that are proceeding before genuinely scientific recommendations can be made. It is sheer presumption to recommend changes in farm practice on the basis of a few Kjeldahl figures.

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The Carbohydrate Constituents of Herbage

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Although an immense amount of routine analytical work has been carried out on forage crops in the past, we are still ignorant, in the main, of what is measured under such headings as crude fibre, total carbohydrate and so on. With the advent of new techniques such as chromatography and desalting by ion-exchange and electrophoretic methods, work is now in progress to fill in these gaps in our knowledge. It is still too early to do more than indicate the probable nature of the carbohydrate components, much less to evaluate them quantitatively, and it may be many years before a complete picture can be drawn. It is perhaps appropriate at this point to emphasize the need for the examination of pure species grown and harvested under controlled conditions so that the seasonal variations of the constituents, for example the fructosans, may be followed.

For convenience we may divide the carbohydrates of herbage into those that play a part in the structure of the plant, and the non-structural components such as the free sugars and oligosaccharides and the reserve polysaccharides.

Structural polysaccharides

Cellulose

The most important structural component from the quantitative standpoint is, of course, cellulose, and although no studies of the fine structure of cellulose specimens obtained from herbage appear to have been carried out, it is reasonable to suppose that there is no essential difference between cotton (or wood) cellulose and the cellulose of herbage. Indirect evidence in favour of this assumption is afforded by the fact that celluloses isolated from such dissimilar sources as the cell-wall of the pear (Hirst, Isherwood, Jermyn & Jones, 1949) and of marine algae (Percival & Ross, 1949) are fundamentally the same as cotton cellulose, being composed of long chains of 1 : 4-linked β-D-glucopyranose residues. One would hesitate to affirm, however,

* Died on 27 September 1951.

that the molecular sizes or chain lengths of all plant celluloses are necessarily the same, and in fact the marine algal cellulose appears to have a shorter chain than standard cotton cellulose.

Pentosans

Xylan. There is abundant evidence that a xylan (or xylans) occurs in herbage because considerable amounts of xylose are produced on the hydrolysis of certain hemicellulose fractions isolated from cocksfoot grass (Buston, 1934), and the amount is possibly of the order of 5–10 %. In no instance, however, has a homogeneous xylan been isolated and its chemical architecture determined. This point is of some importance because it is now becoming clear that several xylans exist. Thus, the one isolated from esparto grass and which has been the most intensively studied consists of 1 : 4-linked β-D-xylopyranose units contained in a singly branched or forked molecule, the branch being formed through a hydroxyl group on $C_{(3)}$ (Chanda, Hirst, Jones & Percival, 1950). The molecule contains some eighty xylose units. The xylan isolated from pear cell-wall (Chanda, Hirst & Percival, 1951) also possesses a forked structure but contains also a D-glucuronic-acid residue as a terminal group, and the molecule is somewhat larger (about 110 units) than that of esparto xylan.

Finally, a xylan has been isolated from the red seaweed *Rhodymenia palmata* which differs from both the previous examples in containing chains of xylopyranose residues linked by both 1 : 4- and 1 : 3-linkages (Percival & Chanda, 1950). With these different examples before us, it would be unwise to speculate too freely on the constitution of herbage xylans, although one would expect the general picture to be similar to that of the first two examples mentioned.

Arabans. No pure arabans have yet been isolated from herbage, although there is evidence for their presence in cocksfoot grass (Buston, 1934) and in clover (Laidlaw & Reid, 1952). Arabans are always associated with pectin (Hirst, 1949), and the arabans isolated from such materials as the groundnut (*Arachis hypogaea*) or sugar beet are known to have highly branched structures. Great difficulty was experienced in isolating the araban specimens for these structural determinations even from sources comparatively rich in the polysaccharide, so that it will necessarily be a difficult matter to determine whether the arabans of herbage conform to the same general pattern.

Galactan

Since the hydrolysis of the holocellulose of pasture grass and of clover gives indications of the presence of galactose it is presumed that a galactan is present. Together with araban a galactan is always found in association with pectin (Hirst, 1942), and evidence has been presented that the polysaccharide in question is composed of D-galactose residues united by β-linkages through the 1 : 4-positions. So far the quantity present in herbage is unknown, and it may be a difficult matter to determine the precise constitution.

Pectin

Although pectic acid undoubtedly exists in herbage (Nanji & Norman, 1928), especially when young, it is not a major constituent and little work appears to have

been carried out on the examination of herbage pectins. Apart from its isolation as calcium pectate, the presence of pectin may be inferred from the fact that arabans and galactans are present and that D-galacturonic acid has been identified on hydrolysis (Buston, 1934). It is of interest to note that the amount of pectic acid present as the methyl ester is greater in grass than in hay, although in both instances the pectic acid appears to be principally in the form of mineral pectates (Buston, 1935). Buston presents evidence that pectin is not a precursor of lignin, and his conclusion, that the hemicelluloses in green unlignified leaves are mainly galactans and arabans, and in older lignified tissues are glucosans and xylans, is of considerable interest, especially in regard to legumes.

Glucosans

There is some evidence that glucosans may be present in herbage, because after the extraction of clover and lucerne with 80 % ethanol to remove sugars and oligosaccharides the aqueous extract can be shown to give some glucose on hydrolysis as well as xylose, arabinose and galactose (Laidlaw & Reid, 1952). It may be recalled that a water-soluble glucosan was isolated from barley roots (Hassid, 1939) in which it appeared that the glucose residues were united by 1 : 6-linkages. It is necessary to keep an open mind as to the source of the glucose in the hydrolysates mentioned above, but in the apparently complete absence of starch in the foliage of herbage the possibility cannot be excluded that a similar glucosan may be present in small amounts in forage plants.

Non-structural carbohydrates

Monosaccharides

Glucose and fructose appear, in small quantities, as herbage constituents and are subject to seasonal variations. In general the fructose content is between 1 and 2 % and the glucose of the order of 1 % (de Man & de Heus, 1949; Laidlaw & Reid, 1952). Preliminary investigations of the seasonal variation have been carried out in Edinburgh and a more systematic investigation is now in progress.

Oligosaccharides

Sucrose. Sucrose is, quantitatively, the most important sugar in herbage, and the amount is surprisingly large, values of 5–6 % having been recorded (de Man & de Heus, 1949; Laidlaw & Reid, 1952).

Melibiose and raffinose. There is evidence from chromatographic examination that small amounts of the reducing disaccharide melibiose (6-[α-D-galactosido]-D-glucopyranose) occur in grass extracts (Laidlaw & Reid, 1952). The presence of this sugar is not unexpected since the trisaccharide raffinose also appears to be present, which by the loss of a fructose residue can give melibiose.

Stachyose. The tetrasaccharide stachyose also appears to be present in the 80 % ethanol extracts of grass. It may be recalled that both raffinose and stachyose have been found in apples, plums and pears (Bradfield & Flood, 1950). Raffinose contains, of course, a sucrose residue and on hydrolysis gives sucrose and galactose or fructose and melibiose, or glucose, fructose and galactose depending on the conditions

employed. Stachyose also on hydrolysis gives glucose, fructose and two molecules of galactose, and although the presence of a sucrose residue in this tetrasaccharide has not been definitely proved, and indeed the precise structure still requires final elucidation, the possible interdependence of all the non-structural components mentioned so far needs little emphasis. The same is true indeed of the highly important fructosan fraction which is the remaining topic for discussion.

Fructosan

From at least two standpoints the fructosan component of the herbage carbohydrates is of the highest importance. In the first place, as far as the metabolism of the plant is concerned it is the reserve carbohydrate, and in the second, I believe that it will be found to play an important part in the making of good ensilage, because, since the fructosans are all constructed from fructofuranose residues, they are by far the most easily hydrolysed of all the polysaccharides.

The experiments of de Man & de Heus (1949) on rye grass show an apparent variation in fructosan content from 5 % when cut on 23 April to 17·6 % when cut on 7 June. In the preliminary experiments in Edinburgh the values determined by chromatographic methods were 6·8 % (3 May) and 12·1 % (6 June) for perennial rye grass and 2 % and 4·9 % for cocksfoot cut on the same dates (Laidlaw & Reid, 1952). It is of great interest to note that no fructosan has been detected in clover or lucerne.

Reference may be made to an important review by Archbold (1940) which, although primarily concerned with the fructosans of such plants as barley and wheat, is of general interest. Several workers have analysed the leaves, stems and ears of wheat, barley and rye at various stages during growth (Colin & Belval, 1922; Belval, 1924*a–c*; Barnell, 1938). Fructosan appeared in the leaf sheaths of wheat in quantities amounting to 0·5 % of the fresh weight on 19 May, increasing to 2·6 % on 11 June. Fructosans were absent in the stems at first, but as the spikes emerged the polysaccharide began to accumulate and reached a maximum at the heading period, reaching a value of 5 % in wheat stalks. The fructosan in the ears, as estimated as soon as it was possible to dissect them, amounted to 6–8 % and this fell continuously during development. Later workers (Archbold, 1938; Barnell, 1938) showed that fructosan was also present in the leaves of barley, although it is thought that the fructosans are produced not in the leaves but in the stems. For wheat and barley it is suggested that the fructosans may be, in part, the precursors of starch (Archbold, 1940). In any case it is clear that the fructosans are stored only temporarily in the stems during a relatively short period when the leaves deliver more sugar than is immediately necessary for the growth of the ear. Although this is not the place to discuss the merits or otherwise of these views, it is clear that from the point of view of plant physiology the measurement of distribution of fructosans in the various parts of the plant is very important.

The chemical constitution of fructosans. Although many specimens of fructosans have been isolated and named (Archbold, 1940) detailed analysis has been confined to but a few, H. H. Schlubach having been the foremost worker in this field.

With grass fructosans the first structural analysis was carried out in Birmingham

(Challinor, Haworth & Hirst, 1934*a*) on a product isolated from rough-stalked meadow grass (*Poa trivialis*). This substance was shown to have a structure represented by a chain of D-fructofuranose units linked through $C_{(2)}$ and $C_{(6)}$, and was quite different from the root-reserve fructosan inulin in which the same units are linked through $C_{(2)}$ and $C_{(1)}$.

The grass fructosan was in fact apparently identical as regards its main structural features, although not necessarily in its molecular size, with the product, levan, which is obtained by the action on sucrose of the micro-organism *Bacillus mesentericus* (Harrison, Tarr & Hibbert, 1930; Hibbert, Tipson & Brauns, 1931; Challinor, Haworth & Hirst, 1934*b*). The soluble fructosan from barley leaves already mentioned (Archbold & Barter, 1935) was also found to contain the same fundamental 2 : 6-linkage (Haworth, Hirst & Lyne, 1937), and this linkage is found also in the fructosans isolated from other grasses, e.g. the so-called phlein from timothy (*Phleum pratense*) (Schlubach & Sinh, 1940), the fructosan secalin from unripe rye (Schlubach & Bandmann, 1939), and pyrosin from the stalks of wheat (Schlubach & Huchting, 1949). So frequently has the 2 : 6-type of linkage been established in fructosans isolated from stems and leaves that it has been proposed (Schlubach & Sinh, 1940) that two main groups of fructosans, called the phlein type and the inulin type, should be considered to exist.

More recent examinations of grass fructosans have been made and the 2 : 6-linkage has been established for all of them. Bell & Palmer (1949) have concluded that the fructosans of Italian rye grass and leafy cocksfoot contain about fourteen fructofuranose units in a chain, although the molecular weights determined by physical methods (ultracentrifuge) show molecules containing about thirty units, which implies branched-chain structures. The fructosan from perennial rye grass (Laidlaw & Reid, 1951) appears to have a chain length of twenty-five to thirty fructose units, and the same is true of a specimen from leafy cocksfoot (Percival & Telfer, 1951). In these instances the results do not suggest that the molecules are branched, and it is clear that further work is necessary to determine whether, by the different methods of isolation adopted by the two schools, different fructosan fractions are obtained.

In the suggested constitution for the perennial rye grass fructosan the molecule is terminated by a sucrose residue. Such a type of end-group was first postulated for inulin (Hirst, McGilvray & Percival, 1950) to account for the results of experiments by methylation and periodate oxidation, and a similar terminal grouping has been postulated for the fructosan triticin of couch-grass rhizomes (Arni & Percival, 1951). This brings me to the point that it is very difficult, if not impossible, to isolate any fructosan that does not yield a small quantity of glucose on hydrolysis, and by the autohydrolysis in water of several fructosans we have found it possible to demonstrate the presence of sucrose. The possibility exists, therefore, that the natural fructosans are built up from sucrose by the attachment of fructofuranose units to either $C_{(1)}$ to give the inulin type, or to $C_{(6)}$ to give the grass-fructosan type of structure. Incidentally it may be pointed out that the stereochemical nature of the two structures is quite different, the former being composed of fructofuranose units arranged one above the other like a pile of plates or the leaves of a book, and the other as an elongated

molecule, facts which surely explain the relative insolubility of inulin in water as compared with the soluble and readily diffusible phlein-type fructosans.

It has been proposed (Palmer, 1951) that the molecular size of a fructosan can be calculated simply by estimating the glucose content on the assumption that one glucose residue is present in a molecule, and good agreement is obtained in most cases.

In connexion with the postulated sucrose (or sucrose-type) end-group, the work of Bacon & Edelman (1951) in Sheffield on the carbohydrates of the Jerusalem artichoke should be mentioned. These workers have demonstrated that at least seven non-reducing components are present with R_F values on the paper chromatogram ranging from that of sucrose to zero, the latter presumably corresponding to inulin. These substances are present in the stems, tubers and roots of the Jerusalem artichoke and in the underground organs of seven other species of Compositae, and when the implications of these results are fully understood there is little doubt that our knowledge of the synthesis and breakdown of fructosans in the plant will be considerably advanced.

In conclusion, although it does not come strictly into the category of herbage, one may be permitted to mention the interesting fructosan triticin isolated from the rhizomes of twitch or couch grass (*Triticum repens* L.). In this polysaccharide the fructofuranose units are linked by both 1, 2- and 2, 6-linkages in equal proportions, and the fructosan is thus a hybrid between the phlein type characteristic of grasses and the inulin type found in tubers such as the dahlia and artichoke (Arni & Percival, 1951). The molecule again appears to contain about thirty units and a terminal sucrose end-group. Whether such a fructosan occurs in stems and leaves is, possibly, doubtful, but its very existence as well as that of others of similarly anomalous structure such as irisin from the wild iris (*Iris pseudoacorus*) makes it even more desirable that a thorough study should be made of the fructosans from sources other than those already mentioned.

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The Utilization of the Minerals, Vitamins and other Constituents of Grass

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Animals that spend their lives on pastures must receive from the herbage all the essential nutrients they require for growth and reproduction. The utilization of the proteins and carbohydrates of grass has already been discussed (Synge, 1952; Percival, 1952), and it now remains to examine the utilization of minerals and vitamins. At the same time, however, the presence of toxic factors in grass and the nutritional hazards which beset the grazing animal must be considered.

Before the adequacy of grasses and clovers in terms of mineral and vitamin content can be assessed, some approximate idea of the requirements of farm livestock for these entities must be obtained. In this respect recommended dietary allowances (Guilbert & Loosli, 1949, 1951) may not provide an adequate assessment, since such requirements of minerals and vitamins tend to be overestimates and include large margins of safety. Minimum requirements (Mitchell & McClure, 1937), expressed as percentages of the dry matter of the ration, should give an indication of the lower limits which are permissible in the composition of the diet. A pasture or grass containing less than this quantity can then be judged to be deficient. This does not mean that deficiency symptoms in their classical sense will arise on such pasture. Animals have considerable abilities to adapt themselves to suboptimal nutrition. These adaptive processes, however, inevitably involve slight declines in growth rate, milk yield, egg production and reproductive performance.

High rates of growth and production not only entail higher requirements per head of stock, but higher concentrations of most nutrients in the dietary dry matter. Thus to produce one egg a day the hen requires 3 % calcium in her diet whereas to produce none she requires only one-tenth this amount. Similarly, the mature cow requires as an absolute minimum 0·17 % phosphorus in her diet if she produces no milk and is not pregnant. She requires half as much again to produce 3 gal. milk a day, and almost double this quantity when lactating during the terminal phases of pregnancy.

Table 1 summarizes data on minimum requirements of Ca and P for farm livestock. Methods and data used for this purpose are given in the footnote to Tables 1 and 2.