
Extraction				
Solvent	I	II	III	Total
Distilled water	42.1	5.3	2.1	49.5
NaOH 0.5%	43.2	5.3	1.3	50.0
NaOH 0.5%*	41.5	13.1	5.3	60.0
NaOH 0.5%	42.8	5.3	5.3	53.4
NaOH 0.5%	41.5	5.3	5.3	52.1
NaOH 0.5%	43.0	5.3	5.3	53.6

* Three 40-ml. extractions

The results obtained indicate that 0.5% NaOH is the strongest dispersing agent. It might be expected, the 0.5% NaOH dispersed no more protein than water. Higher extraction was again obtained with 0.5% NaOH. For reasons stated previously this protein extract cannot be used for accurate analytical work.

Experiment II

The dispersion of alfalfa leaf meal protein in 0.5% NaOH adjusted to various pH values:
The pH was adjusted using dilute HCl. The results are recorded in Table X. The dispersions obtained resemble those reported previously for the dispersion of alfalfa leaf meal in dilute solutions. Over a

pH range of 3.1 to 7.0 no significant difference in dispersion was noted. It would appear that in more acidic solutions protein dispersion is depressed.

TABLE X

The dispersion of alfalfa leaf meal protein
in 6% NaCl solutions adjusted
to various acidities

Protein extracted, %				
Extraction				
pH	I	II	III	Total
2.3	37.5	3.7	1.0	42.2
3.1	43.7	4.4	1.4	49.5
4.1	43.3	4.6	1.3	49.2
5.3	43.1	4.7	1.4	49.2
6.4	43.7	4.8	1.4	49.9
7.0	43.6	4.8	1.4	49.8

The decreased dispersion of the protein in 6% NaCl in this experiment as compared with the dispersion reported in the previous experiment is attributed to the effects of three months' storage.

Extraction with the Waring Blendor

The Waring Blendor effects a very fine division of meals. Protein dispersion obtained using the Waring Blendor was compared with that obtained using the ball mill.

At times of 2.1 to 2.5 no significant difference in absorption was noted. It would appear that in some cases the solution protein degradation is decreased.

TABLE I

The dispersion of silica test with protein
detected in 60 test solutions adjusted
to various acidities

Protein extracted				
Acidity				
Total	III	II	I	IV
47.5	1.0	2.7	30.5	2.5
43.5	1.4	2.4	48.7	3.1
39.5	1.5	2.4	45.3	4.1
40.5	1.4	2.7	48.1	3.3
43.0	1.2	2.8	48.9	2.2
49.5	1.2	2.2	45.5	1.0

The decreased dispersion of the protein in
of HCl in this experiment as compared with the
eration reported in the previous experiment is attrib-
uted to the effects of these various acidities.

Extraction with the various acids

The silica dispersion showed a very fine division of
acids. Protein dispersion showed only one very narrow
and compared with that obtained with the silica.

Material and Method

Idabel peas and ether-extracted linseed oil meal were used. The protein was dispersed by two methods:

1. The meals were ball milled for six hours and the protein extracted in the usual manner by three successive half-hour treatments on the mechanical shaker.

2. The peas were burr milled. The linseed meal was not ground further. The meal and the solvent were introduced into the Waring Blendor, and whipped for a period not exceeding six minutes until the meal was very finely divided. The mixture was transferred to centrifuge bottles and shaken for 25 minutes on the mechanical shaker. The mixtures were centrifuged for 10 minutes at 2000 r.p.m. The supernatant liquid was poured off and the nitrogen content determined on an aliquot. Second and third extractions were made in the usual manner on the mechanical shaker.

The quantities of materials used may be tabulated as follows:

<u>Material</u>	<u>Weight (gm.)</u>	<u>Treatment</u>	<u>Solvent</u>	<u>Volume (ml.)</u>
Idabel peas	6	Ball mill	3% NaCl	50
" "	30	W. Blendor	"	250
Linseed oil meal	2	Ball mill	6% NaCl	100
(oil-free)	5	W. Blendor	"	250

Material and Method

Labels were not used in the first trial. The protein was obtained by the method of...

1. The protein was obtained by the method of... and the protein extracted in the same manner by these successive half-hour treatments of the material.

2. The case was then dried. The finished meal was not found to be... were introduced into the system... period not exceeding six minutes until the meal was very finely divided. The mixture was transferred to two 1000 cc bottles and shaken for 30 minutes on the mechanical shaker. The mixture was centrifuged for 10 minutes at 2000 r.p.m. The supernatant liquid was poured off and the nitrogen content determined on an aliquot. Second and third extractions were made in the same manner on the mechanical shaker.

The quantity of material used may be stated as follows:

Labelled as follows:

Label	Weight (gm.)	Treatment	Solvent	Volume (ml.)
Labelled case	0	Oil-soluble	Oil-soluble	50
" "	50	"	"	"
Labelled oil meal	2	Oil-soluble	Oil-soluble	50
(oil-free)	2	"	"	"

Larger volumes of liquid were found more suitable in the Waring Blendor. Constant meal:solvent ratios were maintained for the individual meals. The results are presented in Table XI.

TABLE XI

A comparison of protein dispersion as affected
by two methods of meal division

Material	Treatment*	Protein extracted, %
Linseed oil meal (oil-free)	1	72.6
	2	76.3
Pea meal (Early Blue)	1	97.4
	2	98.1

- * 1 - Meal finely ground by the ball mill
2 - Meal finely divided by the Waring Blendor

The Waring Blendor showed two advantages:

1. It eliminated the time-consuming operation of ball milling.

2. It gave extraction values equal to, if not greater than, grinding with the ball mill.

It had, however, several disadvantages:

1. The solutions heated while the blendor was in operation. The longer the period of operation the higher the temperature rose. Readings up to 49°C. were recorded.

largest volumes of liquid were found with-
out in the working chamber. Comparison of results
were maintained for the following results. The results
are presented in Table II.

TABLE II

A comparison of results obtained as effected
by two methods of meal division

Material	Experiment*	Results extracted
Mashed oil meal (oil-free)	1 2	98.5 98.7
90% meal (early phase)	1 2	98.5 98.1

* 1 - Meal finely ground by the ball mill
2 - Meal finely divided by the working chamber

The working chamber showed the advantages:
1. It eliminated the time-consuming operation
of ball milling.
2. It gave extraction values equal to, if not
greater than, grinding with the ball mill.
It had, however, several disadvantages:
1. The millstone heated with the digester was
in operation. The longer the period of operation the
higher the temperature rose. Results up to 200° were
recorded.

2. The blades travelling at very high speeds caused extreme foaming in the solution. Foaming and denaturation of protein are directly related.

3. Centrifuging the blended material was very difficult. Two possible explanations for this have been advanced. The particle size may be so reduced by blending that the mixture approached a colloidal solution, or it may be that films of solvent were adsorbed to the surface of the particles, thereby lowering their specific gravity.

2. The disease travelling at very high speeds

greatly reduces the number of the animals. The number and

duration of infection are directly related.

3. Infection of the animal material was very

difficult. Two possible explanations for this have been

advanced. The infection may be so localized or

ing that the animal requires a certain amount of

it may be that the animal is not exposed to the

source of the infection, thereby lowering the

the gravity.

THE ELECTRICAL TRANSPORT METHOD OF BASIC AMINO ACID ANALYSIS

Literature Review and Discussion

Proteins are a group of very complex chemical substances. On hydrolysis they are characterized by the fact that they yield more than twenty alpha-amino acids.

These alpha-amino acids can be divided into three groups: acidic, basic, or neutral, according to their reaction. The reaction is dependent upon the relative strength and number of the basic and acidic radicals present in the individual amino acid. The basic alpha-amino acids are arginine, lysine and histidine. Harrow (11) reports two others--ornithine and citrulline--which are related to arginine but are not considered as occurring naturally in ^{most} plant material.

To date there have been developed five methods of separating the basic group from the remainder of the protein hydrolysate (3). Of these five, two have been adapted to the quantitative estimation of arginine, histidine, and lysine. The other three, as yet, are not satisfactory for the quantitative isolation of the group.

Most of the early work on the basic amino acid content of proteins was based on the use of phosphotungstic acid

THE HISTORICAL DEVELOPMENT OF

AMINO ACID ANALYSIS

INTRODUCTION AND DISCUSSION

Proteins are a group of very complex chemical substances. In hydrolysis they are converted into amino acids. These amino acids may be divided into three groups: acidic, basic, or neutral, according to their reaction. The reaction is dependent upon the relative strength and number of the basic and acidic radicals present in the individual amino acid. The basic amino acids are arginine, lysine and histidine. Harnad (1) reported two other--ornithine and citrulline--which are related to arginine but are not considered as essential nutrients in metabolism. To date seven have been developed five methods of separating the basic from the remainder of the hydrolyzate (2). Of these five, two have been abandoned in the quantitative estimation of arginine, histidine, and lysine. The other three, as yet, are not satisfactory for the qualitative isolation of the group. Most of the early work on the basic amino acid content of proteins was based on the use of phosphomolybdic acid

as a precipitant for this group. It is not completely satisfactory, although it forms an integral part of the Van Slyke distribution method of analyzing proteins. As much as 50% of the cystine present is also precipitated and this necessitates an additional analysis for sulphur. Mitchell and Hamilton (18) discussed the errors involved in the phosphotungstic precipitation. These may be summarized as follows:

1. Tryptophane, phenylalanine, and proline may form a comparatively insoluble phosphotungstate.
2. Completeness of precipitation depends to a large extent upon the conditions of precipitation.
3. Standard correction factors are not entirely satisfactory. The solubility of the bases may vary with their concentration. A correction comparatively small in magnitude may be quite large in proportion when the bases are present in small amounts.
4. Nitrogen may be lost on decomposition of the precipitate.

The second method is that of electrical transport. The first record of the use of an electric current to separate the amino acids into fractions is a U.S. patent taken out in 1912 by Ikeda and Suzuki (8) for the electrical separation of glutamic acid. Foster and Schmidt (8, 9) found that, at pH 5.5, a protein hydrolysate can be separated into three fractions by an electric current. The three bases were carried to the cathode while the more acidic amino acids, aspartic,

as a preliminary to this study. It is not necessarily correct, although it is based on a general view of the way in which the various factors of the system are related. It is not necessarily correct, although it is based on a general view of the way in which the various factors of the system are related.

There are additional factors for which the system is not necessarily correct, although it is based on a general view of the way in which the various factors of the system are related.

Hamilton (19) discusses the errors involved in the above-mentioned analysis. These may be summarized as follows:

1. Hypotheses, assumptions, and results may

be a consequence of the system, and not necessarily correct, although it is based on a general view of the way in which the various factors of the system are related.

2. Completion of the system, and not necessarily correct, although it is based on a general view of the way in which the various factors of the system are related.

3. The system is not necessarily correct, although it is based on a general view of the way in which the various factors of the system are related.

4. The system is not necessarily correct, although it is based on a general view of the way in which the various factors of the system are related.

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The system is not necessarily correct, although it is based on a general view of the way in which the various factors of the system are related.

glutamic, and hydroxy-glutamic acids were carried to the anode. The amino acids which are neutral in reaction were not affected. The method of electrical transport was found applicable to the preparation of pure arginine and lysine in relatively large quantities.

Cox, King, and Berg (6) found the method, with modifications, to be almost entirely satisfactory for the individual isolation of arginine, histidine, and lysine.

Albanese (1) improved upon the methods and the equipment used previously. He reported a method of determining the basic amino acid content of protein hydrolysates, involving the quantitative transport by an electric current, of arginine, histidine, and lysine to the cathode.

The work reported in the following section is an investigation of the efficiency of the electrolytic cell and of subsequent methods of analysis of the catholyte.

Apparatus

Description of the Electrolytic Cell

The apparatus used was built in the laboratory and is similar to that used by Albanese (1). (Plates 1 and 2).

The cell, made of birchwood, consists of three sections. All surfaces are water proofed with successive coats of spar varnish. In addition, the inner and adjoining

glutamic, and glycero-phosphoric acids with details of the method. The amino acids which are neutral in reaction were not attempted. The method of electrical transport was found applicable to the preparation of pure arginine and lysine in relatively large quantities.

Cori, Kline, and Park (3) found the method, with modifications, to be almost entirely satisfactory for the isolation and isolation of arginine, histidine, and lysine. Allmann (1) improved upon the methods and the equipment used previously. He reported a method of determining the basic amino acid content of protein hydrolyzates, involving the quantitative conversion by an electric current of arginine, histidine, and lysine to the acetate. The work reported in the following section is an investigation of the efficiency of the electrolytic cell and of subsequent methods of analysis of the electrolyte.

Apparatus

Description of the Electrolytic Cell

The apparatus used was built in the laboratory and is similar in some respects to Allmann's (1). (Figures 1 and 2). The cell, made of blackwood, consisted of three sections. All surfaces were glass coated with an aqueous solution of silver nitrate. The bottom and side walls

Plate 1
The Electrolytic Cell

Plate 2

Constructional details of the
electrolytic cell

surfaces are coated with wax. The sections are separated by dissimilar membranes, and each resulting chamber has an approximate volume of 125 ml. Surfaces between sections are kept water-tight with rubber gaskets coated with a thin film of paraffin wax. Four long bolts hold the cell tightly together. Taps of rubber hose are used to drain the contents of the anode and cathode chambers.

The use of dissimilar membranes is necessary to prevent endosmotic action. The anodic membrane is similar to that used by Albanese. Linen is coated with gelatin. The gelatin is subsequently hardened by soaking in 4% formaldehyde solution. The wet membrane is stretched tightly over a frame and allowed to dry. The cathodic membrane is cellophane dialysis paper.

The cathode is 45-mesh platinum gauze, two inches by two inches, with wire edges. A two-inch post extends down the middle of the electrode. The anode is cut from one-quarter-inch sheet carbon, two inches by two and one-quarter inches. This arrangement has been found to be quite satisfactory, except that the carbon anode gradually disintegrates, necessitating frequent replacements. The particles of carbon become dispersed throughout the anode chamber and cover the membrane surface. In an attempt to overcome this, glazed brush carbon was tried. This electrode stood up for 15 to 20 hours of operation. Once the outer glazed surface disintegrated the electrode wore away more rapidly than the carbon in regular

use.

The solutions present in the cell heat when subjected to electrolysis. U-tubes containing running water (20°C.) are passed through the anode and cathode chambers to offset this.

Stirring of the solutions in all three compartments is necessary to effect complete separation. This agitation is obtained in the anode compartment by the release of chlorine and in the cathode compartment by the release of hydrogen. The central compartment is stirred with a Cenco variable speed stirrer.

The electrical circuit consists of a 100-watt lamp, a milliamp ammeter, and the cell electrodes connected in series to a 110-volt D.C. line (see Plate 2).

Operation of the Electrolytic Cell

The following procedure, based on that outlined by Albanese, was adopted:

Hydrolysis

A 1- to 2-gram sample of protein is hydrolyzed for 24 hours with 5 ml. per gram of 20% HCl (using all glass apparatus). The hydrolysate is diluted to 100 ml. in a volumetric flask and total N. is determined by the Kjeldahl procedure. An aliquot representing 0.5 to 1.0 grams of protein is used for electrolysis.

Electrolytic Technique

The anode and cathode compartments are filled with distilled water, the middle chamber being only half filled. The current is turned on and the sample is pipetted into the middle compartment, which is then filled with distilled water to the same level as the others. The completion of electrolysis is indicated by current drop as registered on the ammeter.

The contents of the cathode compartment are then run into a 150-ml. beaker. The chamber is washed several times with distilled water and the washings added to the original catholyte. The current is now turned off. The contents of the anode and middle chamber are discarded.

For complete separation of histidine, lysine, and arginine, the foregoing procedure must be carried out twice. The first separation eliminates HCl by electrolysis, the hydrogen being released at the cathode, and the chlorine escaping at the anode. All but the more acidic of the amino acids are carried by electrical transport to the cathode. The transport of the basic amino acids alone is effected by the second separation at pH 5.5. This latter separation is essentially electrodialysis, since it is accompanied by no decomposition.

Electrolytic Treatment

The anode and cathode compartments are filled with distilled water, the anode compartment being only half filled. The current is turned on and the anode is lifted into the middle compartment, which is then filled with distilled water to the same level as the cathode. The migration of electrolyte is indicated by current drop as registered on the meter.

The contents of the cathode compartment are then run into a 100-ml. beaker. The solution is washed several times with distilled water and the washings added to the original cathode. The current is now turned off. The contents of the anode and middle compartments are discarded.

For complete separation of lithium, iodine, and arginine, the foregoing procedure must be carried out twice. The first separation eliminates 90% of lithium, the hydrogen being released at the cathode, and the chlorine escaping at the anode. All but the more soluble of the amino acids are carried by electrical transport to the cathode. The transport of amino acids alone is effected by two current separations at 2.5 v. This latter separation is essential for electrolysis, there is no decomposition of amino acids.

Electrolysis

The current rises rapidly to 750 to 800 milliamps shortly after the sample is introduced and it remains at this point for about six hours. At hourly intervals approximately half the contents of the anode chamber are removed and replaced by distilled water. Electrolysis is complete when the current drops to 100 to 200 milliamps.

Dialysis

The primary catholyte is adjusted to pH 5.6 to 5.8 with dilute H_2SO_4 using a potentiometer equipped with outside electrodes. This solution is introduced into the middle compartment as before. The current rises rapidly to about 450 to 500 milliamps and then drops slowly to 50 to 75 milliamps in about 45 minutes, when the dialysis is complete.

Methods

Nitrogen

Nitrogen was determined by the modified Kjeldahl-Gunning-Arnold method, except where the micro-Kjeldahl method is specified. In the latter method, ammonia was caught in a 2% boric acid solution. The ammonia was titrated with N/100

Electrolysis

The current rises rapidly to 750 to 800 amperes and drops shortly after the anode is introduced and it remains at this value for some time. It slowly increases appreciably with the amount of the anode consumed and reaches a maximum of 1000 amperes at 100 to 120 minutes.

Results

The primary catholyte is analyzed as follows: 0.5 g. with dilute H₂SO₄ using a potentiometer equipped with outside electrodes. This solution is introduced into the anode compartment as before. The current rises rapidly to about 400 to 500 milliamperes and then slowly to 70 to 75 milliamperes in about 45 minutes, when the electrolyte is complete.

Electrolysis

Materials

Electrodes are prepared by the method described in the preceding section. The electrolyte is prepared by dissolving 10 g. of sodium hydroxide in 100 ml. of water. The anode is made of lead. The cathode is made of zinc.

HCl, using a mixed indicator of methyl red and methylene blue.
(27, 31)

Qualitative Tests

The precipitation reaction of the basic amino acids with phosphotungstic acid was used as a general qualitative test.

The diazo reaction, which is sensitive to one part of histidine in 200,000 and the Sakaguchi reaction, positive to one part of arginine in 2,500,000, were used as specific colorimetric tests (14, 35).

The presence of ammonia was detected with Nessler's reagent.

Experimental Results

The Efficiency of Electrical Transport

Two experiments were made to determine the completeness of the transport and of the separation of the basic amino acids in the electrolytic cell.

Experiment I

The dialysis of arginine HCl:

Samples of relatively high and low arginine content were dialyzed. The arginine was precipitated

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the above points. It is very difficult to

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with flavianic acid. A solubility correction for arginine monoflavianate of one gram arginine N per 100 ml. solution was applied. Three solutions containing 12.43 mg., 20.70 mg., and 20.70 mg. arginine nitrogen were dialyzed. The percentage recoveries were 99.4%, 98.6%, and 100.5%, respectively.

Experiment II

The electrical transport of the basic (amino acid) nitrogen of an amino acid mixture:

The mixture simulated a protein hydrolysate containing amino acids of all reaction types. A solution containing 12.48 mg. N as glycine (neutral), 5.42 mg. N as aspartic acid (acidic), 12.64 mg. N as arginine HCl, 5.05 mg. N as lysine HCl, and 4.00 mg. N as histidine HCl (all basic) was introduced into the cell. The electrolysis was complete in one hour; the dialysis in 25 minutes. The resulting catholyte contained 21.79 and 21.87 (duplicate) mg. N as compared with the original 21.74 mg. basic N. The percentage recoveries were therefore 100.2% and 100.6%, respectively

Qualitative tests were made during the transport of Sample I. Negative Sakaguchi, diazo, and phosphotungstic acid tests were obtained on the contents of the central and anode compartments at the completion of electrolysis and dialysis. The final catholyte tested

positive for each of the three reactions.

This experiment indicates that the basic amino acids are transported quantitatively to the cathode chamber and that they may be effectively separated from a protein hydrolysate by electrical transport.

Analysis of the Catholyte Using Various Methods

The current transports ammonia as well as the three basic amino acids to the cathode. Before any subsequent analysis may be made this ammonia must be removed by aerating the catholyte. Ammonia-free air is drawn through the sample at 45°C. for one hour. Analyses have shown no loss of the basic amino acids by this procedure. Samples testing positive for ammonia with Nessler's reagent were aerated. At the end of one hour no ammonia could be detected.

Three methods of analysis were investigated.

Method I

The precipitation method as outlined by Albanese:

Procedure - separation and determination of arginine: To the second catholyte, made acid to methyl red with dilute sulphuric acid and heated on the steam bath, is added flavianic acid solution (1:2 moles per mole of arginine expected). After 10 minutes the mixture

positive for each of the three reactions.

This experiment indicated that the results

which are interpreted qualitatively by the chemical
reactions and that they may be effectively separated from
a complex mixture of chemical reactions.

Analysis of the Catalysts and the Reactions

The current theoretical approach to the study of the

basic nature of the catalysts, their role in the
analysis and the results of the reactions have been

the catalysts. Theoretical results are given in the
appendix to the report. The results have shown no loss of
activity at 450°C. for one hour. The results have shown
the basic nature of the catalysts. The results have shown
positive for reactions with hydrogen and carbon monoxide. At
the end of one hour no reaction could be detected.
These methods of analysis were investigated.

Appendix I

The following section is included for

reference:

Procedures - separation and identification of catalysts
To the present, the catalysts have been separated and
identified by the use of the following methods:
1. The catalysts were separated and identified by the use of
the following methods:
2. The catalysts were separated and identified by the use of
the following methods:
3. The catalysts were separated and identified by the use of
the following methods:

is allowed to cool to room temperature and stored in the ice box for several days. Arginine flavianate is filtered off, dried, and weighed. A solubility correction of 1.0 mg. of arginine nitrogen per 100 ml. of solution is applied to all reported values. The arginine nitrogen value calculated from the monoflavianate (11.48%) is reported as percent of the total nitrogen of the protein.

Electrical removal of flavianic acid: The filtrate after removal of arginine flavianate is introduced into the middle compartment of the cell. A drop of dilute sulphuric acid is added to each of the anode and cathode compartments to increase the initial conductivity. The flavianic acid then passes to the anode, histidine and lysine to the cathode. The time required is approximately one hour. The current rises rapidly to 400 milliamps and then slowly drops to 75 milliamps or lower.

Histidine determination: The alkaline catholyte is made just acid to methyl red with dilute HCl. Saturated mercuric chloride is added (4 moles per mole of histidine expected). Dilute NaOH is added drop by drop until the red color of methyl red is discharged and a pinkish tinge (pH 6.8 to 7.0) appears with phenol red when a white flocculent precipitate

is allowed to cool to room temperature and stored
in the ice box for several days. A typical result
made is shown in Fig. 1. The initial value of
solubility correction of 1.0 m. of organic nitro-
gen per 100 ml. of solution is applied to the
reported values. The organic nitrogen values are
calculated from the concentration (M. %). It is reported
as percent of the total amount of the protein.

Theoretical removal of flavinoids: The theoretical
after removal of organic flavinoids is calculated
from the initial concentration of the salt. A drop of
this solution is added to each of the samples
and suitable components to increase the initial
concentration. The flavinoids with their bases in
the sample, flavinoids and bases in the sample. The
time required is approximately one hour. The con-
centrations remain fairly constant and then slowly
drop to 1/2 of the original or lower.

Flavinoid determination: The standard solution is
made just as the sample and with the same salt. The
three organic nitro-
gen or flavinoid extracted. The initial value is added to
by drop until the color of the sample is changed
changed and a final value (0.5 to 1.0) is
with phenol and water a white precipitate (protein)

forms. The resulting mixture (about 100 ml.) is allowed to stand at room temperature for several hours, and is then centrifuged.

The precipitate is washed twice with about 10 ml. of water, dissolved in dilute HCl, made to volume and aliquots removed for micro-Kjeldahl determinations. The nitrogen content of the whole solution is estimated as histidine N, a percentage of the total N.

Lysine determination: The supernatant fluid and washings from the histidine determination are made to volume and nitrogen determined by the micro-Kjeldahl method. Total nitrogen of this fraction, less nitrogen due to the solubility of the arginine flavianate, is estimated as lysine nitrogen.

Experiment I - The analysis of a basic amino acid mixture:

A solution containing 7.70 mg. N as lysine HCl, 19.98 mg. N as histidine HCl, and 20.54 mg. N as arginine HCl was introduced into the cell.

The dialysis time was sixty minutes. The catholyte was aerated and flavianic acid added. No arginine flavianate was precipitated in a period of five days.

This test was repeated twice with similar results.

forms. The resulting mixture (about 100 ml.) is allowed to stand at room temperature for several hours, and is then centrifuged.

The precipitate is washed twice with about

10 ml. of water, dissolved in dilute HCl, and to

volume and aliquots removed for micro-diffusion determinations. The nitrogen content of the whole solution is estimated as nitride N, a percentage of the total N.

Analysis of the precipitate: The precipitate is washed from the insoluble determination and then to volume and nitrogen determined by the micro-diffusion method. Total nitrogen of this fraction, less nitrogen due to the solubility of the original hydrolyzate, is estimated as organic nitrogen.

Experiment I - The analysis of a basic amino acid mixture:

A solution containing 0.75 mg. N as lysine HCl, 10.35 mg. N as histidine HCl, and 20.54 mg. N as arginine HCl was introduced into the cell. The dialysis time was thirty minutes. The catholyte was removed and the amino acid added. No arginine fluorescence was precipitated in a volume of five days. This time was repeated twice with similar

results.

Experiment II - The electrolysis of a gluten hydrolysate:

The gluten was washed from wheat flour using distilled water, and hydrolyzed with 20% HCl for 24 hours. An aliquot of the hydrolysate, representing 102.8 mgm. N was used. The electrolysis required $6\frac{3}{4}$ hours; the dialysis, 1 hour.

The catholyte was aerated and flavianic acid added. No precipitate formed in a period of 3 days. At the end of 5 days a few crystals were observed. These were of two types: (a) orange platelets; (b) yellow needles. The former fitted the description of arginine monoflavianate; the latter were obviously arginine diflavianate.

Precipitation again was unsatisfactory. The poor results obtained may be due either to one or both of two factors:

1. the pH adjustment using indicators was unsatisfactory;
2. the precipitation was attempted from too dilute a solution with respect to arginine.

A potentiometer equipped with outside electrodes later became available for use. The pH adjustments in the two following experiments were made using this apparatus.

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Experiment III - The effect of pH on the precipitation of arginine flavianate:

A solution of arginine HCl, containing 7.98 mg. arginine nitrogen, was made basic with NaOH, so that the pH on addition of excess flavianic acid was 9.0. Final volume was 40 ml. The solution was gradually made acidic by the addition of dilute sulphuric acid. Arginine flavianate crystals formed immediately at a pH of 3.0.

This procedure was repeated with a weaker concentration of arginine. The solution contained 7.98 mg. arginine nitrogen in a final volume of 80 ml. Arginine flavianate crystals appeared at a pH of 2.9.

With a similar solution of arginine, containing excess flavianic acid and adjusted with dilute sulphuric acid to pH 1.5, no precipitation of arginine flavianate occurred until the pH was raised to 2.0 with dilute NaOH.

These tests would indicate that arginine may be precipitated most readily as the monoflavianate in the pH range 2.0 to 3.0.

Experiment IV - The analysis of an artificial protein hydrolysate:

The artificial hydrolysate contained 1/5 ml. HCl, 12.48 mg. of N as glycine and 5.42 mg. of N

Experiment III - The effect of pH on the reaction

Time of reaction (minutes):

1. Addition of 0.1 ml. of 0.1 M. sodium acetate

0.1 M. sodium acetate, was added to the

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Experiment IV - The effect of pH on the reaction

Time of reaction (minutes):

0.1 M. sodium acetate, was added to the

0.1 M. sodium acetate, was added to the

as aspartic acid. In addition, arginine, histidine, and lysine were added as their HCl salts in the quantities indicated in Table XII.

The electrolysis was complete in $1\frac{1}{2}$ hours; the dialysis in 45 minutes.

The catholyte was made acid to litmus and concentrated in vacuo to 20 ml. Arginine flavianate was precipitated at a pH of 2.8. Recoveries of all fractions, as given in Table XII, are excellent.

TABLE XII

The analysis of an artificial
protein hydrolysate

	Nitrogen, %		Recovery, %
	Theoretical	Experimental	
Arginine	17.96	17.85	99.4
Histidine	7.28	7.31	100.1
Lysine	6.40	6.40	100.0

Method II

This method was intended to eliminate the precipitation of arginine as the monoflavianate.

Procedure - The aerated catholyte is made acid to methyl red with dilute HCl. Saturated mercuric chloride is added, 4 moles per mole of histidine

as separate solid. In addition, arginine, histidine, and lysine were added as their HCl salts in the quantities indicated in Table III. The electrolyte was complete in 1/2 hour; the dialysis in 45 minutes.

The catholyte was made acid to litmus and concentrated in vacuo to 80 ml. Arginine hydrochloride was precipitated at a pH of 8.5. Recovery of all fractions, as given in Table III, was excellent.

TABLE III

Yields of fractions
from electrolysis

Electrolysis			
Theoretical			
Experimental			
Recovery, %			
Arginine	17.50	17.50	99.5
Histidine	7.50	7.50	100.0
Lysine	8.50	8.40	100.0

Method II

This method was intended to eliminate the contamination of arginine as the monohydrochloride.

Procedure - The starting material is used as a neutral red with dilute HCl. The reaction mixture is added, a solution of sodium chloride is added, and the mixture is stirred.

expected. The histidine is precipitated when the pH is adjusted to 6.8 to 7.0, using dilute NaOH. After standing several hours, the mixture is centrifuged. The supernatant liquid is poured off, and the precipitate is washed with two successive 10-ml. portions of water.

The histidine precipitate is dissolved with dilute HCl and made up to volume. The nitrogen is determined by the micro-Kjeldahl method. The filtrate and washings are combined and made up to volume. Two determinations are made: (1) Total N is determined by the micro-Kjeldahl method; (2) Arginine N is determined by the direct method of Plimmer and Rosedale (22). The preliminary step of alkaline aeration is not necessary, since no amide N is present.

Total N - Arginine N = Lysine N.

Experiment I - The analysis of a basic amino acid mixture:

The original solution contained 19.94 mg. of N as arginine HCl, 13.46 mg. of N as histidine HCl, and 7.65 mg. of N as lysine HCl. Electrical transport was completed in 35 minutes. Subsequent analysis showed the catholyte contained 19.58 mg. of arginine N, 13.34 mg. of histidine N, and 7.53 mg. of lysine N. The recoveries were therefore 98.2%,

.

97.9%, and 98.4% respectively.

Experiment II - The analysis of an artificial protein hydrolysate:

The solution before electrolysis contained 13.97 mg. of aspartic acid N, 3.02 mg. leucine N, 9.51 mg. histidine N, 6.19 lysine N, and 20.60 mg. arginine HCl, and 5 ml. HCl. The basic amino acids were present as their HCl salts. The time of the electrolysis was 7 hours; of the dialysis 45 minutes. The aerated catholyte contained 18.71 mg. histidine N. The catholyte of a duplicate contained 26.06 mg. histidine N.

Lysine and arginine determinations were not completed for either sample.

Experiment III - The analysis of a linseed oil meal hydrolysate:

The meal was extracted with ether and hydrolyzed with 20% HCl (5 cc. per gm.) for 24 hours. An aliquot of the hydrolysate representing 158.7 mg. N was used for electrical transport. The electrolysis was completed in $6 \frac{1}{2}$ hours, the dialysis in one hour. The aerated catholyte was analyzed for arginine and histidine.

The meal contained 14.07% of the total N as arginine N, and 7.42% as histidine N. This

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sample was shown, by Method III, to contain 18.96% arginine N and 3.72% histidine N.

Discussion

The excellent results obtained in Experiment I can be explained only by chance precipitation. The other results indicate that arginine is also precipitated by mercuric chloride in the histidine fraction. To obtain specific precipitation of histidine the pH of the solution must be exact. It can be demonstrated that mercuric chloride will precipitate arginine in an acid solution. Application of this method as a standard procedure is too unsatisfactory when indicators are the only available means of pH adjustment. Further work with this method is contemplated; the intention is to use the pH meter to regulate acidity.

Method III

73 This is an application of the Van Slyke nitrogen distribution method of protein analysis (18).

Procedure - The aerated catholyte is made up to a volume of 200 ml. The following determinations are then made:

1. Total nitrogen is determined on 10-ml. aliquots by the micro-Kjeldahl method.

sample was known, by method III, to contain 18.90%
arginine H and 2.72% arginine.

Discussion

The excellent results obtained in experiment I can be explained only by a more exact calculation. The other results indicate that arginine is also precipitated by mercuric chloride in the presence of arginine. To obtain a specific precipitation of arginine the pH of the solution must be exact. It has been demonstrated that mercuric chloride will precipitate arginine in an acid solution. Application of this method as a standard procedure in the analysis of arginine was the only available method at the time. Further work with this method is contemplated; the intention is to use the micro-method to determine activity.

Method III

This is an application of the Van Slyke nitrogen distillation method of protein analysis (12).
Procedure - The sample is weighed in a Van Slyke flask of 200 ml. The following determinations are then made:
1. Total nitrogen is determined on 10-ml. aliquots by the micro-Kjeldahl method.

2. Arginine nitrogen is determined by the method of Plimmer and Rosedale on a 100-ml. aliquot (22).

3. Amino nitrogen is determined on 4-ml. aliquots by the Van Slyke method (34).

The values of histidine nitrogen and lysine nitrogen may be calculated by the following equations:

$$\text{Total N} = \text{arginine N} + \text{histidine N} + \text{lysine N}$$
$$\text{Amino N} = \frac{1}{4} \text{ arginine N} + \frac{1}{3} \text{ histidine N} + \text{lysine N}$$
$$\text{Non-amino N} = \frac{3}{4} \text{ arginine N} + \frac{2}{3} \text{ histidine N.}$$

Experiment I - The analysis of an artificial protein hydrolysate:

The solution contained 1/5 ml. of HCl, 4.00 mg. of histidine N, 12.69 mg. of arginine N, 5.05 mg. of lysine N, 5.42 mg. of aspartic acid N, and 12.48 mg. of glycine N. The electrolysis was complete in 1½ hours; the dialysis in 25 minutes. Two such solutions were electrolyzed. The catholyte from the first was concentrated under reduced pressure at 45°C. to a volume of 50 ml. The second catholyte was aerated for one hour at 45°C.

The analytical results obtained are summarized in Table XIII.

2. Arginine nitrogen is determined by the method of
Hinsman and Hoesly as a 100-ml. aliquot (23).
3. Lysine nitrogen is determined on 4-ml. aliquots by
the Van Slyke method (24).

The values of lysine nitrogen and lysine
nitrogen may be calculated by the following equations:

$$\text{Total N} = \text{arginine N} + \text{histidine N} + \text{lysine N}$$

$$\text{Lysine N} = \frac{1}{4} \text{ arginine N} + \frac{1}{8} \text{ histidine N}$$

$$\text{Lysine N}$$

$$\text{Non-lysine N} = \frac{3}{4} \text{ arginine N} + \frac{5}{8} \text{ histidine N}$$

Experiment I - The analysis of an analytical tri-
tain hydrolysate:

The solution contained 1.5 ml. of 50%
4.00 mg. of histidine N, 12.80 mg. of arginine N,
2.00 mg. of lysine N, 0.40 mg. of aspartic acid N,
and 12.48 mg. of glycine N. The electrolysis was
complete in 16 hours; the nitrogen in 80 minutes.
Two such solutions were electrolyzed. The nitrogen
from the first was concentrated under reduced pres-
sure at 40°C. to a volume of 50 ml. The second
solution was carried for one hour at 40°C.

The analytical results obtained are as-

sembled in Table VII.

TABLE XIII

The analysis of an artificial
protein hydrolysate

	Nitrogen, mg.					
	Theoretical	<u>Experimental</u>		<u>Recovery, %</u>		
		1	2	1	2	
Total basic	21.74	21.79	21.87	100.2	100.6	
Arginine	12.69	12.53	12.60	98.7	99.3	
Histidine + lysine	9.05	9.26	9.27	102.3	102.4	
Histidine	4.00	3.84	4.08	96.0	102.0	
Lysine	5.05	5.42	5.19	107.3	102.8	

Excellent recovery of total basic nitrogen and of arginine nitrogen was obtained with both samples. The distribution of nitrogen between lysine and histidine depends upon the accuracy of the amino nitrogen determination. This was very unsatisfactory with Sample 1. Better results were obtained with Sample 2.

The results showed that the accuracy of this method of analysis is limited by the accuracy of the amino nitrogen analyses. If the latter are inaccurate the percentages of both histidine and lysine are unreliable.

This experiment also demonstrated that basic nitrogen was not lost from the alkaline catholyte either by aerating or by concentrating under reduced pressure at 45°C.

TABLE III

The analysis of the samples of protein hydrolyzates

Thermal treatment		Experimental hydrolysis		Analysis, %	
1	2	1	2	1	2
21.74	21.79	100.0	100.0	21.74	21.79
11.53	11.53	100.0	100.0	11.53	11.53
9.03	9.03	100.0	100.0	9.03	9.03
4.00	4.00	100.0	100.0	4.00	4.00
3.08	3.08	100.0	100.0	3.08	3.08

Protein recovery of total basic nitrogen and of arginine nitrogen was obtained with good results. The distribution of nitrogen between lysine and histidine depends upon the recovery of the amino nitrogen determination. This was very unsatisfactory with sample 1. Better results were obtained with sample 2.

The results showed that the recovery of this method of analysis is limited by the accuracy of the amino nitrogen analysis. If the recovery of the amino nitrogen determination is 100% the recovery of the nitrogen is 100%.

This experiment also demonstrated that basic nitrogen was not lost from the samples hydrolyzed at 100°C or 120°C.

Experiment II - The analysis of gelatin hydrolysates:

Duplicate samples of gelatin were hydrolyzed 24 hours with 20% HCl. The humin was filtered off, and the hydrolysates were made to volume. Humin and hydrolysate fractions were analyzed for nitrogen.

Two aliquots of hydrolysate A, each representing 152.2 mg. nitrogen, and one of hydrolysate B, representing 155.5 mg. nitrogen, were analyzed. The duration of the electrolysis was $4\frac{1}{2}$ hours and of the dialysis 35 minutes for each of the three samples. Table XIV summarizes the results. All calculations were made on the basis: total N = humin N + hydrolysate N.

TABLE XIV

The analysis of duplicate gelatin hydrolysates

	<u>Hydrolysate A</u>		Hydrolysate B
	1	2	
Total basic N, %	27.67	27.45	27.11
Arginine N, %	17.59	17.38	17.22
(Histidine N + Lysine N), %	10.09	10.08	9.89
Histidine N, %	0.77	0.70	0.74
Lysine N, %	9.32	9.38	9.10
Humin N, %	0.57	0.57	1.17

Experiment II - The analysis of gelatin hydroly-

ates:

Duplicate samples of gelatin were hydro-
lyzed 34 hours with 20% HCl. The liquid was filtered
off, and the hydrolyzates were made to volume.
Urea and hydrolyzate fractions were analyzed for
nitrogen.

Two aliquots of hydrolyzate A, each repre-
senting 158.5 mg. nitrogen, and one of hydrolyzate
B, representing 158.5 mg. nitrogen, were analyzed.
The duration of the electrolysis was 4 hours and
of the dialysis 30 minutes for each of the three
samples. Table XIV summarizes the results. All
calculations were made on the basis: total N =
human N + hydrolyzate N.

TABLE XIV

The analysis of duplicate gelatin
hydrolyzates

Hydrolyzate A		Hydrolyzate B	
1	2	1	2
37.67	37.45	37.11	37.11
14.33	14.33	14.33	14.33
10.00	10.00	10.00	10.00
8.00	8.00	8.00	8.00
5.33	5.33	5.10	5.10
0.37	0.37	0.14	0.14
Total basic N, %		Total basic N, %	
Arginine N, %		Arginine N, %	
(Histidine N + lysine N), %		(Histidine N + lysine N), %	
Histidine N, %		Histidine N, %	
Lysine N, %		Lysine N, %	
Human N, %		Human N, %	

The error between internal duplicates was smaller than the error between external duplicates. The latter difference was approximately proportional to the difference between hydrolysates in percentage humin nitrogen. The histidine N + lysine N fractions were in close agreement. The error in the distribution of nitrogen between histidine and lysine was due to the inaccuracies of the amino nitrogen analyses.

The amounts of the three basic amino acids present were similar to the results obtained with gelatin by other investigators.

Experiment III - The estimation of the arginine content of protein hydrolysates by alkaline hydrolysis:

Comparative estimations were made of the arginine content of protein hydrolysates by the alkaline hydrolysis method of Plimmer and Rosedale

- (1) Directly on the hydrolysate (22).
- (2) On the aerated catholyte obtained by electrical separation of the hydrolysate. No preliminary aeration was necessary to eliminate amide nitrogen.

Table XV illustrates the uniformity of the results obtained. Analyses by the second procedure tended to be slightly higher. These results provided an excellent check on the quantitative trans-

port of arginine and on the procedure used for the estimation of arginine in the preceding experiments.

TABLE XV

The estimation of the arginine content of
protein hydrolysates by
alkaline hydrolysis

Protein source	<u>Arginine N as % total N</u>	
	1*	2**
Soybeans	16.1	15.8
Gelatin 1	17.2	17.6
Gelatin 2	17.3	17.4
Peas	15.1	15.4

* Analysis made directly on the protein hydrolysate.

** Analysis made on the aerated catholyte obtained by electrical transport.

port of nitrogen and on the procedure used for the estimation of nitrogen in the preceding experiments.

TABLE IV

The estimation of the nitrogen content of protein hydrolyzates by alkaline hydrolysis

Experiments 1 to 5

Protein source	% N	% N
Boyer's	16.1	16.9
Boyer's 1	17.8	17.3
Boyer's 2	17.8	17.4
Boyer's 3	17.1	16.4

* Analysis made directly on the protein hydrolyzate.
 ** Analysis made on the reduced orthophosphate solution by electrical transport.

THE BASIC AMINO ACID CONTENT OF SOME PLANT PROTEINS

Literature Review and Discussion

Our knowledge of amino acids and of their role in animal nutrition is of recent origin. The vital importance of feed proteins to the animal has, however, been recognized for at least 150 years. The conclusions reached by early investigators were reviewed by Block and Bolling (4).

Francois Magendie (1783-1853), on the basis of livestock feeding experiments, deduced that the nitrogen of the tissues is derived from food nitrogen. The nutritive importance of proteins and the dependence of animals on plants for these substances were pointed out by G. J. Mulder, a Dutch chemist, around 1840. The concept that the protein or nutritive value of a feed could be estimated from the nitrogen content alone appears to have originated with the studies of Boussingault. Leibig supported this hypothesis. He stated in 1851 that vegetable and animal products contained more or less identical protein substances. This view remained dominant until 1897, when Rubner recognized that proteins of varying origin had different biological values. Variations in the amino acid composition of different proteins supported this theory.

THE BASIC AMINO ACID CONTENT OF

FOOD PROTEINS

LITERATURE REVIEW AND DISCUSSION

Our knowledge of amino acids and of their role in animal nutrition is of recent origin. The vital importance of food proteins to the animal has, however, been recognized for at least 150 years. The conclusions reached by early investigators were reviewed by Block and Bolling (4). Francis (5) (1934-1935), on the basis of livestock feeding experiments, believed that the nitrogen of the animal is derived from food nitrogen. The nutritive importance of proteins and the dependence of animals on plants for these substances were pointed out by U. S. Lindner, a Dutch chemist, around 1840. The concept that the nutritive or nutritive value of a food could be estimated from the nitrogen content alone appears to have originated with the studies of Bösenfeldt. In this important work Bösenfeldt stated in 1851 that vegetable and animal products contained more or less identical protein substances. This view remained dominant until 1937, when Butler recognized that proteins of varying origin had different biological values. Variations in the amino acid composition of different proteins suggested this theory.

By 1915, Osborne and Mendel (4) had shown that tryptophane and lysine were indispensable amino acids. Rose (25) specified all the amino acids required for normal growth in the albino rat. Lysine and histidine are both essential; arginine is semi-indispensable. Recent experiments by Holt and Albanese (13) have shown this same group, with the exception of histidine, to be necessary for human nutrition. Histidine deficiency has no effect on the nitrogen balance in man, but a urinary chromogen appears where this amino acid is lacking. Arginine is synthesized by humans and certain rodents but at a rate which does not allow normal sperm production or normal growth (13, 4). In the case of the chick it is an essential amino acid since it cannot be synthesized at all, or only in insufficient amounts (12, 16).

Concurrent with these biological investigations, analytical procedures were developed to determine the amino acid content of proteins. These procedures have proven reasonably accurate on purified proteins, but often failed badly when applied to natural foods. Mitchell and Hamilton (18) summarized the results obtained on the mixed proteins of feeds, using the Van Slyke method of protein analysis. Despite the obvious inaccuracies of the procedure, particularly on this type of material, the results are valuable. Linseed meal protein was reported to contain 7.9% arginine, 3.6% histidine, and 3.1% lysine; soybean protein was reported to contain 7.8% arginine, 3.3% histidine, and 5.2% lysine; and

By 1913, Sabourin and Waller (1) had shown that tyrosine and lysine were indispensable amino acids. These (2) specified all the amino acids required for normal growth in the albino rat. Lysine and histidine were both essential; arginine in semi-indispensable. Recent experiments of Holt and Alpers (3) have shown that some growth, with one exception of histidine, to be necessary for normal nutrition. Lysine deficiency has no effect on the nitrogen balance in man, but a urinary excretion appears when this amino acid is lacking. Arginine is synthesized by humans and certain rodents but not rats which then must obtain some nitrogen from normal growth (3, 4). In the case of the skin, it is an essential amino acid since it cannot be synthesized at all, or only in insignificant amounts (5, 6). Comparison with those of limited investigations, analytical procedures were developed to determine the amino acid content of proteins. These procedures have proven reasonably accurate on purified proteins, but more reliable when applied to natural foods. Methods are available (7) to determine the results obtained in the skin proteins. These, using the Van Slyke method of amino acids, indicate the relative importance of the amino acids, particularly on this type of material, the results are reliable. Human skin protein was reported to contain 7.5% lysine, 2.5% histidine, and 3.1% tyrosine; mouse skin was reported to contain 7.5% lysine, 2.5% histidine, and 3.1% tyrosine; and

alfalfa hay protein was reported to contain 4.4% arginine, 2.3% histidine, and 3.7% lysine.

Procedures based on the isolation of the individual amino acids by precipitation have gradually displaced the Van Slyke method of analysis. These have proven unsatisfactory when applied to whole feeds. The problem of the purification of these materials has been solved in part by treatment with acids, alkalis, or enzymes. Block and Bolling (4) analyzed purified materials and reported that linseed protein contained 6.2% arginine, 1.2% histidine, and 2.5% lysine; and that soybean protein contained 5.8% arginine, 2.3% histidine, and 5.4% lysine.

Tristram (33) analyzed "ether water" protein extracts of the fresh leaves of plants and found that the basic amino acid analysis of leaf proteins exhibit very little variation from species to species or within a species in one season. Lucerne leaf protein contained 7.5% arginine, 1.4% histidine, and 5.8% lysine.

It is quite evident that the amino acid content of plant seeds varies among species. The proteins of the seeds and of the leaves of legumes have a higher lysine content than the proteins of flaxseed and of the cereals. The arginine content of the legume and flaxseed proteins is moderately high, while the histidine content is low.

Rigby (24) reported varying amounts of the sulphur-containing amino acids in wheat gluten owing to different

lithium myristate was reported to contain 2.2% myristic,
2.2% stearic, and 3.7% lauric.

Procedures based on the separation of the individual

amino acids by precipitation have previously been used for
the analysis of lipids. These have proved unsatisfactory
when applied to simple lipids. The problem of the separation
of these materials has been solved in part by treatment with
ethanol, chloroform, or ether. These methods (a) are tedious
and complicated and require the use of large amounts of
solvent, (b) are not quantitative, and (c) are not
specific. The present method is simple, rapid, and
specific.

Tristearin (92) analyzed "ether-soluble" fraction of

lipids of the fresh leaves of glass and water from the same
source and analyzed as lipid, showing identical results.
Variation from results to results is within 1% in the
analysis. The present method contained 2.2% myristic, 1.6%
stearic, and 3.7% lauric.

It is quite evident that the amino acid content of

lipid varies from species to species. The separation of the
amino acids by the method described here is a simple and
rapid method of determining the amino acid content of the
lipids of the leaves of the leaves. The results
obtained are in good agreement with those obtained by
the method of the leaves and the results are in good
agreement with the results of the leaves.

Lipids (93) reported varying amounts of the amino-

acids in the leaves of the leaves of the leaves.

fertilizer treatments. Alten and Haupt (2) found that the tryptophane content of oat straw and English rye grass decreased with increased application of K_2O ; tyrosine in oat straw was little affected by the fertilization; arginine in grass was depressed and in oat straw increased as the K_2O supply was increased. The possibility exists that just as the protein content of seeds may vary with environmental conditions so may the amino acid composition of these mixed proteins.

The work reported in the following section was undertaken with two objects in view:

1. To report the basic amino acid content of the proteins of pea meal, alfalfa leaf meal, linseed oil meal, and soybean meal.
2. To determine to what extent varietal and environmental factors affect the basic amino acid content of the proteins of pea meal.

These two phases of the work are referred to in the following report as Series 1 and Series 2, respectively.

Materials

Series 1

Idabel field peas, Agate soybeans, commercial linseed oil meal, and Ferax alfalfa were analyzed. The alfalfa was 60% in bloom at the time of picking. All samples were coarsely ground in a Wiley mill and then

terrestrial environments. Given and Gault (3) found that the
cryptogamic content of the water and bottom was greatly decreased
after increased application of 100; tyrosine in the water was
little affected by the fertilization; tyrosine in the water was
decreased and in the water increased as the 100 was
increased. The possibility exists that as the tyrosine
content of seeds may vary with environmental conditions so
may the amino acid composition of these seeds and plants.
The work reported in the following section was

undertaken with two objects in view:

1. To report the basic amino acid content of the
protein of pea meal, alfalfa leaf meal, linseed oil meal, and
soybean meal.
2. To determine to what extent variation in
nutritional factors affect the basic amino acid content of the pro-
teins of pea meal.

These two phases of the work are referred to in the
following report as Series 1 and Series 2, respectively.

Materials

Series 1

Labelled field peas, Alaska soybeans, commercial
linseed oil meal, and Vester alfalfa were analyzed. The
alfalfa was 50% in bloom at the time of feeding. All
samples were coarsely ground in a Wiley mill and then

finely ground in a ball mill. The linseed oil meal was extracted with ether to remove the oil. The meals were stored in bottles.

Series 2

Chancellor and Early Blue field peas were obtained from Department of Plant Science experimental plots at Sundre, Edmonton, and Athabaska. These stations represent a diversity of climatic and soil conditions. Sundre and Athabaska are in grey wooded soil areas and Edmonton is in the black soil area. The samples were ground in a burr mill and stored.

Methods

Extraction

The alfalfa meal protein was extracted with 100 ml. of 8% KI per 2 grams of sample; the pea meal protein with 50 ml. of 3% NaCl per 6 grams of sample; the linseed oil meal protein with 100 ml. of 6% NaCl per 2 grams of sample; and the soybean meal protein with 100 ml. of distilled water per 2.5 grams of sample. The dispersion of Series 1 meals was aided by one-half hour shaking on the mechanical shaker; that of Series 2 meals by five minutes in the Waring Blendor and 25 minutes intermittent standing and shaking. The mixtures in both

finely ground in a ball mill. The extracted oil was then
extracted with ether to remove the oil. The solids were
dried in vacuo.

Section 2

Unsubstantiated and false claims have been made
concerning the Department of State's alleged experiments
done at Gurnee, Illinois, and elsewhere. These statements
represent a variety of claims and are completely
untrue and unsubstantiated and in fact would be a gross and
deliberate lie in the face of the facts. The results were
ground in a ball mill and dried.

Section 3

Extraction

The white solid protein was extracted with
100 ml. of 80% for 2 hours of sample; the two were
protein with 50 ml. of 80% and 6 hours of sample;
the liquid oil was extracted with 100 ml. of 80% for
2 hours of sample; and the liquid was extracted with 100
ml. of distilled water for 2 hours of sample. The
distillation of water from the oil was done by one-half hour
distillation on the water-bath; then the oil was
by five minutes in the water-bath and 10 minutes
intermittent shaking and heating. The samples in water

cases were centrifuged for 10 minutes at 2000 r.p.m. and the supernatant liquid decanted. The volume of liquid was measured and the nitrogen content determined. Total soluble nitrogen was calculated on the assumption that the solvent not recovered contained proportionally the same amount of nitrogen as the recovered fraction. This has been shown to give results quite comparable to those obtained by exhaustive extraction. Table XVI compares the calculated total extraction of protein with the actual total extraction of protein obtained with various meals.

TABLE XVI

A comparison of the total protein extracted, calculated and actual, from various meals

Meal	Protein extracted, %		
	Solvent	Calculated	Actual
Idabel peas	3% NaCl	94.3	95.0
Early Blue peas	3% NaCl	96.9	97.4
Chancellor peas	3% NaCl	91.4	92.8
Alfalfa meal	8% KI	60.3	62.4
Linseed oil meal (oil-free)	6% NaCl	74.4	74.6
(natural)	6% NaCl	74.4	74.8

All extractions were made at room temperature.

Dialysis

Protein dispersions in salt solutions were dialyzed 15 hours or longer in cellophane dialysis tubing

cases were centrifuged for 10 minutes at 3000 r.p.m. and the supernatant liquid decanted. The volume of liquid was measured and the nitrogen content determined. Total soluble nitrogen was calculated on the assumption that the solvent not recovered contained proportionally the same amount of nitrogen as the recovered fraction. This has been shown to give results quite comparable to those obtained by exhaustive extraction. Table VII compares the calculated total extraction of protein with the actual total extraction of protein obtained with various media.

TABLE VII

A comparison of the total protein extracted, calculated and actual, from various media

Protein extracted, %		Solvent		Media	
Calculated	Actual	Calculated	Actual	Calculated	Actual
35.0	34.3	35.0	34.3	Label case	
37.4	36.9	37.4	36.9	Early blue case	
38.8	38.4	38.8	38.4	Unfractionated case	
38.8	38.3	38.8	38.3	Label case	
38.8	38.4	38.8	38.4	Label case (oil-free)	
38.8	38.4	38.8	38.4	Label case (neutral)	

All extractions were made at room temperature.

Analysis

Protein determinations in this laboratory were

analyzed 15 hours or longer in duplicate; analysis results

with running water. Removal of the salt is necessary for satisfactory operation of the electrolytic cell.

Hydrolysis

The protein suspensions were now dried in evaporating dishes on the steam bath. The dry material was finely ground with a mortar and pestle. Duplicate samples were hydrolyzed for 24 hours with 5 ml. of 20% HCl per gram of material. An all-glass apparatus was used.

Analysis

The hydrolyzed sample was diluted with water, filtered, and the humin precipitate washed with hot water. The filtrate was made up to 100 ml. in a volumetric flask. Nitrogen in the hydrolysate and in the precipitate was determined.

The basic amino acids of an aliquot of the hydrolysate equivalent to $\frac{1}{2}$ to 1 gram of protein were separated by electrolytic transport. The catholyte was analyzed by the nitrogen distribution method as outlined previously (Method III).

All other nitrogen determinations were made using the modified Kjeldahl method with mercuric oxide as catalyst.

with running water. Removal of the salt is necessary for satisfactory operation of the electrolytic cell.

Hydrolysis

The protein suspensions were now placed in evaporating dishes on the steam bath. The dry material was finally ground with a mortar and pestle. Samples were hydrolyzed for 24 hours with 5 ml. of 60% HCl per gram of material. An all-glass apparatus was used.

Analysis

The hydrolyzed sample was diluted with water, filtered, and the insoluble precipitate washed with water. The filtrate was made up to 100 ml. in a volumetric flask. Nitrogen in the hydrolysate and in the precipitate was determined.

The basic amino acids of an aliquot of the hydrolysate equivalent to 5 to 1 gram of protein were separated by electrolytic means etc. The catholyte was analyzed by the nitrogen distillation method as outlined previously (Method III).

All other nitrogen determinations were made using the modified Kjeldahl method with mercuric oxide as catalyst.

Calculations

Total N = humin N + hydrolysate N.

The percentages of the individual amino acids were determined on the basis, Total N = humin N + hydrolysate N. The following factors used for conversion were calculated on the basis of $N \times 6.25 = \text{protein}$:

% arginine N $\times 0.497 =$ % arginine

% histidine N $\times 0.590 =$ % histidine

% lysine N $\times 0.834 =$ % lysine.

Experimental Results

The humin nitrogen ranged from 1.1% to 4.0% indicating that the protein extracts contained varying amounts of carbohydrate material. A consistently low humin nitrogen content is necessary for accurate analytical work with the amino acids. Large errors between duplicates were encountered in the histidine and lysine estimations. These inaccuracies can be traced partially to the differences between duplicates in the total basic nitrogen. In some instances the lysine values showed an inverse relationship to the histidine estimations. This indicated inaccurate amino nitrogen determinations.

Calculations

Total N = basic N + nitrogenous N.

The percentages of the individual amino acids were determined on the basis, Total N = basic N + nitrogenous N. The following factors used for conversion were

calculated on the basis of N x 6.25 = protein:

1 arginine N x 0.407 = 1 arginine

1 histidine N x 0.530 = 1 histidine

1 lysine N x 0.834 = 1 lysine.

Experimental Results

The amino nitrogen ranged from 1.15 to 2.00 percent-

ing that the protein extracts contained varying amounts of carbohydrate material. A consistently low amino nitrogen content is necessary for accurate calculation with the amino acids. Large errors between duplicate were encountered in the histidine and lysine estimations. These inconsistencies can be traced primarily to the differences between analyses in the total basic nitrogen. In some instances the lysine values showed an inverse relationship to the histidine estimations. This indicated inaccurate amino nitrogen estimation.

TABLE XVII

The basic amino acid content of some plant proteins

Protein source	Protein, %	Protein extracted, %	Sample number	Humin N, %	Arginine, %	Histidine, %	Lysine, %	Total basic N, %
Peas	29.6	94.6	1	1.1	10.6	1.1	9.1	34.2
			2	1.1	10.6	1.5	9.0	34.5
			Average	1.1	10.6	1.3	9.0	34.4
Linseed oil meal	40.9	whole meal	1	5.8	9.4	2.5	5.4	29.5
			2	5.9	9.4	2.6	5.3	29.7
			Average	5.8	9.4	2.6	5.4	29.6
Linseed oil meal	40.9	74.6	1	3.3	9.0	1.8	4.9	27.1
			2	3.4	8.8	2.3	4.3	26.7
			Average	3.4	8.9	2.0	4.6	26.9
Soybeans	49.9	93.0	1	2.3	8.1	1.7	8.8	29.7
			2	2.3	8.0	2.3	8.2	29.9
			Average	2.3	8.0	2.0	8.5	29.8
Alfalfa meal	27.6	62.4	1	3.9	9.7	2.9	4.2	29.4
			2	4.0	9.8	3.6	3.8	30.5
			Average	4.0	9.8	3.2	4.0	30.0

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selected funds are to be used for other purposes.

Series 1 - Results are presented in Table XVII.

High extractions of the proteins of the pea and the soybean meals were obtained. The protein extracted from linseed oil meal and alfalfa meal cannot be considered representative of the total protein in the two meals, but since higher extractions could not be obtained this protein was used in analysis.

The total basic nitrogen content of the pea protein was definitely higher than that of the other plant proteins. The legume seed proteins contained more lysine than the other plant proteins. All the proteins contained small amounts of histidine and relatively large amounts of arginine.

Analyses were made on the whole meals of alfalfa and linseed. The electrical separation of the hydrolysates proved very unsatisfactory, particularly for the alfalfa meal. The results obtained on the oil-free linseed oil meal are included in Table XVII. A higher content of each of the three basic amino acids was obtained in the mixed proteins of the whole meal than in the proteins extracted by 6% sodium chloride.

The general relationship of these results is comparable to that indicated by other investigators, although both the arginine and the lysine results appear to be somewhat higher.

Series I - Results are presented in Table VII.

High extractions of the proteins of the seed and the embryo meals were obtained. The protein extractions from linseed oil meal and alfalfa meal cannot be considered representative of the total protein in the two meals, but since higher extractions could not be obtained this protein was used in analysis.

The total basic nitrogen content of the seed protein was definitely higher than that of the other plant proteins. The legume seed proteins contained more lysine than the other plant proteins. All the proteins contained small amounts of histidine and relatively large amounts of arginine.

Analyses were made on the whole meals of alfalfa and linseed. The electrical separation of the amino acids was very unsatisfactory, particularly for the amino acids. The results obtained on the oil-free linseed meal are included in Table VII. A higher content of each of the three basic amino acids was obtained in the mixed proteins of the whole meal than in the proteins extracted by sodium chloride.

The general relationship of these results is comparable to that indicated by other investigators, although both the arginine and the lysine contents appear to be somewhat higher.

Series 2 - Results are presented in Table XVIII

Higher protein extractions were obtained from all the meals. The extraction of the Chancellor, Sundre, sample was definitely lower than the others, but was quite acceptable. At any one station, higher extractions were obtained from the Early Blue samples than from the Chancellor samples.

The protein content varied as expected, over a wide range. The extremely low protein content of the Athabaska samples may be due to the lack of nodulation on the roots of the plants.

Disregarding the Athabaska Early Blue sample, the results form one general pattern. No varietal differences were found. The histidine content of the proteins of the Edmonton meals was significantly higher than the histidine content of the proteins of the other meals. This difference in the histidine content almost entirely accounted for the higher total basic nitrogen reported for the proteins of the Edmonton meals. The arginine content of the proteins of all meals was remarkably uniform. The lysine content of the protein of the Edmonton meals was approximately 10% lower than that reported for the proteins of the Sundre and Athabaska meals.

The Athabaska Early Blue sample gave unusual results. The total basic nitrogen was the lowest of any

Table 2 - Results are presented in Table 2

These results are presented in Table 2.

The results of the analysis of variance for the protein content of the samples are presented in Table 2. The results show that the protein content of the samples was significantly lower than the control, and the difference was significant. The results also show that the protein content of the samples was significantly lower than the control, and the difference was significant.

The results of the analysis of variance for the protein content of the samples are presented in Table 2.

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Results.

The results of the analysis of variance for the protein content of the samples are presented in Table 2. The results show that the protein content of the samples was significantly lower than the control, and the difference was significant. The results also show that the protein content of the samples was significantly lower than the control, and the difference was significant.

TABLE XVIII

The basic amino acid content of pea proteins

Variety	Station	Protein %	Protein extracted %	Sample number	Humin N, %	Arginine %	Histidine %	Lysine %	Total basic N, %
Early Blue	Edmonton	27.5	92.0	1	1.1	7.8	4.9	8.3	33.9
				2	1.3	7.9	6.3	7.5	35.7
				Average	1.2	7.8	5.6	7.9	34.8
Chancellor	Edmonton	25.5	90.8	1	2.8	7.4	5.9	8.2	34.7
				2	2.8	7.6	5.9	7.8	34.7
				Average	2.8	7.5	5.9	8.0	34.7
Early Blue	Sundre	22.7	92.7	1	3.4	7.8	1.9	9.6	30.4
				2	4.0	7.6	1.9	9.2	29.5
				Average	3.7	7.7	1.9	9.4	29.9
Chancellor	Sundre	18.1	86.6	1	2.2	7.5	1.9	8.9	29.0
				2	2.5	7.5	2.6	8.6	29.7
				Average	2.4	7.5	2.2	8.8	29.4
Early Blue	Athabaska	13.3	96.0	1	3.5	5.7	4.7	7.0	27.8
				2	3.4	5.6	5.3	6.8	28.3
				Average	3.4	5.6	5.0	6.9	28.0
Chancellor	Athabaska	12.1	94.0	1	3.0	7.7	3.1	8.7	31.0
				2	3.1	7.5	2.9	9.0	30.6
				Average	3.0	7.6	3.0	8.8	30.8

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sample. Both the arginine and lysine values were abnormally low. Conversely, relatively high amounts of histidine were present. These results indicate the possibility of unusual environmental factors during growth. It is possible, however, that some unaccounted for error operated with this sample.

Gortner (10) states that in the event that non-protein nitrogenous compounds are present, any method of analysis based upon a nitrogen determination will not necessarily yield accurate values for any particular amino acid. Various nitrogenous bases, such as the betaines choline and guanidine are found in plants. The garden pea contains the betaine trigonelline. The legumes as a family are noteworthy for their alkaloidal content. It may be that these high histidine values were due to the presence of some one or more nitrogenous bases. Despite this, the fact remains that the Edmonton samples are significantly higher in some one or more nitrogenous compounds. It is interesting to note in this connection that the alkaloidal content of plants may be varied by the use of nitrogen and phosphorus fertilizers (10).

Further work is necessary to determine whether the variability in histidine is real or whether some other factor is responsible for the apparent difference. More direct methods of analysis would be preferable and are under investigation.

sample. Both the nitrogen and lysine values were abnormally low. Conversely, relatively high amounts of histidine were present. These results indicate the possibility of unusual environmental factors during growth. It is possible, however, that some unaccounted for error operated with this sample.

Gortner (4) states that in the event that non-protein nitrogenous compounds are present, any method of analysis based upon a nitrogen determination will not necessarily yield accurate values for any particular amino acid. Various nitrogenous bases, such as the betaines choline and guanidine are found in plants. The nitrogen base contains the betaine trigonelline. The legumes as a family are noteworthy for their alkaloidal content. It may be that these high histidine values were due to the presence of some one or more nitrogenous bases. Despite this, the fact remains that the Edmonton samples are significantly higher in some one or more nitrogenous compounds. It is interesting to note in this connection that the alkaloidal content of plants may be varied by the use of nitrogen and other means (5). Fillers (6). Further work is necessary to determine whether the variability in histidine is real or whether some other factor is responsible for the apparent differences. More direct methods of analysis would be preferable and are under investigation.

SUMMARY

The work involved in this research is reported in three sections:

I. The dispersion in various solvents of the proteins of pea meal, commercial linseed oil meal, and alfalfa leaf meal was studied.

1. Maximum dispersion of over 90% of the protein of pea meal was obtained in distilled water and in 3% and 4% concentrations of solutions of sodium chloride, sodium salicylate, and sodium sulphate. Dispersion of the protein of pea meal was depressed in 1% concentrations of these salts.

2. Dispersion of 62.4% of the protein of alfalfa leaf meal was obtained in 8% potassium iodide; dispersion of 76.8% of the protein of "oil-free" linseed oil meal was obtained in 8% sodium salicylate.

3. Dispersion of the proteins of alfalfa leaf meal and linseed oil meal in 6% sodium chloride was little affected by the acidity of the solvent.

4. The Waring Blendor proved only partially satisfactory as an aid to protein dispersion.

II. An investigation of the electrical transport method of basic amino acid analysis was made.

1. An electrolytic cell, built in the laboratory,

EXPERIMENTAL

The work involved in this research is presented in

three sections:

I. The dispersion in various solvents of the proteins of sea
weed, especially cleaned oil seed, and various other seeds and
starches.

1. Maximum dispersion of over 90% of the protein
of sea weed was obtained in distilled water and in 2%
and 4% concentrations of solutions of sodium acetate,
sodium sulfate, and sodium sulphate. Dispersion of
the proteins of sea weed was decreased in 1% solutions
of these salts.

2. Dispersion of 82.4% of the protein of starch
seed was obtained in 2% solutions of sodium acetate, dis-
solved in 1% of the protein of "oil-seed" cleaned oil
seed was obtained in 2% sodium sulphate.

3. Dispersion of the proteins of starch seed
seed and cleaned oil seed in 2% sodium acetate was
little affected by the addition of sea water.

4. The wheat gluten proved only slightly water-
soluble in 1% protein solution.

II. An investigation of the absorption spectrum of
basic amino acid solutions was made.

1. An electrolytic cell, built at the laboratory,

quantitatively separated the basic amino acids from the other constituents of an artificial protein hydrolysate.

2. The catholyte obtained by electrical separation was analyzed for arginine, histidine, and lysine by three methods of analysis. Further work on these methods is contemplated.

III. The basic amino acid content of some plant proteins was determined.

1. Protein extracts of pea meal, linseed oil meal, alfalfa leaf meal, and soybean meal were analyzed. The legume seed proteins were relatively higher in lysine. All proteins were low in histidine and relatively high in arginine.

2. Protein extracts of the pea meals of two varieties, Early Blue and Chancellor, from three stations, Edmonton, Sundre, and Athabaska, were analyzed. No varietal differences were found. The arginine and lysine contents were fairly uniform throughout, but Edmonton-grown samples contained a much larger histidine fraction. The question as to whether this difference in histidine content is real or can be attributed to the presence of nitrogenous bases presents an interesting problem.

quantitatively separated the basic amino acids from the other constituents of an individual protein hydrolysate. 3. The catalytic reaction by electrical separation was analyzed for arginine, histidine, and lysine by means of methods of analysis. Further work on these methods is contemplated.

III. The basic amino acid content of some plant proteins was determined.

1. Protein extracts of pea meal, linseed oil meal, alfalfa leaf meal, and soybean meal were analyzed. The results show that the relative nitrogen content in these all proteins was low in histidine and relatively high in arginine.

2. Protein extracts of the pea meals of two varieties, early blue and Cheyenne, from three locations, Lincoln, Nebraska, and Ames, Iowa, were analyzed. No marked differences were found. The arginine and lysine contents were fairly uniform throughout, but the brown samples contained a much larger histidine fraction. The question as to whether this difference in histidine content is real or can be attributed to the presence of nitrogenous bases present in histidine is not yet decided.

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REFERENCES

1. ALBANESE, A.A. An electrolytic method for the determination of the basic amino acids in protein. J. Biol. Chem. 134:467-482. 1940.
2. ALTEN, F. and HAUPT, W. The connection between mineral fertilization of plants and their content of amino acids. Chem. Abstracts. 34:8151, 1940.
3. BLOCK, R.J. A new method for separation of the basic amino acids from protein hydrolysates. Proc. Soc. Exp. Biol. and Med. 51:252-253. 1942.
4. BLOCK, R.J. and BOLLING, D. Nutritional opportunities with amino acids. Jour. Amer. Dietetic Assoc. 20(2) 1944.
5. CHIBNALL, A.C. The effect of low temperature drying on the distribution of nitrogen in the leaves of the runner bean. Biochem. J. 16:599-607. 1922.

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The writer's thanks and appreciation are extended to the supervisor of this project, Dr. A. G. Webster, for helpful criticism and advice during the progress of the investigation. A. J. Williams assisted in the routine analyses and G. E. Town in the preparation of equipment and photographs. Financial assistance was supplied by a Robert Taylor Research Scholarship, and by the National Research Council, Ottawa.

LITERATURE

1. ALBERTS, A. A. An electrolytic method for the determination of the basic amino acids in proteins. J. Biol. Chem. 154:457-482, 1940.
2. ALLEN, F. and HAMPT, W. The connection between nitrogen fertilization of plants and their content of amino acids. Chem. Abstracts. 34:5151, 1940.
3. BLOCH, R. J. A new method for separation of the basic amino acids from protein hydrolysates. J. Biol. Chem. 154:457-482, 1940.
4. BLOCH, R. J. and MOLLING, G. Nutritional experiments with amino acids. Jour. Amer. Dietetic Assoc. 40:194, 1944.
5. CHESWILL, A. J. The effect of low temperature drying on the distribution of nitrogen in the leaves of the tomato plant. Biochem. J. 18:570-577, 1925.

6. COX, C.J., KING, H. and BERG, C.P. The preparation of lysine, histidine, and arginine from hydrolyzed blood corpuscle paste by electrical transport. Jour. Biol. Chem. 81:755-764. 1926.
7. DERMANSKII, A.V., ANTONOVICH, E.G., and SILAEV, A.B. Peptization of the proteins of the pea. Chem. Abs. 27:884(5). 1933.
8. FOSTER, G.L. and SCHMIDT, C.L.A. The separation of the hexone bases from certain protein hydrolysates by electrolysis. Jour. Biol. Chem. 56:545-553. 1923.
9. _____ The separation of the dicarboxylic acids from certain protein hydrolysates by electrical transport. Jour. Amer. Chem. Soc. 48: 1709. 1926.
10. GORTNER, R.A. Outlines of Biochemistry. 2nd edit. John Wiley and Sons Inc., New York. 1938.
11. HARROW, B. Textbook of Biochemistry. W. B. Saunders Co. Philadelphia, 1944.
12. HEGSTED, D.M., BRIGGS, G.M., ELVEHJEM, C.A., and HART, E.B. The role of arginine and glycine in chick nutrition. Jour. Biol. Chem. 140:191-200. 1941.
13. HOLT, L.E. and ALBANESE, A.A. Convalescence and rehabilitation. Report No.8. Minutes of a conference on amino acid metabolism. Washington, D.C. N.R.C., U.S., July, 1944.
14. JORPES, E. The colorimetric determination of histidine. Biochem. Jour. 26: 1507-1511. 1932.
15. KIZEL, A., AGATOV, P., BEZINER, E. and KASTRUBIN, M. Variations in the amino acid composition of plant proteins and their causes. Chem. Abs. 31:7447(7). 1937.
16. KLOSE, A.A., STOKSTAD, E.L. and ALMQUIST, H.J. The essential nature of arginine in the diet of the chick. Jour. Biol. Chem. 123:691-698. 1938.
17. MILLER, E.C. Plant Physiology. McGraw-Hill Book Co., Inc. New York, 1938.
18. MITCHELL, H.H. and HAMILTON, T.S. The biochemistry of the amino acids. The Chemical Catalogue Co., Inc. New York. 1929.

6. COLE, C.T., KIM, W. and CHAN, S.T. The degradation of tyrosine, histidine, and valine from *Escherichia coli* by electrical transport. Jour. Biol. Chem. 219:585-594, 1966.
7. DEWITT, A.V., LITVIN, S., and SILVER, A.A. Regulation of the permeability of the cell membrane. Jour. Biol. Chem. 241:1041-1048, 1966.
8. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes. Jour. Biol. Chem. 241:543-552, 1966.
9. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes by electrical transport. Jour. Biol. Chem. 241:553-562, 1966.
10. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes by electrical transport. Jour. Biol. Chem. 241:563-572, 1966.
11. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes by electrical transport. Jour. Biol. Chem. 241:573-582, 1966.
12. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes by electrical transport. Jour. Biol. Chem. 241:583-592, 1966.
13. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes by electrical transport. Jour. Biol. Chem. 241:593-602, 1966.
14. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes by electrical transport. Jour. Biol. Chem. 241:603-612, 1966.
15. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes by electrical transport. Jour. Biol. Chem. 241:613-622, 1966.
16. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes by electrical transport. Jour. Biol. Chem. 241:623-632, 1966.
17. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes by electrical transport. Jour. Biol. Chem. 241:633-642, 1966.
18. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes by electrical transport. Jour. Biol. Chem. 241:643-652, 1966.
19. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes by electrical transport. Jour. Biol. Chem. 241:653-662, 1966.
20. FOSTER, C.L. and FOSTER, D.L. The permeability of the cell membrane to certain protein hydrolytic enzymes by electrical transport. Jour. Biol. Chem. 241:663-672, 1966.

19. O'HARA, L.P. and SAUNDERS, F. Studies in proteins. J. Amer. Chem. Soc. 59:352-354. 1937.
20. OSBORNE, T.B. and WAKEMAN, A.J. Protein in green leaves. Jour. Biol. Chem. 42:1-26. 1920.
21. _____ and LEAVENWORTH, C. The protein of the alfalfa plant. Jour. Biol. Chem. 49:63-91. 1921.
22. PLIMMER, R.H. and ROSEDALE, J.L. Direct estimation of arginine. Biochem. Jour. 19:1020-1021. 1925.
23. RICH, C.E. Properties of wheat flour proteins. Cereal Chem. 13:522-541. 1936.
24. RIGBY, F.L. M.Sc. Thesis, U. of A. 1944.
25. ROSE, W.C. The nutritional significance of the amino acids and certain related compounds. Science. 86: 298. 1937.
26. ROXAS, M.L. The reaction between amino acids and carbohydrates as a probable cause of humin formation. Jour. Biol. Chem. 27:71-93. 1916.
27. SANDIN, R.B. and STOVER, N.M. Rapid method for the determination of organic nitrogen in liquids. Can. Jour. Res. 2:264-266. 1930.
28. SMITH, A.K. and CIRCLE, S.J. Peptization of soybean proteins. Ind. & Eng. Chem. 30:1414-1418. 1938.
29. SMITH, A.K., CIRCLE, S.J. and BROTHER, G.H. Peptization of soybean proteins. Jour. Amer. Chem. Soc. 60: 1316-1320. 1938.
30. STAKER, E.V. and GORTNER, R.A. Physico-chemical studies on proteins. Jour. Phys. Chem. 35:(I):1565-1605. 1931.
31. STOVER, N.M. and SANDIN, R.B. Use of boric acid in micro-Kjeldahl determination of nitrogen. Ind. & Eng. Chem., Analytical Edit. 3:250. 1931.
32. TOTTINGHAM, W.E., SCHULZ, E.R. and LEFKOVSKY, S. The extraction of nitrogenous constituents from plant cells. Jour. Amer. Chem. Soc. 46:203-208. 1924.

19. O'HARA, J.E. and SAWHNEY, V. Studies in proteins. 5. Amer. Chem. Soc. 53:352-354. 1937.
20. CARPENE, T.B. and MAXWELL, A.J. Protein in green leaves. Jour. Biol. Chem. 43:1-26. 1950.
21. protein of the alfalfa plant. Jour. Biol. Chem.
43:33-37. 1951.
22. WILLIAMS, R.H. and ROSSMAN, T.L. Direct isolation of arginine. Biochem. Jour. 13:1030-1031. 1953.
23. MEHN, C.E. Properties of wheat flour proteins. Cereal Chem. 13:323-341. 1936.
24. RIGBY, T.L. M.Sc. Thesis. U. of A. 1944.
25. ROSEN, W.C. The nutritional significance of the amino acids and certain related compounds. Science. 50: 398. 1937.
26. FOX, R.H. The relation between amino acids and carbon hydrates as a possible cause of brain formation. Jour. Biol. Chem. 27:71-73. 1916.
27. SANDER, R.B. and STOVER, R.W. Rapid method for the determination of organic nitrogen in lipids. Can. Jour. Res. 3:364-366. 1930.
28. SMITH, A.E. and GIVENS, E.T. Determination of soybean proteins. Ind. & Eng. Chem. 50:1414-1418. 1958.
29. SMITH, A.E., GIVENS, E.T. and BROTHMAN, G.H. Determination of soybean proteins. Jour. Amer. Chem. Soc. 80: 1418-1420. 1958.
30. STARR, E.V. and GOTTWART, R.A. Physical-chemical studies on proteins. Jour. Phys. Chem. 63(1):153-160. 1959.
31. STOVER, R.W. and SANDER, R.B. Use of poric acid in micro-bial determination of nitrogen. Ind. & Eng. Chem. Analytical Ed. 3:230. 1931.
32. TOTTENHAM, W.H., SCOTT, E.R. and IMPROVER, A. The extraction of nitrogenous constituents from plant cells. Jour. Amer. Chem. Soc. 46:203-205. 1924.

33. TRISTRAM, G.R. The basic amino acids of leaf proteins with a discussion of various methods of analyses. Biochem. Jour. 33:1271. 1939.
34. VAN SLYKE, D.D. A method for quantitative determination of aliphatic amino groups. Jour. Biol. Chem:9:185. 1911.
35. WEBER, C.J. A modification of Sakaguchi's reaction for the quantitative determination of arginine. Jour. Biol. Chem. 86:217-222. 1930.

WESTERN, T. D. J. MARTIN, JR. The West's leading source of legal services.
with a division of various services and specialties.
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