THE INSTITUTE OF BREWING RESEARCH SCHEME.

THIRD REPORT ON BARLEY PROTEINS.

THE CHANGES UNDERGONE BY THE NITROGENOUS CONSTITUENTS OF BARLEY DURING MALTING.—I.

By L. R. Bishop.

This report presents for the first time a balance sheet of the changes throughout malting of all the main nitrogenous constituents or groups of constituents in the barley grain. The suggestion of the few analyses of malt available [one by Osborne and Campbell (1) and some by myself (2)] was that it was chiefly hordein which broke down during malting to give salt-soluble products. This study presents a clear picture of what happens, but it is found to be a more complicated process than a simple breakdown of hordein leaving the glutelin unattacked.

To supply the necessary background to this picture it will be of advantage to give a brief summary of some of the relevant facts which have previously been established in the germination of barley and in protein chemistry.

The structure of the barley grain is well-known. During germination the scutellum or inner face of the embryo or germ secretes enzymes (cytase, diastase, proteases, etc.) which attack the materials of the endosperm rendering them soluble so that they are absorbed by the developing embryo. The action of the various enzymes slowly penetrates from the proximal end of the grain near the scutellum to the distal end. The action of the other enzymes probably follows the action of cytase, which renders the cell walls permeable ("modification").

The proteins which are the subject of this study are very complicated bodies built up by linking together of amino-acids. Each protein contains some of nearly every one of the twenty-five or so amino-acids which are known. Each amino-acid is both an acid and a base, and the linking together is effected by the combination of the basic NH_2 (amino) group of one amino-acid with the acidic COOH (carboxyl) group of another amino-acid to give what is known as the peptide group (—CO—NH—CH—). In this way large numbers of molecules of amino-acids are linked together in chains, or more probably complicated structures involving rings, to form the final protein. The completed protein probably has a molecular weight of the order of 20,000 to 60,000.

The two chief proteins of the endosperm are hordein (an alcohol-soluble prolamin) and a glutelin (alkali-soluble). These are attacked during germination first by peptases which commence to reverse the combination process forming bodies (proteoses, peptones, etc.) which are still complicated. These are in turn attacked by tryptases and then ereptases which break them down finally to the constituent amino-acids. Since these compounds are the common basis of all the proteins, the breakdown products at this stage cease to be identifiable as having arisen from one or other of the original proteins of the endosperm. As the breakdown products become less complex they become more soluble and diffusible, and so can be absorbed by the developing embryo and resynthesised to proteins. Brown (3) showed that in this way during malting the nitrogen of the embryo rose from 15 per cent. to 40-50 per cent. of the total nitrogen of the grain.

In this report the word embryo is used to denote the whole of the young growing plant or germ, and so includes both the plumule or acrospire and the rootlets.

The suggestion is made in this report that towards the end of the period on the floor the degradation of proteins in the endosperm and the upgrade processes in the embryo have reached a state of approximate balance. This has been found of great assistance in interpreting the results of germination and the effect of altered conditions during this process on the composition of the malt at the end.

The materials for the investigation were obtained, through the courtesy of Messrs. J. W. Green, Ltd., from malt on the germinating floors and kilns of their maltings at Luton.

Methods.

At each sampling small samples were taken at various places and at various depths on the malting floor or kiln. This sampling was carried out more carefully in Experiments II and III. The mixed sample was immediately taken to the laboratory and in Experiments II and III samples were taken for the estimation of moisture and 1,000 corn weight. The main bulk of the material was dried in vacuo at 40° C. (104° F.) for one hour. It was then cut up coarsely by the knives in the Wiley mill and dried in vacuo again for several hours. The small leakage of air through a tube fitted with a fine capillary tube was used to sweep out the moisture from the oven. This method was adopted since it was the best practical means which could be found for drying as rapidly and completely as possible without causing enzyme action or modification of the physical state of the proteins. The dried material was ground first in a "coffee" mill and then in a "Wiley" mill, using a ½ mm. sieve, and analysed as soon as possible.

Since the formation of the breakdown products is of interest here a full analysis of the protein distribution was carried out, using the methods described in the Second Report (1) (pp. 320-22).

Results.

Owing to the long and involved processes it has only been possible so far to carry out studies of the behaviour of the nitrogenous bodies during three complete maltings. These experiments, however, agree in the general idea which they give of the changes occurring during the operation. Experiments I and II were on English two-rowed barley. They agree closely and are discussed together. Experiment III differed in that it was carried out on a Chilian six-rowed barley of higher nitrogen content and that the temperature at the beginning and end of the period on the floor was much higher.

Diagram I.
Expt. II.

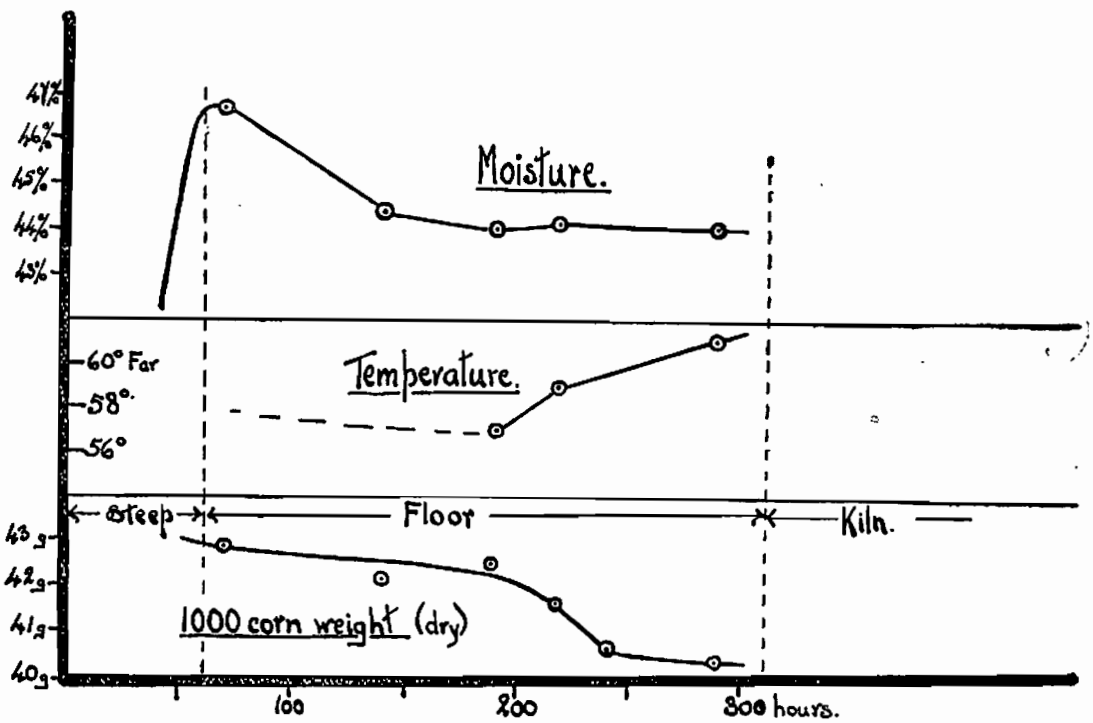
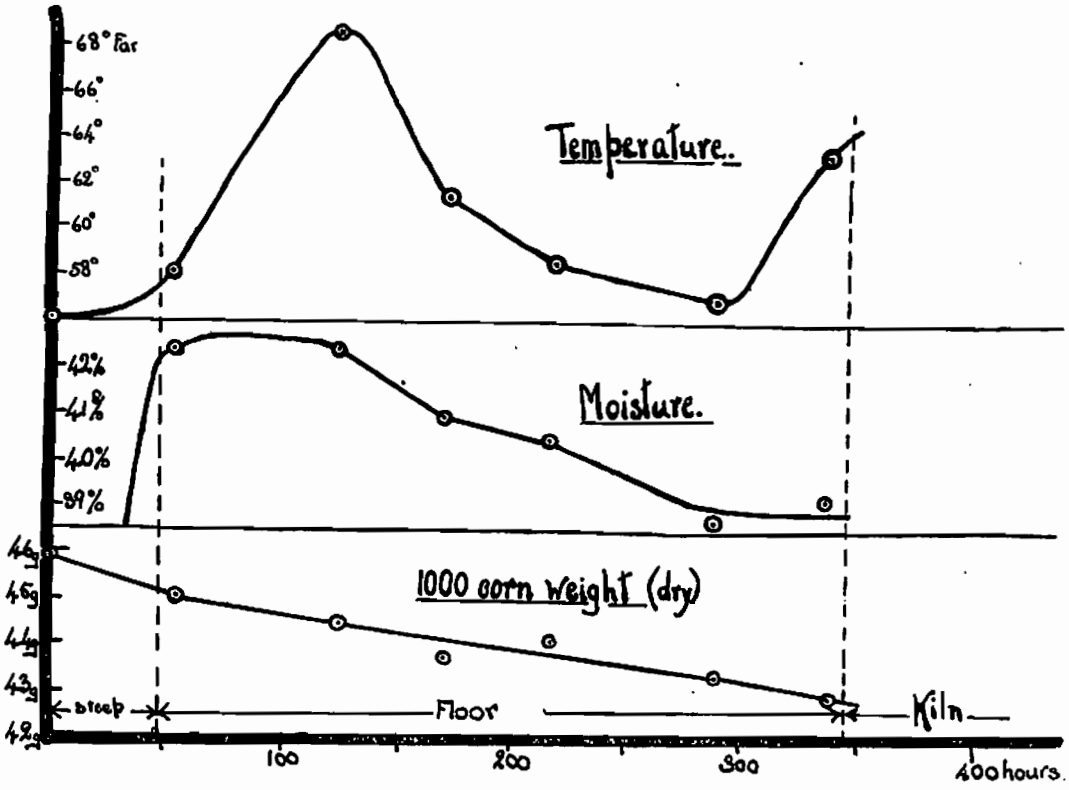


DIAGRAM II.
EXPT. III.



Hence this experiment is discussed separately.

In Experiment I the temperature on the floor remained fairly constant at about 56-60° F. (but notes were not taken). Details of the changes in temperature, moisture and 1,000 corn weight (dry and with rootlets) in Experiments II and III are given in Diagrams I and II. In Experiment II the temperature was fairly constant, while in Experiment III, as noted above, it showed wider fluctuations, and a correspondingly rapid growth of the germ and rootlets took place at the beginning of the flooring period. It is interesting to note that the moisture content while on the floor fluctuates less than might have been expected, especially during the withering stage. The fall in 1,000 corn (with rootlets) weight gives an approximate measure of the amount of respiration and steeping loss.

Tables of the results of the estimations of the amounts of nitrogenous constituents at the various stages are given as an appendix. Unless stated otherwise, the analyses are of the whole grain together with rootlets, if any. The unit in Experiment I is grms. of nitrogen in the form of the various fractions per 100 grms. of dry barley and rootlets. Since carbohydrate material is lost during malting by respiration a better (*i.e.*, constant) basis for comparison is the amount of nitrogen in 1,000 corns (and rootlets), and this is used in Experiments II and III. However, the percentage of the total weight lost by respiration is not large, so that the diagrams of Experiment I are comparable with those of the other two.

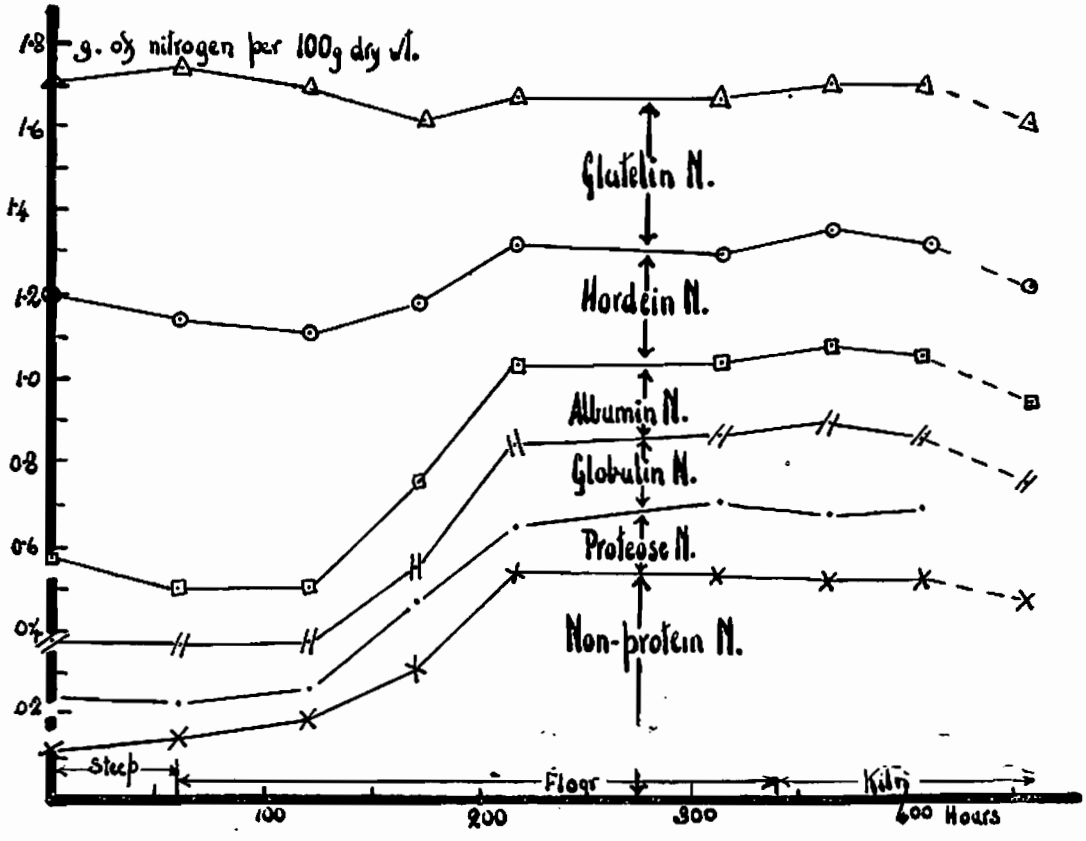
DISCUSSION.

It has been shown in the Second Report (4) (p. 316) that it is probably better to regard the proteins of malt and hence those of intermediate stages as identical with those of barley. This assumption is made in the following discussion.

The changes which occurred in all the

DIAGRAM III.

EXPT. I.—Changes in all Constituents during Malting.



nitrogenous constituents (or groups of constituents) in each experiment are given in Diagrams III, IV, and V. (pp. 326, 327 and 328). At any given time the amount of each of the constituents found is given by the vertical distance between the two curves bounding the space in which its name appears. It is not possible without a great deal of confusion to plot in the usual way all the changes studied on one diagram. The height of the top curve from the base represents consequently the total nitrogen, and this method of plotting gives a clear idea of the proportion of the total nitrogen apparently involved in the changes. The successive plottings in these diagrams may be said to represent a series of "balance sheets" in which is shown the proportion of the total nitrogen which is in each form at successive times.

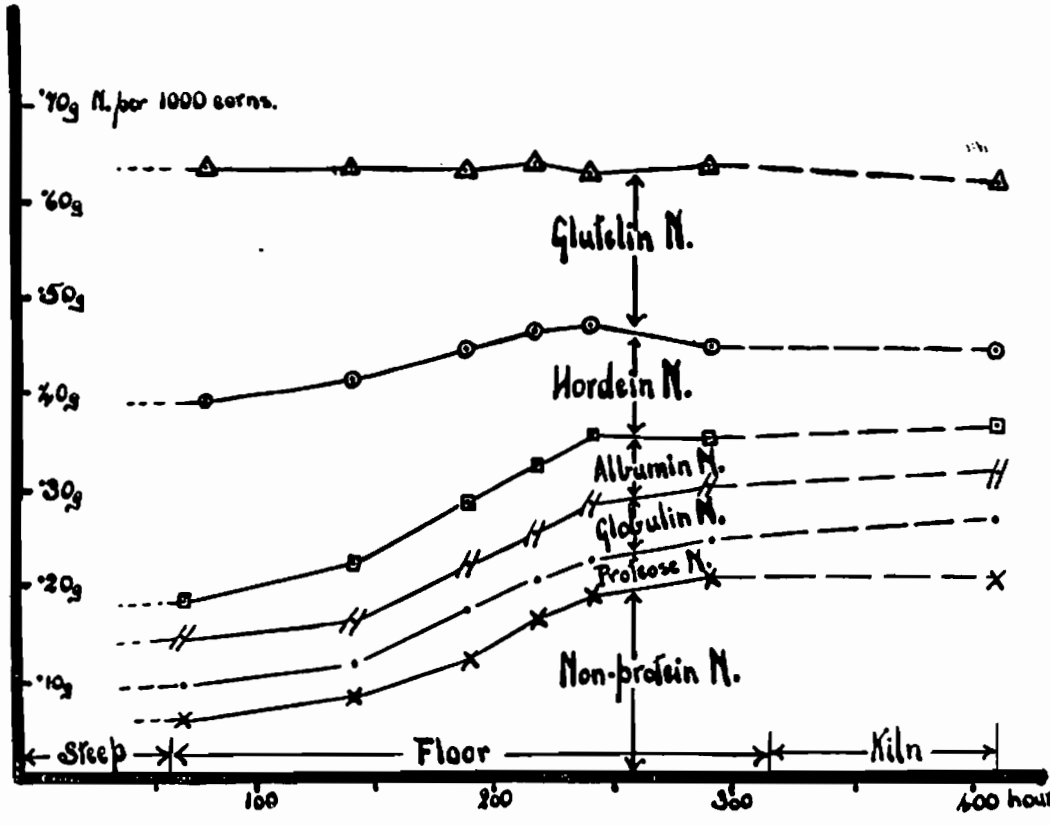
Course of the breakdown of Hordein and Glutelin.

The graphs of the changes in the amounts of the three main fractions in the first two experiments (Diagrams VI and VII p. 328 and 329) indicate that three fairly well defined stages may be distinguished. During the first 100 hours from the beginning of steeping very little happens. There is of course some loss of nitrogen to the steep water, but it is difficult to trace to which fractions this belongs as at the same time there is a commencement of hydrolysis of hordein and glutelin to give salt-soluble compounds.

During the next 100 hours or so there is a very rapid attack on the hordein and glutelin, indicated by the fall of the corresponding curves and the rise of that representing salt-soluble nitrogen. (Diagrams VI and VII.)

DIAGRAM IV.

EXPT. II.—Changes in all Constituents during Malting.



During the rest of the period on the floor hydrolysis *appears* to cease almost entirely in spite of the fact that the substrates do not appear to be exhausted.

The slow initial attack is easily understood, and is due to the time taken by the water to soak into the grain and to the slow initial liberation of the enzymes.

In the second period the rates of breakdown are high. This is more clearly seen in Diagram VIII and IX (p. 330) where the rates of disappearance are plotted. At first glutelin disappears at about the same rate as hordein. After this the rate of hydrolysis of glutelin appears to fall off while that of hordein remains high. Finally, in the third stage the apparent rate of hordein hydrolysis falls off and the amount of glutelin probably increases slightly.

These phenomena can be explained when it is considered that two separate processes are taking place in the germinating grain; (a) hydrolysis in the endosperm of the insoluble proteins (hordein and glutelin) to form soluble degradation products which are transported to the embryo (b) re-synthesis in the embryo to proteins.

Assuming that glutelin is rebuilt in the embryo, then the amount found in the analysis is the result of the equilibrium between the downgrade process in the endosperm and upgrade process in the embryo. Hashitani (5) has shown that malt rootlets contain proteins soluble in 10 per cent. sodium chloride solution (these may be assumed to be albumin and globulin) and in 0.25 per cent. sodium hydroxide solution (probably glutelin) as well as amino-acids

DIAGRAM V.

EXPT. III.—Changes in all Constituents during Malting.

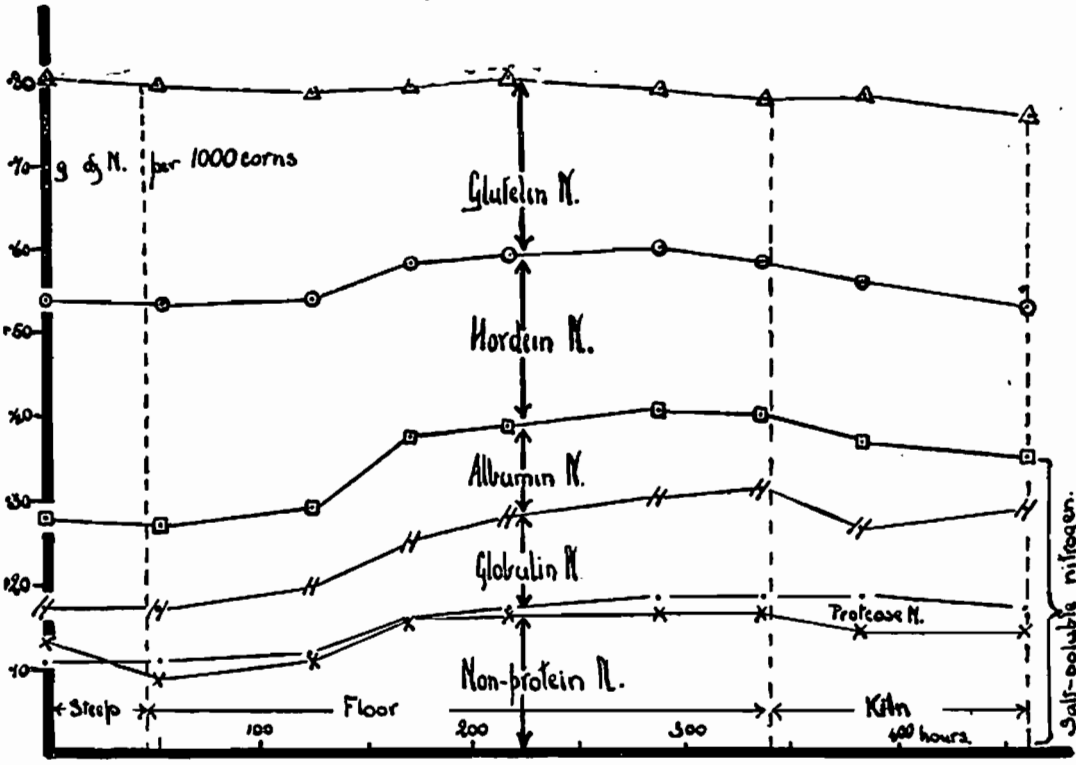


DIAGRAM VI.

EXPT. I.—Changes in Main Fractions during Malting.

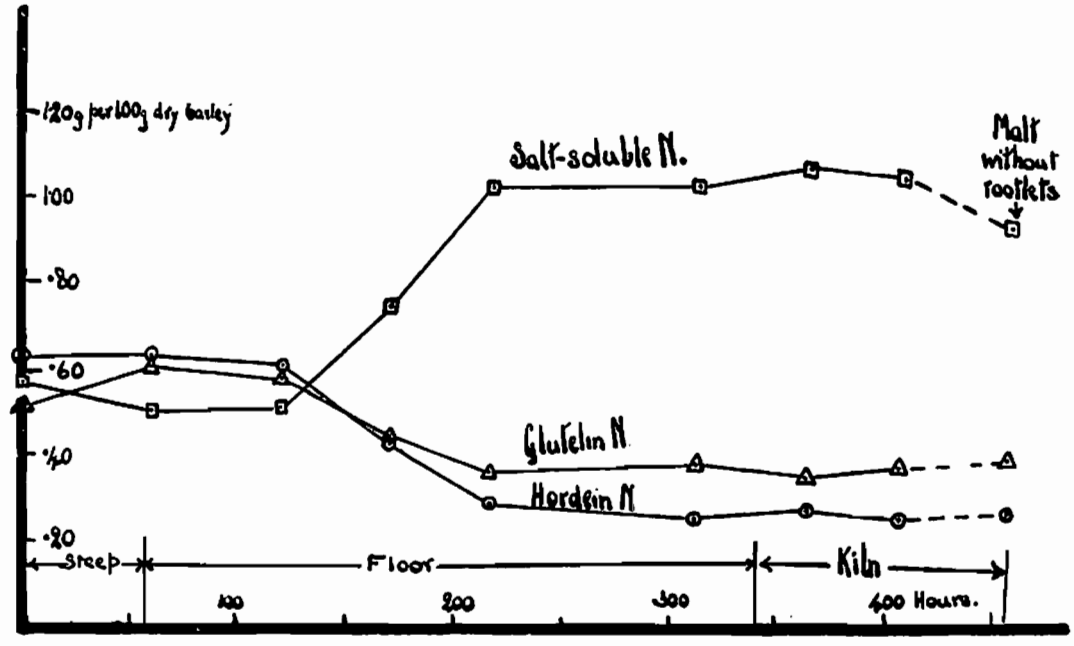
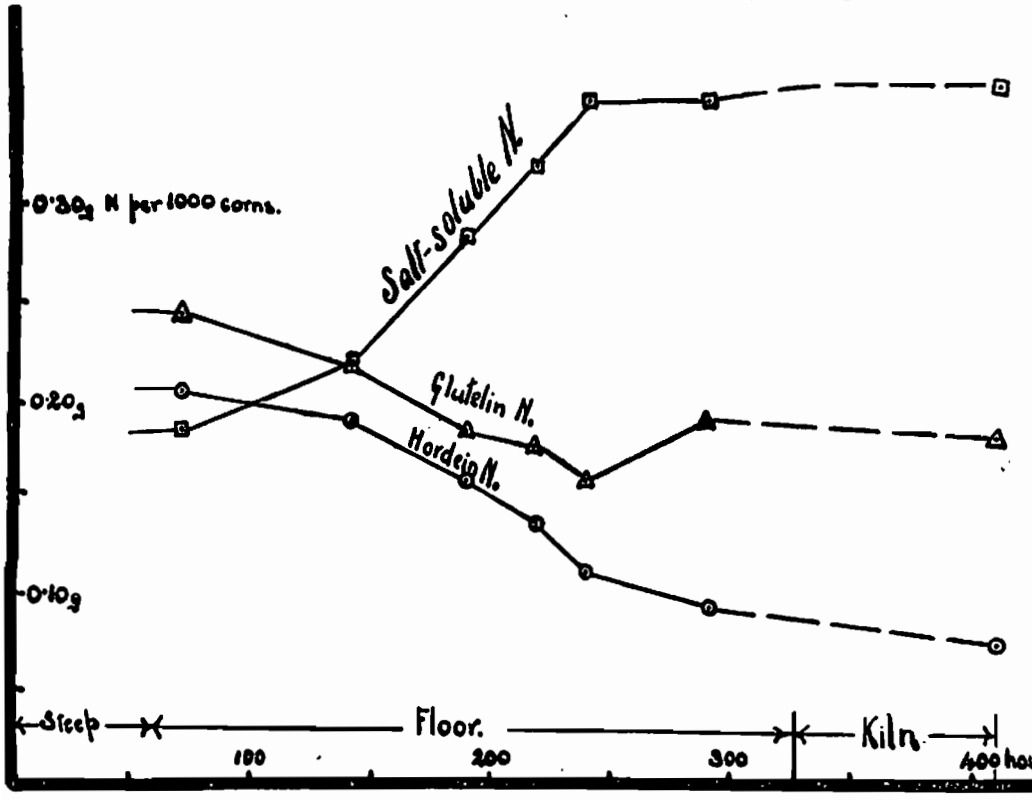


DIAGRAM VII.

EXPT. II.—Changes in Main Fractions during Malting.

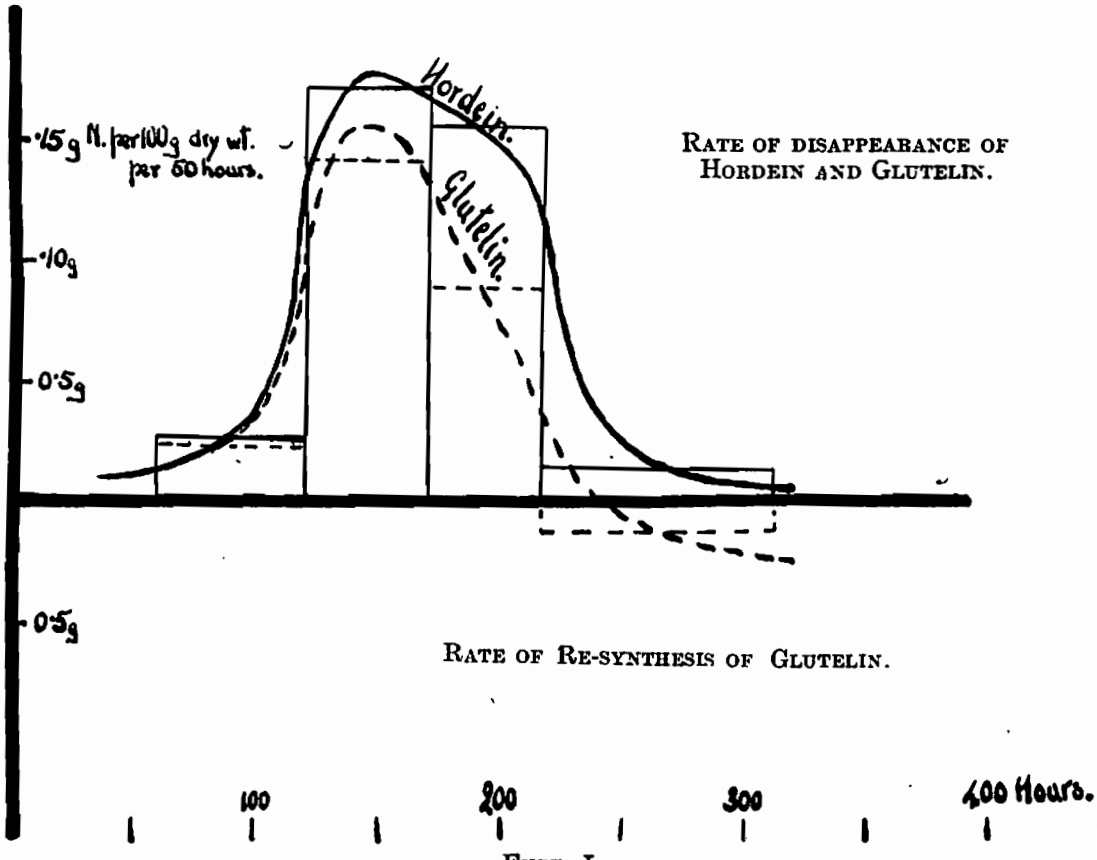


and purine bases. There is thus probably re-synthesis of glutelin in the rootlets and probably, by analogy, in the rest of the embryo also. This offers an explanation of the falling off in the apparent rate of breakdown of glutelin. Chibnall ([6]) has shown that the proteins of vegetative tissues are glutelins, so that such a resynthesis is likely. Brown's ([3]) determinations of the rate of transport of nitrogen from the endosperm to the embryo during malting show that the rate did not fall off much, if at all, during the later stages on the floor. This also would suggest that the equilibrium shown by the analyses is a dynamic one.

It is possible to explain the apparent falling off, in the third stage, of the rate of hydrolysis of hordein in the same way as due to masking by re-synthesis of hordein in the embryo, but this suggestion is very hypothetical. There is no evidence, so far as I am aware, of the occurrence of alcohol-soluble proteins in any of the cereals outside of the endosperm and husk. They have indeed been considered as characteristic reserves of these grains, so that direct evidence must be obtained before this suggestion can be accepted.

The falling off in rate is not accounted for by (*a*) cessation of active breakdown, or (*b*) lack or shortage of hordein to attack. Brown's results mentioned above are against (*a*). Also it was found that water added to a sample in Experiment II, at about 200 hours produced greatly increased growth but no differences from the main bulk in the nitrogen distribution between the different proteins. For this experiment about a kilo of grain was sprinkled plentifully with water, enclosed in a muslin bag as in stocking malting and buried in the "piece." At the close of germination the plumule projected well beyond the length of the corn and the rootlets were more numerous and longer. The very small effect produced on the amounts of the different nitrogenous bodies is illustrated in the following table (Table I, p. 331).

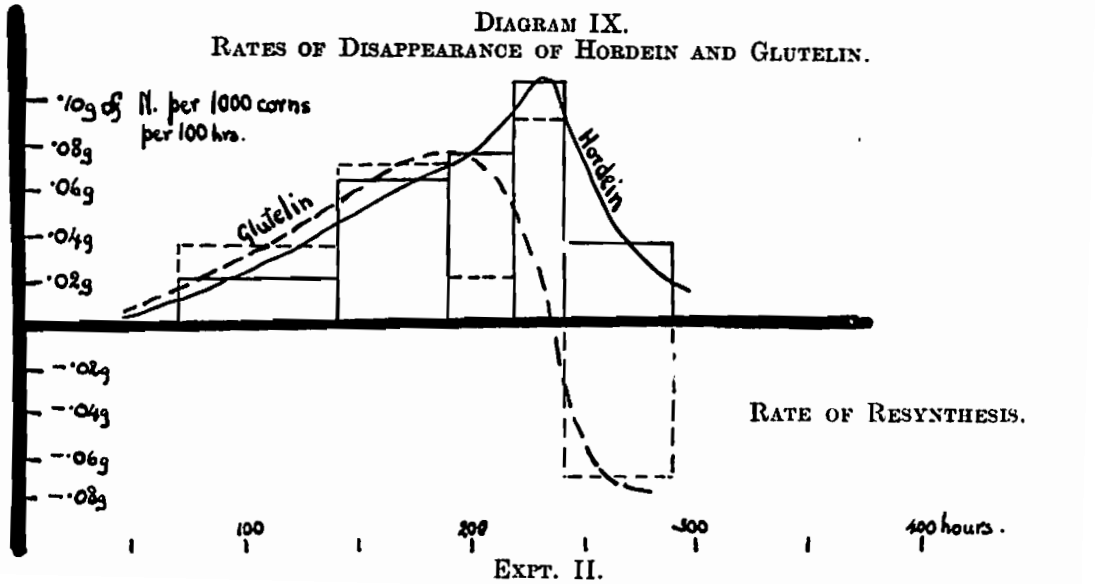
DIAGRAM VIII.



EXPT. I.

DIAGRAM IX.

RATES OF DISAPPEARANCE OF HORDEIN AND GLUTELIN.



EXPT. II.

TABLE 18.

	Nitrogen per 1,000 *Corns in the form of*						
	Salt-sol.	Hordein.	Glutelin.	Albumin.	Globulin.	Proteose.	Non-prot.
200 hours germination without extra water	·350	·095	·192	·051	·054	·044	·207
200 hours germination with extra water	·357	·096	·187	·068	·056	·041	·196

Time is reckoned in all cases from the commencement of steeping.

Against objection (*b*) that there was shortage of hordein to attack, it was found in Experiment I that the amount of hordein not broken down at the end was equivalent to 16.9 per cent. of the total nitrogen while the total nitrogen of the husk at this stage was only 13.9 per cent. of that of the entire grain, so that all of the remaining hordein could not have been protected from attack in the husk.

Since the other explanations of the falling off in the rate of breakdown of hordein fail, it may be assumed for the moment that hordein is re-synthesised in the embryo. It is then possible to produce a general scheme for the protein distribution in any organ of the barley plant during its development.

Meristematic tissue (*i.e.*, that capable of active cell division) is assumed to contain only albumin and globulin. Osborne (7) states that albumin and globulin are the proteins of the embryo of ungerminated wheat grain and that gliadin and glutelin are not present. If this is assumed to be true of the barley embryo then glutelin is synthesised in the developing embryo behind the meristematic regions which contain albumin and globulin. The possibility is now considered that hordein may later be built up in the glutelin containing cells probably only as a temporary stage behind the growing point. This scheme has the advantage that it is also capable of explaining the sequence in which I found the proteins to develop in grain as it was growing in the field (these results will be published later).

Experiment III.

The period of rapid attack here sets in earlier than in the other experiments (Diagram X p. 332) and the rise in salt-soluble nitrogen is shorter and steeper. This is related to the high temperature at this stage and was correlated with rapid growth of rootlets. The graph of the rates of disappearance of hordein and glutelin (Diagram XI p. 332) shows the rapid breakdown, and after this very little appears to happen. The apparent slight alternate synthesis and breakdown of glutelin afterwards may be due to the fluctuations in temperature or to sampling error. There is no marked rise in the glutelin at the end. The rapid growth of the embryo at the beginning may account for the longer period in which there is a state approaching equilibrium, since the rate of synthesis would more rapidly overtake the rate of breakdown. The high respiration rate (deduced from the changes in 1,000 corn weight) and rapid temperature rise shows continued metabolic activity, and is evidence against the sudden cessation of protein breakdown which is the superficial suggestion from the analyses.

Changes in the Salt-soluble Constituents.

These changes are graphed for Experiments I and II in Diagrams XII and XIII (p. 333.) Both peptases and tryptases are present in the germinating barley [Lundin (8).]. Tryptic action is, however, dominant, since nearly the whole of the rise takes place in the non-protein nitrogen.

In Experiment III (Diagram XIV p. 334) the albumin and globulin are seen to be higher in this barley than in the other two barleys, and they increase more through the malting period. The proteose fraction is consistently smaller than in the English barleys, and the rise in non-protein nitrogen is not so marked.

The smaller magnitude of the changes involved, which can be clearly seen from Diagram V (p. 328), indicates that the apparent proteolytic activity is less in this barley; but the real reason may be that the

DIAGRAM X.

EXPT. III.—Changes in Main Fractions during Malting. Chilian Barley.

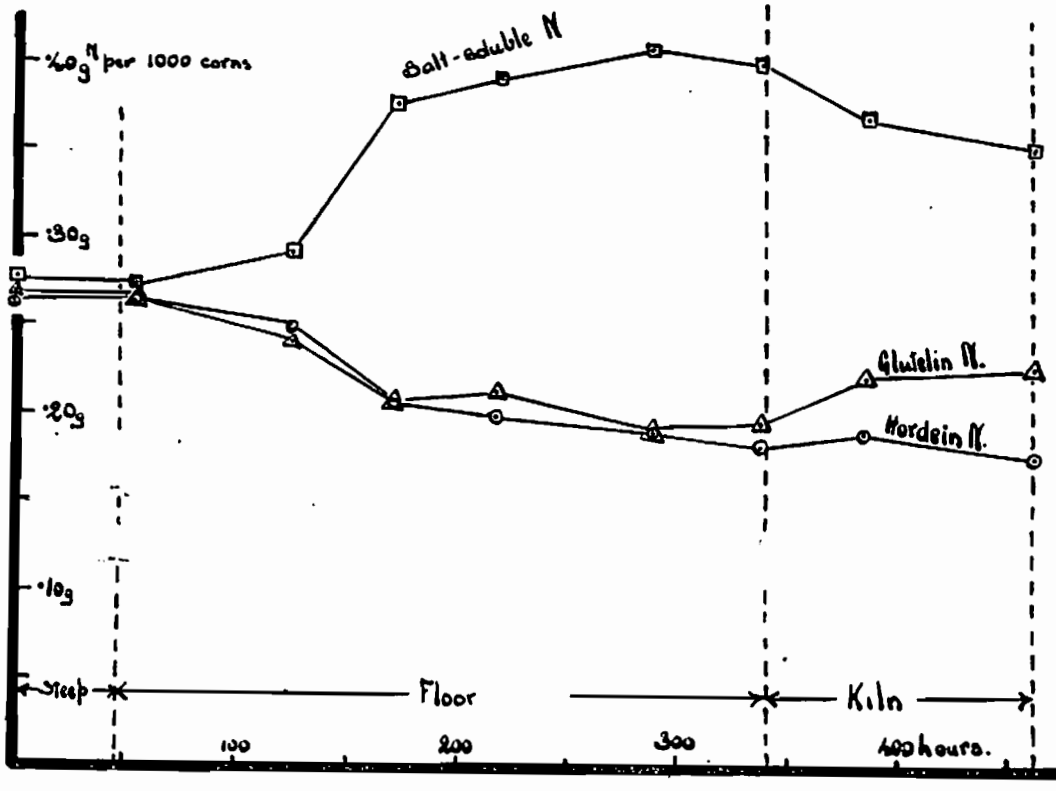
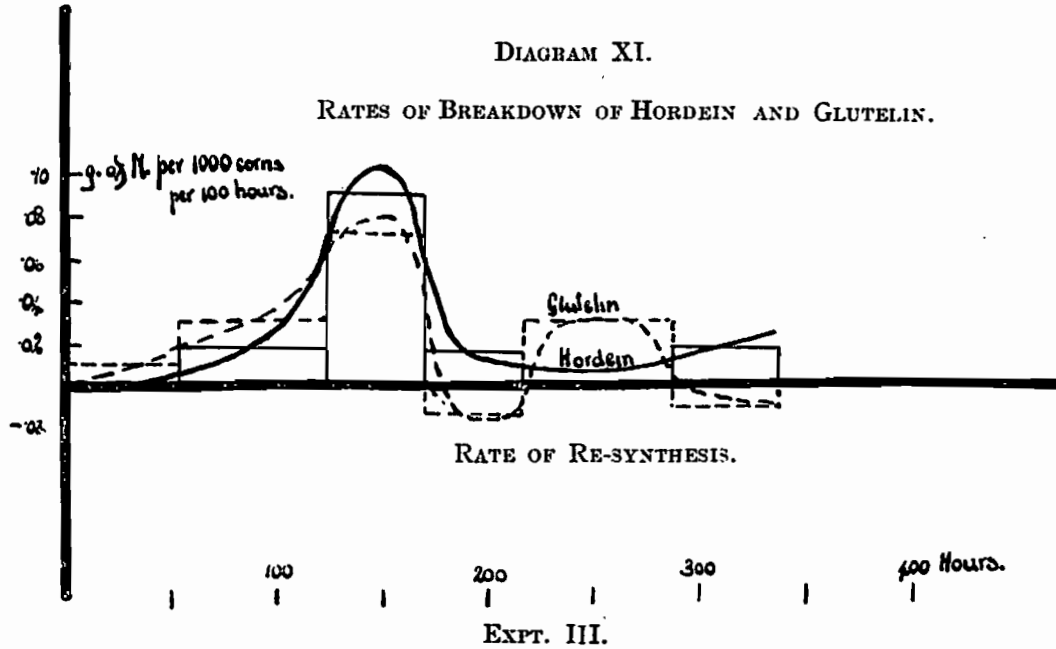


DIAGRAM XI.

RATES OF BREAKDOWN OF HORDEIN AND GLUTELIN.



RATE OF RE-SYNTHESIS.

EXPT. III.

DIAGRAM XII.

EXPT. I.—Changes in the Salt-soluble Constituents during Malting.

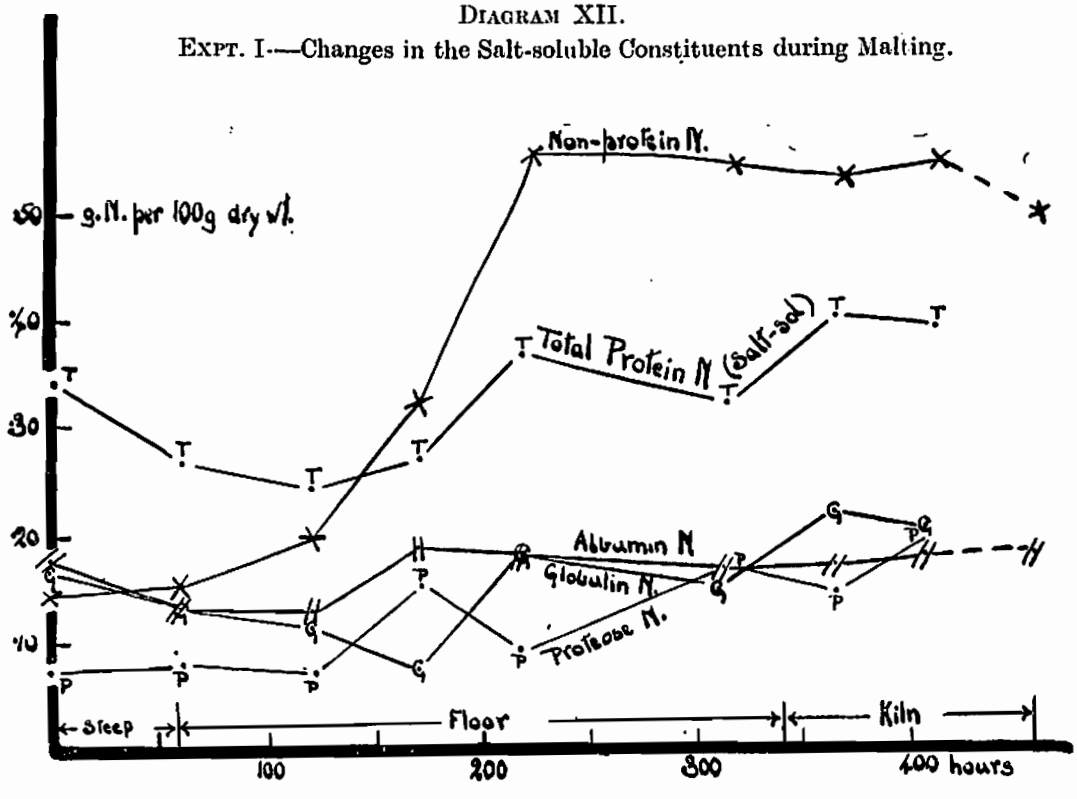


DIAGRAM XIII.

EXPT. II.—Changes in the Salt-soluble Constituents during Malting.

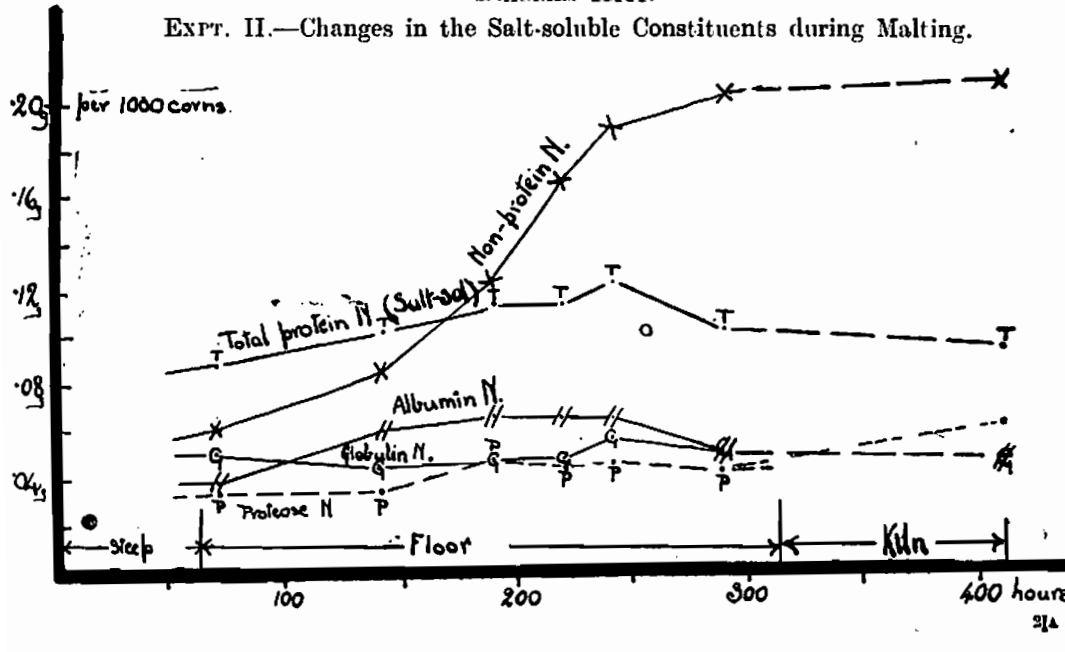
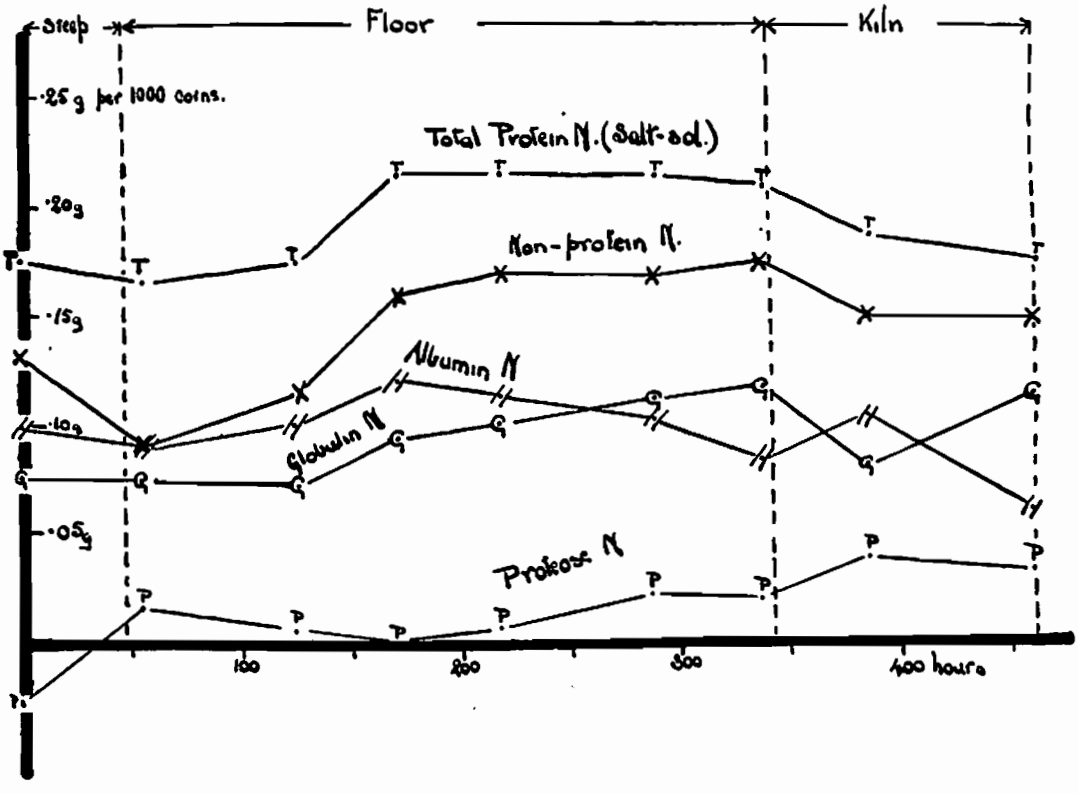


DIAGRAM XIV.

EXPT. III.—Changes in the Salt-soluble Constituents during Malting. Chilian Barley.



quicker development of the embryo allowed it to over take the production of breakdown products at an earlier stage.

It may be noted here that much of the "non-protein" nitrogen consists of organic bases such as betaine, choline, allantoin and hordenine. These must arise (in an as yet obscure manner) directly or indirectly from the breakdown of proteins. Their formation makes an estimation of the amino-acids, not a true measure of the amount of proteolytic activity.

The amount of ammonia was followed in Experiment I by the method of Foreman (9). It increases somewhat during malting, but remains a very small proportion of the total nitrogen, as Windisch and Kolbach (10) found. Foreman's method indicated the absence of ammonia from the barley itself, which is contrary to the findings of other workers.

Changes on the Kiln.

Experiments I and III give most detailed information about this. The most striking thing is that the changes are so small. The apparent change at the end of Experiment I is due to the malt being analysed alone, instead of the malt with its correct complement of rootlets, as has been done in the other two experiments. It is not certain that the small fluctuations which are recorded are significant. It might be expected that when the temperature approached 140° F. denaturation of the albumin would take place. This does not occur to any great extent, and it must be assumed that the drying is so gradual that the tissues have reached a degree of dryness at which the albumin is stable before a sufficiently elevated temperature is reached to cause coagulation. The malts produced were all pale malts. If kilned at a higher temperature, more

serious changes would probably have taken place. Luers and Nishimura [11] also conclude that there is little change in the nitrogenous constituents on kilning pale malts.

In Experiment III in the early stages on the kiln there is a decrease in total protein in the salt solution (*i.e.*, albumin + globulin) and in the non-protein nitrogen, while there is a small increase in proteose. The net result is a reduction of the salt-soluble nitrogen, while the difference appears as glutelin nitrogen.

Effect of Varying Conditions during Flooring on the Composition of the Final Malts.

Experiment II was also made as an experiment on the effect of variation of conditions on the malts produced. The course of the main piece followed normal malting practice, but experimental portions were enclosed in stockings and treated in various ways. They were as far as possible buried in the "piece" to which they belonged.

The following table gives the results of analyses of the final malts (devoid of rootlets).

instance, by accumulation of CO_2 or serious attack by bacteria or fungi) more marked effects would be shown.

If the value obtained for the salt-soluble nitrogen of the stocking malt for 10½ days is assumed to be slightly too low and the glutelin value correspondingly too high, then comparing the stocking malts for 8½ days, 10½ days and 12½ days flooring the general tendency seems to be for the salt-soluble, glutelin and non-protein nitrogen to increase slightly with time and for hordein to decrease slightly. This indicates that the general slope of the curves during the third stage is continued to 12½ days. Kilning is regarded as simply fixing the differences produced. Compared with the above three malts, that receiving less water (48 hour steep), and that receiving more water (from extra sprinkling at 6 days), are both most closely comparable with the malt of the same flooring period (10½ days), and this points to the small effect of the amount of water (within limits) during flooring on the distribution of the nitrogen compounds.

TABLE 2.

Effect of Varied Conditions of Treatment on the Composition of Malts.

	Extract.	N. per 100 grms. dry weight in the form of—						
		Salt-sol.	Hordein.	Glutelin.	Albumin.	Globulin	Proteose etc.	Non-Protein
Normal floor malt, 60 hours steep. 10½ days on floor	100·3	·891	·220	·397	·189	·072	·067	·552
Stocking Malt, 48 hour steep ...	100·6	·844	·231	·396	·182	·087	·091	·436
Stocking Malt, 8½ days on floor ...	98·5	·840	·247	·357	·119	·117	·125	·425
Stocking Malt, 10½ days on floor ...	99·0	·805	·244	·381	·153	·090	·068	·475
Stocking Malt, 12½ days on floor ...	99·6	·887	·207	·372	·091	·217	·065	·518
Stocking Malt, with extra water at 6 days	99·9	·853	·219	·377	·152	·181	·112	·416

I wish to thank Mr. F. E. Day, who determined the extracts.

The idea of a balance between the downgrade processes in the endosperm and the upgrade processes in the embryo explains the smallness of the variations which can be seen among these samples. At the same time, such a theory suggests that if the growth of the embryo is seriously checked (as, for

Comparing the floor malt with the stocking malts the former is seen to correspond most closely with the 12½ day stocking malt which suggests that in the stocking the changes are retarded. These indications from a single experiment are not to be regarded as definite, and they await confirmation. The low extract from the malt receiving 8½ days on the floor shows that modification

was not complete while the differences between the others are not marked.

It seems necessary that the next step in the study should be one with experimental maltings in which all the many factors are controlled. Such experiments would be necessary to understand the effect on the malt and wort composition of various factors, such as variety, moisture content, aeration and temperature at various stages. Such work would be of far greater value if the effects of these conditions on the carbohydrates and enzymes were followed at the same time.

Results of Other Workers.

Loibl [12] and Schjerning [13] have followed the changes during malting of those nitrogen compounds in barley which are extracted by water.

Loibl's estimations of the total soluble and permanently soluble nitrogen confirm the three stages in the attack. The "total soluble nitrogen" probably consists of the albumin, some globulin (owing to the salts present), proteoses, peptones, etc., amino-acids and simple bases. Albumin, part of the globulin, and some of the higher proteoses would be removed on boiling, leaving the remainder as "permanently soluble nitrogen." As stated in the First Report, the amount of globulin dissolved will vary with the amount of salts present and the proportion of grain to water.

Schjerning's results only show a rising period and an equilibrium stage. This is because he never took his second sample until the fourth day. As was stated in the First Report it is not easy to compare with mine the fractions obtained by his precipitation methods.

Kraft [14] has estimated the amount of hordein during a malting by direct alcoholic extraction and precipitation from this by copper hydroxide. His estimate of the nitrogen in this precipitate would be nearer the actual amount of hordein than if he had regarded the total alcohol-soluble nitrogen as hordein. His results also indicate the three stages of the attack on the hordein.

Moritz and Fuller [15] have followed the changes in the amount of amino nitrogen (by titration methods) during malting. They found a rise, and then an equilibrium stage which set in at about the same time as in my third experiment.

Possible Loss of Nitrogen During Malting.

It is well known that some nitrogenous matters are dissolved out during the steeping process, but apparently it has been claimed that there is also a loss subsequently by "respiration." H. van Laer [16] states "parce qu'une certaine quantité de cet élément (N) est éliminé par la respiration—L'élimination de l'azote par respiration est particulièrement élevée quand la germination est forcée par l'élévation excessive de la température, ou des arrosages copieux." No specific reference to original work is given however.

It will be seen that when calculated as nitrogen per 1,000 corns (a constant basis) neither Experiment II nor III give any indication of such a loss of nitrogen, apart from the loss in steeping and a loss at the end of the kilning due to incomplete recovery of rootlets (Table 3, p. 336). Such small haphazard variations as are seen in the intervening period may safely be attributed to small sampling errors.

Summary.

An examination of the amounts of the separate proteins in barley and in the corresponding malt suggests that it is chiefly hordein which has broken down to give salt-soluble constituents. Actually this study shows that as soon as active breakdown commences after steeping, the two insoluble proteins of the endosperm, hordein and glutelin, are broken down at about the same rate, to give salt-soluble products. Then the rate of disappearance of glutelin falls off.

Table 3.

Total Nitrogen per 1,000 Corns (with rootlets).

	Barley	On Floor.						On Kiln.	
Expt. II	—	·635	·634	·631	·642	·627	·638	—	·622
Expt. III	·807	·795	·783	·788	·804	·791	—	·799	·780

Later the rate of disappearance of hordein becomes very small, and the amount of glutelin may increase slightly. At this stage it is kilned.

The falling off in the rate of disappearance of glutelin and the suggestion of a subsequent increase point to a resynthesis of this protein in the embryo. The possibility is suggested that the falling off in rate of disappearance of hordein may similarly be accounted for by resynthesis in the embryo.

The breakdown of hordein and of glutelin gives rise chiefly to the simpler nitrogen compounds comprised in the term "non-protein" nitrogen. Albumin, globulin and proteose increase somewhat, but not very markedly.

In the Chilian barley studied, the albumin and globulin are larger in amount and increase more during malting than in the English barleys. The amount of proteose is less, and the apparent proteolytic activity during malting is not so marked.

The changes in the nitrogen compounds on the kiln when making pale malts are very slight.

Experiments with differing treatment of the same barley on the floor yielded malts the nitrogen distributions of which were very similar. This is accounted for by the tendency to reach, towards the end of the flooring period, a state of balance between breakdown of proteins in the endosperm and resynthesis in the embryo. This equilibrium is not easily disturbed (that is if the growth of the germ is not seriously checked).

There is no evidence in my experiments of any loss of nitrogen during the flooring period (apart from the loss in steep).

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APPENDIX.

Analyses of Barleys during Malting and Kilning.

Table 4. *Experiment I.*
Barley—Garton's Improved.

	Nitrogen grm. per 100 grms. dry weight, including rootlets.								
	Total Nitrogen	Salt Soluble.	Hordein	Glutelin	Albumin	Non-protein.	Total Protein.	Globulin	Proteose
0 hours ...	1·703	·563	·631	·509	·186	·130	·345	·159	·093
60 ,, ...	1·740	·502	·632	·606	·132	·152	·266	·134	·079
120 ,, ...	1·688	·508	·601	·579	·129	·195	·243	·114	·072
170 ,, ...	1·611	·743	·430	·438	·190	·318	·266	·076	·155
216 ,, ...	1·667	1·025	·286	·356	·179	·549	·366	·187	·062
312 ,, ...	1·662	1·031	·253	·378	·171	·532	·322	·151	·170
364 ,, ...	1·689	1·072	·270	·347	·176	·525	·399	·223	·150
406 ,, ...	1·684	1·048	·261	·375	·183	·546	·393	·210	·198
*456 ,, ...	1·597	0·935	·269	·393	·184	·507			

* Finished malt without rootlets. Steep 60 hours. Loaded on kiln at 340 hours.

TABLE 5. *Experiment II.*

English 2-rowed Barley.

		N per 1,000 *corns in the form of*—								
	1,000 corn wts.	Total Nitrogen	Salt Sol.	Hordein	Glutelin	Albumin	Non-protein.	Total Protein.	Globulin	Proteose
70 hours ...	42·9	·635	·185	·206	·245	·030	·062	·090	·051	·035
141 ,, ...	42·2	·634	·223	·191	·220	·060	·086	·103	·044	·035
189 ,, ...	42·6	·631	·286	·160	·186	·067	·125	·116	·049	·049
218 ,, ...	41·7	·642	·324	·138	·180	·067	·167	·115	·048	·045
241 ,, ...	40·7	·626	·356	·113	·159	·067	·191	·125	·058	·048
290 ,, ...	40·4	·638	·350	·095	·102	·051	·207	·105	·054	·044
*410 ,, ...	38·3	·622	·367	·075	·182	·048	·208	·096	·048	·063

Roots included in all cases. *Finished Malt. Steep 60 hours. Loaded on kiln at 314 hours.

TABLE 6. *Experiment III.*

Chilean 6-rowed Barley.

		Nitrogen per 1,000 *corns in the form of*—								
	1,000 corn. wts.	Total Nitrogen	Salt Sol.	Hordein	Glutelin	Albumin	Non-protein.	Total Protein.	Globulin	Proteose
0 hours ...	45 95	·807	·276	·261	·267	·099	·132	·174	·076	·025
53 ,, ...	45·05	·795	·272	·262	·262	·089	·091	·164	·074	·017
124 ,, ...	44·50	·783	·290	·249	·240	·099	·114	·171	071	·008
170 ,, ...	43·73	·788	·375	·207	·207	·120	·159	·213	·093	·003
217 ,, ...	44·18	·804	·393	·199	·213	·111	·168	·212	·101	·009
284 ,, ...	43·41	·791	·410	·192	·190	·102	·167	·212	·111	·023
337 ,, ...	42·91	·779	·402	·183	·195	·085	·173	·207	·117	·021
384 ,, ...	42·67	·782	·370	·191	·222	·104	·150	·185	·081	·040
*460 ,, ...	41·43	·758	·354	·176	·228	·060	·147	·174	·114	·033

Rootlets included in all cases. *Finished Malt. Steep 53 hours. Loaded on kiln at 340 hours.