THE INSTITUTE OF BREWING RESEARCH SCHEME.

FIRST REPORT ON BARLEY PROTEINS.

THE COMPOSITION AND QUANTITATIVE ESTIMATION OF BARLEY PROTEINS.

By L. R. Bishop.

This section of the Barley Research work deals with the nitrogen compounds in barley and malt. The present report is an outline of the preliminary investigations on the barley nitrogen compounds and their behaviour in malting. The aim of the research is to elucidate the part played by the nitrogen compounds in brewing.

1.—Isolation and Composition of the Proteins.

The work of Osborne (2) clarified the position and established the existence in barley of four proteins (three of them clearly defined individuals) together with protein decomposition products such as proteoses, etc.

They are :—

An Albumin, soluble in water (and in dilute salt solutions and alkalis).

A Globulin, soluble in salt solutions (and in alkali).

A Gliadin (Hordein) soluble in 75 per cent. alcohol (and in alkali).

A Glutelin (not purified), soluble in alkali in the absence of salts.

Subsequent work has not affected this division seriously, and the classification of the individuals may be taken as fairly well established.

Osborne applied specific names to the albumin (leucosin) and globulin (edestin) believing them, on the basis of elementary analysis, to be identical with corresponding proteins in other plants. Analysis shows that the relative amounts of the constituent amino-acids in the barley proteins probably differ from those of other plants, *e.g.*, Edestin of barley differs from that of hemp seed and leucosin of wheat from that of barley. Hence these specific names will not be employed and the proteins will be referred to as barley albumin and globulin.

Osborne (2) and some later workers characterised the proteins by their elementary composition, *i.e.*, by the percentages of carbon, nitrogen, hydrogen and oxygen. These vary so little between different proteins that very accurate estimation is necessary and even then elementary composition cannot be regarded as a good test of individuality. Methods have since been evolved for characterising the individuality of proeirns by the amounts of various amino-acids to groups of amino-acids which they contain. Some of these methods have been used in the present investigation.

The results obtained by various workers on barley proteins by these methods are summarised below.

Hordein.—Of the four proteins of barley this is the easiest to prepare pure, and hence has been investigated more than the others. A number of analyses are given by previous workers. Osborne and Harris (3) obtained the following Hausmann numbers :—

Amide N	23·3	per cent.
Humin N	1·3	per cent.
Basic N	4·5	per cent.
Non-Basic N	70·0	per cent.

Osborne and Clapp (4) and also Kleinschmidt (5) subjected hordein to an analysis by the ester method, with the following results, which show the order of agreement of the analyses of various authors :—

	Osborne and Clapp (1907).	Kleinschmidt (1907).
Glycocoll	0·00	0·00
Alanine	0·43	1·34
Valine	0·13	1·40
Leucine	5·67	7·00
Proline	13·73	5·88
Phenylalanine ..	5·03	5·48
Aspartic Acid ..	—	1·32
Glutamic Acid ..	36·35 (43·20 later)	41·32
Serine	—	0·10
Cystine	doubtful	—
Tyrosine ..	1·67	4·00
Oxyproline ..	doubtful	—
Arginine	2·16	3·14
Histidine	1·28	0·51
Lysine	0·00	0·00
Ammonia	4·87	4·34
Total ..	71·32	75·83

Luers (6) and Hoffman and Gortner (7) have published Van Slyke analyses :—

		Luers.	Hoffman and Gortner
Amido N		23·00%	23·38%
Humin N		1·70%	1·44%
Basic	Cystine N	1·58%	1·38%
	Arginine N	5·00%	6·22%
	Histidine N	0·93%	10·36X%
	Lysine N	0·18%	3·02%
Non-Basic	Amino N of Filtrate	53·85%	50·41%
	Non-amino N of Filtrate	12·49%	3·65X%

These may be summarised for comparison with Osborne and Harris's results above as:

	Luers.	Hoffman and Gortner.
Amido N	23·00%	23·38%
Humin N	1·70%	1·44%
Basic N	7·69%	20·98%
Non-basic N	66·34%	54·06%

Proline tends to precipitate as phosphotungstate and appear in the analysis as histidine instead of appearing as non-amino nitrogen. This probably explains the results of Hoffman and Gortner, as their histidine and non-protein nitrogen values differ widely from those suggested by other methods. I am trying to overcome this difficulty.

The following figures have been published on the basis of colorimetric determinations:—

Tryptophane.	Cystine.	
0·45	1·47	Jones, Gersdorff and Moeller (8).
1·06	1·55	

Albumin.—I have prepared this in a fairly pure state and analysed it by the Van Slyke method. The results compare tolerably well with those of Luers and Landauer (9).

TABLE I.

	Luers & Landauer.	Author.
Amido N	9·40%	9·29%
Humin N	1·10%	2·29%
Cystine N	1·47%	—
Arginine N	11·64%	18·31%
Histidine N	4·48%	0·20%
Lysine N	8·44%	9·67%
Amino N of Filtrate	60·44%	58·93%
Non-amino N of the Filtrate	2·67%	2·54%

Globulin.—This has been studied, but I am not yet satisfied with the purity of the specimens I have prepared and analysed. No details of its composition have been published apart from its elementary composition.

It is not considered that present methods for separating these proteins (albumin and globulin) are satisfactory, and I am trying to improve the methods with a view to obtaining data which are less approximate.

Glutelin.—Osborne was unable to obtain this pure. A method, which is discussed later (page 109), has recently been worked out.

Jones and Gersdorff (10) and (11) have examined the proteins of wheat bran, and consider them different in composition from those of the endosperm. If this is confirmed then a similar difference might occur between the proteins of barley endosperm and husk. However, as Jones and Gersdorff attempted to obtain their proteins quantitatively as well as pure, the purity is open to doubt.

II.—Quantitative Estimation of the Proteins.

For purposes of quantitative estimation the criterion of solubility in specified solvents seems to give the sharpest separation and it is on this basis that most methods for estimating cereal proteins have been worked out.

Schjerning (12) attempted the separation by precipitation with heavy metal salts. This process involves adsorption and salt formation, and the precipitations are usually balanced reactions. The evidence seems to suggest that his precipitates are not definite separations of individual proteins. His claim that albumin I. and II. correspond to Osborne's globulin and albumin is based on a single experiment (13), in which he obtained approximately equal amounts of nitrogen in his precipitates, and in these proteins estimated in a manner somewhat similar to Osborne's method. Schjerning's immense masses of data (*Compt. rend. Trav. Lab.* Carlsberg) are consequently of questionable value.

In the following consideration of methods, experience with wheat will be drawn upon, since the problem of the estimation of wheat proteins has received a good deal of attention recently, and the problems are very similar; so that this work helps to throw light on the methods to be employed in barley.

Various papers on wheat protein estimation have been summarised by Bailey and Blish (14) and Sharp and Herrington (15).

It becomes clear from these summaries, that, as might be expected, the characteristic solvent for a particular protein does not extract that protein alone. For instance, Osborne (2) realised quite clearly that alcohol extracts other substances in addition to the alcohol-soluble protein, so that even in most of his qualitative preparations he extracted the barley or wheat flour with sodium chloride solution before the alcohol extraction. Despite this, nearly all the workers since that time (1895), in attempting even quantitative estimations of alcohol-soluble proteins of wheat and barley, have extracted the flour directly with alcohol. The fact that H. T. Brown (16) used alcoholic extraction as a method of separating amides, amino-acids and "unclassified nitrogen" from barley, makes it clear that the alcohol-soluble protein is not the only nitrogen compound in barley which dissolves in alcohol.

The albumin and proteoses, amino-acids, etc., cannot be extracted by water before extracting the globulin by salt solution, since barley contains free salts such as phosphates which dissolve some of the globulin.

The best method is to extract first with salt solution and then with 70 per cent. (by volume) alcohol. However, the alcohol-soluble protein is soluble to a small extent in water and salt solutions. Osborne investigated this and states (2) "It is the opinion of the writer that the hordein of barley is decidedly less soluble in water than the gliadin of wheat." Consequently, the problem of sharp separation of proteins in barley appears to be easier than with wheat.

Bailey and Blish (14) attempted to ascertain the solubility of gliadin in their salt solution extracts of wheat by an ingenious method, which is of no value in this case; nevertheless, as this method is of value in the alcohol extract it will be explained in detail.

It depends on the fact that gliadin of wheat has a very high percentage of amide nitrogen (25·52 per cent. is quoted as the best determination), while the amide nitrogen of wheat albumin is 6·8 per cent. and of the globulin 7·7 per cent. Amide nitrogen determinations of different workers on the same protein in the Van Slyke process agree much more accurately than do any of the other figures, so this appears to be the most accurate character which can be determined chemically. Bailey and Blish argued that the ratio of amide to total nitrogen (ammonia percentage) of the salt solution should be that of the mean of the percentages in albumin and globulin. This mean was adjusted to allow for the relative amounts of albumin and globulin. Higher percentages would then indicate the amount of gliadin. This neglects the fact that approximately half of the salt extract consists of proteoses, amino-acids and other nitrogenous compounds, and consequently the method is of no value in this case.

In attempting to estimate the solubility of hordein in salt solutions, I used two methods, both of which indicated that it was low. Details are given in the following section.

After the salt extraction it seems possible to extract the alcohol-soluble protein quantitatively by hot alcohol, as the only nitrogen compound present. The purity is indicated by the ammonia percentage method of Bailey and Blish which can be applied here. Bailey and Blish obtained with wheat 25·57 per cent. of ammonia in the alcohol extract after salt extraction (value quoted for pure gliadin 25·52 per cent.).

The ammonia percentage of the alcohol extract determined by myself for barley agrees closely with that for pure hordein.

	Ammonia % of Extract.	Values for pure hordein.
Barley "B"	23·3	Osborne(2) 23·3%
		Luers (6) 23·00%
Orwell Barley, 1925	23·2	Hoffman & Gortner(7) 23·38%

Hence the total nitrogen of the alcohol extract may be taken as a measure of the hordein present.

Undoubtedly the alcohol extracts bodies of the lecithin type, which contain some nitrogen. However the amount of these appears to be small both from the evidence above and from the fact that only small amounts of nitrogen were obtained by ether extraction of the residue of evaporated alcoholic extracts.

Sharp and Herrington (15) show that, after salt extraction, hot alcohol removes more nitrogen from wheat flour than cold, and

since Bailey and Blish (14) show this belongs almost entirely to gliadin, it follows that the hot alcohol extraction, without decreasing the purity of the product, is more efficient than cold alcohol in extracting the alcohol-soluble protein.

Glutelin by Difference.—After the removal of salt-soluble and then alcohol-soluble nitrogen the remaining nitrogen probably represents fairly pure glutelin (which has, however, been denatured by the alcohol and cannot be extracted). That this is so with wheat is indicated by the ammonia per cent. of the residue which was found by

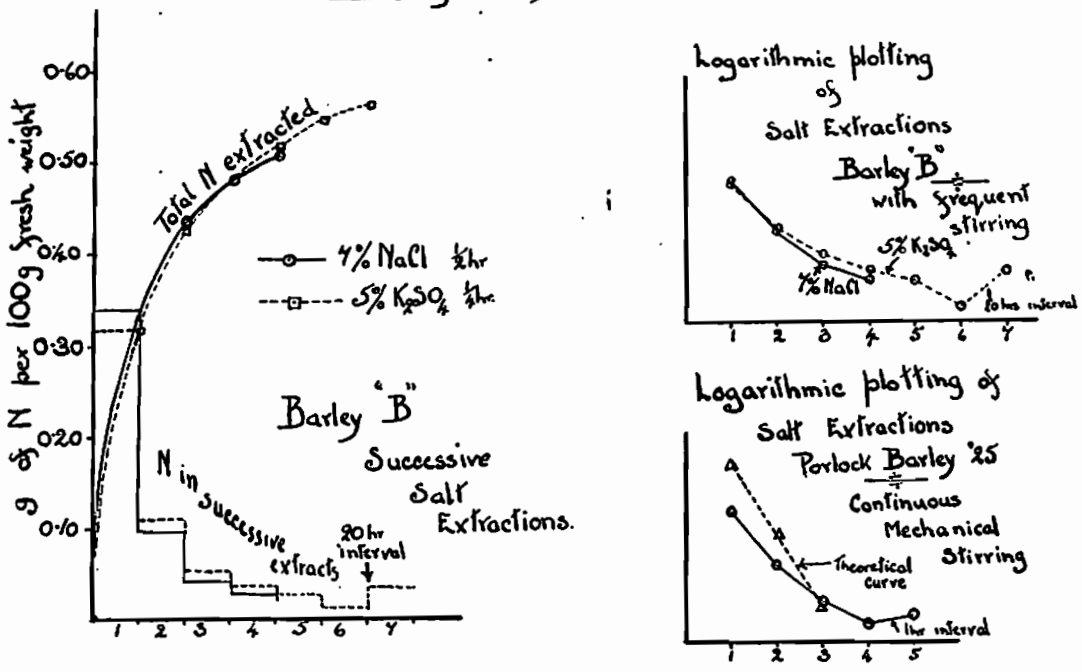
Experimental.

The preparation of albumin for Van Slyke analysis was made in the usual way, that is, by ammonium sulphate precipitation of sodium chloride solution extracts of barley. The precipitate was extracted with water to remove albumin and the solution then dialysed and the albumin separated by coagulation at 70°C.

The liquids were filtered at each stage and the protein washed with alcohol and ether, and dried in vacuo.

As stated above, this method is not very satisfactory, and it is hoped to improve it.

Diagram I.



Bailey and Blish (14) to be 18·6 per cent., agreeing with Osborne's value of 18·8 per cent. for pure wheat glutelin.

American workers (17) have also found rough agreement between their methods for estimating wheat glutelin directly and the estimation by difference.

Similar agreement for amounts of glutelin in barley estimated by direct and indirect methods is shown below. (Diagram V.)

Salt Extraction.

The results of the successive salt extractions of barley are given in Diagram I.

Note.—The percentages of nitrogen for barley "B" are calculated as percentages of the fresh weight of the sweated barley. The nitrogen percentages of all other barleys are calculated on dry matter.

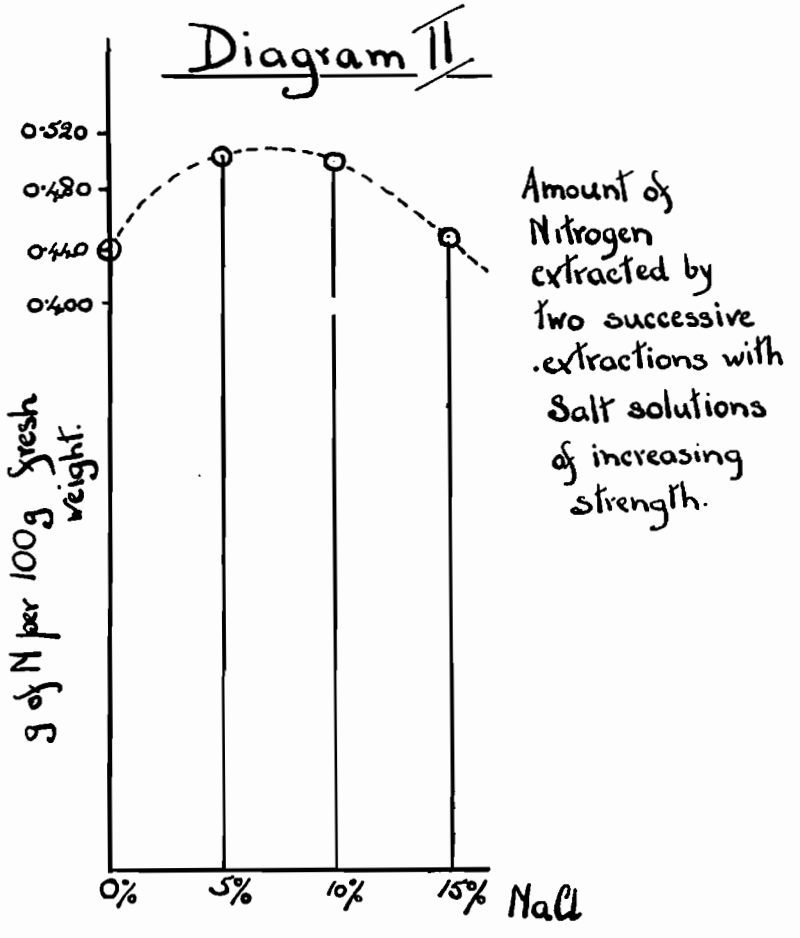
The residual solubility of nitrogen is low compared with the total amount extracted.

This solubility is due to residual albumin and globulin, to proteolysis, and to solubility of hordein and glutelin. Hence none of these factors appears to interfere seriously. The extra amount obtained by bacterial action, etc., after 20 hours is not large.

For the direct estimation of hordein solubility, preparations of hordein were made from barley previously extracted by salt solution. The hordein was precipitated in a finely divided condition and washed on the centrifuge with water to remove alcohol.

The solubilities were as follows :—

	Grms. Hordein N per 100 ccs. of solution.	Amount of Hordein N which would be dissolved in 5 extractions of 70cc.
Water ..	0·0030	0·010
1% NaCl ..	0·0030	0·010
8% NaCl ..	0·0024	0·003
16% NaCl ..	0·0016	0·006
0·15% K_2SO_4	0·0023	0·008
5·0% K_2SO_4	0·0024	0·008



Equal amounts of hordein were added to the solutions and left to stand in the presence of toluene for 20 hours with occasional stirring. The amount of nitrogen in a known volume of the filtered solution was determined by Kjeldahl.

The amount dissolved by 350 cc. of 5 per cent. K_2SO_4 (0·008) is equivalent to 3 per cent. of the hordein nitrogen or low nitrogen barleys, and 1 per cent. on high nitrogen barleys.

Determinations of the amount extracted

by two successive extractions showed that the maximum amount of nitrogen was extracted by sodium chloride between 5 per cent. and 10 per cent. strength.

5 per cent. potassium sulphate extracted about the same amount of nitrogen as 7 per cent. sodium chloride (see Diagram I.). Hence 5 per cent. potassium sulphate was adopted as a standard, for convenience in the subsequent Kjeldahl process.

The salt extracts were too gummy to filter, and it was found necessary to centrifuge them. Even after centrifuging, filtration was very slow and the clogged filter probably acted as an ultra-filter, thus retaining proteins in true solution. Consequently the salt and alcohol extracts were centrifuged from the barley meal and the extracts poured off from the compact mass. Under these circumstances the values for the amounts extracted will probably be rather high, due to protein matter in suspension, while in the filtered extract they will be too low.

Salt extract barley "B."	
Centrifuged extract	0·499 grm. N/100 grms. of sweated barley.
,, ,, filtered..	0·473 grm. N/100 grms. of sweated barley.
Difference	0·026 grm. 5% of amount extracted.

With the amounts employed in the standardised method (70 cc. of solution to 10 g. of barley) it was found that 12 grms. of solution were retained by the barley centrifuged off at 2,500 revs. per min. Hence at least three extractions would be necessary to reduce the remaining salt-soluble nitrogen to a negligible amount. It will be seen from Diagram I. that five extractions are necessary in practice owing to the slowness of the diffusion of the proteins from the particles of barley.

Continuous shaking in a mechanical shaker was found to increase the efficiency of the extraction. This can be seen in the steeper slope of the logarithmic plottings of Porlock barley compared with those of barley "B" (Diagram I.).

The solubility appears to be affected by the fineness and evenness of grinding, so that these must be standardised. An adjustable large cone coffee mill proved more satisfactory than most mills.

The p_H of the potassium sulphate solution used was between 5 and 6. This is the isoelectric region of hordein and glutelin at which they are most insoluble.

Fractionation of Salt Soluble Substances.—Luers and Landauer (18) have shown that p_H 4·6 is the isoelectric point of barley albumin, and this has been adopted as the point for the best coagulation of albumin. The solutions were heated to 82°C. till coagulation was complete (20 minutes). Recently, however, it has been found that the values obtained in this way for albumin were too high, since the acidity of the sodium acetate-acetic acid buffer caused some precipitation of globulin which cannot be filtered or centrifuged off completely. Hence it would be preferable to make the estimation of albumin on water extractions of barley.

Barley "B."	
Albumin N for water extraction ..	0·073% N/100 sweated barley.
"Albumin N" 5% salt ,, ..	0·125% N/100 sweated barley.

The albumin precipitate is too light to centrifuge off and must be filtered off for the nitrogen estimation.

Total Protein.—The amount of nitrogen in the form of fully built up proteins can be estimated approximately by precipitation with 5 per cent. trichloracetic acid or 5 per cent. salicyl sulphonic acid (sulpho-salicylic acid). The amount of nitrogen precipitated depends on the concentration of the precipitant, so that only comparative results can be obtained by using standardised conditions.

Barley "B."	
5% Trichloracetic acid N=...	0.290% sweated barley
3% Salicyl-sulphonic acid N=	0.250% ,, ,,
Total nitrogen of salt solution N=	0.498% ,, ,,

These results indicate that approximately half the nitrogen of the salt extract represents fully built up proteins.

Diagram III. shows roughly the proportions of the different nitrogen compounds in the salt extract.

Non-protein Nitrogen.—This was estimated by the method of Blish (19) as modified by Olsen and Bailey (20). The solution is made alkaline by N/10 sodium hydroxide. Two drops of phenol-phthalein solution

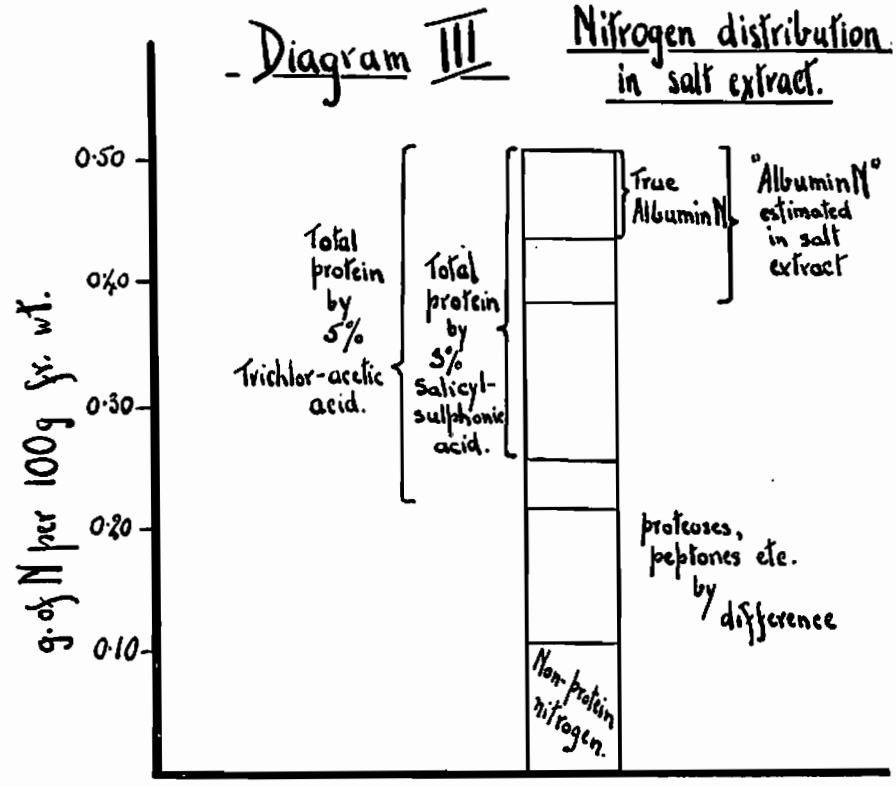
are added and N/10 copper sulphate is then run in until the violet colour of the solution just changes permanently to green. This ensures precipitation at a constant p_H (8·0). Proteins, proteoses, and some amino-acids are removed as copper compounds by centrifuging.

The precipitation is an equilibrium reaction depending on the amount of precipitant, as is shown by the following figures.

Barley 166 cc. (Archer, 1924 crop, N.I.A.B.)

The precipitate from 140 cc. salt extract was dissolved in 20 cc. N/10 alkali made to 140 cc. with 4 per cent. potassium sulphate salt solution and re-precipitated by N/10 $CuSO_4$.

Nitrogen in precipitate.	Nitrogen in solution.
0.374 grm. N/100 grms. of dry barley	0.022 grm. N/100 grms. dry barley = 5.6%



Barley 167cc. (Archer 1924 crop N.I.A.B.) N/100 grms. dry barley.

	Protein N	Non-protein N	Total N
140 cc. salt ex. 14 cc. N/10 NaOH ..	0·385	0·130	0·515
140 cc. salt ex. 30 cc. N/10 NaOH ..	0·425	0·078	0·503
	Total nitrogen direct		0·509

The copper hydroxide-protein precipitate is somewhat soluble in the salt solution in which it is precipitated. Hence comparative results only can be obtained by using standardised conditions.

The non-protein nitrogen from Blish's work (19) consists roughly of lower polypeptides, some amino-acids, asparagine and what H. T. Brown termed " unclassified nitrogen," which he showed probably contained some betaine and choline (16). By analogy with the results of Kiesel (21) on rye, compounds such as xanthine, guanine, adenine, hypoxanthine, tetramethylene-diamine, may be present.

Alcohol Extraction.—Figures of the ammonia percentage given in the previous section (page 103) indicate that the nitrogen extracted (after salt extraction) probably represents nearly pure hordein.

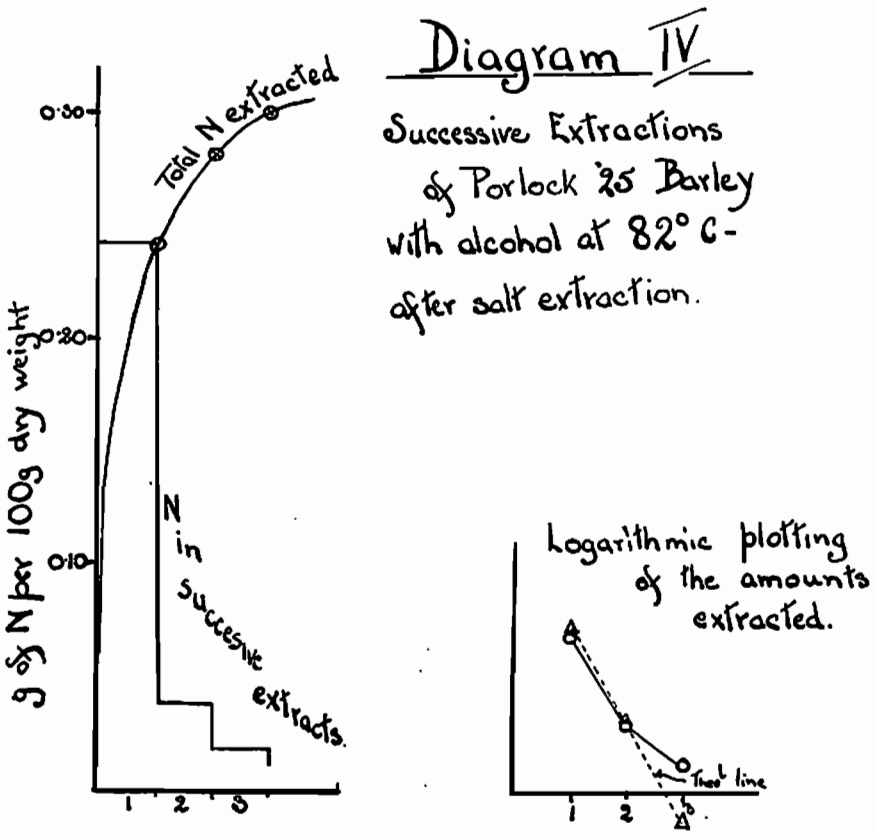
The alcohol-soluble protein is not very soluble in cold alcohol, when the barley has been extracted first with salt solution.

50-60 per cent. alcohol at 19° C. dissolves 0·12 per cent. of hordein nitrogen per 100 cc.=approx. 0.68 grms. hordein per 100 cc.

2,500 revs. per min. So that under the standard conditions (60 cc. of alcohol to 10 grms. of barley) three extractions would be necessary theoretically to extract nearly all the nitrogen. Successive extractions shewed that this number was sufficient in practice.

Glutelin.—Osborne (2) was unable to purify specimens of the alkali-soluble protein of barley, and pure preparations have not yet been made so that its properties are unknown.

In the quantitative experiments it has been



So that hot alcohol must be used for the extraction. Bailey and Blish's (14) method of extracting wheat with hot alcohol under pressure, to prevent loss of solvent, appeared to be better than refluxing as being less likely to cause denaturation.

The number of extractions necessary for the complete extraction of the alcohol-soluble nitrogen under these conditions was determined. Eight grms. of alcohol are retained by 10 grms. barley centrifuged off at

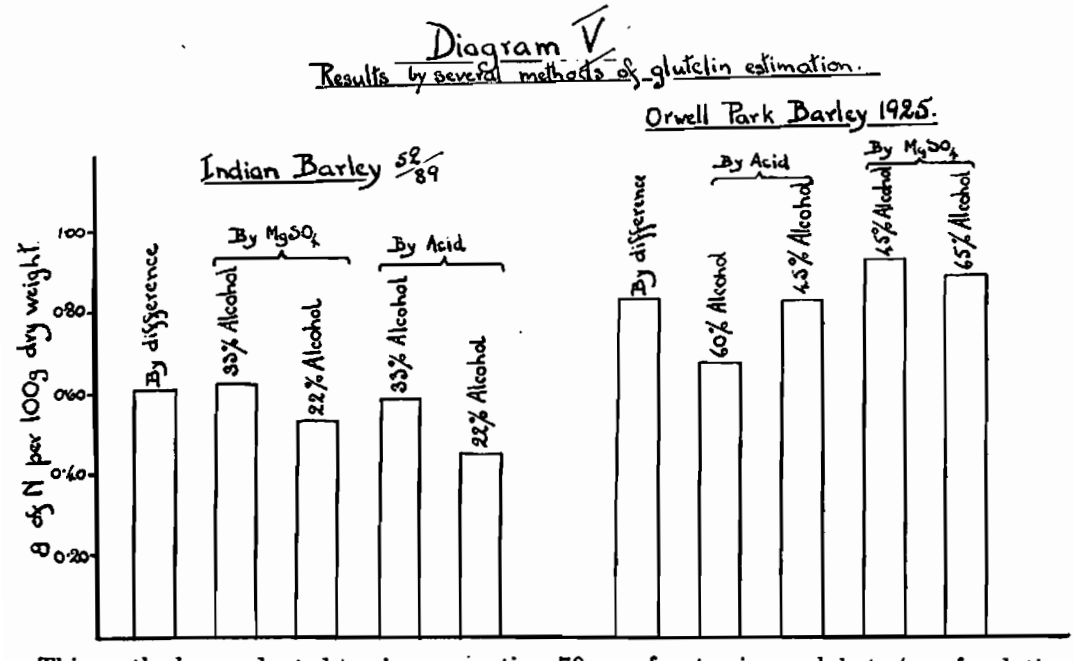
estimated by difference under conditions comparable with those which when used with wheat probably give a fairly good measure of glutelin nitrogen.

Two methods which have been used for the direct estimation of wheat glutelin have been applied to barley.

(a) Blish and Stanstedt (22) dissolved all the proteins of wheat in dilute alkali, added alcohol to keep the gliadin in solution, filtered the solution clear and precipitated the

glutelin by adding acid until the isoelectric point p_H 6 was reached. The amount of nitrogen in the precipitate was then estimated. I have applied this method to barleys, using various strengths of alkali and alcohol.

(b) Csonka and Jones (23), in order to obtain pure preparations of the glutelins, treated wheat, rice and oat flour with alkali, added alcohol, filtered and precipitated the glutelins, which are sensitive to the presence of salts, by the addition of small amounts of ammonium sulphate.



This method was adapted to give a quantitative estimate of the glutelin present in barley. Magnesium sulphate solution was used as the precipitant instead of ammonium sulphate.

Some of the results by both these methods are summarized above (Diagram V).

These results show that there is a fairly good agreement between the estimation by difference and the direct estimation.

Further study of the methods will probably lead to an accurate method for the direct estimation of barley glutelin.

III.—Details of the Method Used for the Quantitative Estimation of Barley Proteins.

About 50 grms. of barley is carefully cleaned of extraneous matter and finely ground in a coffee mill, the resulting flour being well mixed to reduce sampling errors.

Moisture.—This was determined in duplicate on 5 grm. samples which were dried at 98° C. for four hours in a current of heated dry air (Siau oven).

Total nitrogen was determined in duplicate on 2 grm. samples.

Salt Extraction.—This is usually carried out with two or four samples at once. Exactly 10 grms. is weighed out into a 100 cc. centrifuge tube. The flour is stirred with 70 cc. of potassium sulphate (p_H of solution 5-6) corked and shaken in a mechanical shaker for 15 minutes. The pairs of tubes are then balanced by the addition of water and centrifuged at 2,500 revs. per min. for 5 minutes. The supernatant liquid is filtered through cotton wool into a 500 cc. graduated flask. The residue in the centrifuge tube is again extracted in the same way and the process repeated to give five extractions in all. The resulting extracts are made up to 500 cc., and 100 cc. is taken for the determination of total nitrogen present.

Albumin N.—200 cc. of the salt extract is buffered to p_H 4·6 with 20 cc. of buffer solution (equal volumes of normal solutions of sodium acetate and acetic acid) and heated to 82° C. for 20 minutes in a tube carefully

cleaned by dichromate and sulphuric acid. Under these conditions the albumin coagulates into large particles which do not adhere to the tube. The precipitate is filtered off on a coarse filter paper, and the nitrogen in the precipitate determined by Kjeldahl. (Note that the albumin by this method is probably too high, for the reason explained above, page 106.

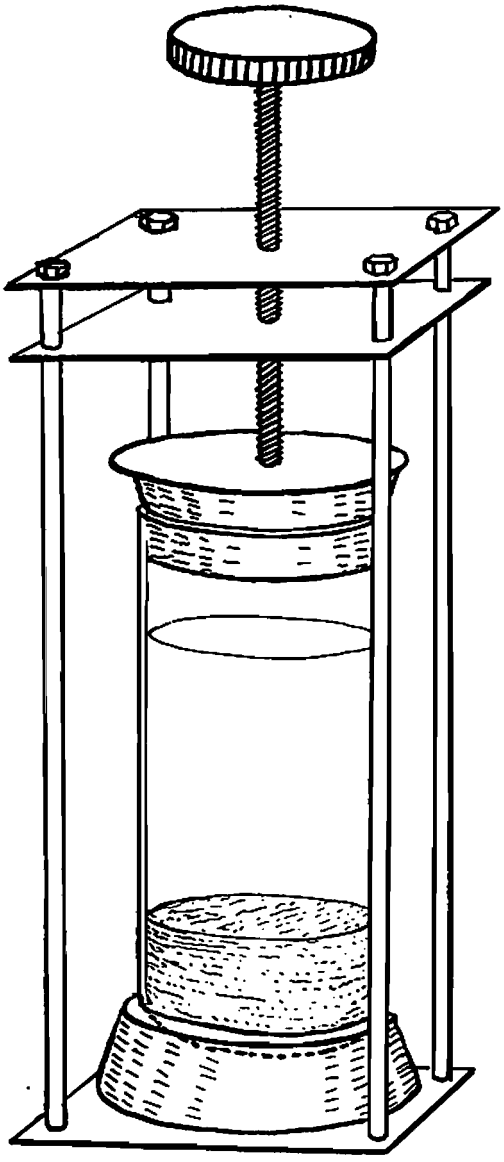


DIAGRAM VI.

APPARATUS FOR ALCOHOLIC EXTRACTION UNDER PRESSURE.

Non-protein Nitrogen.—70 cc. of the salt extract is put into each of two 100 cc. centrifuge tubes and 10 cc. of N/10 sodium hydroxide added to each. Two drops of phenol-phthalein are added and N/10 copper sulphate run in from a pipette, with stirring, until the violet colour just changes permanently to green. After standing a few minutes the tubes are balanced and the precipitate centrifuged off. The nitrogen in the precipitate is determined by Kjeldahl and represents "protein" nitrogen. That in solution is determined and represents "non protein" nitrogen. The sum of the two is a check on the accuracy of the estimation of total nitrogen of the salt extract and of the "protein" and "non-protein" nitrogen.

Alcohol-soluble Nitrogen. Hordein Nitrogen.—The salt-extracted barley is mixed with 60 cc. of 70 per cent. (by volume) ethyl alcohol. The tube is closed with a rubber stopper and is screwed tightly in the small brass apparatus shown in the diagram.

The tube is then put in a bath, maintained at 82° C. and is shaken at frequent intervals. At the end of 30 minutes the tube is taken out, allowed to cool a minute, unscrewed, balanced, and centrifuged while hot for one minute at 2,500 revs. per min. The alcohol extract is poured into a 250 cc. graduated flask containing a few drops of strong alkali, which prevents deposition of protein from the cooling solution. Two further extractions are made under the same conditions and the total extract allowed to cool and made up to 250 cc. Total nitrogen is determined in duplicate on 100 cc. lots of the solution.

Glutelin Nitrogen.—This is determined by subtracting the sum of the salt and alcohol-soluble nitrogen from the total nitrogen of the sample.

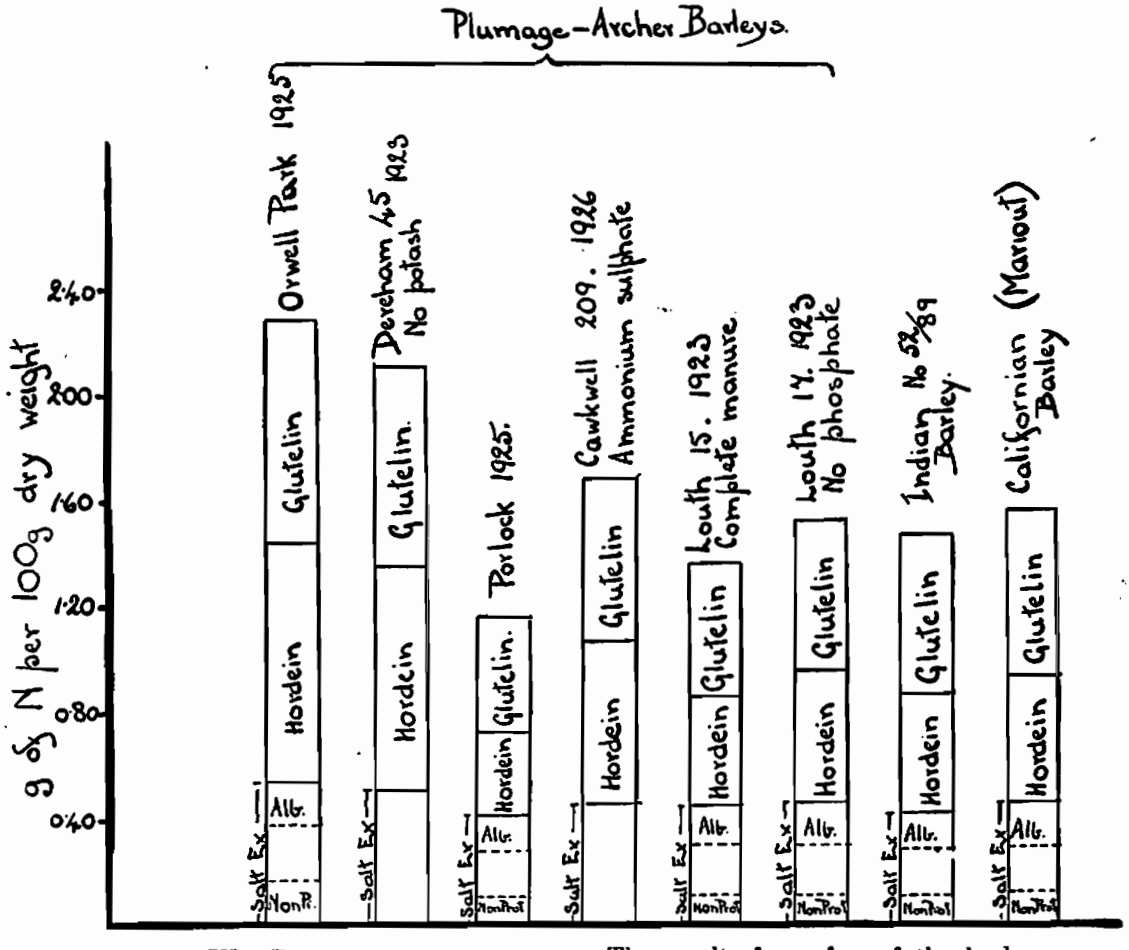
The successive salt extractions are carried through as quickly as possible after one another and similarly the estimations on the solutions are carried out immediately, to minimise bacterial action and denaturation. After the last salt extraction the barley residue is at once mixed with the first lot of alcohol. It is well to carry through this extraction quickly to reduce the possibility of denaturation. The Kjeldahl determination on the extract should be done soon after

the solution is made up to avoid the possibility of the loss of some nitrogen as ammonia.

The above set of processes is designed to estimate as accurately as possible single proteins or a definite group of proteins and further accuracy has been obtained by standardised procedure.

Among them may be mentioned the Louth barleys which gave better malts than was expected from the appearance of the barley. Porlock barley was the sample with the lowest nitrogen content, and Orwell Park that with the highest during the whole of the experiments.

Diagram VII



IV.—Results.

A selection was made of "good" and "bad" barleys from the samples of Plumage-Archer barleys grown under the Institute scheme. Among those chosen were examples which departed from the general trend of nitrogen content with valuation, so that their total nitrogen content was not sufficient to explain the valuation.

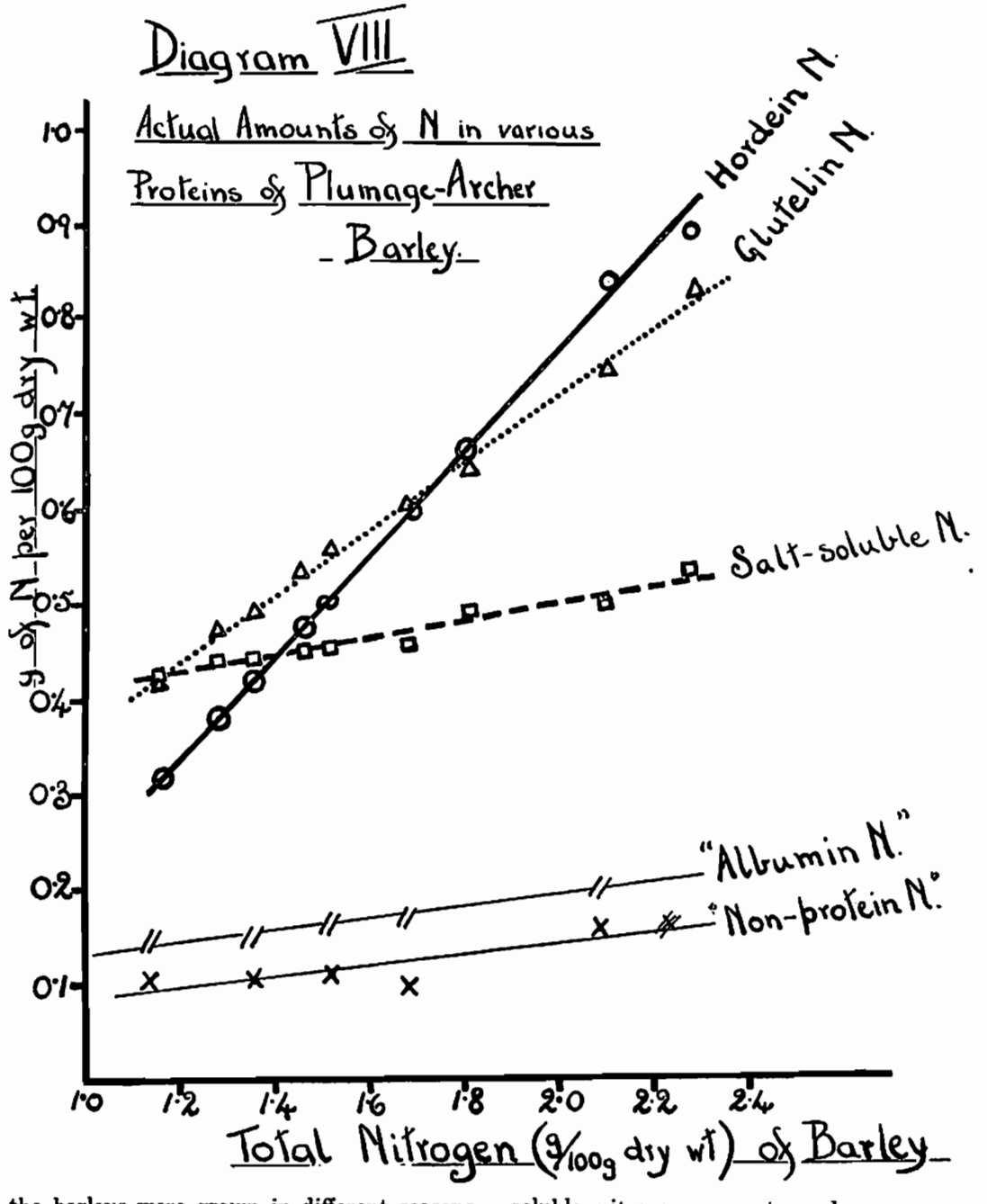
The results for a few of the barleys are shown diagrammatically above (Diagram VII.).

It is clear that no striking irregularities are to be seen in the proportions of different proteins.

When the amounts or percentages (Diagrams VIII. and IX.) of nitrogen in the different protein fractions are plotted against

the total nitrogen in the barley an evident regularity runs through the group.

It will be noticed from Diagram IX. that the percentage amount of glutelin remains constant while that of hordein increases with increasing total nitrogen, and the salt



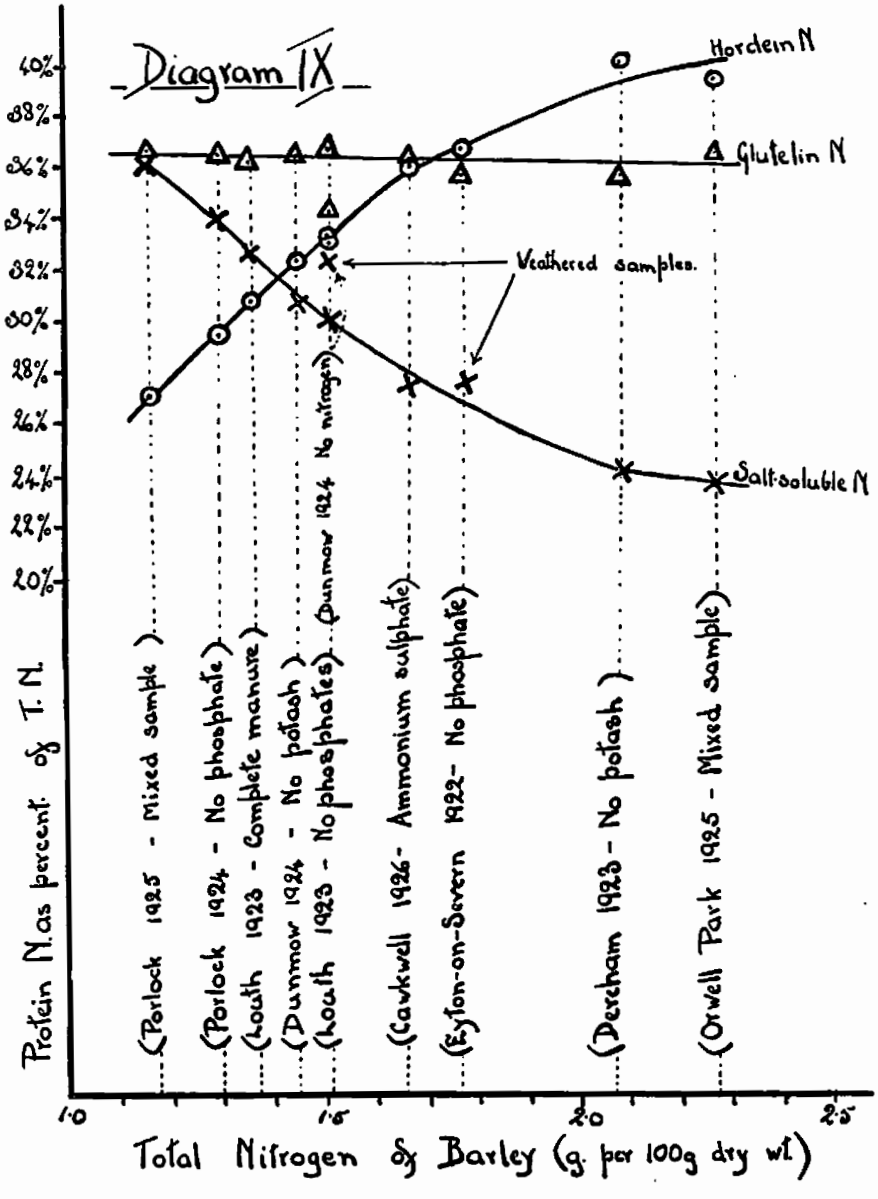
soluble nitrogen percentage decreases correspondingly. As might be expected, the "albumin" and "non-protein" nitrogen

the barleys were grown in different seasons and that the soils and the manuring were very varied. This diagram also shows that

curves behave similarly to the salt-soluble nitrogen curve. Beaven (25) found that the percentage of alcohol-soluble nitrogen increased in greater proportion than the total nitrogen, which is in agreement with the above conclusion.

and are affected only indirectly by conditions such as soil, climate and manuring.

Section XI. (pp. 118-123) of Mr. Hulton's Report (24) summarises previous work and theories on the relation of the amounts of the various nitrogenous constituents to



For the barleys shown in the curves it follows that the relative proportions of the different nitrogenous substances at maturity depend on the total nitrogen of the grain, what is referred to as "quality" in barley.

The total nitrogen of the mature barleys used in the above experiments is then a

good measure of the amounts of the separate constituents, and differences in "quality" between these barleys could not be explained as due to differences in amounts of individual proteins apart from the variation in total nitrogen. The total nitrogen content, as is well known, is widely influenced by soil and season. Inside the grain, however, the proportions of constituent proteins may be pictured as balanced, so that any given amount of total nitrogen would at maturity have settled down to predictable amounts of each constituent.

Plottings of results with other barleys together with the Plumage-Archer curves are given in Diagram X.

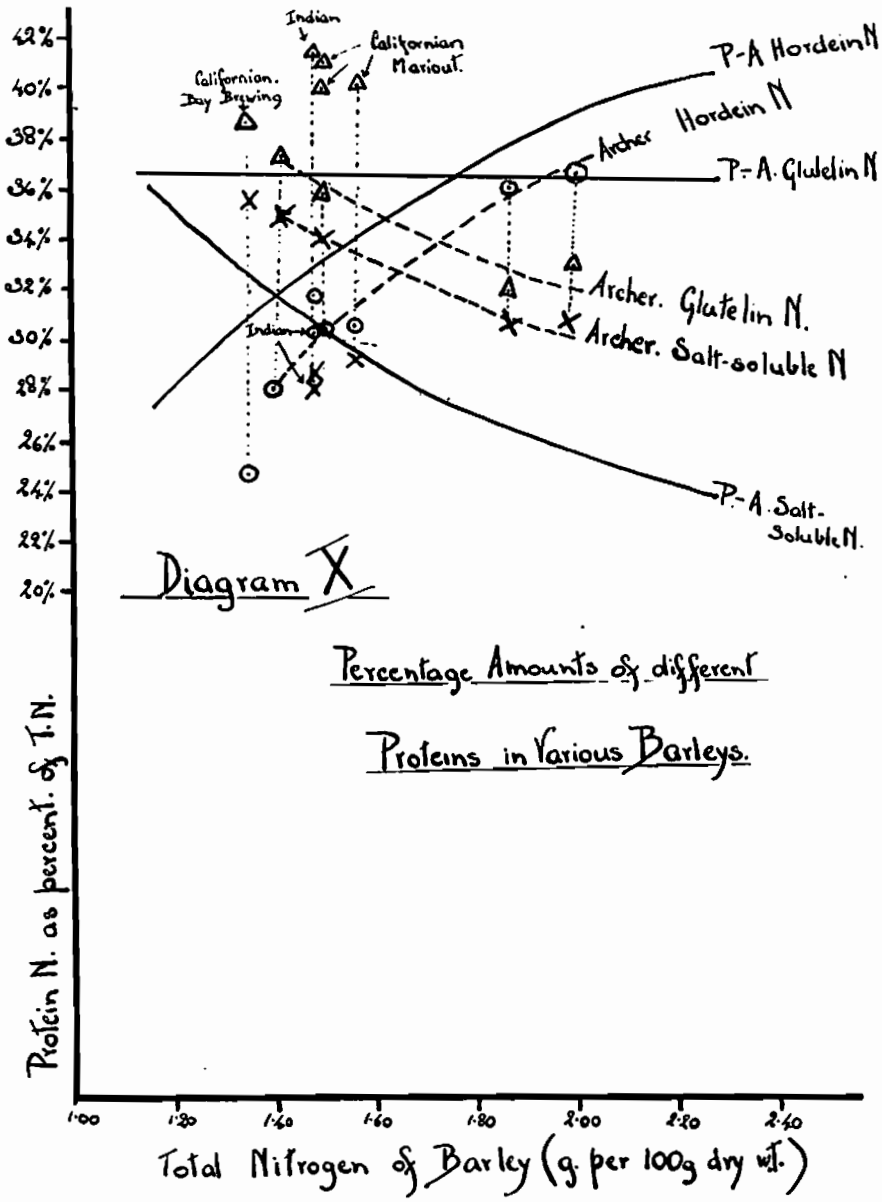


Diagram X

Percentage Amounts of different Proteins in Various Barleys.

The smoothed curves for the results of the Plumage-Archer barleys have been given for comparison—"P.-A. salt-soluble," etc.

They indicate that other varieties of barley may have curves similar to those of

Plumage-Archer. The glutelin percentage of Indian and Californian barleys appears to be higher than that of English barleys, while the percentage of salt and alcohol-soluble fractions is smaller.

Beaven (25) noted that the percentage of alcohol-soluble nitrogen is generally lower with six-rowed varieties.

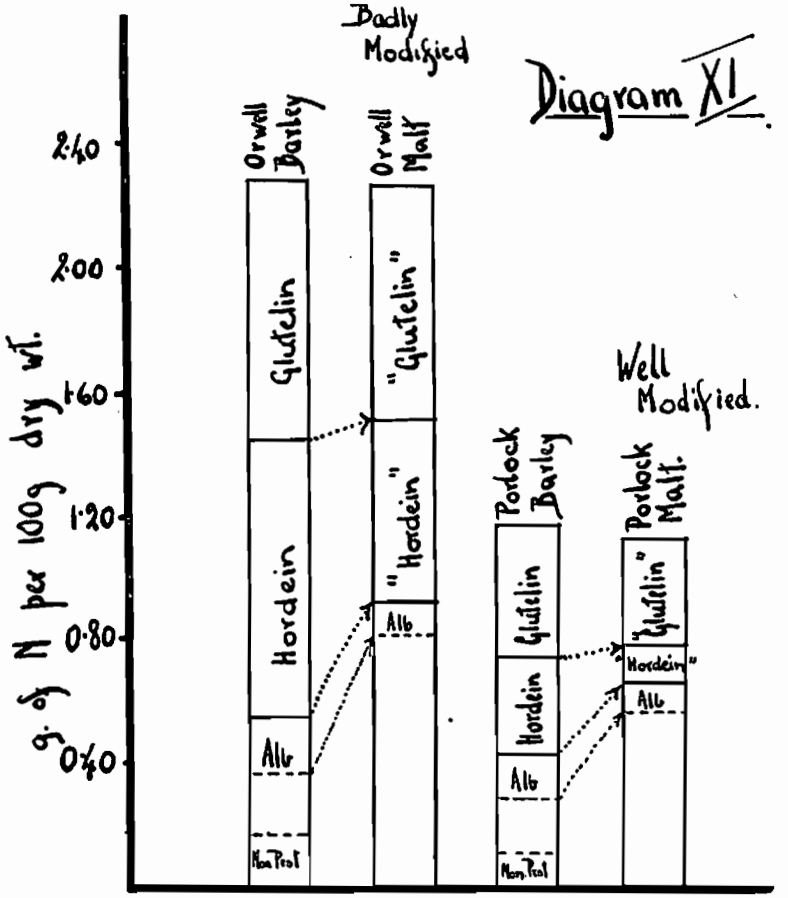
Various possibilities are suggested by these results. (1) If wheats show a similar behaviour it might be possible to cross varieties possessing other desirable characters with varieties having a high glutelin percentage, since this protein is chiefly responsible for the baking quality of bread.

(2) Some preliminary experiments indicate that hordein is the chief protein attacked during malting. (See Diagram XI).

This agrees with a comparison of Osborne's (2) and (26) estimates of the proteins in barley and malt, and also with the work of Kraft (27).

Barleys which have a high glutelin content would then have less protein already salt and water-soluble, and less hordein to break down into such compounds. So that barley varieties with a high glutelin content may be of value in brewing in being equivalent to nitrogen diluents. This deduction is at present very problematical.

My work does not appear to support the suggestion that wide variations in the alcohol-soluble protein may account for differences in "quality" between barleys of the same variety. A great many other factors enter into the determination of "quality." In particular the proteins are broken down by enzymes, and different enzymes (peptase, tryptase, etc.) break them down to different stages—proteoses or peptones, or right down to amino-acids. So that the amount and nature of the proteolytic enzymes will have a large share in determining the nature of the resulting nitrogen



compounds in the wort and beer. Hence the amounts of the different proteolytic enzymes and the acidity conditions under which they act will be important factors in "quality" from the nitrogen standpoint.

Comparison with Results of Other Workers.

In Diagram XII. the results for hordein (hot alcohol extraction after salt extraction) are compared with the results of other workers who extracted directly with alcohol, either in the cold, H. L. Hind (28), Beaven (25), or when warm, Murphy (29), or by boiling under a reflux condenser (unpublished data by courtesy of H. L. Hind). In addition to the hordein, alcohol dissolves other nitrogen compounds. So that boiling alcohol gives a higher result than the hordein estimation; but the small solubility of hordein results in it being incompletely dissolved by cold alcohol, so that the total amount of nitrogen dissolved by this is less than the amount of hordein nitrogen alone.

From a short account (30) of work by Prior on Austrian barleys, it is probable he adopted methods similar to those above. As indicated on the diagram, his estimates of the alcohol-soluble protein cover a wide range.

Recent work in Japan on the amounts of proteins in three varieties of barley has been mentioned in an abstract (31), which is not full enough for an opinion to be formed of the value or bearing of the work.

Summary and General Conclusions.

The main object of the present work was the estimation of the various proteins in barley. A study was made of the conditions which were necessary for doing this. As a result, a method was developed and standardised. The albumin, globulin and breakdown products of protein are first extracted from a sample of barley by salt solution. In this fraction, "albumin nitrogen" and "non-protein nitrogen" are estimated. After the salt extraction hordein is extracted by hot alcohol. The nitrogen in this extract probably represents approximately pure hordein. The remaining nitrogen, after the salt and alcohol extractions, probably represents pure glutelin. Estimations of the amounts of glutelin by two direct methods show tolerable agreement with the estimation by difference

Direct determination of the alcohol-soluble nitrogen in barley without the previous treatment with salt solution does not satisfactorily estimate the amount of hordein.

Results are given of estimations by the above method of selected samples of Plumage-Archer barley grown under the Institute's scheme in different years, on different soils, and with different manurings.

In these samples, the total nitrogen varied from 1·2 per cent. to 2·3 per cent.

(*a*) The percentages of glutelin remained constant at 36 per cent. of the total nitrogen, whatever this amount.

(*b*) The percentage of hordein increased as the total nitrogen increased, rising from 26 per cent. of the total nitrogen when 1·2 per cent. of nitrogen was present to 40 per cent. of the total nitrogen when 2·3 per cent. of nitrogen was present.

(*c*) The percentage as salt-soluble nitrogen fell from 36 per cent. to 24 per cent. as the total nitrogen increased from 1·2 per cent. to 2·3 per cent.

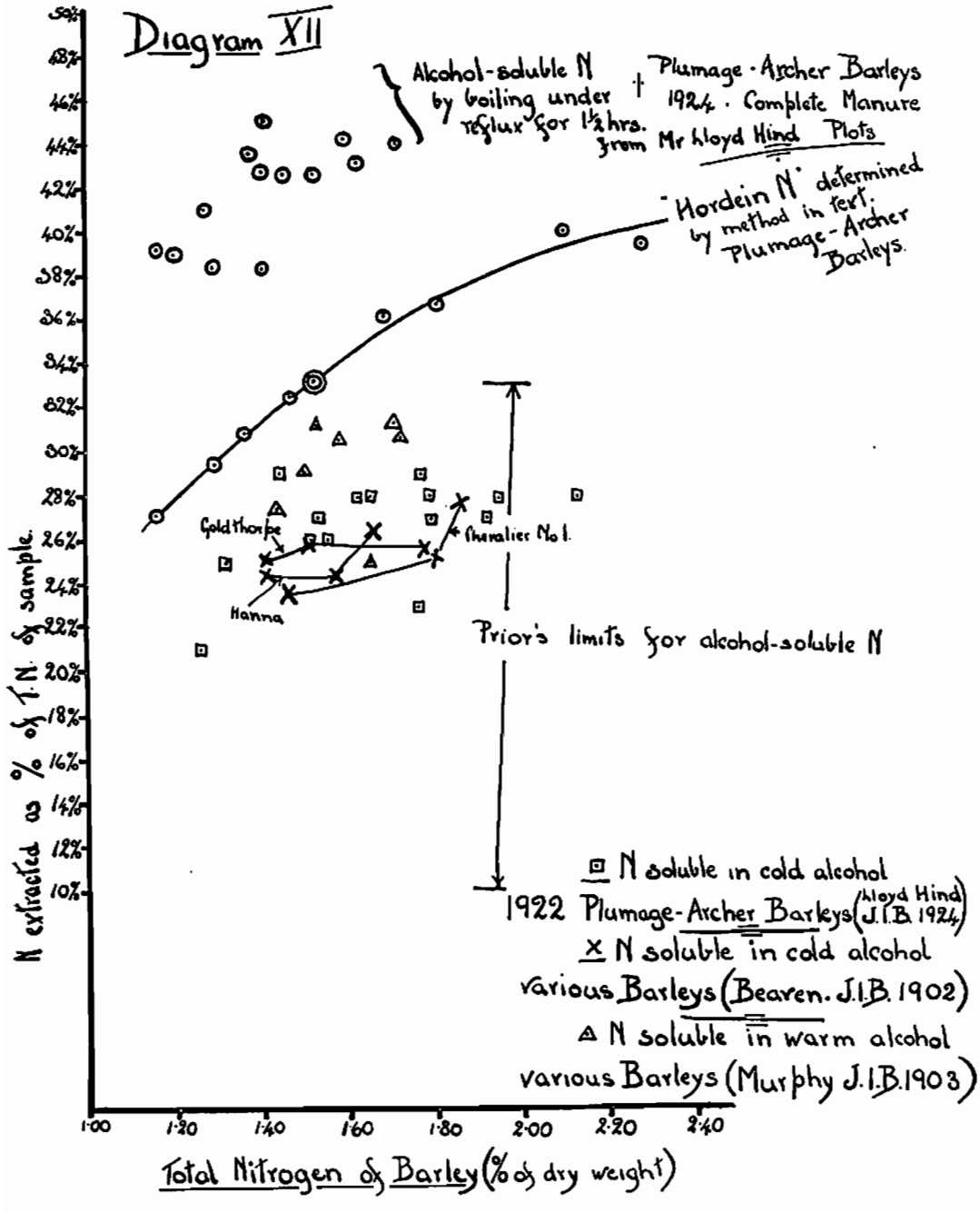
The analytical figures for mature samples fell on smooth curves between these points, whatever the history of the barley, indicating that for these samples external conditions, while altering the total amount of nitrogen in the grain, appeared to have no influence on the proportions of the proteins in the mature barley. There appears to be a balance between the various proteins which adjusts itself according to the total amount of nitrogen present. For these samples, therefore, total nitrogen is a good measure of the amounts of individual proteins, and, further, the varying "quality" of the mature samples of comparable barleys having equal nitrogen content, is not due to variations in the amounts of individual proteins, such as hordein. The amounts of the various proteolytic enzymes may, however, play an important part in determining "quality."

Other barleys, not from the Institute's set, gave curves of apparently similar type, but somewhat different numerical values.

Some preliminary experiments on malt indicate that hordein is the chief protein which is attacked during malting, breaking down to give salt-soluble compounds. The amount of albumin also appears to fall slightly. A possible bearing of this is that barleys having more glutelin, *i.e.*, the protein which appears *not* to give rise to soluble

nitrogen, may be of value as nitrogen diluents.

The existing methods of isolating and



Hordein, having an exceptionally high proline content, cannot be analysed satisfactorily by the present Van Slyke process; studying the various nitrogen compounds from barley have been investigated, but they have not proved altogether satisfactory. it is hoped to improve the methods for isolating the albumin and globulin.

I wish to acknowledge valuable advice

from Dr. A. C. Chibnall and from Mr. H. J. Page, B.Sc., during the course of the work, and from Sir John Russell, D.Sc., and Mr. H. L. Hind, B.Sc., F.I.C., in the preparation of this paper.

REFERENCES.

(1) Sir E. J. Russell and H. M. Lancaster. Reports (*this Journ.*, 1924, *et seq.*)
(2) T. B. Osborne (*J. Amer. Chem. Soc.*, 1895, **17**, 539.)
(3) T. B. Osborne and I. F. Harris (*ibid.*, 1903, **25**, 323.)
(4) T. B. Osborne and S. H. Clapp (*Amer. J. Physiol.*, 1907, **19**, 117.)
(5) A. Kleinschmidt (*Z. Physiol. Chem.*, 1907, **54**, 110.)
(6) H. Luers (*Bio. Chem. Z.*, 1922, **133**, 603.)
(7) W. F. Hoffman and R. A. Gortner (*Colloid Symposium Monograph II.*, 1925. 209.)
(8) D. B. Jones, C. E. F. (Gersdorff and O. Moeller (*J. Biol. Chem.*, 1924-25, **62**, 183.)
(9) H. Luers and M. Landauer (*Bio. Z.*, 1922, **133**, 598.)
(10) D. B. Jones and C. E. F. Gersdorff (*J. Biol. Chem.*, 1923-1924, **58**, 117.)
(11) D. B. Jones and C. E. F. Gersdorff (*ibid.*, 1925, **64**, 241.)
(12) H. Schjorning (*Z. Analyt. Chem.*, 1898, **37**, 413.)
(13) H. Schjerning, C. R. Carlsberg (*Lab.*, 1909, **8**, 266.)
(14) C. H. Bailey and H. J. Blish (*J. Biol. Chem.*, 1915, **23**, 345.)
(15) P. F. Sharp and B. L. Herrington (*Cereal Chem.*, 1927, **4**, 249.)
(16) H. T. Brown (*Trans. Guin. Res. Lab.*, 1903-6.)
(17) D. Coleman (*Cereal Chem.*, 1927, **4**, 321.)
(18) H. Luers and M. Landauer (*Z. f. Electrochem.*, 1922, **28**, 341.)
(19) M. J. Blish (*J. Biol. Chem.*, 1918, **33**, 551.)
(20) A. G. Olsen and C. H. Bailey (*Cereal Chem.*, 1925, **2**, 76).
(21) A. Kiesel (*Z. Physiol. Chem.*, 1924, **135**, 61.)
(22) M. J. Blish and R. M. Stanstedt (*Cereal Chem.*, 1925, **2**, 57.)
(23) F. Csonka and D. B. Jones (*J. Biol. Chem.*, 1927.)
(24) H. F. E. Hulton (*this Journ.*, 1922, **28**, 33.)
(25) E. S. Beaven (*ibid.*, 1902, **8**, 542.)
(26) T. B. Osborne and G. F. Campbell (*J. Amer. Chem. Soc.*, 1896, **18**, 542.)
(27) W. Kraft (*Z. ges. Brauw.*, 1910, **33**, 193-205.)
(28) H. L. Hind (*this Journ.*, 1924, **30**, 969.)
(29) A. J. Murphy (*ibid.*, 1903, **9**, 557.)
(30) E. Prior (*Z. f. ges. Brauw.*, 1906, **29**, 613.)
(31) Y. Nakamura (*Bull. Agric. Chem. Soc.* (Japan), 1926, **2**, 155. *J. Amer. Chem. Abstr.*, 1927, **21**, 2295.)