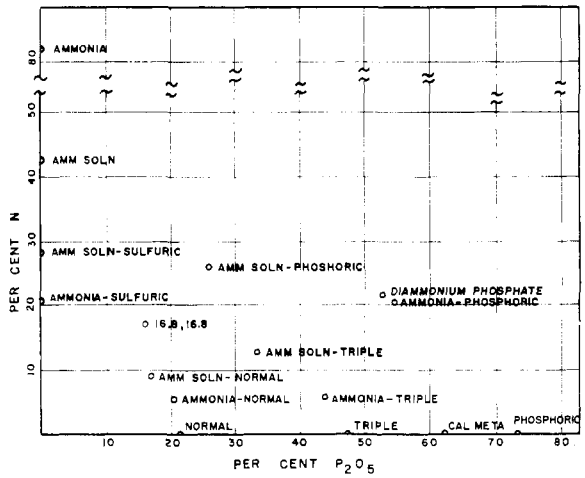

Figure 2. Analysis, on bone-dry basis, of reaction products and of raw materials

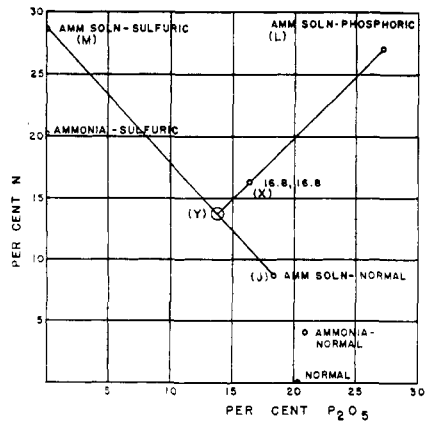

Figure 3. Determination of proportions of the desired raw materials from 16.8–16.8 point, *X*

analyses are on a bone-dry basis. Taking the 13–13–13 as an example, the grade to be formulated is a 16.8–16.8–0. As the initial step, the 16.8–16.8 point is plotted (Figure 3) and the proportions of the desired raw materials are determined in the following manner:

Construct a line between the points epresenting combinations *M* and *J*. Draw a second line from the point representing combination *L* through the point representing the desired composition, to an intersection with the first line. For convenience, designate this intersect point *Y*. Measure the line segment *MY* and divide this length by the total length of the line, *MJ*, times 100, to determine the per cent of combination *J* in the composition represented by point *Y*. Similarly, the length of the line segment *YJ* divided by *MJ*, times 100, equals the per cent of combination *M* in that same composition. With these calculations, point *Y* represents a composition of 78.8% combination *J* and 21.2% combination *M*.

The same method used to determine the composition represented by point *Y*, may be used to determine the composition represented by point *X*. The line segment *XY* divided by the total length of the line *LY*, times 100, is the per cent of combination *L* contained in 16.8, 16.8. This is determined to be 24.1%. The 16.8, 16.8 is composed of 24.1% of combination *L* and 75.9% of composition *Y*. Composition *Y* has already been shown to contain 78.8% of combination *J* and 21.2% of combination *M*. Therefore, 16.8, 16.8 is composed of 24.1% combination *L*, 59.8% combination *J*, and 16.1% combination *M*. These percentage figures may be used to calculate the formula of the 16.8–16.8–0 base, which, with the addition of potash, will produce the 13–13–13. The quantities obtained are identical to those obtained using the algebraic method.

The initial calculations required to prepare the tables and graphs are somewhat lengthy; however, they should be useful over a period of time, as there is little variation in the analysis of the raw materials for a given plant.

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FORAGE CROP CONSTITUENTS

The Isolation and Analysis of Hemicelluloses of Brome Grass

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HEMICELLULOSES are closely associated with cellulose in plants; in forage grasses they amount to 35% to 40% of the dry matter of the cell walls. When separated from the cellulose, they appear to be mixtures and extremely complex. Their chemical study begins with their separation as a crude mixture and the identification and quantitative determination of the sugars and uronic acids which they contain. Considerable progress has been made in the study of hemicelluloses in wood and in such agricultural by-products as straw and corncobs. In some cases, hemicelluloses, which seem to have molecular identity, have been isolated from the mixtures (*1*, *3*). In forage grasses, however, little work has been done. Binger, Sullivan, and Jensen (*5*) found four sugars—xylose, glucose, arabinose, and galactose—and at least one uronic acid in the

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Hemicelluloses were studied in the leaves, stems, roots, and flowering heads of brome grass (*Bromus inermis*) at several stages of maturity. They were prepared from holocelluloses by extraction with hot water and with dilute alkali. The water-extractable hemicelluloses contained four sugars, two uronic acids, and four unidentified substances. Xylose was the most common sugar, arabinose was second, and glucose and galactose were in lesser quantities. In qualitative examination of chromatographs of the uronic acids, galacturonic appeared to be more abundant than glucuronic. The alkali-soluble hemicelluloses contained only xylose and glucose.

hemicelluloses from orchard grass. The present work continues this study. The hemicelluloses of brome grass (*Bromus inermis*) have been isolated from various organs, and their basic units have been identified and determined quantitatively.

Methods

The plant material consisted of leaves, stems, and roots of brome grass harvested on May 18, 1955, at the boot stage or just prior to complete emergence of the head; and of leaves, stems, roots, and heads harvested 4 weeks later during flowering; and of second growth foliage from the same plants on August 30. These were dried at 80° C. for 2 hours and then at 40° to 50° C. overnight, or until air dry, and ground in a Wiley mill to pass a 20-mesh screen. The plants were of two genetic clones which were kept separate. Both clones were studied but only one is reported here in detail.

The plant samples were analyzed as follows: Moisture, ash, and crude protein were determined by standard methods (*4*). Lignin was determined by the method of Ellis, Matrone, and Maynard (*10*) and cellulose by the method of Crampton and Maynard (*7*), each with minor modifications. Acid-hydrolyzable carbohydrate was determined by treating an alcohol-extracted sample of grass with 1.0*N* sulfuric acid for 2.5 hours in a boiling water bath and, after neutralization, by measuring the reducing power by a copper method.

The isolation and analysis of hemicellulose involve three general steps: preparation of holocellulose, removal of hemicellulose from the holocellulose, and analysis of hemicellulose for its components.

Preparation of Holocellulose. Under the conditions formerly used for the preparation of hemicellulose, filtrations were extremely slow, particularly with samples of immature grass high in protein. The removal of most of the protein by pepsin made filtration and washing after delignification much more rapid, and the holocellulose when prepared contained less protein and less lignin. Treatment with ammonium oxalate to remove pectin was considered unnecessary, as the yield of holocellulose on a protein-, ash-, and lignin-free basis was essentially the same whether ammonium oxalate was used or not. The conditions of delignification were essentially those of Binger, Sullivan, and Jensen (*5*), who modified slightly those of Whistler, Bachrach, and Bowman (*16*).

The method for the preparation of holocellulose follows:

A 25-gram batch of grass was extracted for 30 hours with benzene-alcohol (2.5 to 1 v./v.) in a Soxhlet apparatus. The residue, after air drying, was digested with 250 ml. of 1% pepsin in 0.1*N* hydrochloric acid for 30 hours at 38° C., the mixture was filtered, and the residue was washed on a sintered filter with hot water. The residue was then treated a second time with pepsin and washed again. To the wet residue in a 1-liter beaker were added, in order, 625 ml. of water, 2 ml. of glacial acetic acid, and 7.5 grams of technical sodium chlorite. The mixture was stirred, covered with a watch glass, and heated in a bath at 85° C. under a well-ventilated hood. Three more additions of the same quantities of acetic acid and chlorite were made at 15-minute intervals. The mixture was stirred frequently to prevent frothing, and water was added to replace that lost by evaporation. After the fourth 15-minute digestion (total time 1 hour), the mixture was cooled to 10° C. and filtered. The residue was washed six times with ice water—i.e., it was stirred on the filter each time with ice water with the suction off. The residue, holocellulose, was then washed with alcohol and ether and dried.

Preparation of Hemicelluloses. Treatment of the holocellulose with hot water and then with cold dilute alkali removed over 80% of the hemicellulose. The water extracts were acid, pH 3.5 to 4.0, and gave no precipitate when neutralized. The alkaline extracts gave only insignificant quantities of precipitates when neutralized. Both alcohol-insoluble and alcohol-soluble hemicelluloses were obtained from the water extracts and also from the alkaline extracts.

The procedure for the preparation of hemicellulose follows:

A 30-gram sample or less of holocellulose was heated with constant stirring for 24 hours in 900 ml. of water in a boiling water bath, filtered, and the residue was washed with hot water. The filtrate and washings constituted extract 1. A second extraction under the same conditions gave extract 2, and a third and a fourth extraction combined gave extract 3 + 4. The residue was extracted with 0.5% potassium hydroxide at room temperature for 24 hours with constant stirring, and, after filtration, the process was repeated with 1.5% potassium hydroxide. Each of the five extracts was neutralized to pH 7.0, concentrated to a small volume, dialyzed, again concentrated, and finally poured into 10 volumes of alcohol. The precipitated hemicelluloses were collected by centrifuging, washed with alcohol and ether, and dried. The five alcoholic supernatant liquids were combined and evaporated to dryness to yield an alcohol-soluble hemicellulose. Preliminary work had shown that most of this came from the water-extractable portion.

Table I. Chemical Composition of Source Material, Brome Grass, on Oven-Dry Basis

Plant Part	Stage of Maturity	Protein, %	Lignin, %	Ash, %	Cellulose, %
Leaves	Boot	18.3	6.2	8.7	26.4
	Flowering	12.5	6.9	8.4	28.4
Stems	Boot	7.2	6.7	6.5	34.3
	Flowering	2.8	11.7	4.2	39.5
Roots	Boot	4.6	16.2	10.9	32.4
	Flowering	4.0	15.6	9.4	30.4
Heads	Flowering	13.1	11.4	4.7	25.2
Leaves	Aftermath	13.0	6.7	9.1	29.6

Table II. Yields of Holocellulose and Cellulose per 100 Grams of Grass

Plant Part	Stage of Maturity	Yield Holocellulose, G. From 100 G. brome grass	Yield Holocellulose, G. Corrected[a]	Yield Cellulose in Holocellulose, G.	Cellulose in Holocellulose, %	Yield Cellulosic Residue, G.
Leaves	Boot	44.0	40.6	24.3	59.8	26.1
	Flowering	49.1	45.6	27.0	59.2	28.0
Stems	Boot	59.6	57.5	33.0	57.4	35.0
	Flowering	70.5	68.2	38.9	57.0	40.2
Roots	Boot	68.7	59.6	30.6	51.3	34.6
	Flowering	61.0	55.3	29.3	53.0	31.2
Heads	Flowering	53.2	49.3	24.6	49.9	26.3
Leaves	Aftermath	49.2	45.2	28.1	62.2	28.8

[a] On protein-, ash-, and lignin-free basis.

Table III. Yields of Crude Hemicellulose from Water and Alkali Extracts per 100 Grams of Grass

Plant Part	Stage of Maturity	Water Extracts, G. 1	Water Extracts, G. 2	Water Extracts, G. 3 + 4	Alkali Extracts, G. 0.5%	Alkali Extracts, G. 1.5%	Alcohol-Soluble, G.
Leaves	Boot	6.8	2.4	0.9	0.8	0.4	1.0
	Flowering	8.8	2.2	0.8	0.7	0.4	1.5
Stems	Boot	7.7	4.4	1.3	0.7	0.5	1.9
	Flowering	11.6	4.1	1.1	0.6	0.7	2.6
Roots	Boot	11.5	4.1	1.3	1.3	0.7	2.5
	Flowering	10.1	1.8	1.0	0.9	0.7	1.1
Heads	Flowering	10.3	1.4	0.6	0.7	0.5	0.8
Leaves	Aftermath	6.5	2.0	0.8	0.8	0.5	0.7

Table IV. Yields of Hemicellulose Estimated by Different Methods, per 100 Grams of Grass

Plant Part	Stage of Growth	Calcd. Hemicellulose, G.	Acid Hydrolyzable In grass[a], g.	Acid Hydrolyzable In holocellulose[a], g.	Sum of Hemicellulose Crude preparations, g.	Sum of Hemicellulose Components[b], g.	Acid Hydrolyzable Remaining in Cellulosic Residue[a], G.
Leaves	Boot	14.2	12.0	10.4	12.3	8.1	2.8
	Flowering	17.2	14.1	12.1	14.4	9.2	3.4
Stems	Boot	23.2	16.7	14.3	16.4	10.3	4.3
	Flowering	28.7	16.3	16.3	20.7	12.3	4.7
Roots	Boot	27.2	19.5	15.1	21.4	13.0	3.8
	Flowering	24.9	18.9	16.2	15.5	9.4	3.3
Heads	Flowering	24.1	20.3	16.9	14.4	10.2	3.2
Leaves	Aftermath	15.6	15.0	11.5	11.4	7.5	3.4

[a] Reducing power as glucose × 0.9.
[b] From Table V.

Analyses of Hemicelluloses. The procedure for the analysis of the hemicelluloses for their components was as follows:

About 100 mg. of hemicellulose was hydrolyzed with 3% sulfuric acid for 12 hours at the temperature of boiling water. After neutralization with barium carbonate and concentration, the solution was diluted to 5 ml. with dilute alcohol so that the final alcohol concentration was about 10%. Identification of the sugars was made by descending paper chromatography with the solvent mixture of Whistler and Kirby (*17*) and the developing spray of Partridge (*14*). This aniline-hydrogen-phthalate spray is not satisfactory for quantitative purposes because it destroys the reducing power of the sugars. For quantitative measurement, separation was carried out on Whatman No. 1 paper, 7.5 × 22.5 inches, by a single run of 48 hours with ethyl acetate–pyridine–water (8 to 2 to 1 v./v.), according to Whistler and Kirby (*17*). The sheets after drying were sprayed on both sides with a solution of 40 mg. of bromocresol purple, 100 mg. of boric acid, and 7.5 ml. of aqueous 1% borax, all in 100 ml. of methanol, according to the procedure of Gardner (*12*). The sheets when dried at 105° C. for 1 to 2 minutes became yellow but on cooling became blue, leaving yellow spots where the sugars were present against a blue background. A spot containing as little as 10γ of sugar was visible under these conditions. The spots were outlined with a pencil before the colors faded and were cut out, as were blanks from the same sheet. The sugars were eluted with 1 ml. of water as described by Dimler, Schaefer, Wise, and Rist (*8*) and their reducing power was determined according to Nelson (*13*), with final reading in a Coleman, Jr., spectrophotometer at 530 mμ. The indicator was not destructive to the sugars and did not interfere with their determination other than requiring a reading at 530 mμ instead of at 500 mμ as specified by Nelson (*13*). Because of the high proportion of xylose to other sugars, this was better determined with a smaller aliquot on a separate chromatograph.

Analysis for Uronic Acids. A separate chromatograph was made for the qualitative detection of uronic acids as follows:

Following the procedure of Fischer and Dörfel (*11*), the chamber was equilibrated overnight with ethyl acetate–pyridine–water (40 to 11 to 6 v./v.), and separation was made by two runs of 24 hours each—1 hour drying between—with ethyl acetate–pyridine–acetic acid–water (5 to 5 to 1 to 3 v./v.). The papers were dried in air, sprayed according to Timell, Glaudemans, and Currie (*15*) with a solution of 3 grams of *o*-aminobiphenyl and 1.3 ml. of 85% phosphoric acid in 100 ml. of acetic acid. On drying, the uronic acids appeared as orange to purple spots.

Quantitative determination of total uronic acid was made by heating about 1 gram of hemicellulose with 12% hydrochloric acid and measuring the carbon dioxide evolved, following the directions of Whistler, Martin, and Harris (*18*).

Results and Discussion

The composition of the source material appears in Table I.

Holocellulose was prepared from each plant part. The yields of holocellulose on the basis of 100 grams of dry grass appear in the first column of figures in Table II. Each holocellulose contained 0.2 to 1.1% protein, 1.0 to 2.5% lignin, and 2.2 to 10.0% ash. The Crampton-Maynard or "true" cellulose contents (Table II, column 4) were determined on the holocellulose and are lower than those in Table I, which were determined on the grass. Apparently about 4% of the cellulose was removed by the chlorite treatment or so modified that it was dissolved by the nitric-acetic acid treatment in the Crampton-Maynard method.

The percentages of cellulose in corrected hollocellulose are in the fourth column. In the first sample of leaves at the boot stage, for example, 59.8% of the corrected hollocellulose is true cellulose. The remaining 40.2% of the holocellulose, or 16.3% of the grass, is

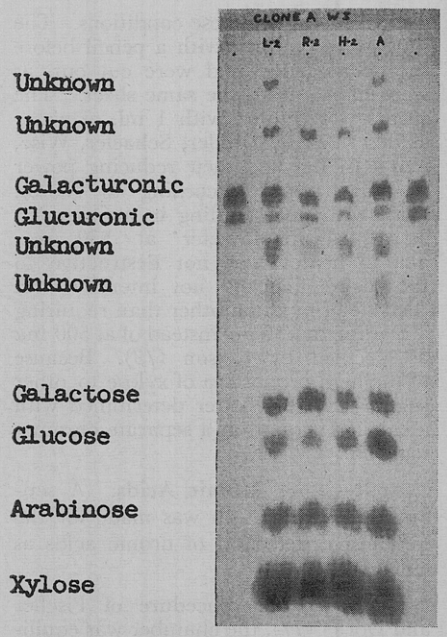

Figure 1. Sample chromatograph. Separation by the Fischer and Dörfel reagent

L-2. Leaves at flowering stage
R-2. Roots at flowering stage
H-2. Heads
A. Aftermath

Table V. Sugar Content of Hemicelluloses, in Per Cent of Hemicellulose Preparations

Extracting Medium	Galactose	Glucose	Arabinose	Xylose	Uronic Anhydride	Galactose	Glucose	Arabinose	Xylose	Uronic Anhydride
	Leaves, Boot Stage					Leaves, Flowering Stage[1]				
Water 1	4.6	5.6	9.7	50.0	9.6	4.4	3.5	8.1	49.6	10.4
Water 2	3.6	4.8	5.2	53.0	7.3	3.2	4.0	3.8	55.4	6.8
Water 3 + 4	1.4	11.9	1.8	47.0	*[a]	1.0	10.5	1.4	50.8	*
0.5% KOH	T[b]	5.9	T	5.3	*	T	6.9	T	7.6	*
1.5% KOH	T	14.3	T	37.9	*	T	11.1	T	53.0	*
	Stems, Boot Stage					Stems, Flowering Stage				
Water 1	3.5	10.0	6.7	46.8	8.4	2.1	5.7	3.8	52.8	8.4
Water 2	2.5	6.7	3.1	53.8	7.1	1.6	4.4	1.0	47.8	8.3
Water 3 + 4	1.0	14.4	0.9	46.1	*	0.8	10.0	0.3	44.3	*
0.5% KOH	T	11.4	T	11.1	*	T	10.8	T	19.2	*
1.5% KOH	T	16.5	T	49.8	*	T	8.7	T	63.5	*
	Roots, Boot Stage					Roots, Flowering Stage				
Water 1	9.0	3.4	10.4	47.7	8.1	9.3	3.0	9.9	44.6	5.8
Water 2	5.6	4.5	5.0	45.5	6.3	5.2	5.7	3.8	42.3	4.9
Water 3 + 4	1.6	10.7	1.2	36.5	*	1.1	12.4	0.9	37.3	*
0.5% KOH	T	5.3	T	7.7	*	T	4.8	T	5.9	*
1.5% KOH	T	10.3	T	45.1	*	T	8.5	T	40.5	*
	Heads					Aftermath				
Water 1	3.5	3.8	6.5	61.4	6.1	5.8	10.4	9.1	44.0	10.2
Water 2	2.1	4.7	2.7	52.9	5.2	4.4	12.4	4.3	46.0	6.6
Water 3 + 4	0.8	11.0	1.1	51.9	*	1.0	17.1	1.4	44.5	*
0.5% KOH	T	3.5	T	4.0	*	T	8.2	T	3.4	*
1.5% KOH	T	8.8	T	61.3	*	T	14.9	T	40.2	*

[a] * not determined.
[b] T, trace or none.

some form of hemicellulose carbohydrate. It was the isolation of this material which was attempted. The residues (Table II, column 5) contained an average of 85% cellulose, and on hydrolysis yielded an average of 11% reducing sugar.

In the leaves and in the stems, the yield of holocellulose increased between the two stages of growth, but in the roots, the yield decreased during the same period. The relative proportion of hemicellulose to cellulose did not change as the plant matured, but differed according to plant part, as there was a greater proportion of hemicellulose in the heads and roots than in the leafy samples. Another clone of brome grass gave slightly lower yields of holocellulose, but the relative differences between parts and between stages of growth were essentially the same.

Hemicelluloses were prepared from each holocellulose. The weights of the hemicellulose preparations are given in Table III, on the basis of 100 grams of grass. More than half the total hemicellulose was found in the first hot-water extract. The fourth hot-water extract yielded negligible quantities, so that the third and fourth extracts were combined. The other clone of brome grass gave similar yields of hemicellulose.

The total quantity of hemicellulose in the grass was estimated by various methods (Table IV). The calculated hemicellulose (first column), was obtained by subtracting cellulose from corrected holocellulose. The validity of these percentages depends upon the stability of both cellulose and hemicellulose during the pepsin and chlorite treatments. The acid-hydrolyzable carbohydrate in the grass was determined from the reducing power after hydrolysis (second column). It is based on total reducing power calculated as glucose although other sugars are present. There is disagreement between these two methods, especially in the results of analysis of root material, but in the other clone, agreement was better. High lignification may possibly prevent complete acid hydrolysis of hemicellulose to reducing sugars. When the acid-hydrolyzable carbohydrate was determined on the holocellulose (third column), it was lower than when determined on the grass, owing to losses during the preparation of the holocellulose.

The sum of the weights of the crude hemicellulose preparations (fourth column) is often less than the preceding values. Some hydrolysis of hemicellulose may have occurred during the hot-water extraction, and portions were lost during dialysis. If this were true, hemicelluloses may not be truly water soluble as the hot-water extractions may have been essentially acid hydrolyses, the acidity being caused by the release of acetic acid absorbed during the chlorite treatment. This loss might have been prevented by buffering the water during extraction. The fifth column gives the sum of the individual sugars and uronic acids which appear in subsequent tables.

The last column contains the acid-hydrolyzable material remaining in the cellulosic residue and which may be added to the figures in the fifth column to give an approximate measure of all the hemicellulose recovered as sugar. The hemicelluloses, taking any method of calculation, increased from the boot to the flowering stage in the leaves and stems but decreased during the same interval in the roots. The order of increase or decrease was the same for the hemicellulose as for the cellulose so that the relative proportions persisted.

The hemicelluloses, on hydrolysis, gave four sugars—xylose, galactose, arabinose, and glucose—which separated easily on the chromatograms and were readily identified. A typical chromatogram appears in Figure 1. The identification of the slower moving uronic acids was more difficult as there were four major uronic acid spots and several minor ones. Two of the major spots were identified as galacturonic and glucuronic acids by comparison with known acids. The R_G values—the fraction of the distance traveled by glucose—of these were 0.33 and 0.38, respectively, as compared with 0.32 and 0.42 given by Fischer and Dörfel (*11*). The presence of both these acids is unusual as most hemicelluloses contain only the latter. Other relatively heavy spots at R_G 0.48 and 0.60 were unidentified, but each when eluted, hydrolyzed further, and chromatographed, gave evidence of xylose. Two slowly

Table VI. Average Sugar Content of Hemicellulose Preparations, as Per Cent of Total Sugars Excluding Uronic Acids

Extracting Medium	Stage of Growth	Galactose	Glucose	Arobinose	Xylose
			LEAVES		
Water	Boot	5.9	8.7	12	74
	Flowering	5.9	6.3	11	77
Alkali	Boot	T	34	T	66
	Flowering	T	25	T	75
			STEMS		
Water	Boot	4.6	14	7.4	74
	Flowering	3.2	9.0	4.6	83
Alkali	Boot	T	33	T	67
	Flowering	T	19	T	81
			ROOTS		
Water	Boot	11	6.5	13	70
	Flowering	13	6.5	13	68
Alkali	Boot	T	25	T	75
	Flowering	T	23	T	77
			HEADS		
Water		4.4	5.9	7.8	82
Alkali		T	17	T	83
			AFTERMATH		
Water		7.4	17	11	65
Alkali		T	38	T	62

moving spots occurred, one at R_G 0.17 in all samples and another at R_G 0.07 in all except the roots. The unidentified spots may be either aldobiuronic acids or methylated uronic acids.

The quantitative yields of the sugars and uronic acids, in per cent of the hemicellulose preparations, are given in Table V, the sugars being determined by their individual reducing power, but the uronic acids, in toto, by decarboxylation. Xylose makes up about three fourths of the total sugar found in most preparations. The other three sugars occur in all water-extractable hemicelluloses, in measurable quantities but in varying relative proportions from sample to sample. The alkali-extractable hemicelluloses contain only glucose and xylose in measurable amounts; the other sugars and uronic acids occur in quantities too small for accurate determination.

Table V also shows that, proceeding from the first to the last water-extractable hemicellulose, xylose does not show any quantitative change but galactose and arabinose decrease and glucose increases. Uronic acids also decrease. The 0.5*N* potassium hydroxide–extractable hemicellulose contains little total sugar and probably includes some dissolved matter other than hemicellulose, as protein or lignin.

No consistent differences are noted in the composition of hemicelluloses in the same part of the plant between the early stage of growth and the later stage, but some differences can be noted between one part of the plant and another. In the water-extractable hemicellulose, leaves are higher in galactose and arabinose but lower in glucose than the corresponding samples of stems. The aftermath foliage is higher in glucose than foliage of the first cuts. Roots are higher in galactose than leaves but are otherwise like the leaves. The heads are like the leaves. In another clone, only the water-extractable hemicelluloses were analyzed and found to contain a slightly larger proportion of glucose in the leaves and stems at both stages of maturity.

The average sugar contents are summarized in Table VI, with each of the four sugars as per cent of total sugar (excluding uronic acids). Impurities in the preparations may therefore be overlooked and comparisons made between samples. Distinct but small differences occurred in composition of hemicelluloses from different plant parts. Aftermath was unusually higher in glucose and lower in xylose than first-cut foliage. Stems were higher in glucose than other parts on the same sampling date, but roots were distinctly higher in galactose and lower in glucose. More mature stems and leaves had higher xylose contents and lower contents of other sugars, especially glucose. The sugar content of the hemicelluloses of the roots did not change during the period when the tops were maturing.

The hemicelluloses from brome grass, as prepared and analyzed, were probably mixtures, and some, at least, belong to the polyuronide hemicelluloses. One is not justified in assuming the composition of a molecule of hemicellulose on the basis of the quantitative amounts of sugars reported here. For example, the first water extract of leaves at the boot stage contained polyuronide hemicellulose with xylose, arabinose, uronic acid, galactose, and glucose in the relative proportions 10:2:2:1:1. But in the second water extract, the relative proportion of arabinose changed to 1. The third and fourth water extracts showed further changes, and the relative proportions approached those present in the alkaline extracts. The last contained only glucose and xylose, and uronic acids in amounts too small for accurate measurement. The simplicity of composition of the alkali-soluble hemicellulose—assuming that uronic acids are not present—resembled that of xylans commonly associated with cellulose.

This, apparently, is a complex mixture of hemicelluloses of varying solubility. Those more readily dissolved by hot water contain all the sugars and uronic acids, even if no one molecule contains them all. Those removed by longer extraction with water are not so diverse in composition. Those removed by alkali may be xylans and glucosans, but they were dissolved with dilute alkali at room temperature while xylans are usually removed with stronger alkali or more drastic treatment. All the cellulosans, if present, were not removed. The residues remaining after the 1.5% alkali extraction contained—in addition to cellulose—some carbohydrate material, which amounted to 3 to 4% of the original grass and which, on hydrolysis, yielded reducing sugars. The differences in composition between parts of the plant also suggest that the mixtures are not alike; the roots in particular have a sugar pattern different from those of the other organs.

Other workers have reported the composition of hemicelluloses from grasses. Adams (*2*) isolated a hemicellulose from wheat leaf which contained xylose, arabinose, and glucuronic acid in the molecular proportions of 30 to 3 to 1; and one from wheat straw with similar proportions, 32 to 5 to 3 (*7*). Ehrenthal, Montgomery, and Smith (*9*) isolated one from wheat straw containing xylose, arabinose, and glucose, the first in greatest quantity. The hemicelluloses of esparto grass were composed predominantly of xylose (*6*). The results obtained here with brome grass are not at variance with the few contributions that have been made with related plants.

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BIOCHEMISTRY OF MYOGLOBIN

Production and Identification of a Green Pigment Formed during Irradiation of Meat Extracts

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Studies of the conditions under which the maximal discoloration of heme pigments occurs, by ionizing radiations, show a maximal relative production of green pigment with respect to red at pH 5.3. This production could be greatly enhanced by the addition of cysteine or other sulfhydryl reagents and inhibited by the addition of aldehydes. This evidence, plus the reactions it undergoes with dithionite and carbon monoxide identifies the green heme pigment as sulfmyoglobin. Evidence is presented that the sulfmyoglobin complex breaks down under either mild oxidizing or reducing conditions to form either metmyoglobin or myoglobin. The protein moiety of the green pigment is partially denatured, and the production of the green pigment is postulated to be one of the steps in the irradiation destruction of muscle color.

THE PRINCIPAL FORM of the heme pigments, myoglobin and hemoglobin, after irradiation is a bright red compound, which is spectrally similar to the oxygenated form of the pigments (*3*, *15*). This pigment is relatively stable and imparts a very satisfactory color to the irradiated meat in which it predominates. However, under certain conditions other pigments occur, principally the met or ferric form of the heme compounds. In this oxidation state, the heme pigments are brown, with absorption bands at 507 and 635 for myoglobin and 500 and 630 for hemoglobin. This condition imparts a brownish or sometimes greenish cast to the meat. The met form is an important component of the heme pigments in irradiated tuna and pork (*15*) and was observed by Hannan (*5*) and Ginger, Lewis, and Schweigert (*3*) in irradiated meats in which the oxygenated form predominated before irradiation.

While these two pigments are the major contributors to the color of irradiated meat, there is yet another detectable pigment produced, characterized by an absorption band in the 615- to 620-mμ region. This green pigment, under some conditions, contributes to the color of the irradiated product, particularly in comminuted meats (*3*). Although the greatest quantities are produced when meats or extracts thereof are irradiated in the presence of air, close examination of the spectra of meat samples irradiated in the absence of air show a slight absorption in the 615-mμ region. The identity of this green pigment has not been established, and it appeared possible that the isolation or identification of the compound could give some insight into the reactions which take place during irradiation, with particular reference to the changes which the heme pigments undergo.

Two chromatographically distinct compounds have been identified previously (*4*) from the acid-acetone cleavage of irradiated meat extract pigments. One had an R_f of about 0.75 and appeared to be protohematin, while the other compound had a somewhat lower R_f and fluoresced in the red portion of the spectrum. The fluorescence marks the pigment as an iron-free porphyrin, but whether it was produced as a result of gamma irradiation or was produced from hematin by the isolation procedure was not established.

The irradiation of beef muscle extracts resulted in color changes similar to