OXIDATION AND METHYLATION
OF THE
ACCESSIBLE FRACTION OF CELLULOSE

A Thesis

by

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Submitted to the Faculty of Graduate
Studies and Research in partial fulfilment of the requirements of the
degree of Doctor of Philosophy

McGill University
October 1952

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Professor C.B. Purves for his continual inspiration and his ever available friendly counsel and interest throughout the course of the present research.

The author also wishes to express grateful acknowledgment of financial assistance from the Department of Veterans' Affairs, from the Allied Dye and Chemical Corporation in the form of a Fellowship in 1949-1950, and from the Pulp and Paper Research Institute of Canada in the form of grants in the summers of 1949, 1950 and the spring and autumn of 1951.

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GENERAL INTRODUCTION

Interpretation of numerous X-ray studies has shown that cellulose consists of crystalline regions embedded in a matrix of more randomly distributed amorphous material. Since both the proportions and the detailed structure of these regions have great influence on physical properties, such as tenacity and flexibility, the adsorption of liquids and gases and on the ability of the fibers to swell in liquids, their measurement is of considerable interest. A chemical analogy of the strictly physical definition, divides the fiber into accessible and inaccessible regions, the accessible portion being defined as that into which the reagent can penetrate. Since the amount of accessible material may be increased by swelling or decreased by crystallization depending on the nature of the reaction, the estimates from various reactions usually differ. Nor will the amorphous region necessarily coincide with the accessible, the one being arrived at by a truly physical measurement, the other resulting from the imposition of a chemical reaction upon an already complex physical system.

This concept of accessibility, however, continues to arouse wide interest since the processing of cellulosic materials, of major importance to most countries, necessarily depends upon the chemical behavior of the fibers, rather than on their ultimate X-ray pattern.

The first part of the present research consists of a study of the amount of various celluloses accessible to oxidation by a non-swelling solution of chromium trioxide used under strictly standardized conditions. The effects on accessibility of solvent exchange from water to organic liquids like benzene, of drying from benzene, and of slight mechanical pressure have been noted, and the accuracy of the method greatly improved. A technique for determining the true dry weight of a benzene-wet sample without prior drying has made it possible to determine the accessibilities of three types of swollen linters. These accessibilities have then been compared with those found by another non-swelling method involving the methylation of samples previously impregnated with a solution of thallous ethylate in benzene.

The second part of the work was devoted to a more detailed study of the superficial methylation produced by thallous ethylate followed by dimethyl sulphate in benzene. In spite of severe technical difficulties caused by degradation during methylation and by occluded thallium salts, some information was obtained concerning the distribution of methoxyl groups within the glucose units, and along the lengths, of the macromolecules in the accessible portions of a highly swollen cellulose sample.

HISTORICAL INTRODUCTION

In the present work no attempt will be made to cover the vast historical background on which present day concepts of the physical and chemical nature of cellulose are based. Well-known text books (1)(2) perform this function. Only the more pertinent topics will be discussed in some detail, and even in this narrower field, the following short sections on the molecular and crystalline structure of cellulose depend extensively on the recent review by Hermans (3).

By 1935, the chemical unit of cellulose was well established as a linear polymer of anhydroglucopyranose units in which an oxygen bridge of the β-configuration joined together the 1 and 4 carbon atoms of adjacent hexose units. Such a thread molecule is reproduced in Fig. 1 using the type of plane projection introduced by Haworth (4). The pyranose residues on each end of the formula each differ from the anhydroglucose repeating unit in having 4 rather than 3 free hydroxyl groups, one of which is aldehydic in character. It follows that as the length of the chain increases, the determination of these end groups becomes progressively more difficult (5)(6). Staudinger has defined cellulose as embracing the higher members of a whole range of polymeric homologues of which glucose represents the first and cellobiose the second member. The degree of polymerization represented by n in Fig. 1 thus varies over a wide range and is dependent on the history of the fiber and its source. Typical chain length distribution step curves (Fig.2) for cellulose from aspen wood (a) and cotton (b) are described by Heuser and Jörgensen (7).

Fig. 1, however, tells little about the spatial configuration of the cellulose molecule. This aspect is reviewed in considerable detail by Hermans (3) and is based primarily on geometric considerations. The similarity of the hexose repeating unit containing 5 carbon and 1 lactonic oxygen atom to cyclohexane allows the unit to take two limiting structures, an "armchair" form and a "bath" form, in both the α-configuration (starch) and the β-configuration (cellulose). The "arm chair" form is rigid and best exemplifies the linear character of cellulose for when each second unit is rotated through 180° on its longitudinal axis, the glucosidic oxygen bridge being retained in a relatively strain-free position, the polymer then extends unilaterally. The consequences of these deductions are indicated in Fig. 3 (3) which represents the cellobiose unit. The nuclear atoms of the ring are now distributed in two parallel planes and make up a band approximately 9 A° in width and 4.5 A° in thickness. A noteworthy feature, however, is that all the hydroxyl groups project from the sides of the band and form two hydrophilic faces, while the hydrogen atoms projecting above and below the planes provide hydrophobic regions. A second polymer chain, on approach, will then have definite stimuli for parallel orientation and on addition of other macromolecules build up a

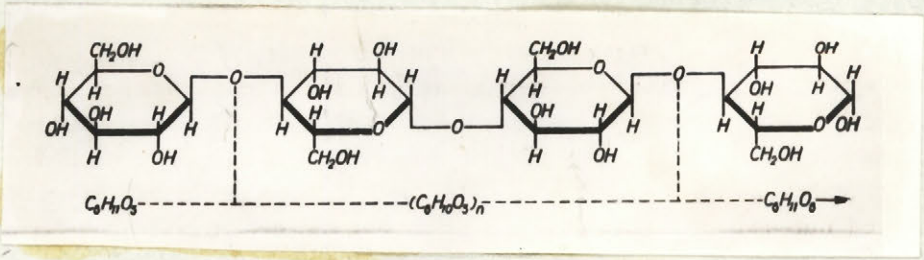


FIG. 1 The cellulose macromolecule

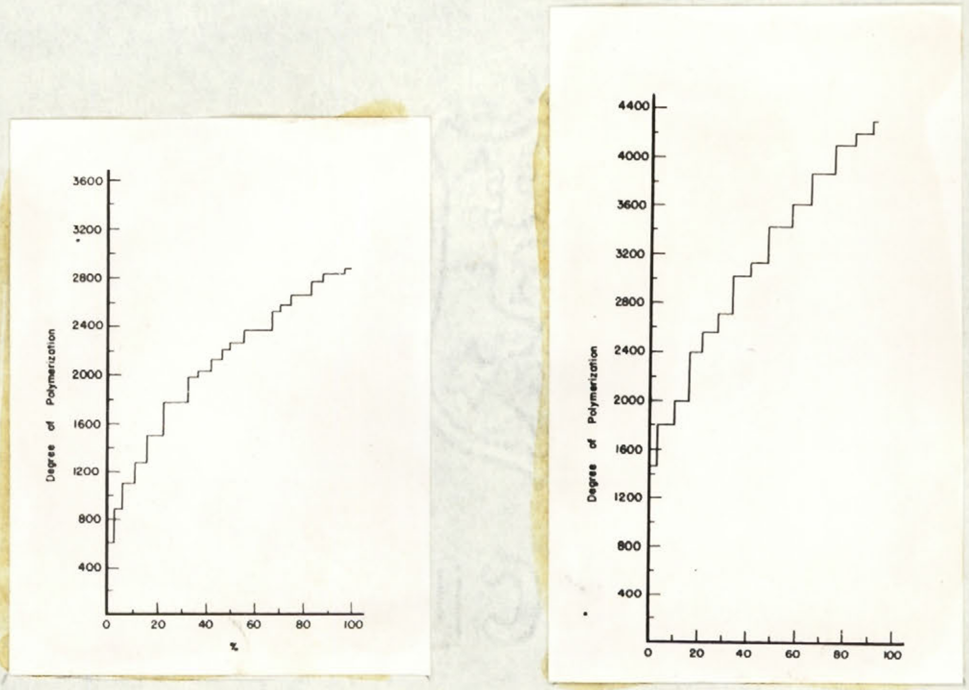
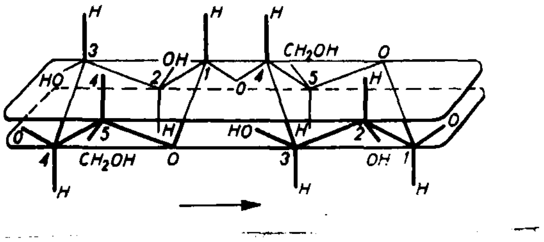


FIG. 2 The chain length distribution of
(a) Aspenwood cellulose
(b) Cotton from unopened boll.

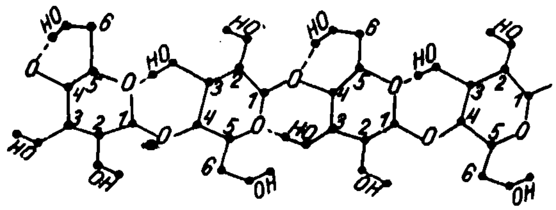
regular crystalline structure. Hermans states that these contrasting fields of force in the cellulose molecule may explain in some degree its insolubility. On the other hand, starch in the α-configuration can best be visualized using the "bath" form. This conformation would allow the molecule to assume a spiral form having five or six glucose units per coil.

Hermans states that "there can be no question of a chain with all the ring planes parallel". The idealized version in Fig. 3 must be refined further before spatial dimensions in agreement with X-ray data (p. 8) are possible. To provide a strain-free glucosidic link and the proper unit length, the chain must be buckled in its vertical aspect only or have a moderate zig-zag in both the vertical and horizontal planes. In the latter case, the ring planes form an angle of not more than 10°30' with the horizontal plane and give a fiber period of the correct length. A top view of a Stuart model of such a chain is reproduced in Fig. 4. Here the distances (2.5-2.6 A°) between the hydroxyl groups and the lactonic and glucosidic oxygen atoms are such that all the hydroxyl groups except those in the 2-position are capable of hydrogen bond formation within the chain itself, thus adding to the rigidity and insolubility of the chain.

The X-ray spectrography of cellulose from almost all natural sources, whether cotton, wood pulp or the product of a microorganism, reveals the same structure for the crystalline portions and such samples are called native cellulose or cellu-



<u>FIG. 3</u> Nuclear frame of a cellobiose unit.



<u>FIG. 4</u> Plan view of a Stuart's model of the cellulose chain with probable intramolecular hydrogen bonds.

lose I. The familiar monoclinic unit cell, a crystallographic representation of the smallest unit which can produce the known X-ray diffraction pattern, is illustrated in Fig. 5. Its parameters enclose four anhydroglucose units, mounted on digonal screw axes of alternating polarity. This crystallographic b axis defines the length of the unit cell, that of a cellobiose unit, and practically coincides with the fiber axis in well-oriented celluloses such as ramie fiber. In this direction the glucose units extend to the full polymer length and are held together with a covalent bonding energy of approximately 50 Kcal per mole, giving to the cellulose its tensile strength. In plan, the top of this unit has a slope and is bounded by the a and b axes meeting at an angle of 84°. The a axis, the dimension of breadth, is in fact the effective width of an anhydroglucose unit. In this plane the oxygen atoms of adjacent glucose units are closer together (2.5 A°) than would be expected if Van der Waal's forces alone were operating. Hydrogen bonds of 15 Kcal per glucose unit are therefore postulated. The c axis is the depth of two anhydroglucose units. The distances separating the atomic centres of adjacent chains in this plane are relatively great (3.1 A°) and the binding forces are probably solely of the cohesive or Van der Waal's type. Cellulose therefore assumes the composite properties of a chain and a layer lattice. The dimensions of this unit cell, derived by Meyer and coworkers on the basis of Haworth's model of cellobiose, are: $\beta = 84°$; $a = 8.35$ A°; $b = 10.3$ A°; $c = 7.9$ A°. More recent figures given by Kiessig for the unit cell of ramie fiber were $\beta = 84.5°$;

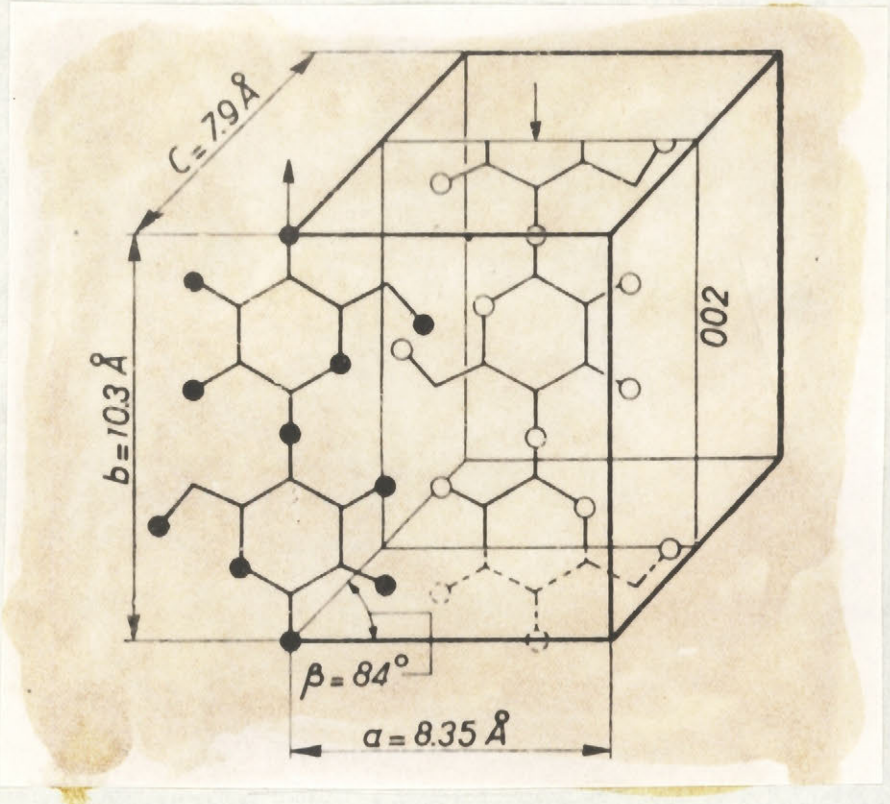


FIG. 5 Unit cell of cellulose.

a = 8.17A°; b = 10.31 A°; c = 7.84 A° (8). The results arrived at by the X-ray diffraction technique (9)(10)(11) are a compromise between physical difficulties, such as obtaining a cellulose target in which the crystallite orientation is consistent, and present concepts of the chemical nature of the units involved, and should not be considered final. Peirce (12) observes that the above unit cell of cellulose is essentially orthorhombic, whereas the pattern reveals a monoclinic lattice. To introduce a crystalline feature capable of maintaining the distortion to the monoclinic habit, Peirce postulates chelate bonding between hydroxyl groups in one of the four glucose units in each cell.

The Fine Structure of Cellulose

The polymer thread and the unit cell are the defined individuals on which the macrostructure of cellulose, such as constitutes at least 98% of the purified cotton fiber, is based. This macrostructure is differentiated by measurements of a physical nature, such as moisture absorption, heat of wetting, density and X-ray diffraction, into crystalline and amorphous regions. The proportion of each depends on the type of cellulose and the method by which it is determined, as can be seen in Table I.

The crystalline region is frequently defined as that fraction which gives rise to a coherent X-ray diffraction pattern. Since a depth of 10 to 20 layers of oriented polymers

TABLE I

PERCENTAGE OF CRYSTALLINE MATERIAL IN VARIOUS CELLULOSES(13)

Method of Determining Crystallinity	Type of Cellulose		
	Native Cotton	Pulps	Regenerated
X-ray analysis	70 ± 2	...	39 ± 3
Sorption isotherms	68	...	35
Density determination	60	50	25
Recrystallization of amorphous cellulose	...	...	35

must be present to give a well defined diffraction pattern, it follows that in those regions that produce the diffuse background on the film, the macromolecules are in greater disarray. Mark (14) in his discussion of fiber structure, reviews the different interpretations of these and other data with their pictorial representations. The micelle theory (circa 1927) in which cellulose was built up of brick-like crystallites, having voids between, into which gases and liquids could penetrate, soon gave way to the fringed micelle theory of Astbury (15). In this theory the crystallites are no longer discreet individuals with definite bounds but become those ill-defined regions in which sufficient long macromolecules are orientated to a maximum degree. These points are brought out in Fig. 6 which Campbell (16) has used to show that the total area in cellulose available for adsorption and wetting is very great when compared to the external surface of the fiber. Here the macromolecules are in

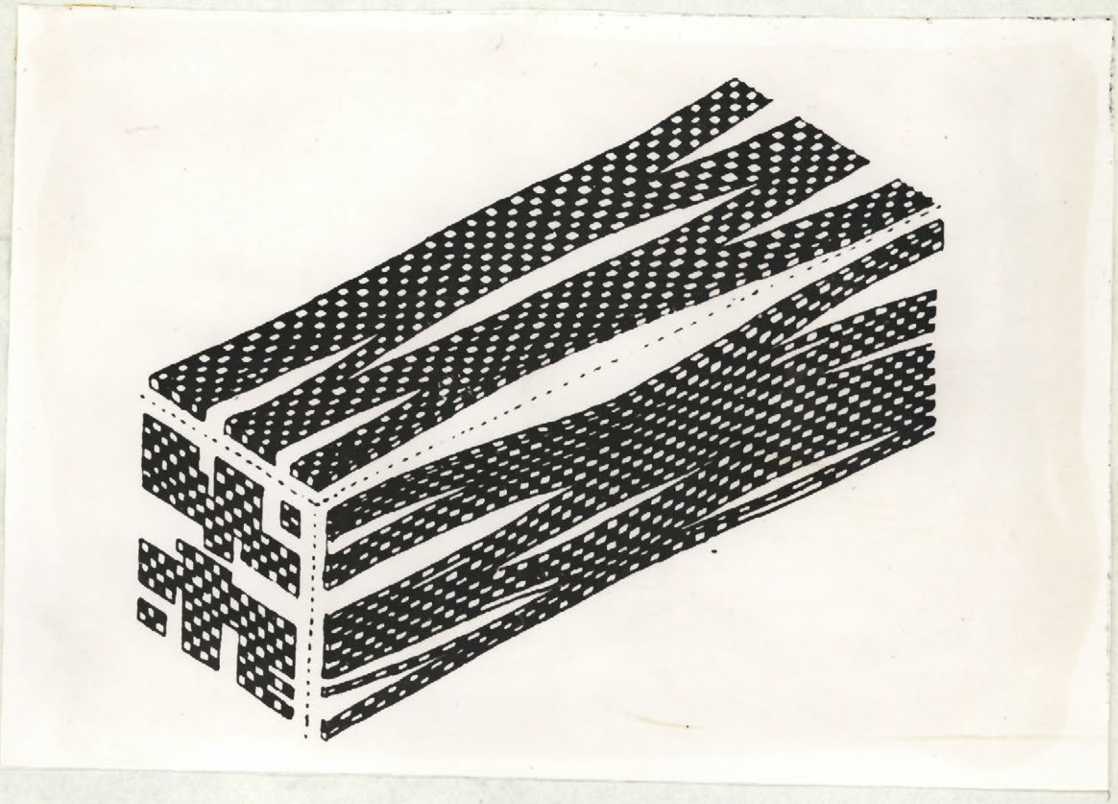


FIG. 6 Concept of structure in fibril layers. Unit parts of cellulose molecules regularly spaced. No definite positions of ends of cellulose chains. Crystal structures bonded to each other by branching.

considerable alignment, have no definite ends and may wander through several crystalline regions. In the amorphous portion, the state is not complete disorder but a lower order of orientation in the lateral dimension of the chain bundles.

The following generalized definition of the micellar structure of cellulose, provided by Keays (17), may be compared with Fig. 7 by Frey-Wyssling (18).

Crystalline Regions

At intervals throughout the cellulose structure the three-dimensional arrangement of the glucose anhydride unit is repeated in such a manner as to build up localities sufficiently large to give coherent X-ray diffraction patterns. It is assumed that the crystallites are not all of the same length or of uniform cross-section, that the crystalline-amorphous interface is not sharp, and that a fraction of the crystallites may protrude in the form of fringes into the amorphous region.

Amorphous Regions

The amorphous regions extend between the crystallites and act as a "grundmasse" for them. The cellulose chains are more or less randomly kinked and in a state of more or less random orientation. In short, the amorphous component may comprise an infinite variety of structures ranging from a few parallel long-chain bundles, or embryonic crystallites, to very short chain segments, with completely random orientation.

It is interesting to note that Svedberg (19) has isolated crystalline particles of cellulose having the same dimensions (500 A° in length by 50 to 100 A° in width) as the minimum figures found by X-rays (20) for the crystallites *in situ*. Svedberg believes these units to be the micelles themselves. The cellulose samples were first nitrated without degradation after which chain length distribution curves were obtained by a combination of fractional precipitation, ultracentrifugal sedimentation and osmotic measurements. Native cotton as well as processed cellulose gave a maximum at a degree of polymerization of 70 to 100 and after acid hydrolysis most of the cellulose was found in the range 100-200. It is evident that a polymer chain having a degree of polymerization of 3000 or 15000 A° will in all probability pass through many crystallites.

The data on the diameter of the crystallites, obtained by the usual wide angle X-ray diffraction, have been recently augmented by Heyn (21) who has studied the diffraction and scattering of X-rays within very small angles adjacent to a primary beam passed through a carefully aligned fiber bundle mounted at right angle. Since change in the intermicellar distances in the cellulose by swelling leads to a corresponding decrease in the extent of scattering, Heyn concludes that the average period might be a measure of the lateral centre to centre distance of the crystallites. Selected values from this work appear in Table II.

The intermicellar spaces are accessible to swelling media. In addition, native (but not artificial) fibers have

TABLE II

SCATTERING AND APPROXIMATE IDENTITY PERIOD FOR DIFFERENT FIBERS

	Ramie	Viscose	Mercerized Cotton	Cotton
Maximum scattering angle	1°18'	1°9'	51'	36'
Smallest period (in A°)	34	38	51.5	73
Average period (in A°)	68	73	95	14.6

a system of longitudinal microscopic and submicroscopic faults permeating the structures from primary wall to lumen, which are accessible to colloidal dyestuffs. This aspect has been studied in great detail by Frey-Wyssling who deposited gold and silver metal in the interstices and then subjected the fibers to X-ray measurements. He concludes that the larger capillaries are about 2500 A° in length and have a maximum diameter of 400 A° (22). The finer capillaries, having a diameter of about 100 A° (23), are assumed to be holospaces between and parallel to the fibril bundles.

The fuzz hairs, up to 10 mm. in length and averaging 0.2 mm. in diameter, remaining on the cotton seed after the lint fibers have been removed by ginning, are termed cotton linters. Their morphological structure, reviewed by Hock (24) and Hermans (25) consists of a thin outer membrane, called the primary wall, of secondary walls and the central channel or lumen. In the primary wall the cellulose macromolecules are probably in crosswise arrangement and occlude pectic materials and waxes. This tough unreactive skin (26) is not able to swell and when the secondary wall cellulose is mercerized will strip back or burst the skin to give "balloons" and "dumb bells". Schramek and Stenzel (27) who removed this cuticle from spruce summerwood tracheids by cautious treatment with sodium hydroxide and carbon disulphide, have been able to unroll spirally a portion of the secondary wall which had longitudinal but no lateral fibrillar structure. In cotton the fiber windings are in the outside secondary wall and may have a S or a Z configuration which can reverse its direction several times along the fiber length. The main portion of the secondary wall consists of day and night growth rings in which the cellulose macromolecules are oriented at approximately 45° to the fiber axis. No decision has yet been reached as to whether or not a definite supermolecular unit is responsible for the microscopically observable fibrous structure of the secondary wall cellulose. Morehead (28) has disintegrated these fibrils, using ultrasonic vibration, and by electron microscopy demonstrated the existence of minute fibrillae but gave no average dimensions. The results of Kinsinger and Hock (29) are representative of a great volume of work on these fibrillae. Using the metallic shadow casting technique, they obtained electron photomicrographs of beaten natural fibers, in which the fibrils have diameters ranging from 90 to 400 A° and showed a periodic variation at intervals of about 150A° along the fiber axis.

The scientifically popular diagrammatic representation of Frey-Wyssling in Fig. 8 (30) approximates our present knowledge of the structure of cellulose fibers.

The Effect of Alkali on Cellulose

Of the many double compounds known to be formed when an alien molecule penetrates the crystal lattice of cellulose, only those produced by sodium hydroxide and water are of immediate interest. After studying the X-ray diffraction patterns of cellulose specimens exposed to alkali over a wide range of concentration (0 to 50%) and temperature (-20° to 100°) Sobue, Kiessig and Hess (31) published a rather complex phase diagram which contains native cellulose and four allotropes of sodium cellulose. The work of Sisson and Saner (32) carried out under the same conditions, presents a simpler picture.

The changes taking place in the a c plane of the lattice under the action of alkali and water in our range of interest are represented diagrammatically in Fig. 9 which is taken from Meyer, Misch and Badenhuizen (33). When cellulose I (native cellulose) or cellulose II is completely mercerized at ordinary temperature, sodium cellulose I is formed. When freed of alkali by washing with cold water, the undried fiber provided Sakurada and Okamura (34) with a completely new X-ray pattern. This modification has been termed "water cellulose" since each glucose unit corresponds to 4 moles of water of which one is contained

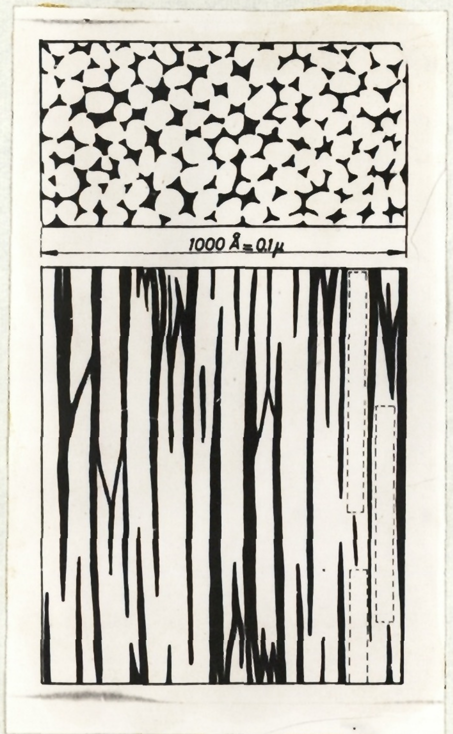


FIG. 7 Diagram of micellar texture of cellulose fibers.

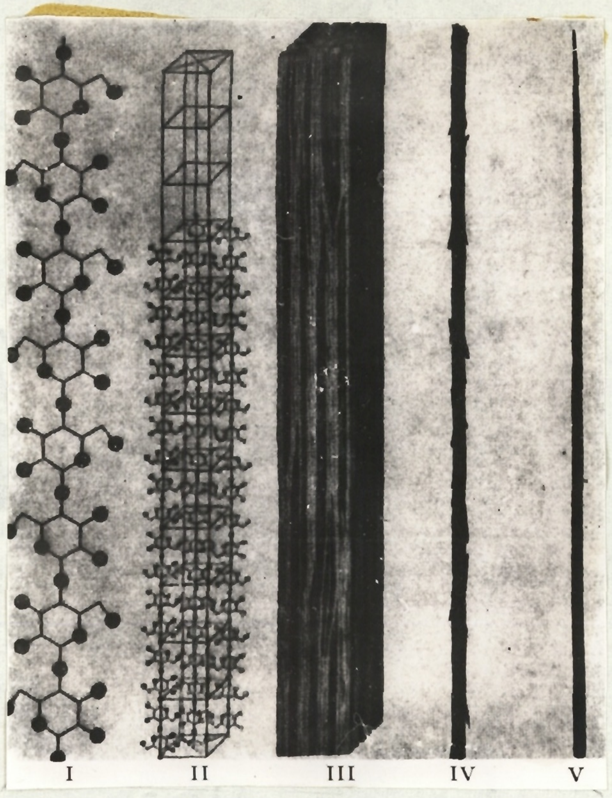
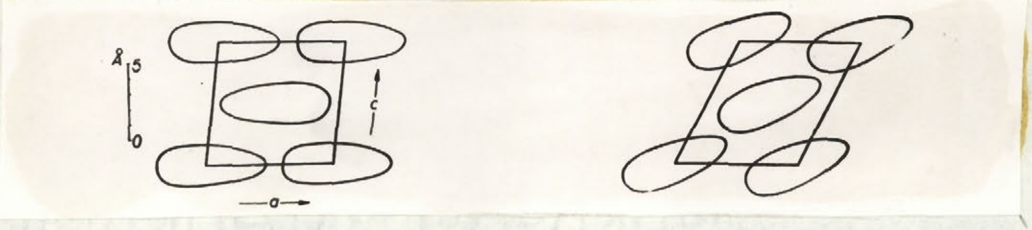


FIG. 8 Diagrammatic representation of the entire structure of the cellulose fiber.

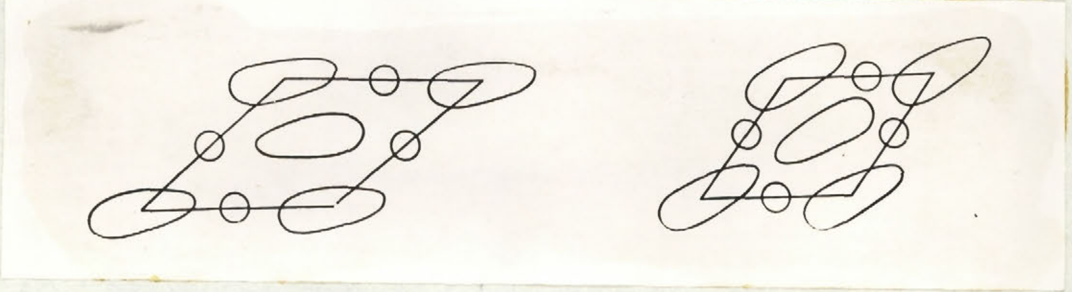
I Cellulose chain $M = 300 \times 10^6:1$
II Cellulose lattice $M = 100 \times 10^6:1$
III Micellar structure $M = 10 \times 10^6:1$
IV Fibril $M = 10^5:1$
V Flax fiber $M = 10^4:1$

in the crystal lattice. Meyer, Misch and Badenhuizen (33) have proved the identity of "water cellulose" with the cellulose hydrate shown in Fig. 9d and showed furthermore that this labile structure loses water readily on standing. Hermans and Weidinger (35) later found that cellulose hydrate II (i.e. water cellulose) having an approximate composition of one and one-third moles of water per glucose unit, was slowly transformed to cellulose hydrate I (with one-third mole of water per glucose unit) slowly on standing in water at room temperature, and rapidly by boiling in water for a short period (36). Careful drying of the hydrate yields cellulose II which on contact with moist air reverts to the hydrate I form. The formation of two hydrates can be visualized as involving a stretching of the cell dimensions perpendicular to the 101 plane (top left to lower right in any unit cell in Fig. 9) with a separation of the hydroxyl groups of adjacent chains. The increases in the unit cell volumes in passing from cellulose II to hydrate I and to hydrate II are 5.7% and 22.6% respectively. On a macroscale this increase is observed as a tendency for the fiber length to decrease, with an increase in lateral dimensions, and an overall increase in volume. By varying the sodium hydroxide concentration and the reaction temperature over a wide range, Richter and Glidden (27) have shown conclusively that swelling was greatest for any given temperature at 10 to 12% concentration and increased with a decrease in temperature. The greatest weight increase recorded for a moist cotton linters specimen was 1800%, at their lowest temperature (5°) and at 10%



Cellulose I (native)
a = 8.35Å
c = 7.9Å
b = 84°

Cellulose II
(hydrate cellulose)
a = 8.14
c = 9.14
B = 62°



Alkali cellulose I
a = 12.8
c = 13.2
B = 40°

Cellulose hydrate
a = 10.0
c = 9.8
B = 52°

FIG. 9 Diagram of lattice types

caustic concentration. Saito (38) states that the point of maximum swelling, regardless of temperature or concentration, corresponds to an adsorption of one mole of sodium hydroxide per cellobiose unit. The swelling and solubility of many types of cellulose in 10% caustic soda was comprehensively studied by Eisenhut (39). The selected values in Table III show that swelling is considerable, that the solubility of each specimen is highest at the lowest temperature and that a quite different temperature-solubility increment exists for the various types of cellulose.

TABLE III

SWELLING AND SOLUBILITY OF DIFFERENT CELLULOSE FIBERS[a] (39)

	Swelling (%)	Solubility in 10% caustic		
		30°	15°	5°
Cotton linters	45	0.7	1.5	3.1
Spruce sulphite pulp	45	13.6	19.	22.
Viscose staple from linters	80	0.9	7.5	56.

(a) degree of polymerization approximately 775

From such data and from the many studies of sorption isotherms, differential heats of wetting and similar physical phenomena, there is today general agreement that the swelling of cellulose in alkali or water involves the following steps (40) (cf 41).

1) When cellulose I (native cellulose) is exposed to water, an essentially topochemical reaction first occurs. The crystal lattice remains intact while the hydrate I is formed throughout the truly amorphous portion, and the hydrate II as well, in those regions where the cellulose chains have considerable freedom of movement. Heat is evolved during the initial stage but on further addition of water the fiber becomes saturated and the evolution of heat diminishes towards zero.

2) When cellulose II (regenerated cellulose) takes up a small amount of water, a competition of hydrate formation will occur between the crystalline and amorphous regions. When sufficient water has entered, the lattice form will be hydrate I, the amorphous portion will be hydrate II. The free energy of hydration is not sufficient to expand the lattice to the hydrate II dimensions.

3) When the crystallites have been expanded previously by drastic treatment such as low temperature mercerization or xanthation, water can be substituted in the unstable lattice at low temperature. The lattice will then be bydrate II while in the amorphous regions the fully hydrated cellulose chains are "dissolved" in the absorbed water.

The Density of Cellulose in Various Modifications

Closely related to the topic just discussed, is the measurement of that volume in a visible fiber which is actually occupied by cellulose, or in practice, the determination of the density. Accurate microscopic measurements showed that the density of the cell walls of cotton should be 1.15 (42), whereas in fact the densities obtained in benzene were approximately 1.5. It was thus evident that even non-polar liquids like benzene were able to penetrate into ultramicroscopic spaces in the fiber structure.

The variation in the density of cellulose as determined by different methods, which is illustrated in Table IV (43), has caused much speculation (e.g. 44). To account for the high density of 1.61 in water, as compared with the figure of 1.59 calculated as the density of the unit cell, massive compression of the water has been postulated (45).

TABLE IV

DENSITIES OF CELLULOSE FIBERS IN VARIOUS BUOYANCY AGENTS

	Medium Used			
Material	Helium	Toluene	Water	Heptane
Cotton	1.567	1.550	1.6095	1.540
Mercerized cotton	1.550	1.536	1.6066	...
Viscose rayon	1.548	1.543	1.6084	1.516

Hermans, Hermans and Vermass (46) commented that the macroscopic concepts of density cannot be applied in all cases to systems such as cellulose, where the "pores" decrease in size from microscopically-observable holes to spaces of the same order of magnitude as the molecules of the buoyancy medium. Of the limiting case they state (41): "The problem may be compared to that which would arise if one wished to estimate the density of a pile of spheres by means of a medium consisting of smaller spheres, knowing only the weight of the smaller spheres included in the unit of volume. The results depend upon the relative dimensions of the two kinds of spheres and is governed by the rules of spherical packing. If the spheres constituting the medium are small enough to penetrate <u>even</u> into the voids between the larger spheres, it will moreover be found that the density of the pile of spheres is suddenly far greater" (as is the case with water) "than when such penetration cannot take place" (as in the case with polar liquids). In developing and supporting their case with numerous other experimental data, they are able to explain the variations in densities found in the different buoyancy media and conclude that the most desirable information is given by non-polar liquids such as benzene.

They also point out that when the percentage of amorphous material in the cellulose has been increased as by swelling, the <u>apparent</u> density will be higher than the true density since the greater penetration of the medium into the amorphous portions is concomitant with a decrease in the apparent specific volume, cellulose hydrate II weighed in water at 4-5° having a density of 1.642 (45).

The Accessibility of Cellulose

At present the terms "accessible" and "inaccessible" are used in apposition to "crystalline" and "amorphous" the former terms describing the availability of the cellulose to chemical reagents, while the latter are based on physical measurements and mathematical analysis. The "accessible" fraction is not necessarily identical with the "amorphous" fraction but will be similar, since in the more loosely packed, randomly distributed amorphous regions, penetration and diffusion of reagents will be faster than in crystalline localities. The critical regions, to which are due the discrepancies in these values, are probably the micellar fringes where the crystallites taper off into leaflets of lower order of crystallinity. The leaflet macromolecules, held together by Van der Waal's forces and hydrogen bonds, might then behave as inaccessible material in the initial stages of chemical reactions, yet will not contribute to the X-ray diffraction pattern because of their low order of crystallinity. On the other hand, another large portion of the leaflets would occur on their extensive surfaces, and therefore be accessible.

It is to be expected that celluloses of different origin will vary in the degree and kind of order throughout the fiber. Furthermore, the cellulose fiber should be considered to be a system in a state of physico-chemical equilibrium, whose properties can be changed, possibly in ways not yet reduced to precise measurement, by the addition or subtraction of any form of energy. The action of swelling agents such as water or sodium hydroxide in the preferred state of native cellulose, cellulose I, has been discussed. The result of drastic mechanical action such as grinding or beating is well known. Cellulose is defibrillated by ultrasonic vibration (49) and destroyed after sufficient exposure in the electron microscope (50). After probable degradation by sunlight in the mature boll (51, 52), cotton is ginned, digested in mild alkali, bleached and dried (53). Subsequent heat treatment, or drying at atmospheric pressure or in vacuum from water, or after solvent exchange, also changes the properties of the fiber measurably (17).

It does not seem probable that accurate differentiation of the segments of a single macromolecule into two distinct states can be made, nor that the absolute structure in situ of the cellulose specimen will be quantitatively reproduced by the means at our disposal. However, portion of the cellulose accessible to a given reagent under the conditions of the experiment will be a definite empirical quantity which will reflect the physico-chemical conditions in the fiber. Many of the methods used to determine the accessible portion depend on the interpretation of reaction rate plots obtained by treatment of the cellulose in a large excess of reagent. The general form of such a rate curve, shown in Fig. 10 indicates the initial fast reaction which is attributed to readily accessible material, and the later more linear portion which results from a slower attack on the non-accessible regions.

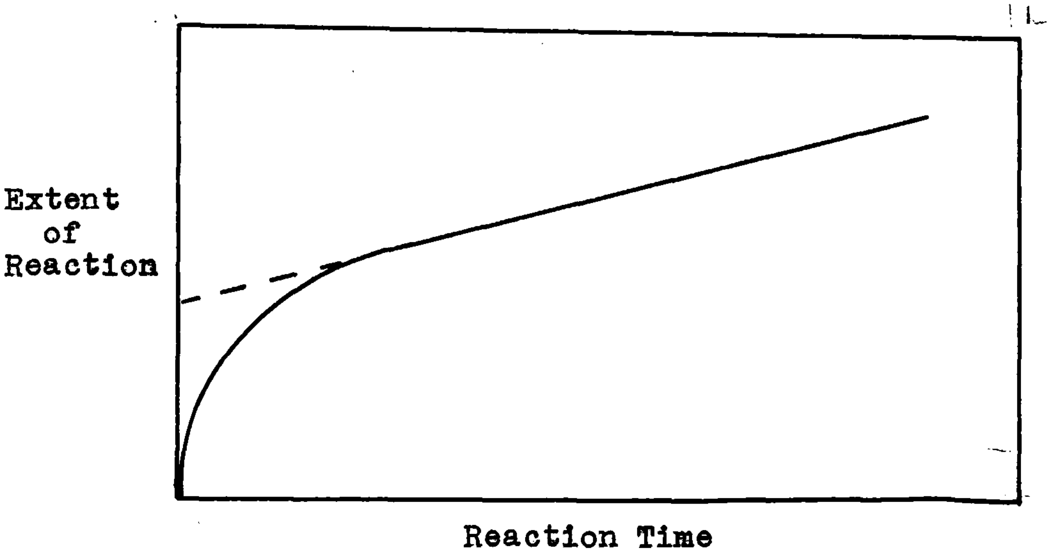


FIG. 10 Ideal Reaction-Rate Curve in the Measurement of Cellulose Accessibility.

Chemically, cellulose behaves like an alcohol and a glycoside. Attack on the glucosidic link, as by the hydrolytic oxidation method introduced by Nickerson (54) alters the structure markedly, since the freed lengths of macromolecules or leaflets align themselves to give regions of higher crystalline order, resulting in a lower accessibility value. It would appear more desirable to measure the accessibility of the hydroxyl groups as by deuterium exchange (55), thallation-methylation (56) or by reaction with sodium (57) in which the reactivities of the primary and secondary hydroxyl groups are similar, or by the formic acid method (58) which is based on the higher reactivity of the primary hydroxyl group. The effect of solvent should also be noted, since the molecular dimensions of the reactant or its solvent carrier may be a limiting factor, while in aqueous or other swelling media, the accessibility values tend to be high owing to the increase of the accessible fraction during the reaction. The periodate oxidation technique (59) will also give a value for accessibility based on cleavage of glycol groups in the desorganized regions, while the action of chromium trioxide (60) is probably more complex.

Although experimental details have not been disclosed, the formic acid determination of accessibility reported by Tarkow and Stamm (61 cf. 58) is of much interest. This method is based on the ability of formic acid, in excess and under rigid conditions, to react only with the primary hydroxyl groups of polyanhydroglucoses. When the residual mixture was heated under reflux with an excess of acetic anhydride, one mole of carbon monoxide was evolved per mole of formic acid remaining after the esterification. When the initial formic acid content for a series of glucose solution was plotted against the experimental value for combined formic acid, a curve was obtained that became horizontal when the formation of glucose diformate was complete, only the 4 and 6 hydroxyl groups having reacted. Similar curves were plotted for various cellulosic materials, the horizontal portion of the curve in each case giving a value for combined formic acid that was lower than the figure of 28.7% found for starch (calculated for starch monoformate as per cent by weight of original material 28.4%). The accessibilities listed in Table V column 2, which were calculated using the starch value of 28.7 as equivalent to 100% accessibility, are then the percentages of primary hydroxyl groups and therefore the anhydroglucose units,

units, available for reaction with the small highly reactive molecule of formic acid. The author (58) states that this acid does not hydrolyse alpha methyl glucoside (and by analogy will not cleave the cellulose macromolecule and thus allow recrystallization) but, however, swells cellulose appreciably more than does water. Table V shows that the values obtained are in good agreement with the data from moisture regain and deuterium exchange during which the cellulose undergoes intermicellar swelling to approximately the same degree. Fig. 11 taken from Tarkow (58) shows that the accessibility value was independent of the moisture content of the same cellulose and that there was a linear relationship between the formic acid accessibility and per cent moisture absorption for a given series of celluloses,

TABLE V

ACCESSIBILITY OF VARIOUS CELLULOSES IN SWELLING SYSTEMS

Sample	Formic Acid (ref. 58)	Moisture Regain (ref. 62)	D_2O Exchange (ref. 55)
Cotton	28	44	44
Wood pulp	35 - 47	53	55
Mercerized cotton	52		
Mercerized wood pulp	..	69	74
Fiber G[(a)]	65	72	67
High tenacity rayon	77	87	86

(a) A rayon of the high tenacity type.

which passed through the origin of the plot.

A similar linear relationship between the accessibilities of cellulose samples and their ability to absorb water vapour had been noted by Howsmon (62). This author standardized the equilibrium moisture regain method as follows:

(1) Samples (1 g.) of cellulose were heated for 5 hours at 105°C., cooled and weighed in small weighing bottles.

(2) They were then conditioned at 58% relative humidity and 75° F. for the time required to give constant weight.

(3) The difference between this weight and the dry weight was expressed as per cent moisture regain.

(4) This regain was corrected by a factor which was the average regain for standard rayon in sixteen determinations divided by the rayon regain as a control sample (Table VI, col. 2).

(5) The sorption ratio of each cellulose was the moisture regain of the sample divided by the moisture regain of cotton (Table VI, col. 3).

(6) The accessibility of each sample, given in Table VI col. 4 was obtained when the sorption ratio was multiplied by the accessibility of cotton found by Mark using deuterium exchange (55)(63).

(7) The density of each sample, in column 5 Table VI, was determined by the procedure of Hermans (64).

The moisture regain was therefore related to accessibility in an empirical manner only. The results are in accord with modern concepts of sorption, whereby the initial strong sorption of water by cellulose is caused by hydrogen bonding of the water molecules to the accessible hydroxyl groups, which may be situated in the "amorphous" region and on the surface of the crystallites. That the density also is dependent on the crystalline-amorphous ratio is evident from the inverse relationship between accessibility and density.

TABLE VI

MOISTURE REGAIN, ACCESSIBILITY AND DENSITY OF CELLULOSE FIBERS(62)

Sample	Moisture Regain (58% R.H.)	Sorption Ratio	Accessibility	Density (20°C.)
Cotton	6.70	1.00	44	1.544
Wood pulp	8.05	1.20	53	1.541
Mercerized wood pulp	10.45	1.56	69	1.534
Fiber G	11.00	1.64	72	1.525
Textile rayon	12.00	1.79	79	1.522
High tenacity rayon	13.25	1.98	87	1.509

The deuterium exchange determination of accessibility is founded on the report by Champetier and Viallard (65) that deuterium oxide would replace the hydrogen atoms of all hydroxyl groups in cellulose with deuterium after a sufficiently long time. In the initial experiments of Badgley, Frilette and Mark (55) 7 g. of conditioned wood pulp was soaked in 30 ml. of 37% deuterium oxide, thermostated at 25°C. After one hour, 10 ml. was withdrawn from the glass stoppered test tube and the density of the liquid was determined by pycnometer. The liquid was then returned to the test tube. This procedure was repeated hourly and the results shown in Fig. 12 were obtained. The initial fast reaction took place in the first hour and decreased rapidly to zero order kinetics. Extrapolation of the linear branch of the conversion curve to zero time, showed 34% of the hydrogens on hydroxyl groups to be exchanged. The following possibilities were presented.

(1) All primary hydroxyl groups exchange much faster than secondary hydroxyl groups and are therefore used up first. The extrapolation should then intersect the ordinate at 33% exchange.

(2) All hydroxyl groups in the disordered localities are exchanged faster than all hydroxyl groups inside the micelle. The experimental value would then be a measure of the amount of easily accessible material.

(3) It is possible that all hydroxyl groups in the disordered areas and on the surface of the crystallites exchange rapidly.

(4) It is possible that only the primary hydroxyl groups which are easily accessible exchange rapidly while all others undergo a slower reaction.

In a later paper Frilette, Hanle and Mark (63) describe the precautions taken to eliminate exchange of deuterium with the moisture of the air during experiments and the technique of stirring which eliminates the risk of too slow diffu-

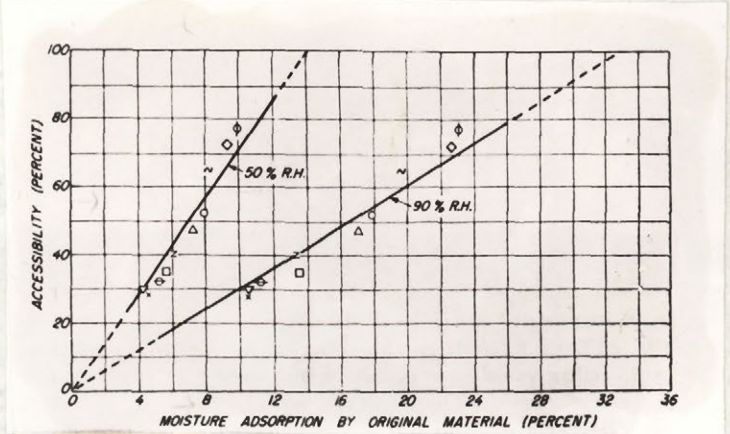


FIG. 11 Relationship between accessibility and moisture adsorption.

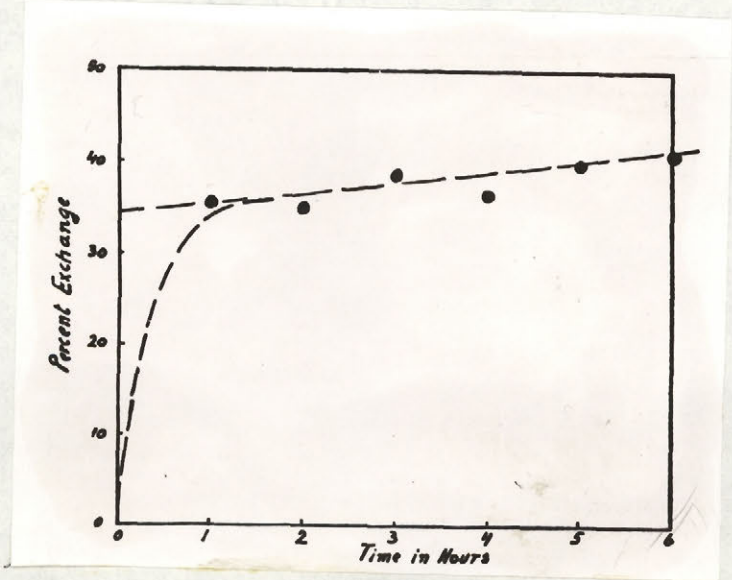


FIG. 12 Rate of hydrogen - deuterium exchange of a sulphite pulp sample.

sion of the heavy water into the fiber mass. Using cotton linters as a standard, rate curves were plotted for pH 6 at 25° and 70° and for pH 3.9 and 10.5 at 25°. The accessibility values ranged from 52 to 63%. It should be noted that at 70° and pH 6, and at 25° and pH 3.9, the linear portion of the curves has a zero slope, indicating that 63% of the hydroxyl groups are <u>all</u> that will react with deuterium. A similar horizontal plane was noted in the curves for the formic acid procedure (58). Under certain experimental conditions, apparently, penetration or diffusion of deuterium and formic acid definitely stops, and does give a sharp distinction between at least two characteristics of cellulose. The authors (63), working at 25° and pH 6, then obtained the values for a series of celluloses whose accessible fraction had been measured by the modified (66) Nickerson (54) method. From these accessibility values, given in Table VII, only the suggestion "that all hydroxyl groups in the disordered areas and on the surface of the crystallites exchange rapidly" remains tenable.

The determination of the accessibility of cellulose by the hydrolytic oxidation technique was developed by Nickerson (54)(67)(68), together with Haberle (69)(70), and was modified by Conrad and Scroggie (66) among others. The measurement was based on the rate of evolution of carbon dioxide from a boiling solution, 0.6 M in ferric chloride and 2.4 N in hydrochloric acid, containing the cellulose sample. Since the rate of gas evolution was dependent on temperature, pressure and concentration of the

TABLE VII

ACCESSIBILITIES BY DEUTERIUM EXCHANGE AND NICKERSON DEGRADATION(63)

Sample	Accessibility (%)	
	D_2O	$HCl-FeCl_3$
Cotton	41-46	...
Cotton linters	61	5
Wood pulp, beech	54	12
" " , pine	55	10
" " , hemlock	55	9
"Cordura" rayon	86	30
Fiber G(a)	67	15
Textile rayon	68	27

(a) A rayon of the high tenacity type

reactants, it was necessary to standardize the reaction conditions rigidly. The cellulose specimens were sometimes vacuum dried over phosphorus pentoxide, but were usually air dried. The plots of carbon dioxide evolved versus time are of the general form given in Fig. 10 but show a gentle subsidence of rate rather than a noticeable superimposition of two reactions, one fast and one slow. They were consequently harder to interpret and various methods were devised for overcoming this difficulty, each being a comparison of some function of the cellulose curve such as its slope, or area subtended at each time, with that of a glucose control. Nickerson and Haberle interpreted their curves as showing 3 rather distinct regions in the cotton linters: an amorphous fraction, made up 3.3% by weight of the original cellulose and was converted to carbon dioxide in 4 minutes; a mesomorphic region, in which the material was semi-crystalline, made up a further 3% and was hydrolysed in 30 minutes; the crystalline region comprising the rest of the cellulose was attacked at a steady rate. The viscosity of the cellulose dropped rapidly during the initial stages of hydrolysis indicating cleavage of the linear macromolecules that passed through the amorphous region, and after recovery the samples showed a decrease in moisture regain, pointing to loss of the avidly hygroscopic localities. In the later stages neither viscosity nor moisture regain changed markedly. There was a further similarity in the hydrocelluloses since the viscosities indicated average crystallite lengths of 244 to 283 glucose units for unmercerized cellulose and of 110-179 for mercerized cellulose (71) values which were in reasonable agreement with X-ray data. Representative values of accessibility found by this method, given in Table VII, show the effect of recrystallization on the availability of the anhydroglucose units as noted by Howsmon (62) and others. Hermans (72) found that when viscose rayon was boiled in 2.5 N sulphuric acid, the crystallinity as measured by X-ray increased from an initial value of about 40% to the final value of about 50% within 30 minutes. The same effect was noted when rayon was mercerized but has not yet been explained (73). Conrad and Scroggie (66) used a modified ferric chloride - hydrochloric acid method, which gave a maximum error of 8 to 10% in precision, in evaluating the characteristics of various celluloses which are used in the viscose industry. The accessibility values for pulps of lower alpha-cellulose content and containing non-cellulosic carbohydrates (Table VIII) are high (cf. Nickerson, 67); mercerization and drying of linters through solvent exchange increase accessibility, probably by reducing the tendency for hydrogen bonding in the disordered areas.

TABLE VIII

ACCESSIBILITY OF RAYON GRADE WOOD PULPS AND LINTERS(66)

Cellulose	Alpha Cellulose (%)	Accessibility (%)
Pulp from Beech wood	89	11.5
Pulp from Southern Pine	93	10.5
High alpha content pulp	95	7.5
Cotton linters	99	5.3
Cotton, mercerized	..	14.5
Cotton, solvent exchanged	..	7.5

The purely oxidative reagent, periodic acid, the chemistry of which was reviewed by Jackson (74), was used by Goldfinger, Mark and Siggia (59) to estimate the accessible fraction of several celluloses. The rate curves obtained by oxidizing the sample in a solution of potassium metaperiodate acidified with sulphuric acid to $pH < 1$, showed an initial fast reaction and a subsequent zero order reaction, that could be readily extrapolated after 24 hours. The initial fast reaction was attributed to the rapid conversion of the glycol groups in the accessible domain to aldehyde groups, but the resulting dialdehyde cellulose underwent a slow further oxidation by the excess periodic acid. The requirement of more than one mole of periodate per mole of glycol unit was supported by Hudson and Jackson (75), who found that cellulose consumed 1.87 moles of periodate after 37 days. Timell (76) in more recent work, used a 0.05 M solution of trisodium paraperiodate buffered with acetic acid to pH 4 and confirmed the findings of Goldfinger et al (59), that diffusion of the oxidizing solution into the crystalline regions is the rate-controlling step in the slow reaction. He also pointed out that recrystallization had probably taken place, since periodate accessibilities given in Table IX were similar to the Nickerson accessibilities in Table VII. The high accessibilities of materials dried from homopolar solvents, in relation to direct drying from water, was also emphasized.

When native cellulose is immersed in aqueous solutions, the amorphous portions are hydrated and <u>inter</u>micellar swelling takes place. Liquid ammonia, on the other hand, like caustic soda of mercerizing strength, causes <u>intra</u>micellar swelling. The ammonia cellulose formed can exchange each hydrogen atom on the hydroxyl groups through the cellulose structure with atoms of metallic sodium. Timell (77) using a laborious and rigidly controlled technique, found that the rate of exchange

TABLE IX

ACCESSIBILITY OF VARIOUS CELLULOSES ESTIMATED BY PERIODATE OXIDATION

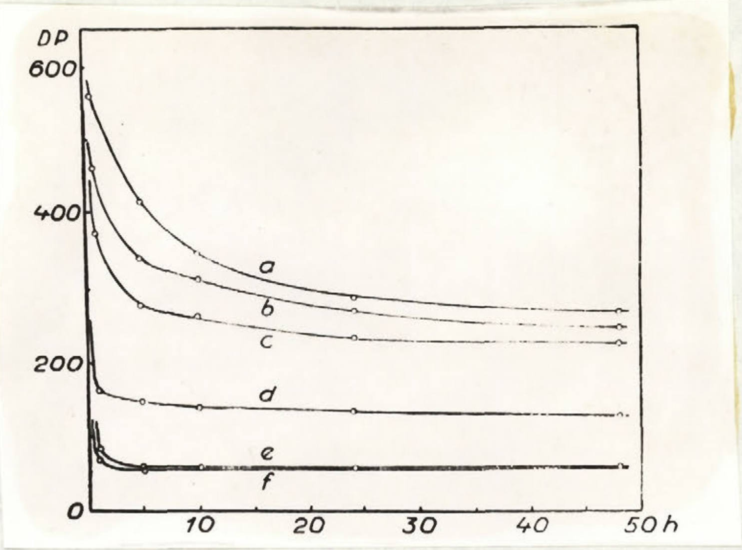
Material	Accessibility (%) Goldfinger et al. (ref. 59)	Accessibility (%) Timell (ref. 76)
Cotton linters	6.0	6.2
Viscose rayon		18.0
Sulphite pulp:		
α-content, 94%		7.0
α-content, 90%		8.6
α-content, 88%		9.5
Regenerated cellulose:		
dried from water	7.0	8.0
dried from water - acetone - benzene	19.5	14.2

showed the superimposition of a fast and a slow reaction. By extrapolation of the linear segment he obtained accessibility values of 39% for cotton linters, 43% for sulphite pulp and 58% for viscose rayon, which were in accord with the figures derived by deuterium exchange (55) given in Tables V and VII.

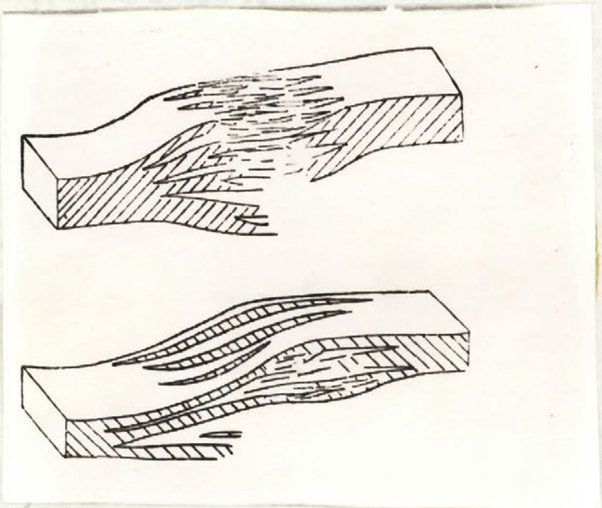
It has been pointed out that during swelling in caustic soda and during acid hydrolysis, the structure of cellulose is altered in a remarkable fashion. Although the data derived from such reactions cannot describe the original arrangement in the cellulose, it will probably be true that such data are dependent on the original fine structure, and reflect in some detail the size of the crystallites, the degree of lateral order in the transition regions, and to some extent, the magnitude and reactivity of the original amorphous region. The inferences about the original structure that can be drawn from such data are discussed in an excellent paper by Jörgensen (78). Series of samples of wood pulps, ranging in their average degree of polymerization from 1950 to 500, and of cotton linters, were swollen in water, and in 2, 4, 6, 8 and 10% sodium hydroxide solutions at 3°C. for 2 hours. The samples were then washed free of alkali, solvent exchanged and air-dried. Each sample was then hydrolysed (cf. 69, 70) in 2.5 N sulphuric acid at 97° in a special apparatus that provided a continuous flow of fresh acid. The amount of reducing sugar in the effluent was determined as glucose at intervals and plotted as dissolved material against time. In other studies the hydrolysis was stopped after increasing time intervals and the unhydrolysed material recovered for moisture regain and viscosity determinations, giving curves typified by Fig. 13. When the fibers were swollen in water, only cotton linters hydrolysed to the limiting degree of polymerization of 130 to 140 in 4 hours. For wood pulps this point, given by the horizontal portion of curves in Fig. 13, was reached only after many hours and was dependent on the process of manufacture, being 300 for the high D.P.pulp and 160 for the lowest D.P. pulp. The difficulty in reaching this low D.P. for pulp, as contrasted with linters, shows that the supermolecular arrangement between the crystallites cannot be the same. The differences are summarized up pictorially in Fig. 14 by modifying Hermans' concept (69). The intercrystalline arrangement in the linters allows ready penetration and rapid cleavage to fragments, the degree of polymerization of which is not noticeably affected by prolonged treatment. It is assumed that in the pulps the interconnecting transition cellulose has considerable lateral order and is more slowly hydrolysed. As the swelling prior to hydrolysis is increased until 10% caustic soda is used, the limit D.P. decreases to 45-60 for pulps and to 80 for cotton linters. The leaflet transition areas in the linters were viewed by Jorgensen as relatively short and tattered, while in the same regions of the pulps the crystallites were more deeply fissured and the semi-crystallites had fewer fringes. The other data fitted in with this representation and indicated that the dense crystalline regions in pulps were of smaller dimensions than those in cotton.

The methods by which accessibility can be determined without necessarily swelling the cellulose prior to or during the reaction are few in number: methylation with thallous ethylate and methyl iodide or with diazomethane or oxidation with chromium trioxide in anhydrous media. These methods, it would seem, eliminate the swelling variable concealing the in situ structure of the cellulose. The thallation-methylation and chromium trioxide techniques have been used in this research and will be discussed in detail.

The thallation-methylation of Purves and coworkers (56)(79)(80) is based on the fact that thallous ethylate, a strong base like sodium ethylate, is soluble in organic liquids



<u>FIG. 13</u> Drop in degree of polymerization during heterogeneous hydrolysis of a pulp in a: water, b: 2% NaOH, c: 4% NaOH, d: 6% NaOH, e: 8% NaOH, f: 10% NaOH.



<u>FIG. 14</u> Proposed arrangements for the intercrystalline structure in cotton linters (top) and wood pulp fiber (bottom)

and the solution reacts quantitatively or nearly so with some fructosides in alcoholic solution (81). Cellulose in an organic solution containing excess thallous ethylate forms the thallous cellulosate

$$\text{Cell-OH} + \text{TlOC}_2\text{H}_5 \longrightarrow \text{Cell-OTl} + \text{C}_2\text{H}_5\text{OH}$$

which, when heated with an excess of methyl iodide or sulphate is converted to a partially methylated cellulose, having a

$$\text{Cell-OTl} + \text{CH}_3\text{I} \longrightarrow \text{Cell-OCH}_3 + \text{TlI}$$

methoxyl group in each hydroxyl group that was originally in contact with the ether, benzene or other non-swelling liquid used as carrier for the thallous ethylate. Harris and Purves (79) showed that no penetration of thallous ethylate into the crystallites occurred because the X-ray diffraction patterns obtained from a mercerized ramie fiber before and after thallation were almost identical, and because thallation of finely powdered sucrose was small. The thallous cellulosate obtained after reacting mercerized ramie fiber in ether, 0.1 N in thallous ethylate, decomposed catalytically and the thallium content was variable. After methylation, however, average methoxyl values of 1.70% were obtained which, when corrected for ether occluded even after 19 hours drying at 98° in vacuo, were reduced to 1.45%. This value corresponds to methylation of 2.5% of all the hydroxyl groups in cellulose. Harris and Purves also noted that approximately half the methoxyl containing material was removed when the superficially methylated fibers were extracted with water.

This method was then applied by Assaf, Haas and Purves (56) for the measurement of the accessible material (or internal surface) in a number of mercerized samples. The cotton linters were swollen in 10% caustic at 5°C. (37) and, after washing with water and de-ashing with 1% acetic acid, were solvent-exchanged through methanol into anhydrous benzene. Although great care was taken during the slow removal of benzene under vacuum, the samples differed considerably in their accessibilities (Table X). To avoid changes in the specimen over a period of time, triplicate samples were treated with 0.1 N thallous ethylate in a variety of solvents and methylated simultaneously in the apparatus illustrated in Fig. 15 to give methoxyl contents that agree to within ± 10% of the mean. The points shown in Fig. 16 result from plotting the molecular volume of the solvent against the respective methoxyl percentage, which is the left-hand ordinate. The right-hand ordinate expresses this methoxyl scale in terms of hydroxyl groups substituted. Since the percentage of methoxyl , X, in the partially methylated cellulose was calculated from the weight of cellulose before methylation, on which basis trimethyl cellulose has a methoxyl content of 57.4%, the percentage of hydroxyl groups substituted in the cellulose was given by 100X/57.4 and was termed the per cent accessibility.

It is apparent that the methoxyl value serves to delineate the penetration of the solvent into the fiber, and that in the case of the normal ethers the relationship is linear. It was suggested that the absolute "amount of amorphous cellulose be defined as the percentage accessible to a liquid of zero molecular volume and with no tendency to swell the cellulose or penetrate its crystallites". The accessibility values given in Table X were obtained by extrapolating the methoxyl values,found for several swollen cellulose preparations examined by thallous ethylate dissolved in various ethers, to zero molecular volume, and show that this method was very sensitive to minor change in the fiber structure. The same authors (56) expressed these changes as a change in colloidal surface (see Table X) by assuming on the basis of X-ray data, that a single glucose unit had an area of 52×10^{-16} cm^2 (82). One gram of cellulose as a continuous unimolecular film would then have an area of $52 \times 10^{-16} \times 6.02 \times 10^{23}/162$ or 1.87×10^{7} cm^2.

The second non-swelling reaction system was also formulated and adapted to the measurement of accessibility by Purves and co-workers. In 1943, Gladding and Purves (83) found that a solution of chromium trioxide in acetic acid and acetic anhydride (4 vols. to 1 vol.) did not oxidize powdered starch at 20°C hardly oxidized dry unswollen cotton linters but rapidly oxidized dry swollen linters. The oxycellulose from previously swollen samples when recovered had a blue tinge indicating the presence of trivalent chromium. When less than 0.3 atom of oxygen had been consumed per glucose unit by the cellulose, a quantitative yield of fibrous product was usual. At higher levels

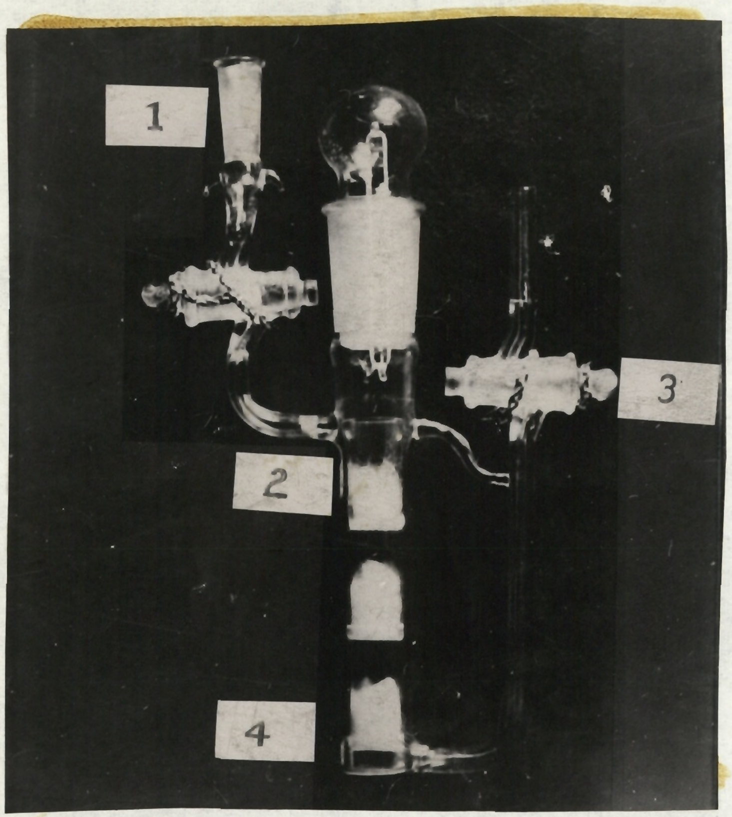


FIG. 15 Apparatus used for small-scale thallation of cellulose.

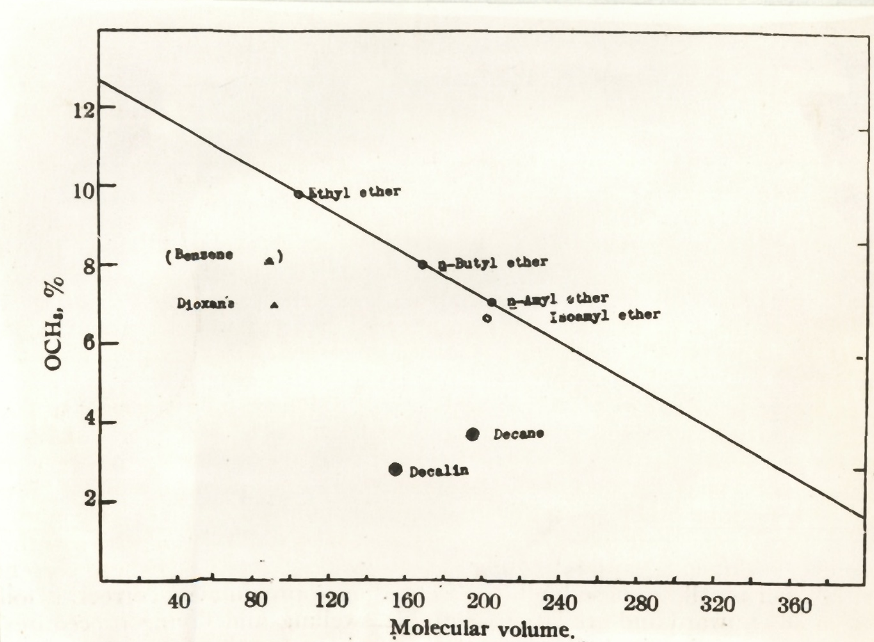


FIG. 16 The superficial methylation of a uniform, swollen cellulose by thallous ethylate dissolved in various liquids.

TABLE X

THALLOUS ETHYLATE ACCESSIBILITIES FOR VARIOUS CELLULOSES(83)

Cellulose	Accessibility (%)	Colloidal surface ($cm^2 \times 10^4/g.$)
Unswollen linters	0.4	8
Unswollen ramie[a]	0.25	5
Linters[b]	22.	420
Linters[b]	17.	330
Linters[b]	9.	170
Linters[b]	27.	520
Linters, regenerated[a,b]	33.	60
Ramie [a,b]	18.	360

(a) Values taken from (76).
(b) Swollen in caustic soda and dried through solvent exchange

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of oxidation however, this product was a powder and the yield was slightly low, indicating considerable degradation. Approximately twice as many carbonyl as carboxylic acid groups were formed and the rate of oxidation plot showed an initial fast and later slow reaction, indicating that the oxidation was limited essentially to the amorphous region.

This oxidation was adopted by Glegg (80), working in this laboratory, to the measurement of accessibility. When small samples of cellulose were examined at 20°C., the rate curve could be readily extrapolated to zero time to give the accessibility as moles of chromium trioxide per anhydroglucose unit. The values obtained are given in Table XI together with the thallous ethylate accessibilities of the same samples.

TABLE XI

COMPARATIVE ACCESSIBILITIES OF VARIOUS CELLULOSES (60)

Cotton Linters	Accessibility (%)	
	Moles CrO_3 Consumed per Glucose Unit	Fraction Accessible to TlOEt
I[a]	0.31	0.34
II[a]	0.58	0.38
III[a]	0.53	0.40
IV[a], 3.80% water removed	0.20	0.35
V[a], 11.60% water removed	0.05	0.05
VI[a], 7.78% water removed from II	0.31	0.33
VII unswollen	0.003	0.005

(a) Swollen in caustic, neutralized with acetic acid, solvent-exchanged, vacuum dried.

The chromium trioxide accessibility was found to be independent of the concentration of the oxidizing reagent but highly dependent on the pre-history of the cellulose. Batches having the same accessibility were not prepared and samples which when dried, were exposed to moisture prior to redrying decreased in accessibility. Since the initial fast reaction was essentially complete in the first 5 minutes, and since the linear portion of the curve was usually attained in 30 minutes, Jörgensen (84) also in this laboratory, used the oxidation values at this time as representing the accessibilities of the cellulose sample.

The Jörgensen Accessibility used in the present research, is defined "as the atoms of oxygen consumed per anhydroglucose unit when small (0.5 - 1 g.) samples of cellulose are oxidized in acetic acid - acetic anhydride solution (4:1) containing approximately 1.5 g. of chromium trioxide in 200 ml., for 30 minutes at 20°C.". Using this measurement, Jörgensen found the following accessibilities: cellulose hydrate II, 1.65; cellulose hydrate I, 1.21; cellulose II, 0.61; native cotton linters, 0.24.

The Distribution of Substituents in a partially Methylated Cellulose

In the later stages of the present research, the problem of the distribution of methoxyl groups in a superficially methylated cellulose, prepared by thallation and methylation of cellulose hydrate II, was touched upon. Of the several aspects of this problem, attention will be directed to the question of distribution of methoxyl groups along the cellulose macromolecule and within the anhydroglucose residues, recently reviewed in great detail by Timell (54). Timell also combined the techniques of Purves and collaborators for the estimation of substituents in the second, third and sixth positions of the glucose residues with the methods of Traube and co-workers (85) for determining the amounts of mono-, di-, tri- and unsubstituted residues. He thus obtained the complete distributions for a number of cellulose ethers. A mathematical analysis of the kinetics of such reactions had already been made by Spurlin (86) and the experimental was compared with the mathematical distribution to give further information on reaction rates and the reactivities of the three kinds of hydroxyl groups. Ultimately, the confirmation of these findings probably rests on the quantitative separation, isolation and identification of each glucose and substituted glucose component of the cellulose structure. Since there are eight possibilities, one trisubstituted, three disubstituted, three monosubstituted and one unsubstituted glucose residue, each of which may have an alpha or beta configuration after hydrolysis or methanolysis, the problem is extremely difficult. In this connection, it would seem that the complementary techniques of flowing and paper chromatography, particularly those developed by the English group of Flood, Hirst, Jones and Wadman hold especial promise (98)(99).

In spite of the complexity of the substituted cellulose system, several papers have been published recently in which the average distribution of methyl (87)(88), ethyl (89) and xanthate (90) groups between the second, third and sixth positions of the glucose units and along the cellulose chain, has been determined within experimental error. Each is based on the methods introduced by Cramer and Purves (91) for cellulose acetate and Mahoney and Purves (92)(93) for methyl and ethyl ethers of cellulose. A portion of the summary from the work of Mahoney and Purves (92) is reproduced to show the utility of these methods, the details of which will be discussed immediately.

"1. A technical ethyl cellulose, averaging 2.48 ethoxyl and 0.52 hydroxyl groups per glucose residue, was found by oxidation with lead tetraacetate to have 0.01 glycol unit in the second and third positions. The corresponding mixture of ethyl glucopyranosides was oxidized with periodate and had 0.25 to 0.29 glycol unit distributed between the 2,3 and 3,4 positions. Oxidation of the corresponding mixture of reducing sugars with lead tetraacetate revealed the presence of 0.13 to 0.15 mole of glycol in the 1,2 position. The combined results showed that 0.13 to 0.15 hydroxyl was unsubstituted in the second position of the ethyl cellulose and 0.24 to 0.28 mole in the third."

"2. The rate at which the ethyl cellulose was esterified in a homogeneous pyridine solution at 20° by a twelve-fold excess of p-toluenesulphonyl chloride (tosyl chloride) was followed by sulphur and alkoxyl analyses for four months, or until the esterification was complete. Samples, removed at intervals, were converted to the 6-iodo derivative by heating with sodium iodide in acetonyl-acetone. The analyses showed that 0.124 mole of hydroxyl group was present in the sixth or primary position of the original ethyl cellulose."

Interpretation of the tosylation rate curve showed a fairly rapid reaction with the 0.151 mole of hydroxyl group in the second position, with a very slow tosylation of 0.245 mole in the third position while the first order rate constants for this reaction were in the ratio of 2.3 for the second, 0.07 for the third and 15 for the sixth position.

In their second paper (93) Mahoney and Purves showed that certain conclusion could be drawn on the type of reaction involved in esterification by determining free primary hydroxyl groups and also the amount of free glycol units. Knowing the total alkoxyl substitution by standard determinations and the primary hydroxyl substitution, the sum (H) of the free hydroxyl groups in the second and third positions was known. It was further shown that if the cellulose had been homogeneously dispersed, so that each hydroxyl unit had the same chance of etherification, the probability of a 2,3-glycol unit was $H^2/4$; under the same circumstances, if substitution at one of the two secondary positions in a particular glucose unit eliminated the chance of the hydroxyl group in the other position reacting, the probability was H-1. In the case where portions of the chain were exposed to reaction, while others were not, i.e. a heterogeneous reaction, the expression became H/2. Substitution of the experimental value of H in these expressions gave calculated values for 2,3-glycol units which were checked against those found by periodate oxidation. (Table XII).

TABLE XII

COMPOSITION OF SOME CELLULOSE ETHERS(93)(94)

Sample	Substituent	Substitution (moles)			Unsubstituted 2,3 Glycol			
		Total	Primary	Secondary	Found	$H^2/4$	H-1	H/2
A	Ethyl	2.48	0.88	1.60	0.01	0.04	0	0.20
B	Methyl	1.85	0.51	1.34	0.32	0.11	0	0.33
C	Methyl	0.59	0.26	0.33	0.83	0.70	0.67	0.84
D	Ethyl	0.58	0.29	0.29	0.75	0.73	0.71	0.86
E	Ethyl	1.20	0.36	0.84	0.52	0.34	0.16	0.58
F	Ethyl	0.71	0.30	0.41	0.74	0.63	0.59	0.80

Comparison of column 6 with columns 7, 8 and 9 shows that, for ethers B, C, E and F the experimental value agreed best with H/2, indicating a heterogeneous substitution, while for ether D, which had been alkylated while homogeneously dispersed in a quarternary ammonium base, the distribution of substituents along the chain agreed with $H^2/4$, or was very uniform. Although sample A, like B, C, E and F had been prepared heterogeneously by heating alkali cellulose in an excess of alkyl chloride, the agreement with $H^2/4$ (or H-1) showed that in its later stages at least the alkylation was in a substantially homogeneous system. It was assumed that the substitution became high enough for the partly alkylated product to be effectively dispersed in the excess alkyl halide.

The enthusiasm for these methods of Purves and coworkers is tempered only slightly by the fact that the tosylation and iodination reactions must be used under quite rigidly controlled conditions. Oldham and Rutherford (95) had found that when the tosyl esters of simple glycosides were heated with sodium iodide in acetone, the ester of the primary hydroxyl groups was quantitatively replaced with iodine while the esters of the secondary hydroxyls remained unaffected. This fact was used by Cramer and Purves (91) with cellulose acetates and Mahoney and Purves (92)(93) with cellulose ethers, as an aid in estimating the distribution of substitution on the different hydroxyl groups. In their earlier work, these investigators esterified ether A in Table XII with 12.5 molar equivalents of tosyl chloride dissolved in pyridine over a period of 4 months at room temperature. At intervals, portions of the gel were cut away and the ethoxyl and sulphur contents of the dry, purified solid were determined. After the partly tosylated samples had been heated in acetonyl acetone containing sodium iodide for 2 hours at 115°, the mixture was poured into aqueous acetone. The iodinated material was recovered by filtration and analysed for sulphur, ethoxyl and iodine. The results showed that while esterification was completed only after 147 days, the tosyl groups replaceable by iodine reached a constant maximum of 0.124 mole per glucose unit 2 hours after the beginning of the esterification. In their later paper (93) which dealt with the less highly substituted ethers B to F in Table XII the tosylation and iodination conditions were essentially unaltered. In these cases, however, Mahoney and Purves analysed at 100 and 200 hours of esterification and found a systematic decrease of approximately 0.01 to 0.03 mole of iodine per glucose unit in that interval.

Malm, Tanghe and Laird (96) studied rather thoroughly the effect of various factors particularly time, on the progress of tosylation and iodination, when the general procedure of Mahoney and Purves was followed. The specimens used were cellulose acetates having from 0.38 to 1.27 unsubstituted hydroxyl groups per anhydroglucose residue. The tosylation-time curves s showed a rapid reaction which was complete after 3 to 4 hours, followed by a slow reaction which became linear after 8 to 24 hours. While normally, this linear portion of the curve had a slight positive slope, in a single case the slope was negative. This effect was traced to a displacement of tosyl by chlorine, due to the influence of pyridine hydrochloride, during tosylation. The chlorine introduced was replaced with iodine during iodination to give, in this case, a molecular amount of iodine introduced which exceeded the amount of tosyl present. In all other cases, as the time of tosylation was increased, increasing amounts of iodine were introduced when the mixed esters were iodinated. This increase in iodine content could not be explained by the replacement of chlorine alone and was due to replacement of tosyl groups in the secondary hydroxyl positions. Furthermore, the authors found that although esters with a low tosyl content gave a slight and regular increase in iodine content as conditions were made more drastic, substances having high tosyl contents were even more readily substituted to an excessive degree by iodine. They also found that although traces of free iodine were occluded in the samples isolated after iodination, the amount (0.6-0.8%) which was removed by soaking this specimen in sodium thiosulphate was not sufficient to materially affect the results. They concluded that the method of tosylation and iodination did not give exact results in the determination of primary hydroxyl groups in these cases and recommended that the estimation be carried out under strictly controlled conditions. Their results however confirmed the general findings of Purves and collaborators.

Timell (97) carried out similar investigations on cellulose ethers, particularly on a propyl cellulose having 1.67 propyl and 0.30 primary hydroxyl groups per glucose unit. When the propyl cellulose was tosylated at 0, 15 and 25°C., the rate plots rose sharply during the first day and fell off to a slight linear increase after that time. Extrapolation to zero time gave three different values, the first being lower, the second and the third increasingly higher than the known primary hydroxyl content. The curves could be correlated with the amounts of specific hydroxyl groups only by mathematical analysis, similar to that of Mahoney and Purves (92). To determine the effect of degree of tosylation on the values of primary hydroxyl groups estimated by iodination, Timell prepared two tosylated propyl celluloses: one esterified for 24 hours at 25°C., contained 0.46 tosyl group per glucose unit, the second, after 8 days at the same temperature, contained 0.75

tosyl group. When the first tosyl ester was iodinated in acetone containing sodium iodide at 120°, the iodine was consistently 0.29 atom (known 0.30) for from 1 to 10 hours of heating but decreased thereafter. The 2-hour iodination of Purves and co-workers therefore appeared suitable for ethers having low tosyl contents. In contrast, the highly tosylated material gave a value of 0.33 atom introduced after 15 minutes' condensation and increased thereafter. This author (97) also showed that under ordinary circumstances, the replacement of tosyl groups by chlorine atoms during tosylation could be neglected, since it primarily occurred on the primary hydroxyl group and was substituted with iodine on iodination. The amounts of chlorine introduced, were in any case, not sufficient to explain the more than theoretical iodine values and Timell supported Malm, Tanghe and Laird in the susceptibility of highly tosylated secondary hydroxyl groups to iodination. He concluded that in a determination of the primary hydroxyl constituent of a cellulose ether, short esterification periods, consistent with quantitative tosylation of the primary hydroxyl group, were desirable and should be followed by an iodination limited to 2 hours at 120°.

EXPERIMENTAL

Preparation of Solvents

Absolute Methanol

Commercial methanol was dried by the method of Lund and Bjerrum (100) using magnesium turnings in excess. The methanol was distilled from the solid residue of magnesium hydroxide and magnesium methylate.

Absolute Ethanol

Absolute industrial ethyl alcohol (95%) was dehydrated by the above procedure (100).

Chloroform

Technical grade chloroform was distilled and the fraction distilling at 61-62° was stored for short periods only in brown bottles.

Anhydrous Acetic Acid

Glacial acetic acid was dried according to Hutchinson and Chandler (101) using chromium trioxide. The mixture was heated under reflux for 4 hours and the fraction distilling at 118° collected.

Acetic Anhydride

Commercial grade acetic anhydride was distilled and the fraction coming over at 138.5-140° was accepted and stored.

Anhydrous Benzene

Reagent grade, thiophene-free benzene was dried over sodium wire. The large quantities of benzene used in solvent-exchange, thallation and methylation were recovered. After a short storage period the thallous ethylate decomposed and formed a black sludge. The supernatant liquid was neutralized with sodium bicarbonate, washed with water and shaken several times with concentrated sulphuric acid until the acid layer was almost colorless. After neutralizing with saturated sodium bicarbonate solution and washing with water, the benzene was left in contact with calcium chloride for 24 hours. After distillation and storage over sodium wire, each batch had the same refractive index (1.4971) as dry, thiophene-free, reagent grade benzene.

Analytical Methods

Methoxyl Determination

Both the Pregl and the Vieböck and Schwappack apparatus were used in determining the methoxyl content by a somewhat modified Clark procedure (102). An aged stock solution of bromine, potassium acetate and acetic acid, prepared according to the instructions of Hoffman and Wolfrom (103) was used in the receivers in preference to a freshly prepared solution. Samples were added to the distilling flask only after the mixture of 10 ml. of "Merck Reagent Hydriodic Acid for Methoxyl Determination" and 0.2 g. of purified red phosphorus or 2.5 ml. of redistilled phenol had been heated by themselves at reflux temperature for 20 min. Under these conditions, no blank was obtained when phenol was used. While red phosphorus gave a blank of 0.12 ml. of 0.04 N thiosulphate, it was quite satisfactory under optimum conditions. Six determinations were then possible when 10 mg. samples weighed to 0.01 mg. were used.

Test for Thallium

The presence or absence of thallium was determined by the following semi-micro test (104). To 4 drops of the solution, acidified with 2 drops of 10% sulphuric acid, was added an excess of fresh 10% potassium iodide. The formation of a yellow precipitate insoluble in 0.1 N thiosulphate indicated the presence of thallium. The limit of sensitivity for the test was given as 1 mg. per 100 ml. of solution.

The micro determination of thallium present in various cellulosic fractions by the combustion method (105) was not possible. No constant weight could be obtained since the thallium vaporized at the required temperature.

Determination of Iodine

The procedure described by Mahoney and Purves was used (92). Samples (0.05 g.) contained in 100 ml. distilling flasks were soaked in 25 ml. of warm ethanol for 10 minutes before 1 g. of reagent grade potassium hydroxide pellets was added. During the subsequent one hour reflux period, the solution was swirled occasionally to wash the sides of the flask. The brown solution was then diluted to 100 ml., poured into a 500 ml. Erlenmeyer flask, and made acid to litmus by the dropwise addition of 10% sulphuric acid. Twenty ml. of 1 N calcium hypochlorite solution was added and the solution boiled for 10 minutes after which 2 ml. of 20% sodium formate was added and the solution reboiled briefly (106). After cooling, the solution was acidified with 5 ml. of 10% sulphuric acid. Excess potassium iodide was added and the liberated iodine titrated with 0.04 N thiosulphate.

$$\text{Per cent iodine} = \frac{V \times N \times 126.9 \times 100}{1000 \times 6 \times \text{sample wt.}}$$

where V was the volume in ml.
and N the normality of the thiosulphate.

Estimation of Reducing Power

The procedure of Shaffer and Somogyi (107) was used in this determination. On each day, duplicate estimations were made with distilled water for the blanks, with a standard glucose solution containing approximately 1.0 mg. per 5 ml. and with the unknown. The reducing power of the unknown was estimated as glucose, using the volumes of 0.05 N thiosulphate required in the final iodometric titration.

$$\text{Reducing power of unknown (mg. glucose)} =$$

$$\frac{(\text{Vol. for blank} - \text{Vol. for unknown})}{(\text{Vol. for blank} - \text{Vol. for glucose})} \times \text{mg. glucose in 5 ml.}$$

Use of Ion Exchange Resins

Amberlite Ion exchange resins IR-120-AG (a sulphonic acid type) and IRA-410 (a strong base type) were gifts of Rohm and Haas Co., Philadelphia. These resins did not absorb methylated sugars (108) and were used to remove inorganic salts, principally of thallium, from the soluble methylated cellulose fraction and the methyl glucoside derived therefrom. Columns (18" x 2" diameter) containing three-quarters of a pound of these resins were regenerated and exhausted 5 times with alternate use of 10% hydrochloric acid and 10% sodium hydroxide. The regenerated column was then washed with distilled water until the effluent was neutral to litmus, before the contaminated fraction in aqueous solution was passed at a rate of 50 drops per minute first through one column and then the other. The columns were then washed with distilled water, the effluent being added to the concentrate before the columns were regenerated and rewashed to neutrality. The fraction was then repassed until the final effluent was neutral (thallium-free). In this manner the largest fraction (27.5 g.) was freed of thallium methyl sulphate in four passes through each column, 2.3 g. of methyl cellulose being recovered. Since the regeneration of the sulphonic acid type column with hydrochloric acid liberated the very slightly soluble thallous chloride, the column was never freed completely of thallium salts which moved further down the column on each successive run.

Preparation of Cellulose Samples

Extraction of Cotton Linters

High-grade cotton linters, a gift of the Hercules Powder Co., through the courtesy of Dr. Spurlin, were supplied with these specifications: cook number 05708, March 4, 1948; viscosity: 440 sec. in 2.5 g. conc.; soda soluble content: less than 2%; ash content: 0.3; color: 0.2. These linters, used by other investigators (17)(109)(110) in this laboratory, were freed from fats and other soluble impurities by extracting 40 g. lots in a 2 liter Soxhlet apparatus with constant boiling 2:1 benzene-ethanol for 48 hours. After drying in air for several days under protection from dust, the linters were stored in air-tight containers.

Moisture Content of Dewaxed Linters

The procedure for obtaining the precise bone dry weight of cellulose for the determination of moisture content and densities was as follows. Quadruplicate samples of approximately 0.8 g. were placed in previously tared, oven dried weighing vials (8.5 cm. x 1.5 cm.) having female covers. After the vials were weighed to the fourth decimal place, short lengths of fine Nichrome wire were inserted between the vial and the cover and the drying was carried out in a Cenco vacuum oven at 105° under 2 mm. pressure. After 12 hours the vials were removed from the oven and placed in a desiccator over phosphorus pentoxide to cool. When cool the lids were lifted slightly, allowing the wire segments to drop out, and the lids were then seated firmly in place, this operation taking approximately 5 seconds. The vials were then reweighed, the wires re-inserted and the vials replaced in the oven. When the weights in two consecutive weighings did not vary by more than 0.00029, the samples were considered bone-dry. A total drying time of 30 hours fulfilled this condition. The moisture contents of the different lots varied between 2.7 and 6.0%.

Preparation of Alkali for Mercerization

A 35% solution was made up by dissolving 483 g. of Nichols reagent-grade sodium hydroxide in 700 ml. of previously boiled distilled water. After cooling the solution was made up to one liter and allowed to stand for 48 hours to precipitate sodium carbonate. The supernatant liquid was then decanted, filtered through glass wool and stored at room temperature in a brown soft-glass bottle that had been carefully lined with paraffin. As required, 250 ml. was removed and diluted to one liter with boiled distilled water. When necessary the caustic strength was adjusted so that the final solution always contained 116-123 g. of sodium hydroxide per liter. This 10-11% solution was stored in a paraffin lined bottle at -7°C.

Alkali Soluble Content of Cotton Linters

The cells illustrated in Fig. 17 were used to find the percentage of cotton linters soluble in 10-11% alkali at 0-3°C. One gram samples were weighed into four tared cells with glass wool plugs and sintered glass disks protecting the

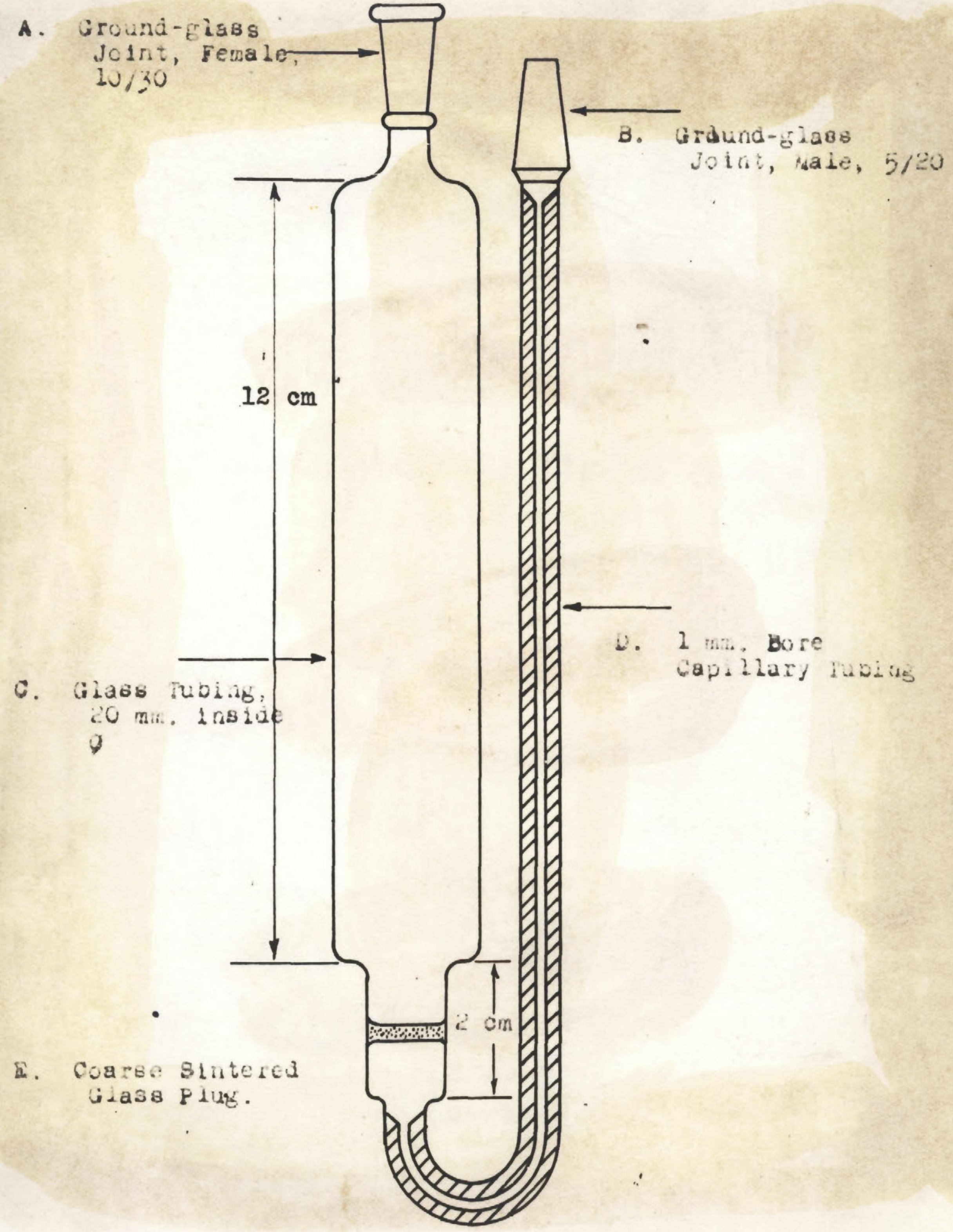


FIG. 17 Reaction Cell Used for Alkali Solubility of Cellulose

outlet B. The cells were then placed in an ice water bath, 35 ml. of 10-11% alkali was added and the contents stirred occasionally during the 2.5 hour mercerization period at 0-3°C. The alkali was then withdrawn by the application of suction to B, replaced with ice water and the contents stirred. The washing was repeated at 15-minute intervals until the effluent was neutral. Ice cold 10% acetic acid was then added and the cellulose rewashed to neutrality after 15 minutes. The vessels were then dried *in vacuo* at 105° to constant weight (48 hours). Table XIII gives the details and the reproducibility of the estimation.

TABLE XIII

ALKALI SOLUBILITY OF COTTON LINTERS

		Cellulose Content		
Sample No.	Sample Weight[a] (g.)	Before Swelling[b] (g.)	After Swelling[c] (g.)	Soluble (%)
1	1.0431	0.9905	0.9727	1.80
2	1.0178	0.9665	0.9485	1.86
3	1.0585	1.0052	0.9865	1.86
4	0.9959	0.9457	0.9292	1.74
			Average	1.82

(a) Air-dry weight of linters
(b) Column 2 minus moisture content of 5.04%.
(c) Dried *in vacuo* at 105°C. to constant weight.

Preparation of the Cellulose Modifications

The swelling of the dewaxed, air-dried cotton linters in alkali was accomplished by the procedure of Richter and Glidden (37). The swollen fibers were then washed free of alkali with ice water (111). The simple, convenient apparatus used consisted of a vertical Pyrex tube of 10 cm. diameter and 50 cm. length tapering at the base to a large bore stop-cock. A Buchner filter plate seated on a bed of fine Pyrex glass wool served as a screen to prevent loss of fine particles, this filtering system being tared before insertion. To facilitate the withdrawal of liquid a length of gum rubber tubing passed from the stop-cock to a large suction flask. The stop-cock being open, the column was placed in an ice-water bath. Thirty grams of the dewaxed air dired cotton linters of known moisture content was placed on the filter plate and one liter of ice cold 10-11% (always over 10% when the moisture content of the fibers was taken into account) caustic solution added. By occasional stirring of both the ice bath and the gel-like mixture, the system was kept at 0-3° for 2 1/2 hours. A water pump vacuum was then applied to the suction flask and about 500 ml. of caustic withdrawn and one liter of ice-cold distilled water added to the column. To prevent the formation of many dense pellets in the final product, it was essential that the removal of caustic be gradual and that the mass be kept in suspension by stirring on each addition of ice water. During the next three washings with liter portions of ice water, approximately one liter of solution was removed after a 15 minute soaking period, leaving adequate liquid in contact with the whitening fibers. The cellulose was freed of alkali in this manner, the seventh effluent usually being neutral to litmus. The material was then de-ashed by soaking in 10% ice cold acetic acid for 20 minutes and again washed with ice water till neutral. The product obtained was termed Cellulose Hydrate II in accordance with Hermans' nomenclature (35).

Cellulose Hydrate I was prepared by boiling the Hydrate II for 30 minutes in the final neutral water.

The preparation of water-swollen linters consisted of soaking the dewaxed, air-dried linters in ice water (0-3°) for 2 1/2 hours. The fibers were then de-ashed and washed till free of acetic acid at this temperature.

Solvent-Exchange Process

The process of solvent-exchange, essentially that used by Gladding and Purves (83) followed immediately after the swelling of the linters, and was carried out in the mercerization apparatus. The ice water was drawn off (without however prolonging the suction so that the fiber surfaces became dry), one liter of ice cold 50% acetic acid was added and the mixture stirred. After 20 minutes the liquid was withdrawn, a fresh liter of cold anhydrous acetic acid was added and the cooling bath removed. The exchange was continued until the effluent acetic acid, after a one hour contact period, had the same refractive index as anhydrous acetic acid. The same procedure was followed in the second stage, using cold anhydrous, thiophene-free benzene. Usually 8 liters of acetic acid and a similar quantity of benzene was used. To cut down the preparation of large quantities of anhydrous solvents a counter-current principle was adopted. The second and subsequent portions of solvent on each stage were labelled and stored for another run, in which only a single fresh portion of each solvent at the end of each stage was required.

When carefully treated the cellulose in benzene was almost transparent, the appearance of white clumps indicating incomplete solvent-exchange at the first stage. Methanol could be substituted for acetic acid but was not used when the product was to be analysed for methoxyl content.

The unmatted benzene-wet material above the porous plate in the apparatus was then quickly transferred to glass containers with ground glass stoppers and the vessels filled with anhydrous benzene. The weight of cellulose in storage was then found by air drying the previously tared filter disk and glass wool screen, deducting the weight of trapped cellulose from the weight of linters used, and subtracting the independently known moisture content and the caustic soda solubles (1.82%).

The rather complete removal of unbound water and acetic acid by the above procedure is indicated by the fact that aliquots of the supernatant benzene from 5 batches of cellulose hydrate II, one of hydrate I and one of water-swollen cellulose, all had the same refractive index as anhydrous benzene after storage of one year.

The Oxidation of Cellulose

Oxidation of the Solvent by Chromium Trioxide

Gladding and Purves (83) used chromium trioxide dissolved in a mixture consisting of 4 vols. of anhydrous acetic acid to 1 vol. of redistilled acetic anhydride. To study the stability of this system, a 200 ml. glass stoppered volumetric flask, containing a known weight of chromium trioxide and thermostated at 20 ± 0.1°, was filled to the mark with the solvent. After all solid had dissolved, 5 ml. aliquots of the somewhat red solution were removed and titrated iodometrically with 0.1069 N sodium thiosulphate solution, and the amount of chromium trioxide reduced then calculated. The details of two runs are given in Tables XIV and XV.

TABLE XIV

RATE OF REDUCTION OF CHROMIUM TRIOXIDE BY SOLVENT

Time (hours)	Thiosulphate Consumed[a] (ml.)	Difference (ml.)	CrO_3 Reduced m. moles	CrO_3 Reduced m. moles per hour
0	7.11[b]	....	....	.....
1	7.10	0.01	0.014	0.014
22.5	6.86	0.25	0.36	0.016
28.5	6.78	0.33	0.47	0.016
48	6.58	0.53	0.76	0.016

(a) Normality = 0.1069
(b) Initial concentration of CrO_3
was 1.01 g. per 200 ml.

TABLE XV

RATE OF REDUCTION OF CHROMIUM TRIOXIDE BY SOLVENT

Time (hours)	Thiosulphate Consumed[a] (ml.)	Difference (ml.)	CrO_3 reduced m. moles	CrO_3 reduced m. moles per hour
0	13.76[b]	....	....	.....
5	13.71	0.05	0.076	0.014
6	13.60	0.16	0.23	0.038
23	13.36	0.40	0.57	0.025
48	12.94	0.82	1.17	0.024
167	11.08	2.68	3.82	0.023
191	10.65	3.11	4.43	0.023

(a) Normality = 0.1069
(b) Initial concentration of CrO_3
was 1.96 g. per 200 ml.

Effect of Benzene on the Oxidizing Medium

To elucidate the effect of benzene remaining in the cellulose specimen, upon the oxidizing medium, 5 ml. of anhydrous, reagent grade, thiophene-free benzene was added to 195 ml. of the chromium trioxide mixture at 20 ± 0.1°. Aliquots of 5 ml. were removed from the solution at 20° at intervals and titrated iodometrically with 0.1 N thiosulphate.

TABLE XVI

REDUCING EFFECT OF BENZENE ON CHROMIUM TRIOXIDE

Time (hours)	Thiosulphate Consumed[(a)] (ml.)	Difference (ml.)	CrO_3 reduced m. moles	m. moles per hour
0	7.11[(b)]	....	....	.....
1.5	6.97	0.14	0.20	0.13
3	6.88	0.23	0.30	0.11
4.5	6.85	0.26	0.37	0.082
22.5	6.41	0.70	1.00	0.045
28.5	6.34	0.77	1.10	0.039
48	6.00	1.11	1.53	0.033

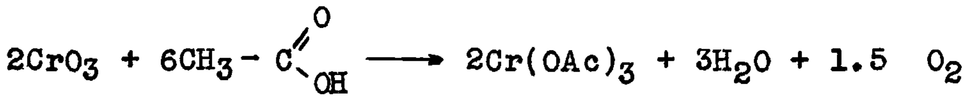
(a) Normality = 0.1069
(b) Initial concentration of CrO_3 was 1.01 g. per 200 ml.

Chromium Trioxide Oxidation of Cellulose

Preliminary studies on the oxidation of cellulose for extended periods by the chromium trioxide reagent were carried out in the manner of Glegg (80). The unswollen, dewaxed, air-dried samples I, II and III (I and II - cotton linters; III - Novocell pulp) were prepared for oxidation by soaking for 12 hours in distilled water in a 3" x 1 1/2" diameter weighing bottle. The water was then removed by applying suction to a 1 1/4" diameter filter stick and replaced with 4:1 acetic acid - acetic anhydride after 3 hours. Sample IV (collapsed cellulose hydrate II) had been dried *in vacuo* over paraffin and phosphorus pentoxide after mercerization and solvent-exchange in the prescribed manner. In this case the preparation for oxidation consisted of soaking the sample overnight in anhydrous acetic acid followed by solvent-exchange with 4:1 acetic acid - acetic anhydride. The final solvent was then removed by filtration and the sample transferred to 200 ml. of the oxidizing medium of known strength at zero time. Aliquots of 5 ml. were withdrawn at intervals and titrated iodometrically with 0.1 N thiosulphate.

The results are presented in Tables XVII, XVIII, XIX, XX. Columns 1 and 2 are self explanatory. The figures in column 3 were obtained by multiplying the corresponding figure in column 2 by the number of aliquots remaining in the flask at that time. Column 4 (the total amount of chromium trioxide reduced) resulted from the summation of the corresponding figure in 3 and the moles of chromium trioxide reduced to that time in all preceeding aliquots. Column 5, Table XVII, was derived from column 4 by deducting the moles of chromium trioxide reduced by the acetic acid - acetic anhydride solvent at any time "t" by use of Fig. 19 and gives the moles of oxidant reduced by the cellulose. Columns 6 and 7 were the result of converting moles of chromium trioxide reduced in 4 and 5 into atoms of oxygen absorbed per anhydroglucose unit.

If 1.87×10^{-3} mole of chromium trioxide were reduced by 0.4000 g. of cellulose, the accessibility in atoms of oxygen absorbed per glucose unit was $\frac{0.00187}{0.4000} \times 162 \times \frac{3}{2}$ the factor 3/2 being determined by the equation



$$2CrO_3 + 6CH_3-C\overset{O}{\underset{OH}{\lessgtr}} \longrightarrow 2Cr(OAc)_3 + 3H_2O + 1.5\ O_2$$

In Tables XVIII, XIX and XX no correction has been made for the effect of solvent on the oxidant.

TABLE XVII

CHROMIUM TRIOXIDE OXIDATION OF UNSWOLLEN COTTON LINTERS (I)

	Chromium Trioxide Reduced				Accessibility[d]	
Time (hours)	Moles per Aliquot[c] $x\ 10^{-5}$	Moles at time "t" $x\ 10^{-3}$	Total Moles $x\ 10^{-3}$	By Cellulose[a] $x\ 10^{-3}$	Un-corr.	Corr.
0[b]	....	....	....	....	....	....
0.25	0.99	0.396	0.396	0.396	0.20	0.20
0.50	1.94	0.758	0.768	0.767	0.39	0.39
0.75	2.17	0.825	0.854	0.854	0.43	0.43
1.00	2.33	0.861	0.912	0.896	0.46	0.45
1.25	2.54	0.906	0.980	0.968	0.50	0.49
1.50	2.48	0.868	0.967	0.954	0.49	0.48
1.75	2.63	0.895	1.02	1.00	0.52	0.51
2.00	2.67	0.881	1.03	1.02	0.52	0.52
2.25	2.71	0.866	1.04	1.03	0.53	0.52
2.75	2.90	0.849	1.10	1.08	0.56	0.55
3.25	2.97	0.392	1.13	1.10	0.57	0.56
3.75	3.01	0.873	1.14	1.12	0.58	0.57
4.25	3.05	0.854	1.15	1.14	0.59	0.58
48	11.86	3.20	3.53	2.68	1.79	1.39
72	17.47	4.54	5.04	3.74	2.56	1.90
96	22.08	5.51	6.18	4.48	3.14	2.28
120	25.20	6.05	6.94	4.87	3.53	2.47

(a) Solution contained 0.5036 g. of dewaxed, air-dried cotton linters (0.4790 g. cellulose).

(b) Initial CrO_3 concentration was 1.25 g. per 205 ml. or 2.61 g. CrO_3 per gram of cellulose.

(c) Aliquots of 5 ml. titrated with 0.1144 N thiosulphate.

(d) Atoms of oxygen per glucose unit

TABLE XVIII

CHROMIUM TRIOXIDE OXIDATION OF UNSWOLLEN COTTON LINTERS (II)

	Chromium Trioxide Reduced			Accessibility[c]
Time (hours)	Moles per Aliquot[b] x 10^{-5}	Moles at Time "t" x 10^{-3}	Total Moles x 10^{-3}	Uncorrected
0[a]	....	....	.....	....
0.25	2.44	0.976	0.976	0.39
0.50	2.78	1.08	1.10	0.43
0.75	3.66	1.39	1.44	0.57
1.00	3.17	1.17	1.26	0.50
1.25	3.55	1.28	1.40	0.55
1.50	3.89	1.36	1.52	0.60
1.75	4.08	1.39	1.58	0.63
2.0	4.38	1.45	1.68	0.67
2.5	4.50	1.44	1.72	0.68
3.0	4.69	1.45	1.78	0.70
3.5	4.69	1.42	1.79	0.71
4.0	4.84	1.40	1.82	0.72
4.5	4.99	1.40	1.87	0.74
5	5.53	1.49	2.01	0.80
6	5.41	1.41	1.98	0.78
7	5.99	1.50	2.12	0.84
24	12.44	2.99	3.68	1.46
72	30.99	7.10	7.91	3.14
96	36.29	7.96	9.08	3.60
120	45.53	9.56	11.04	4.38

(a) Solution contained 2.34 g. CrO_3 and 0.6482 g. of air dry cotton linters (0.6149 g. cellulose per 205 ml. or 3.81 g. CrO_3 per gram of cellulose.

(b) Aliquots of 5 ml. titrated with 0.144 N thiosulphate.

(c) Atoms of oxygen per glucose unit.

TABLE XIX

CHROMIUM TRIOXIDE OXIDATION OF NOVOCELL PULP (III)

	Chromium Trioxide Reduced			Accessibility(c)
Time	Moles per aliquot(b) x 10^{-5}	Moles at time "t" x 10^{-3}	Total moles x 10^{-3}	Uncorrected
0(a)	.....	.....	.....	....
10 mins.	1.39	0.556	0.556	0.33
20 "	2.17	0.847	0.862	0.50
30 "	2.82	1.07	1.11	0.65
45 "	3.85	1.42	1.48	0.86
60 "	3.78	1.36	1.46	0.85
90 "	4.38	1.53	1.67	0.98
17.5 hrs.	14.0	4.76	4.94	2.89
19 "	14.4	4.74	5.06	2.96
20.5 "	15.0	4.79	5.26	3.08
42 "	24.0	7.43	8.05	4.71
43 "	24.6	7.39	8.25	4.82
45 "	25.1	7.28	8.38	4.90
69 "	28.1	8.44	9.80	5.74
121 "	33.8	9.12	10.99	6.42

(a) Solution contained 1.425 g. of CrO_3 and 0.4408 g. of air dried Novocell pulp (0.4152 g. of cellulose) per 200 ml. of solution i.e. 3.43 g. of CrO_3 per gram of cellulose.

(b) Aliquots of 5 ml. titrated with 0.1069 N thiosulphate.

(c) Atoms of oxygen per glucose unit.

TABLE XX

CHROMIUM TRIOXIDE OXIDATION OF SWOLLEN, DRIED COTTON LINTERS (III)

Time	Chromium Trioxide Reduced: Moles per Aliquot[(b)] x 10^{-5}	Chromium Trioxide Reduced: Moles at Time "t" x 10^{-3}	Chromium Trioxide Reduced: Total Moles x 10^{-3}	Accessibility[(c)] Uncorrected
0[(a)]	....	....	....	....
5 mins.	1.32	0.528	0.528	0.28
15 "	3.04	1.18	1.19	0.62
30 "	4.23	1.62	1.67	0.87
45 "	5.65	2.09	2.18	1.14
60 "	5.84	2.11	2.26	1.18
90 "	7.06	2.47	2.67	1.39
2 hrs.	7.88	2.68	2.95	1.54
3 "	10.10	3.33	3.78	1.97
4 "	10.51	3.36	3.82	1.99
20 "	18.78	5.82	6.38	3.33
44 "	26.58	7.97	8.72	4.55
116 "	35.94	10.42	11.43	5.96

(a) Initial solution contained 1.610 g. of CrO_3 and 0.4659 g. of swollen, solvent-exchanged vacuum dried cotton linters, i.e., 3.45 g. of CrO_3 per gram of cellulose.

(b) Aliquots of 5 ml. titrated with 0.1069 N thiosulphate.

(c) Atoms of oxygen per glucose unit.

Jörgensen Accessibility Measurement

The Jörgensen modification (84) of Glegg's accessibility measurement (80) consisted of finding, in terms of atoms of oxygen required per anhydroglucose unit, the amount of oxidant consumed by the cellulose in 30 minutes at 20°C. In the present procedure, 7.5 g. of the slowly soluble chromium trioxide was dissolved overnight in one liter of a mixture of anhydrous acetic acid and redistilled acetic anhydride (4 vols. to 1 vol.). A 200 ml. volumetric flask was then filled to the mark with solution at 20 ± 0.1° and three 5 ml. aliquots were removed and titrated iodometrically with 0.1 N thiosulphate, just before the introduction of the sample.

Prior preparation of the cellulose (approximately 0.5 g.) consisted of solvent-exchanging dry unswollen fibers through water (12 hours) then anhydrous acetic acid to acetic acid - acetic anhydride, while swollen fibers dried from benzene, or swollen fibers immersed in benzene, were solvent-exchanged through anhydrous acetic acid (2 hours) to the final solvent. The filter stick and weighing bottle already described for these manipulations and for removing the excess liquid were used.

Using tweezers, the moist sample was placed on a small glass wool plug at the base of the neck of the flask, and at zero time was pushed into the slightly red oxidizing solution. Small particles in the weighing bottle were washed into the oxidizing medium with 4:1 acetic acid - acetic anhydride sufficient to make the total volume up to 200 ml. The flask contents were shaken vigorously at short intervals and after 30 minutes at 20 ± 0.1°, three 5 ml. aliquots of the now dark colored solution were withdrawn and titrated.

When it became obvious that pushing the swollen fibers and the glass wool plug down the narrow neck of the volumetric flask exerted sufficient compression to lower the accessibility of the sample, the procedure was somewhat modified. After three 5 ml. aliquots had been withdrawn to give the initial oxidizing strength, the remaining liquid was poured into a 250 ml. wide mouthed glass-stoppered flask. The moist sample, displacing from 2-4 ml. of solution, was then dropped into the oxidizing solution and the residual fibers in the weighing bottle washed into the flask with 12 ml. of the 4:1 acetic acid - acetic anhydride. The accessibility values in Tables XXI and XXII were estimated in this manner.

In a typical experiment 0.5952 g. of cellulose was oxidized. Triplicate 5 ml. aliquots of the original oxidizing solution consumed 12.63, 12.67, 12.70 or an average of 12.67 ml. of 0.09 N thiosulphate. Its strength, when cellulose and washings were added was 12.67 x 37/40 or 11.72 ml. After the oxidation the final three aliquots were equivalent to 11.15, 11.06 11.14 or an average of 11.12 ml. Hence the reduction in oxidant per 5 ml. aliquot--11.72 - 11.12--0.60 ml. thiosulphate;

the moles of oxidant consumed = $\frac{0.60 \times 0.0900 \times 40}{1000 \times 3}$ = 0.0072; the atoms of oxygen required per anhydroglucose unit =

$$0.0072 \times \frac{162}{0.5952} \times \frac{3}{2} = 0.30.$$

In a general formula the accessibility was given by $\frac{V \times N \times 3.24}{W}$ where V was the reduction in oxidant per 5 ml. aliquot in terms of thiosulphate, the normality of which was N corresponding to a sample weight of W grams. The factor 3.24 is equivalent to $\frac{40 \times 162 \times 3}{1000 \times 3 \times 2}$. The accessibility values, presented in Tables XXI and XXII, show that the method has considerable precision.

TABLE XXI

CHROMIUM TRIOXIDE ACCESSIBILITIES OF VARIOUS CELLULOSE MODIFICATIONS

Sample No.	Wt. in benzene(a) (g.)	Benzene		Calcd. Wt.(b) in Air (g.)	Thiosulphate(c) (ml.)	Accessibility(d)
		Density	°C.			
Water-swelled cellulose (Expt. 400)						
1	0.1317	0.8872	21.5	0.3004	0.16	0.18
2	0.1207	0.8872	21.5	0.2774	0.11	0.13
3	0.1166	0.8871	21.6	0.2660	0.08	0.10
					av. =	0.14 ± 0.04
Cellulose Hydrate I (Expt. 368)						
1	0.2163	0.8767	22.0	0.4712	1.41	0.983
2	0.2427	0.8767	22.0	0.5285	1.55	0.976
3	0.2036	0.8767	22.0	0.4436	1.29	0.967
4	0.2554	0.8763	22.3	0.5552	1.66	0.991
					av. =	0.979 ± 0.012
Cellulose Hydrate II (Expt. 354)						
1	0.2673	0.8765	22.2	0.5816	2.89	1.68
2	0.2613	0.8765	22.2	0.5685	2.88	1.71
3	0.1963	0.8765	22.2	0.4271	2.09	1.65
4	0.2069	0.8765	22.2	0.4502	2.19	1.64
5	0.1729	0.8765	22.2	0.3762	1.86	1.66
					av. =	1.67 ± 0.04

(a) Weight of vial and sample in benzene (about 8 g.) minus weight of vial in benzene.

(b) Assuming the density: Expt. 400 - 1.562; Expt. 368 - 1.621; Expt. 354 - 1.623.

(c) Normality: Expt. 400 - 0.1025; Expt. 368 - 0.1025; Expt. 354 - 0.1045.

(d) Atoms of oxygen per glucose unit after oxidation at 20° for 30 minutes.

TABLE XXII

CHROMIUM TRIOXIDE ACCESSIBILITIES OF VARIOUS CELLULOSE MODIFICATIONS

Sample No.	Wt. in Benzene(a) (g.)	Benzene		Calcd. Wt.(b) in Air (g.)	Thiosulphate(c) (ml.)	Accessibility(d)
		Density	°C.			
Cellulose Hydrate II (Expt. 380)						
1	0.1294	0.8755	23.1	0.2812	1.47	1.73
2	0.1374	0.8755	22.9	0.2987	1.55	1.71
3	0.1601	0.8755	22.9	0.3480	1.86	1.76
					av. =	1.73 ± 0.03
Cellulose Hydrate II (Expt. 410)						
1	0.2042	0.8767	22.0	0.4516	1.39	1.04
2	0.1999	0.8767	22.0	0.4422	1.28	0.98
3	0.1962	0.8773	21.4	0.4345	1.25	0.97
					av. =	1.00 ± 0.04
Cellulose Hydrate II (Expt. 430)						
1	0.1004	0.8765	22.2	0.2133	0.20	0.32
2	0.1140	0.8771	21.6	0.2424	0.28	0.40
3	0.1474	0.8771	21.6	0.3134	0.30	0.33
					av. =	0.35 ± 0.05
Cellulose Hydrate II (Expt. 450)						
1	0.1266	0.8775	21.2	0.2766	0.67	0.82
2	0.1389	0.8775	21.2	0.3035	0.77	0.85
3	0.1308	0.8775	21.2	0.2858	0.73	0.86
4	0.1167	0.8775	21.2	0.2550	0.30	0.40(d)
					av. =	0.84 ± 0.01
Cellulose Hydrate II (Expt. 460)						
	0.1308	0.8782	20.5	0.2851	1.65	1.95

......

TABLE XXII (Cont'd)

CHROMIUM TRIOXIDE ACCESSIBILITIES OF VARIOUS CELLULOSE MODIFICATIONS

Sample No.	Wt. in Benzene(a) (g.)	Benzene Density	Benzene °C.	Calcd. Wt.(b) in Air (g.)	Thiosulphate(c) (ml.)	Accessibility(d)
Cellulose Hydrate II (Expt. 470)						
	0.1179	0.8729	25.5	0.2551	1.37	1.81

(a) Weight of vial and sample in benzene (about 8 g.) minus weight of vial in benzene.

(b) Assuming the density: Expt. 380 - 1.622; Expt. 410 - 1.600; Expt. 430 - 1.656; Expt. 450 - 1.618; Expt. 460 - 1.623; Expt. 470 - 1.623.

(c) Normality: Expt. 380 - 0.1020; Expt. 410 - 0.1040; Expt. 430 - 0.1060; Expt. 450 - 0.1040; Expt. 460 - 0.1040; Expt. 470 - 0.1040.

(d) This value excluded in averaging.

The Launer Determination of Total Cellulose

The procedure of Launer (112) was assessed using unmercerized linters and the oxycellulose recovered after oxidation with chromium trioxide. The sample (0.3 g.) was dissolved in 75 ml. of 12 molar sulphuric acid and 25 ml. of potassium dichromate solution (1.835 N) was added to the clear solution. The beaker was then covered with a watch glass and the solution heated at 140-150°C. for 10 minutes, cooled and made up to 500 ml. Aliquots of 25 ml. were then titrated iodometrically with 0.1 N thiosulphate. The equivalent weight of the anhydroglucose unit was taken as $\frac{162.1}{24}$ since $C_6H_{10}O_5 + 6\ O_2 \longrightarrow 6\ CO_2 + 5\ H_2O$, so that one ml. of dichromate oxidized 0.0124 g. of cellulose. If 21.96 ml. were consumed in the oxidation of 0.3 g. of cellulose then the sample contained $\frac{21.96 \times 0.0124}{0.3} \times 100 = 90.8\%$ cellulose.

Representative results from the oxidation of cotton linters are reproduced in Table XXIII.

TABLE XXIII

LAUNER OXIDATION OF DEWAXED, AIR-DRIED COTTON LINTERS

Sample Weight	Moisture (%)	Launer Cellulose (%)	Recovery (%)
0.2981	4.88	94.05	98.93
0.2920	4.88	95.06	99.94
0.2834	4.88	95.51	100.39
0.2830	4.88	94.89	99.77
0.3594	4.41	95.20	99.61
0.3054	4.88	93.65	98.53

The possibility of obtaining the original cellulose content of samples that had been oxidized for 30 minutes in chromium trioxide, by a Launer oxidation of the residual isolable material was investigated. After the accessibility estimation the heterogeneous mixture was immediately drowned in 400 ml. of distilled water, recovered on a filter and washed to neutrality in weighed sintered glass filters. The green-tinged, degraded fibers were then dried and subjected to the potassium dichromate oxidation, using appropriate amounts of reagents. The results given in Table XXIV include the amount of cellulose oxidized by chromium trioxide .

TABLE XXIV

ESTIMATION OF CELLULOSE BY CHROMIUM TRIOXIDE AND LAUNER OXIDATIONS

Sample No.	Weight (a) (g.)	Accessibility (b)	Weight of Cellulose Oxidized by		Total Oxidized(c) Cellulose (g.)	Weight Accounted for (%)
			CrO_3 (d)	$K_2Cr_2O_7$		
1	0.5172	1.01	0.0436	0.4197	0.4633	89.5
2	0.3671	1.16	0.0356	0.2902	0.3258	88.7
3	0.5670	1.10	0.0520	0.4471	0.4991	88.2
4	0.4174	1.19	0.0413	0.3187	0.3600	86.4
5	0.4704	1.28	0.0502	0.3881	0.4383	92.2
6	0.4753	1.44	0.0573	0.3731	0.4304	90.6

(a) Corrected for moisture content and alkali solubles after swelling.

(b) Atoms of oxygen per glucose unit after 30 minutes of chromium trioxide oxidation at 20°C.

(c) Total of columns 4 and 5.

(d) Calc. on basis of 12 atoms of oxygen required to oxidize one glucose unit.

Determination of the Densities of Various Cellulose Modifications in Benzene

By Pycnometer

The use of pycnometers for the determination of the density of a solid as described by Russel (113) proved unsatisfactory since it was impossible to add the sample without occluding bubbles of air which were difficult to displace. Later it was known that procedure of pushing the fibers through the pycnometer orifice would have collapsed the structure. When applied to samples of a single cellulose hydrate II (Expt. 354) densities ranging from 1.38 to 1.71 were obtained.

By Hydrostatic Balance

The densities were finally determined in benzene by the procedure used by Hermans for the determination of the density of cellulose in water (114). A Voland chemical balance reading to 0.1 mg. was converted to a hydrostatic balance by replacing the left-hand pan and stirrup with a lead counterbalance having two hooks, one for attachment to the knife edge stirrup, the other to provide a means of suspension of the object to be weighed. A 100 ml. beaker, insulated with asbestos to minimize temperature fluctuation in the weighing medium, was kept filled at constant level with anhydrous benzene in the 7-inch space beneath the counterbalance. The baskets in which the fibrous samples were weighed were tared 90 x 15 mm. glass vials having ground-glass female covers. These vials were suspended from the counterbalance by a single strand of Nichrome wire, the lower section of which formed a tight circle to hold the vial. The vial, without cover, was then immersed in benzene, weighed and the temperature of the benzene read to the nearest 0.1°C. The vial and wire were then unhooked and suspended in other benzene containing the bulk cellulose to be examined and samples of 0.2 to 0.6 g. were transferred. This transfer to the vial was made beneath the benzene surface to prevent the occlusion of air bubbles and exposure to moisture. The vial and the cellulose sample were then weighed and the temperature of the benzene in the beaker recorded. Owing to the free evaporation of benzene in the beaker, the temperature of the bath was always somewhat lower than that of the cellulose. A period of 30 minutes was essential before equilibrium was established. Since the temperature in the benzene bath sometimes shifted by 1.5° during these two weighings, the vial weight then became inaccurate and the sample had to be discarded. In order to overcome the lack of a constant temperature room, in later experiments, one vial was calibrated and used in all weighings. However this procedure also led to noticeable error since small particles of cellulose adhered in subsequent transfer to the second vial in which the sample was dried.

The vial was then capped with a short length of Nichrome wire inserted to allow the passage of benzene vapour, and placed in a vacuum desiccator over phosphorus pentoxide. After slow removal of observable benzene by application of a water pump vacuum, the samples were prepared for drying in a

Cenco Constant Temperature Oven fitted with a chamber having a rubber gasketed vacuum closure.

In order to screen the fibers from contamination in the oven, a 250 ml. beaker was inverted over a 100 ml. beaker holding 4 vials. The annular gap was then packed quite tightly with surgical cotton batting. These beakers were then placed in a 600 ml. beaker and the space between the 250 ml. and 600 ml. beakers packed with absorbent cotton. The protected samples were then heated in the oven at 105° at 1 mm. for 30 hours. After cooling in a desiccator over phosphorus pentoxide, the beakers and batting were removed. The vial covers were then lifted slightly, allowing the wire to drop out, and then pressed firmly into place. The vials and the uncolored and presumably uncontaminated fibers were then weighed.

The density of the cellulose was then calculated from the equation

$$d_c = \frac{G_1 d_b}{G_1 - G_2}$$

where d_c = density of the cellulose,
d_b = density of the benzene,
G_1 = bone dry weight of the sample
and G_2 = weight of the sample in benzene.

Detailed examples of the method are in Table XXV, XXVI and XXVII.

Conversely, since $G_1 = \dfrac{G_2\, d_c}{d_c - d_b}$, if the density of the cellulose was known, the cellulose content of samples wet with benzene could be determined without subjecting the fiber to drying processes, by weighing the sample in benzene.

TABLE XXV

DENSITIES OF VARIOUS CELLULOSE MODIFICATIONS IN BENZENE

Sample No.	Weight in Benzene(a) (g.)	Benzene		Dry Weight(b) (g.)	Density (calcd.)
		°C.	Density		
Water-swelled Cellulose (Expt. 400)					
1	0.2697	24.3	0.8742	0.6135	1.560
2	0.2500	24.0	0.8745	0.5671	1.564
3	0.1085	20.5	0.8785	0.2477	1.562
4	0.1468	20.5	0.8785	0.3363	1.559
5	0.1297	20.5	0.8785	0.2962	1.563
6	0.1542	20.5	0.8785	0.3521	1.563
				av. =	1.562 ± 0.003(c)
Cellulose Hydrate I (Expt. 368)					
1	0.3242	23.3	0.8753	0.7066	1.618
2	0.2670	21.7	0.8770	0.5991	1.619
3	0.1888	20.0	0.8788	0.4113	1.625
4	0.2168	21.8	0.8769	0.4727	1.620
5	0.2053	21.9	0.8768	0.4463	1.622
6	0.2704	20.0	0.8788	0.5899	1.623
				av. =	1.621 ± 0.004(d)
Cellulose Hydrate II (Expt. 354)					
1	0.1918	22.8	0.8758	0.4163	1.624
2	0.1808	22.8	0.8758	0.3900	1.632
3	0.2121	22.8	0.8758	0.4605	1.624
4	0.1514	22.2	0.8765	0.3295	1.622
5	0.1697	20.0	0.8788	0.3702	1.623
6	0.1399	22.6	0.8760	0.3062	1.613
				av. =	1.623 ± 0.010(e)

(a) Weight of vial and sample in benzene (about 8 g.) minus weight of vial in benzene.
(b) Dried for 30 hours at 105° and 1 mm. of pressure.
(c) Other determinations: 1.574; 1.578; 1.595 (all discarded).
(d) Other determinations: 1.613; 1.630; 1.620; 1.627
(e) Other determinations: 1.623; 1.623; 1.653. (last discarded).

TABLE XXVI

DENSITIES OF VARIOUS CELLULOSE MODIFICATIONS IN BENZENE

Sample No.	Weight in Benzene[a] (g.)	Benzene		Dry Weight[b] (g.)	Density (calcd.)
		°C.	Density		
Cellulose Hydrate II (Expt. 380)					
1	0.1246	19.8	0.8791	0.2725	1.620
2	0.1639	19.8	0.8791	0.3594	1.616
3	0.1507	19.8	0.8791	0.3273	1.629
4	0.1463	20.0	0.8788	0.3188	1.624
				av. =	1.622 ± 0.007
Cellulose Hydrate II (Expt. 410)					
1	0.1894	20.4	0.8784	0.4216	1.595
2	0.2944	20.7	0.8780	0.6529	1.599
3	0.1878	21.1	0.8776	0.4179	1.594
4	0.1843	21.5	0.8772	0.4071	1.603
				av. =	1.600 ± 0.007[c]
Cellulose Hydrate II (Expt. 430)					
1	0.1753	21.5	0.8772	0.3769	1.646
2	0.1414	21.6	0.8771	0.3005	1.656
3	0.1509	21.6	0.8771	0.3195	1.662
4	0.1580	21.7	0.8770	0.3346	1.662
				av. =	1.656 ± 0.010
Cellulose Hydrate II (Expt. 450)					
1	0.1833	21.8	0.8769	0.3987	1.623
2	0.2036	21.6	0.8771	0.4437	1.621
3	0.2360	21.5	0.8772	0.5171	1.614
4	0.1988	21.6	0.8771	0.4351	1.615
				av. =	1.618 ± 0.004

(a) Weight of vial and sample in benzene (about 8 g.) minus weight of vial in benzene.

(b) Dried for 30 hours at 105° and 1 mm. pressure.

(c) Other determinations: 1.607; 1.598; 1.605.

TABLE XXVII

DENSITIES OF VARIOUS CELLULOSE MODIFICATIONS IN BENZENE

Sample No.	Weight in Benzene(a) (g.)	Benzene		Dry Weight (g.)	Density (calcd.)
		°C.	Density		
Cellulose Hydrate II (Expt. 380)					
1	0.1251	22.7	0.8759	0.2829(a)	1.570
2	0.1629	22.7	0.8759	0.3667(a)	1.576
3	0.2123	22.7	0.8759	0.4782(a)	1.575
4	0.1484	22.7	0.8759	0.3354(a)	1.571
				av. =	1.573 ± 0.003
Cellulose Hydrate II (Expt. 410)					
1	0.1966	22.7	0.8759	0.4458(a)	1.567
2	0.1884	22.2	0.8764	0.4246(a)	1.576
3	0.2127	22.6	0.8761	0.4795(a)	1.575
4	0.2003	22.7	0.8759	0.4504(a)	1.577
					1.574 ± 0.007
Cellulose Hydrate II (Expt. 410) Unprotected					
1	0.1884	22.5	0.8761	0.4166(b)	1.599
2	0.2026	22.7	0.8759	0.4527(b)	1.585
3	0.1966	22.7	0.8759	0.4458(b)	1.567
4	0.1884	22.2	0.8764	0.4246(b)	1.576
				av. =	1.582 ± 0.017

(a) Samples dried to constant weight at 65° and 1 mm. pressure under protection from contamination.

(b) Samples dried to constant weight at 105° and 1 mm. pressure without protection from contamination.

Methylation of Cellulose

Preparation of Thallous Ethylate Solution

The method of Assaf, Haas and Purves (56) was modified slightly. The 500 ml. Erlenmeyer flask used in this procedure had a side arm with stop-cock and was completely covered with black electrician's tape to prevent access of light. The 24/40 top opening was stoppered with a ground glass joint having a bubbling tube which extended to the bottom of the flask. The joints were lubricated with Dow Corning silicone grease to prevent "freezing" under the action of the strong alkali. To the flask was added a considerable quantity (5-15 g.) of pure sliced thallium metal from which most of the oxide layer had been removed by scraping. After adding 250 ml. of absolute ethanol, the bubbler was tied firmly in position and a rubber balloon, filled with oxygen, fitted tightly over the orifice of the bubbler. The flask was then placed on a shaker and agitated in a horizontal plane, the balloon being refilled when necessary. After 12 hours the apparatus was removed, the stop-cock opened and the thallous ethylate solution expelled into a 500 ml. brown bottle covered with black tape. The last few ml. sometimes contained a brown sludge and were discarded. The thallous ethylate - ethanol solution was then chilled overnight at -5°C. during which time large colorless plates of thallous ethylate were deposited. The supernatant mother liquor was drained into a second taped brown bottle. The mother liquor from a previous run had been added to the Erlenmeyer flask containing thallium metal and in this 12 hour interval a fresh thallous ethylate solution had been prepared. This solution was now added to the thallous ethylate crystals, chilled and the cycle continued until a considerable quantity of crystalline thallous ethylate was on hand. After the crystals had been drained from ethanol as well as possible they were dissolved in anhydrous benzene and the strength of the solution found by titration with standard acid. The concentration was then adjusted to about 0.5 N by dilution with anhydrous benzene. After approximately 15 cycles the accumulated contamination in the mother liquors decomposed the thallous ethylate as it was formed. For this reason the liquors were discarded after 10 cycles and replaced with fresh anhydrous ethanol.

Accessibility by Thallation - Methylation Procedure

Triplicate small samples (0.05 - 0.2 g.) of the swollen cellulose stabilized in benzene were weighed on the hydrostatic balance and quantitatively transferred to the small porous cups used by Assaf, Haas and Purves (56) in similar experiments. The cells, taped to prevent access of light and each containing three cups (see Fig. 15) were then filled with 0.5N thallous ethylate in benzene. After standing in the dark for 24 hours at room temperature the depleted solution was sucked off through the stopcock (3) and replaced with fresh 0.5 N thallous ethylate. When a further 48 hours had elapsed the solution was withdrawn as before and the thallated cellulose washed with 5 volumes of anhydrous benzene at one hour intervals with a minimum exposure to light and with a sparing use of the water pump. The samples were then prepared for methylation by adding to each cell 15 ml. of benzene containing a large excess (3 ml.) of freshly distilled methyl iodide. The large ground glass stopper of each cell was then replaced with a standard taper reflux condenser fitted with a calcium chloride drying tube. After closing the stopcocks, the cells were immersed in a water bath maintained at 70°, the highest temperature possible without the formation of bubbles which dislodged small particles of cellulose from the cups. After 3 hours the cells were cooled, the condenser replaced with the stopper and the methyl iodide solution removed. After 4 washings with benzene at one hour intervals the cells, filled with benzene, were heated at 70° for one hour to leach out the last traces of methyl iodide, were cooled and the benzene drawn off. The cups with the orange tinted methylated cellulose, were then placed in order in a vacuum oven and dried at 80° and 2 mm. pressure to constant weight. The contents of each cup were then transferred to stoppered vials, the encrusted cups weighed and the weight of methylated cellulose and the thallous iodide contaminant obtained. Duplicate samples from each vial were then removed and the methoxyl content determined using the factor

$$\frac{\text{Initial weight of cellulose}}{\text{Total weight of methylated material}} \times \text{Weight of methoxyl sample}$$

to give the cellulosic content of each sample for the methoxyl analysis. Results showed a high degree of reproducibility, all being in the range ± 2% of average values; and it was not necessary to perform all six analyses in most cases. The results of these determinations are given in Tables XXVII and XXIX.

Evaluation of Methyl Chloride as a Methylating Agent

(a) At Room Temperature. Fifty ml. of 0.587 N (0.0294 mole) thallous ethylate in benzene was added to a clean large thick walled Pyrex bomb of the Carius type, taped to exclude light. After cooling the tube in a dry ice chloroform bath, approximately 5 ml. of liquid methyl chloride (0.0059 mole) was added from chilled graduated cylinder. The bomb was sealed while in the cooling bath and allowed to stand at room temperature for 15 days. After being cooled again, the bomb was opened and the methyl chloride allowed to evaporate at room temperature. Titration of the residue showed that 25 ml. of 0.475 N thallous ethylate (0.0119 mole) remained. The reaction was therefore not quantitative under these conditions.

(b) At 70°C. This experiment was carried out in a 6" x 3" stainless steel bomb which was kept vertical. The top of the cylinder was grooved to seat the lead gasketed inner lid of the same diameter which had similar grooves. The external vertical face of the cylinder was provided with threading onto which the outer U-shaped cover was screwed to its limit. The dome of this outer cover was pierced with a heavy threaded bolt, which, when tightened, exerted considerable pressure on the inner lid and thus made the grooved seal gas-tight. To the glass liner, of 80 ml. capacity, was added 64 ml. of 0.590 N thallous ethylate (0.0378 mole). The cylinder and contents

TABLE XXVIII

THALLATION-METHYLATION OF VARIOUS CELLULOSE MODIFICATIONS

Cup	Sample in Benzene Weight (g.)	Sample in Benzene °C.	Calcd. dry Weight(a) (g.)	After TlOEt-MeI (g.)	Sample for OCH_3 Weight (g.)	Sample for OCH_3 Cellulose(b) (mg.)	OCH_3(c) (%)
Water-Swelled Cellulose (Expt. 400)							
Bottom	0.0752	22.2	0.1714	0.4843	0.0162	5.74	8.98
					0.0149	5.27	....
Middle	0.0493	22.2	0.1123	0.3262	0.0241	8.31	9.10
					0.0187	6.48	....
Top	0.0412	22.2	0.0939	0.2817	0.0201	6.70	9.00
					0.0177	5.90	9.10
						av. =	9.04 ± 0.06
Cellulose Hydrate I (Expt. 368)							
Bottom	0.0627	22.3	0.1365	0.6000	0.0367	8.35	14.9
					0.0400	9.11	14.6
Middle	0.0358	22.3	0.0780	0.3575	0.0242	5.28	14.8
					0.0332	7.24	14.9
Top	0.0536	22.3	0.1167	0.5222	0.0314	7.01	14.6
					0.0318	7.10	14.5
						av. =	14.7 ± 0.2

....

TABLE XXVIII (Cont'd)

THALLATION-METHYLATION OF VARIOUS CELLULOSE MODIFICATIONS

	Sample in Benzene				Sample for OCH_3		
Cup	Weight (g.)	°C.	Calcd. dry Weight(a) (g.)	After TlOEt-MeI (g.)	Weight (g.)	Cellulose(b) (mg.)	OCH_3(c) (%)
Bottom	0.0515	22.7	0.1119	0.5561	0.0265	5.33	17.6
					0.0265	5.33	17.7
Middle	0.0388	22.4	0.0843	0.3981	0.0195	4.13	17.1
					0.0212	4.48	16.9
Top	0.0282	22.3	0.0613	0.2968	0.0232	4.79	16.3
					0.0208	4.29	17.1
						av. =	17.1 ± 0.8

(a) Calculated using the density of benzene at temperature of weighing, the respective cellulose density: 400 - 1.562; 368 - 1.621; 354 - 1.623 and data in Column 2.

(b) Calculated from Columns 5 and 6.

(c) Thiosulphate in final titration was 0.03356 N.

TABLE XXIX

THALLATION-METHYLATION OF VARIOUS CELLULOSE MODIFICATIONS

Cup	Sample in Benzene		Calcd. dry Weight$^{(a)}$ (g.)	After TlOEt-MeI (g.)	Sample for OCH_3		$OCH_3$$^{(c)}$ (%)
	Weight (g.)	°C.			Weight (g.)	Cellulose$^{(b)}$ (mg.)	
Cellulose Hydrate II (Expt. 380)							
Bottom	0.0324	22.3	0.0705	0.3588	0.0269	5.28	17.5
					0.0327	6.42	17.3
Middle	0.0390	22.3	0.0849	0.4286	0.0332	6.58	17.9
					0.0318	6.30	17.6
Top	0.0473	22.2	0.1030	0.5314	0.0313	6.07	17.6
					0.0188	3.64	17.7
						av. =	17.6 ± 0.3
Cellulose Hydrate II (Expt. 410)							
Bottom	0.0554	21.2	0.1227	0.5199	0.0350	8.26	16.9
					0.0335	7.90	17.2
Middle	0.0567	21.2	0.1256	0.5162	0.0447	10.9	16.5
					0.0325	7.90	16.4
Top	0.0665	21.0	0.1473	0.5978	0.0509	12.5	16.2
					0.0501	12.3	16.3
						av. =	16.6 ± 0.6

....

TABLE XXIX (Cont'd)

THALLATION-METHYLATION OF VARIOUS CELLULOSE MODIFICATIONS

	Sample in Benzene				Sample for OCH_3		
Cup	Weight (g.)	°C.	Calcd. dry Weight(a) (g.)	After TlOEt-MeI (g.)	Weight (g.)	Cellulose(b) (mg.)	OCH_3(c) (%)
Cellulose Hydrate II (Expt. 430)							
Bottom	0.0328	22.2	0.0697	0.2860	0.0152	3.71	12.1
					0.0182	4.44	11.8
Middle	0.0467	22.2	0.0992	0.4177	0.0231	5.49	13.0
					0.0227	5.39	11.2
Top	0.0512	22.2	0.1088	0.5122	0.0196	4.16	14.2
					0.0209	4.44	14.3
						av. =	12.8 ± 1.6

(a) Calculated using the density of benzene at temperature of weighing, the respective cellulose density, 380 - 1.622; 410 - 1.600; 430 - 1.656 and data in Column 2.

(b) Calculated from Columns 5 and 6.

(c) Thiosulphate in final titration was 0.03356 N.

were then cooled and 3 ml. (0.035 mole) of methyl chloride added. The bomb was then made gas-tight and submerged in a water bath at 70° for 24 hours. After removal from the bath the bomb was cooled to room temperature and the dome screw cautiously loosened to allow methyl chloride to escape. When opened, the bomb contained approximately 40 ml. of 0.520 N thallous ethylate (0.0208 mole) plus a voluminous deposit of thallous chloride.

Hydrolysis of Methylated Cellulose in the Presence of Thallous Iodide

A water-soluble methylated cellulose was hydrolysed with sulphuric acid by the Monier-Williams procedure (115) in the presence of thallous iodide. The iodide was prepared by dissolving 5 g. of thallous sulphate in 10 ml. of 10% sulphuric acid and adding excess of 20% potassium iodide solution. The dense orange precipitate was recovered, washed and dried. A 1.2534 g. sample was added to 0.5430 g. thallium-free, methylated cellulose (MeO, 10.57%) dissolved in 5 ml. of 72% sulphuric acid. After 3 days at room temperature the solution was diluted at 475 ml. and heated under reflux for 48 hours. The solution was then neutralized with barium carbonate and filtered. A 5 ml. aliquot showed the solution to have a Shaffer-Somogyi alkaline copper reducing power equivalent to 0.1062 g. of glucose. From the remaining 510 ml. of solution, 0.4588 g. of clear syrup (MeO, 8.07, 8.04%) was obtained by evaporation and drying. This syrup was equivalent to a recovery of 85.4% on a weight basis but to only 65% on a methoxyl basis.

Large-Scale Thallation and Methylation (Fig. 21)

The 250 ml. separatory funnel and the 725 ml. reaction chamber of the apparatus (Fig. 18) used in these experiments were covered with black tape to exclude light during the thallation. Into the reaction vessel, which would hold approximately 20 g. of swollen cellulose without packing, was introduced 10.8 g. (0.067 mole) of cellulose hydrate II (Expt. 460) in benzene. After the filter stick had been worked gently to the bottom of the flask and the separatory funnel was clamped in position, the benzene was expelled through stopcock D by passing in dry nitrogen under pressure through stopcock C with B closed. Stopcock D was then closed, A and B were opened and 475 ml. of 0.55 N (0.26 mole) crystal clear thallous ethylate was drawn into the reaction chamber by application of mild suction at C. After standing in the dark for 24 hours at room temperature in the closed system, two 5 ml. aliquots of the depleted liquor were withdrawn from the central region of the fibrous mass and titrated. The residual normality of 0.25 indicated the reaction of 0.14 mole of thallous ethylate with the cellulose. The partially exhausted solution was expressed under dry nitrogen and replaced with 450 ml. of 0.45 N (0.20 mole) concentration. After a further 10 hours at room temperature, the titration of aliquots showed the solution to be 0.41 N. The maximum consumption of thallous ethylate was therefore 0.16 mole. The slightly yellow fibers, now occupying approximately two-thirds of their original volume, were washed with four 450 ml. volumes of anhydrous benzene at 30 minute intervals.

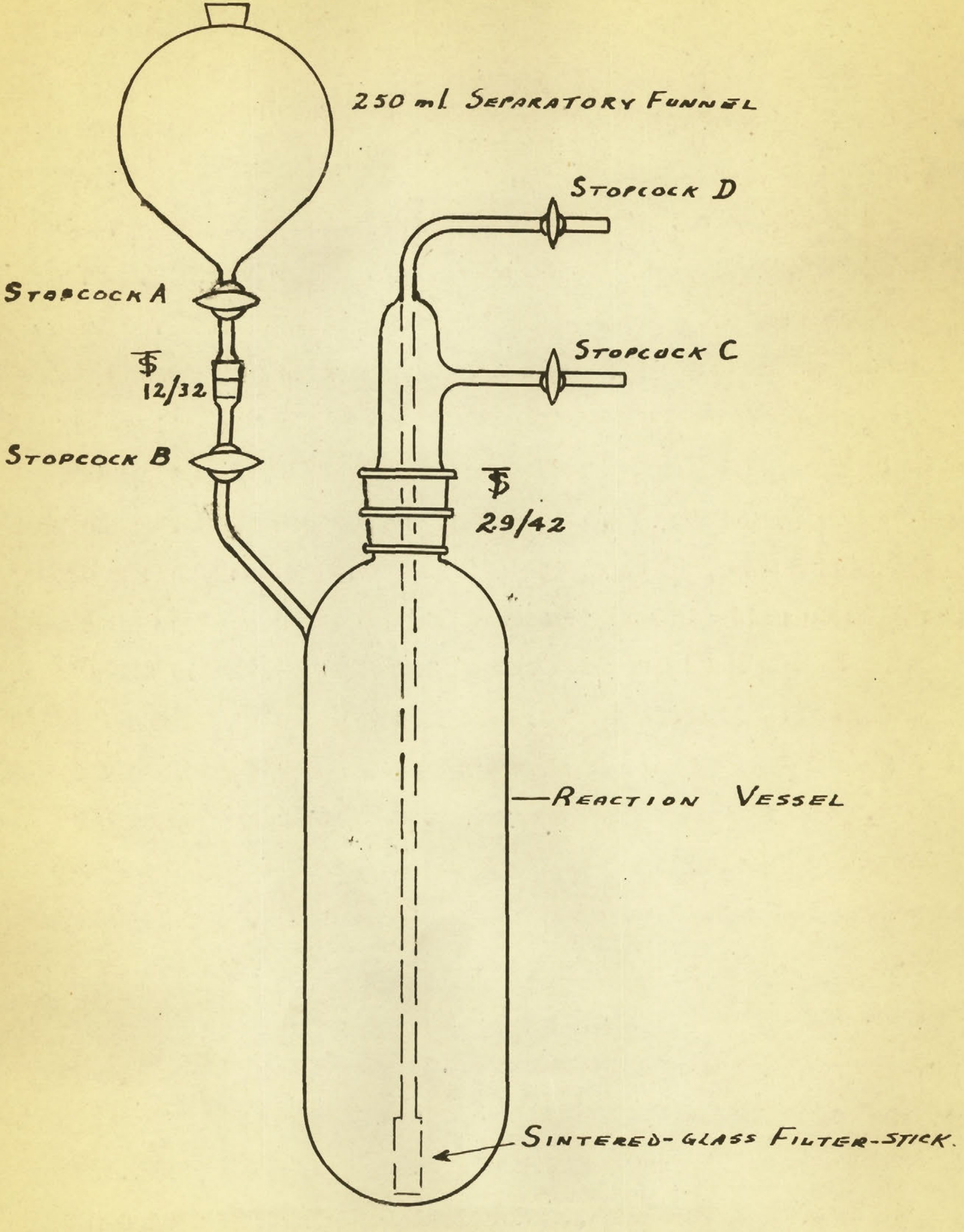


Fig 18 : Apparatus for Large-Scale Thallation and Methylation

The more concentrated liquors were stored in the cold in taped bottles for further use.

Redistilled dimethyl sulphate, (32 ml. or 0.34 mole) in 450 ml. of anhydrous benzene was then added to the benzene wet fibers. The filter stick assembly was replaced with a standard taper condenser which had a calcium chloride tube protecting the top opening. The mixture was heated at reflux on a water bath for 90 minutes, cooled to room temperature and the benzene, containing the excess dimethyl sulphate, was then sucked into a large filter flask. The partially methylated cellulose was now impregnated with white thallium salts, the space occupied had decreased considerably but much of the fibrous character was retained. These white solids were washed with four 450 ml. portions of anhydrous benzene and dried <u>in vacuo</u> overnight over phosphorus pentoxide and paraffin chips at room temperature. The dry weight was 47.0 g.

In an almost identical experiment 18.3 g. (0.113 mole) of cellulose hydrate II (Expt. 470) having an accessibility of 1.81 used a maximum of 0.15 mole thallous ethylate over two 24 hour contact periods with about 0.5 N solution. The benzene washed thallium cellulosate was heated under reflux with 53 ml. (0.50 mole) of dimethylsulphate in 450 ml. anhydrous benzene for 90 minutes. After removal of the benzene solubles, 70.7 g. of vacuum dried degraded white powder was obtained.

In the subsequent processing of the contaminated methyl cellulose fractions from these two separate reactions (460 and

470), the former will be used as an example for experimental details. Essential data for 470 will appear in brackets.

Benzene Soluble Fraction To the 2.5 liters of somewhat brown benzene solution was added a slurry of 6 g. of barium carbonate in 200 ml. of distilled water. After removal of the benzene by distillation at 40°C. under vacuum, the aqueous concentrate was heated under reflux for 2 hours in the presence of excess barium carbonate. The solids were then removed by filtration and discarded. The filtrate was extracted with six 100 ml. volumes of redistilled chloroform but with considerable difficulty since mixing of the phases produced a rather stable milky emulsion that was not broken by heat, cold, electrolytes or by the addition of some other solvents. The emulsion when centrifuged, however, at 3000 r.p.m., divided into three layers. The clear, chloroform layer was pipetted off and retained and the operation repeated 4 times. Cautious vacuum distillation of the middle jelly-like layer and the top aqueous layer was accompanied by excessive foaming but led to the isolation of 32.9 g. (Expt. 470, 37.2 g.) of barium methyl sulphate containing a little thallium methylsulphate.

The chloroform soluble material was recovered after distillation of the solvent, as a honey-colored syrup weighing 0.1811 g., having a methoxyl content 36.4, 36.6% and containing some thallium. This syrup was united with a similar fraction (MeO, 36.3, 35.9%) from Expt. 470 weighing 0.4386 g., and the aqueous solution was cleared of inorganic salts by passage through

Amberlite ion exchange resins IR 120 and IRA 410. The neutral effluent was evaporated in a tared 100 ml. round bottom flask and dried to constant weight at 60° under 2 mm. The yield of 0.5715 g. of clear, friable thallium-free solid (MeO 37.10, 37.10) was distributed in the proportion of the original material (Expt. 460, 0.168 g.; Expt. 470, 0.404 g.).

Benzene Insoluble Material The 47.0 g. of benzene extracted, dried solids (Expt. 470, 70.7 g.) were re-extracted with anhydrous methanol in a 250 ml. Soxhlet extractor. The readily soluble acidic extractives were neutralized at once by 5 g. of solid barium carbonate which was present in the distilling flask. Since the heavy precipitate caused severe bumping, the contents of the flask were replaced with fresh anhydrous methanol after one hour. After 24 hours' extraction, the contents of the extraction thimble were dried in vacuo at room temperature to a constant weight of 8.31 g. (Expt. 470, 16.98 g.). The methoxyl content of the white mixture of fibers and powder was 17.2, 16.7% (Expt. 470, 9.12, 8.95%).

Methanol Soluble Fractions After the addition of a slurry of 6 g. of barium carbonate in 200 ml. of distilled water, the methanol extract (550 ml.), was evaporated to 200 ml. at atmospheric pressure. The solids were removed on a filter, washed carefully with water and discarded. The neutral filtrate was then extracted with six 100 ml. volumes of chloroform but with considerable difficulty. On agitation an emulsion was formed, that was completely stable for 48 hours and was not broken by heat or cold or by addition of solvents or electrolytes. This emulsion was partially broken by centrifuging at 3000 r.p.m. and the clear chloroform layer was withdrawn by pipette and counter extracted with water. The chloroform soluble materials, recovered as a white flecked syrup by evaporation were then dried to constant weight at 60° in vacuo. This syrup (0.1378 g.) contained much thallium and was united with similar extracts from Expt. 470 (0.4336 g.).

The residual contents of the centrifuge tubes and the aqueous washings of the chloroform extract were united and freed of chloroform by heating on a steam bath, the evaporation being hastened by jets of compressed air playing on the liquid surface. The concentrate (200 ml.) was then extracted continuously with butanol at approximately 62° in a 250 ml. liquid-liquid extractor operating under water pump vacuum. After 36 hours, the extraction was stopped and the butanol extract, containing a considerable amount of white solids, was dried to constant weight in vacuo at 60° after vacuum distillation of the butanol. The 5.32 g. of amorphous residue (Expt. 470, 6.78 g.) consisted of white crystals in a matrix of yellow syrup and contained appreciable quantities of thallium.

The chloroform and butanol extracts of the methanol soluble material were united and the aqueous solution was passed through the IR-120 and IRA-410 ion-exchange columns until neutral and free from thallium. After evaporation of the water and drying of the residue to constant weight at 60° in vacuo, 1.2568 g.

of methylated cellulose was obtained as a water white, easily crushed solid having a methoxyl content of 33.07, 33.16%. This product was divided in proportion between Expt. 460, 0.5519 and Expt. 470, 0.706 g. and was termed the "Methanol Soluble Extractable Fraction".

The Methanol Soluble Non-extractable Fraction was recovered from the butanol-water phases remaining in the body of the continuous extractor. The mixture was evaporated to dryness under an air jet and the solid, heavy residue brought to constant weight (27.43 g.) *in vacuo* at 62°. (In Expt. 470, the material charred and frothed on drying in the vacuum oven and the remains weighed 39.62 g.).

When the thallium-containing solids had been dissolved in water, freed of inorganic salts by ion-exchange, the organic matter was isolated and dried to a colorless, transparent, tough film (2.3015 g.) having a methoxyl content of 29.43, 29.41%.

Methanol Insoluble Material A weighed amount of the white semi-fibrous powder (8.31 g.) was placed in a 16 inch length of cellophane dialysis tubing, having a surface area of 2 1/2 sq. inch per inch. A one-holed rubber stopper was inserted in the open end and the membrane bound tightly to it. The sac was then suspended in 4 liters of distilled water and water added to the dialysis sac to the desired level. Penetration of water into the powder was rather slow and was accompanied by swelling of the fibers, the final product being a heterogeneous cloudy colloidal suspension. Tests were made for thallium in the dialysate when this was replaced with fresh distilled water at the end of each 12 hour period. After 84 hours of dialysis, no positive test for thallium was obtained on aliquots both from the dialysate and from the solution within the sac. After a further 24 hours of dialysis, the contents of the sac were filtered through a tared sintered glass funnel with little difficulty. The insoluble portion was washed well with water and dried at 105° and 2 mm. pressure to constant weight. The product (7.58 g.) was a translucent, fibrous almost unbreakable disk which had a methoxyl content of 16.38, 16.40% and gave no test for thallium.

The water soluble material from the sac had marked foaming properties and was evaporated at 60° in a tared round bottom 100 ml. flask with the aid of a gentle jet of compressed air. After drying to constant weight at 60° under high vacuum, a tough, transparent thallium-free film (0.5444 g.), having a methoxyl content of 26.18, 26.60% was obtained.

The highly colloidal material remaining in the sac after the dialysis in Expt. 470 (16.98 g.) was extremely difficult to filter but was divided into 1.4692 g. of water soluble friable fibers containing 15.74, 15.77% methoxyl, and a water insoluble fraction weighing 9.42 g. (methoxyl 12.30, 12.32%) which could be crushed in a mortar.

The combined dialysate (32 liters) was evaporated quickly over a Meeker burner with the help of air jets. The incrustation of white thallium salts was washed before transferring the concentrate to a tared 100 ml. flask for further concentration at 60°. The product remaining after drying in vacuo at 60° was a moist colorless gel, weighing 0.5621g., which contained thallium. This fraction, united with a similar fraction from Expt. 470, weighing 0.9559 g., was dissolved in water and the solution de-ionized with the resins IR 120 and IRA 410. The resulting neutral product was concentrated in a tared flask and dried at 60° in vacuo to a tenacious gel weighing 1.3217 g. and with a methoxyl content of 23.80, 24.78%. This was distributed between Expt. 460, 0.4900 g., and Expt. 470, 0.8317 g.

Methanolysis of Partly Methylated Celluloses

The water insoluble methyl cellulose fractions from Expts. 460 and 470 were subjected to methanolysis by the method of Irvine and Hirst (116). Small samples (1.2 g.) were sealed in Pyrex tubes each containing 25 ml. of 1.37% hydrogen chloride in anhydrous methanol. The tubes were inserted in small steel bombs with a cushion of methanol and were rotated for 7 days at 128° in a special constant temperature bath (117). The undissolved material remaining after this treatment was recovered and washed with methanol in tared sintered glass filter cups. The dry mass consisted of undegraded fibers mixed with an appreciable amount of brown thallium salts. (Methoxyl content, Expt. 460, 5.32, 5.34%; Expt. 470, 4.56, 4.37%).

The brown methanol filtrate was passed through ion exchange columns of IR-120 and IRA-410 until neutral and thallium free. When concentrated and dried at room temperature *in vacuo* over phosphorus pentoxide a colorless syrup was obtained. Methoxyl content: Expt. 460 - 34.51, 34.54%; Expt. 470 - 34.40, 34.20%.

TABLE XXX

METHANOLYSIS OF PARTIALLY METHYLATED CELLULOSES

		Expt. 460		
		Weight (g.)	Methoxyl (%)	Methoxyl (g.)
Methanolysis product:	insoluble	0.8602	5.33	0.046
	soluble	1.4069	34.52	0.485
Total recovery		2.2671		0.531
Methyl cellulose sample		2.3836	16.39	0.391

		Expt. 470		
		Weight (g.)	Methoxyl (%)	Methoxyl (g.)
Methanolysis product:	insoluble	1.7418	4.46	0.078
	soluble	1.3914	34.30	0.478
Total recovery		3.1332		0.556
Methyl cellulose sample		3.7022	12.31	0.456

Tosylation of Methylated Celluloses

The tosylation of the partially methylated cellulose fraction from Expts. 460 and 470 and of the methylated methyl glucosides from their water insoluble fractions was carried out by a modification of the procedure of Mahoney and Purves (92). Small samples (0.15 - 0.50 g.) of these dissimilar products were dissolved or swollen in anhydrous pyridine (2 ml. per 0.1 g.) at room temperature until the most insoluble specimen no longer seemed to swell. p-Toluene sulphonyl chloride (tosyl chloride), purified to the correct melting point by two recrystallizations from ether, in a ratio of 12.5 moles per mole of free hydroxyl group in the sample, was dissolved in anhydrous pyridine (2 ml. per g. of tosyl chloride). The tosyl chloride solution was then added to the sample at approximately 5°C. These solutions were then allowed to stand for 12 hours at 22 ± 1°, those containing undissolved material being continually tumbled during this period. The brown solutions were then "drowned" in 300 ml. of ice cold water containing 10% of acetone and were allowed to stand for a minimum time of one hour.

The solubility of the product decreased with decrease in the methoxyl content of the original material: the highly methylated samples giving solutions, those of intermediate methoxyl content giving unfiltrable gums, while those containing the least methoxyl produced filtrable solids. Depending on its characteristics, the product was then isolated by extraction, filtration, or a combination of these techniques, and analysed for methoxyl content. The solids were recovered in tared sintered glass filter crucibles, were washed with acetone-water and dried to constant weight. In extractions the aqueous solution was shaken with four 75 ml. volumes of benzene. The benzene extract was then washed twice with 50 ml. of 10% hydrochloric acid, twice with 50 ml. of saturated sodium bicarbonate solution and with water. The aqueous solution was then resaturated with chloroform, hydrochloric acid, and bicarbonate in the same manner. The combined benzene and chloroform extracts were evaporated and the residues dried at room temperature over phosphorus pentoxide in vacuo

Iodination of Tosylated Products

The iodination of the tosylated products was carried out by the procedure of Mahoney and Purves (92) with acetonyl acetone as the solvent for the sodium iodide with the exception of the methyl methylglucoside in which acetone was used (95).

To 0.2-0.5 g. samples was added 25 ml. of a solution of 250 ml. of redistilled acetonyl acetone containing 10 g. of sodium iodide. After heating the mixture for 2 hours at 117°, the brown liquid was poured into 200 ml. of ice cold water-acetone (10-1). The solubilities of the iodinated products were similar to those of the parent tosylated methyl celluloses and the isolation was completed in the same manner. The removal of acetonyl acetone products that had to be recovered by extraction was at 80° and 2 mm. pressure and gave brown syrups. The methyl methylglucosides iodinated in acetone were extracted with benzene and chloroform and were also back-extracted with 1 N sodium thiosulphate. All products were then analysed for methoxyl content and iodine content.

Periodate Oxidations of partly Methylated Celluloses

The various fractions from Expts. 460 and 470 and of the methylated methyl glucosides from their non dialysable, water insoluble constituents were oxidized with periodate according to Mahoney and Purves (92). Small samples (0.05-0.15 g.) were weighed into 50 ml. volumetric flasks and dissolved or swollen with 5 ml. of distilled water. After 24 hours the flasks were filled with a solution of 0.05 M sodium paraperiodate containing 22.2 ml. of acetic acid per liter (i.e., pH 4) and maintained at 21 ± 1° during the reaction period. At intervals, 5 ml. aliquots of the solution and the blank were removed and titrated with 0.01 N sodium arsenite as originated by Fleury and Lange and described by Jackson (118). This titration depended on the fact that at 20° and pH 9, the periodate ion, but not the iodate ion formed in the oxidation, was capable of oxidizing arsenite to arsenate.

$$IO_4^- + AsO_3^{\equiv} \longrightarrow IO_3^- + AsO_4^{\equiv}$$

When excess of arsenite was added, titration of the unoxidized excess with standard iodine made it possible to calculate the amount of periodate remaining at any time. In a typical experiment, an 0.0665 g. sample of methyl cellulose of substitution 2.32, corresponding to 0.342 millimole of base molecular weight 194.5, was oxidized with 45 ml. of 0.05 M periodate in presence of 5 ml. of water, under the same conditions as a blank solution containing 45 ml. of periodate and 5 ml. of water. After 60 hours an excess (i.e., about 50 ml.) of 0.01 N arsenite solution was added to a 5 ml. aliquot. The arsenite equivalent of the periodate aliquot was found to be 44.64 ml. by titrating with 0.01 N iodine. Similarly an aliquot of the blank periodate solution was equivalent to 46.15 ml. of 0.01 N arsenite. The difference (46.15 - 44.64) of 1.51 ml. corresponded to the periodate that had been reduced by the sample in that time. This amount was $\frac{151 \times 0.01 \times 10}{2}$ or 0.075 millimole for the entire sample, corresponding to $\frac{0.075}{0.342}$ or 0.22 mole per mole of methylated cellulose. The results of these oxidations are summarized in Tables XXXI, XXXII, XXXIII.

TABLE XXXI

OXIDATION OF PARTLY METHYLATED CELLULOSES WITH PERIODATE

Methyl Cellulose				Oxidation		
D.S.	Base Mol. Wt.	g.	m. moles	Hours	m. moles KIO_4	Moles KIO_4 per mole
Benzene Soluble Fraction						
2.32	194.5	0.0665	0.342	60	0.075	0.219
				84	0.066	0.193
				108	0.065	0.190
				156	0.059	0.173
				204	0.063	0.184
Methanol Soluble Extractable Fraction						
2.04	190.5	0.1009	0.529	60	0.104	0.197
				84	0.102	0.193
				108	0.101	0.191
				156	0.104	0.197
				204	0.102	0.193
Methanol Soluble Non-extractable Fraction						
1.77	186.8	0.0975	0.522	60	0.082	0.157
				84	0.075	0.143
				108	0.072	0.143
				156	0.074	0.143
				204	0.067	0.128
Dialysate Fraction						
1.43	182.0	0.0850	0.467	60	1.49	3.19
				84	1.49	3.19
				108	1.48	3.18
				156	1.48	3.18

TABLE XXXII

OXIDATION OF PARTLY METHYLATED CELLULOSES WITH PERIODATE

Methyl Cellulose				Oxidation		
D.S.	Base Mol. Wt.	g.	M. Moles	Hours	M. Moles KIO_4	Moles KIO_4 per mole
Non-dialysable, water soluble fraction (Expt. 460)						
1.57	183.9	0.0587	0.319	60	0.158	0.445
				84	0.161	0.504
				108	0.159	0.496
				156	0.151	0.473
				204	0.156	0.489
Non-dialysable, water insoluble fraction (Expt. 460)						
0.94	175.1	0.0956	0.546	60	0.115	0.217
				84	0.132	0.242
				108	0.146	0.267
				156	0.174	0.318
				204	0.222	0.406
Non-dialysable, water soluble fraction (Expt. 470)						
0.89	174.4	0.1241	0.712	60	0.255	0.358
				84	0.274	0.385
				108	0.265	0.372
				156	0.280	0.393
				204	0.288	0.404
Non-dialysable, water insoluble fraction (Expt. 470)						
0.69	171.7	0.1686	0.982	60	0.200	0.204
				84	0.235	0.239
				108	0.265	0.270
				156	0.300	0.306
				204	0.384	0.391

TABLE XXXIII

OXIDATION OF THE METHYL METHYLGLUCOSIDES WITH PERIODATE

Methyl Methylglucoside				Oxidation		
D.S.	Base Mol. Wt.	g.	M. Moles	Hours	M. Moles KIO_4	Moles KIO_4 per Mole
From Non-dialysable Water Insoluble Fraction (Expt. 460)						
2.38	213.3	0.0995	0.469	60	0.418	0.895
				84	0.427	0.914
				108	0.433	0.928
				156	0.452	0.967
				204	0.481	1.03
From Non-dialysable Water Insoluble Fraction (Expt. 470)						
2.19	210.7	0.0989	0.468	60	0.475	1.01
				84	0.507	1.08
				108	0.470	1.00
				156	0.485	1.03
				204	0.504	1.07

Chain Length Distribution of a Partly Methylated Cellulose

In the following investigation of the non-dialysable, water insoluble fraction from Expt. 460, the nitration, fractionation and viscosity determinations with the derived curves were completed by Timell, then at Syracuse University and only the methoxyl determinations and the calculations based thereon were contributed by the writer.

Timell first ground the hard horny methyl cellulose until it passed through a 40-mesh screen. After 3 days drying in vacuo over phosphorus pentoxide at room temperature, the powder weighed 4.437 g. and had a methoxyl content of 16.38, 16.40.

A sample (3.191 g.) was nitrated as described by Timell and Purves (119) in a mixture of phosphoric anhydride - phosphoric acid - nitric acid (10:26:74), prepared according to the directions of Alexander and Mitchell (120). When the almost clear solution, produced by 5 hours' nitration at approximately 15°C. was poured into a saturated sodium chloride - water solution at -6°C., the methyl cellulose nitrate precipitated and was recovered on a tared Pyrex glass filter funnel in which it was stabilized and dried (121). The dry material (4.437 g. or yield of 139% by weight) had a methoxyl content of 8.28%. The methyl cellulose nitrate, 4.004 g., was dissolved in 200 ml. of acetone in a 500 ml. Erlenmeyer flask, fitted with a rubber stopper through which a stirrer and a burette entered. Dissolution took place rapidly, indicating the very low D.P. of the sample. A slight haze persisted, and the insoluble material was removed by the centrifuge before the fractionation was started. It amounted to 35.3 mg. The real amount fractionated was thereby reduced by 35.3 mg. to 3.969 g. The fractionation which divided the methyl cellulose nitrated into 12 fractions was carried out as described by Timell and Purves (119). The total weight of the fractions, 3.765 g. corresponding to a yield of

95% was a reasonable value. Timell then determined the viscosity of each sample in acetone, using an Ostwald viscometer which required 194 seconds for the efflux of the pure solvent. The kinetic correction of only 0.75%, calculated according to Schultz was always applied. The intrinsic viscosity was calculated from experimental data with the help of Huggin's equation and with the value (0.41) for k' found earlier by Timell and Purves (122) to be valid for methylated cellulose nitrates. For converting the intrinsic viscosity values to degree of polymerization, the formula D.P. $= k \, [\eta]$ was used, in which k was a constant amounting to about 100 for cellulose nitration in acetone at 25°C. These data appear in Table XXXIV while Table XXXV gives the data for the construction of integral weight - distribution curve (Fig. 24). Table XXXVI includes the methoxyl contents of the various fractions and their methoxyl degree of substitution calculated on the basis of a total substitution of 2.90, which value was obtained by Timell and Purves (123) quite consistently when methyl cellulose was nitrated by this procedure.

TABLE XXXIV

FRACTIONATION OF METHYLATED CELLULOSE NITRATE

Fraction No.[a]	Weight (mg.)	Mg. in 100 ml. Acetone	Time (sec)	η_{sp}	$[\eta]$ corr.
1	86.1	43.6	239.5	0.2397	5.04
2	355.5	46.4	216.5	0.1170	2.42
3	299.7	51.3	213.6	0.1031	1.94
4	445.3	44.8	206.8	0.0680	1.49
5	500.7	55.4	205.1	0.0592	1.05
6	370.2	47.3	202.3	0.0447	0.94
7	303.3	47.2	201.2	0.0390	0.82
8	407.2	45.0	198.5	0.0251	0.56
9	194.9	73.2	200.3	0.0344	0.47
10	217.9	80.2	200.0	0.0328	0.41
11	122.6	100.4	197.2	0.0184	0.18
12	461.1	100.0	194.7	0.0055	0.06
Total	3,764.5			Average	1.09
Original Sample		65.8	207.3	0.0706	1.05

(a) The fractions are numbered from 1 to 12 in the order in which they were precipitated, i.e., the most soluble fraction is number 12.

TABLE XXXV

DATA FOR INTEGRAL CHAIN LENGTH DISTRIBUTION CURVE

Fraction No.[a]	$P \triangle n$	$\sum P \triangle n$	P
1	12.23	12.23	6
2	3.28	15.51	18
3	5.78	21.29	41
4	5.20	26.49	47
5	10.78	37.27	56
6	8.07	45.34	82
7	9.83	55.17	94
8	13.29	68.46	105
9	11.83	80.29	149
10	7.98	88.27	194
11	9.44	97.71	242
12	2.29	100.0	504

(a) The order of numbering is the reverse of Table XXXIV. Here, number 1 is the most soluble fraction.

TABLE XXXVI

VARIATION OF METHOXYL SUBSTITUTION WITH CHAIN LENGTH OF NON-DIALYSABLE, WATER INSOLUBLE FRACTION (EXPT. 460)

Fraction No.	Weight (mg.)	Methoxyl (%)		Methoxyl (mg.)	Methoxyl D.S.(b)	D.P.
1	86.1	2.38,	2.41	2.07	0.22	504
2	355.5	3.45,	3.47	12.28	0.32	242
3	299.7	3.97,	3.99	11.94	0.36	194
4	445.3	4.42,	4.37	19.65	0.40	149
5	500.7	5.51,	5.51	27.75	0.49	105
6	370.2	5.87,	5.88	21.72	0.52	94
7	303.3	7,04,	7.04	21.28	0.62	82
8	407.2	8.72,	8.69	35.41	0.75	56
9	194.9	9.85,	9.81	19.15	0.85	47
10	217.9	12.28,	12.35	26.84	1.03	41
11	122.6	16.01,	15.98	19.60	1.30	18
12	461.1	19.48,	19.50	89.90	1.54	6
Total Recovery	3,764.5			307.59		
Original Sample	3,969.0	8.35		331.2(a)	0.72	105

(a) With proportionate loss of weight in each fraction, 3,764.5 mg. should give 314.0 mg. methoxyl, therefore, 98% of methoxyl groups were accounted for.

(b) Calculated on basis of total substitution of 2.90.

RESULTS AND DISCUSSION

The Preparation of Cellulose of Known, High Accessibility

Attention was at first confined to the preparation in larger quantity (20 g.) of a cellulose that had high and, if possible, reproducible accessibility as measured by oxidation with chromium trioxide. Glegg (80) had found that when 1 to 2 g. of chromium trioxide, dissolved in 4:1 acetic acid - acetic anhydride was in contact with 1 to 2 g. of cellulose at 20°, the oxidation rate curve became linear in about one hour and could be extrapolated back to zero time quite satisfactorily after 3 hours to give values of accessibility in terms of moles of chromium trioxide reduced per glucose unit. These values agreed within ± 10% for samples of the same cellulose and ranged from 0.01 for dry cotton linters to 0.7 for swollen samples dried from benzene. The swollen samples had been mercerized in 10% caustic soda at 0-5°C. for 3 hours, neutralized with acetic acid, solvent exchanged into benzene and dried from benzene _in vacuo_. Keays (17) noticed that similar preparations of cellulose still cont~~ained~~ benzene (0.61%). Jörgensen's modification of the Glegg method meant that the 30 minute intercept of the rate curve was accepted as the accessibility value, rather than the value derived by extrapolation. Jörgensen found the reproducibility of the intercept method to be within ± 5% with undried samples.

There were two known factors that might affect the position or slope of the oxidation rate curve and hence the intercept or extrapolated value of accessibility; the first being the slow reduction of chromium trioxide by the acetic acid - acetic anhydride noted by Gladding and Purves (83); and the second the unknown effect of benzene in the samples. The effect of the solvent on chromium trioxide is given in Tables XIV and XV and in Plots I and II in Fig. 19, which show that the rate of reduction was dependent on, and almost directly proportional to, the concentration of chromium trioxide for 48 hours at least. Plot I shows that when 1.01 g. (10.1 m. moles) of chromium trioxide was dissolved in 200 ml. of acetic acid - acetic anhydride (4 vols. to 1 vol.) at 20°C., the rate of reduction was 0.016 m. moles per hour. For Plot II, in which the initial concentration was almost doubled (19.6 m. moles) 0.024 m. mole of the chromium trioxide was reduced per hour. Since the rate was the same at all times, the imposition of this reaction on the cellulose oxidation increases the slope of the later linear portion of the cellulose oxidation curve but not its extrapolated value. This inference is illustrated in Fig. 20 where Plot I was experimental and Ia was obtained by deducting the moles of chromium trioxide reduced by the solvent from the total reduction. This correction was determined at any time by the initial concentration of chromium trioxide and was given by Fig. 19. The value at the 30 minute intercept was high by a non-detectable amount, being only 0.007 m. mole of chromium trioxide, equivalent to approximately 0.005 ml. of 0.1 N thiosulphate. Oxidation of

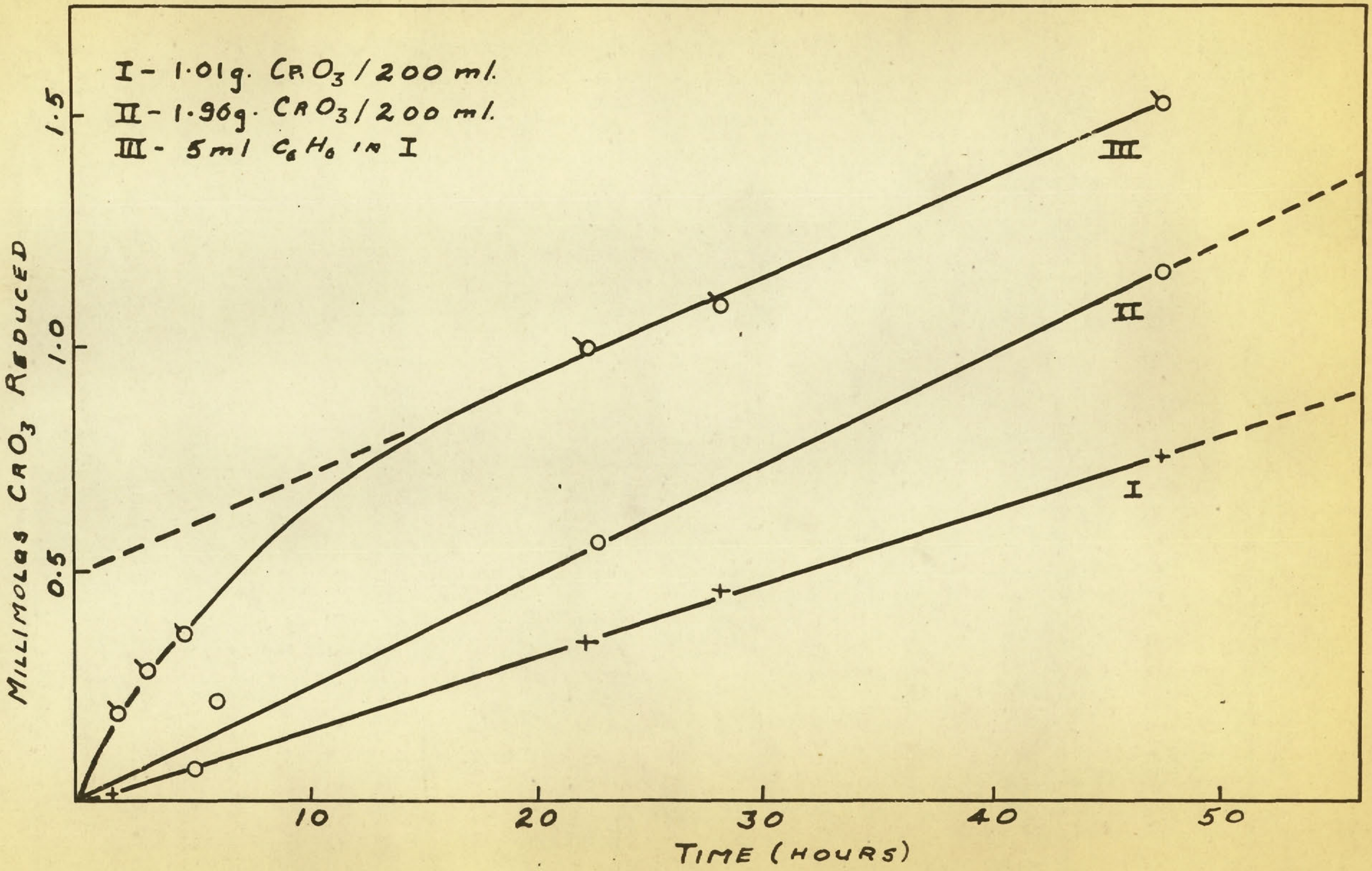


Fig. 19 : The Reduction of Chromium Trioxide by Solvent and by Benzene

solvent had therefore no appreciable effect on accessibility values. When 5 ml. (55 m. moles) of anhydrous, thiophene-free, reagent grade benzene was introduced into 195 ml. of the oxidizing solution containing 1.01 g. (10.1 m. moles) of chromium trioxide, the data given in Table XVI andPlot III of Fig. 19 were obtained. It was evident that some fraction of the benzene reduced 0.5 m. mole of chromium trioxide in approximately 15 hours, after which time the linear portion was practically parallel to Plot I. This result would be expected, for Plot I referred to the same initial concentration of chromium trioxide but benzene was absent. The linear portions of the accessibility plots for linters having some residual benzene occluded would therefore be higher than, and parallel to, the true position. Calculation showed that the amount of displacement that could be expected was negligible. A swollen sample weighing 2 g. and containing 2% of benzene (about 0.5 m. mole) would reduce only 0.004 m. mole of chromium trioxide in 15 hours because of the benzene content. This amount would not be detected.

In another preliminary study, some cellulose samples were oxidized with chromium trioxide solution for 120 hours to observe the general trend of the over-all oxidation. These data, collected in Tables XVII to XX, are reproduced in Fig. 20. Plots I and II were obtained when dry cotton linters were oxidized with 2.61 g. and 3.81 g. of chromium trioxide per gram of cellulose respectively. Although the accessibility of the linters did not appear to be independent of the concentration of oxidant

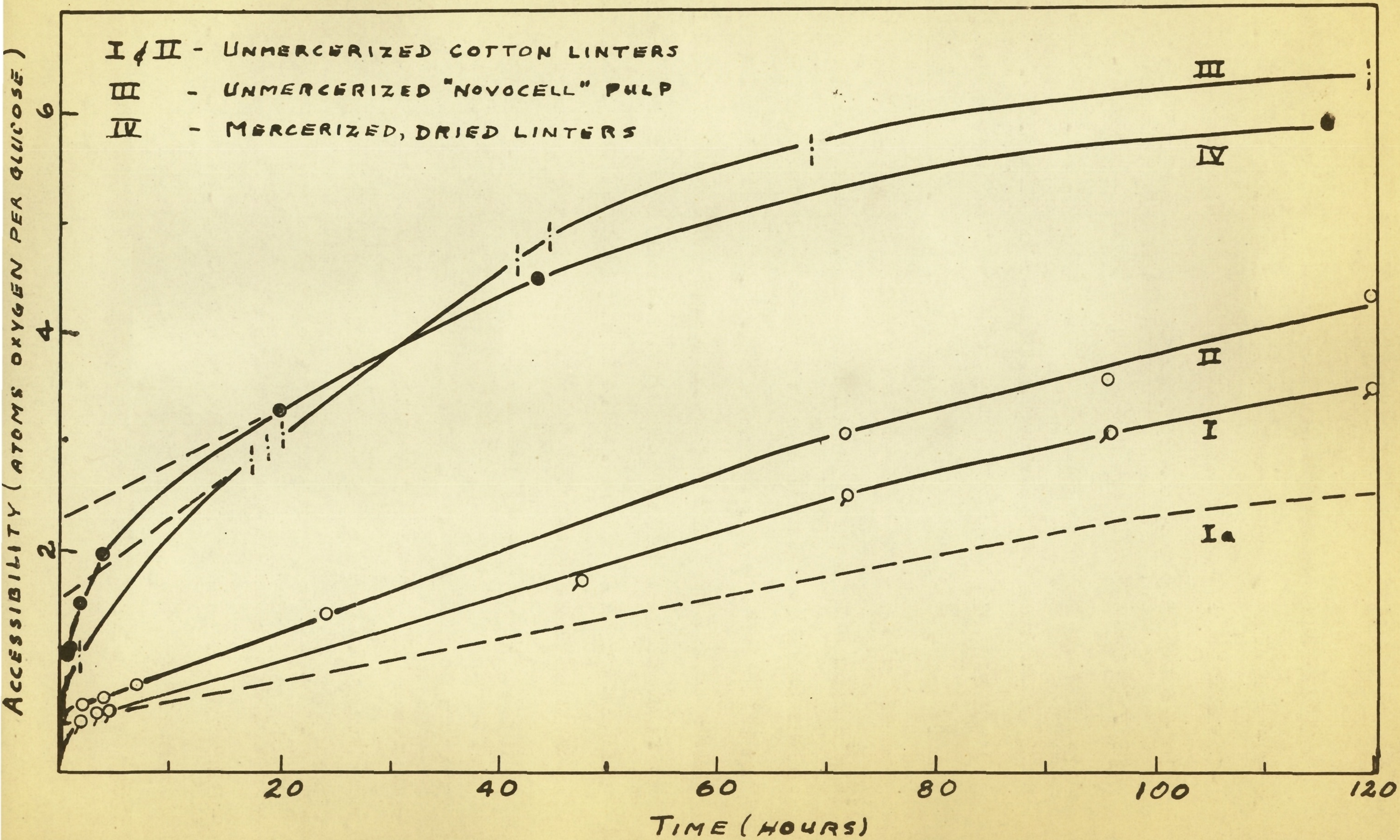


FIG. 20: CHROMIUM TRIOXIDE OXIDATION OF COTTON LINTERS

as Glegg maintained (80), Jörgensen accessibilities of 0.39 and 0.43 were within ± 5% of the mean value of 0.41. Jörgensen (126) gave a value of 0.24 for cotton linters after swelling in water for 12 hours, while Glegg (80) found that unswollen linters dried through solvent-exchange consumed 0.005 atom of oxygen per glucose unit. The average value of 0.41 for linters might be compared with an intercept value of 0.65 for a purified wood pulp (Table XIX or Plot III) and for 0.87 for a swollen, dried sample of linters (Table XX and Plot IV). The pulp, while having somewhat more accessibility than the dry linters, had less than the swollen linters. As the oxidation continued, however, the pulp was oxidized at a considerably faster rate than the swollen linters and consumed more oxidant, although the initial concentration of both samples was 3.43 g. of chromium trioxide per gram of cellulose. These results are in accord with Jörgensen (78), who considered the crystallites of pulp to be more "frayed" than the crystallites of linters and hence to be more subject to continued attack.

The Jörgensen measurement was used from this time on, as the standard test of accessibility, its particular advantage over the method used by Glegg being that it gave the required information in only 30 minutes by iodometric titrations at the beginning and end of the oxidation. This statement of course neglects the time of 2 to 12 hours spent in solvent-exchanging the samples into the oxidizing solvent for either method. As Jörgensen pointed out, the rapid oxidation in the accessible portion was nearly complete after 30 minutes and the corresponding accessibility values would be nearly correct. In addition, Jörgensen had shown that cellulose hydrate II had an accessibility of 1.55 to 1.72 for preparations from cotton, cotton linters and spruce holocellulose, all reproducible well within ± 5%. This value was considerably higher than that for the most accessible cellulose prepared by Glegg (0.97).

The word "reproducible" has been emphasized, since it represents a very elusive characteristic in cellulose. This remark can best be illustrated by the work of Assaf, Haas and Purves (56) and Glegg (80), since in each case cotton linters were swollen by the method of Richter and Glidden (37), the caustic soda being neutralized with acetic acid at 0 to 5°C. and the neutral fibers solvent-exchanged to benzene. The linters were then dried by removal of the benzene under vacuum. Assaf, Haas and Purves had from 3 to 27% of accessible material as determined by thallous ethylate, while Glegg found his samples to contain 30 to 40%. Brown (109) pointed out that the accessibility was highly dependent on the method of removal of caustic from the swollen fibers, noting that Glegg neutralized the cellulose - caustic soda slurry directly with acetic acid and got highly accessible material, while Assaf, Haas and Purves obtained less available material when they diluted the alkali and washed the swollen cellulose before de-ashing with acetic acid. In none of these cases was it claimed that several specimens of cellulose had been prepared with the same accessibility.

The figure of 1.65 atoms of oxygen per glucose unit for cellulose hydrate II from cotton linters, quoted by Jörgensen, could then represent a maximum value. The other lower values obtained by other workers would merely indicate the various states of collapse which the dynamic cellulose structure passed through as the drying conditions were varied somewhat in time, pressure or temperature. That collapse had taken place was pointed out by many writers, but the original expanded state of the fibers had never been measured. With the objective, then, of preparing swollen cotton linters, with a reproducible Jörgensen accessibility of 1.65, the conditions of mercerization, etc., were standardized as strictly as was feasible. The cotton linters had been used previously by other workers in this laboratory, including Brown (109), Keays (17), Jörgensen (84) and Timell (110). Timell found the average viscosity degree of polymerization to be between 1580 and 1720.

After the linters had been dewaxed by extraction with 2:1 benzene-ethanol, they were air dried and placed in an air tight container from which batches were removed as required. Every portion taken from the same container was considered to have the same moisture content, which varied from 2.7 to 6% for the different preparations. Although the moisture determinations were carried out with great care in manipulation and weighing before and at intervals during the drying at 105° and <u>in vacuo</u>, the results were frequently erratic. Consequently it was sometimes necessary to repeat the determination several times before acceptable results were obtained. In the more recent work on the determination of the density of swollen cellulose which required a similar drying procedure, many disturbing and fluctuating results were obtained initially. The samples were finally protected by a double filtering system of cotton batting, so that when the vacuum was released, the gases flowing into the samples were cleansed of the volatile contaminants in the oven. The results were then very consistent and it is suggested that moisture determinations under the same conditions would be reproducible and precise.

During this research, 12 batches of swollen cellulose each containing approximately 20 g. of cotton linters, plus numerous small samples, were prepared. In each case the mercerization was carried out at 3°C. with a maximum temperature variation of ± 2° during 2.5 hours. The concentration of alkali was consistently between 116 g. and 123 g. of sodium hydroxide per liter of solution. These conditions were known to give maximum swelling (37). The alkali was then washed from the cellulose by repeated periods of soaking in ice cold water, the temperature at all times being between the 3 ± 2° limit, the sample being hereby converted into the cellulose hydrate II state (34)(35). It was found that when the alkali concentration was diminished too rapidly in the initial stages of washing, or if the mass was insufficiently stirred during this period, the almost colorless gel tended to separate into lumps. These lumps could not be dispersed by stirring at later stages and the final product contained many hard white pellets which would create a diffusion problem in later studies. With care, the fibers obtained were fairly well separated. Under these swelling conditions 1.82% of the cotton linters was soluble in the alkaline solution (Table XIII). This figure was in the range of solubility given in the specifications of the linters i.e., less than 2%, and would be predicted from the work of Eisenhut given in Table III. Keays (17) reported that between 1.51 and 1.75% of these linters was soluble under the foregoing conditions.

After the cellulose hydrate II had been de-ashed with ice cold 10% acetic acid and washed to neutrality again, it was, on occasion, converted to hydrate I by boiling for a short time in the final wash water (124). All preparations were then carefully solvent-exchanged at as low a temperature as possible, through anhydrous acetic acid to anhydrous benzene. The exchange was continued at each stage until the refractive index of the liquid that had been in contact with the linters had regained its original value. This procedure was simple and quite satisfactory. The refractive index of the benzene, in contact with masses of solvent-exchanged celluloses, did not vary during a year of storage, an indication that all mobile water or acetic acid had been removed.

The Effect of Drying on the Accessibility of Cellulose

The Jörgensen method was used to delineate the effect of drying on the accessibility of cellulose, particularly the effect of slow removal of benzene from samples swollen and solvent-exchanged into benzene. Table XXXVII summarizes the results for a cellulose hydrate II. The fibers were drained and samples of the benzene wet material were placed in a desiccator over phosphorus pentoxide and paraffin shavings.

TABLE XXXVII

EFFECT OF SLOW REMOVAL OF BENZENE ON THE ACCESSIBILITY OF CELLULOSE HYDRATE II

Sample No.	Weight (g.)	Drying		Thiosulphate[b] (ml.)	Accessibility[c]
		months	mm.[a]		
1	0.4659	6	200	...	0.87[d]
2	0.4804	8.5	115	1.03	0.62
3	0.4838	8.5	115	1.18	0.71
4	0.5725	8.5	115	1.38	0.71
5	0.5809	12	60	0.36	0.19
6	0.5919	12	60	0.69	0.34

(a) Over P_2O_5 in desiccator at pressure of mercury noted.

(b) Difference between initial and final titration of 5 ml. aliquots in terms of 0.090 N thiosulphate.

(c) By CrO_3 in 30 minutes at 20°C. as atoms of oxygen per glucose unit.

(d) From Table XX.

The desiccator was then stored while evacuated slightly, and approximately each week the vacuum was slightly increased. The last two samples, from which benzene had been removed at 60 mm. pressure, had lost about half their measured original accessibility. Table XXXVIII gives the effect of more rapid removal of the benzene from a partially collapsed cellulose hydrate II. A portion of a large preparation in benzene, of accessibility 1.0, was quickly drained and placed in a desiccator over phosphorus pentoxide and paraffin chips, and dried at water pump pressure with a calcium chloride drying tube in the line for 96 hours, and for a further 48 hours under Hy-vac pressure. At intervals four samples were removed, two for accessibility measurement and two for drying at 2 mm. pressure and 65°C. to determine their solvent content. Table XXXVIII reveals the large decrease in accessibility occasioned by the removal of benzene and explains the lack of reproducibility in samples prepared in this manner by other workers.

TABLE XXXVIII

EFFECT OF MORE RAPID REMOVAL OF BENZENE ON ACCESSIBILITY OF CELLULOSE HYDRATE II

Drying Data		Accessibility Data			
		Weight			
Weight (g.)	Benzene(a) (%)	(g.)	Corr.(d) (g.)	Thio(b) (ml.)	Accessibility (c)
Never dried(e)					1.04, 0.97
Dried at 20° for 24 hours					
0.6897	2.89	0.4310	0.4187	0.37	0.30
0.6767	2.80	0.5288	0.5137	0.59	0.39
Dried at 20° for 48 hours					
0.5900	1.83	0.6111	0.6000	0.48	0.27
0.4855	1.81	0.4210	0.4133	0.25	0.21
Dried at 20° for 144 hours					
0.4271	1.80	0.3813	0.3744	0.19	0.17
		0.2881	0.2829	0.11	0.13

(a) After further drying for 24 hours at 65° and 2 mm. pressure. Weight changed by 0.0001 g. or less after further 24 hours.

(b) Normality = 0.1045.

(c) CrO_3 for 30 min. at 20° as atoms of oxygen per glucose unit.

(d) By subtracting benzene content given in column 2.

(e) From Table XXII, Expt. 410.

Table XXXIX is included to show the negligible accessibility of samples, swollen to the cellulose hydrate II form and then dried directly from water at 105° and 2 mm. pressure. This treatment obliterated the accessible surface completely in four instances while two samples still retained some accessibility (0.76).

TABLE XXXIX

EFFECT OF DRASTIC REMOVAL OF WATER ON ACCESSIBILITY OF SWOLLEN CELLULOSE

Sample No.	Weight[(a)] (g.)	Thiosulphate[(b)] (ml.)	Accessibility[(c)]
1	0.4372	0.98	0.76
2	0.4123	0.88	0.76
3	0.3995	0.00	0.00
4	0.6627	0.01	0.00
5	0.9905	0.00	0.00
6	0.9665	0.05	0.00

(a) After drying at 105° at 2mm. to constant weight.

(b) Thiosulphate normality = 0.1042.

(c) Jörgensen accessibility

The Effect of Solvent-Exchange and Storage in Benzene on the Accessibility of Cellulose Hydrate II

The cells illustrated in Fig. 15 were extremely useful in these small scale experiments, since the mercerization and solvent-exchange operations were readily carried out by proper manipulation of the stopcocks. Initially a small Pyrex glass wool plug was inserted at the base of the cylinder to retain fine particles of cellulose. Both the cellulose and glass wool plug were placed in the narrow neck of the volumetric flask and were pushed into the oxidizing solution for measurement of the Jörgensen accessibility. The results are given in Table XL. All samples of dewaxed, air dried cotton linters were mercerized and washed as already described to give cellulose hydrate II. Samples 1 and 2 were exchanged from water, through acetic acid to the 4:1 acetic acid - acetic anhydride mixture immediately. Samples 3 and 4 were exchanged through acetic acid to benzene. When the refractive index of the benzene effluents became constant, they were immediately exchanged back through acetic acid to the oxidizing solvent. Samples 5 and 6, 7 and 8 were solvent-exchanged through acetic acid to benzene until the refractive index was constant and remained in benzene for 2 days and 14 days, respectively before the accessibility was measured.

The results showed that neither solvent-exchange nor storage in benzene was effective in reducing the accessibility. It was noted later that the accessibility of a cellulose hydrate II

TABLE XL

THE EFFECT OF SOLVENT-EXCHANGE AND STORAGE IN BENZENE ON THE ACCESSIBILITY OF CELLULOSE HYDRATE II

	Sample		Thiosulphate	
Number	Weight (g.)	Cellulose (g.)[(a)]	(ml.)	Accessibility[(b)]
No Solvent-Exchange				
1	0.5142	0.4794	1.91	1.45
2	0.5092	0.4748	1.53	1.17
Solvent-Exchanged into Benzene				
3	0.5588	0.5209	2.09	1.46
4	0.4959	0.4623	1.70	1.33
Solvent-Exchanged into Benzene and Stored for 2 Days				
5	0.5045	0.4704	1.77	1.28
6	0.5098	0.4753	2.02	1.46
Solvent-Exchanged into Benzene and Stored 14 Days				
7	0.4465	0.4162	1.75	1.43
8	0.4629	0.4316	1.83	1.44

(a) Column 2 minus 5.04% moisture and 1.82% alkali soluble.

(b) By the Jörgensen method.

stored in benzene for 16 months had not changed from the original value. However, when the hydrate II is kept in water at room temperature, the crystal lattice slowly changes to the cellulose hydrate I form (36). The replacement of the polar water molecule with the homopolar benzene certainly stabilizes the cellulose hydrate II lattice although just how is not known, since it is hard to visualize the benzene (molecular volume of 89) replacing the water molecule (molecular volume of 18) in the lattice. On the other hand, if water remains in the cellulose hydrate II lattice after solvent-exchange into benzene, one must imagine that the benzene molecules in the accessible regions act as props to keep the lattice in its expanded form. The benzene is possibly oriented in layers along the hydrophobic faces and in this manner separates the fringes of crystallites into layers.

The Effect of Manual Pressure on the Accessibility of Cellulose Hydrate II

In one series of experiments more than 30 separate small samples were individually swollen, solvent-exchanged and oxidized. While the values obtained were between 1.01 and 1.46 the precision was poor and it seemed probable that some slight variation in the preparation of the sample was responsible. This point was checked by weighing 6 samples of a large cellulose hydrate II preparation in benzene (Expt. 354) by means of the hydrostatic balance. The contents of each sample vial were emptied into a large weighing bottle and the vial washed free of residual fibers with anhydrous acetic acid. The fibers were then solvent-exchanged through acetic acid to acetic acid - acetic anhydride and the accessibilities were completed in the usual fashion using the 200 ml. volumetric flask in the reaction. The prepared samples were placed on a glass wool plug at the base of the narrow neck and were pushed into the reaction mixture at zero time. Sample 4 lodged in the neck and considerable pressure was applied in forcing the sample into the reaction mixture. Although the reaction mixture was shaken vigorously from time to time, the accessibility of sample 4 was noticeably less than those of samples 1, 2 and 3 (Table XLI). Sample 5 was then introduced into the oxidizing mixture with no pressure on the fibers, and sample 6 was deliberately lodged in the flask neck. The accessibilities fluctuated between 1.81 and 1.43 when the samples were introduced normally; when the samples were deliberately compressed, the accessibility fell almost 50% from 1.81 to about 1.00. These results show quite conclusively the sensitivity of the cellulose hydrate II structure to collapse under even mild pressure.

At this point the procedure for measuring accessibility was modified by substituting a wide mouthed flask for the volumetric flask. The values obtained hereafter (see Tables XXI and XXII) were highly reproducible; five determinations giving 1.67 ± 0.04 atom of oxygen per glucose unit for the cellulose used in the preceding compression studies.

The Use of the Hydrostatic Balance

As was stated at the beginning of this discussion, the first stage of this research was to prepare a large batch of cellulose of known high accessibility. It was shown that the procedure of drying cellulose hydrate II from benzene made a large portion of the cellulose structure inaccessible to the

TABLE XLI

EFFECT OF PRESSURE ON THE ACCESSIBILITY OF CELLULOSE HYDRATE II

Sample No.	Weight in Benzene[(a)] (g.)	Benzene °C.	Benzene Density	Calcd. Weight[(b)] in air (g.)	Thiosulphate (ml.)	Accessibility[(c)]
Samples measured without deliberate compression						
1	0.2592	21.0	0.8777	0.5646	2.39	1.43
2	0.2184	21.1	0.8776	0.4757	2.48	1.74
3	0.1971	21.3	0.8774	0.4288	1.98	1.56
5	0.2171	21.2	0.8775	0.4729	2.54	1.81
Samples measured after compression						
4	0.2658	21.2	0.8775	0.5790	1.57	0.92
6	0.2309	19.5	0.8791	0.5036	1.54	1.03

(a) Weight of vial and sample in benzene (about 7.5 g.) minus weight of vial in benzene.

(b) Assuming density of cellulose was 1.623. (Table XXI, Expt. 354).

(c) After oxidation with CrO_3 for 30 minutes at 20°. Thiosulphate normality = 0.1045 N.

chromium trioxide reagent, while on the other hand, the accessibility could be retained indefinitely if the cellulose hydrate II was maintained in a benzene-wet condition. It therefore appeared desirable to determine accessibilities on samples that had never been dried, and to overcome the problem of accurately determining the true dry weight of such a sample after withdrawal from a much larger quantity of a benzene-wet preparation. The assumption that the accessibility would be, e.g. 1.65, by analogy with a small sample prepared in a seemingly identical fashion did not seem warranted.

Investigation showed that cotton linters could be estimated almost quantitatively (Table XXIII) by oxidation with potassium dichromate according to the procedure of Launer (112). Samples of cellulose hydrate II were subjected to the Jörgensen accessibility measurement, and the residual solids were removed by filtration. These solids were then oxidized by the Launer method, and the total consumption of oxidant in both oxidations accepted as a measure of the dry weight of the cellulose. Table XXIV shows that approximately 10% of the cellulose was unaccounted for. Gladding and Purves (83) noted that at high levels of oxidation with chromium trioxide, a portion of the cellulose was not recovered. It therefore was likely that about 10% of the oxycellulose was soluble. This method of estimating cellulose therefore could not be used.

The problem was solved by extending the use of the hydrostatic balance, as applied by Hermans (114) to determine the density of cellulose fibers in water, to measure their weight in benzene, having first found the density of that particular modification in benzene. To determine the density, four to six samples were weighed by the hydrostatic method given in the Experimental Section, these operations taking less than one hour per sample. The benzene was then removed under mild vacuum and the samples were dried in a Cenco vacuum oven at 105°C. and 1 to 2 mm. pressure till constant weight was attained. Knowing the bone dry weight of cellulose (G_1), the weight of the same sample in benzene (G_2) and the density of the benzene (d_b) in which the sample was weighed, the density (d_c) of the particular sample was given by

$$d_c = \frac{G_1 \, d_b}{G_1 - G_2} \quad .$$

Once the density was known, the samples to be used in accessibility measurements could be weighed on the hydrostatic balance and the weight in benzene converted to the weight of cellulose in the sample, since

$$G_1 = \frac{G_2 \, d_c}{d_c - d_b} \quad .$$

The results given in Tables XXV and XXVI show that the method used was very precise. The values obtained with seven different cellulose preparations were in all cases within ± 0.7% of their average value. Water-swollen cellulose had a density of 1.562; cellulose hydrate I, 1.621; and the best value for cellulose hydrate II was 1.623 (Expts. 354 and 380). These values are compared in Table XLII with average values of densities reported in the literature from experiments using different buoyancy media.

TABLE XLII

DENSITIES OF CELLULOSE MODIFICATIONS IN VARIOUS BUOYANCY MEDIA

Cotton	Buoyancy Medium			
	Helium	Benzene	Water	Benzene After Solvent-exchange
Native	1.56(a)	1.55(b)	1.61(a)	1.56
Mercerized	1.55(a)	1.53(c)	1.61(a)	....
Cellulose Hydrate I	....	....	....	1.62
Cellulose Hydrate II	....	....	1.64(b)	1.62

(a) From Davidson (45).
(b) Hermans (124).
(c) Stamm and Seborg (125).

These figures show that the density in benzene of the water-swollen cellulose used in the present research agrees well with determinations in helium or benzene. The densities of cellulose hydrate I and II in benzene lie between the densities determined in water for native cotton and cellulose hydrate II. Possibly the most noteworthy observation is the rather large spread (1.56 to 1.62) in the densities of native cotton and cellulose hydrate II, determined in benzene after solvent-exchange, in contrast with a variation of 1.61 to 1.64 when weighed in water. Qualitatively this discrepancy means that a small amount of additional water can fill spaces in the cellulose when the latter changes from native to cellulose hydrate II, but that these same changes allow the benzene molecule to enter in considerable quantity. Table XXVII shows the range of values obtained when the benzene was not completely removed, or when the fibers became contaminated during drying. The cellulose hydrate II (Expts. 380 and 410) with densities of 1.622 and 1.600, gave values of 1.573 and 1.574 when the benzene was removed at 65° under 1 mm. pressure until constant weight was reached. In these two cases reproducibility was good. When the fibers were not protected during drying at 105° *in vacuo*, erratic values as typified by Table XXVII Cellulose Hydrate II (Unprotected) were obtained, ranging from 1.576 to 1.599 for samples carried through the drying procedure together.

The Chromium Trioxide Accessibility of Various Celluloses

Once reliable density figures for the various cellulose preparations stabilized in benzene were obtained, further small samples were withdrawn from the large masses were weighed on the hydrostatic balance and subjected to oxidation with chromium trioxide. Great care was taken in handling the fibers so that collapse due to rough treatment could be eliminated. The Jörgensen accessibilities (Tables XXI and XXII) were most gratifying and confirmed the reliability of the weighing procedure and the reproducibility of the oxidation. The range of error of ± 0.04 atom of oxygen per glucose unit was equivalent to ± 1 drop of 0.1 N sodium thiosulphate in the final titration and gave promise of even better results when lower concentrations of thiosulphate were used.

The Jörgensen accessibility values obtained in the research are presented in Table XLIII.

Jörgensen himself working on the same cotton linters, found that 0.24, 1.21 and 1.65 were consistent estimations for cotton linters, cellulose hydrate I and cellulose hydrate II respectively (84). Jörgensen stated the accessibilities as "moles chromium trioxide per glucose unit" and these values have been converted to "atoms oxygen" and appear in brackets. There is reason to believe (127) that Jörgensen's units were actually "atoms of oxygen" so that the unconverted values also are listed in Table XLIII. Because of this element of doubt no comparison could be made except that in both the Jörgensen series and the present work, the accessibility increased from cotton linters to cellulose hydrate I to cellulose hydrate II. For reasons that will be discussed later, only preparations 354, 380, 460 and 470 were considered to be cellulose hydrate II and even these varied from 1.67 to 1.95, indicating variations in structure in these hydrates II which might have occurred during swelling. Although Richter and Glidden (37) and others (38) (39) contended that maximum swelling of cellulose occurred at approximately 5°C. with 10 to 11% caustic (120 g. per liter).

TABLE XLIII

THE DENSITY AND ACCESSIBILITIES OF VARIOUS CELLULOSE MODIFICATIONS

		CrO_3 Accessibility		Thallous Ethylate Accessibility	
Expt. No.	Density(a)	Atoms Oxygen per G.U. (b)	(%) (c)	OCH_3(d)	(%)(e)
Water Swollen Cellulose					
400	1.562	0.14	1.2	9.04	15.7
Jörgensen		0.24 (0.36)			
Cellulose Hydrate I					
368	1.621	0.98	8.2	14.7	25.6
Jörgensen		1.21 (1.82)			
Cellulose Hydrate II					
354	1.623	1.67	13.9	17.1	29.8
Jörgensen		1.65 (2.48)			
380	1.622	1.73	14.4	17.6	30.7
410	1.600	1.00	8.3	16.6	28.9
430	1.656	0.35	2.9	12.8	22.3
450	1.618	0.84	7.0		
460	1.623(f)	1.95	16.2	21.0(g)	46.1
470	1.623(f)	1.81	15.1		

(a) From Tables XXV and XXVI. (b) From Tables XXI and XXII.
(c) Calculated on basis of 12 atoms of oxygen = 100% accessibility.
(d) From Tables XXVII and XXIX.
(e) Calculated on basis of 57.4% methoxyl = 100% accessibility.
(f) Density assumed on basis of 354 and 380.
(g) From Table XLVI; methoxyl content based on weight of methylated sample; 45.6% MeO = 100% accessibility on this basis.

Saito (128) claimed that the concentration for maximum swelling at 0° was 30 to 40 g. of sodium hydroxide per liter. Dilution then, as occurred when the 10% alkali was washed out of the cellulose, would expand the fiber still further and the accessibility of the end product would be dependent on the rate at which the caustic had been removed, as well as on the temperature. The accessibilities, herein reported, seem to indicate that cellulose hydrate II is an indistinct stage of the "solution" of the dynamic cellulose structure in water, rather than a definite state, even while definite stoichiometric relationships exist in the crystallite. For this reason the writer prefers to consider 12 atoms of oxygen per glucose as representative of 100% accessibility rather than 1.65 which Jörgensen postulated at 100% accessibility i.e., cellulose hydrate II was a definite state of maximum accessibility. The per cent accessibility data in Table XLIII Column 4 were in accordance with the equation

$$C_6H_{10}O_5 + 6\ O_2 \longrightarrow 6\ CO_2 + 5\ H_2O$$

and were similar to values obtained by the Nickerson hydrolytic - oxidation method (Table VII). By analogy and by reason of the heterogeneous action of the chromium trioxide, it is reasonable to assume that glucosidic bonds were cleaved with subsequent recrystallization in the mesomorphic regions.

Accessibilities by the Thallous Ethylate Method

The cellulose preparations from Experiments 354 to 430, of known density in benzene and of known reactivity to chromium trioxide were further characterized by thallation and methylation. Triplicate samples (0.1 g.) of each batch, always wet with benzene, were weighed on the hydrostatic balance, with great care since the wet weight in benzene amounted to only 0.05 g. on a balance reading to tenths of a milligram. The samples were then transferred to porous cups and placed in the thallation apparatus (Fig. 15) in which they were exposed to the 0.4 N thallous ethylate for varying lengths of time. When the depleted thallous ethylate was withdrawn after one hour and replaced with fresh thallous ethylate for a further one hour, the methoxyl contents obtained after methylation with methyl iodide, were erratic. For example, the variation was from 6.5 to 12.6% methoxyl for the same preparation. When the initial thallation period was extended to 24 hours and the second thallation to 48 hours, the results were notably better (Tables XXVIII and XXIX). It appeared, therefore, that diffusion of the thallous ethylate into the interstices of the fibers was the rate controlling step. Assaf, Haas and Purves (56) and later Glegg (80) were able to avoid this difficulty, since they thallated swollen samples that had been thoroughly dried from benzene under vacuum. These workers placed the dry samples in the thallation apparatus, evacuated the system and allowed the thallous ethylate to be drawn in under vacuum, aiding the penetration of the reagent into the fibers. This technique may partly explain the fact that Glegg's methoxy values were from 30 to 40%, whereas in the present series the range was 20 to 30%, with one notable exception (Table XLIII, Experiment 460). Apart from

Experiment 430 the methoxyl values found in the triplicate estimations were reproducible to ± 5%. The methoxyl percentage of the water-swollen cellulose and the cellulose hydrate II (Expt. 354), 9.0 and 17.1%, were close to those, 9.8 and 17.8%, found by Reeves and Thompson (129) for moisture conditioned, unmercerized cotton and for mercerized cotton, respectively. These authors, however, carried out their superficial methylations with 7 treatments with 0.5 M to 0.9 M solutions of diazomethane in ether.

A study of the data collected in Table XLIII shows that there was no correlation between the density and the accessibility of the cellulose. The accessibility of each cellulose was in the same rough order by either the chromium trioxide or the thallous ethylate method. Glegg using these two measurements found no definite correlation. While the order of accessibilities was roughly the same by both methods, there was a marked difference in the magnitudes. When, for example, water-swollen cellulose was compared with cellulose hydrate II (354) the change by the chromium trioxide method was from 1.2 to 13.9% or by a factor of about twelve; by the thallous ethylate method the change was less than two-fold, from 15.7 to 29.8%. A possible explanation for these differences would relate the molecular volumes of the solvents and reactants to the variation in molecular dimensions within the fiber in changing from the water-swollen to the hydrate II form. The benzene, having a molecular volume (M.V.) of 84, carried the thallous ethylate molecule (M.V., 69) to 15% of the hydroxyl groups in the water-swelled cellulose; in the hydrate II form a further 15% were contacted. The chromium trioxide (M.V., 69) however was dissolved in acetic anhydride (M.V., 94) and acetic acid, which because of a tendency to dimerize would have a maximum molecular volume of 104 (i.e. twice the molecular weight divided by the density). The acetic acid was only able to carry the chromium trioxide (or chromium ion) to 1.7% of the oxidizable positions, but in the hydrate II form contacted twelve times the number. It would seem, therefore, that the dimensions of space in the accessible region were critical between 80 and 100 as molecular volumes. In contrast, however, Assaf Haas and Purves found that benzene carried thallous ethylate to 8% of the hydroxyl groups while ether, with a molecular volume of 104, carried the thallous ethylate to 10%.

The Experiment numbers in column 1 of Table XLIII are in chronological sequence. Each represents the processing of approximately 20 g. of cotton linters to one of three cellulose modifications which were finally stabilized by solvent-exchange and were stored in benzene. As each batch was prepared, small samples were removed for density determinations. The density being known, further small samples were oxidized with chromium trioxide to ascertain whether or not the Jörgensen accessibility was sufficiently high (1.6 to 1.7) to warrant the thallation and methylation of the remainder. The results for Expts. 410, 430 and 450 showed that these batches could not be used; the accessibilities ranged from 0.35 to 1.00; the densities varied from 1.600 to the extremely high value of 1.656, whereas the usual value was 1.623. Subsequent treatment of small samples with thallous ethylate and methyl iodide showed that their accessibilities by this method had also decreased. Since the procedures by which cellulose hydrate II had been prepared and measured had not been varied, these results were very surprising.

A systematic attempt to discover the cause of the difficulty led to the conclusion that the Jörgensen procedure was not at fault, since the oxidation of preparation 354, prepared more than 16 months before, showed the accessibility of 1.67 to be unchanged. Since the anhydrous acetic acid had solidified during the solvent-exchange of some of these samples, it was possible that the growth of the crystals had affected the structure of the cellulose. Four small (0.4 g.) samples of dewaxed, air-dried cotton linters were therefore processed as described in the Experimental Section for the preparation of cellulose hydrate II. Two of these samples were from the same lot as Expt. 450 and two were donated by Mr. G. Moulds. During solvent-exchange, the acetic acid in contact with one sample of each pair was allowed to freeze. These two samples gave Jörgensen accessibilities of 0.93 and 0.86, while the unfrozen samples gave similar values (0.90 and 0.75). These low accessibilities were therefore not due to distortion of the cellulose structure on solidification of the acetic acid. Since the pairs of samples had been thoroughly dewaxed by different workers, the similar low accessibilities were not likely to have been caused by failure to complete this operation, and by a variable and partial "water-proofing" of the fibers against the swelling action of the alkali.

The mercerizing solution, although of the correct concentration when used (116 to 123 g. per liter) and containing no sodium carbonate, had been stored since the beginning of the research as 35% caustic soda in a large soft-glass bottle that had been carefully lined with paraffin wax. When a fresh solution of 35% alkali was made up, linters mercerized under the conditions required for the formation of cellulose hydrate II had the desired accessibility of 1.67 atoms of oxygen absorbed per glucose unit.

It thus appeared likely that some change had occurred in the caustic soda during prolonged standing, and that this change was possibly, although not probably, an accumulation of sodium silicate. Unfortunately, this hypothesis was not tested at the time by analysing the residual alkali and cellulose samples for silica. It was, however, quite clear that the Jörgensen estimation was very sensitive to obscure changes which took place within the fiber, and gave values whose variation in magnitude could not be ignored. The variation in the thallous ethylate accessibilities were of a much smaller order.

Methylation of the Accessible Fraction of Cellulose Hydrate II

Unlike most methods of determining accessibility, which are based on the extrapolation and interpretation of reaction rate plots, the method of Purves and coworkers (56)(79) involves the preparation of a superficially methylated cellulose and the determination of its methoxyl content. Since the excess thallous ethylate used is dissolved in a non-swelling carrier liquid, the colloidal state of the sample should not be greatly altered during the measurement. The method involves only the assumptions that all hydroxyl groups accessible to the carrier liquid become completely thallated, and that the subsequent methylation converts the same hydroxyl groups, and no others, completely to methyl ethers. It therefore seemed reasonable to assume that the distribution of these methoxyl groups along the cellulose macromolecules and within the individual glucose residues would throw much light on the nature of the accessible portion of the original cellulose.

The next stage in this research was accordingly to methylate by the thallous ethylate procedure approximately 20 g. of highly accessible cellulose hydrate II, and to recover the methylated cellulose quantitatively and free from impurity (Fig. 21). The considerable quantities of thallous ethylate required were prepared by modifying slightly the procedure of Assaf, Haas and Purves (56) to afford additional precautions against decomposition by agents like moisture, light and carbon disul-

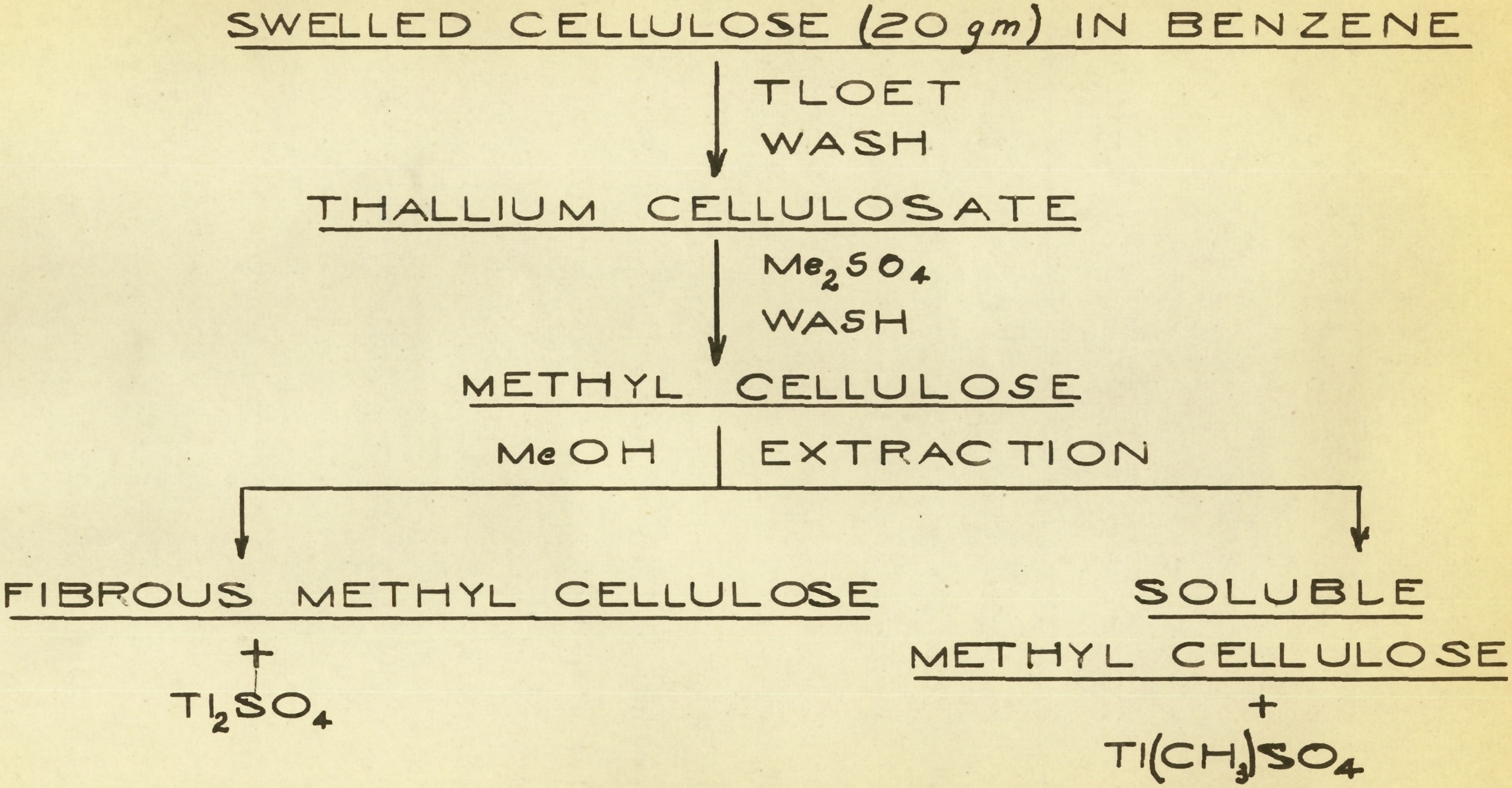


FIG 21: FLOW DIAGRAM OF THE PREPARATION AND ISOLATION OF METHYLATED CELLULOSE FRACTIONS

phide. When the proper precautions were taken, 500 ml. of stock solutions of 0.5 N thallous ethylate in benzene were successfully kept in the cold as water-white liquids for long periods. An investigation of the methylating agents showed that methyl chloride did not react quantitatively with the thallous ethylate in sealed vessels under the vapour pressure of methyl chloride, either at room temperature for 15 days or at 70° for 24 hours. Excess methyl iodide, on the other hand, reacted very readily, when thallous cellulosate was heated at 70° in benzene but the partly methylated cellulose was impregnated with the very insoluble, orange thallous iodide. When such a preparation was hydrolysed in 72% sulphuric acid, according to the procedure of Monier-Williams (115), the yield of purified syrup was 85% on a weight basis but only 65% on a methoxyl basis. It followed that under the conditions of hydrolysis some hydrogen iodide had been produced from the thallous iodide with subsequent demethylation. For these reasons and because of the higher solubility of thallous sulphate, dimethyl sulphate was used as the methylating agent.

The thallation and methylation reactions were carried out very simply with the aid of the apparatus illustrated in Fig. 18. Cellulose hydrate II (10 to 20 g.) of known accessibility and wet with benzene was transferred to this apparatus with minimum exposure of the fibres. After carrying out the thallations with two treatments with 500 ml. of 0.5 N thallous ethylate in benzene, the excess was removed and the slightly yellow fibers, now occupying approximately two thirds of their original volume, were washed with benzene. Methylation was promptly carried out with a solution of dimethyl sulphate in 450 ml. of benzene, care being taken to keep the amount (two moles per mole of hydroxyl group in the original cellulose) always sufficient to replace all the thallium occluded in or reacted with the cellulose. This excess of dimethyl sulphate, used to assure complete methylation, caused degradation of the fibers when the mixture was heated at reflux. The mass of fibers now had a chalky appearance and had settled still further. After withdrawing the sometimes slightly brown dimethyl sulphate solution, the heavy white solids were washed with benzene several times to remove all traces of dimethyl sulphate. These solutions were retained since they contained the "Benzene-Soluble Fraction" of the methylated cellulose. After drying in vacuo overnight, the solids were extracted with anhydrous methanol for 24 hours to remove the "Methanol-Soluble Fraction". After drying again to constant weight, the more or less fibrous white powder was dialysed against distilled water until there was no detectable amount of thallium ion in an aliquot removed from the dialysis sac. The recovery of solid material is given in Table XLIV. The data show that when the reflux period was reduced from 3 to 1.5 hours, the recovery of thallium-free methylated cellulose from the dialysis sac was almost doubled. Excluding Expt. 430 which was prepared with stored alkali and known to be very different in reactivity, the figures show that better recovery ran parallel to the higher accessibility probably because more thallous cellulosate involved more methylation and less dimethyl sulphate remained to degrade the fibers. The most

TABLE XLIV

EXTRACTION OF THE CRUDE METHYL CELLULOSE

			Weight of Material Recovered after			Insoluble Methylated Cellulose	
Expt. No.	Cellulose (g.)	Accessibility[(a)]	C_6H_6 Extn. (g.)	MeOH Extn. (g.)	Dialysis (g.)	Wt.[(b)] (%)	MeO (%)
354[(c)]	18.4	1.67	....	....	5.9	32	5.5
380[(c)]	22.5	1.73	69.9	20.2	9.4	42	5.3
430[(d)]	14.6	0.35	61.4	16.0	10.9	75	11.8
460[(d)]	10.8	1.95	47.0	8.3	8.1	75	17.0
470[(d)]	18.3	1.81	70.7	17.0	10.9	60	12.7

(a) By CrO_3 method. See Tables XXI and XXII.
(b) Percentage of original cellulose in dialysis sac.
(c) Refluxed with dimethyl sulphate (2 moles per mole hydroxyl) for 3 hours.
(d) Refluxed with dimethyl sulphate (2 moles per mole hydroxyl) for 1 1/2 hours.

noticeable point, however, was the large mass of solids remaining on the fibers after the benzene extraction (column 4) but which were removed in the subsequent extraction with methanol. This salt, thallous methyl sulphate, was removed in the first minutes of extraction with methanol. The remaining thallous sulphate was only slightly soluble in hot methanol but in one case (cf. Table XLIV Expt. 460 Cols. 5 and 6) was almost completely removed when extracted for the standard 24 hour period. In general, about

5 times as much thallous methyl sulphate as thallous sulphate was formed. In a separate experiment, 0.021 mole of dimethyl sulphate was heated under reflux in benzene for 24 hours with 0.044 mole of thallous ethylate. After this time only 0.023 mole of thallous ethylate had been consumed indicating the formation of about (0.023-0.021) 0.002 mole of thallous sulphate and 0.021 mole of thallous methyl sulphate.

The low recoveries listed in Table XLIV indicated the amount of degraded partly methylated cellulose that was soluble in the benzene or methanol, or was dialysable. This amount was, however, very small in comparison with the large amounts of thallium salts and the separation of the methyl cellulose proved to be a difficult and unsatisfactory process.

The "Benzene Soluble Fraction" contained most of the residual dimethyl sulphate together with small amounts of thallous methyl sulphate and highly methylated cellulose. In the early attempts to isolate the organic constituent, the strongly acidic character of dimethyl sulphate was not fully appreciated. After neutralization of the benzene solution with barium hydroxide at room temperature, the mixture on evaporation yielded a charred mass. When the benzene was removed at 40° under vacuum from an aqueous suspension containing solid barium carbonate, the dimethyl sulphate was converted to barium methyl sulphate and the solution remained neutral as the water was distilled. Towards the end of this evaporation, which was accompanied by generous frothing, the residue blackened slightly. In the final procedure a slurry of barium carbonate in water was added to the solution, the benzene was distilled at 40° under vacuum, the aqueous mixture was heated under reflux to destroy last traces of dimethyl sulphate and the solids were removed by filtration and discarded. Attempts at extracting the methyl cellulose from this aqueous concentrate with chloroform provided the writer with exceptionally stable emulsions which could not be broken. By centrifuging the emulsion at 3000 R.P.M., a small amount of clear chloroform layer was formed and removed by pipette. The remaining jelly-like layer and aqueous phase were then re-extracted. The chloroform soluble material was recovered as a clear honey-colored syrup containing some thallium (Table XLV).

The procedure with the "Methanol-Soluble Fraction" was identical with the final procedure for the "Benzene-Soluble Fraction" except that thallium methyl sulphate was present in the former rather than the barium analogue. The properties of the solutions containing the alkali methyl sulphates were similar, however, in their ability to give tenacious foams in aqueous solutions and suspensions with chloroform. The chloroform extract, recovered by centrifuging the suspension, was distilled and yielded small quantities of syrup containing much thallium (Table XLV). In a further attempt to isolate the methyl cellulose which was believed to be present in the "methanol-Soluble Fraction" the mixture remaining in the centrifuge cups after removal of the chloroform layer, was freed of chloroform. The aqueous concentrate was then continuously extracted with butanol at 62° for 36 hours. When the butanol extract was distilled a mass of thallium salts embedded in a syrup resulted (Table XLV).

At this time, samples of Amberlite Resins IR-120-AG (a sulphonic acid type) and IRA-410 (a strong base) were received through the courtesy of Rohm and Haas, Co., Philadelphia. Yorston (108) had found that only small quantities, at most, of methylated sugars were absorbed by these resins when solutions of the sugars containing inorganic salts were de-ionized. Since the "Benzene-Soluble Fractions" from Expts. 460 and 470 were small in quantity and were similar in methoxyl content, they were united and their aqueous solution was freed of thallium salts by passage through columns of the Amberlite base and acid exchange resins in turn, regenerating the columns with acid or alkali, and recycling the solutions until all thallium had been removed and the effluent was neutral. The liquor was then distilled and the methylated cellulose was obtained in a clear, solid form. The results of treating each of the fractions containing methyl cellulose in the manner described is given in Table XLV.

The syrup isolated by extracting the "Methanol-Soluble Fraction" with chloroform and butanol was called the Methanol-Soluble Extractable Fraction and was purified by ion exchange technique. The residual aqueous portion, containing the Non-Extractable Fraction was now taken to dryness to find the total weight of solids. These solids were redissolved and passed

TABLE XLV

DATA ON THE ISOLATION OF SOLUBLE METHYL CELLULOSE BY ION EXCHANGE (a)

Fraction (b)		Before Ion Exchange	After Ion Exchange	
		Weight (g.)	Weight (g.)	MeO (%)
Benzene soluble fraction				
	Expt. 460	0.181(c)		
	Expt. 470	0.439(d)		
		0.620	0.572	37.1
Methanol soluble fraction				
Extracted with chloroform from water				
	Expt. 460	0.138		
	Expt. 470	0.434		
		0.572		
Extracted with butanol from water				
	Expt. 460	5.32		
	Expt. 470	6.78		
		12.10		
Total extractable fraction		12.67	1.257	33.1
Non extractable	Expt. 460	27.43	2.302	29.4
Dialysis Product				
Dialysable	Expt. 460	0.562		
	Expt. 470	0.956		
		1.518	1.322	23.8

(a) Amberlite ion exchange resins IR-120, IRA-410.
(b) From original weight of cellulose of 10.8 g. (Expt. 460) and 18.3 g. (Expt. 470).
(c) OCH_3, 36.5% (d) OCH_3, 36.1%

through the columns IR-120 and IRA-410 until thallium-free and neutral. This aqueous fraction contained the great proportion of the methanol-soluble material, both inorganic and organic, removed in the original extraction. Unfortunately, this non-extractable portion of the Methanol Soluble Fraction of Expt. 470, after processing still contained acidic material and was charred when dried at 60° in vacuo.

The benzene-extracted, methanol-extracted solid from Expt. 460, a semi-fibrous powder containing thallous sulphate swelled and dissolved somewhat in distilled water to give a heterogeneous cloudy suspension. After being dialysed, the dialysate, approximately 32 liters, was concentrated and finally dried. The combined dialysates from the parallel Expt. 460 and 470 (Table XLV) were then passed through the ion-exchange columns and the effluents evaporated to give a clear tenacious gel. Meanwhile the contents of the dialysis sac had been separated into a water-soluble and a water-insoluble component by means of a sintered glass filter. These fractions were then dried.

In retrospect, the ion-exchange resins IR-120 and IRA-410, could have been used to recover the benzene- and methanol-soluble methylated cellulose fractions without any prior processing, since these resins are not soluble in organic solvents. Since the thallium salts of the mineral acids were sparingly soluble (1 to 5%) (130) the thallium removed from the Amberlite resin IR-120 during regeneration with 10% hydrochloric acid was deposited around the granules of resin. As the column was regenerated and exhausted the thallous chloride moved down the column. It would appear, therefore that the effluent liquid from IR-120 should be checked closely when thallium is being removed. In the case of the dialysate, although the final effluent was neutral, the syrup reported here as pure was later found to contain thallium. The peculiar results obtained when this fraction was tosylated and iodinated, and also when oxidized with periodate, were probably connected with this impurity.

Table XLVI summarizes the data on the various fractions of partly methylated cellulose obtained in Expts. 460 and 470. In Expt. 460 the methylated cellulose was recovered in 96.8% yield, 11.55 g. of material having an average methoxyl percentage of 21.01 being produced from 10.8 g. of cellulose. The fractions C, D and E of Expt. 460 totaled 10.34 (87%) so that the error introduced by allocating the weight of purified methyl cellulose (fractions A, B and H) in proportion to the weights of original samples (Table XLV) cannot be great.

It is immediately evident from Table XLVI that a fractionation of the methylated cellulose according to the degree of substitution had taken place, since the D.S. decreased from 2.32 for Fraction A to 0.94 or 0.69 for the non-dialysable, water-insoluble fractions (E and G). A fractionation according to ascending chain length also occurred, since the material isolated in Expt. 460 changed from Fraction A, a friable powder, through films of increasing toughness (those from Fractions C and D could

TABLE XLVI

FRACTIONS RECOVERED AFTER LARGE SCALE THALLATION-METHYLATION EXPERIMENTS

	Fraction	Yield[(a)] (%)	MeO (%)	D.S.	Appearance
Expts. 460 + 470					
A	Benzene soluble	1.9	37.10	2.32	Fine clear powder
B	Methanol soluble, extractable	4.3	33.11	2.04	Fine clear powder
C(b)	Methanol soluble, non-extractable	21.3	29.42	1.77	Colorless tough film
H	Dialysate	4.5	24.29	1.43	Colorless tenacious gel
Expt. 460					
D	Non dialysable, water soluble	5.0	26.39	1.57	Very tough film
E	Non dialysable, water insoluble	69.3	16.60	0.94	Horney fibrous mass
I	Methyl methylglucosides	....	34.52	2.37	Colorless syrup
Expt. 470					
F	Non dialysable, water soluble	8.0	15.75	0.89	White powder
G	Non dialysable, water insoluble	51.5	12.31	0.69	Friable mass
J	Methyl methylglucosides	....	32.30	2.19	Colorless syrup

(a) As percentage of original cellulose (Expt. 460 = 10.8 g.; Expt. 470 = 18.3 g.) The combined weight of methyl cellulose fractions from Expt. 460 was 11.55 g.

(b) Expt. 460 only.

not be crushed but could be torn) to the fibrous horny mass recovered as the water-insoluble portion (Fraction E). In Expt. 470 where the effective concentration of dimethyl sulphate was higher than for Expt. 460, the chain length of the fractions was shifted toward lower degrees of polymerization; Fractions F and G being a glass-like solid and a white mass, respectively, each of which could be pulverized quite readily.

A comparison of the data on the Water-Insoluble fractions (Table XLIV) indicated that the more severe hydrolysis conditions served to shear away the more highly methylated chain segments from the crystallites; the mildest conditions used (Expt. 460) giving a 75% yield of material containing 17% methoxyl, while the most drastic hydrolysis (Expt. 354) left a residue amounting to 32% of the original weight but containing only 5.5% methoxyl groups. If the hydrolysis of the glucosidic linkages took place after the thallium alcoholate had been converted to methoxyl groups, the effect of the recrystallization normally encountered (cf. Nickerson) during chain cleavage was eliminated. The Water-Insoluble Fraction of the highly degraded methyl cellulose then represented the crystallites remaining in the fiber after swelling to the cellulose hydrate II state. Studies of chain length distribution by electron microscopy and other methods would give some idea of their dimensions.

to p. 165.

The Distribution of Methoxyl Groups in the Superficially Methylated Cellulose

The final phase of this research was devoted to studying the distribution of the methoxyl groups, with which the accessible hydroxyl groups of the cellulose hydrate II had been "tagged". Initial plans contemplated quantitative recovery and identification of each glucose and methylated glucose residue, obtained by the methanolysis (116) or hydrolysis (115) of the superficially methylated cellulose. The paper chromatographic technique of Jones et al (98)(99)(131) which required only small amounts of material was to have played a major role in the proposed separations. A sample of water-insoluble methylated cellulose, purified by dialysis, was hydrolysed with 72% sulphuric acid by the procedure of Monier-Williams (115). The clear methyl glucose syrups recovered still contained thallium. Drops of an aqueous solution of the syrup were placed on Whatman No. 1 filter paper and developed with a butanol-water system. Duplicate strips were dried and sprayed with ammoniacal silver nitrate, and aniline phthalate in glacial acetic acid (132). Although the spot for the glucose control was quite apparent after either spray, only faint coloration appeared for the methyl glucose spots. It was concluded that the ubiquitous thallium salts were causing gross interference and this method of separation was discarded. The result, however, would probably have been more encouraging if obtained after, instead of before, the discovery that thallium contaminants were readily removed from the soluble fractions by the Amberlite exchange resins.

Methanolysis, by the method of Irvine and Hirst (116) gave the first indication that the dialysis was not able to remove the last traces of thallium salt, presumably sulphate, from the Water-Insoluble, Non Dialysable Fraction. When 1.2 g. samples were heated for 7 days at 128° in anhydrous methanol containing 1.37% of hydrogen chloride, a considerable amount of material remained undissolved (cf. 57, 90). The solids consisted of white fibers and brown thallium salts. The occlusion of these thallium salts would seem to indicate that during the methylation, the cleavage of glucosidic links had permitted some recrystallization of the cellulose, with imprisonment of the thallium compounds. The regions in which the thallium was trapped were apparently not re-opened by swelling during the subsequent dialysis for 84 hours in water at room temperature.

The second approach to discovering the distribution of the methoxyl groups was by the tosylation-iodination technique. The selection of reaction conditions was considered critical, since the samples to be esterified ranged in their degree of methoxyl substitution from 2.3 down to 0.7 mole per glucose unit. Both Malm, Tanghe and Laird (96) and Timell (57) pointed out the risks of over-tosylation with subsequent over-iodination, when determining free primary hydroxyl groups in alkyl celluloses of low substitution. Their results, together with those of Hackett and Downing (133) on the tosylation of the free 3-hydroxy group in diacetone galactose, led to the decision to employ the pyridine-tosyl chloride-free hhdroxyl ratio given by Mahoney and

Purves (92) for 12 hours at room temperature.

Previous workers seem to have tosylated either simple compounds such as sugars or alcohols (and to have isolated the ester by extraction) (95) or celluloses having a fairly high degree of polymerization in which case the product was collected on a filter. In the present series the variations in the physical properties of the tosylates were considerable, ranging from highly methylated short-chain material, that was totally soluble, through oils and gums, to longer chain fractions of low methoxyl content and high crystallinity which were insoluble. The data for these tosylations are given in Table XLVII and Table XLVIII, together with the iodination data and the method of isolation (whether by extraction, filtration or a combination of both) which was the same for both reactions. The recovery of materials, based on methoxyl content before and after tosylation, varied between 80 and 100%, and in each case the weight of tosylated substance exceeded the original sample weight. It was strangely high for Fraction H, the dialysate, presumably because of the conversion of occluded thallium salts to the p-toluenesulphonate, but re-extraction with chloroform and benzene failed to decrease the apparent yield.

The tosylated methyl celluloses were then iodinated by the procedure of Mahoney and Purves (92) in which the specimen was heated for 2 hours at approximately 115° in acetonylacetone containing an excess of sodium iodide. The solution was then poured into ice cold water and the iodinated products were

TABLE XLVII

DATA FOR TOSYLATION AND IODINATION

	Fraction				
	A (a)	B (ab)	C (ab)	D (b)	E (b)
Original Methoxyl Substitution	2.32	2.04	1.77	1.57	0.94
Tosylation					
Sample weight (g.)	0.1469	0.2840	0.4304	0.1985	0.4610
Methoxyl content (%)	37.10	33.11	29.42	26.39	16.39
Amount recovered (g.)	0.2174	0.3788	0.5483	0.3165	0.6542
Methoxyl content (%)	23.79	22.18	20.77	14.95	10.71
Methoxyl recovery (%)	94.8	89.4	89.7	90.4	92.7
Iodination					
Sample weight (g.)	0.1954	0.2438	0.2805	0.1944	0.3046
Amount recovered (g.)	0.2327	0.2711	0.2461	0.1410	0.2701
Methoxyl content (%)	15.72	9.98	21.90	16.73	11.97
Methoxyl recovery (%)	79.4	50.2	93.0	81.2	99.0
Iodine content (%)	6.75	5.27	19.70	23.53	15.77
Primary Hydroxyl Groups	0.244	0.263	0.388	0.538	0.301
Ratio Primary to Secondary OCH_3	0.48	0.57	0.53	0.41	2.9

(a) Tosylates isolated by extraction with chloroform and benzene.
(b) Tosylates isolated on a filter.

TABLE XLVIII

DATA FOR TOSYLATION AND IODINATION

	Fraction				
	F (b)	G (b)	H (a)	I (a)	J (a)
Original Methoxyl Substitution	0.89	0.69	1.43	2.37	2.19
Tosylation					
Sample weight (g.)	0.4098	0.5768	0.2952	0.3105	0.2302
Methoxyl content (%)	15.75	12.31	24.29	34.52	32.30
Amount recovered (g.)	1.3269	1.4179	1.2534	0.7080	0.4491
Methoxyl content (%)	4.51	5.04	5.51	12.60	13.22
Methoxyl recovery (%)	92.7	100.0	96.5	83.2	79.8
Iodination					
Sample weight (g.)	0.3369	0.3105	0.4665	0.6627	0.4148
Amount recovered (g.)	0.1458	0.2003	0.4634	0.4227	0.4356
Methoxyl content (%)	5.65	6.58	0.64	15.89	12.31
Methoxyl recovery (%)	54.2	86.5	11.5	80.6	97.9
Iodine content (%)	11.09	9.83	25.10	18.62	13.98
Primary Hydroxyl Groups	0.425	0.248	13.65	0.679	0.608
Ratio Primary to Secondary OCH_3	1.9		...	0.30	0.49

(a) Tosylates isolated by extraction with chloroform and benzene.

(b) Tosylates isolated on filter.

recovered by repeating the filtration or extraction procedures successfully used on the parent tosyl compound. For undetermined reasons, the methoxyl recoveries from Fractions B, F and H were only 50, 54 and 11.5% respectively. The percentage of iodine in the rather red residues was then determined (92). During the iodination, the iodine should replace only the tosyl group at the primary hydroxyl position, and the resulting iodocelluloses should be colorless. Malm, Tanghe and Laird (96) investigated the amount of free iodine present in their samples and stated that the results were hardly influenced by soaking the fibers in sodium thiosulphate solution. Neither Timell or Mahoney and Purves took this precaution. In the present case, only the partly tosylated methyl glucosides were washed with thiosulphate solution before the iodine determination and all the brown color disappeared. Although all samples should have been treated in the same manner, it was not likely that the omission of this precaution seriously invalidated the iodine contents found.

When x, y and z represented the average substitution of methoxyl, tosyl and iodine, respectively, in the cellulose derivative, the base molecular weight was
$111 + 45x + 171y + 127z + 17(3 - x - y - z)$ and its content of methoxyl groups and iodine atoms was $31x$ and $127z$, respectively. By manipulating simultaneous equations connecting the above relationships with the per cent analyses it was readily

shown that

$$z = \frac{31 \text{ x } x \text{ x } \% \text{ iodine in sample}}{127 \text{ x } \% \text{ methoxyl in sample}}$$

and hence the mole substitution (z) of iodine and the amount of unsubstituted primary hydroxyl group per glucose residue could be calculated. The values for the various methyl celluloses are given in Tables XLVII and XLVIII.

When the moles of free primary hydroxyl groups were known, subtraction from unity gave the number of primary hydroxyl positions that presumably were methylated. Subtraction of the latter quantity from the total methoxyl substitution of the original methyl cellulose then revealed the total substitution in the two secondary positions. Thus from the first column of Table XLVII, (1 - 0.244) or 0.756 primary and (2.32 - 0.756) or 1.564 secondary methoxyl groups were present. The ratios of the primary to the secondary substitutions, in this case 0.756/1.564 or 0.48, are recorded in the bottom lines of the Tables. When it is remembered that there are two secondary for each primary position and that the ratio corresponding to random substitution would be 0.38, it is at once evident that methylation in the primary position was favoured in almost all fractions, and was almost or completely restricted to the primary positions in the non-dialysable water insoluble fibrous fractions E and G.

As the total substitution increased in the more soluble fractions, the methylation of the secondary hydroxyl groups became more significant. Since the mixtures I and J of partly methylated methyl glucosides were prepared from the methylated cellulose fractions E and G their ratios of primary to secondary alkylation should be identical to those of the fractions from which they were prepared after allowance was made for the extra substitution of unity introduced by the glycosidic methoxyl group. The ratio of 0.30 found for I was obviously low, perhaps because the recovery of methoxyl groups in the iodination was only 80.6%. The result of 0.44 for Fraction J, with a recovery of 97.9%, was more satisfactory. Comparison of the ratio of 1.9 from Fraction F (Expt. 470, recovery 54.2%) with the value 0.41 from the similar fraction D (Expt. 460, recovery 81.2%) suggests that material highly methylated in the secondary positions had been lost in the former case. The absurd result of 13.65 moles of primary hydroxyl group recorded for the dialysable fraction (H) probably reflected its gross contamination with thallium salts. The above defects in the experimental data precluded any attempt to interpret the results on a quantitative basis.

To gain information about the distribution of the methoxyl groups along the cellulose macromolecules, the unsubstituted 2,3-glycol groups in the various fractions were estimated by the method of Mahoney and Purves (92). Small samples were oxidized with aqueous sodium paraperiodate at pH 4 and the consumption of periodic acid was followed by titrating the residual periodate with sodium arsenite. These operations were carried out under stringently controlled conditions and since only the later horizontal portions of the oxidation rate plots were desired, titrations were made only after 60, 84, 108, 156 and 204 hours. The experiments given in Tables XXXI, XXXII and XXXIII are summarized in Figures 22 and 23 and the final count of glycol groups is recorded in Column 5 of Table XLIX. Since one glucose residue could have only one unsubstituted glycol unit at most, the value of 3.19 found for the "dialysate" fraction H was impossibly high and was rejected. This was the fraction contaminated with thallium salts, which presumably were oxidized by the periodate to the thallic condition.

As Mahoney and Purves (92) explained, if H moles of unsubstituted hydroxyl group were distributed between the second and third positions of a cellulose derivative in a purely random way, the chance of a given glucose residue having both positions unsubstituted (i.e., containing a glycol grouping) had a maximum value of $H^2/4$; if the introduction of a substituent in one of the two positions prevented the substitution of the other, the chance was H - 1; and if the unsubstituted hydroxyl groups concentrated in localized groups along the macromolecule, the chance of a glycol unit was H/2. Columns 6, 7 and 8 of Table XLIX recorded the chances calculated from the observed secondary substitution given in column 4. Comparison of the glycol units found (column 5) with the calculated probabilities shows that the glucose residues in the methyl cellulose A were

TABLE XLIX

DISTRIBUTION OF HYDROXYL GROUPS IN ETHERS A - H

	Moles Hydroxyl Groups(a)			Moles Glycol Groups			
Ether	Total	Primary	Secondary (b)	Found	$H^2/4$	H - 1	H/2
A	0.68	0.24	0.44	0.19	0.05	0.00	0.22
B	0.96	0.26	0.70	0.19	0.12	0.00	0.35
C	1.23	0.39	0.84	0.14	0.18	0.00	0.42
D	1.43	0.54	0.89	0.49	0.20	0.00	0.45
E	2.06	0.30	1.76	0.16	0.77	0.76	0.88
F	2.11	0.43	1.68	0.34	0.71	0.68	0.84
G	2.31	0.25	2.06	0.15	1.06	1.06	1.03
H	1.57	13.65	....	3.19	....	....	....

(a) Moles per glucose residue.
(b) Total secondary hydroxyl (H) found by total minus primary.

for the most part entirely substituted, or unsubstituted, in the secondary positions. In this fraction, as well as in the water soluble but non dialysable fraction D, the results suggested chain segments highly substituted in what had been the amorphous region and nearly unsubstituted where they had been shielded from thallation. Fraction C, in contrast, was substituted to a lower degree but with random arrangement along the chain, while fraction B occupied an intermediate position. Fractions E, F, G, which were of low substitution and of a higher degree of polymerization, apparently resisted complete penetration by the periodate solution and their glycol counts were low.

There was no doubt about the unreliability of the estimation in the case of sample G which retained the theoretical maximum number of two secondary alcohol groups, and therefore one mole of glycol instead of the 0.15 mole found.

The glycol contents of the partly methylated methyl glycosides I and J (Fig. 23) could not be interpreted on the same basis as those of the other fractions, because the glycosides always contained an extra free hydroxyl group in the fourth position. When this was taken into account, the values of 0.85 for fraction I and of 1.0 for fraction J did not appear unreasonable, although the continuing slow oxidation of the latter fraction remained unexplained.

The Chain Length Distribution of a Partly Methylated Cellulose Fraction E

The author's erstwhile Colleague, Timell (134) was kind enough to include the non dialysable, water insoluble methylated cellulose E from Expt. 460 in his study of the chain length distribution of superficially methylated cellulose (110). The study involved nitrating the sample without degradation, fractional precipitation of the nitrated methyl cellulose and the determination of the intrinsic viscosity of each subfraction. These data, given in Tables XXXIV and XXXV, also in Fig. 24, are presented in this thesis as a matter of record but should not be considered as a contribution by the author. Timell found that the chain length of Fraction E varied from 6 to 504 glucose

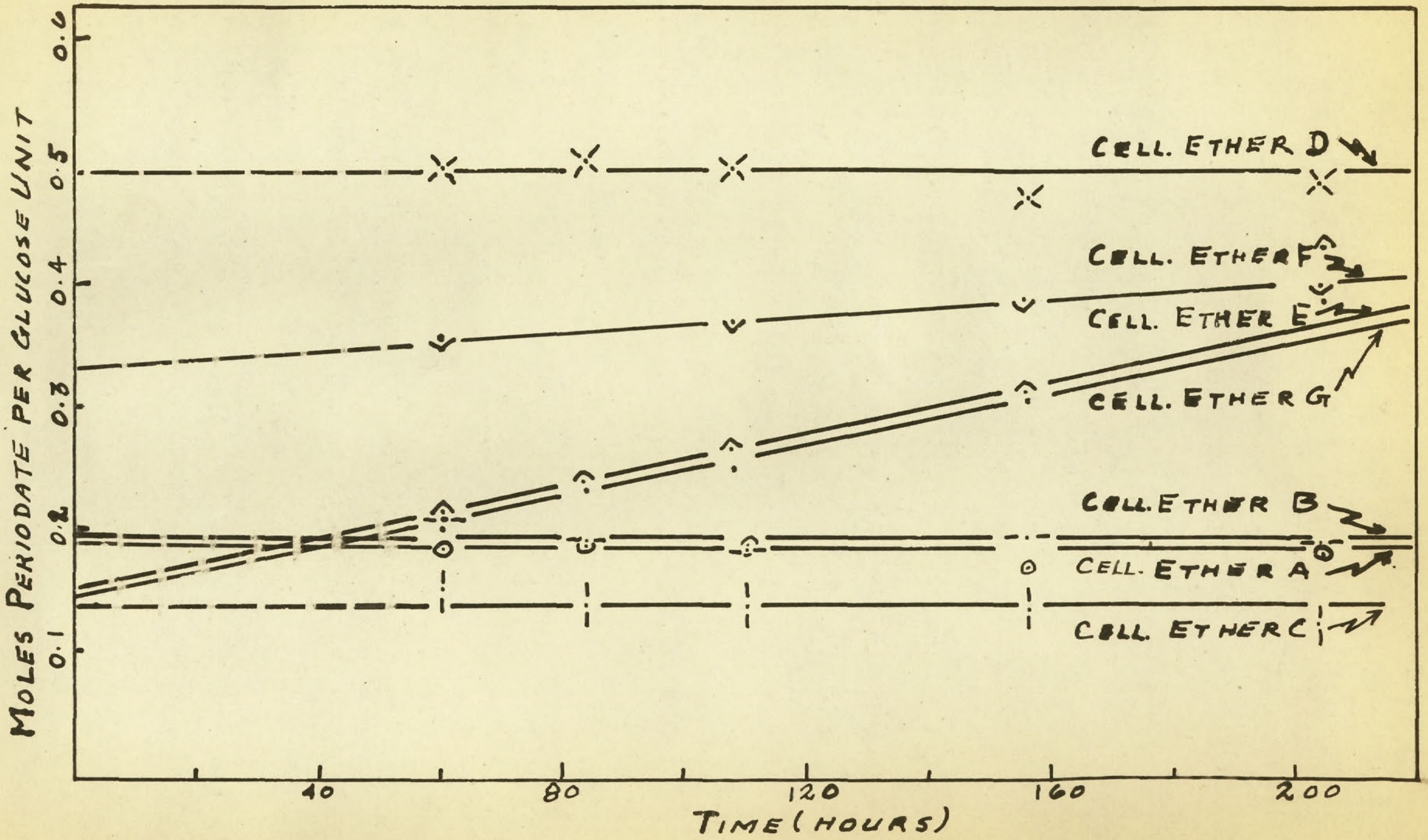


Fig. 22 : Periodate Oxidation—Rate Curves for Methylated Celluloses

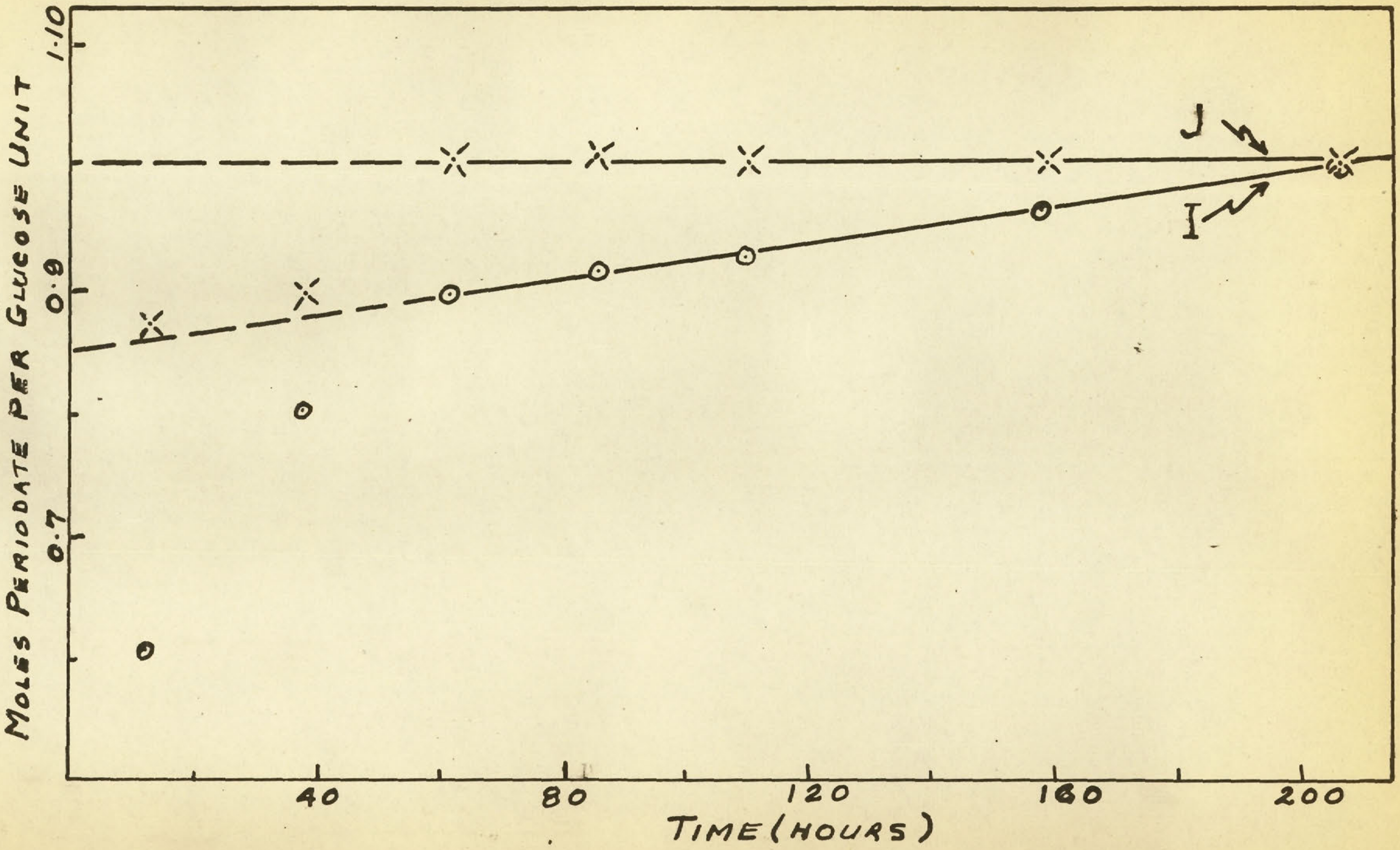
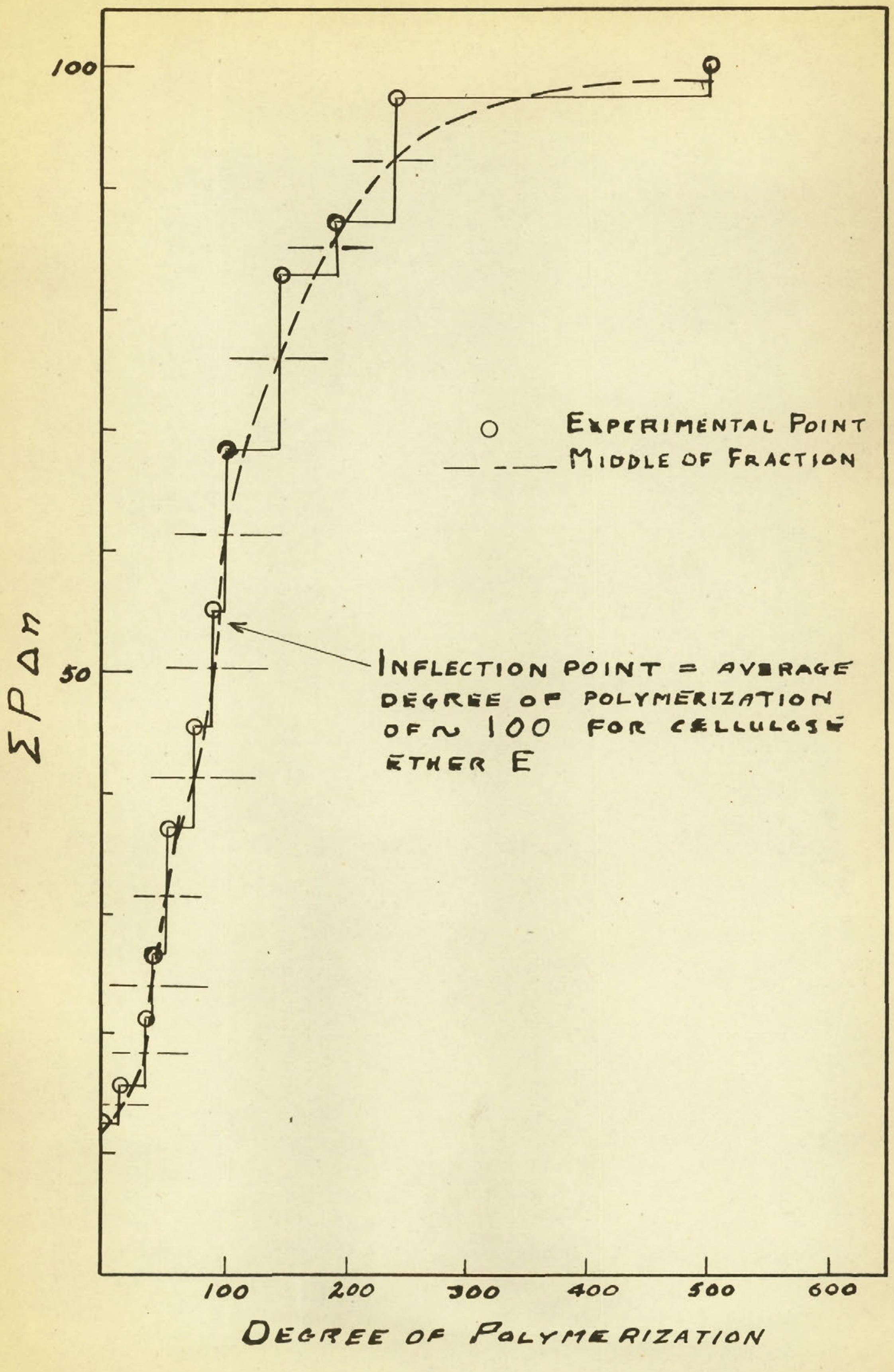


Fig 23: Periodate Oxidation—Rate Curves for Methyl Methylglucosides



INFLECTION POINT = AVERAGE DEGREE OF POLYMERIZATION OF ~ 100 FOR CELLULOSE ETHER E

FIG. 24: INTEGRAL CHAIN-LENGTH DISTRIBUTION CURVE

units, with an average D.P. of approximately 100. Although the increasing solubility of the other fractions from Expt. 460 might be attributed to their increasing methoxyl content, their other physical characteristics (Table XLVI) also pointed to a progressive decrease in average chain length. The drastic hydrolytic action of the dimethyl sulphate during methylation of the thallous cellulosate became very obvious in light of the fact that the average chain length of the original cotton linters was approximately 1650.

Timell also noted that about 1% of the nitrated methyl cellulose was insoluble in acetone. When the sub-fractions from Timell's chain length distribution studies arrived in the author's hands this material was found to be 1.21% of thallium salts, and it was possible to correct the methoxyl content of the original sample from 16.39 to the true value of 16.60%. The author then found the methoxyl contents of the twelve subfractions supplied by Timell, these data being given in Table XXXVI. Since the sum of the methoxyl contents in these fractions amounted to 307.6 mg., while the original amount was 314.0 mg., 98% was accounted for and the accuracy of the methoxyl determination verified.

By using 2.90 as the total substitution of methoxyl and nitrate groups per glucose unit, which was usual as Timell and Purves had shown previously (123), it was possible to calculate from the observed percentage of methoxyl, the methoxyl substitution of each of Timell's twelve subfractions (Table

XXXVI). This substitution increased from 0.22 for the longest chain having a D.P. of 504, to 1.54 for the chains of D.P. 6, the short-chain subfractions making up by far the biggest fraction. These results, plotted in Fig. 25 were in qualitative agreement with the findings of Timell and Purves (110) that the accessible fraction of alkali cellulose superficially methylated with dimethyl sulphate in benzene contained highly methylated short-chain constituents.

The general trendsof the chain length study of Fraction E were not inconsistent with the view that it retained some of the crystalline character of the original swollen linters. Since the interior of such crystallites would be little affected by degradation or superficial methylation, the average methoxyl content decreased as the degree of polymerization rose (Fig. 25). The rapid change in slope between D.P. 80 to 120 indicated that most of the crystallites were of that length, which was previously suggested by Svedberg (19) and by X-ray data (20). If the data in Table XXXVI are accurate, a chain passing through a single crystallite having a length of 100 glucose units would have an average methoxyl substitution of 0.5 or would contain 50 methoxyl groups. If the ends of the chain are assumed to be totally substituted, about 84 glucose units will remain unsubstituted in the interior of the crystallite.

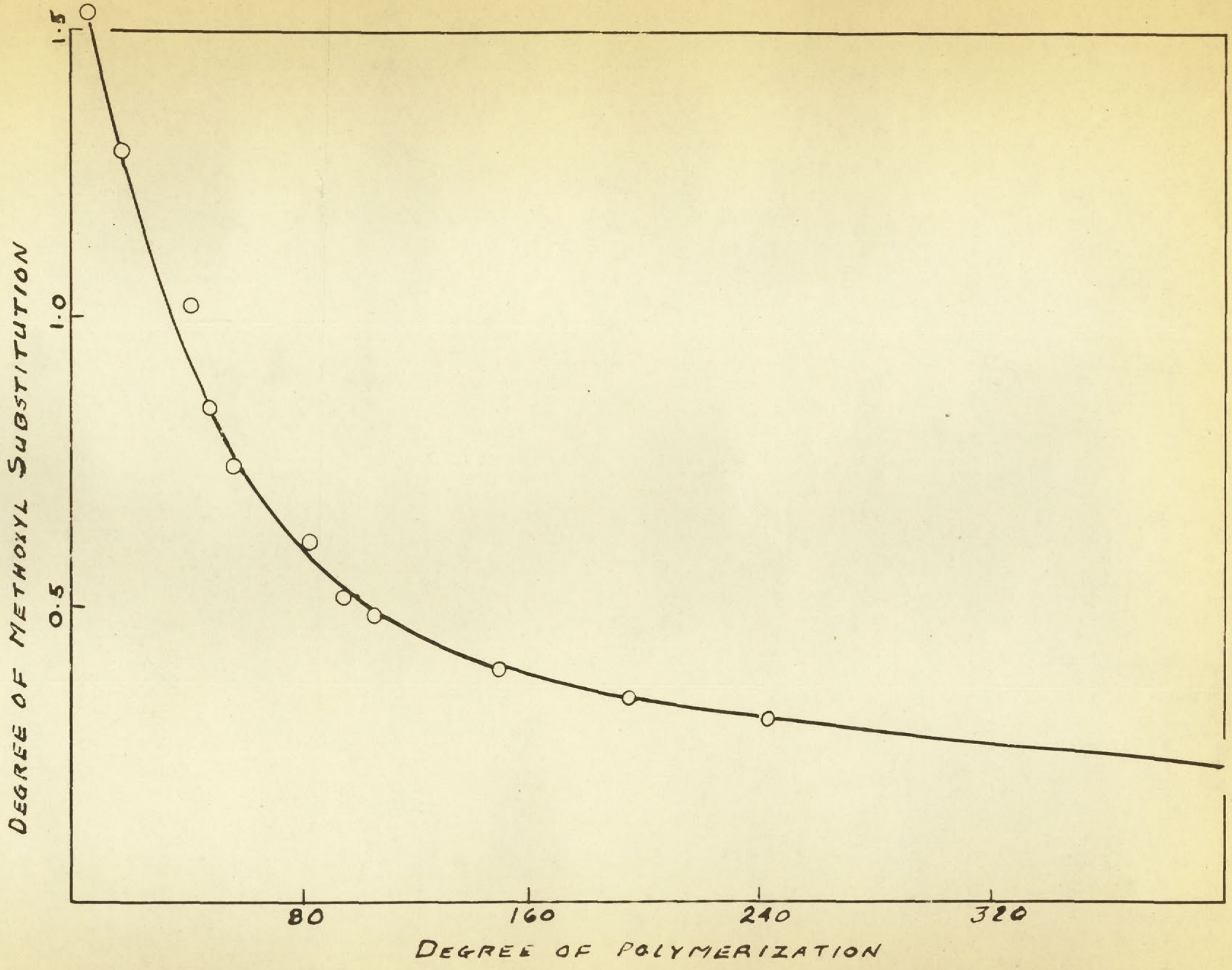


Fig. 25: Variation of Methoxyl Substitution with Chain-Length in Cellulose Ether E

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. Three previously known modifications of dewaxed cotton linters were prepared under quite similar conditions. Linters that had been swollen to a maximum in 10 to 11% sodium hydroxide solution at 0 to 5°C., washed free of alkali with ice water, de-ashed and solvent-exchanged near zero through anhydrous acetic acid to anhydrous benzene, were termed "cellulose hydrate II". When the linters, mercerized and de-ashed under the same conditions, were boiled for 30 minutes in the final neutral water and then solvent-exchanged, the product was called "cellulose hydrate I". Cotton linters, swollen in distilled water at 0 to 5°C. for 2.5 hours, de-ashed and solvent-exchanged into anhydrous benzene, was termed "water-swollen cellulose".

2. The Jörgensen accessibility, defined as the atoms of oxygen consumed per anhydroglucose unit when cellulose was oxidized with chromium trioxide dissolved in acetic acid - acetic anhydride (4 vol. to 1 vol.) at 20° for 30 minutes, was used as the standard measurement of the accessibility of the celluloses.

3. Using cellulose hydrate II, it was shown for the first time that drying from benzene under slight vacuum at room temperature for one year decreased the accessibility from 0.87 to approximately 0.25; drying from benzene under water pump

vacuum at room temperature caused a decrease from 1.00 to 0.15 in 144 hours. The fact that drying directly from water under 1 mm. pressure at 105° reduced the accessibility almost to zero was confirmed.

4. Cellulose hydrate II was found for the first time to retain its original accessibility during solvent-exchange and, when stored in anhydrous benzene, retained the initial accessibility even after one year.

5. Cellulose hydrate II was found for the first time to be very sensitive to pressure, mild compression decreasing the accessibility from approximately 1.7 to approximately 1.0.

6. The densities of the cellulose modifications in benzene, determined by means of a hydrostatic balance, were shown for the first time to be: water swollen cellulose, 1.56; cellulose hydrate I, 1.62; cellulose hydrate II, 1.62. This determination gave precision of greater than 1%, but the densities of the different cellulose hydrate II preparations varied from 1.600 to 1.656 under certain conditions of preparation.

7. The density of the cellulose modification being known, further small samples of the various preparations were weighed on the hydrostatic balance, to give the weight of cellulose in a sample wet with benzene, without the necessity of prior drying. When the specimens were weighed in this manner and compression was avoided, the Jörgensen accessibilities of

all preparations were highly reproducible, the limiting factor being the conditions of a final iodometric titration. Previous values found for water swollen cellulose, cellulose hydrate I and cellulose hydrate II were revised to 0.14, 0.98 and from 1.67 to 1.95 atoms of oxygen per glucose unit respectively.

8. The accessibilities of almost all preparations, expressed as per cent of the total hydroxyl groups methylated, were also estimated by the thallous ethylate procedure. Samples weighed on the hydrostatic balance had highly reproducible methoxyl contents. The accessibilities of water swollen cellulose, cellulose hydrate I and uncontaminated cellulose hydrate II were 15.7, 25.6 and from 29.8 to 46.1% by this method. No accurate correlation existed between the chromium trioxide and the thallous ethylate accessibilities.

9. Batches of cellulose hydrate II, weighing from 10 to 20 g., were methylated superficially by means of thallous ethylate and dimethyl sulphate. The product was recovered in six fractions, varying in methoxyl content from 37.1 to 16.6%, each of which was contaminated with large quantities of thallium salts. Four of these fractions were recovered free from thallium by the use of dialysis and ion-exchange technique. The recovery of the methylated fractions was 96.8% of the theoretical yield.

10. The distribution of the methoxyl groups in each of the partly methylated cellulose fractions was studied by the tosylation-iodination procedures, with the result that a pre-

ferred methylation of the primary hydroxyl groups in most fractions of the superficially methylated cellulose was detected. Oxidation of the least methylated fractions with aqueous potassium periodate gave untrustworthy results for their content of unsubstituted 2,3 glycol groups, but the results for two more highly methylated, shorter chain fractions suggested that most of the 2,3 positions were either completely methylated, or not at all.

11. In collaboration with Dr. T.E. Timell the chain-length distribution of one of the least degraded, least methylated fractions, still non dialysable and water insoluble, was determined. The methoxyl substitution of the sub-fractions increased from 0.22 to 1.54 as their chain length decreased from 504 to 6.

12. Although unexpectedly severe degradation during the methylation with dimethyl sulphate, great difficulty in eliminating thallium salts from the products and small amounts of the individual fractions impaired the quantitative aspects of the work reported under 10 and 11, their general trend was not inconsistent with what is known of the fine structure of cellulose.

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