# INSTITUTE OF BREWING RESEARCH SCHEME.

## Fourth Report on Barley Proteins.

## THE PROTEINS OF BARLEY DURING DEVELOPMENT AND STORAGE AND IN THE MATURE GRAIN.*

### The Composition and Quantitative Estimation of the Barley Proteins.—III.

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In my first report I gave the results of estimations—by a method proposed there—of the amounts of proteins in a number of Plumage-Archer barley samples. This particular set showed a very definite and regular relationship between the amounts of the different proteins, and suggested a similar type of relationship for other varieties. Since that time, this suggestion has been extensively studied, and tested by methods which have been improved in detail.

It will be seen later that the present results place the suggestion on a wider and firmer basis. A very pertinent point then emerges, if these regularities exist, how do they arise? In order to study this point, attention was directed to the development of the barley grain on the plant, since it is during this period that the greater part of the protein formation might be expected to take place. As the result of studies of samples of developing grain, taken during the summers of 1928 and 1929, and of a number of other samples this report presents an outline of the changes in the amounts of protein during development, and also of the subsequent maturation changes. Studies are also given of the proteins of mature grain of different varieties, and these suggest interesting varietal differences and similarities.

As the importance of the nitrogen compounds in practice becomes clearer, so the necessity becomes greater for a sound understanding of the behaviour of barley proteins, and it was to this end that the research was directed. This Report is chiefly a presentation of experimental data, the theoretical considerations arising from them will be dealt with in a later paper.

### Section I.

### Changes of the Proteins during the Development and Storage of Barley Grain.

A preliminary development experiment was carried out in the summer of 1928, and a more detailed experiment in the summer of 1929. The results are regarded as giving valuable confirmation of the regularities indicated by the studies of protein amounts in mature grain.

#### Experimental Methods.

The variety chosen for development study in 1928 was Standwell, and in 1929, Plumage-Archer. The chosen plot of barley on the Rothamsted Experimental Farm was watched until the first day on which anthers emerged from the developing grain. On this day several thousand of the ears, with anthers showing, were then marked by loosely tying red wool below the ears (method of Miss Brenchley, *Ann. Bot.*, 1912, 903). The assistance in this of a number of members of the Rothamsted staff is gratefully acknowledged. These marked ears were all of the same physiological age, and samples from these ears may be regarded as showing the development of a single ear or grain.

At intervals of a few days samples of several hundreds of these ears were taken at random over the whole plot, and in 1929 duplicate samples were taken to estimate the combined sampling and analytical errors. The results show that these were small.

The samples were rapidly taken to the laboratory. The fresh weight of a hundred ears was determined. The awns were cut off, and the grain separated from the ears.

* Note.—With the consent of the Research Fund Committee part of the data in this Report was incorporated in a Thesis presented to the University of Cambridge for the degree of Ph.D. A copy of this thesis was deposited in the University Library on 23rd March, 1929.

Blind corns were removed, and the thousand corn weight was determined in duplicate. The grain was then dried for 1 hr. at 40° C. in a vacuum oven. It was then removed, and put several times through a Wiley mill without a sieve, so that every grain was cut across by the knives. This greatly hastened drying, which was continued in the vacuum oven until the material was dry (*circa* 9 per cent. moisture). In the second season the drying arrangements were more elaborate. A higher vacuum was obtained and a current of air from a fine inlet swept through the drying material. The results indicate that enzyme action in the second set was checked before much change took place, but probably the arrangements in the first year were insufficient to do this entirely. The drying to 9 per cent. is necessary to check enzyme action, and to ensure sufficient and comparable fineness of grinding.

The dried material was carefully ground very fine, in a coffee mill (grinding twice), and then reground in a Wiley mill with a half mm. sieve.

The shortened form of extraction was employed (this *Journ.*, 1929, 316), but in the second season total protein in the salt extract was also estimated (p. 321, *ibid.*)

Since the amounts of carbohydrate and of nitrogen change rapidly, the grain itself is the only convenient unit as a basis for calculation. Consequently, results are given as weights of nitrogen per thousand corns and this unit is retained throughout the paper.

In 1929 the development of the proteins was followed also by cytological studies. Constant thickness microtome sections were cut of the grain from each sampling. These were stained by Millon's reagent and iodine, under constant conditions. Millon staining was made on:

(1) Sections direct (giving salt-soluble proteins, hordein and glutelin.)
(2) After salt extraction (giving hordein and glutelin), and
(3) After salt and alcohol extraction giving glutelin alone.

There is no direct method of staining individual proteins, so this technique was devised to localise the position of the proteins in the grain.

## Results.

The results for the two years are given as amounts per thousand corns in Tables I. and II., and in Diagrams I., II. and IV.

Table I.

*Development of Proteins in Standwell Barley.*
Anthers emerged July 10th, 1928 = 0 days.

| Age in days. | Grm. of Nitrogen per 1,000 corns. | | | |
|---|---|---|---|---|
| | Total. | Salt-Sol. | Hordein. | Glutelin. |
| 7 | 0·421 | 0·216 | 0·089 | 0·116 |
| 10 | 0·509 | 0·240 | 0·137 | 0·132 |
| 14 | 0·669 | 0·272 | 0·218 | 0·179 |
| 18 | 0·757 | 0·294 | 0·272 | 0·194 |
| 24 | 0·821 | 0·298 | 0·296 | 0·227 |
| 30 | 0·901 | 0·318 | 0·337 | 0·246 |
| | Total nitrogen per 100 grms. | Re-analysis after 20 months' storage. | | |
| 7 | 1·516 | 0·176 | 0·092 | 0·153 |
| 10 | 1·563 | 0·207 | 0·138 | 0·163 |
| 14 | 1·619 | 0·242 | 0·213 | 0·213 |
| 18 | 1·640 | 0·276 | 0·266 | 0·216 |
| 24 | 1·700 | 0·262 | 0·293 | 0·266 |
| 30 | 1·830 | 0·294 | 0·317 | 0·290 |

## Discussion.

The figures for both years show that the nitrogen and total dry matter in the grain increase regularly with time. The rate of entry is rapid at first and declines steadily. The nitrogen percentage remains approximately constant (see Diagram I.) which shows that nitrogen compounds and carbohydrates (which constitute the major part of the remainder) enter the grain in approximately constant relative proportions during the period studied.

In Diagram II. are given the curves for the amount of the proteins per thousand corns in developing Standwell grain. It is clear here and in developing Plumage-Archer (Diagram IV.,) that salt-soluble nitrogen is high at first, glutelin increases steadily throughout and hordein most rapidly in the later stages. By comparison of Diagram II., with that for mature grain (Diagram III.) it becomes clear also that the relationships seen in mature grain are the result of the developmental sequence common to the variety. Within each variety at

TABLE II.

*Analyses of Development Samples Plumage-Archer.*

*Another emergence, July 12th, 1929=0 days.*

| | | Initial Moisture. % on dry | Final Dry Matter % | 1,000 Corn Weight. grms. | Weights of Nitrogen in grm. per 1,000 Corns. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Total. | | Salt Sol. | Total* Protein. | Non-Protein.† | Hordein. | Glutelin. |
| Sample I. | A | 219·6 | 93·69 | 14·03 | 0·220 | α | 0·153 | 0·071 | 0·087 | 0·011 | 0·056 |
| 3 days | B | 222·7 | 94·01 | 14·08 | 0·212 | α | 0·149 | 0·065 | 0·085 | 0·011 | 0·052 |
| Sample II. | A | 182·0 | 92·55 | 24·64 | 0·354 | α | 0·186 | 0·070 | 0·116 | 0·060 | 0·108 |
| | | | | | | β | 0·159 | 0·044 | 0·117 | 0·065 | 0·130 |
| 7 days | B | 180·5 | 92·62 | 24·74 | 0·355 | α | 0·192 | 0·068 | 0·124 | 0·061 | 0·102 |
| | | | | | | β | 0·164 | 0·045 | 0·119 | 0·067 | 0·124 |
| Sample III. | A | 136·2 | 93·05 | 32·70 | 0·438 | α | 0·256 | 0·144 | 0·115 | 0·093 | 0·089 |
| | | | | | | β | 0·218 | 0·102 | 0·115 | 0·102 | 0·118 |
| | | | | | | γ | 0·194 | 0·083 | 0·111 | 0·109 | 0·135 |
| 10 days | B | 138·0 | 93·30 | 33·15 | 0·460 | α | 0·262 | 0·140 | 0·124 | — | — |
| | | | | | | β | 0·252 | 0·126 | 0·126 | 0·100 | 0·108 |
| | | | | | | γ | 0·200 | 0·082 | 0·120 | 0·117 | 0·143 |
| Sample IV. | A | 102·2 | 91·64 | 39·29 | 0·543 | α | 0·235 | 0·113 | 0·122 | 0·158 | 0·150 |
| | | | | | | β | 0·215 | 0·091 | 0·121 | 0·158 | 0·170 |
| 14 days | B | 104·2 | 92·25 | 39·41 | 0·554 | α | 0·253 | 0·129 | 0·124 | 0·162 | 0·139 |
| | | | | | | γ | 0·206 | 0·087 | 0·118 | 0·174 | 0·174 |
| Sample V. | A | 100·2 | 93·09 | 42·34 | 0·614 | α | 0·253 | 0·135 | 0·119 | 0·204 | 0·157 |
| 17 days | B | 99·00 | 93·20 | 42·51 | 0·605 | α | 0·250 | 0·134 | 0·120 | 0·200 | 0·155 |
| | | | | | | β | 0·230 | 0·109 | 0·123 | 0·197 | 0·178 |
| | | | | | | γ | 0·215 | 0·106 | 0·110 | 0·210 | 0·180 |
| Sample VI. | A | 85·80 | 92·84 | 44·36 | 0·644 | α | 0·247 | 0·129 | 0·117 | — | — |
| | | | | | | β | 0·246 | — | — | 0·221 | 0·177 |
| 21 days | B | 87·42 | 92·56 | 45·35 | 0·668 | α | 0·253 | 0·134 | 0·121 | 0·233 | 0·182 |
| | | | | | | γ | 0·229 | 0·127 | 0·101 | 0·215 | 0·224 |
| Sample VII. | A | 48·09 | 92·39 | 46·00 | 0·688 | | 0·225 | 0·121 | 0·102 | 0·230 | 0·233 |
| 28 days | B | 43·60 | 92·60 | 46·42 | 0·713 | α | 0·226 | 0·122 | 0·104 | 0·259 | 0·228 |
| | | | | | | β | 0·231 | — | — | 0·258 | 0·224 |
| | | | | | | γ | 0·217 | 0·125 | 0·093 | 0.264 | 0·232 |

* Total Protein=total fully built up protein in salt extract.

† Non-Protein here=usual non-protein plus proteose.

any given nitrogen content there is a tendency for the proteins in developing grain to settle down toa very definite equilibrium. During development, synthesis does not keep pace with the rate of entry of nitrogen into the grain, hence maturation changes ensue.

The hordein curves of mature and developing grain are so closely similar that they may be regarded as indentical. The salt-soluble nitrogen is higher than in mature grain and the glutelin correspondingly lower. Subsequent analyses (20 months later) of the same samples showed that salt-soluble nitrogen and glutelin had also settled down to the values given by mature grain (see the second part of Table I. p. 337).

The same behaviour is shown by the 1929 set of Plumage-Archer samples. These were

rapidly dried and so, it is considered, retained in almost the state at which they existed at the moment of sampling. At the time of rapid entry of nitrogen a large proportion of the nitrogen was in the form of simple compounds (non-protein and proteose nitrogen) in which form the nitrogen enters the grain or is first synthesised. Synthetic changes continued in the dried and ground grain, but at a very reduced rate, requiring months instead of hours to reach equilibrium. After several months storage the proportions approached those found in the mature grain of the same total nitrogen content per thousand corns. This can be seen on comparing Diagrams IV. and V. of the developing and mature grain, respectively. Many successive analyses were made of the development samples after intervals of time; these are given in full for the B samples for salt-soluble and glutelin nitrogen in Diagram VI. (p. 343). The duplicate analyses of samples A and B are given in full in Table II., and it will be seen that these agree so closely that the combined sampling and analytical errors are negligible and these conclusions are, therefore, sound.

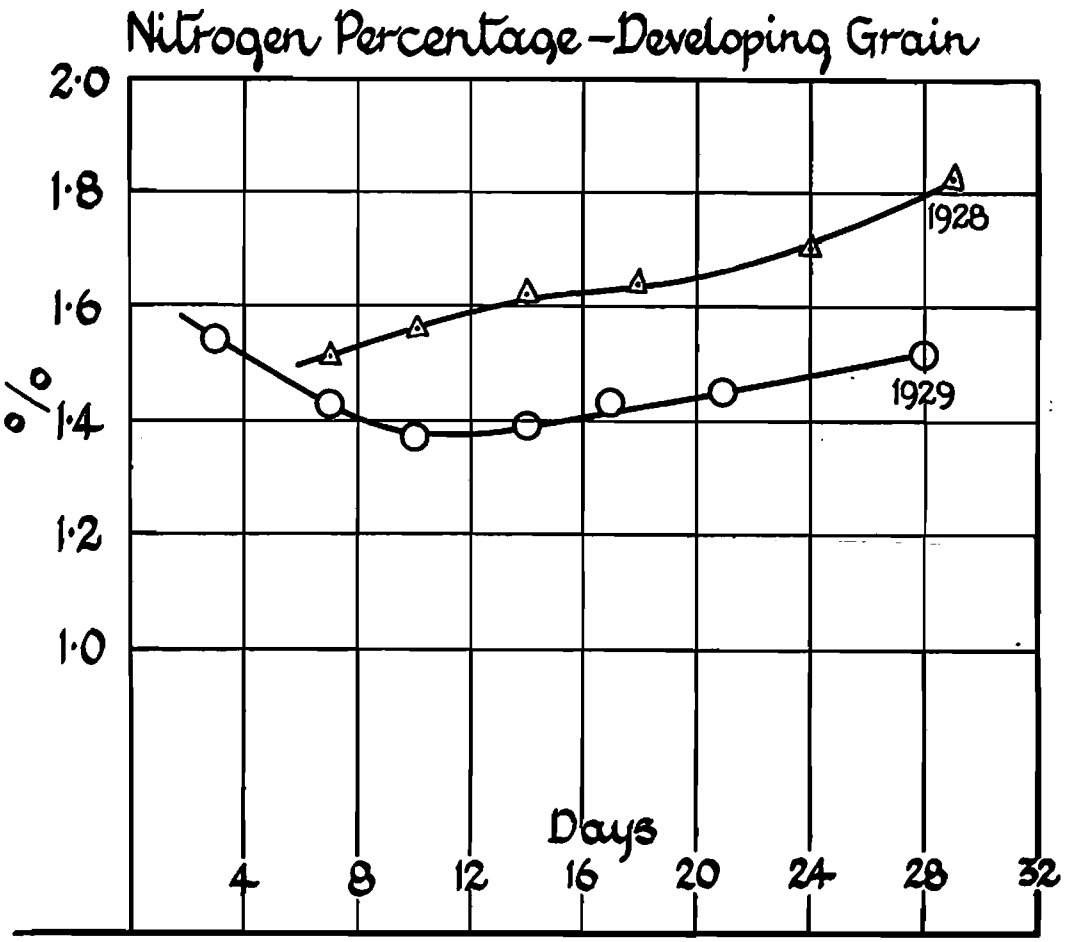



DIAGRAM I.

In this table are given the successive analyses of each of the samples (α, β and γ). The first (α) is an analysis immediately after collection, the second (β) a re-analysis after several weeks and the third (γ) a re-analysis after several months.

Clearly the nitrogen in the grain settles

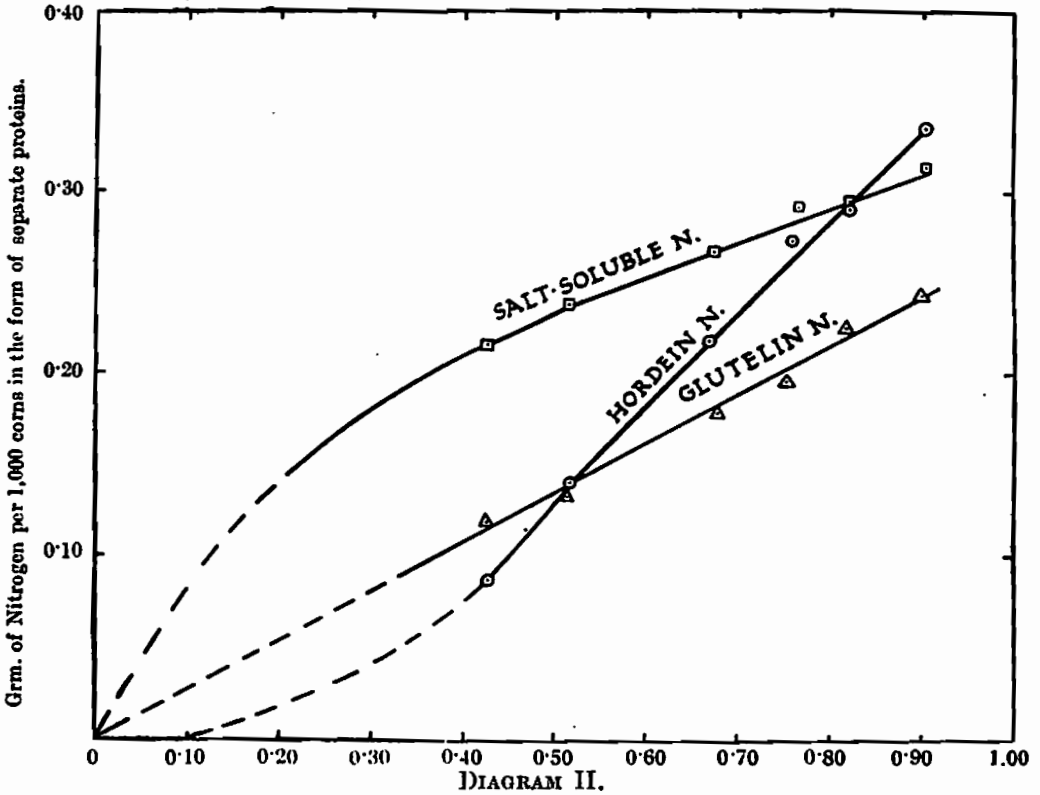



DIAGRAM II.

Analyses of Mature Standwell Grain.

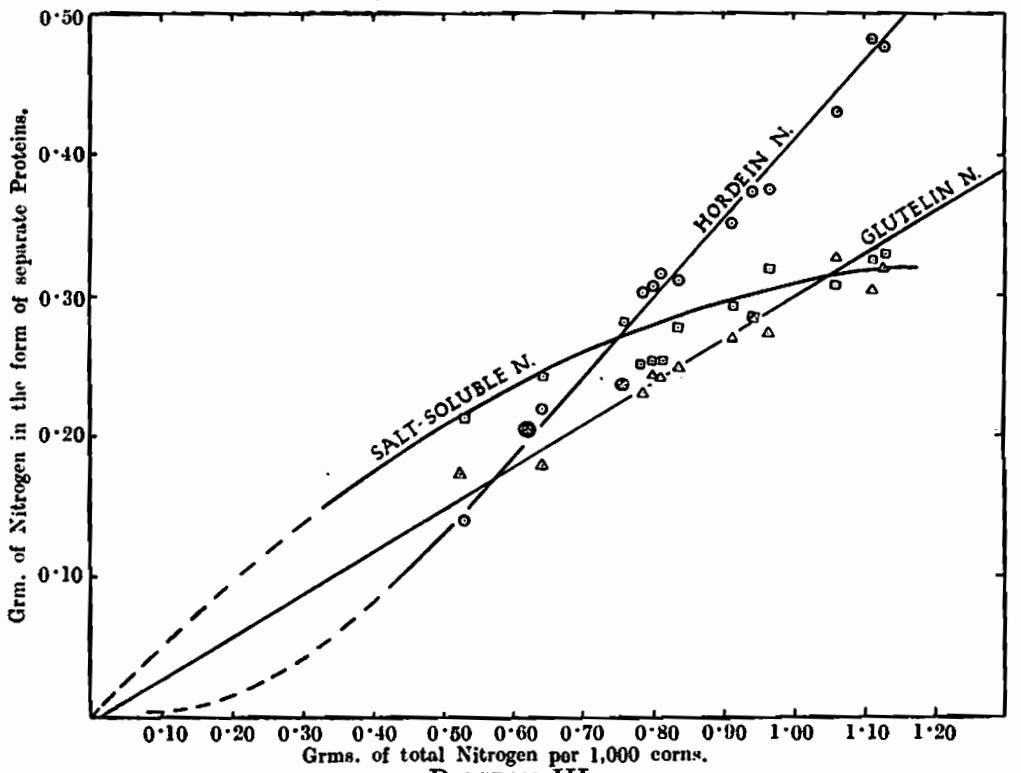



DIAGRAM III.

PLUMAGE, ARCHER.

Changes during storage of development samples. Curves from mature grain analyses.

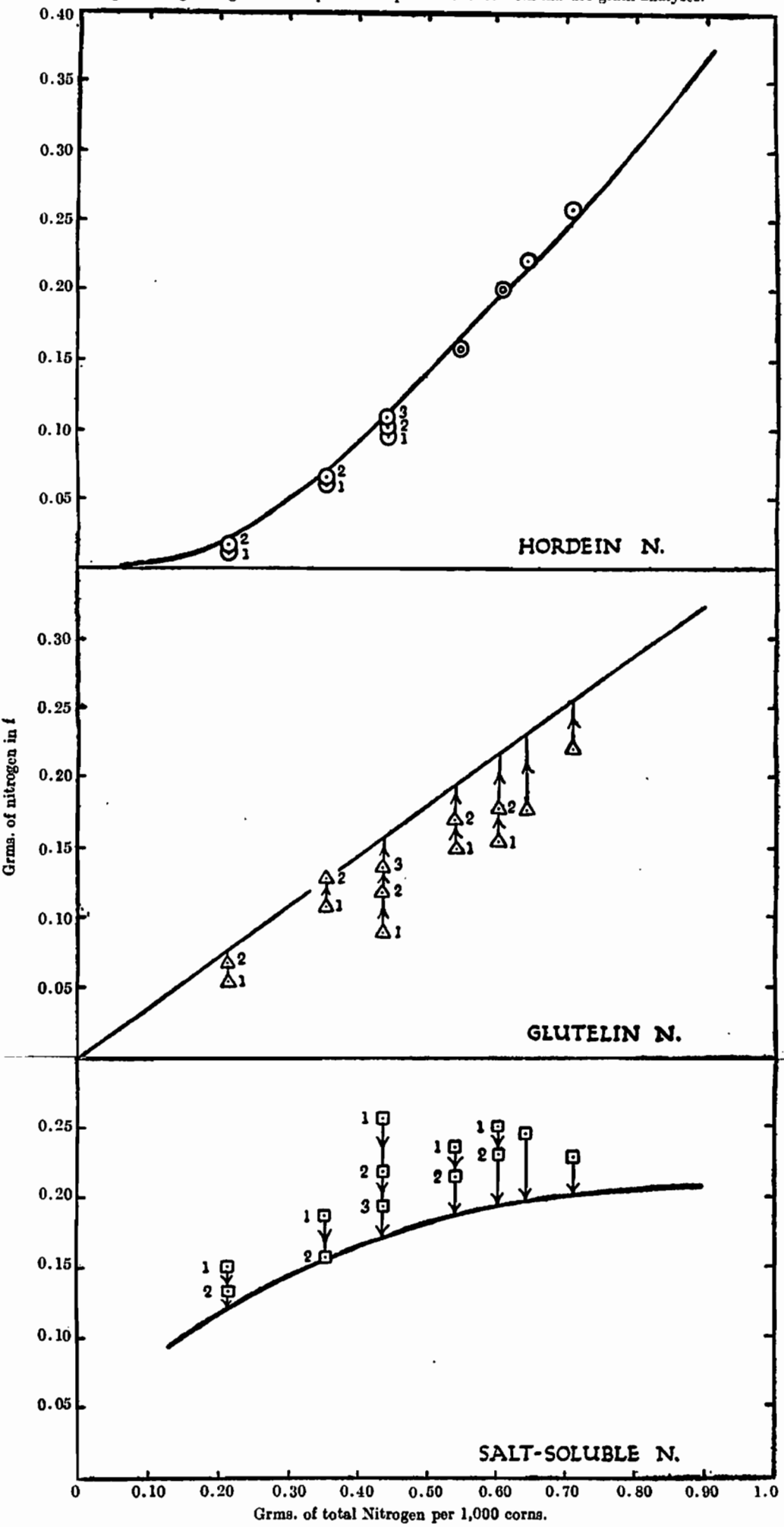



DIAGRAM IV.

down to definite proportions regulated by the total nitrogen content, either in grain at any stage during development or in the final grain. Also it reaches the same final point whatever the moisture content (unless this is sufficient to cause germination). It also reaches the same equilibrium whether ground or unground.

Further, it can be seen that for a given total nitrogen content a different equilibrium is reached for Plumage-Archer than for Standwell. For instance, at 0·50 grm. of nitrogen per thousand corns the following amounts of nitrogen are found :—

| | Salt-sol. | Hordein. | Glutelin. |
|---|---|---|---|
| Plumage-Archer .. | 0·175 | 0·140 | 0·185 |
| Standwell .. .. | 0·24 | 0·13 | 0·13 |

The equilibrium is only reached in harvested grain after considerable storage periods, data showing this will be given later. The regularities which I have found in mature grain are only seen if this has been stored for a very considerable period so that all the samples analysed have settled down to the final equilibrium. It is not safe to study the regularities in grain less than a year old.

SIGNIFICANCE OF THE MATURATION CHANGES.

The demonstration of such maturation changes naturally raises the question of the relation of these to the simultaneous maturation change noticed by the maltster—the increase in germinative power in grain stored after harvest. The two may be related, but I prefer to make no suggestion of relationship. The main part of the increase in germinative power is probably closely related to increase in oxygen permeability of the husk (see G. T. Harrington, *J. Agric. Res.*, 1923, **23**, p. 79). Although there is probably also a change in the germ itself (W. Windisch, *Woch. Brau.*, 1905, 89). How far these changes are related to changes in the *composition* of the proteins is doubtful.

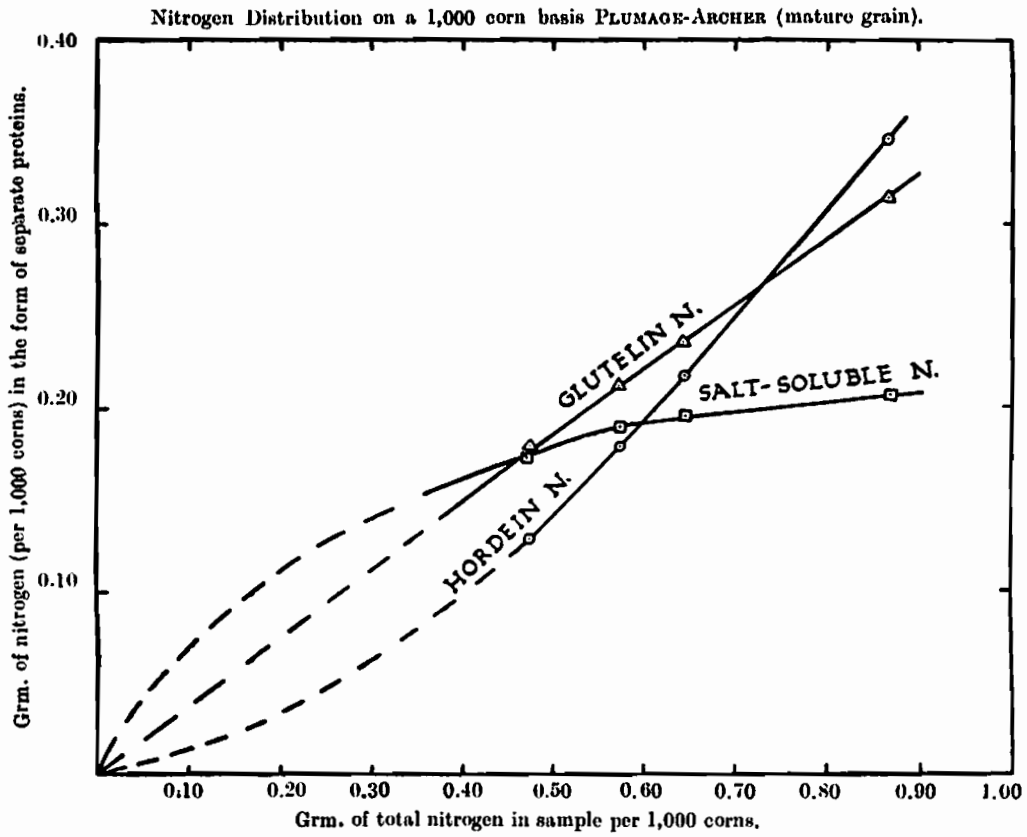



DIAGRAM V.

### The Best Basis for Comparison.

The hordein value is always near its final value which suggests that the rate of formation is much more rapid than with glutelin. The former is, therefore, more reliable in comparing analyses.

Throughout this paper the considerations are based on weights of nitrogen per thousand corns since this is regarded as a better basis for calculation than amounts per hundred grams of dry weight. The thousand corn weights were all close together in the early samples studied so that the regularities were apparent on either basis. In the development samples and in samples of Standwell grain which were deliberately separated into grain of widely different thousand corn weights, it appears clear that the thousand corn weight is the better basis.

### Cytological Studies.

It may be argued that the changes in the amount of the proteins during development may be due to successive development of different parts of the grain. For instance, if the salt-soluble nitrogen were chiefly in some part of the grain which developed early then this would account for its preponderance in the early stages. This suggestion is nullified, however, by cytological studies carried out in 1929. These show that not all, but the main part of the proteins is in the periphery of the endosperm and that all three, salt-soluble, hordein and glutelin are there together, and are not segregated in different organs.

*The equilibrium between the proteins is therefore probably an equilibrium within the cell where they exist together.* This is supported also by the fact that the equilibrium is reached in ground samples.

### Diagrammatic Space Model.

The relations between development and maturity in high and low nitrogen barleys are shown diagrammatically in a space model. (See Diagram VI. p. 343).

In this diagram the heights represent weights per 1,000 corns of nitrogen in the form of the different proteins. These are superimposed so that the top surface measures the total nitrogen. The sides show development with time in high and low nitrogen barleys and the end represents and explains the resulting relation between mature samples of different nitrogen contents.

### Analogy with the Proteins of Wheat.

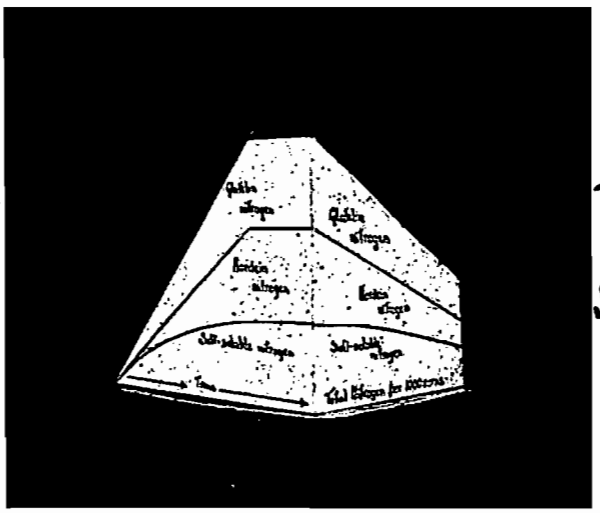
If the relationships suggested here for barley are valid then it might be expected that analogous regularities would be found in wheat. The available data for the proteins of wheat was therefore examined. H. E. Woodman and F. L. Engledow (*J. Agric. Sci.*, 1924, 563), used a method of estimation of the salt-soluble, alcohol-soluble and glutelin nitrogen which probably gave a good relative measure of the amounts of these proteins present, although only about 80 per cent. of the proteins was accounted for. When their results (per hundred ears) for the development of the proteins in wheat grain are plotted they give a set of curves very similar in type to the Plumage-Archer development curve (Diagram IV.). The proportion of salt-soluble nitrogen in their results is high at first and increases slowly only later in development. The glutelin increases steadily throughout. Gliadin (the alcohol-soluble protein of wheat, corresponding to hordein in barley) increases rapidly in the later stages. In the last analyses (after the yellow-ripe stage) salt-soluble nitrogen decreases in amount. The only difference from my results with barley is that in these analyses gliadin and not glutelin increases as a result of the decrease of salt-soluble nitrogen during maturation.

The analyses of developing wheat grain by C. E. Mangels and T. E. Stoa (*Cereal Chem.*, 1928, 385) are unfortunately given only as percentages and without a constant basis (such as a hundred ears or a thousand grains). Nevertheless they show the same general type of relationship.

The results of E. Grewe and C. H. Bailey (*Cereal Chem.*, 1927, 230) have also been studied. These authors analysed a number of samples of wheat flour by similar methods. When plotted the scatter of the points is greater than with the present barley analyses. Yet the same general type of relationship (as seen in Diagram VIII., First Report, this *Journ.*, 1928, 113) is clearly visible. The glutelin percentage remains constant, the gliadin proportion increases with increasing total nitrogen content (per 100 grams dry weight) with a corresponding decrease in the salt-soluble nitrogen. The salt-soluble nitrogen is a much smaller proportion of the whole in wheat than in barley.

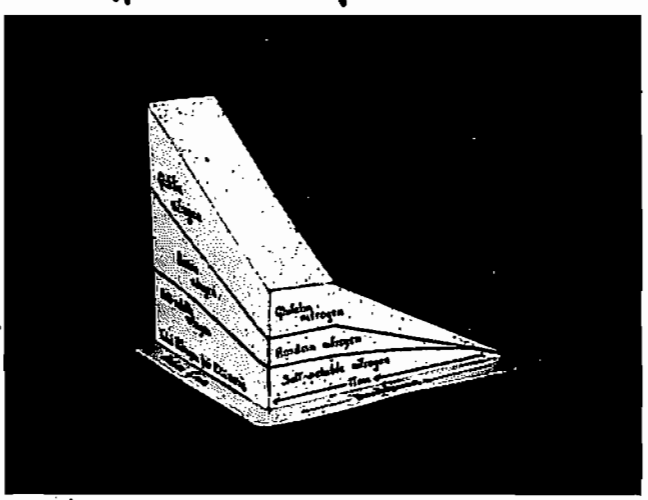
# Inter-relations of amounts of total nitrogen during development and at maturity.

Development
High total nitrogen.




Diagrammatic Space Model.

Mature
grain samples.



Development
Low total nitrogen.

DIAGRAM VI.

All this evidence suggests that the same type of relationship holds for wheat as for barley. This support gives broadness and soundness to the conclusions drawn from the analyses given here.

## Section II.

## VARIETAL DIFFERENCES AND SIMILARITIES.

### Methods.

The grain was dried at 38° C. to about 10 per cent. moisture; and it was then ground twice, as fine as possible, in a special coffee mill, and reground in the Wiley mill, using a ½ mm. sieve. This ensures a fine grind which is comparable in different samples.

Estimations of moisture, thousand corn weight, total nitrogen, salt-soluble nitrogen, hordein nitrogen and glutelin nitrogen were made as described in the Second Report (this *Journ.*, 1929, 316).

### Results.

The results of analyses of mature Plumage-Archer, Standwell, Archer and F.112 barleys are given in Tables III., IV., V. and VI. respectively and in Diagrams III., V., VII. and VIII., in all cases as weights in grams per thousand corns.

### Discussion.

The curves for mature Standwell and Plumage-Archer grain given in Section I. are sufficient to demonstrate that with the mature grain the protein equilibrium is both regular within the variety and differs considerably in two different varieties, Plumage-Archer and Standwell. Diagrams III. and V.

Curves for other varieties given here and others still being studied also suggest that varieties differ in the equilibrium position for a given amount of total nitrogen, so that these proportions may be characteristic and regular for each variety, or group of varieties. A. Szilvinyi (*Woch. Brau.*, 1930, 3) who has examined *one* sample of each of eight varieties by my method concludes that with these there is no regular relation between the quantities of each of the proteins and the total nitrogen. This is exactly what is to be expected in his results if varieties differ in their characteristic proportions of proteins.

It can be seen from Diagrams VII. and VIII. that, in the varieties Archer and F.112 (a race of *Hordeum hexastichum* raised by Dr. E. S. Beaven), here also the proteins are regular in amount within the variety and differ greatly between the two varieties. The values for Archer given in the First Report (this *Journ.*, 1928, 101) are slightly incorrect owing to differing fineness of grind.

Table III.

*Analyses of Plumage-Archer Mature Grain.*

| Plumage-Archer Barley. | Weight of 1,000 Corns Dry. Grms. | Dry Matter % | Grms. of Nitrogen per 1,000 Corns. | | | |
|---|---|---|---|---|---|---|
| | | | Total. | Salt Ext. | Hordein. | Glut. |
| Porlock '25 mixed .. .. | 40·97 | 87·72 | 0·474 | 0·171 | 0·128 | 0·175 |
| Orwell '25 mixed .. .. | 38·28 | 86·12 | 0·872 | 0·208 | 0·344 | 0·320 |
| Louth '23 No. 15 .. .. | 42·42 | 87·52 | 0·578 | 0·189 | 0·178 | 0·211 |
| Louth '23 No. 17 .. .. | 42·95 | 87·98 | 0·654 | 0·197 | 0·216 | 0·241 |

By comparing the curves for the four varieties given here, it will be seen that the following statements are true for all the varieties studied. The salt-soluble nitrogen forms a greater proportion of the whole at low nitrogen contents than at the high, *i.e.*, it increases in amount less rapidly than the others. The change in proportion is regular with the increase in nitrogen content. On the other hand the hordein nitrogen is a low proportion of the whole at low total nitrogen contents and increases rapidly and regularly with increase of total nitrogen. The rise in hordein nitrogen percentage corresponds exactly to the fall in salt-soluble nitrogen percentage, so that the percentage of the remainder (glutelin) remains constant.

Varieties differ, however, in the proportion of each protein at any given total nitrogen content. The easiest to consider is glutelin since the proportion remains constant within each variety for all total nitrogen contents. Thus Plumage-Archer has 36 per cent. of glutelin, F.112 has 41 per cent.,

TABLE IV.

*Analyses of Mature Standwell Grain.*

| | Weights of nitrogen in grms. per 1,000 corns. | | | | 1,000 Corn weight. Grms. |
|---|---|---|---|---|---|
| | Total. | Salt. Sol. | Hordein. | Glutelin. | |
| Leegomery 1925 Medium .. .. | 0·528 | 0·214 | 0·141 | 0·173 | 39·52 |
| Leegomery 1927 Medium .. .. | 0·621 | 0·204 | 0·208 | 0·209 | 37·52 |
| Cannington 1928 Medium .. .. | 0·641 | 0·242 | 0·220 | 0·179 | 39·44 |
| Leegomery 1925 Large .. .. | 0·757 | 0·282 | 0.237 | 0.238 | 49·91 |
| C4 Medium .. .. .. .. | 0·784 | 0·251 | 0·302 | 0·231 | 41·21 |
| C2 Medium .. .. .. .. | 0·800 | 0·252 | 0·305 | 0·243 | 40·55 |
| C3 Medium .. .. .. .. | 0·810 | 0·251 | 0·316 | 0·243 | 40·75 |
| A4 Medium .. .. .. .. | 0·835 | 0·278 | 0·309 | 0·248 | 43·76 |
| Leegomery 1927 Large .. .. | 0·912 | 0·292 | 0·350 | 0·270 | 48·51 |
| C1 Medium .. .. .. .. | 0·941 | 0·283 | 0·373 | 0·285 | 43·50 |
| Cannington 1928 Large.. .. .. | 0·964 | 0·318 | 0·373 | 0·273 | 50·28 |
| C4 Large .. .. .. .. | 1·060 | 0·305 | 0·430 | 0·325 | 50·13 |
| C3 Large .. .. .. .. | 1·113 | 0·327 | 0·481 | 0·305 | 50·83 |
| C2 Large .. .. .. .. | 1·123 | 0·328 | 0·474 | 0·321 | 50·23 |

Medium and large refer to the graded grain sizes. A4, C3, etc. refer to plots in the Rothamstead top dressing experiment of 1928.

TABLE V.

*Analyses of Mature Archer Barleys.*

*N.I.A.B. Samples.*

| | Wt. of 1,000 Corns Dry. Grms. | Dry Matter. % | Grms. of Nitrogen per 1,000 corns. Total. | Salt-sol. | Hordein. | Glutelin. |
|---|---|---|---|---|---|---|
| 160 Mkt. Weighton, 1923 .. .. | 39·00 | 87·56 | 0·595 | 0·201 | 0·183 | 0·211 |
| 170 Norwich, 1923 .. .. .. | 39·16 | 88·94 | 0·718 | 0·220 | 0·257 | 0·241 |
| 167c Newton St. Faith, 1924 .. .. | 34·95 | 88·00 | 0·515 | 0·185 | 0·152 | 0·178 |
| 169c Newton St. Faith, 1924 .. .. | 34·69 | 88·02 | 0·476 | 0·174 | 0·132 | 0·170 |

and Standwell *circa* 30 per cent. Archer with 35 per cent. of glutelin, is not clearly distinguishable from Plumage-Archer. (See diagram IX, p. 348).

Hordein nitrogen can be measured much more accurately than the other proteins. This is because in immature barley it reaches its equilibrium value much more quickly than the other proteins. The value is therefore not affected by immaturity. It is also not so much affected by the fineness of grinding of the sample.

The hordein value is therefore the most accurate and it would be preferable to use this for comparisons. It will be seen, however, that there is little, if any, difference in hordein content between the English two-rowed barleys; the differences here are largely differences in salt-soluble and glutelin nitrogen. The six-rowed barley F.112 differs markedly from the others in its hordein content.

The analyses of the F.112 and Standwell barleys have been repeated and the standard errors of the means for the proteins, when calculated as a percentage of the total nitrogen are given in Table VII. It will be seen that the standard error for hordein is even lower than that for total nitrogen, each determined by the usual Kjeldahl method.

The second analyses of the Standwell barleys were kindly carried out by Miss D. Marx, M.Sc., a year after my analyses. The mean difference in hordein nitrogen found is 0·1 per cent. of the total nitrogen. It therefore appears that the personal error in the estimations is negligible.

Values of three times the standard error are significant. The differences recorded

TABLE VI.

*Analyses of Mature F.112 Grain.*

| Barley Source. | Weights of Nitrogen in grms. per 1,000 corns. | | | | 1,000 corn weight. grms. |
|---|---|---|---|---|---|
| | Total. | Salt-Sol. | Hordein. | Glutelin. | |
| 144 Small, 1926 .. .. .. .. | 0·288 | 0·112 | 0·055 | 0·121 | 20·80 |
| Beaven's (1) .. .. .. .. | 0·384 | 0·147 | 0·087 | 0·150 | 32·63 |
| 142c, 1927 .. .. .. .. | 0·443 | 0·161 | 0·102 | 0·180 | 33·57 |
| Good Easter, 1928 .. .. .. | 0·456 | 0·176 | 0·090 | 0·190 | 37·24 |
| 141, 1926 .. .. .. .. | 0·459 | 0·168 | 0·100 | 0·191 | 34·65 |
| 195c, 1927 .. .. .. .. | 0·460 | 0·170 | 0·105 | 0·185 | 34·28 |
| 144 Medium, 1926 .. .. .. | 0·493 | 0·187 | 0·103 | 0·203 | 35·31 |
| 190c, 1927 .. .. .. .. | 0·532 | 0·174 | 0·138 | 0·220 | 36·06 |
| Beaven's (3) .. .. .. .. | 0·545 | 0·184 | 0·147 | 0·214 | 33·22 |
| 144, 1927 .. .. .. .. | 0·554 | 0·180 | 0·141 | 0·233 | 38·75 |
| Beaven's (6) .. .. .. .. | 0·623 | 0·188 | 0·181 | 0·254 | 31·88 |
| Beaven's (5) .. .. .. .. | 0·664 | 0·202 | 0·182 | 0·280 | 38·57 |

Glutelin thus appears as the best and simplest thing to compare in different varieties and the glutelin contents of the different varieties are plotted in Diagram IX (p. 348); they show marked differences between the different varieties.

### ACCURACY OF THE ESTIMATIONS.

The differences of a few per cent. in the glutelin on which the arguments above are based, raise the question of the accuracy of the method, which if good enough, makes the differences significant and the conclusions based on these sound.

The accuracy has already been considered in the Second Report (this *Journ.* 1929, 316), but more detailed study is now necessary.

TABLE VII.

Variety F.112.

STANDARD ERROR (S) OF MEANS OF ANALYSES.

| | |
|---|---|
| Thousand Corn Weight (Triplicates) | S = 0.1gg = 0.52% |
| Dry Matter (Duplicates) ... ... | S = 0.045% |
| Total Nitrogen (Duplicates) ... | S = 0.006 = 0.4% |
| Salt Soluble Nitrogen (Dupl.) ... | S = 0.4% of total Nitrogen |
| Hordein Nitrogen (Dupl.) ... ... | S = 0.3% ,, ,, ,, |
| Glutelin (Dupl.) ... ... ... | S = 0.5% ,, ,, ,, |
| Standwell Hordein (Dupl.) ... ... | S = 0.2% ,, ,, ,, |

Nitrogen Distribution on a 1,000 corn basis. ARCHER BARLEY (mature samples).

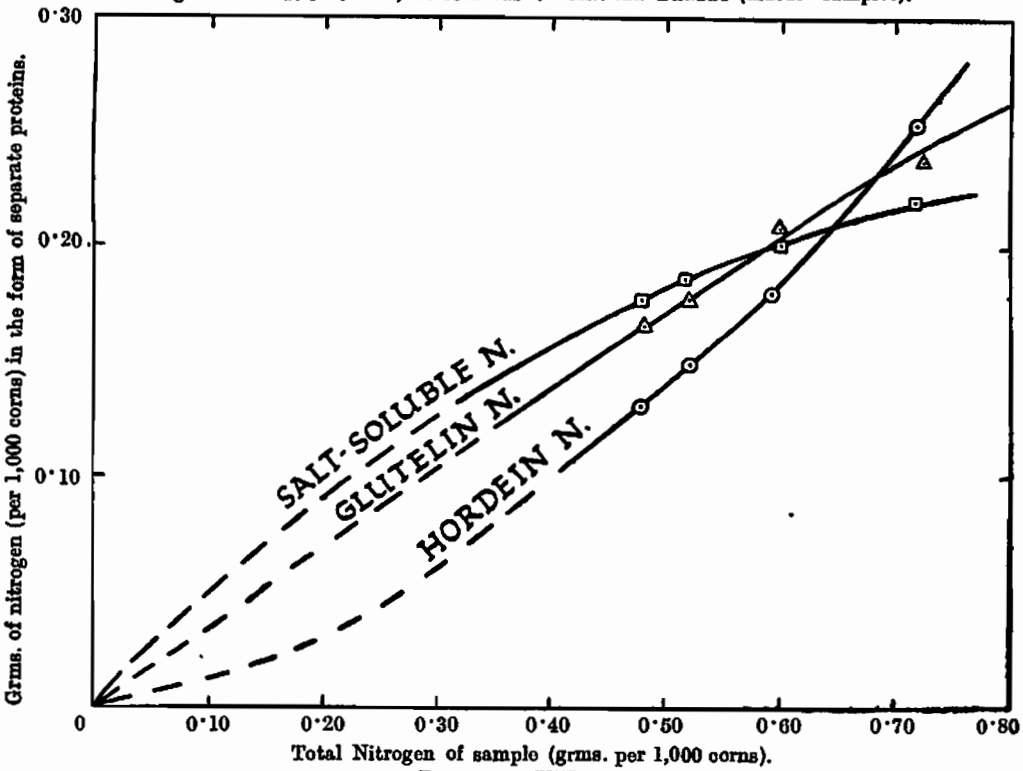



DIAGRAM VII.

F. 112. Analyses of mature grain.

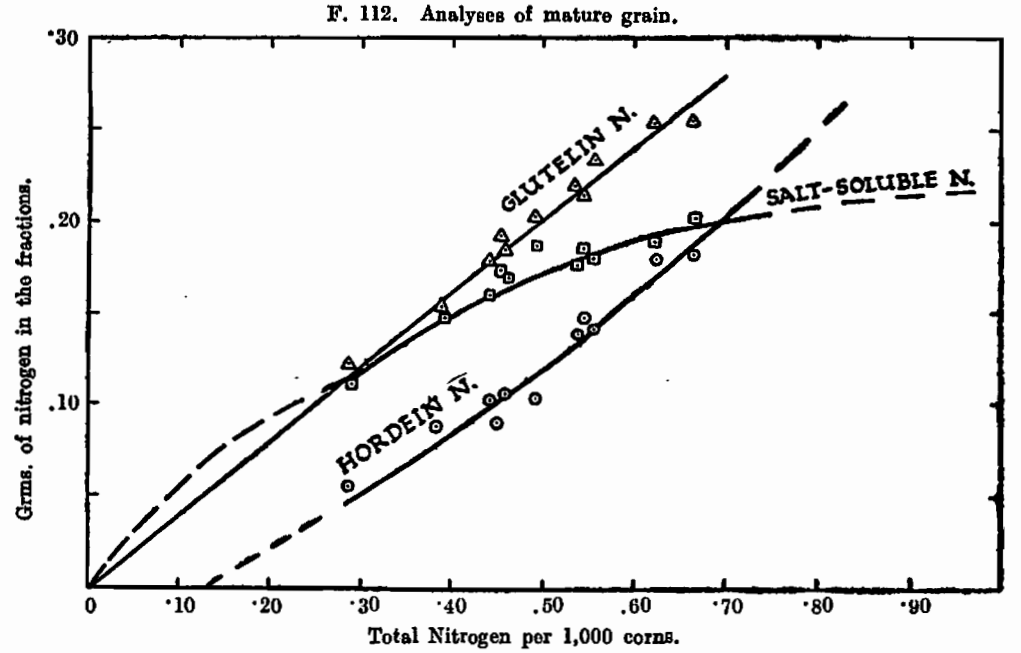



DIAGRAM VIII.

are hence manifestly real and therefore there are very significant differences between varieties in this respect.

*The relationship between the quantities of the individual proteins and the total nitrogen is characteristic of the variety.*—It does not necessarily follow that it will be possible to demonstrate significant differences between every pair of varieties.

HYBRIDIZATION.

The question of the mode of inheritance of these differences is obviously raised. What, for instance, is the relation of the protein proportions in Plumage-Archer to its parents Plumage and Archer? This aspect is at present under investigation.

MATHEMATICAL SUMMARY.

It is suggested that in mature grain of any one variety, or pure line, the protein proportions may be represented as follows in amounts of nitrogen per thousand corns:—

$$S = aN - bN^2$$
$$H = pN + qN^2$$
$$G = xN$$

Where S = salt-soluble nitrogen, H = hordein nitrogen, G = glutelin nitrogen and N is the total nitrogen per thousand corns.

The values of the factors (a, b, p, q, and x) are regarded as characteristic of the variety.

SUMMARY.

*Within each separate variety the weights of the individual proteins are simple regular functions of the total nitrogen content per thousand corns.* In each variety studied the percentage of salt-soluble nitrogen on total nitrogen decreases with increase of the total nitrogen. The hordein nitrogen percentage shows a corresponding increase so that the glutelin percentage remains constant throughout.

The curves for different varieties are therefore all similar in form, but they differ in actual magnitude. These differences are, in most of the cases studied, so much larger than the analytical errors that they are undoubtedly significant and are characteristic of the variety. *The relationship between the quantities of the individual proteins and the total nitrogen is characteristic of the variety.*

If they are allowed time to reach the natural equilibrium, exactly the same proportions of the proteins are found in samples of develop-

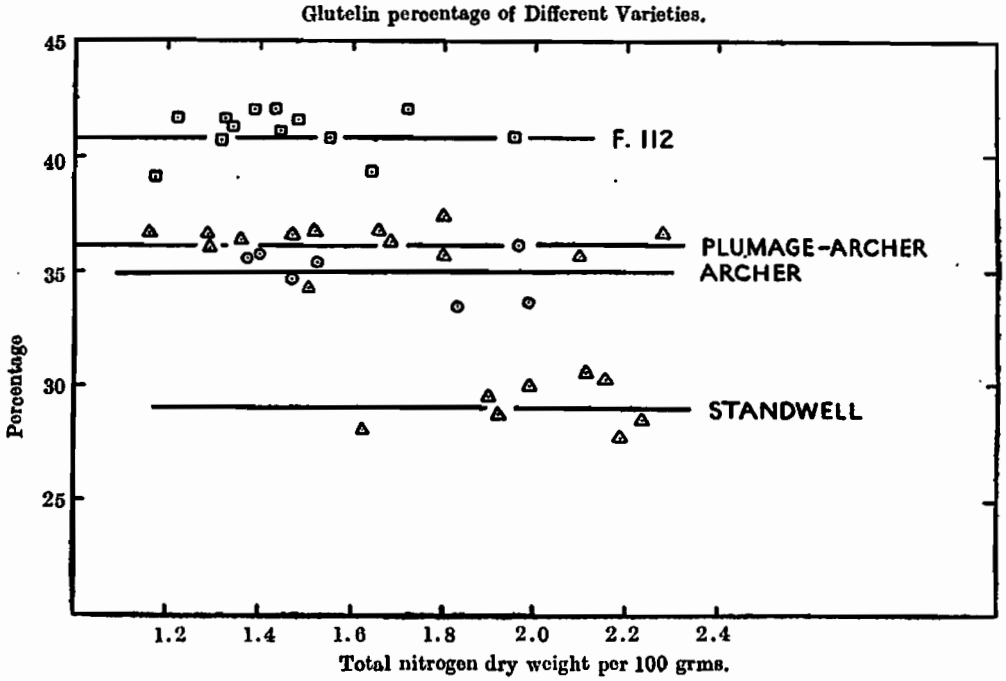



DIAGRAM IX.

ing grain as in mature grain of the same total nitrogen content per thousand corns. However, when freshly taken, development samples and immature grain contain a larger proportion of simple compounds which have entered the grain but have not been synthesised to the equilibrium point. *Development of the proteins in the barley grain is essentially a synthesis, of the simple compounds which enter it, up to a definite equilibrium point controlled only by the total nitrogen content and the variety.*

Salt-soluble, hordein and glutelin nitrogen are congregated chiefly, but not entirely, in the periphery of the endosperm and it is suggested that the regularity in the quantities of these proteins is due to a type of mass action equilibrium within the individual cell.

I gratefully acknowledge the following:—Help with the analyses from Miss D. Marx, M.Sc. and Mr. F. E. Day, B.Sc., F.I.C., samples of grain from Dr. E. S. Beaven and the National Institute of Agricultural Botany, and advice from Prof. A. C. Chibnall.

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# INSTITUTE OF BREWING RESEARCH SCHEME.

## NOTE ON THE PHOSPHORIC ACID OF BARLEY GRAIN.

By E. M. Crowther, D.Sc., F.I.C.

Determinations of phosphoric acid in the dry matter of barley grain were made on 127 samples taken from 4 series of field experiments conducted under the Institute of Brewing Research Scheme. (See Barley Research Reports, this *Journ.*, 1923, 624; 1924, 624; 818, 969; 1925, 104, 548, 601; 1927, 104; 1928, 307, 321, 436.) The samples were selected so as to investigate the influence of soil, season, varieties, and manuring. All of them had been previously valued and analysed by the usual methods of barley and malt analysis, so that it was possible to examine the association of variations in phosphoric acid content with those of other important characteristics. The finely ground barley grain was moistened with calcium acetate solution and carefully ashed. The residue was evaporated to dryness with nitric acid and taken up again in nitric acid. The phosphoric acid was determined by the Lorenz method, the detailed procedure for precipitation and drying of the phosphomolybdate being that elaborated by Neubauer for seedling analysis, (*Cf.* H. Neubauer *Z. Pflanz. Dung.*, A., 1923, 329. *Arbeiten der Zuckerfabrik Kleinwanzleben* III, 1927). The results of the four series are discussed briefly in turn. A more general discussion of the relation of soil conditions to nutrient uptake and barley yield and quality is given by L. R. Bishop in this issue.

*Series* 1.—General manurial trials at Rothamsted, Wellingore, Woburn, Eyton, and Orwell Park centres, 1922 to 1925, and Porlock, 1923-24 (98 samples). There are unfortunately several gaps in the series, where the whole of the stored sample had been used up in earlier analyses, and a rigid statistical analysis is not, therefore, attempted. The average manurial effects are shown at the bottom of Table I., and for comparison the average nitrogen contents for the same plots are given. It is at once obvious that the fertilisers have had little effect on either, and that the effect on $P_2O_5$ per cent. is even less than that on N per cent. There is some evidence that nitrogenous manures slightly reduce the $P_2O_5$ per cent. The $P_2O_5$ content of the three sets of plots receiving nitrogen (average 0·950 per cent.) is slightly below that of the two sets without nitrogen (average 0·969 per cent.). All fertiliser mixtures reduced the nitrogen content of the grain and the combination of potash and phosphate gave the lowest N per cent. but the highest $P_2O_5$ per cent.

Wide variations in soil and season produced no very great differences in $P_2O_5$ per cent., the extreme values for the individual plots being 1.18 per cent. and 0·74 per cent. In Table II. the values for the 5 plots at each centre in each year have been averaged to obtain more representative values after eliminating manurial effects. The variations from year to year are about the same as those from centre to centre. No general relationship was found between $P_2O_5$ content and any one of the following:—Yield, barley valuation, malt valuation, nitrogen content, 1000 corn weight, diastatic power, cold water extract and extract calculated on