Seasonal changes in lignin and cellulose content of Montana range grasses
by Leonard F Gieseker, Jr

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of Master of Science in Chemistry
Montana State University
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Abstract:
Lignin in Gallatin Valley grasses increased with advancing season, beginning at about five per cent in May, and rising to a high of eighteen per cent for Bromus inermis at the end of August, contrasted with fourteen per cent for Bromus marginatus. The wheatgrasses ranged in between, with Agro-pyron pauciflorum remaining lower in lignin than crested wheatgrass during the first half of the season. Cellulose content closely paralleled lignin content. Grasses at Judith Basin followed a similar course, but matured about a month earlier.

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Approved:

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ABSTRACT

Lignin in Gallatin Valley grasses increased with advancing season, beginning at about five per cent in May, and rising to a high of eighteen per cent for Bromus inermis at the end of August, contrasted with fourteen per cent for Bromus marginatus. The wheatgrasses ranged in between, with Agropyron pauciflorum remaining lower in lignin than crested wheatgrass during the first half of the season. Cellulose content closely paralleled lignin content. Grasses at Judith Basin followed a similar course, but matured about a month earlier.

# INTRODUCTION

Grasses are without doubt the most important plants for man's welfare of all plants grown in the world, because he lives chiefly upon grasses and the animals who depend on them for food. Range land is vastly important for the production of these animals, and in the United States alone it represents approximately forty per cent of the country's total area. Without grasslands, man would have to restrict his home and activities to an amazingly small portion of the land he now inhabits on this earth, and human civilization would be similarly limited.

Wheat and corn are only two of the many grasses that are of great importance in the United States. However, without even these two our entire pattern of life would have to be changed, because they play enormously important parts in our present-day life.

Montana is composed primarily of range land, as the amount under cultivation is insignificant as compared with the total acreage used for grazing purposes. If the people of Montana are to prosper, it is necessary for them to have at their disposal all possible information regarding Montana range grasses, since these grasses are of vital importance to them.

One way of obtaining a greater understanding of grasses is by a chemical study. By determining the various chemical constituents of grasses it is possible to probe into their inner structures, and many times variations there are apparent which are not visible to the eye. More particularly, an analysis of their lignin and cellulose contents will offer specific information regarding their nutritive value. From a nutritional point of view a

knowledge of the seasonal changes of the lignin and cellulose contents of Montana range grasses should be a definite aid, as with this knowledge it is possible to compare the shifting seasonal feed values of the grasses studied. Such a comparison should naturally lead to a wiser utilization of Montana range land, and it is hoped that this will eventually be the case.

# REVIEW OF LITERATURE

## A. History of Feed Analyses

Shortly after the beginning of the nineteenth century Davy 23/11/ valued feeding stuffs according to the amount of extract that was removed by digestion with hot water. In 1809 Thaer 91/ introduced his system of hay equivalents, using meadow hay as the standard. He estimated that 90 pounds of dry hay, or clover, or vetch, or alfalfa, or sainfoin, or 200 pounds of potatoes, or 266 pounds of carrots, or 350 pounds of rutabagas with tops, or 460 pounds of beets with tops, or 525 pounds of radishes or 600 pounds of white cabbage were equal to 100 pounds of meadow hay in feeding value. These hay values were an advance over the older methods of valuation, but the failure to consider the protein fraction of the feed led to discordant results. Other observers obtained different hay values, and so a mass of contradictory tables began to accumulate in the literature. Boussingault, 9/ in 1844, proposed the valuation of cattle feeds upon the basis of their nitrogen content as compared with ordinary hay of 1.34% nitrogen content.

It was the great service of Henneberg to break away entirely from the old hay equivalent conception and to base the valuation of feeding stuffs on the percentage of the three major nutritive constituents: proteins, fats and carbohydrates. Crude fiber, assumed to be indigestible, was determined by a modification of the old Einhof procedure, which consisted of removing the more easily-hydrolyzed cellulose constituents with successive portions of boiling dilute acid and alkali. The sum of the percentages of moisture,

crude fat, crude protein (N x 6.25), ash and crude fiber subtracted from 100 gave the percentage of nitrogen-free extract. The crux of the whole N-free extract problem is in the nature of the substances removed by the boiling acid and boiling alkali treatment in the determination of crude fiber. Questions of terminology bothered Henneberg, 45/ as he stated that the substance called wood fiber is evidently not always the same substance, since the product obtained from the same feeding stuff varies with different processes of mechanical comminution. For this reason he considered it desirable to give the residue obtained by the treatment with acid and alkali a less definite designation than wood fiber (i.e., cellulose). This is especially necessary since the residues obtained from different substances by exactly the same process have a very different composition (clover hay residue, for example, is quite different from that of wheat straw or from that of the feces of an animal fed clover hay, etc.), and are essentially unlike the pure wood fiber or cellulose of the chemist. He felt it necessary, therefore, to propose for the residue in question the name "crude fiber." For a similar reason the names "N-free extract" and "crude protein" were devised.

The old Henneberg method of feed analysis has been used since 1866 with very few changes. It is still the standard method of feed analysis. The Henneberg system of analysis is as follows:

1. Crude fat (ether extract).
2. Crude fiber (extractions, dilute alkali and acids).
3. Ash.

4. Protein (N x 6.25).

5. Nitrogen-free extract (by difference).

After a study of the above system of analysis one will find that not one of the above existing so-called fractions is a pure compound, but each is a mixture.

## 1. Ether Extract 13/

True fats (triglycerides) and fatty acids which are utilized by animals as sources of energy and of the essential fatty acids.

Phospholipids, sterols, waxes and resins, and plant pigments. This group may make up as high as forty per cent of the ether extract, and with the exception of phospholipids and carotene, appear to be of little use to the animal.

Fat-soluble vitamins A (carotene), D, E and K. Essential dietary constituents.

## 2. Crude Protein (N x 6.25)

The Kjeldahl method for determining crude protein in feeds is really only a method for determining total nitrogen. Since many purified proteins have been found to contain 16% nitrogen, 100 divided by this figure gives 6.25, which is the customary factor used for expressing results as crude protein. The requirements of an animal are for certain amino acids which are constituents of proteins. Of more than thirty amino acids which have been identified so far, approximately ten have been shown by Rose 60/ to be essential for normal growth and maintenance of rats. That this requirement

of the rat cannot be taken too literally for other species of animals was recently shown by Almquist, who found that glycine is an essential amino acid for chicks. 2/ Previous to this time glycine has always been considered to be a non-essential amino acid.

Regarding ruminants (the type of animals with which we are concerned), there is a serious question as to whether the amino acid content of the feed (in other words, the quality of the protein) is of very great significance. This is because these herbivorous animals depend largely for their nutrients upon the action of microorganisms which not only break proteins down into amino acids, but are also able to synthesize essential amino acids which are then used by the animal. An example of this is the utilization of urea for cattle. Urea is a simple organic nitrogen compound which is not known to have any food value itself, but which can be used by microorganisms in the rumen in synthesizing essential amino acids. For this reason it seems probable that an extensive survey of the amino acid content of feeds for ruminants would be of dubious value, and the total nitrogen determination is probably satisfactory for most purposes.

### 3. Ash

This includes all mineral elements except that portion which escapes during ignition, particularly sulfur, chlorine and iodine.

The importance of mineral elements in the nutrition of animals has been known for a long time. However, until recent years the study of minerals in this relation has been limited to a few of the major elements, specifically calcium, phosphorus, sodium, potassium, magnesium and chlorine. These are

"major" elements only in the sense that they are required in relatively large quantities by the animal. Of no less importance are the so-called "minor" elements, which are required in smaller quantities but are none the less essential. Iodine, for instance, needs be present in the plant only in quantities measured in parts per billion, and yet a deficiency of iodine in plant material used as feed causes profound changes in the animals thus deprived of this element.

Specific knowledge of the nutritional significance of minerals is due primarily to research within the last quarter of a century. It is now known that in addition to the "major" elements mentioned above, iodine, iron, sulfur, manganese, copper, zinc and cobalt are essential elements in nutrition. It is probable that further work will add more minerals to the list, possibly including silicon, fluorine, boron, bromine, aluminum, arsenic or nickel. All of these are found regularly in the animal body.

### 4. Crude Fiber
### (Extraction with dilute acid and alkali)

Cellulose 6/ is a major constitudent of the crude fiber fraction. The cellulose in plant cell walls is practically indigestible by carnivora and digestible to a widely variable extent by omnivora and herbivora. Cellulose utilization by any animal is dependent on intermediary action by bacteria of the digestive tract for its degradation to glucose. Cellulose is an important source of energy to herbivorous animals.

Only part of the lignin is found in the crude fiber fraction. The remainder is found in the nitrogen-free extract. Lignin is indigestible it-

self and also inhibits the digestion of cellulose. It was originally assumed that the crude fiber fraction was indigestible and the nitrogen-free extract digestible.

One can see from the above that this is decidely not the case. Also, the fact that the crude fiber contains variable amounts of lignin makes any assumptions drawn from the crude fiber content of plant materials of little value.

### 5. Nitrogen-free Extract (Obtained by difference)

The fact that this fraction is obtained by difference is only one of the objections to its use. It is customarily assumed that nitrogen-free extract is a measure of the digestible energy-supplying material of feed. The nitrogen-free extract contains most of the lignin, which is approximately 98 per cent indigestible by animals. To make matters even worse, a high lignin content 11/74/ may prevent the digestion of the easily-digestible portions of the plants by incrusting them and thus keeping them from coming into contact with intestinal bacteria and digestive juices. Thus it is possible for them to pass through and remain intact in the feces of the animal.

The nitrogen-free extract also contains hemicellulose, starches and sugars which are more or less easily digested and are energy-supplying materials.

The hemicelluloses 74/ are widely distributed in the vegetable kingdom and next to cellulose are undoubtedly the most abundant of the materials of plant origin. Hemicellulose may be defined as carbohydrate substances that,

in their natural state, are insoluble in boiling water but are soluble in dilute aqueous solutions of alkalis. Upon heating them with dilute acids at atmospheric pressure, hemicelluloses afford simple sugars and frequently also uronic acids. In connection with certain types of hemicellulose two terms have recently come into use; namely, "polyuronides" and "cellulosans." The term "polyuronides" is formed by the conjugation of certain sugar acids (glucuronic and galacturonic acids) with sugars. While most of the hemicelluloses are polyuronides, it must be emphasized that not all polyuronides are hemicelluloses, for pectins, gums and mucilages also contain uronic acids.

The term "cellulosans" refers to those hemicelluloses that are usually free of uronic acid and are intimately associated with cellulose.

An excellent review of the present knowledge of hemicelluloses is found in Norman's book, Biochemistry of Cellulose, Polyuronides, Lignin etc.

From the above discussion of the Henneberg system of feed analysis one would assume that some other method of analysis would be much better. This is indeed the case. A complete analysis of feeds in which one would analyze for the various carbohydrates (for example, pentosans, starches, hemicellulose, etc.) would be very long and tedious and could only be performed in a few cases. It would also be impracticable where a large number of analyses were needed. Taking this into consideration, a system of analysis which overcomes the faults of the crude fiber method and yet is simple enough for routine use has been presented by Norman. He suggested the following procedure:

1. Ether extract.
2. Crude protein (N x 6.25).
3. Cellulose.
4. Lignin.
5. Ash.
6. Easily-digested carbohydrates (by difference).

This system discards a fractionation which is wholly arbitrary and without biological significance in favor of a separation of the feed into groups which can be truly evaluated from a nutritive standpoint.

The determination of plant cellulose, for instance, offers the advantage that it is more specific than the crude fiber fraction. It is known that cellulose per se is almost completely digestible in the ruminant.

The determination of lignin is a definite step forward, since lignin itself is at least 98% indigestible and also definitely retards the digestibility of other constituents.

## B. Lignin

The function of lignin in the plant may be to give strength and rigidity to the cell wall. Unlignified material usually is soft and contains a high percentage of water. It also breaks much more easily than lignified material. On the other hand, a high percentage of lignin will often make the woody material brittle. Lignin is extremely resistant to decay and bacterial action. Wood buried at least 500,000 years has been found and on analysis much of the lignin was in an apparently unaltered state.

The lignin in animal metabolism is very significant, since it is extremely resistant to digestion by enzymes. When it is fed to dogs 21/ there is a partial breakdown of the lignin, as evidenced by an increase in the amount of benzoic acid eliminated as hippuric acid and also a loss in methoxyl content of the lignin from the feces. It is postulated that such breakdown of lignin as does occur is probably not due to bacterial action but that it takes place in the stomach, possibly through some enzyme present in the gastric juice. In the human body there is no evidence at present that any of the lignin ingested can be utilized.

Lignin is the incrusting part of plant and wood tissue which is characterized by its phenolic characteristics and by its methoxyl content, and is that portion of plant tissue recovered by the 72 per cent sulfuric acid or fuming hydrochloric acid method for determination of lignin. Recent work 22/59/61/ has further subdivided the incrusting or fiber-bonding materials in wood into two fractions, the lignin-bonding material and the non-lignin-bonding material. This is of great interest, since the more general definition of lignin must be revised to take this into consideration. Since no clear-cut definition of lignin has been offered, this attempt of further subdivision of plant-incrusting materials may lead to confusion in terminology. Experimental work has indicated fairly definitely that both lignin and another material are concerned in the bonding together of wood fibers. The non-lignin bonding material has been examined in holocellulose sections of wood; it has been found that treatment with dilute solutions of sodium hydroxide will remove it and so permit maceration. The lignin is removed by

chlorination of the wood samples and washing with water. Both the lignin and non-lignin bonding material must be removed before fibers will separate; the removal of either is not sufficient to cause such separation. The nature of this non-lignin bonding material has not been definitely worked out but may consist of hemicellulose consisting of xylan, uronic acid and probably glucosan, together with other components whose exact nature is not known.

Schulze 84/ is generally credited with the introduction of the term "lignin" into plant chemistry, 73/ as it was early recognized that there was present in plants a woody "lignified" portion.

By treating wood with nitric acid and potassium hydroxide Payne 70/ obtained a more or less pure cellulose and pointed out that some substance had been removed which was richer in carbon than cellulose. This substance has been named lignin.

Lignin 69/ is a substance or mixture of substances which composes the more resistant or woody portions of plant tissue. It should be pointed out clearly that lignin is a vague, crude, general term and covers the woody portion of plants much as the word "protein" covers the living nitrogenous portion of living plants. The chemistry of lignin today is in the state that the chemistry of proteins was in twenty years ago.

Thus, in the determination of lignin when one investigator reports a percentage of lignin in a sample and another, by a different method of analysis, reports a different percentage in the same sample, no one at present can say which one is correct. To overcome this difficulty one chooses a method of analysis of lignin that best suits his needs. In the determina-

tion of lignin in feed one chooses a method that best represents that portion of the plant that is indigestible by animals, as determined by digestion trials. With this in mind a brief discussion of the chemistry of lignin will be presented here.

The lignin present in woody material may be chemically combined with carbohydrates. There are two views held at present as to the linkage of lignin with the carbohydrate material; one is that there is an ester-like 26/100/ union between an acidic group in the lignin and an hydroxyl of the carbohydrate, while the other view 51/38/62/ is that an ether-like linkage exists between lignin and the carbohydrate. Due to the fact that lignin is more difficult to separate in some plants than in others, it is possible that either one or both linkages are present in various plants.

There has been considerable speculation as to the precursors of lignin. Some of the probably precursors are: cellulose, 36/ soluble carbohydrates, pentoses, methyl pentoses,53/54/55/78/79/85/ hexoses and pectin.24/ Some of the early investigators of lignin even went so far as to state that lignin of phenolic character was not present in plants and wood, but formed as a result of the action of chemical reagents on the carbohydrates. This viewpoint has been definitely discarded;67/ however, it has been recognized that various methods for the determination of lignin 64/ may form insoluble condensation products from the carbohydrates of plants. These carbohydrate condensation products seldom amount to as much as 10 per cent of the total lignin determined.

Lignin shows a number of color reactions.76/ Nearly one hundred years

ago, Runge 83/ found that pine oil, when treated with phenol and hydrochloric acid, assumed a greenish-blue coloration, and when treated with aniline-sulfate, a yellow color was produced. Both organic and inorganic reagents have been employed. Among the former, "phloroglucinol reagent" or "Wiesener's reagent," which gives a reddish-violet coloration with lignified material, has been widely used in testing for lignin. Colors in general are produced when lignified tissues are treated with phenols and with amines. The color reactions given by lignified material with aromatic compounds are probably due to an aldehydic substance which is closely associated with the lignin complex. In using color tests for lignin one must realize they may be misleading.35/ A critical survey should precede the use of any color test for lignin to be sure that it is specific for lignin and not due in part to other constituents of the plant.

According to Harris,43/ lignin can be isolated only with the application of hydrolytic reactions. Even the so-called "native lignin" (Klason), the alcohol-soluble lignin obtained from wood without the use of added acid, was found to be a result of the action of hydrolytic enzymes.

The quantitative estimation of lignin is divided into two classes: the direct and the indirect methods.

## Indirect Methods

Schulze 84/ oxidized lignified plant material with nitric acid and potassium chlorate and assumed the loss in weight represented lignin. This method is only of historical interest, as the results obtained are too high;

not only is the lignin completely oxidized, but also the hemicelluloses and perhaps some of the cellulose is also destroyed. Benedikt and Bamberger 6/ determined the number of methoxyl groups in wood samples and computed the percentage of lignin in the sample. This method is based on the assumption that in lignified material lignin is the only substance containing methoxyl groups and the methoxyl content in lignin from various sources is the same. Cross, Bevan and Briggs 20/based their method on the reaction between lignin and phloroglucinol. Due to the combination of phloroglucinol with the furfural-yielding groups, this method gives too high a result. The method of Seidel 86/ is based on the fact that when nitric acid is added to lignin, oxides of nitrogen are liberated, and may be determined by titration with permanganate. The method of Waentig and Gierisch is based on the fact that lignin takes up a considerable quantity of chlorine. By determining the amount of chlorine adsorbed the percentage of lignin is calculated. Mehta 62/takes advantage of the fact that a solution of phosphotungstic and phosphomolybdic acids in phosphoric acid is a very sensitive reagent for detecting minute amounts of aromatic substances containing hydroxyl groups. Eichler 25/ regards fluorescence as a very good method of estimation of lignification, and he regards it as a more sensitive method than color reactions.

## Direct Methods

The direct methods for quantitative estimation of lignin are divided into two classes, those which depend upon dissolving cellulose and other carbohydrates out and leaving the lignin as an insoluble residue, and those

which dissolve out the lignin and thus separate it from the cellulose and other carbohydrate material.

The sulfuric acid method 28/55/68/57/ is most commonly used today. However, there are several objections to it. It is based on the fact that cold 72 per cent sulfuric acid will hydrolyze cellulose. Usually this is preceded by an extraction with water to remove water- and alcohol-soluble portions of the woody material. If one adds a formaldehyde solution to the 72 per cent sulfuric acid, the time required for the hydrolysis of the carbohydrates is considerably reduced.82/ The objections to this method are the possibility of incomplete hydrolysis of the carbohydrates, the formation of condensation products of the carbohydrates and contaminating the lignin residue with nitrogenous complexes.

Norman and Jenkins 65/ point out that fructose, xylose, arabinose, and polysaccharides give a solid residue on standing in 72 per cent sulfuric acid and, therefore, interfere with the lignin determination by this method. The time of treatment with 72 per cent sulfuric acid can be regulated so that this error can be reduced to the point that it is not a serious factor.

The fuming hydrochloric acid method 97/81/8/ method is similar to the 72 per cent sulfuric acid method except that fuming hydrochloric acid is used. This method is subject to two errors:75/ one, incomplete hydrolysis of proteins and two, formation of reversion products of humus-like material when the hydrolyzed product is diluted with water before filtration.

The lignin in a liquefied material can be removed by heating with alkali under pressure, and acidifying.62/ This method gives values that are too

low owing to the partial degradation of the lignin by sodium hydroxide solution heated to a comparatively high temperature.

For industrial purposes delignification is brought about by heating wood with acid sulfites under pressure, producing soluble lignin sulfonic acid, which is sold commercially as the basic calcium salt.

Whatever method is used, the finely-ground plant material must first be freed from various "extractives" such as fatty substances, resins and volatile oils. Ether and ethanol, or acetone and ethanol, or a 1:2 alcohol-benzene solution are used for this extraction. This may be followed by an extraction with hot or cold water. In some cases a preliminary treatment with 5 per cent sodium hydroxide is suggested to remove the gummy substances, but in using this there is always the danger of losing some of the lignin. It is not necessary in the determination of lignin in grasses.

There is as yet no method by which lignin can be isolated unchanged. As a result of acid treatment,58/ lignin may polymerize, resinify, condense, or carbonize.

Whatever method is employed, a lignin preparation is obtained which is no longer identical with the natural lignin. Also, it should be pointed out that the lignin obtained by different methods of analysis may not be similar in composition. Hilpert 48/ points out that when one considers the lignin isolated by the two different methods to be identical, one is overstepping the limits of tolerance in composition further than is customary in chemistry. Even should two lignins agree in the methoxyl content, one can still not draw conclusions as to identity in composition.

Lignin can be isolated from wood by heating with alcohol and hydrochloric acid.39/33/42/48/ In place of ethanol other hydroxyl compounds can be used, such as butyl and amyl alcohols, ethylene glycol, the monomethyl ether of ethylene glycol, and glycerol α-monochlorohydrin. In all cases a product is obtained containing the alkyl or aryl group in combination with the lignin. It has been suggested that a product in the nature of an acetal is formed, but this has not been definitely proved.

Lignin can also be extracted from wood with phenols.12/52/95/ These combine chemically with the lignin, yielding so-called "phenol lignin." The reaction product is filtered, and the filtrate steam distilled to remove the excess phenol. The lignin remains in the distilling flask.

Lignified material such as wood and straw, when heated several hours with acetyl bromide, dissolves completely. When this solution is poured in ice water, a precipitate of bromo lignin separates out.

A rather pure lignin material has been prepared by extracting with diazotized sulfanilic acid in the presence of sodium hydroxide solution. The dissolved lignin was precipitated with β-naphthylamine hydrochloride or with p-bromoaniline hydrochloride. The objectionable feature of this method is the fact that part of the cellulose is also dissolved by the diazobenzenesulfonic acid treatment.

The constituent groups of lignin have been carefully studied by many investigators.41/72/71/30/31/32/ The presence of methoxyl groups ($-OCH_3$) has been definitely established. Since the methoxyl groups are not readily split out, they are probably attached as an ether linkage rather than an

ester linkage. Also, it is thought that the methoxyl groups are attached to aromatic nuclei. The rate of removal of methoxyl groups in lignin with hydriodic acid was found to correspond closely to that of vanillin.

No definite content of methoxyl groups in lignin has been ascertained, as it varies with the source of the lignified material and also with the method employed for the isolation of the lignin.

The presence of hydroxyl groups in lignin is indicated by the fact that it can be acetylated and alkylated. Alcoholic groups, as well as phenolic groups are present.

Wood and other lignified materials, when distilled with dilute mineral acids, give formic and acetic acids. This indicates that lignin may contain acetyl and formyl groups, although their presence in lignin has not been definitely established.

There is also evidence that carbonyl groups are present in lignin. As lignin preparations reduce Fehling's solution, aldehydic groups may be present. But it must be remembered that other substances besides aldehydes reduce Fehling's solution. Indefinite condensation products are obtained with phenylhydrazine and semi-carbazide. It may be that the formaldehyde arises from the methylene dioxide group ($O-CH_2-O$) present in the lignin molecule, as compounds containing this group are known to yield formaldehyde when they are distilled with hydrochloric acid.

The formation of rather unstable lignosulfonic acids by the interaction of lignified material with sulfurous acid and bisulfites may be due to an ethylenic bond 55/ in lignin.

Data on the halogenation of lignin 29/ are rather inconclusive as sub-

stitution as well as addition of bromine no doubt takes place.

Lignin can be acetylated 47/ with acetic anhydrides and pyridine or acetyl chloride. It appears, however, that isolated lignin must be so altered by the hydrochloric acid separation method that its activity toward acetylation is diminished. A benzoyl derivative of alkali lignin has been prepared by treating the lignin with benzoyl chloride and pyridine. Results of the analysis of the benzolated product for carbon and hydrogen indicate the presence of four benzoyl groups.

Lignin can be readily alkylated 88/86/34/ by the usual alkylating reagents. One method is to suspend lignin in 10 per cent sodium hydroxide solution and add dimethyl sulfate, keeping the temperature at 60° C. The methylated lignin in some cases exhibits double refraction.

By chlorination Cross and Bevan 18/ obtained a substance which they represented by the formula $C_{19}H_{18}Cl_4O_9$. This substance dissolves in alkaline solutions and in glacial acetic acid and alcohol. By a drastic chlorination using antimony pentachloride containing a small amount of iodine, perchloroethane and hexachlorobenzene were obtained. Lignin will also absorb bromine, which indicates it may have one or several ethylenic bonds. Also, when lignin is brominated, a loss of methoxyl takes place to the extent of 30 per cent of the original methoxyl content. Since all the methoxyl groups could not be removed by the bromination, it is probably that all the methoxyl groups in lignin are not similarly combined.

Lignin can be nitrated 89/ very easily. An amorphous nitro substance can be obtained by nitrating lignin with 5 N nitric acid. This indicates

that lignin has a phenolic structure.

When wood and similar lignified materials are heated with a solution of sulfurous acid and acid sulfites, as in the sulfite process for pulping wood, the lignin goes into solution, leaving the cellulose in more or less pure state. The solution containing the lignin (or waste sulfite lignin from pulp mills) has been the subject of numerous investigations. There is at present a disagreement among investigators as to the mechanism of the reaction between lignin and sulfurous acid and bisulfites.

When lignin is subjected to even mild oxidation, complete disruption of the molecule takes place and simple degradation products are obtained. Among the oxidation products 78/92/40/56/57/3/ found were formic and oxalic acid; also succinic acid, malonic acids, and adipic acid. In some cases of oxidation small quantities of the aromatic acids have been obtained, such as benzoic, phthalic, isophthalic, trimellitic, hemimellitic, prehnitic, pyromellitic, benzene pentacarboxylic, and mellitic acids. A recent patent indicates that vanillin is a result of oxidation of lignin under certain conditions.

No definite compounds have been isolated by the reduction of lignin which are of much aid in determining the structure of lignin.

Lignin has been demethoxylated by heating with dilute acids under pressure. There was evidence also that the product here had undergone considerable internal change, as many of its reactions had changed. Lignin isolated from corn cobs has been shown to be entirely free from furfural-yielding substances. Hydrolysis of lignin with hydrochloric acid gives no reducing su-

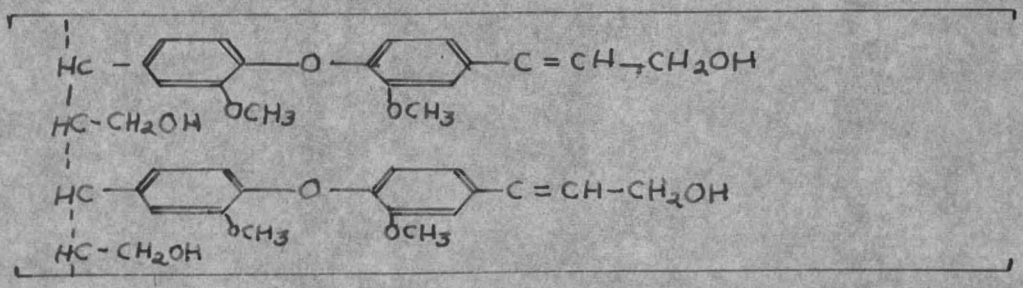
gar but the formation of a hydrazone melting at 155° C. indicates the presence of arabinose.

The fusin of lignin 27/ with alkali yields in nearly all cases protocatechuic acid, catechol, oxalic and other simple aliphatic acids. Vanillic acid has also been reported as one of the degradation products. In addition, there is always formed a dark, amorphous, humin-like degradation product called lignic acid.

## The Structure of Lignin

It is impossible to give a structural formula for lignin, since lignin has never been isolated in the pure state. The lignin obtained by one method of separation differs from the lignin obtained by any other method. Nearly all recent workers agree that coniferyl alcohol or coniferyl aldehyde or some closely-related compound is the essential grouping in the lignin. Of the many formulas presented for lignin, only two will be presented here.

Kurschner's formula for lignin:




Kurschner suggests 58/ that lignin is a polymer of the glycoside coniferin. However, since no n-hexyliodide was isolated from the reduction products, it seems unlikely Kurschner's formula will hold.

Freudenberg's lignin:

He suggests twelve molecules of caffeic alcohol condense with themselves to form primary lignin having the formula $C_{120}H_{144}O_{37}$ with the structure indicated.32/ Freudenberg notes that in this formula the linkages are depicted as taking place in the para position, although a part of the units may be linked in the meta position. Phillips 72/ suggests that the fundamental units are guaiacol and n-propyl-guaiacol, where the hydrogen in the n-propyl side chain is substituted by alcohol hydroxyl groups.

## Cellulose

### Cellulose Discussion

In the case of most plant materials in which cellulose plays a structural role, the cellulose does not occur in a pure or easily-purified condition, but is found in the most intimate association with other cell-wall constituents. Separation and isolation is a matter of considerable difficulty. This led in the past to the idea of compound celluloses, as lignocellulose, pectocellulose, cutocellulose, etc. However, the modern concept of the steric structure of the cellulose molecule rules out the possibility of cellulose acting as a structural constituent while in chemical combination with any other large molecule. Cellulose derivatives, when they do exist, appear to lose the physical properties and structural strength which characterize cellulose in its skeletal role, and assume the physical properties of a mucilage. Hence the theory that compound celluloses occur in the plant skeleton is untenable.

No single treatment is known which, without attacking the cellulose, will remove the encrusting materials, chiefly lignin and hemicellulose. The best method at present is alternately exposing material to chlorine and extraction with hot sodium sulfite solution. The residue thus freed of lignin and hemicellulose is taken as the cellulosic aggregate of the material.

Cotton cellulose is, in fact, pure glucose polysaccharide, and cannot be regarded as a typical plant cellulose. Norman uses the analogy of judging mineral ores against a vein of native metal. Plant "cellulose" is a mixture.

By treatment of cellulose in the cold with 17.5 per cent NaOH, a residue, termed $\alpha$cellulose, is obtained which, although not absolutely free from associated polysaccharides, is taken as representing true cellulose for industrial purposes. The strong alkaline extract is acidified and the precipitate which is formed is termed $\beta$cellulose. The remaining cellulose in solution is termed $\gamma$cellulose.

Cellulosan implies a hexosan or pentosan occurring intermixed with the true cellulose in the natural cellulosic aggregate. The associated polysaccharide found in most structural cellulose is xylan.

Thus, when one speaks of natural cellulose, one means plant cellulose or (true cellulose + cellulosan).

Bearing in mind the above definition of natural cellulose, any method of determining cellulose must yield a residue containing all the cellulosans since this fraction is to be regarded as an integral part of the cellulosic framework.

The natural celluloses of plant materials are much less resistant to the action of acids and alkalis than is cotton cellulose. This is probably due to the infusion of xylan chains of shorter length than the pure cellulose chains, which are more easily hydrolyzed than the pure cellulose.

Norman states: "There is a possibility which has not been considered by workers on cellulose; namely, that there may be mixed chains of xylan an cellulose, or, more accurately, of xylose and glucose. The presence of a few such mixed chains might account for the extreme stability of a small proportion of the xylan, which resists extractions by long boiling with 10 per cent sodium hydroxide."

It is suggested that the chains are not a jumble, but that a short chain of xylose is connected to a chain of glucose units. In other words, it is unlikely that several glucose molecules, then a xylose molecule, then several glucose molecules, are hooked together at random.

Bacteriological studies employing natural celluloses in place of the highly-purified cellulosic substrates customarily used might yield results of considerable interest. It seems likely that the ability of bacteria to use cellulose in the presence of other available carbohydrates will be more common than generally supposed. An analogy here would be the case of an animal which, if fed entirely on starch, would die, but which would live and prosper and be able to utilize starch if it were fed to the extent of 50 per cent of his diet along with other necessary ingredients. The use of pure cellulose probably has led many bacteriologists to erroneous conclusions when attempting to determine if cellulose is attacked by certain organisms.

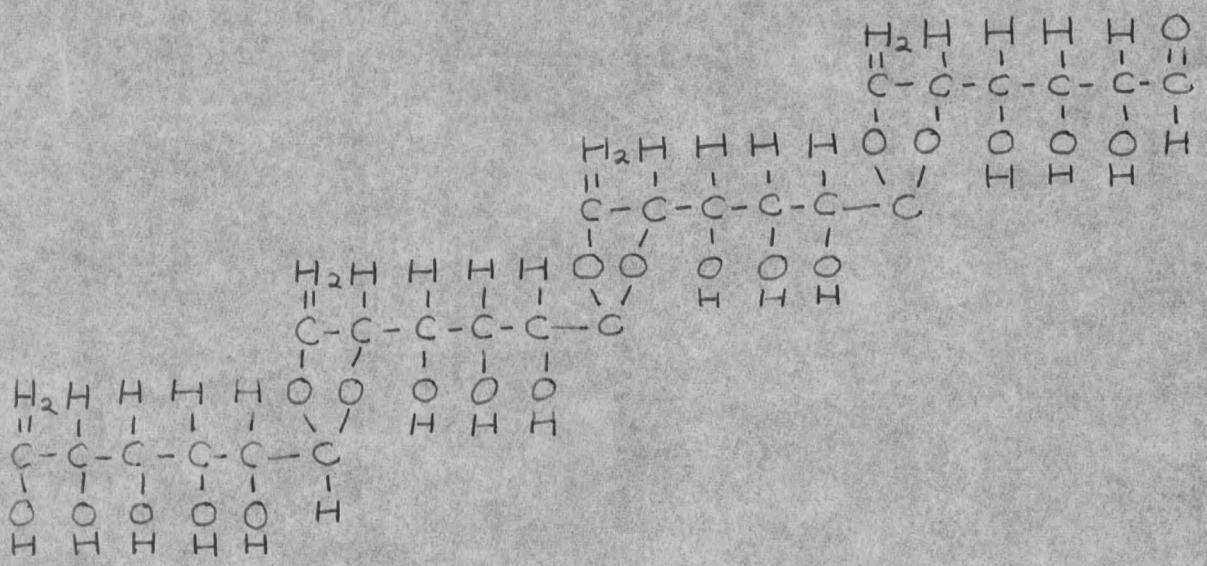
## The Chemical Constitution of Cellulose

Anselme Payne 70/ is usually credited with the first isolation of cellulose in 1838. In 1895 Tollens 46/ proposed a formula for cellulose that was generally accepted until advancing research pointed out certain properties of cellulose that the Tollens formula could not explain.

At the time that Tollens suggested his formula for cellulose the following facts were known concerning cellulose:

1. The product of hydrolysis of cellulose is glucose.
2. Acetolysis yields a tri- or disaccharide.
3. There are probably not more than three hydroxyls which react as alcoholic groups.
4. There is little indication of free aldehyde group.

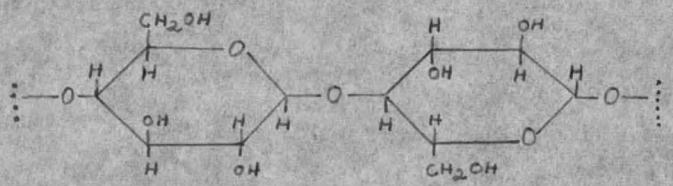
Tollens thought, thus, that cellulose was a long chain of glucose anhydrides linked together through glucose anhydride units.



The amorphous nature of cellulose, its insolubility and other properties have placed it in a class of substances of a high molecular weight. This formula shown is only a small portion of the total molecule of cellulose.

Tollens had a definite idea as to how the glucose anhydrides are linked together, as shown by his formula.

Since the time of Tollens' proposed formula there has accumulated in the literature a large number of structural formulas for the cellulose molecule. At present one can probably say that the best one is very similar to Tollens' original formula except that the amylene oxide, glucose, is present, and that linkage occurs at the 1-4 positions.



The Degradation of Cellulose by Biological Processes

Cellulose, since it is present in many plants in a considerable percentage, plays a very important part in many biological activities. Nature has provided for the destruction of cellulose by the activities of microorganisms; i.e., fungi and bacteria. By controllling the conditions of biological destruction (fermentation), valuable products such as alcohols, acids, etc. may be obtained. This forms the basis of important branches of industrial utilization of cellulose wastes.

Fermentation of cellulose eventually results in a number of gases, depending upon the types of microorganisms used. Four kinds of fermentation can be differentiated.

1. Methane fermentation, brought about by the action of sewage bacteria, produces chiefly methane, with smaller amounts of carbon dioxide and the lower fatty acids (from formic to butyric).

2. Hydrogen fermentation, produced by Bacterium fermentationis, yields mainly hydrogen, with smaller amounts of carbon dioxide and the lower fatty acids mentioned in (1).

3. Methane-hydrogen fermentation, produced by various thermophilic bacteria, such as are capable of assimilating nitrogen from the air or other nitrogen sources, yields nitrogen and carbon dioxide.

4. Nitrogen fermentation, produced by denitrifying bacteria, such as are capable of assimilating nitrogen from the air or other nitrogen sources, yields nitrogen and carbon dioxide.

As to the formation of methane, it is assumed by Symons and Buswell and others that primarily carbon dioxide and hydrogen are liberated, and these then react to form methane and water, with organic acids as intermediates. Although without question organic acids are intermediates it seems highly improbable that carbon dioxide and hydrogen, once liberated, would again react to form methane.

The process of fermentation is preceded by hydrolysis through hydrolytic enzymes. It has been possible to direct this process so that either cellobiose or glucose prevails by allowing either of the enzymes, cellobiase or

cellulase, to act. This may be accomplished by adjusting temperature conditions to the optimum and by arresting the process of fermentation by means of antiseptics. In view of this, the assumption may be made that the biological process passes through the same stages as cellulose subjected to hydrolysis by a chemical process in the laboratory. It appears likely that the bacteria also effect a gradual shortening of the cellulose chain, and that with refined methods oligosaccharides and even fractions of higher molecular weights might be isolated. Steps in this direction may be seen in the work which led to the recognition that cleavage of the longer chains (from cellulose down to the water-soluble dextrins) is apparently effected exclusively by cellulase whereas the oligosaccharides undergo scission chiefly under the action of cellobiase. Thus one may distinguish between " -glycopolysaccharase" and " -glucoöligosaccharase."

However, no connection is apparent between observations of this kind and the fact that some bacteria cease to act after they have shortened the cellulose chain to a certain extent. It would appear that accumulation of degradation products at this stage inhibits further growth of bacteria.

The number of isolated and well-defined bacteria capable of cellulose destruction has greatly increased during recent years, and very interesting results of extensive research work, especially in the field of soil microbiology, are available.

Allen and Carlson 1/ found that wild rats could not digest cellulose, and that in all cases the wild rats died of starvation or enteritis. Enzyme preparations from the animal digestive tract had no digesting effect on cel-

lulose from celotex.

The carnivorous animals cannot digest appreciable quantities of cellulose. The ruminants are, however, able to utilize a large portion of the cellulose. Jacques 51/ found that Plectridium celluloyticum isolated from the rumen of cattle ferments cellulose with the formation of fatty acids. The total quantity of fatty acid formed represented 80 per cent of the cellulose.

Woodman and Stewart 49/ investigated the action of thermophylic cellulose-splitting bacteria on the fiber of a number of typical feeding stuffs. They stated that a small amount of lignocellulose was probably responsible for a distinct lowering of digestibility, caused not entirely by the presence of lignocellulose, but also by the manner of its deposition within the cell walls. Also, they demonstrated that during the growth of perennial rye grass the increase in lignin content was associated with reduced digestibility, a conclusion which is in harmony with other feeding trials. Later investigation has substantiated their findings, with the exception of their use of the term "lignocellulose." It has not been proved farily definitely that lignocellulose as a compound does not exist, and that the decrease in digestibility of the cellulose is due in main to the manner of deposition of lignin in the cell.

Pryanishnikov 77/ found the digestibility of roughage may be influenced by the following factors: the presence of lignin, the content of silicon in the cell walls, the different micellar conditions of cellulose and the modifications in the character of the close connections between cellulose and

incrustants.

Waksman 93/ states that when lignin is removed from plant materials the rate of cellulose decomposition in the ruminant increases but the presence of only 8 per cent lignin was found to be sufficient to reduce the rate of cellulose decomposition by 50 per cent. A reduction of the lignin content to 1.5 per cent enables the cellulose to decompose as readily as in the pure form. He considers the depressing effect of lignin on cellulose decomposition to be due to the manner of combination with cellulose, which is protected from rapid attack by saprophytic microorganisms.

Crampton has made extensive studies on the effect of lignin on digestibility of grasses. 13/14/15/16/17/ He states that the nutritive value of herbage may change appreciably in a ten-day period in the summer. In midsummer the availability of the carbohydrate fraction of the herbage is materially reduced. This is due to the amount and kind of deposition of the lignin, which affects the digestibility and nutritive values of herbage.90/ Crampton also suggests the use of rabbits as a substitute for steers in digestibility trials, as there is a high correlation between the two species in case of digestibility of crude protein and lignin.

It is of interest to note that pepsin, rennin, catalase and other enzymes can be removed from solutions by a single filtration through cotton, or to a certain extent by filter paper, due to adsorption of enzymes by cellulose.44/ For further information on cellulose digestion the following articles are referred to: 98/99/100/10/.

It has been found that in termites that cause destruction of wood the

cellulose is destroyed by bacteria within the intestines of the termites.5/

The Degradation of Cellulose by Acids

Hydrolysis 37/ with mineral acids yields glucose to a less extent than does hydrolysis with organic acids. If the hydrolysis is not complete a number of intermediate products result. They are composed of broken chains of the cellulose molecule. This is probably caused by the fact that the cellulose molecule is so large that hydrolysis must start on the surface and penetrate inward. Thus, if one were to stop the hydrolysis before it was complete one would find a mixture of compounds with the formula $(C_6H_{12}O_6)_X$. The "X" in this formula would vary from one to the number representing the true cellulose molecule. Just how many glucose units from a cellulose molecule can not be accurately determined, and it is very probable that the number varies in celluloses of different plants.

The intermediate products of the degradation of cellulose can best be obtained by heating with dilute acids. By heating cellulose fiber with dilute hydrochloric acid a product is obtained which resembles oxycellulose in appearance and behavior. Thus the cellulose emerges from the treatment as a weakened fiber which may easily be rubbed to a powder between the fingers. It is termed hydrocellulose. In the formation of hydrocellulose the only change conceivable is a shortening of long chains. Under the hydrolyzing effect of the acid, a number of oxygen bridges may break up, resulting in a saccharide having a pronounced reducing effect, due to the presence of more aldehyde groups.

In heating hydrocellulose with dilute alkali (4-6 per cent sodium hydroxide), the raw hydrocellulose preparations may be divided into two components. The filtrate shows pronounced reducing power on Fehling's solution; thus it must contain the lower degradation products. The residue, however, only shows a very slight reducing power, which means it contains the higher degradation products, and is also soluble in higher alkali concentrations (8 per cent sodium hydroxide). The purified alkali-soluble hydrocellulose has been termed "celluloseA."

It is possible by use of mineral acid to convert 99 per cent of the original cellulose into alkali-soluble cellulose. Organic acids such as formic, acetic, and oxalic may also be used for conversion.

With concentrated acids such as sulfuric, nitric, hydrochloric, hydrofluoric, or phosphoric, cellulose swells considerably, becomes peptized, and finally dissolves completely. Usually, immediately after preparation the solution shows reducing power toward Fehling's solution. Upon dilution with water immediately after dissolving and with thorough cooling, the greater part of the cellulose may be regenerated in the form of white flakes which show the behavior and X-ray diagram of mercerized cellulose. The preparation obtained is often termed "amyloid," merely because it gives the same blue coloration with iodine in the presence of traces of sulfuric acid as starch (amylum) does.

On standing of the cellulose dissolved in acid, degradation proceeds, and by the addition of alcohol a precipitate characterized by its high reducing power may be obtained. It is partly or entirely soluble in water and

has the name "cellodextrin." It has played a great part in the endeavors to isolate homogeneous intermediates in the course of the degradation of cellulose. In the light of modern conception of the chemical constitution of cellulose, cellulose dextrin is far from being a homogeneous product. It is rather regarded as a mixture of more or less shortened glucose anhydride chains; in other words, a mixture of oligosaccharides of varying chain length, the longest link of which may comprise thirty or less glucose anhydrides. Since the molecules of smaller size crystallize, the whole mass appears crystalline. Certain biose anhydrides which at one time were claimed to have been isolated as homogeneous degradation intermediates, also apparently represent mixtures of oligosaccharides of various chain lengths similar to cellulose dextrins.

## Methods of Analysis of Cellulose

Although numerous methods for the determination of cellulose appear in the literature the general procedure is quite similar in all of them. Lignin, pectin and related compounds are removed by oxidation with chlorine gas, nitric acid, hypochlorite solution or other reagents which will cause solution of the resistant components of fibrous material. A weak hydrolysis may or may not be employed, depending on what portion of the pentosans and hemicellulose one wishes to consider as being a component part of the "plant cellulose."

The Cross and Bevan 19/ method of determination of cellulose was one of the first methods of determination of cellulose to be extensively used in

plant analysis. It is the chlorination method. It depends upon oxidation without hydrolysis and does not remove the pentosans or hemicellulose. Many other methods are a modification of the original Cross and Bevan method. The Konig 57/ method involves digestion at 137° with glycerol and $H_2SO_4$. Waksman and Stevens 94/ remove the lignin from the organic matter with acid sodium sulfite solution, followed by extraction of the cellulose with ammoniacal copper solution and its precipitation with hydrochloric acid. Ost and Wilkening 68/ use hydrolysis of the cellulose-containing material, previously freed from sugars, starches and hemicellulose with concentrated acids, then completing the hydrolysis to glucose by boiling with the dilute acid. The cellulose content is calculated from the amount of reducing sugar formed. The Norman and Jenkins 66/method is based on the assumption that the cellulosans or polysaccharide material associated with "pure cellulose" must remain intact in any satisfactory method of cellulose estimation. The method proposed for straws and woods involves first two treatments with neutral hypochlorite and then three or more with acid hypochlorite, each followed by boiling with sodium sulfite. Williams and Olmstead 96/ and others first free the fiber as completely as possible from the starch, protein and fat by enzymic digestion.

Recently the monoethanolamine method of Reid, Nelson and Aronovsky 80/ has attracted the attention of investigators. It is a rapid method and consists of refluxing two hours with monoethanolamine which decomposes the major portion of the lignin in plant material and removes the less-resistant pentosans, but has little effect on the $\alpha$-cellulose, either in plant material or in the isolated form.

## STATEMENT OF PROBLEM

The problem for this thesis consisted of the determination of ash, ether extract nitrogen, lignin and cellulose in several range grass species, and in addition, a study of the methods of analysis for lignin and cellulose.

## EXPERIMENTAL WORK

The grass samples were collected from Experiment Station plots in Gallatin and Judith Basin Counties. These collections were made at one-week intervals, beginning May 14, 1940, and lasting until September 15, 1940.

The annual growth cycle 4/ of these grasses was divided into four definite developmental stages and tend to offer a convenient basis for presenting experimental data. These stages are:

A. Snow disappearance to flower stalks first in evidence.

B. Flower stalks first in evidence to heads fully out.

C. Heads fully out to flowers in bloom.

D. Flowers in bloom to seeds fully ripe.

E. Seeds shattering.

### List of Grasses Collected and Analyzed

Gallatin County

Agropyron cristatum (L.) Gaertn., Standard crested wheatgrass

Agropyron cristatum, Fairway crested wheatgrass

Agropyron pauciflorum (Schwein) Hitch., slender wheatgrass

Bromus inermis Leyss., smooth brome grass

Bromus marginatus Nees., mountain brome grass

Elymus junceus Fisch., Russian wild-rye

Judith Basin County

Agropyron cristatum, Standard crested wheatgrass

Agropyron cristatum, 19537, Standard crested wheatgrass (selection)

Agropyron cristatum, M 24-3, Standard crested wheatgrass (selection)

Agropyron cristatum, Fairway crested wheatgrass

Agropyron cristatum, 1350, Fairway crested wheatgrass (selection)

Elymus junceus, Russian wild-rye

The grasses from Gallatin upon collection were immediately brought into the laboratory and placed in an oven at 50° C. until dry. They were dried as quickly as possible to prevent any avoidable change in their composition. The grasses collected at Judith Basin, due to the distance of that station from our laboratory, were only air-dried before shipping, and oven-dried after arrival.

After drying, the grasses were ground in a Wiley mill until they passed through a one-half millimeter screen. All analyses were run in duplicate.

Moisture was determined by placing a 2-gram sample for 5 hours in a vacuum oven at 100° C. and 25 mm. This was used in calculating all other analyses on an oven-dry basis.

Ash was determined by heating a 1-gram sample for one hour at 600° C. in a muffle furnace.

Total nitrogen determined by the Kjeldahl method was multiplied by 6.25 to give the crude protein content.

Ether extract was determined by extracting a 1-gram sample overnight with anhydrous ether and weighing the extracted material, which was dried for one hour at 100° C. after the ether had been evaporated off on a steam plate.

"Other carbohydrates" was determined by subtracting the total ash, ether extract, protein, lignin and cellulose from 100.

## Lignin Procedure

The method used for the determination of lignin was that of Crampton and Maynard, 17/ which is a modification of the 72 per cent sulfuric acid method. Weigh out an air-dry 1-gram sample of grass or feces and extract with ether for 16 hours by means of a Bailey-Walker extractor. Transfer the dried residue into a 50-cc. glass-stoppered Erlenmeyer flask and add 40 cc. of a 2.0% solution of pepsin in 0.1 N HCl. Digest overnight at 40° C. Recover the undigested residue by filtration through 200-mesh bolting silk in a Gooch crucible. In the analysis of feces, use a No. 54 Whatman filter paper instead of bolting silk. Wash successively with hot water, hot alcohol, hot benzene, hot alcohol and ether. Transfer the washed residue to a 100-cc. beaker, and allow the ether to evaporate. Moisten the residue with 4 cc. of 40% formaldehyde. Then add 4 cc. of 72% $H_2SO_4$, allowing it to penetrate the sample (2 minutes). Add 6 cc. of concentrated $H_2SO_4$ and stir vigorously with a glass rod to aid in solution, which should be complete in from 10 to 15 minutes. Partly immerse the beaker in cold water bath to prevent the temperature from rising above 70° C. When sample is dissolved, stir in 35 cc. of a granulating reagent consisting of a 1:6 mixture (by volume) of chloroform and glacial acetic acid, and pour the whole into 500 cc. distilled water in an 800-cc. beaker. Boil gently until the chloroform has been driven off (15 minutes), after which the solution should clear and the lignin settle in granular form. Filter (using asbestos) in a Gooch crucible with suction. Wash in not less than 200 cc. of 5% HCl. Dry at 110° C. and determine lignin by loss on ignition.

Discussion of the Crampton-Maynard Method for Lignin Determination

This method consists of solution of the sample in concentrated $H_2SO_4$ after pretreatment to remove fats, sugars and proteins which otherwise would interfere with the lignin values obtained.

The special problem in the analysis of forage and feces lies in the removal of the protein without a simultaneous removal of a part of the lignin. According to present information, lignin is soluble in varying degrees in dilute alkali (hot or cold), boiling water and dilute mineral acid (1.25%) at boiling temperature. To overcome this difficulty, enzyme digestion has been used. Various enzymes have been used. Williams and Olmstead, 96/ working with human diets and feces, proposed the use of pancreatin in a solution buffered at pH 8. The objection to the use of pancreatin is the fact that lignin is slightly soluble in a pancreatin solution buffered at a pH of 8; also, enzyme digestion residue is not completely soluble in concentrated sulfuric acid. Using this method in a steer digestion trial, 25 per cent more lignin was recovered in the feces than was consumed!

Pepsin, on the other hand, is active in acid medium, and it has not yet been shown that lignin is soluble in dilute mineral acid at temperatures at which this enzyme is active. The effectiveness of pepsin, according to Horwitt,50/ in removing nitrogen from spinach leaves, was about 89 per cent of the total nitrogen. It is assumed that the protein is reduced by pepsin digestion to a level where it will no longer seriously interfere with the lignin determination.

The problem of completely and rapidly dissolving the undigested residue

in the strong acid was solved by Ross and Hill,82/ who found that lignified tissue would dissolve promptly (10-15 minutes) in 72% $H_2SO_4$ if first moistened with formalin. The use of formaldehyde has not been accepted by some investigators, due to the fact that formaldehyde condensation products may be formed leading to high lignin values. With the sample in contact with the acid for so short a time, condensation of the carbohydrates (pentoses and hexoses) which might add to the lignin value is presumably largely avoided.

Accepting the hypothesis that lignin is not utilized by the animal, the practical usefulness of any lignin method would be indicated by a lignin balance trial. Crampton and Maynard found practically quantitative recovery of the dietary lignin, 97.8% in rabbits and 99.3% in steers, using their method.

The most serious objection to the determination of lignin in feed analyses is that the present methods of lignin determination are so long and time-consuming. If a rapid method of estimation of lignin of suitable accuracy could be found, the determinations of lignin and cellulose would no doubt be more generally accepted as a replacement of the Henneberg (crude fiber) method.

## Cellulose Procedure

The method used in the determination of cellulose was a modification of the Crampton and Maynard method.17/ It consists of treating plant material with a boiling mixture of nitric acid and acetic acid. The nitric acid

oxidizes the lignin and related compounds so that they go into solution. The acetic acid hydrolyzes the less-resistant carbohydrates. A modification of this method was chosen because it compares favorably with results of other cellulose methods; in addition, it is a very rapid method. Since there is a variation in the amount and composition by the different methods a method was chosen which was suitable for use in animal studies, one that gave a high recovery of non-starchy hexosans.

The details of the procedure used are as follows: Place a 1-gram, air-dry sample in a 6-inch Pyrex centrifuge tube. Add 15 cc. of 80% acetic acid and 1.5 cc. conc. $HNO_3$. Place a water-filled Battersea crucible, top diameter 30 mm., into the neck of the centrifuge tube. This serves as a simplified reflux condenser. Boil gently for 20 minutes on a sand bath. Cool centrifuge tubes with cold water and remove Battersea crucibles. Add about 20 cc. of alcohol, mixing well with stirring rod. Centrifuge until precipitate settles (5 minutes). Decant the liquid. Wash (in centrifuge tube) successively with alcohol (2 washings), hot benzene, hot alcohol, ether. Dry residue in centrifuge tube at room temperature until no noticeable ether odor remains, and place in oven at 100° C. for one hour.

Remove precipitate from the centrifuge tubes and weigh. Calculate cellulose as loss on ignition.

Our experience has shown this method to give reproducible results. The only objection is that it is rather difficult to remove the cellulose quantitatively from the centrifuge tubes. This difficulty arises from the fact that the cellulose becomes electrically charged and is attracted by the glass. This may be partly overcome by brushing a rubber policeman with a camel's-

hair brush, causing the cellulose to be attracted more to the rubber policeman than to the glass.

In the method of determination of cellulose which was used, in which the cellulose is dissolved by acetic acid solution containing nitric acid, by refluxing and then precipitating in alcohol, it is probably that we are dealing here with cellulose dextrins as a final product.

TABLE I.

RESULTS OF RANGE-GRASS ANALYSES*

| Sample Number | Kind of Grass | Date Collected | Growth Stage | Moisture (dry basis) | Total Ash | Ether Extract | Crude Protein(6.25) | Lignin | Cellulose | Other Carbohydrate |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Std. | 5/16 | $A_1$ | 5.4% | 10.0% | 4.8 % | 26.4 % | 5.3 % | 20.2 % | 33.3 % |
| 2 | inermis | 5/15 | $A_1$ | 5.9 | 11.7 | 5.0 | 25.9 | 5.2 | 23.2 | 29.0 |
| 3 | Fwy. | 5/16 | $A_1$ | 6.2 | 10.1 | 4.5 | 25.1 | 5.7 | 22.5 | 32.1 |
| 4 | margin. | 5/16 | $A_1$ | 6.4 | 13.6 | 5.3 | 25.4 | 5.1 | 21.6 | 29.0 |
| 5 | pauci. | 5/16 | $A_1$ | 6.3 | 10.6 | 5.5 | 24.3 | 5.3 | 21.1 | 33.2 |
| 6 | junc. | 5/22 | $A_2$ | 6.2 | 10.5 | 3.0 | 24.3 | 6.5 | 23.6 | 32.1 |
| 14 | junc. | 4/18 | $A_2$ | 3.7 | 9.4 | 3.9 | 35.5 | 4.4 | 18.8 | 28.0 |
| 20 | junc. | 5/22 | $A_3$ | 6.5 | 10.8 | 3.3 | 26.7 | 6.8 | 20.7 | 31.7 |
| 29 | Fwy. | 5/31 | $A_1$ | 6.8 | 9.7 | 4.5 | 19.0 | 7.5 | 25.5 | 33.8 |
| 30 | Std. | 5/31 | $B_1$ | 5.4 | 8.7 | 4.3 | 22.5 | 7.2 | 25.2 | 32.1 |
| 31 | margin. | 5/31 | $B_1$ | 6.7 | 10.1 | 4.4 | 19.7 | 6.1 | 26.8 | 32.9 |
| 32 | inermis | 5/31 | $B_1$ | 6.4 | 10.9 | 5.0 | 22.9 | 6.0 | 27.2 | 28.0 |
| 33 | pauci. | 5/31 | $A_1$ | 4.7 | 9.5 | 5.2 | 20.3 | 6.7 | 24.8 | 33.5 |
| 34 | junc. | 6/6 | $C_2$ | 6.8 | 10.1 | 3.4 | 23.8 | 8.8 | 24.6 | 29.3 |
| 41 | margin. | 6/12 | $D_1$ | 6.1 | 10.5 | 3.3 | 17.0 | 9.1 | 28.6 | 31.5 |
| 42 | inermis | 6/12 | $D_1$ | 6.2 | 9.6 | 4.9 | 21.8 | 8.5 | 28.8 | 26.4 |
| 43 | pauci. | 6/12 | $A_1$ | 5.4 | 8.7 | 4.7 | 16.2 | 7.4 | 28.1 | 34.9 |
| 44 | Std. | 6/12 | $C_1$ | 6.1 | 8.6 | 4.5 | 23.3 | 8.9 | 27.6 | 27.1 |
| 45 | Fwy. | 6/12 | $C_1$ | 5.4 | 9.0 | 3.8 | 16.7 | 9.3 | 27.9 | 33.3 |
| 52 | junc. | 6/19 | $D_2$ | 5.6 | 8.6 | 3.1 | 17.4 | 10.8 | 29.2 | 30.9 |
| 54 | junc. | 6/6 | $B_3$ | 5.9 | 8.7 | 3.2 | 19.3 | 9.0 | 23.2 | 36.6 |
| 55 | Fwy. | 6/6 | $B_3$ | 8.2 | 7.8 | 3.5 | 14.6 | 9.4 | 26.0 | 38.7 |
| 62 | 19537 | 6/6 | $B_3$ | 5.7 | 7.7 | 4.3 | 12.0 | 8.0 | 24.5 | 43.5 |
| 63 | 1350 | 6/6 | $B_3$ | 7.2 | 10.0 | 4.9 | 11.2 | 8.2 | 23.6 | 42.1 |
| 65 | Std. | 6/6 | $C_3$ | 6.9 | 7.8 | 2.5 | 15.9 | 13.1 | 30.0 | 30.7 |
| 66 | M24-3 | 6/6 | $B_3$ | 7.5 | 8.0 | 3.4 | 12.4 | 9.6 | 23.9 | 42.7 |
| 67 | Fwy. | 6/26 | $D_1$ | 6.5 | 8.5 | 4.4 | 15.5 | 11.6 | 29.1 | 30.9 |
| 68 | Std. | 6/26 | $D_1$ | 5.9 | 6.9 | 3.7 | 13.9 | 13.2 | 32.4 | 29.9 |
| 69 | margin. | 6/26 | $D_1$ | 5.6 | 8.6 | 3.3 | 11.9 | 11.2 | 29.1 | 35.9 |
| 70 | inermis | 6/26 | $D_1$ | 6.3 | 8.9 | 4.2 | 14.1 | 11.2 | 31.1 | 30.5 |
| 71 | pauci. | 6/26 | $C_1$ | 6.0 | 9.7 | 3.9 | 13.8 | 10.2 | 31.6 | 30.8 |
| 76 | 1350 | 6/19 | $C_3$ | 6.8 | 7.3 | 3.9 | 10.1 | 9.5 | 26.4 | 42.8 |

TABLE I (continued)

| Sample Number | Kind of Grass | Date Collected | Growth Stage | Moisture (dry basis) | Total Ash | Ether Extract | Crude Protein (6.25) | Lignin | Cellulose | Other Carbohydrates |
|---|---|---|---|---|---|---|---|---|---|---|
| 77 | junc. | 6/19 | $D_3$ | 6.4 % | 9.2 % | 3.2 % | 16.0 % | 11.8 % | 27.6 % | 32.2 % |
| 79 | Fwy. | 6/19 | $C_3$ | 8.1 | 5.4 | 2.7 | 9.0 | 13.2 | 29.9 | 39.8 |
| 82 | 19537 | 6/19 | $C_3$ | 6.9 | 10.7 | 3.5 | 8.7 | 11.5 | 28.2 | 37.4 |
| 83 | Std. | 6/19 | $C_3$ | 6.2 | 7.7 | 2.9 | 14.1 | 13.3 | 31.0 | 30.7 |
| 84 | M24-3 | 6/19 | $C_3$ | 8.2 | 8.6 | 3.5 | 10.0 | 11.0 | 28.2 | 38.7 |
| 92 | junc. | 7/3 | $D_2$ | 5.0 | 10.6 | 2.7 | 17.8 | 11.4 | 28.7 | 28.8 |
| 97 | Fwy. | 7/10 | $D_1$ | 5.8 | 7.8 | 3.7 | 12.4 | 11.6 | 31.1 | 33.4 |
| 99 | margin. | 7/10 | $D_1$ | 6.1 | 10.1 | 3.3 | 8.2 | 10.8 | 29.8 | 37.8 |
| 101 | inermis | 7/10 | $D_1$ | 6.0 | 10.6 | 4.4 | 13.5 | 10.5 | 27.9 | 33.1 |
| 103 | pauci. | 7/10 | $C_1$ | 4.8 | 7.7 | 3.5 | 9.6 | 10.5 | 32.8 | 35.9 |
| 105 | Std. | 7/10 | $D_1$ | 4.9 | 7.3 | 3.8 | 12.5 | 12.8 | 31.4 | 32.2 |
| 107 | 19537 | 7/3 | $D_3$ | 4.6 | 8.6 | 2.8 | 7.5 | 12.1 | 28.0 | 41.0 |
| 111 | M24-3 | 7/3 | $D_3$ | 4.5 | 3.6 | 3.6 | 5.1 | 14.1 | 30.8 | 42.8 |
| 112 | junc. | 7/3 | $D_3$ | 5.6 | 11.0 | 3.3 | 11.5 | 10.9 | 30.2 | 33.1 |
| 116 | Std. | 7/3 | $D_3$ | 4.8 | 7.8 | 2.9 | 7.6 | 13.0 | 31.7 | 37.0 |
| 117 | 1350 | 7/3 | $D_3$ | 5.9 | 7.1 | 4.0 | 8.0 | 11.7 | 28.5 | 40.7 |
| 119 | Fwy. | 7/3 | $D_3$ | 5.0 | 8.2 | 4.2 | 6.3 | 12.7 | 29.9 | 38.7 |
| 131 | junc. | 7/17 | $D_2$ | 3.6 | 12.5 | 4.0 | 18.8 | 10.3 | 26.7 | 27.7 |
| 133 | Fwy. | 7/24 | $D_1$ | 6.1 | 8.8 | 3.5 | 7.6 | 12.4 | 30.8 | 36.9 |
| 135 | Std. | 7/24 | $D_1$ | 6.0 | 6.9 | 3.5 | 9.1 | 13.7 | 33.7 | 33.1 |
| 137 | pauci. | 7/24 | $D_1$ | 6.2 | 7.2 | 2.9 | 5.7 | 13.3 | 36.6 | 34.3 |
| 139 | margin. | 7/24 | $E_1$ | 6.1 | 9.4 | 2.6 | 7.3 | 13.2 | 34.7 | 32.8 |
| 141 | inermis | 7/24 | $D_1^+$ | 6.3 | 11.6 | 4.2 | 10.4 | 12.6 | 32.2 | 29.0 |
| 150 | junc. | 7/30 | $E_2$ | 5.0 | 15.1 | 4.4 | 20.4 | 9.4 | 26.1 | 24.6 |
| 153 | Fwy. | 8/7 | $D_1$ | 4.6 | 9.4 | 3.5 | 8.4 | 11.8 | 32.1 | 34.8 |
| 154 | Std. | 8/7 | $D_1$ | 4.6 | 6.7 | 3.7 | 9.0 | 13.6 | 34.7 | 31.3 |
| 155 | pauci. | 8/7 | $D_1$ | 4.3 | 8.0 | 4.1 | 5.7 | 13.3 | 35.8 | 33.1 |
| 156 | inermis | 8/7 | $D_1$ | 4.5 | 8.7 | 2.7 | 7.8 | 15.4 | 37.0 | 28.4 |
| 157 | margin. | 8/7 | $E_1$ | 4.6 | 9.6 | 3.7 | 5.7 | 11.7 | 32.2 | 37.0 |
| 160 | 1350 | 7/17 | $D_3$ | 4.8 | 8.2 | 3.5 | 5.5 | 11.8 | 30.4 | 36.6 |
| 162 | Std. | 7/17 | $D_3$ | 2.6 | 6.0 | 4.1 | 5.2 | 12.3 | 31.6 | 40.8 |
| 163 | Fwy. | 7/17 | $D_3$ | 4.4 | 6.2 | 3.2 | 9.2 | 13.7 | 32.2 | 35.5 |
| 165 | M24-3 | 7/17 | $D_3$ | 4.0 | 7.5 | 3.5 | 4.8 | 11.8 | 30.7 | 41.7 |
| 167 | 19537 | 7/17 | $D_3$ | 6.4 | 7.6 | 3.6 | 6.5 | 12.7 | 29.9 | 39.7 |

TABLE I (continued).

| Sample Number | Kind of Grass | Date Collected | Growth Stage | Moisture (dry basis) | Total Ash | Ether Extract | Crude Protein(6.25) | Lignin | Cellulose | Other Carbohydrates |
|---|---|---|---|---|---|---|---|---|---|---|
| 170 | junc. | 7/17 | $E_3$ | 4.8 % | 13.8% | 4.3 % | 7.5 % | 11.4% | 31.8% | 31.2 % |
| 173 | 19537 | 7/31 | $D_3$ | 7.8 | 7.3 | 4.1 | 6.9 | 15.3 | 35.1 | 31.3 |
| 175 | Fwy. | 7/31 | $E_3$ | 3.8 | 10.0 | 3.5 | 5.3 | 13.9 | 33.6 | 33.7 |
| 177 | 1350 | 7/31 | $E_3$ | 4.2 | 8.6 | 2.3 | 5.0 | 14.1 | 32.0 | 38.0 |
| 179 | M24-3 | 7/31 | $D_3$ | 4.4 | 9.8 | 3.7 | 8.0 | 13.6 | 28.5 | 36.4 |
| 180 | junc. | 7/31 | $E_3$ | 5.5 | 13.7 | 4.1 | 6.3 | 11.9 | 35.8 | 28.2 |
| 182 | Std. | 7/31 | $D_3$ | 4.3 | 7.3 | 3.2 | 6.9 | 14.2 | 33.1 | 35.3 |
| 185 | junc. | 8/14 | $E_2$ | 4.1 | 14.0 | 4.0 | 20.8 | 7.9 | 24.8 | 28.5 |
| 193 | Std. | 8/22 | $E_1$ | 4.4 | 7.5 | 3.1 | 9.1 | 14.0 | 36.3 | 30.0 |
| 194 | Fwy. | 8/22 | $E_1$ | 4.7 | 9.0 | 2.7 | 8.6 | 13.7 | 35.6 | 30.0 |
| 195 | margin. | 8/22 | $E_1$ | 4.4 | 9.9 | 2.8 | 6.2 | 12.4 | 34.5 | 34.2 |
| 196 | inermis | 8/22 | $E_1$ | 4.1 | 7.1 | 2.3 | 4.6 | 16.0 | 39.6 | 30.4 |
| 197 | pauci. | 8/22 | $E_1$ | 4.4 | 8.1 | 3.5 | 5.6 | 13.7 | 37.3 | 31.8 |
| 198 | junc. | 8/28 | $E_2$ | 3.8 | 12.9 | 4.2 | 16.6 | 9.5 | 29.9 | 26.9 |
| 205 | Std. | 9/4 | $E_1$ | 3.5 | 5.5 | 2.4 | 6.1 | 15.6 | 40.4 | 30.0 |
| 206 | Fwy. | 9/4 | $E_1$ | 5.6 | 8.6 | 2.8 | 9.0 | 14.1 | 36.9 | 28.6 |
| 207 | inermis | 9/4 | $E_1$ | 4.5 | 8.9 | 2.4 | 6.2 | 17.8 | 41.3 | 23.4 |
| 208 | margin. | 9/4 | $E_1$ | 4.7 | 9.2 | 2.5 | 5.5 | 13.3 | 38.5 | 31.0 |
| 209 | pauci. | 9/4 | $E_1$ | 4.1 | 7.7 | 2.9 | 4.3 | 14.0 | 39.7 | 31.4 |

Sub-1 = Ft. Ellis, Gallatin County
Sub-2 = Campus, Gallatin County
Sub-3 = Judith Basin County

*The collection of grasses and some of these analyses were performed by members of the Chemistry Staff as a part of regular range grass projects, and are included here for the sake of completeness.

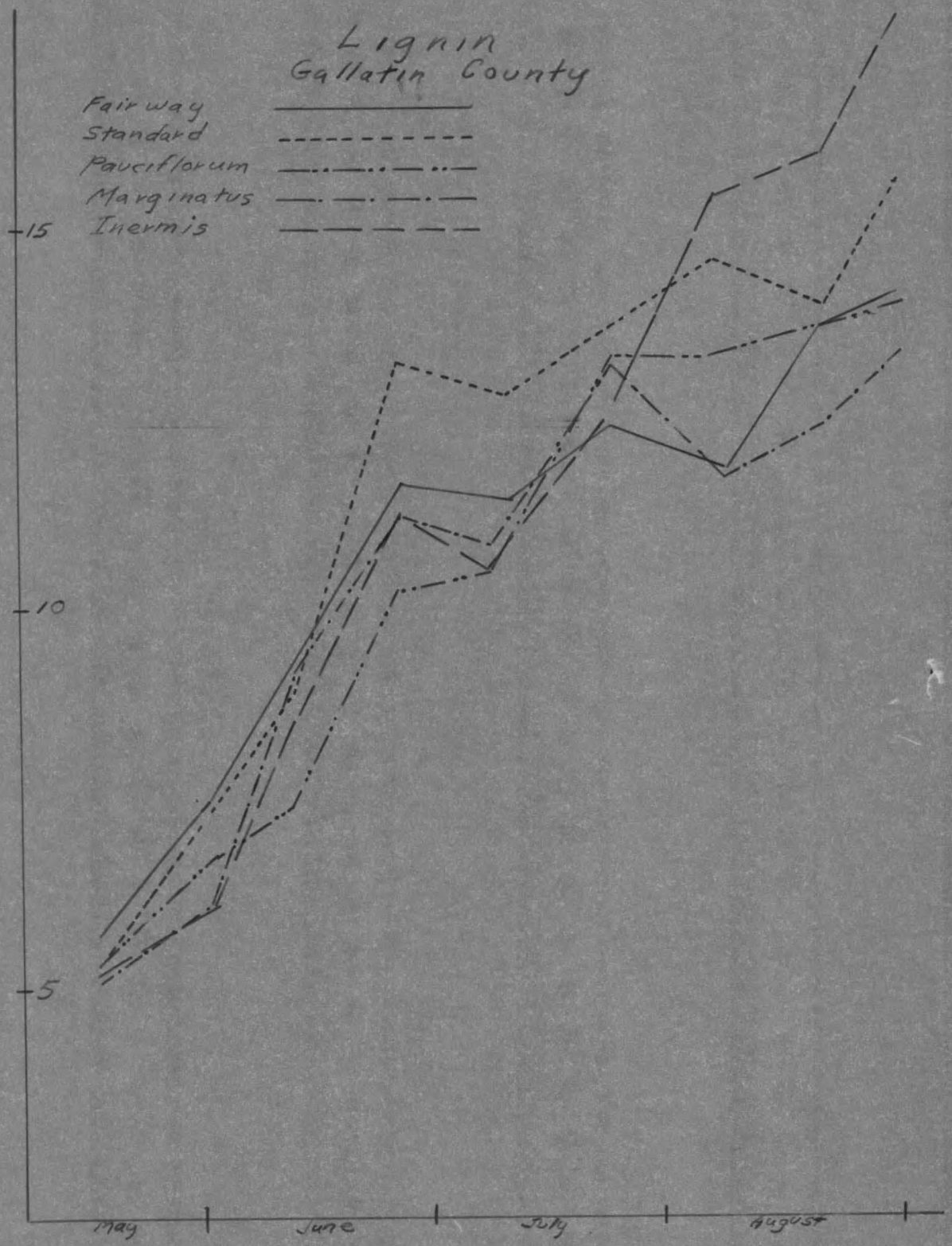

Lignin
Gallatin County
Fairway
Standard
Pauciflorum
Marginatus
Inermis
15
10
5
May
June
July
August

Figure 1.

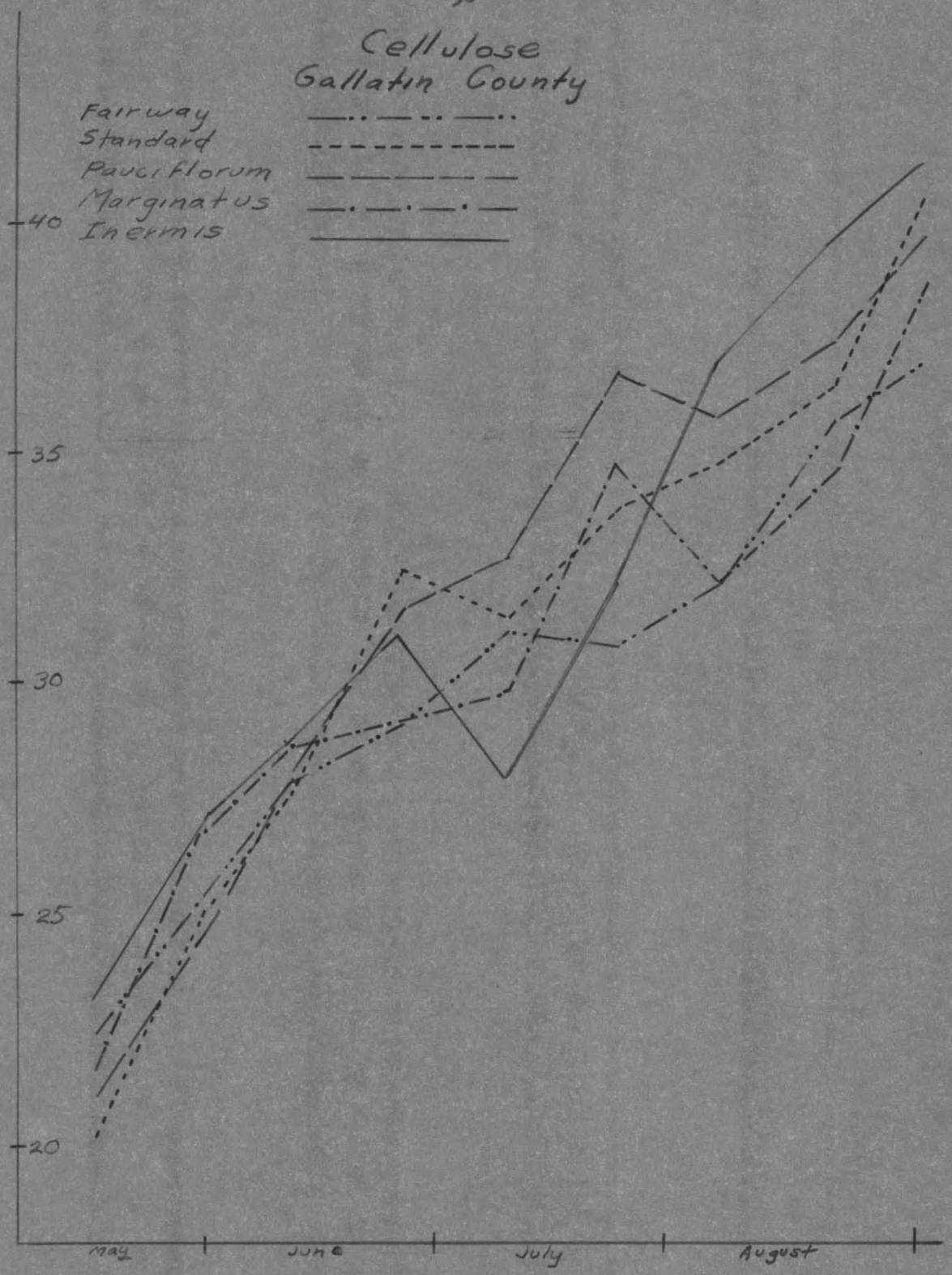

Cellulose
Gallatin County
Fairway
Standard
Pauciflorum
Marginatus
Inermis
40
35
30
25
20
May
June
July
August

Figure 2.

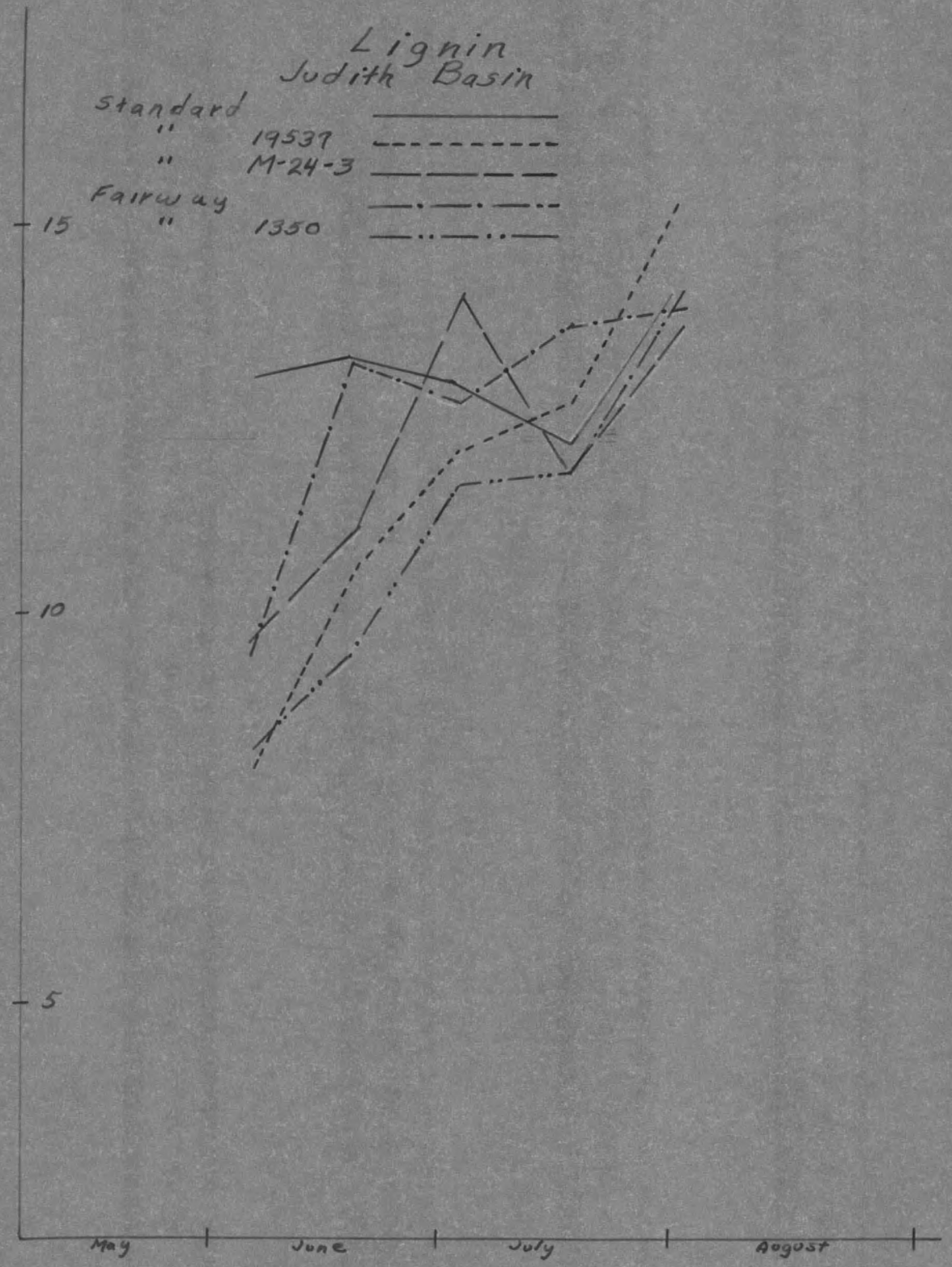

Lignin
Judith Basin
Standard
"
"
Fairway
"
19537
M-24-3
1350
15
10
5
May
June
July
August

Figure 3.

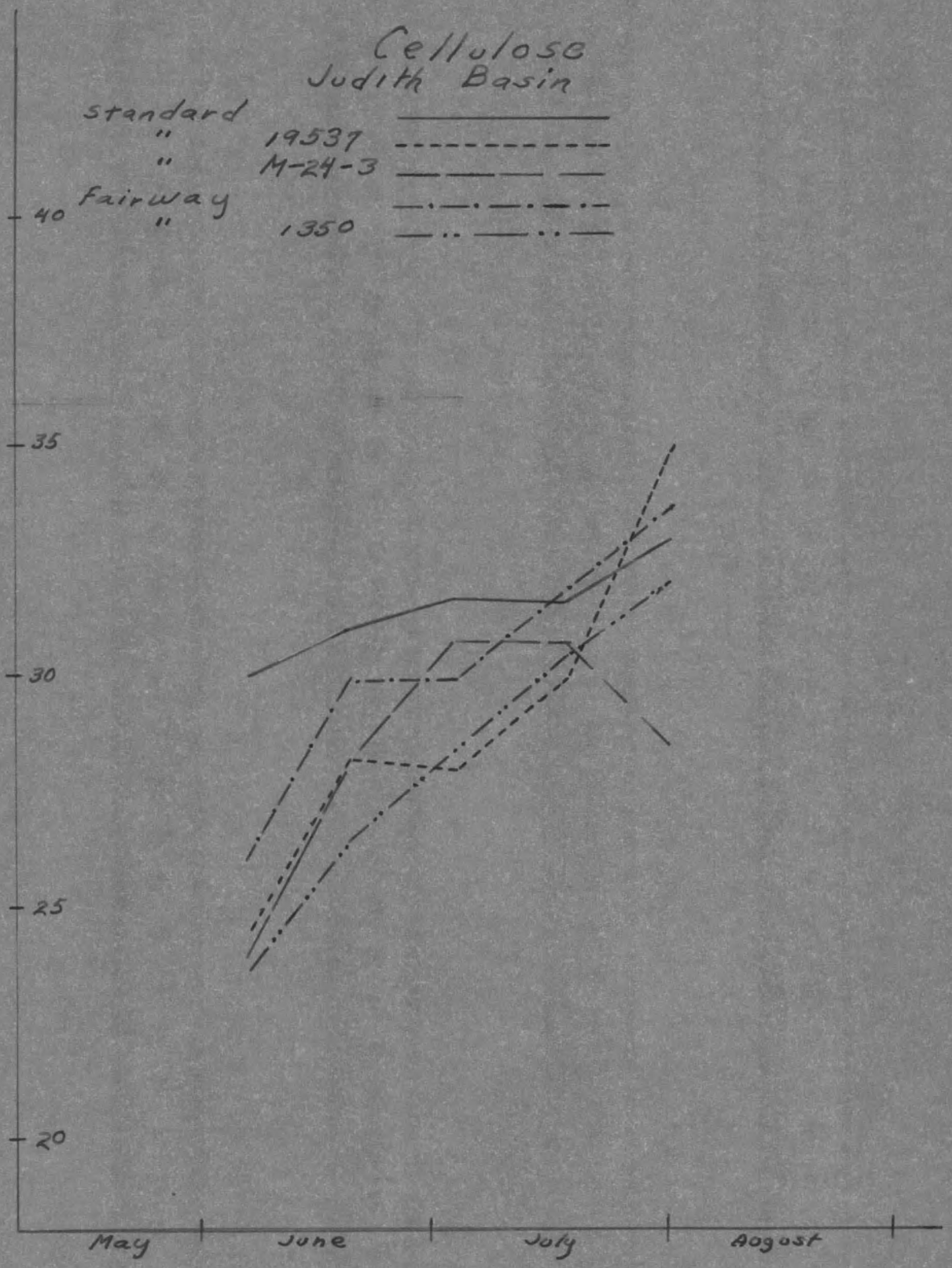

Cellulose
Judith Basin
standard
"
"
Fairway
"
19537
M-24-3
1350
40
35
30
25
20
May
June
July
August

Figure 4.

## EXPERIMENTAL RESULTS

### Lignin Content of Gallatin County Grasses

The lignin content in the five Gallatin County grasses (figure 1) increased from 5-6 per cent in the first collections on May 16 to 13-18 per cent on the last collection, September 4, 1941. It was interesting to note that the lignin content of the grasses increased more or less uniformly until June 26. On the next collection, July 10, there was a drop in lignin content of the four grasses that were in stage D. Only pauciflorum, which was in stage C, showed a slight increase in lignin over the previous collection date. A decrease in the lignin percentage does not necessarily mean that any lignin, once formed in the plant, is decomposed; it is more probable that the plants synthesized other constituents a great deal faster. Thus, although there may have been an actual increase in total lignin in a plant, if, simultaneously, the plant may have synthesized a large quantity of carbohydrates, the lignin content on a percentage basis would show a decrease.

### Comparison of the Lignin Content of Bromus marginatus and Bromus inermis

There was a close parallel in growth stages in inermis and marginatus. Both matured early, having reached stage D by the latter part of June, and remained in stage D until early in August. There was a close similarity in lignin content until stage E. During this period the lignin content of inermis rapidly increased. Inermis, on September 4, showed the highest lignin content of all grasses analyzed . Marginatus did not show this tendency,

and at the end of the growing season had the lowest lignin content of the grasses plotted in figure 1.

Comparison of Agropyron cristatum selections, Fairway and Standard

Fairway and Standard reached the same growth stages at about the same time. The lignin contents of these grasses were nearly the same during the early growth stages (A, B and C). Shortly after July 1, both grasses were in stage D. From this time on, Standard had a considerably higher lignin content than Fairway. Fairway and Standard reached stage D about two weeks after the brome grasses.

Agropyron pauciflorum

The remarkable feature of pauciflorum was that it matured late in the season, not reaching stage D until early in August. As might be expected, the pauciflorum had the lowest lignin content of the grasses tested during June and July.

Elymus junceus

Junceus was sampled from the campus plots, while the other Gallatin County grasses were grown at Fort Ellis. It was not possible to compare it with the Fort Ellis grasses unless other variables besides seasonal change were considered. It is of interest to note, however, that the lignin content increased from 4.4 per cent on May 18 to 11.4 per cent on July 3. Thereafter, there was a slight decrease in lignin content. The reason for the apparent decrease in percentage of lignin was not determined.

## Cellulose in Gallatin County Grasses

The cellulose content of the five Gallatin county grasses plotted on figure 2 increased from 20-24 per cent on May 16 to 37-42 per cent on September 4. There was a marked tendency for the cellulose to increase along with the lignin. On July 10 when the lignin content showed a decided drop, the percentage of cellulose in inermis and Standard likewise decreased. On August 7 there was a decided drop in both lignin and cellulose in marginatus. With the exception of pauciflorum, which had a low lignin and a high cellulose percentage in mid season, there was a general tendency for lignin content to parallel cellulose content. Inermis, at the end of the season, was highest in both cellulose and lignin. Standard, in the latter part of June, was highest in both cellulose and lignin. Inermis, in the first part of July, had the lowest cellulose and lignin contents of any of the grasses. What significance this correlation may have is not known except that the formation of lignin closely parallels the formation of cellulose. This may have value in determining the precursors of lignin.

Early in the season the cellulose content of Elymus junceus was about the same as the other Gallatin County grasses. Here again, the cellulose content closely paralleled the lignin, and on September 4, had 29.9 per cent cellulose, which was several per cent lower than the other Gallatin County grasses grown at Fort Ellis.

## Lignin Content of Judith Basin Grasses

Six grasses from Judith Basin were analyzed; sampling began on June 6

and continued at two-week intervals until July 31. The grasses at Judith Basin matured earlier than those in Gallatin County. At the time of the first collection on June 6, most of the grasses were already in or past stage B. It is to be regretted that data on the early stages of these grasses cannot be presented. The lignin content of five Judith Basin grasses appears in figure 3. Fairway, Selection 1350, had a definitely lower lignin content than Fairway until the last sampling (July 31), at which time the lignin content of both were about equal. Selection 1350 had the lowest average lignin content during the entire season, in comparison with the other four grasses studied.

Agropyron cristatum Standard and Selections 19537 and M24-3

Standard had a higher lignin content than the other selections when first collected on June 6. This was probably due to the fact that the Standard samples were more mature and were already in stage C when first collected, while the other selections were still in stage B. It is doubtful if other variations in lignin content were significant. In fact, the earlier values for lignin may all be questioned, due to the fact that the samples from Judith Basin contained more or less old grass from the previous season.

In general, the lignin values of the Judith Basin grasses in June were higher than those from Gallatin County at the same time. Late in the summer all grasses had very nearly the same lignin content except inermis in Gallatin County, which had several per cent higher lignin content than any other grass.

## Cellulose Content of Judith Basin Grasses

The similarity of the cellulose and lignin content of these grasses to the cellulose and lignin content of the Gallatin County grasses was pronounced. They were nearly the same. Standard had a much higher cellulose content when compared with the other grasses on June 6. Selection 19537 showed the highest cellulose content on July 31. Fairway, throughout the season, had several per cent more cellulose than Selection 1350, as noticed in figure 4.

In general, the Judith Basin grasses had a slightly lower cellulose content than the Gallatin County grasses.

## Elymus junceus - Judith Basin

The lignin content of junceus May 22 was 6.8. It gradually increased to 11.8 per cent on June 19 and throughout the rest of the season was nearly constant. At the end of the season Elymus junceus had several per cent less lignin than the other Judith Basin grasses. The cellulose rose gradually from 20.7 per cent in the latter part of May to 35.8 per cent early in August. Here the general tendency for lignin to closely parallel cellulose content was not apparent.

Comparing junceus from Gallatin County and Judith Basin, the lignin content until June 19 was practically the same. Thereafter, the lignin content in Gallatin County junceus decreased about 2 per cent, while in Judith Basin County it remained constant at about 11.5 per cent. The cellulose of junceus in Gallatin County was several per cent higher than Judith Basin

early in the season. This was reversed in mid-season, and on July 31 the Judith Basin junceus had 35.8 per cent cellulose--about 10 per cent more cellulose than the Gallatin County junceus.

## DISCUSSION

The chemical analysis of various Montana grasses has been undertaken with the hope that the results will have value in predicting feeding values. A complete chemical analysis of grasses at present is not possible, as our knowledge of plant constituents is far from complete. Also, if one were to analyze one grass in as much detail as present knowledge permits, it is doubtful if one could complete such an analysis in several years' time. Therefore, it seemed desirable to limit the analysis of grass to a few plant constituents that are of importance in predicting feeding values, understanding, however, that one, by interpretation of results, is not presenting a complete picture. Attempts were made, therefore, to choose a method of analysis which would have the greatest biological significance, and which would be capable of completion in a reasonable amount of time. For reasons given in the introduction, the methods of feed analysis (crude fiber, nitrogen-free extract) as presented by the Association of Official Agricultural Chemists were not used. The analysis of grasses was confined to six plant constituents; namely, moisture (for dry basis), total ash, ether extract, crude protein, lignin, cellulose and other carbohydrates. The moisture determination was necessary in order to calculate the other constituents from an air-dry basis to a dry basis. The "other carbohydrate" fraction was determined by difference.

A brief discussion of the factors that will affect the growth and composition of range grasses is perhaps necessary before any interpretation of results is attempted. Atkinson and Woodward 4/ point out that many factors

influence the growth and composition of pasture herbage. The chief factors are climate, seasonal variation, species, soil type, soil fertility, fertilization and frequency of cutting. Here again, it became necessary to segregate the factor of seasonal change and discuss it in as great detail as the analysis permits. Probably only when each factor affecting growth and composition of forage plants is understood will it be possible to have a complete picture of plant life.

In predicting feeding values by chemical analysis a study of the grass or feeding material is only half the picture. A study of the animal is just as important. There is a great deal of variation in animals of different species in their ability to digest certain plant constituents. Even in animals of the same species, all do not have the same ability for assimilation of food. When one is feeding mixed rations the digestibility of the different constituents is sometimes materially changed.

The Crampton and Maynard method for determination of lignin is satisfactory in that it is possible to obtain reproducible results. This method gives approximately one hundred per cent recovery of lignin in the feces. One main objection to this method is that it is very long and time-consuming. It is doubtful, therefore, if the determination of lignin will be put into practice by those running large numbers of feed analyses unless a more rapid method with suitable accuracy is found.

It is generally accepted that lignin is indigestible in itself, and also, by incrusting or some other means, it prevents the digestion of other plant constituents which otherwise would be utilized. Lignin may hinder

digestion by inactivating the enzymes by some means. It is indeed likely that phenolic groups or others present in the lignin complex have a toxic effect on the digestion enzymes. Waksman 93/ found that the presence of only 8 per cent lignin was sufficient in ruminants to reduce the rate of cellulose decomposition by fifty per cent. If lignin determinations are to be of real value in feed analysis, a great deal of similar research must be carried on.

Until the time that knowledge of lignin in relation to feed analyses is put on a quantitative basis, its usefulness is restricted. It is probable that lignin is a mixture of different compounds. When the chemistry of lignin is further elucidated, the part that lignin plays in restricting digestibility can be put on a sound chemical basis.

The crampton and Maynard method for cellulose was not changed in principle; however, by use of centrifugal separation it was possible to shorten the time required for each determination.

As previously stated, plant cellulose is a mixture. The chemical and nutritive properties of mixtures are difficult to determine accurately. The effect of lignin on the digestibility of plant cellulose is a complicating factor.

Percentages of cellulose and lignin were determined by loss of weight on ignition. The percentage of ash was about 1 per cent of the total weight. The ash probably contained, in main, silicon dioxide. A question naturally arises as to whether the ash present was due to adsorption during analysis or was ash originally present as a constituent part of the lignin and cel-

lulose. If low-temperature ignitions were employed to ash the cellulose and lignin residues, phosphorus might possibly be found. Phosphorus, if present in the lignin and cellulose ash, should be critically examined.

The different sugars present in grasses are of much interest, since they provide the main source of energy for animals. The determination of the various pentoses and hexoses in the water-soluble hemicellulose, and cellulose fractions, might lead to valuable information from a nutritional standpoint. No doubt the cellulose in the grasses analyzed contained pentoses (probably zylose). Further investigation of the carbohydrates of these grasses is desirable.

The determination of cellulose and lignin is a definite step forward; the foregoing discussion was presented to point out that research in this field is only in its infancy.

## SUMMARY

Lignin analyses on grasses from Gallatin Valley indicated a similarity of all species tested, during May and June, 1940. By the end of August, however, inermis had reached a high of 17.8 per cent and on this basis was probably the least-digestible of the grasses. Contrasting with this was marginatus, which ended with a lignin content of only 13.3 percent.

Standard cristatum showed a tendency to contain more lignin that Fairway cristatum, and on this basis would be judged slightly inferior. Lignin values for these two grasses fell between those for the two brome grasses.

Pauciflorum showed a definite superiority over the other Agropyrons tested in that it maintained a lower lignin content during May and June.

At Judith Basin the five cristatum selections sampled matured a month earlier than cristatum did in Gallatin Valley, but followed a similar path of increase in lignin content. The special selections of cristatum contained slightly less lignin than the regular plots. Here, as in Gallatin Valley, Fairway contained less lignin than Standard.

Cellulose ranged from approximatey 20 to 40 per cent, the changes in cellulose closely paralleling changes in lignin content as the season advanced.

ACKNOWLEDGMENT

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