THE INSTITUTE OF BREWING RESEARCH SCHEME.

REGULARITIES IN THE CARBOHYDRATE COMPOSITION OF BARLEY GRAIN.

By L. R. Bishop, M.A., Ph.D. and D. Marx, M.Sc.

In the course of investigations on the proteins indications were obtained that there exist in the carbohydrate composition of barley grain regularities which are similar to those demonstrated among the proteins. (L. R. Bishop, this *Journ.*, 1930, 336.) It therefore became desirable to ascertain whether the indications were correct; for, if these regularities could be established in carbohydrate composition, the results would be of considerable importance both in their practical application and in the study of barley metabolism.

The obvious method of attack on this problem was to estimate the quantities of the individual carbohydrates in a number of samples of barley, and in part this has been done. However, this would give results of biochemical interest only, but this work sought to obtain at the same time results of direct practical application by considering, in addition, empirical divisions of the carbohydrates of barley. The empirical estimations included are those for "brewers' extract," crude fibre and a new estimation designated the "insoluble carbohydrate" which aims, as far as possible in a single rapid estimation, to measure the carbohydrate fraction complementary to extract—that of "spent grains." Objections may be raised to the use of such empirical estimations, but experience suggests that, under their appropriate standardised conditions, they give definite, reproducible fractions of the carbohydrates present. While it may be pointed out that the "scientific estimations" of starch, cellulose, lignin and hemicelluloses* are open to similar objections; for a consideration of these estimations shows that in every case they, too, depend on the use of standardised conditions and an empirical factor. In addition the "scientific" estimations are slow and the entities they claim to estimate are by no means well defined. From the results it will be seen that both types of estimation—the "scientific" and the "empirical"—support the existence of carbohydrate regularity.

* 'Hemicellulose' includes pentosans, hexosans and polyuronides which are soluble in mild alkali and hydrolysed by dilute acids as defined by:—
L. F. Hawley and A. G. Norman. (J. Ind. and Eng. Chem. 1932, 1190.)

This word regularity will be used throughout and it may be pointed out here that it is not confined to that special case of a regular relation—proportionality.

SECTION 1.

Methods and Results.

The "insoluble carbohydrate" method was worked out as a quick, and reproducible, estimate of the carbohydrates in barley corresponding, in part, to those in spent grains; details of this method are given in Appendix I and results in Appendix II. Estimations of pentosan content (Kröber method) and crude fibre were made on a number of samples. In certain cases estimations were made of the cellulose content (method of S. H. Jenkins; *Biochem, J.*, 1930, 1428), lignin (strong sulphuric acid method), ash and fats. The primary data are given in Appendix II, together with determinations of moisture and nitrogen contents and thousand corn weights of the barleys and extracts of the corresponding malts by the Institute of Brewing Standard Method.

SECTION 2.

Discussion.

As in the protein researches (L. R. Bishop. *loc. cit.*), it has been found with the carbohydrates that the regularities it is proposed to discuss hold much more accurately when quantities are calculated on a thousand corns* as a unit rather than on a hundred grams dry weight. With mature barleys the difference between the two methods is distinct but not

* The word corn is used instead of grain, since the latter may be confused with a unit of weight.

RELATION BETWEEN HEMICELLULOSE (PENTOSANS) AND TOTAL CARBOHYDRATE.

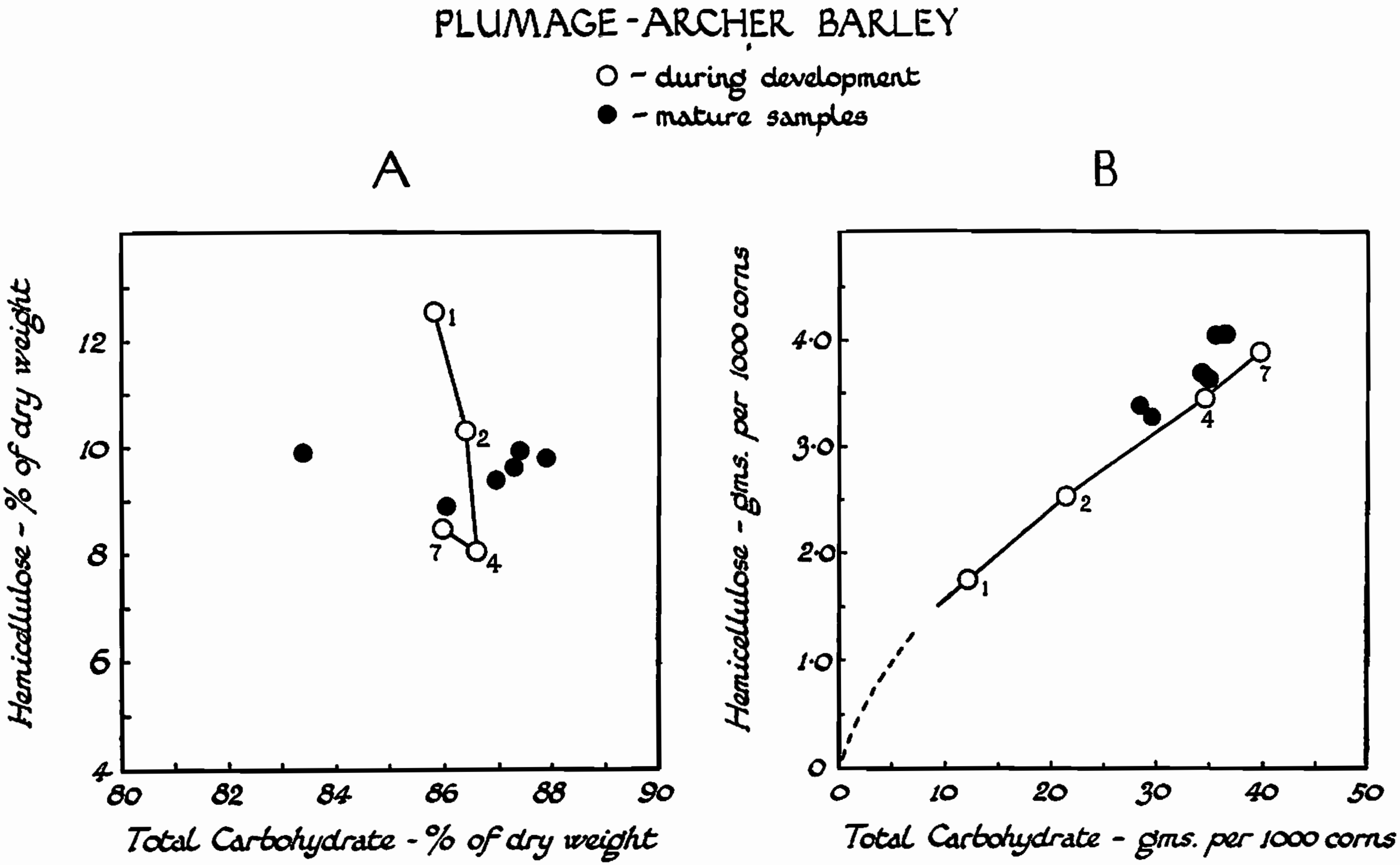

DIAGRAM I.

large, when, however, the wider range provided by immature barleys is taken into account the contention is proved, as is illustrated in Diagram 1. The same results plotted in (Ia) on a dry weight basis show no relation, and in (Ib) a regular relation on a thousand corn basis. So that, unless otherwise stated, comparisons will be made in this discussion on quantities of nitrogen or carbohydrate per thousand corns. It follows from these results, and similar ones for the proteins, that the natural plant unit is the individual corn, not a unit of weight such as a hundred grams, which is unnatural to the plant but is chosen because it is natural to the analyst.

In Diagram 1b quantities of pentosan (referred to here and in Diagram II as hemicellulose) have been plotted against the total carbohydrate. The latter figure has been obtained by difference: the quantity of protein present (nitrogen %× 6·0) together with a constant 5 per cent. for ash and fats has been subtracted from 100 per cent. While this is not strictly accurate, it is close to the truth, and should there be in fact departures these would tend to mask the regularities which are nevertheless clearly shown in the following discussion.

Diagram 1b establishes the regular relation of pentosan to total carbohydrate in Plumage-Archer variety; for any given amount of total carbohydrate, there is a definite proportion in the form of pentosan both during development and at maturity. Data are given below which show that other carbohydrate constituents of Plumage-Archer show a regular relation to the total carbohydrate. It is also shown that similar regularities hold for other varieties, but although similar they are not identical and there are differences between the corresponding quantities in different varieties. The similarities in type of relation and quantitative differences between varieties are all the more interesting because they are closely comparable with the similarities and differences which have already been demonstrated among the proteins. (L. R. Bishop. *loc. cit.* and further work to be published). The main varietal difference in carbohydrate composition is that between two-row and six-row barleys. This is shown in Diagram II for a number of carbohydrate fractions of barley taking Plumage-Archer and Spratt-Archer as representatives of two-row barleys and Atlas and F.112 of six-row barleys.

From the practical standpoint the carbohydrates of barley may be broadly divided into two groups (a) those which after malting and mashing produce extract and (b) those which do not.

They are :—(a) starch and sugars (b) cellulose and lignin. The hemicelluloses are to some extent rendered soluble.

The other empirical fractions measured are made up, as far as carbohydrates are concerned, from the following constituents of barley :—

Insoluble carbohydrate :—Cellulose and part of the lignin, Crude fibre :—chiefly cellulose.

Later on in the paper, the extract from a malt is assumed to be a measure of starch content in its barley. This is probably at least as true as the claim made by Schéele and Svensson (*Svenska Bryggare Föreningens*, 1927, 43 and 251) and earlier workers that the estimation of starch gives a measure of extract in the malt, an assumption which is the converse of that made in this paper. Either assumption is in agreement with the general " carbohydrate regularity " principle.

The results in Diagram 2 show that the various fractions of the carbohydrates which have been measured ascend regularly with the total carbohydrate and that there is a regular difference between two and six-rowed barleys. It is in these relations that the close and striking analogy with proteins is most clearly evident: a consideration of them however necessitates a comparison between carbohydrates and proteins.

Analogy Between the Proteins and Carbohydrates of Cereals.

Individual proteins of the groups, albumin, globulin, glutelin and nucleo-protein, occur in the cells of most parts of nearly all plants and may be regarded as the normal cell proteins. Similarly, the carbohydrates, comprising sugars, hemicelluloses, cellulose and lignin are also normal cell constituents. On the other hand, the alcohol-soluble proteins are a distinctive group found only in cereal grains. They are insoluble in water and salt solutions, while as has been shown, they occur in greater amounts in barleys of higher nitrogen content, for instance in young developing Plumage-Archer grain three days after flowering only 5 per cent.

THE CARBOHYDRATES OF TWO-ROW AND SIX-ROW BARLEYS.

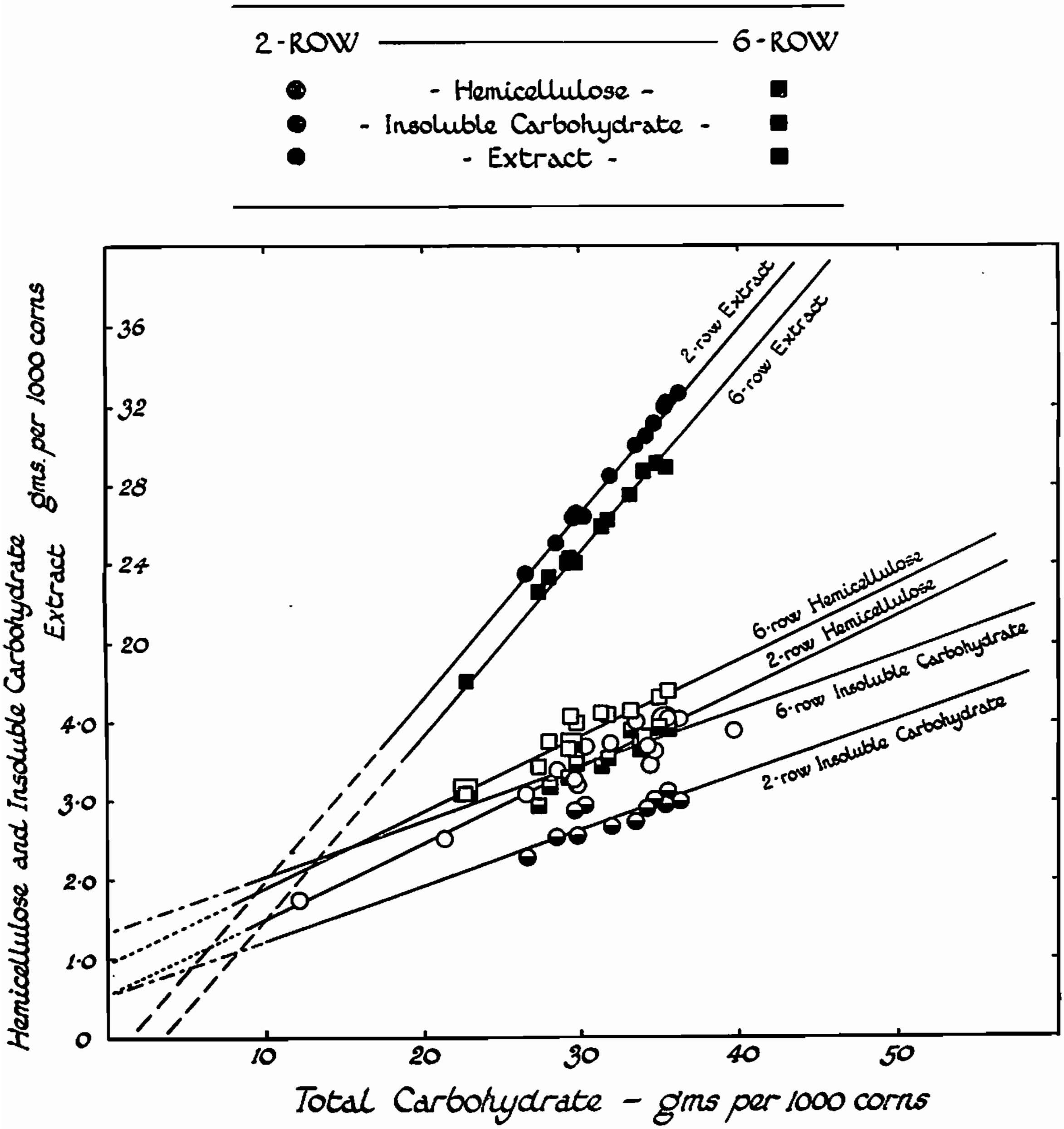

DIAGRAM II.

Changes in Protein Distribution.

Number.	Total Nitrogen per 1000 corns. grm.	Percentages on Total Nitrogen. "Reserves." Hordein.	"Cell Proteins." Glutelin + salt soluble.
		Two-row. Plumage-Archer (P) and Standwell (S.).	
P. dev. 1	0·216	5·1	94·9
P. dev. 2	0·354	18·2	81·8
Porlock '25	0·474	27·0	73·0
P. dev. 4	0·548	30·2	69·8
P. 15 '23	0·578	30·8	69·2
P. 17 '23	0·654	33·0	67·0
P. 60 '24	0·671	33·9	66·1
P. dev. 7	0·700	35·0	65·0
Orwell '25	0·872	39·5	60·5
S. C1 M.	0·941	39·7	60·3
S. C4 L.	1·060	40·6	59·4
S. C2 & 3 L.	1·118	42·7 ↓	57·3 ↑
		Six-row. F.112 (F), Atlas (A) and Vaughn (V).	
F. 144 '26	0·288	19·1	80·9
A. 2 '31	0·348	23·0	77·0
A. 22 '31	0·378	24·6	75·4
F. 142c '27	0·443	23·0	77·0
F. 195c '27	0·460	22·8	77·2
F. 190c '27	0·532	25·6	74·4
F. B.5	0·664	27·4	72·6
A. 23 '31	0·780	30·2	69·8
V. 7 '31	0·879	35·5 ↓	64·5 ↑

In this table each barley is referred to by the year of growth and the same reference number as that used in previous reports in this series and in the "Ten Years' Report on the Barley Researches" (this "Journ.," 1933, 287). P. dev. nos. 1-7 refer to barley taken at various stages of development; for the carbohydrate analyses of these barleys we are very much indebted to Dr. A. G. Norman, F.I.C.

nitrogen* and the difference between two and six-rowed barleys.

It is evident from Table 1 that, similarly, with an increasing total carbohydrate content the percentage of extract rises, and that of cellulose and pentosan falls; further, that, at any given quantity of total carbohydrate, six-row barleys (compared with two-row) yield less extract and contain correspondingly more pentosan, insoluble carbohydrate and crude fibre.

Having shown the broad difference between two and six-row varieties, the differences between individual varieties have next to be considered; do they differ significantly from one another, apart from the broad difference between two-row and six-row varieties? Here the limits of accuracy of most methods of estimation are approached, and it is necessary to resort to statistical methods to decide whether the small differences found are likely to be real or due to chance. The following table shows the results of such a study.

* It is hoped that at the same time two common misinterpretations of the protein results will be checked (*a*) that which translates the "regular relation of hordein to total nitrogen" into "a constant percentage of hordein on total nitrogen"; (*b*) that in which two-row barleys are stated to have a "higher percentage of hordein than six-row barleys" without the necessary qualification "at any given quantity of total nitrogen."

of the nitrogen was in the form of hordein while there was 40 per cent. in the highest nitrogen Plumage-Archer barley (Orwell Park, 1925) examined. In malting the chief source of nitrogen for the germ appears to be the alcohol-soluble protein nitrogen. The facts, therefore, suggest that the alcohol-soluble proteins function as reserve or storage proteins, as Beaven noticed (this *Journ.*, 1902, 561).

Starch, which is generally accepted as the storage carbohydrate, therefore corresponds to the alcohol-soluble proteins. The evidence given here suggests that, in exact agreement also, the proportion of starch, as measured by extract, increases with increase of the total quantity of carbohydrate, just as the percentage of hordein increases with increase of total nitrogen. As more total carbohydrate, or protein, is available, so a greater proportion is converted to starch, or hordein, for storage and this increase takes place regularly.

The analogy with the proteins may be pressed still further for just as six-row varieties have a smaller proportion of their total nitrogen in the form of reserve hordein at any given total protein content, so these same varieties have a smaller proportion of reserve carbohydrate at any given total carbohydrate content.

These points are illustrated in Table I.

Whilst most of the protein data has been published before, in a different form, it is quoted here as it demonstrates the rise in hordein percentage with increasing total

TABLE 1.

CHANGES IN CARBOHYDRATE AND PROTEIN DISTRIBUTION WITH TOTAL QUANTITY.

Changes in Carbohydrate Distribution.

Barley number.	Total Carbohydrate per 1000 corns. grms.	Percentages on Total Carbohydrate.				
		"Reserves."	"Cell Constituents."			
		Extract of malt.	Pentosan.	Insoluble Carbohydrate.	Crude Fibre.	Cellulose.
	Two-row. Plumage-Archer (P) and Spratt-Archer (S.).					
P. dev. 1	12·1	—	14·5	—	—	12·5
P. dev. 2	21·3	—	11·8	—	—	10·4
S. 164 '28	26·5	88·0	11·5	8·6	7·1	—
P. 43 '23	28·5	87·5	11·9	8·9	—	—
P. 164c '28	29·6	88·7	11·0	9·7	7·5	—
S. 106c '30	29·8	88·8	10·7	8·6	—	—
S. 507 '28	30·3	86·1	12·1	9·6	—	—
S. 171 '27	31·9	89·3	11·7	8·3	6·8	—
S. 106c '29	32·5	89·4	12·0	8·2	6·6	—
P. dev. 4	34·2	—	10·1	—	—	10·0
P. 158c '25	34·2	89·0	10·8	8·4	—	—
P. 29 '23	34·7	89·5	10·3	8·6	—	—
S. 112c '30	35·4	90·0	11·3	8·3	—	—
P. 192c '28	35·5	90·5	11·4	8·8	—	—
Porlock '25	36·3	89·4	11·1	8·2	6·9	—
P. dev. 7	39·7	—	9·8	—	—	8·6
	Six-row. F. 112 (F.) and Atlas (A.).					
F. 144 '26	22·6	79·9	13·7	13·8	—	
F. 195c '27	27·2	82·8	12·6	10·7	9·6	
F. 189c '27	28·0	83·4	13·3	11·3	8·5	
F. 190c '27	29·2	82·2	12·6	11·3	8·7	
A. 5 '31	29·3	82·7	13·9	12·7	—	
A. 8 '31	29·6	81·1	13·4	11·6	—	
F. 91 '29	31·3	82·2	13·1	11·0	8·5	
A. 1 '31	31·7	82·5	12·8	11·2	—	
A. 23 '31	33·1	82·8	12·5	11·8	—	
F. 92 '29	34·0	84·1	11·2	10·9	—	
A. 22 '31	34·9	83·2	12·3	11·3	—	
A. 9 '31	35·5	83·6	12·4	11·0	—	

TABLE 2.

SIGNIFICANCE OF VARIETAL DIFFERENCES IN CARBOHYDRATE COMPOSITION.

Table of varietal constants in Equations of type :—

$$X = a + b\ T$$

Where a = the varietal constant, X = H, I or E in grms. per 1,000 corns and T = total carbohydrate in grms. per 1,000 corns.

		Pentosan (H).	Standard Error.	Insoluble Carbo-hydrate (I).	Standard Error.	Extract (E).	Standard Error.
Two-row	Standwell a=	+ ·5		+ ·5		— ·8	
	Spratt-Archer a=	+ ·6		+ ·5		—1·3	
	Plumage-Archer a=	+ ·5		+ ·6		—1·3	
	B·244 a=	—	±0·16	+ ·5	±0·14	—2·0	±0·34
Six-row	Indian .. a=	+ ·9		+1·1		—2·4	
	F·112 .. a=	+ ·9		+1·3		—3·0	
	Atlas .. a=	+1·0		+1·5		—3·5	
	b all varieties ..	0·09626		0·0899		0·9300	

TABLE 3.

COMPOSITION OF THE INSOLUBLE CARBOHYDRATE AND OF SPENT GRAINS OF DIFFERENT VARIETIES.

	Ash.	Ether soluble.	Total dilute acid extract.	Lignin.	Acid-insoluble proteins.	Pento-sans.*	Cellu-lose.	Total.
			As percentages on dry matter.					
Two-row malt spent grains ..	4·6	6·4	28·8	17·3	11·8	9·8	16·7	95·4
B·244 malt spent grains	5·6	5·1	34·0	15·5	11·2	8·5	16·3	96·2
Insoluble carbohydrate	1·0	8·0	4·5	10·7	0·8	3·9	62·7	91·6
			Calculated back to original malt or barley as 100 per cent.					
Two-row malt spent grains	1·1	1·5	6·8	4·1	2·8	2·3	4·0	—
B·244 malt spent grains	1·6	1·4	9·4	4·3	3·2	2·4	4·5	—
Insoluble carbohydrate	0·1	0·6	0·3	0·8	0·1	0·3	4·7	—

* Apart from those included in acid extract and cellulose.

These results suggest it is possible that some two-row barley varieties differ from one another in their content of these carbohydrate groupings, but the differences are not significant. Similarly, there are insignificant differences among six-row barleys which are, nevertheless, consistent. The results given in Table 2 of a previous paper (L. R. Bishop and F. E. Day, this *Journ.*, 1933, 545) establish varietal differences in extract. There owing to the large number of analyses available the proof is definite ; Standwell, for instance, is significantly higher in extract than Plumage-Archer and Spratt-Archer. And probably correlated with this is the fact that in the Table above Standwell is lower than the others in pentosan and insoluble carbohydrate contents. Similarly, among the six-row barleys, the order in all three sets of analyses is—Indian, F. 112, Atlas—which suggests that the observed differences are real.

While the results in Table 2 leave the question of individual varietal differences unsettled, they establish quite conclusively the broad difference between two-row and six-row barleys.

These results are regarded as placing on a firm basis what it is proposed to refer to as the "carbohydrate regularity principle," implying a regular relation between the individual carbohydrates and the total, a relation which is characteristic for each variety or group of varieties, but differs between them.

The whole of the evidence presented in an earlier paper (L. R. Bishop and F. E. Day, this *Journ.*, 1933, 545 ; Prediction of Extract II) is a further argument in favour of the carbohydrate regularity principle. There it was shown conclusively that each variety yields a quantity of extract which is constant, apart from variations caused by protein content and thousand corn weight,* and that there are significant differences between the constants of different varieties. Confirmation of the principle is also to be found in a subsequent paper (this *Journ.*, 1934, 75), where it has been applied to a practical problem with successful results.

The special case of B.244, a two-row-six-row hybrid, merits attention. In its insoluble carbohydrate and its hordein content, it behaves like its two-row parent. As malt, its extract approaches, but falls short of that of the two-row barleys, while in its percentage of permanently soluble nitrogen and cold water extract it possesses six-row characters. Analyses suggest the probable reason why B.244 fails to come up to the two-row standard in extract is that it has a higher content of some carbohydrate related to starch but not rendered soluble in mashing, and the variety probably corresponds to six-row varieties in this respect.

The evidence bearing on this is given in Table 3, which also brings out another point.

It will be seen from the upper part of the table that some 5 per cent. is not accounted for in these analyses, but the figures are closely comparable. New methods in preparation will considerably improve the absolute value of future figures, particularly those for lignin.

The lower part of the table shows that the insoluble carbohydrate fraction includes all of the cellulose and part of the fats and lignin of spent grains.

It was found that 21 per cent. of the two-rowed and 25 per cent. of the B.244 spent grains were soluble in boiling water, and that the main bulk of this extract was not pentosan or protein. It came, it is presumed, chiefly from carbohydrate which is soluble in boiling water or dilute acid, but is not rendered soluble by malt diastase. If B.244 has a higher proportion of this carbohydrate than have two-row barleys, the low extracts of the former variety are explained. Calculated on original malt, B.244 has 2·6 per cent. more of its spent grains soluble in boiling dilute acid than has the two-rowed spent grains, which corresponds to a lowering of 3·4 lb. in extract, while the average deviation of B.244 from its predicted extract was—3.3 lb.; this is because B.244 has a low insoluble carbohydrate content like that of two-row barleys.

This section may be summarised by stating that during development or at maturity each variety has a definite "carbohydrate pattern." When the quantity per corn (or per thousand corns) is taken as a unit, then with increasing total carbohydrate all the separate carbohydrates increase and increase regularly. The storage carbohydrates increase more rapidly than the "cell carbohydrates."* In two-row barleys

* It may be noted here that the thousand corn weight factor is an approximate method of stating the rise in extract percentage with increasing total carbohydrate per corn since a heavier corn implies (generally speaking) more carbohydrate per corn (or per thousand corns). If extract increases accurately with total carbohydrate then formula (b) below should be more accurate than (a), which becomes an empirical first approximation to the same truth.

(*a*) $E = A - bN + cG$.

(*b*) $E_4 = a_1 + b_1T$.

Where E_4 = grms. of extract per 1000 corns,
A and a_1 = varietal constants,
b, c and b_1 represent the appropriate factors,
and T = total carbohydrate in grams per thousand corns.

If equation (*b*) be converted to the usual units, it will be found that the reciprocal of G not G itself should be used.

For any single sample:—

$$E_4 = a_1 + b_1 \ T$$

$$E = E_4 \times \frac{100}{G} \times \frac{1}{0{\cdot}763} =$$

$$\frac{100 \times a_1}{\cdot 763} \times \frac{1}{G} + \frac{100 b_1}{\cdot 763} \times \frac{T}{G}$$

which suggests that extract is a function of $\frac{1}{G}$ and the percentage on dry weight of total carbohydrate. The latter decreases with increase of the nitrogen %. Therefore E may be tested as a function of $\frac{1}{G}$ and N %.

This was done with the "six variety" results and the following equations obtained:

(*c*) $E = A - 10{\cdot}20N + 0{\cdot}176\,G$........ $\pm 0{\cdot}95$.

(*d*) $E = a_2 - 10{\cdot}11N - 0{\cdot}272\,Z$........ $\pm 0{\cdot}86$.

Where (A) and (a_2) are varietal constants and Z is the reciprocal of the average weight of a single corn (*i.e.*, $\frac{1000}{G}$)

The expectations are fulfilled in that (*d*) is more accurate than (*c*) but the difference is not significant.

This accounts for the decreasing effect of increase of thousand corn weight in the higher ranges, which is noted in Prediction of Extract I (this *Journ.*, 1930, 421).

* With the carbohydrates the relations might be explained as the relation between internal volume of cells (starch) which would increase more rapidly than surface carbohydrates (cellulose, pentosan) if decided by physical factors only. On the other hand, this explanation will not deal with the proteins where microscopic examination shows all to be intimately associated. (L. R. Bishop, this *Journ.*, 1930, 336.)

the storage carbohydrates regularly form a larger proportion of the whole than they do in six-row barleys.

In every respect the statements in the last paragraph are equally true if the word protein is substituted all the way through for carbohydrate. The full proof of this behaviour for proteins is available and will be published in a further paper. A generalisation of both sets of results is given in diagrammatic form in Diagram III.

Such behaviour is considered to support the view, advanced previously in connection with the proteins, that during the formation of the grain there is a dynamic equilibrium of a mass action type between the different carbohydrates, through the intermediary of enzymes and simple sugars. With desiccation in the ripening grain the dynamic equilibrium becomes converted to a static one. Thus one curve will define the proportion to the total of any particular carbohydrate either during the development of an individual grain or in a set of mature samples of grain.

Varietal Individuality.

Recent work (L. R. Bishop and F. E. Day.

GENERALISED DIAGRAM OF CARBOHYDRATE AND PROTEIN RELATIONS AND INTER-RELATIONS.

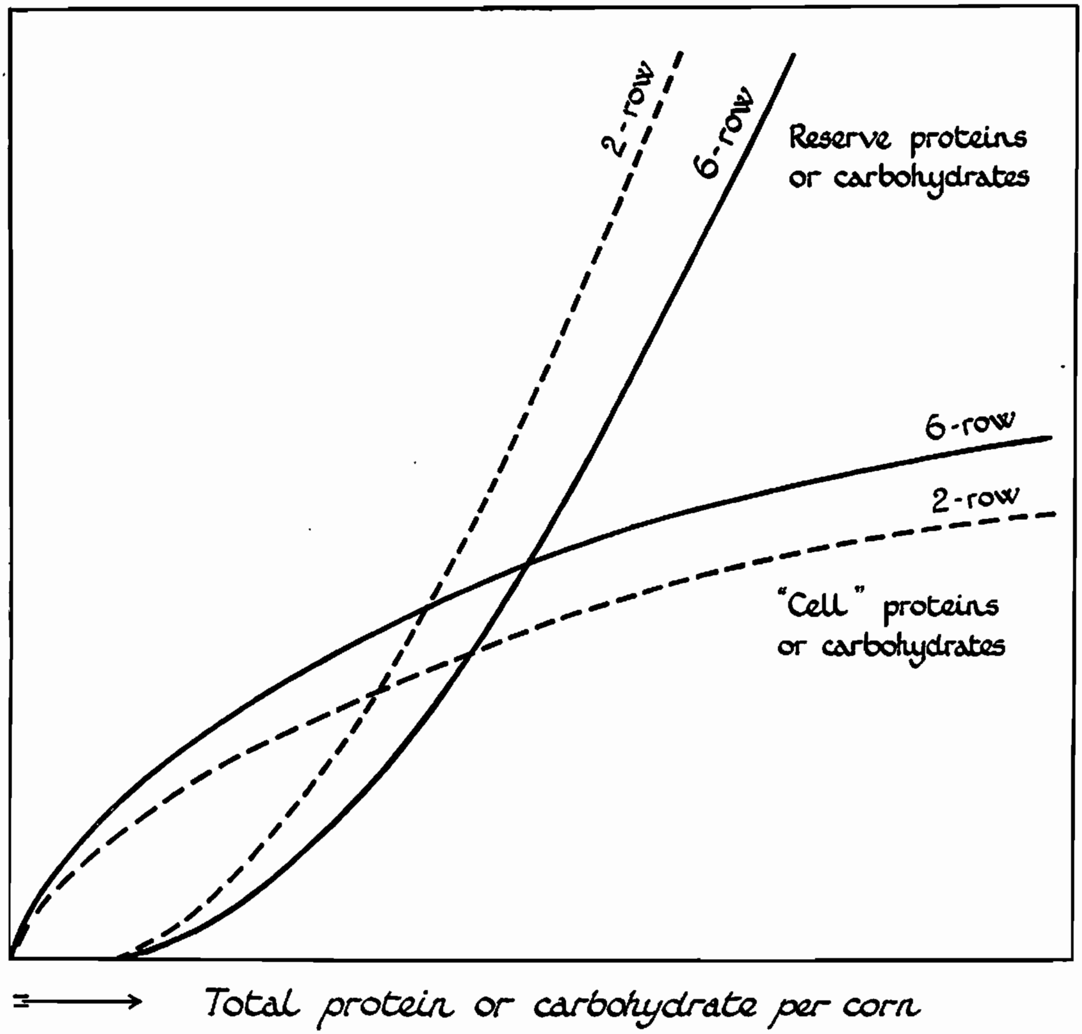

DIAGRAM III.

this *Journ.*, 1933, 545: E. J. Russell and L. R. Bishop, *ibid.* 287,) has done a good deal to clarify the bio-chemical characteristics of barley varieties and the insoluble carbohydrate and pentosan contents add to the list of properties of a barley grain, which, like the properties of a crystal, can be given as definite measurements characteristic of the variety. As we have seen the actual insoluble carbohydrate per 100 grms. dry weight varies with the thousand corn weight as well as with the variety, but that, if the insoluble and total carbohydrate are calculated as so many grams per thousand corns then the relation between the two is characteristic of the variety. For approximate purposes the insoluble carbohydrate may be given as a percentage of the total although the percentage varies somewhat with the latter. (See Table I.)

Table 4 gives the percentages of the insoluble carbohydrate measured in different varieties and the percentages of pentosan both on total carbohydrate and both for comparison with the extract constant which is similarly characteristic of the variety. As the insoluble carbohydrate and pentosan contents rise from variety to variety so the extracts decrease correspondingly.

An implication of this work is that there are similar small and regular differences in composition between the extracts of different varieties and that within each variety the carbohydrate composition of the extract is closely similar; this is, of course, apart from variations introduced by subsequent conditions, such as in mashing.

When this work was commenced, a study of the literature showed the prevailing ideas were that irregularity not regularity prevailed in the barley grain. Metaphorically speaking, the barley grain was looked on as a householder whose income (of proteins and carbohydrates) was spent according to the dictates of fancy or the weather. Now the barley grain is to be looked on as the (economist's) ideal householder. Of the total income, a fixed proportion is expended on walls and lining (cellulose, hemicellulose and glutelin), strengthening material (lignin), available goods (sugars, albumin, globulin and proteoses) and a regular proportion is put in the bank for storage (starch and hordein). If the income is increased this efficient householder increases all allotments but more especially his reserves in the bank.

There is, however, one small qualification to these broad general statements for external conditions during and after harvest can probably do something, though not a lot, to affect the proportions of the carbohydrate and disturb the regularity. Statistical analysis of the data suggests that this occurs but that its effect is small. It is indicated also in Diagram 1b, which shows that developing barley is probably slightly lower in hemicellulose content than at maturity.

Malting and Carbohydrate Regularity.

The analogy between the proteins and carbohydrates can be seen also in malting.

When the proteins are broken down they yield intermediate products (proteoses and peptones) and finally amino-acids. However the individual amino-acids do not accumulate to any great extent, instead, they are converted to asparagine which is a simple form of dipeptide. In an exactly analogous way the carbohydrates of barley are broken down to intermediate, and finally the simple, constituent sugars; here again these do not

Table 4.

Varietal Carbohydrate Characteristics.

Variety.	Insoluble Carbohydrate as percentage of Total Carbohydrate.	Pentosan as percentage of Total Carbohydrate.	Extract Constant. (A)*.
Standwell	8·3 ± 0·1	11·0 ± ·1	109·7 ± 0·4
Spratt-Archer	8·6 ± 0·2	11·6 ± ·2	108·6 ± 0·1
Plumage-Archer	8·8 ± 0·2	11·1 ± ·2	108·3 ..
Indian	11·1 ± 0·2	12·8 ± ·3	104·8 ± 0·2
F·112	11·5 ± 0·4	12·8 ± ·4	103·0 ± 0·3
Atlas	11·6 ± 0·1	12·9 ± ·3	101·5 ± 0·3

* in equation $E = A - 10{\cdot}5\,N + 0{\cdot}2\,G$.

accumulate, but are converted to a disaccharide—cane sugar. For instance, Loibl (Zeit. ges. Brau. 1923, **46**, pp. 30, 37, 45, 61, 69), found during malting a rise of 5 per cent. in cane sugar, 2½ per cent. in reducing sugars and a fall of 6½ per cent. in starch.

Both in protein and in carbohydrate metabolism, Nature confronted by the multitudinous complexities of her own arrangements seems to choose one simple path from among the many possible.

Malt Analyses.

The carbohydrate regularity of different varieties suggests itself as the explanation of the varietal regularities seen in malt analyses. The cold water extract and the percentage of permanently soluble nitrogen are affected considerably by the physiological condition of the barley and the malting treatment, as has been shown, but these analyses also show a marked varietal individuality which suggests a carbohydrate effect. Below in Table 5 is given a list of the average insoluble carbohydrate, cold water extract and permanently soluble nitrogen of six examples of each of seven varieties malted in a comparable manner.

TABLE 5.

VARIETAL AVERAGES FOR INSOLUBLE CARBOHYDRATE, COLD WATER EXTRACT AND PERMANENTLY SOLUBLE NITROGEN.

	Insoluble Carbohydrate.*	Cold Water Extract.**	Permanently soluble Nitrogen.†
Two-row—			
Standwell ..	7·0	23	37
Spratt-Archer	7·4	20	35
Plumage-Archer	7·6	22	36
B.244	7·6	17	28
Six-row—			
Indian ..	9·5	20	29
F.112	9·9	20	32
Atlas	9·6	16	28

* Percentage on barley.
** Percentage on malt.
† Percentage on barley nitrogen.

At first sight the hypothesis suggests itself that the greater amount of insoluble carbohydrate, as cell walls, in six-row barleys retards enzymic attack during malting giving as a result lower cold water extract and permanently soluble nitrogen percentage. However, the six-row × two-row hybrid, B.244, shows that the story is probably not as simple as this; for, despite a low percentage of insoluble carbohydrate, it has a low percentage of cold water extract and permanently soluble nitrogen percentage.

Again, another possible explanation of the higher percentage of permanently soluble nitrogen in two-row barleys (compared with six-row) is as follows: Hordein is *apparently* (see L. R. Bishop, this *Journ.*, 1929, 323) the chief protein broken down during malting and at any given total nitrogen content per thousand corns two-row barleys have more hordein than six-row and so should yield more permanently soluble nitrogen. This is an untenable explanation for two reasons. (*a*) Table 1 shows that high nitrogen six-row barley would be required to have a larger percentage of permanently soluble nitrogen than low nitrogen two-row barley, and (*b*) B.244, which has the high hordein content of two-row, yields the lower, six-row, permanently soluble nitrogen percentage.

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SUMMARY.

In each variety the individual carbohydrates of barley grain increase regularly with the total carbohydrate. There are small differences between individual varieties which are more marked in the general distinction between two- and six-row barleys. The carbohydrates of extract ("reserve carbohydrates") increase more rapidly than the remaining carbohydrates ("cell carbohydrates") with increase of total carbohydrate. The unit on which these relations show most accurately is for quantities calculated at so much per corn (or per thousand corns).

In each of the above respects the behaviour of the carbohydrates parallels that of the proteins. Both suggest the regularities result from equilibria of a mass action type during development of the grain.

The similarity between the proteins and carbohydrates also holds in malting, during which the proteins are broken down to give asparagine and the carbohydrates to sucrose.

APPENDIX I.

I. Methods for Estimation of "Insoluble Carbohydrate."

The barley is first sieved and separated from fibrous contamination.

Grinding. The sample is ground in a Wiley mill with a 1 mm. sieve and the ground material very thoroughly mixed. It is necessary to break the material into small even lumps small enough to be easily attacked by the reagent but leaving a residue large enough to be retained by the filter without choking. This the Wiley mill with a 1 mm. sieve does successfully. An analysis by sieving of a typical sample showed the following distribution in particle size. (Table 6.)

Table 6.

Particle Size by Two Methods of Grinding.

	Wiley mill, 1 mm. sieve.	Coffee mill.
>2 mm.	0	5 per cent.
1—2 mm.	0	13 ,, ,,
0·5—1 mm.	55 per cent.	48 ,, ,,
0·1—0·5 mm.	32 ,, ,,	25 ,, ,,
<0·1 mm.	13 ,, ,,	9 ,, ,,

Trials of material ground in the ordinary coffee mill showed that duplicates do not agree so well as those from material ground in the Wiley mill.

The two essentials for accurate estimation appear to be:

1. Evenness of particle size in the ground material,
2. Accurate standardization of conditions, particularly of the rate of boiling.

Details of Method. A 5 grm. portion of the barley is weighed out, and transferred to a 500 cc. Kjeldahl flask. The neck of the flask is bound with asbestos string to protect the hands from heat. Two hundred cc. of 0·5 per cent. sulphuric acid is brought to the boil under a reflux condenser, poured on to the barley with shaking to avoid lumps. The flask is fitted to another reflux and heated so as to cause it to boil in about 30 seconds. Steady boiling at a standard rate is continued for exactly ten minutes with occasional shaking. The flask is removed and 20 cc. of 9·50 per cent. sodium hydroxide solution (sp. gr. 1·100) added from a pipette, the flask being shaken meanwhile. This alkali neutralises the 0·5 per cent. acid and leaves 0·5 per cent. NaOH in the resulting 220 cc. of solution. The flask is put back under the reflux and brought to the boil. The whole operation of neutralising and bringing to the boil occupies one minute Boiling is continued for exactly 10 minutes. The contents are filtered on double No. 41 Whatman filters on a Buchner funnel with suction. The flask is then rinsed out with hot distilled water on to the filter which is thoroughly washed by successive portions of hot water and allowed to suck dry.

The double filters have previously been cut to equal weight (±0·0005 grm.) and are a size larger than the funnel, *e.g.*, 11 cm. papers on a 10 cm. funnel. They must be neatly fitted in.

The papers are dried in an electric oven for four hours* at 110°C. in a current of air, cooled in a desiccator and weighed with the second filter paper on the other pan of the balance. Duplicate analyses are made and the results calculated per 100 grms. dry matter.

Tests of the method showed it to give closely agreeing duplicates.

* Since this pre-drying with gentle heat on an electric hot plate has been used until "hand dry." One hour in the oven is then sufficient.

APPENDIX II.

Table 7.

Analyses of Barleys and Malts Studied.

Analyses are on dry weight, and are the mean of duplicate determinations, except the pentosan figures and some of those for extract.

Barley.								Malt Extract.		
Variety.	Number.	Dry Matter %	Nitrogen %	1000 corn weight grams	Pentosan %	Crude fibre %	Insoluble carbohydrate %	Analysis	Prediction (1)*	Difference
				Six Variety Set.						
Standwell ..	161, '25	86·80	1·37	42·2	9·2	5·44	7·07	102·6	102·6	0
	161, '26	87·76	1·69	38·5	9·9	5·6	7·12	100·8	99·6	+1·2
	161, '27	87·46	1·78	41·1	9·0	5·65	6·71	101·2	100·0	+1·2
	491/4, '28	90·67	2·12	46·4	9·3	5·74	7·18	96·8	95·6	+1·2
	140, '31	89·32	1·58	40·7	9·4	—	6·93	103·4	101·1	+2·3
	150, '31	88·56	1·92	54·6	9·2	—	7·08	100·1	97·6	+2·5
Spratt-Archer ..	171, '27	89·26	1·60	37·3	10·0	5·80	7·14	99·8	100·3	—0·7
	164, '28	88·17	1·34	30·5	10·0	6·2	7·50	100·4	101·7	—1·3
	507, '28	89·80	2·15	36·7	10·0	—	7·95	93·2	93·1	+0·1
	106c, '29	91·52	1·47	38·8	10·3	5·72	7·06	101·0	101·7	—0·7
	106c, '30	88·68	1·63	35·0	9·1	—	7·31	99·1	99·6	—0·5
	112c, '30	89·06	1·36	39·6	9·8	—	7·20	102·3	102·3	0
Plumage-Archer..	29, '23	87·66	1·49	40·3	8·8	—	7·44	100·9	100·5	+0·4
	43, '23	87·44	1·94	34·2	9·9	—	7·44	95·7	96·5	—0·8
	Porlock '25	87·86	1·18	41·3	9·8	6·02	7·24	103·0	103·8	—0·8
	158c, '25	88·00	1·35	43·5	9·4	5·63	7·36	101·4	102·0	—0·6
	164c, '28	88·50	1·28	33·9	9·6	6·55	8·44	101·4	99·6	+1·8
	192c. '28	88·50	1·27	40·6	9·9	—	7·68	103·6	101·8	+1·8
Indian	1, '29	88·94	1·18	33·4	11·2	—	9·28	99·8	98·1	+1·7
	10, '29	89·02	1·38	29·8	11·2	6·89	9·36	96·1	96·1	0
	18, '29	92·04	2·05	29·2	11·3	—	10·06	87·4	88·1	—0·7
	19, '29	89·56	1·52	35·4	10·4	7·08	8·91	95·3	96·1	—0·8
	21, '29	89·68	1·24	28·3	10·5	—	10·11	96·0	95·2	+0·8
	23, '29	89·56	1·32	35·3	11·3	7·06	9·52	98·1	96·2	+1·9
F.112	144, '26	88·22	1·40	26·1	11·8	—	11·92	90·7	88·4	+2·3
	189c, '27	89·78	1·48	32·8	11·8	7·35	9·72	94·1	94·2	—0·5
	190c, '27	88·04	1·53	34·0	10·8	7·50	9·68	92·4	93·3	—0·9
	195c, '27	87·06	1·30	31·2	11·0	8·38	9·37	94·6	97·2	—2·6
	91, '29	87·86	1·38	36·1	11·4	7·35	9·52	93·5	95·5	—2·0
	92, '29	88·78	1·29	39·0	9·8	—	9·47	96·2	96·6	—0·4
Atlas	1, '31	87·58	1·40	36·6	11·1	—	9·71	93·6	95·0	—1·4
	5, '31	87·90	1·57	34·2	11·9	—	9·71	92·8	93·4	—0·6
	8, '31	87·70	1·80	35·2	11·3	—	9·72	89·5	91·1	—1·6
	9, '31	87·92	1·42	41·1	10·7	—	9·55	94·8	94·9	—0·1
	22, '31	87·70	1·28	40·0	10·8	—	9·84	95·2	95·8	—0·6
	23, '31	88·60	1·89	39·5	10·5	—	9·10	90·8	92·2	—1·4

* From Equation (1) in a subsequent paper (Prediction of Extract III, this *Journ.*, 1934, 81).

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