HYDROGEN EXCHANGE BETWEEN CELLULOSE AND WATER

by

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FOREWORD

This thesis describes an investigation of i) the accessibility of hydrocelluloses and oligosaccharides ii) the recrystallization of amorphous cellulose and iii) the molecular changes occurring during cyclic drying and wetting at high temperatures. The presentation of the work is made as a series of Parts suitable for publication and with a General Introduction and Concluding Discussion.

The contents appear as follows:

Part I: a brief survey of the fine structure of cellulose and the molecular changes occurring under various conditions with special emphasis on the hydrogen exchange reaction between cellulose and water;

Part II: a study of i) the accessibility of hydrocelluloses and oligosaccharides ii) the recrystallization occurring during heterogeneous acid hydrolysis and iii) the effect of acid hydrolysis on crystallites;

Part III: a study of the exchange of hydroxyl hydrogens in cellulose during cyclic wetting and drying at elevated temperatures;

Part IV: a general discussion of the results, suggestions for future work, and a list of claims to original research;

APPENDIX I: a study of the recrystallization of amorphous cellulose;

APPENDIX II: additional details of the experimental techniques and the methods of calculation;

APPENDIX III: a study of the effect of interfiber bonding on the accessibility of cellulose.

SUMMARY

The fine structure of cellulose was studied by means of the hydrogen exchange reaction between cellulose and water. The accessibility of cellulose remained constant even after prolonged heterogeneous acid hydrolysis indicating that the cellulose may be completely crystalline. Accessibility measurements made on the oligosaccharides (cellobiose to cellopentaose) suggest that the accessibility is related to the perfection of the crystal lattice and does not measure the crystalline-amorphous ratio as previously believed.

The amount of hydroxyl groups becoming inaccessible during treatment with mineral acids containing tritiated water showed that a slight yet significant amount of recrystallization occurs during acid hydrolysis.

Cyclic drying and wetting studies conducted at elevated temperatures (above 100°C) showed that the number of cycles required for the complete exchange of hydroxyl hydrogens in cellulose decreases rapidly with increasing temperature. At 190°C there is complete exchange in one cycle.

Studies on the kinetics of recrystallization of amorphous cellulose showed that appreciable crystallization occurs only at 100% RH. The amount of crystallization occurring at lower relative humidities is slight yet significant.

All these results are discussed in relation to the current theories on the structure of cellulose.

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PART I

GENERAL INTRODUCTION

GENERAL

The organization of cellulose at the molecular level is commonly acknowledged to play an important role in its behaviour and although widely studied, is still imperfectly understood. In this thesis the hydrogen exchange reaction between cellulose and water has been used to study the fine structure and reactivity of cellulose. This is in continuation of the studies initiated by Lang and Mason[1] and later pursued by Sepall and Mason[2]. Extensive surveys of present knowledge of the field of cellulose structure have been given in many texts[3,4]. In this section only those aspects of the structure of cellulose which are relevant to the present work are briefly reviewed.

The chemical structure of cellulose is now well established. The cellulose molecule is a linear polysaccharide composed of glucopyranose residues linked by 1-->4 β-glycosidic bonds. The degree of polymerization is not known with certainty but recent research suggests that it may be as high as 15,000[5].

The organization of the long chain molecules in cellulose fibers has been studied by a variety of methods, of which the most fruitful has been X-ray diffraction. The X-ray diffraction patterns of cellulose display both the relatively sharp interferences typical of diffraction by a crystalline array and also a more diffuse liquid-like scattering. The notion therefore arose that cellulose fibers consist of highly ordered (crystalline) and less well ordered (amorphous) regions. Although the less well ordered regions may range from imperfectly crystalline material to completely amorphous material, it has been convenient for many purposes to regard cellulose as consisting of two distinct phases, one crystalline and the other amorphous.

The observed diffraction behaviour of cellulose has been widely interpreted in terms of a theory known as the "fringed micelle" concept[6].

It is assumed that the molecules order themselves only along a portion of their lengths, the remainder meandering through entangled amorphous regions perhaps fitting into other crystallites further along their lengths. The fiber is thus visualized as a random assembly of such crystalline and amorphous regions. The relative amounts of the total crystalline and amorphous regions determines the degree of crystallinity of the fiber.

The methods used to determine the degree of crystallinity of cellulose may be divided into two groups, namely, physical and chemical. The physical methods are based upon the assumption that crystalline and amorphous cellulose have different physical properties. Some of the chemical methods involve the study of the kinetics of some specified reaction, which generally proceeds initially at a rapid rate, followed by a slower one. The assumption is made that the initial rapid rate involves reaction in the amorphous regions and the lower rate involves attack on the crystallites themselves. Other chemical methods involve reactions with reagents which react with a limited percentage of the total number of hydroxyl groups present. It is assumed here that the reagent is unable to penetrate the ordered regions and the extent of reaction is a measure of the accessible cellulose present.

The accessibility of cellulose has been measured by determining the extent of reaction under standard conditions by a number of methods of which the most important are (a) hydrogen exchange with water[7], (b) heterogeneous acid hydrolysis[8,9], (c) thallation[10], (d) periodate oxidation[11], (e) esterification with formic acid[12] and (f) reaction with sodium in liquid ammonia[13]. The results obtained by these methods differ from each other, and are believed to be dependent on the experimental conditions and the size of the reactants[10].

In the present investigation the hydrogen exchange method was

employed since it has a negligible effect upon the cellulose structure.

When cellulose is treated with deuterium oxide (D_2O) the following reaction occurs:

$$C_6H_{10}O_5 + 3D_2O \longleftrightarrow C_6H_7D_3O_5 + 3HOD$$

Tritiated water (HTO) also reacts in a similar manner. The fraction of hydroxyl groups exchanging under standard conditions is hereafter referred to as the "accessibility" and is denoted by the symbol A.

The experimental techniques which have been used in the present study for measuring hydrogen exchange between cellulose and water are essentially the same as those described by Sepall and Mason[2] and are summarized below.

1. Exchange with D_2O - Gravimetric Method

This involves determining the increase in mass of the samples following exchange with pure D_2O. Four days of drying under vacuum (1μ Hg) at 75°C was followed by four days of deuteration at a given relative humidity. The unreacted and adsorbed D_2O was removed by a 4-day evacuation (1μ Hg) at 75°C. The percent accessibility A_1, was then calculated from the following relation

$$A_1 = \frac{100\,\Delta\omega}{FS}$$

where F (equal to 0.0185 for cellulose) is the fractional increase in mass after all the hydroxyl hydrogens in the sample have been exchanged by the deuterium; S is the mass of the sample and $\Delta\omega$ is the increase in mass resulting from exchange of hydrogen by deuterium. Although the method is time-consuming and requires large samples, it is absolute.

2. Exchange with Tritium

This also involves essentially three steps; 8 hours of tritiation

is followed by 14 hours of evacuation at 1μ Hg pressure and 75°C. The tritium radioactivity in the sample is then determined by the use of a methane-filled windowless proportional counter[14]. The accessibility $\underline{A}_2$ was obtained by comparison with the count rate of a standard sample (cellophane), the accessibility of which had previously been determined by the gravimetric deuterium exchange method under similar conditions of temperature and relative humidity.

Several investigators[15-19] have attempted to obtain information on the fine structure of cellulose by studying the kinetics of heterogeneous acid hydrolysis. The hydrolysis causes cleavage of the glycosidic bonds in the cellulose chains. The rate of hydrolysis, which is initially rapid, gradually slows down to a more or less constant rate. The degree of polymerization at this stage, known as the "levelling-off" DP, varies with different celluloses. The course of the hydrolysis has generally been interpreted in terms of the fringed micellar model. The hydrolysis is assumed to take place most rapidly in the amorphous regions. The highly ordered crystalline regions are more resistant to attack and are thus hydrolyzed more slowly.

If during hydrolysis the amorphous regions are indeed selectively attacked, and if the accepted crystallinity of cellulose fibers (40-70%) [3,20,21] is valid, then the partially hydrolyzed cellulosic residues should show an increase in crystallinity. A number of attempts to demonstrate such an effect have been reported, but the results do not seem to resolve the question.

Using X-ray methods, Hermans and Weidinger[22] showed that there was a slight increase in the crystallinity of Ramie during hydrolysis. On the other hand, wood cellulose showed no change in crystallinity[23]. McKeown and Lyness[24] measured the X-ray crystallinity and density of

hydrolyzed wood and cotton cellulose and found no change.

In the present investigation native and regenerated celluloses were hydrolyzed for various periods of time down to the micellar stage and the accessibility was measured by the gravimetric deuterium exchange method. In all cases the accessibility was found to remain constant during hydrolysis.

It seems possible to interpret this observation as indicating that the description of the structure of cellulose in terms of the fringed micellar concept is an oversimplification. Gjönnes et al.[25] have shown that the intensity distribution of native celluloses can be completely explained without introducing the concept of an amorphous phase. This would mean that cellulose is essentially completely crystalline, and that the diffuse X-ray scattering which had previously been regarded as coming from a separate amorphous phase, derives in fact from lattice imperfections of various kinds. However, the question then arises as to the meaning of the accessibility and degree of crystallinity which varies widely from one type of cellulose to another. An attempt to resolve this question has been made by measuring the accessibility of oligosaccharides which can readily be obtained in a form that is essentially completely crystalline.

It has often been suggested that recrystallization occurs during the hydrolytic depolymerization of cellulose chains. On the basis of X-ray data, Ingersoll[26] proposed that relatively mild hydrolysis might cause the crystallization of cellulose chains simultaneously with chain splitting. Howsmon[27] gave moisture regain, weight loss, and X-ray data showing that the sharpening of the X-ray diagram could not be explained by the very small loss in weight, due presumably to the removal of amorphous cellulose. Howsmon also compared physical and chemical methods

for characterizing cellulose fine structure and proposed that hydrolytic methods give high values for the crystallinity of cellulose because additional crystallization of the chains occurs on hydrolysis.

Brenner, Frilette, and Mark[28] came to a similar conclusion on the basis of specific volume and density measurements made on hydrolyzed cellulose. More recently, using improved X-ray techniques to follow changes in crystallinity on hydrolysis, Hermans and Weidinger[22] obtained data which appeared to further confirm the belief that with regenerated cellulose, at least, crystallization of the cellulose occurs simultaneously with chain splitting on acid hydrolysis. Recently it has been suggested [17,29] that the amount of recrystallization during hydrolysis is of the order of 2-5 per cent.

In the present investigation a study was made of the effect of acid hydrolysis upon the formation of inaccessible regions in cellulose. Cellulose, whose available hydroxyl hydrogens had previously been exchanged with tritium, was hydrolyzed in acid containing tritiated water. It was assumed that the exchanged hydroxyl groups in the crystallites are immobile and cannot be re-exchanged unless the crystallites are disrupted, and further that it is recrystallization that causes the tritiated hydroxyl groups to become immobile.

It has been demonstrated by Mann and Marrinan[30] that when cellulose is deuterated and subsequently rehydrogenated by immersion in water complete rehydrogenation is not possible as some of the deuterium is very tenaciously retained. The retained deuterium is held in OD groups located in crystalline regions. It was suggested that the resistant groups are formed by a change in the cellulose structure, at least a part of which is accounted for by recrystallization. Lang and Mason[1] showed by repeatedly wetting and drying cellophane in HTO that inaccessible

hydroxyl groups were progressively exchanged with little change in the accessibility. The irreversible exchange of hydrogen during drying was therefore attributed to molecular rearrangements which cause a partial interchange of cellulose between accessible and inaccessible forms. In a continuation of these experiments Sepall and Mason[2] showed that all the inaccessible hydroxyl hydrogens could be exchanged, and they proposed that molecular rearrangements introduced by swelling in the amorphous regions are also responsible for disrupting and reforming ordered regions.

Sepall and Mason[2] also studied the effect of temperature (25-100°C) on the exchange in the inaccessible regions. It was found that the number of wetting-drying cycles required for complete exchange of the inaccessible hydroxyl groups decreased as the temperature of wetting is raised. It was suggested that the crystallites may be continuously disrupted and reformed by the thermal energies of the molecules, an effect which is enhanced by the presence of liquid water because it lowers the second order transition temperature and thereby permits molecular rearrangement to occur more readily.

In the present investigation similar studies were conducted at more elevated temperatures.

It has been shown by Hess et al.[31] and by Hermans[32] that when dry native cellulose fibers are treated in a vibrating ball mill, the X-ray diagram characteristic of the fibers soon disappears; the crystalline interferences fade and are replaced by a broad diffuse ring, indicating that the crystalline lattice is destroyed by the mechanical impact of the balls on the fiber.

When the amorphous grinding product is treated with water it recrystallizes yielding X-ray pattern of cellulose II. In the present

investigation this process of recrystallization has been studied in some detail.

The recrystallization of amorphous cellulose by non-aqueous liquids seems to have received little attention. It has been shown[33-35] that the sorptive capacity of cotton fibers for normal alcohols decreased as the chain length of the alcohol increases. It is now generally accepted that cellulose shows appreciable swelling only in polar liquids, and in order to increase molecular order the swelling medium must be capable of promoting hydrogen bonding.

The objects of the work described in the following pages have been:

(1) to obtain information on the fine structure of cellulose,

(2) to study the changes in molecular order in cellulose during drying at elevated temperatures, and

(3) to study recrystallization effects in cellulose induced by water and various organic liquids.

The principal reason for carrying out the work was to provide background information on the reactivity of cellulose and the interaction between cellulose and water, a problem which has been extensively investigated in this laboratory[1,2].

The subject matter is presented in three parts. The first deals with:

(1) A study of the reactivity of hydrolyzed cellulose using the exchange reaction between cellulose hydroxyl hydrogens and heavy water (D_2O) and radioactive or tritiated water (HTO).

(2) A study of the effect of acid hydrolysis upon the formation of inaccessible regions in cellulose.

(3) The rate of hydrolysis of the inaccessible regions in previously

tritiated cellulose.

(4) The accessibility of crystalline and amorphous oligosaccharides.

(5) An interpretation of the experimental results in terms of the fine structure of cellulose.

The second part describes a study of the irreversible exchange of hydrogen in the drying of cellulose at elevated temperatures.

The third part consists of three appendices: Appendix I deals with the recrystallization of amorphous cellulose, Appendix II describes in detail the experimental procedures and methods of calculation employed, and Appendix III gives the effect of interfiber bonding on the accessibility of cellulose.

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PART II

THE ACCESSIBILITY OF HYDROCELLULOSES AND OLIGOSACCHARIDES BY HYDROGEN EXCHANGE

ABSTRACT

The accessibility of hydrocelluloses and oligosaccharides was measured by hydrogen exchange methods. It is shown that the accessibility of various celluloses remains constant during prolonged heterogeneous hydrolysis. This is at variance with current views on the fine structure of cellulose (Fringed Micellar Theory), on the basis of which a substantial decrease in accessibility would be expected during hydrolysis.

Accessibility measurements on amorphous and crystalline oligosaccharides suggest that the accessibility may be related to the perfection of the crystal lattice and does not measure the crystalline-amorphous ratio as previously supposed.

The amount of hydroxyl groups which become inaccessible in cellulose during treatment with acids containing tritiated water suggests that a slight but significant amount of recrystallization occurs during acid hydrolysis.

From a study of the rate of hydrolysis of the inaccessible regions in previously tritiated cellulose, it was found that the crystallites are only slightly attacked during hydrolysis.

Finally, the results are discussed in relation to current theories on the fine structure of cellulose.

INTRODUCTION

The interaction of acids with cellulose leads to the formation of hydrocelluloses through cleavage of the glycosidic bonds in the cellulose chains. For many years considerable interest[1-12] has centered on this reaction as a means of obtaining information on the molecular morphology of cellulose. Kinetic studies have shown that under heterogeneous conditions the reaction takes place in two stages. In the initial fast phase the chain length, loss in weight and sorptive capacity for water vapour decreases rapidly to a so-called "levelling-off" value which varies with the biological origin of cellulose. In the subsequent stage the reaction proceeds at a much reduced rate. These observations have generally been interpreted on the basis of the well-known "amorphous-crystalline" or "fringed-micelle" model; the hydrolysis has been assumed to take place most rapidly in the amorphous regions. The crystalline regions are more resistant to attack and are thus hydrolyzed more slowly.

If the action of acids on cellulose leads to a progressive removal of the amorphous phase it might be expected to result in a gradual increase in the proportion of crystalline matter. Several attempts have been made to demonstrate such an effect. Hermans and Weidinger determined the X-ray crystallinity of hydrocelluloses obtained from native and regenerated celluloses[13]. For native celluloses there was essentially no change in crystallinity but for regenerated cellulose (rayon) an appreciable increase was found. Davidson[10] also found little change in the crystallinity of cotton cellulose in heterogeneous acid hydrolysis; he further observed that the boiling of hydrocelluloses with 1% NaOH, which caused a loss of weight of about 50 per cent, had no

effect on the X-ray diffraction diagrams. Quite recently McKeown and Lyness[14] made more elaborate measurements of the X-ray crystallinity of various hydrocelluloses. They found that even peptized cellulose, commonly called "micelles", widely considered to be completely crystalline and to be closely related to the crystallites present in the original cellulose, have the same X-ray crystallinity as unhydrolyzed specimens. In this connection Nelson's[15] study of the apparent activation energy of hydrolysis of various celluloses is also of interest; it was concluded that the apparent activation energy is practically the same in the amorphous and crystalline regions.

These results suggest that the presently accepted concept of the structure of cellulose as a system of disordered and entangled chains with local order is only an approximation of reality.

The main purpose of the present investigation was to study the reactivity of hydrocelluloses with the use of the exchange reaction between cellulose hydroxyl hydrogens and heavy water (D_2O) and radioactive or tritiated water (HTO). The results show that the percentage of hydroxyl groups exchanged in cellulose under standard conditions, defined here as the "accessibility", remains the same even after prolonged heterogeneous hydrolysis.

When cellulose is treated with deuterated or tritiated water, some of the exchanged deuterium or tritium becomes incorporated into regions where rehydrogenation is not possible[16-18]. The phenomenon has been attributed to recrystallization which, it has been suggested, also occurs in the early stages of the heterogeneous hydrolysis of cellulose[19-21]. It was therefore of interest to examine the effect of hydrolysis upon the creation of these resistant or inaccessible groups. The assumption was made that if inaccessible groups are formed

during hydrolysis a certain proportion of the cellulose hydroxyl groups will become incorporated into crystallites. Accordingly, if all the accessible cellulose hydroxyl groups are tritiated and the specimen subjected to hydrolysis, some of the tritiated hydroxyl groups will become incorporated into the newly formed crystalline regions and will not be rehydrogenated on subsequently washing with water. These reacted inaccessible hydroxyl groups could then be determined by measuring the residual tritium radioactivity. From these studies it was found that a slight yet significant amount of inaccessible regions are formed during acid hydrolysis.

To obtain information on the behaviour of the crystallites towards hydrolysis a study was made of the rate of hydrolysis of the inaccessible regions in previously tritiated cellulose. The assumption was made that hydroxyl groups in crystalline regions will not exchange. It was found, by measuring the loss in tritium radioactivity as a result of rehydrogenation taking place during hydrolysis, that the crystallites are only slightly attacked during hydrolysis.

As mentioned above the accessibility of cellulose is constant during hydrolysis. A possible interpretation of this observation is that cellulose is not composed of separate crystalline and amorphous phases but may in fact be completely crystalline. However, the question then arises as to the significance of the terms degree of crystallinity and accessibility. In an attempt to elucidate this question a study was made of the accessibility of oligosaccharides prepared in various ways.

As the oligosaccharides can be obtained as single crystals, accessibility measurements on them may provide information on the significance of accessibility and its relation to the fine structure of cellulose.

EXPERIMENTAL

Materials and Methods

The samples listed below were used.

1. Cotton linters: dewaxed by alcohol-benzene extraction.
2. Wood cellulose: softwood, acetate grade wood pulp.
3. Fortisan: supplied by the Amcel Co. Inc., New York.
4. Saponified cellulose triacetate: saponification was carried out by two methods as follows:

   (a) aqueous media: by the use of 2 per cent potassium hydroxide solution in aqueous methanol (50:50 v/v). The sample was subsequently washed with glacial acetic acid and water.

   (b) non-aqueous media: by the use of 1 per cent sodium methylate in anhydrous methanol. The saponified material was then washed with anhydrous methanol.

Hydrolysis was carried out at 50°C, in glass-stoppered Erlenmeyer flasks using 2N hydrochloric acid for wood cellulose and 1N sulphuric acid for all other celluloses. For 15 g. of cellulose 500 cc of the acid was used. The products obtained after various periods of hydrolysis were washed with water until free of acid. They were then dried under vacuum at 50°C. For the cotton and wood cellulose, sols of cellulose "micelles" were obtained by peptization with distilled water after drastic hydrolysis. The method used was similar to that described by Rånby[22]. The cellulose was hydrolyzed with boiling 2.5N sulphuric acid for about 16 hours, and the resulting hydrocelluloses were washed with water until peptization occurred. The sols so obtained were separated from the non-peptized residue by centrifugation, reduced in volume on a vacuum evaporator and dehydrated by freeze-drying. The

peptized as well as non-peptized residues were finally dried under vacuum at 50°C.

The average degree of polymerization of the hydrolcelluloses was evaluated by determining the intrinsic viscosities in cadoxene (for wood cellulose) and cuene (for all other samples). The viscosity average degrees of polymerization ($\overline{DP}_v$) were approximated from the relations derived by Henley[23].

The accessibility of the samples was determined by the gravimetric deuterium exchange method. This involved determining the increase in mass of the sample following exchange with deuterium oxide (99.7% isotopically pure) using an apparatus and conditions similar to those of Sepall and Mason[24]. Four days of drying under vacuum (1μ Hg) at 75°C was followed by 4 days of deuteration at an arbitrarily chosen relative humidity of 100%. The unreacted and adsorbed D_2O was then removed by a further 4-day evacuation at 1μ Hg. The per cent accessibility (A_1) was calculated from the relation

$$A_1 = \frac{100 \times (\Delta\omega)}{FS}$$

where F (= 0.0185 for cellulose) is the fractional increase in mass after all hydroxyl hydrogens in the sample have been exchanged by the deuterium; S is the mass of the sample and $\Delta\omega$ is the increase in mass resulting from exchange of hydrogen by deuterium. All weights were corrected for changes in air buoyancy.

The experiments to study the effect of hydrolysis upon the formation of inaccessible regions were performed with cotton linters and saponified cellulose triacetate (obtained from the non-aqueous saponification medium as described above) as follows. The samples (0.5 g.) were treated with 100 cc of tritiated water (HTO) (total activity 2 milli-

curies) for about 8 hours and then subjected to hydrolysis with 100 cc of 1N sulphuric acid containing HTO (total activity 2 millicuries) for various periods at 50°C. Subsequently the acid was decanted off and the samples washed with distilled water until acid-free. The samples were then dried at room temperature. Blank runs involving treatment with HTO only under otherwise identical conditions were also made. The percentage of hydroxyl groups which became inaccessible or immobile during hydrolysis was determined by measuring the tritium radioactivity in the samples using a methane-filled windowless proportional counter described by Sepall and Mason[25]. The samples were used in sheet form of superficial density of about 3-5 mg./cm.^{2} The percentage exchange (W) was obtained by comparison with the count rate of a standard sample, the accessibility of which had been determined by the gravimetric D_2O exchange method under conditions of temperature and relative humidity identical to those used in measuring the sample radioactivity. Cellophane, at 25°C and 100% RH was used as the standard sample. The accessibility determined by the deuterium exchange method has been found to be in good agreement with that obtained from the tritium exchange method[24] (see also Appendix II).

The quantities measured were $\underline{W}_n$, $\underline{W}_{1H}$ and $\underline{W}_H$, and are defined as follows: $\underline{W}_n$ represents the net inaccessible hydroxyl groups formed during n cycles of wetting in HTO and drying. $\underline{W}_{1H}$ represents the net inaccessible hydroxyl groups produced during water immersion and acid hydrolysis; while $\underline{W}_H$ is the net inaccessible hydroxyl groups caused by acid hydrolysis alone. All quantities are expressed as per cent of the total hydroxyl group.

In studying the rate of hydrolysis of inaccessible regions, all the hydroxyl hydrogens were exchanged with tritium by cyclic drying

and wetting in HTO[18]. The exchange was brought about in inverted U tubes at 120°C. As shown elsewhere[26] about 20 cycles are needed for complete tritiation at this temperature. A sample of 0.75 g. was treated with 8 cc of HTO, with total activity of 8 millicuries, large enough so that dilution effects could be neglected. The sample and HTO were placed in either arm of the U tube, which was then evacuated and sealed off. The drying of the sample was brought about by freezing the HTO contained in one arm, the other arm being maintained at room temperature. After the exchange had been completed, the tubes were broken open and the samples exposed to 75% RH to rehydrogenate the labile tritiated hydroxyl hydrogens. The tritium, which became inaccessible or non-labile, was measured by the solid-counting technique using a methane-filled windowless proportional counter as described earlier (for further details see Appendix II). The samples were then hydrolyzed using 1N sulphuric acid at 50°C for various intervals of time. Blank runs were also made, involving immersion in distilled water only, under otherwise identical conditions. The net amount of tritium thus retained was measured again by the same solid-counting technique.

In the experiments with oligosaccharides cellobi-, -tri-, -tetr-, and -pentaose were used. The cellobiose was highest purity, crystalline, from Eastman Organic Chemicals.

The others were prepared from acid hydrolysates of cellulose by ethanol-water gradient elution from chromatographic columns composed of a stearic acid (1%) treated mixture of charcoal and celite. The method was the same as that described by Miller et al.[27], except that all quantities were doubled. The identity of the oligosaccharides was confirmed through paper chromatographic and X-ray diffraction studies by comparison with standard samples.

The oligosaccharides were obtained in three forms as follows: (i) freeze-dried from water, (ii) crystallized from water-ethanol and (iii) crystallized by slow evaporation from water-ethylene chlorohydrin (50:50 v/v) mixtures.

The accessibility of the samples was determined by the tritium exchange method using a methane-filled windowless proportional counter[25]. Measurements were made at various relative humidities obtained by circulating water at definite temperatures through a water jacket around the counter and the tritiated water reservoir. The samples were examined as pellets of superficial density 3-5 mg./cm.^{2} prepared in a Carver laboratory press at pressures of 2000-5000 lb./in.^{2} Unlike cellulose with which about 8 hours of tritiation is required, the oligosaccharides needed about 24 hours for complete exchange of all the accessible hydroxyl groups. Evidence for this is given in Appendix II. After evacuation to remove the unreacted tritiated water, the activity of the sample was estimated by measuring the tritium radioactivity.

In certain cases the crystallinity of samples was compared using a relative X-ray method based upon that of Hermans[28]. The scattering from a nickel foil placed between the specimen and detector was used as a reference for the incident X-ray intensity. The ratio of a specimen interference, I_{hkl}, and the intensity of a reference line from the nickel foil is a constant independent of the incident X-ray intensity.

Thus $$I_{hkl} \Big/ I_{ref} = K_{hkl}$$

For a series of specimens differing only in crystallinity K_{hkl} gives a measure of crystallinity, provided the quantity of specimen irradiated is the same in each case. The measurements were made in a

Geiger counter diffractometer at several levels of incident intensity and K_{hkl} was evaluated from the slope of the straight line obtained by plotting I_{ref} against I_{hkl}. (For further details see Appendix II).

RESULTS

(a) Hydrocelluloses

The accessibility A_1 and viscosity average degree of polymerization of the various hydrocelluloses are given in Table I. In Fig. 1 the DP values are plotted against time of hydrolysis for cotton linters, wood and Fortisan, respectively. The curves show the characteristic rapid initial decrease in DP to the levelling-off value. Clearly, extensive hydrolysis has occurred. The loss in weight at the levelling-off stage corresponded to about 2 to 5%. In Table I the DP values after 24 hours of hydrolysis are representative of the levelling-off values, and are within the range previously reported by other authors for similar celluloses[9,14,22, and 29]. The accessibility values also showed good agreement with results obtained for similar samples by other authors[17,18]. It is quite apparent, however, that within the limit of experimental error there is no change in accessibility even after prolonged and drastic hydrolysis. Even the peptized celluloses have the same accessibility as the unhydrolyzed celluloses. Obviously these results are not in accord with the fringed micelle model. The results obtained in the study of the effect of hydrolysis upon the formation of inaccessible regions are given in Table II. In Fig. 2 $\underline{W}_{1H}$ is plotted as a function of the duration of hydrolysis. It is seen that after an initial rapid rise the value of $\underline{W}_{1H}$ tends to an asymptotic limit after about four hours. It will be noted that there is a finite value of $\underline{W}_{1H}$ at zero time of hydrolysis amounting to about 0.2% in the case of cotton and about 0.45% for saponified cellulose; evidently this represents the inaccessible tritiated hydroxyls formed during immersion of the specimen in tritiated water and subsequent

TABLE I

ACCESSIBILITY OF HYDROCELLULOSES

Gravimetric D_2O Exchange Method

TREATMENT	COTTON LINTERS		WOOD		FORTISAN		SAPONIFIED CELLULOSE TRIACETATE	
	Accessibility A_1 %	$\overline{DP}_v$	Accessibility A_1 %	$\overline{DP}_v$	Accessibility A_1 %	$\overline{DP}_v$	Accessibility A_1 %	$\overline{DP}_v$
Unhydrolyzed	40	1920	58	2160	69	430	79	-
30 Minutes	40	1090	58	330	68	340	-	-
2 Hours	41	880	57	330	69	110	-	-
24 Hours	39	740	58	310	67	100	80	-
Unpeptized	40	200	57	150	-	-	-	-
Peptized	41	190	59	76	-	-	-	-

FIGURE 1

Curves showing change of $\overline{DP}_v$ with time of hydrolysis.

1. Cotton cellulose (1N H_2SO_4 at 50°C.)
2. Wood cellulose (2N HCl at 50°C.)
3. Fortisan (1N H_2SO_4 at 50°C.)

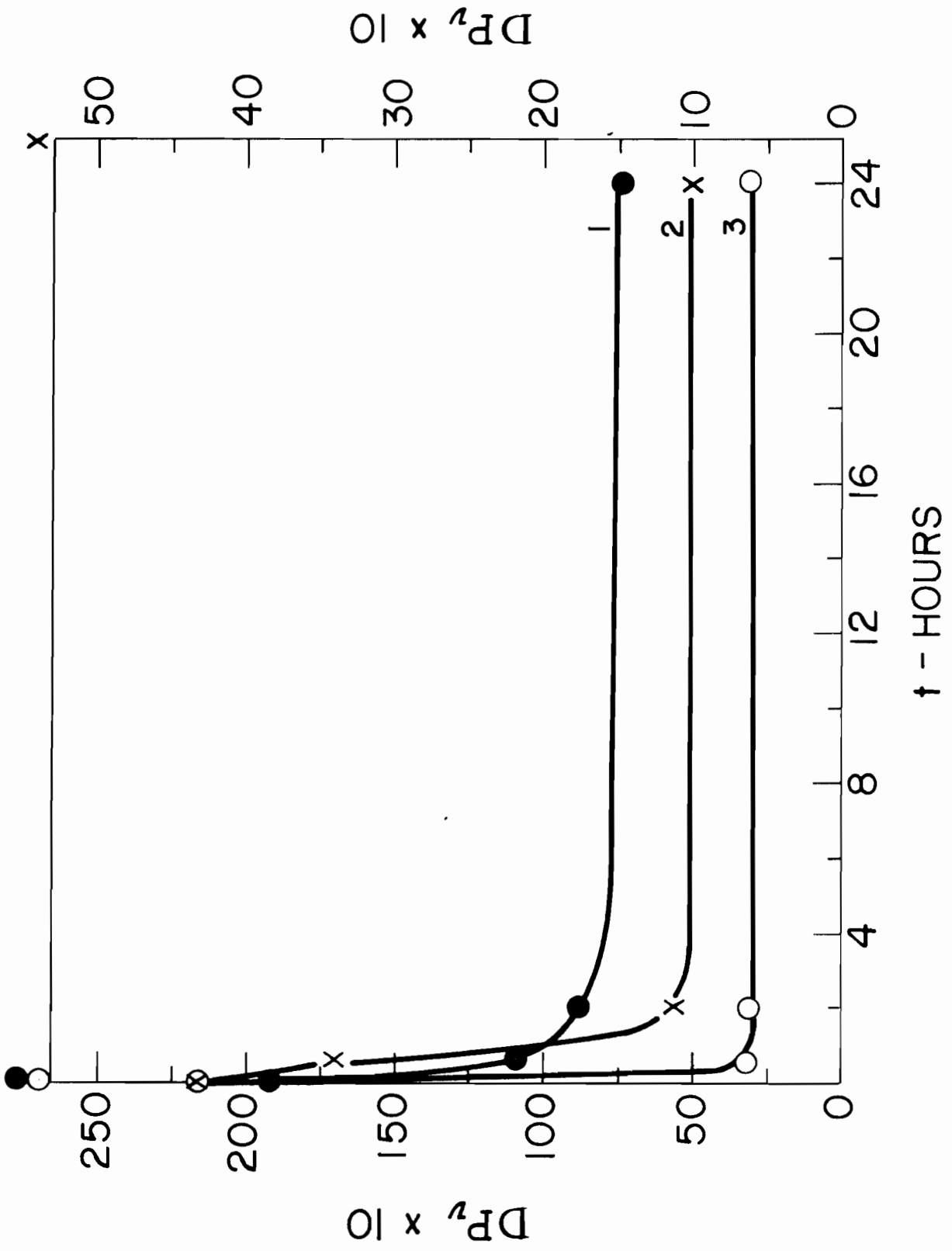

DP$_v$ x 10
250
200
150
100
50
0
DP$_v$ x 10
50
40
30
20
10
0
0
4
8
12
16
20
24
t - HOURS
1
2
3

TABLE II

INACCESSIBLE HYDROXYL GROUPS FORMED

IN THE ACID HYDROLYSIS OF CELLULOSE

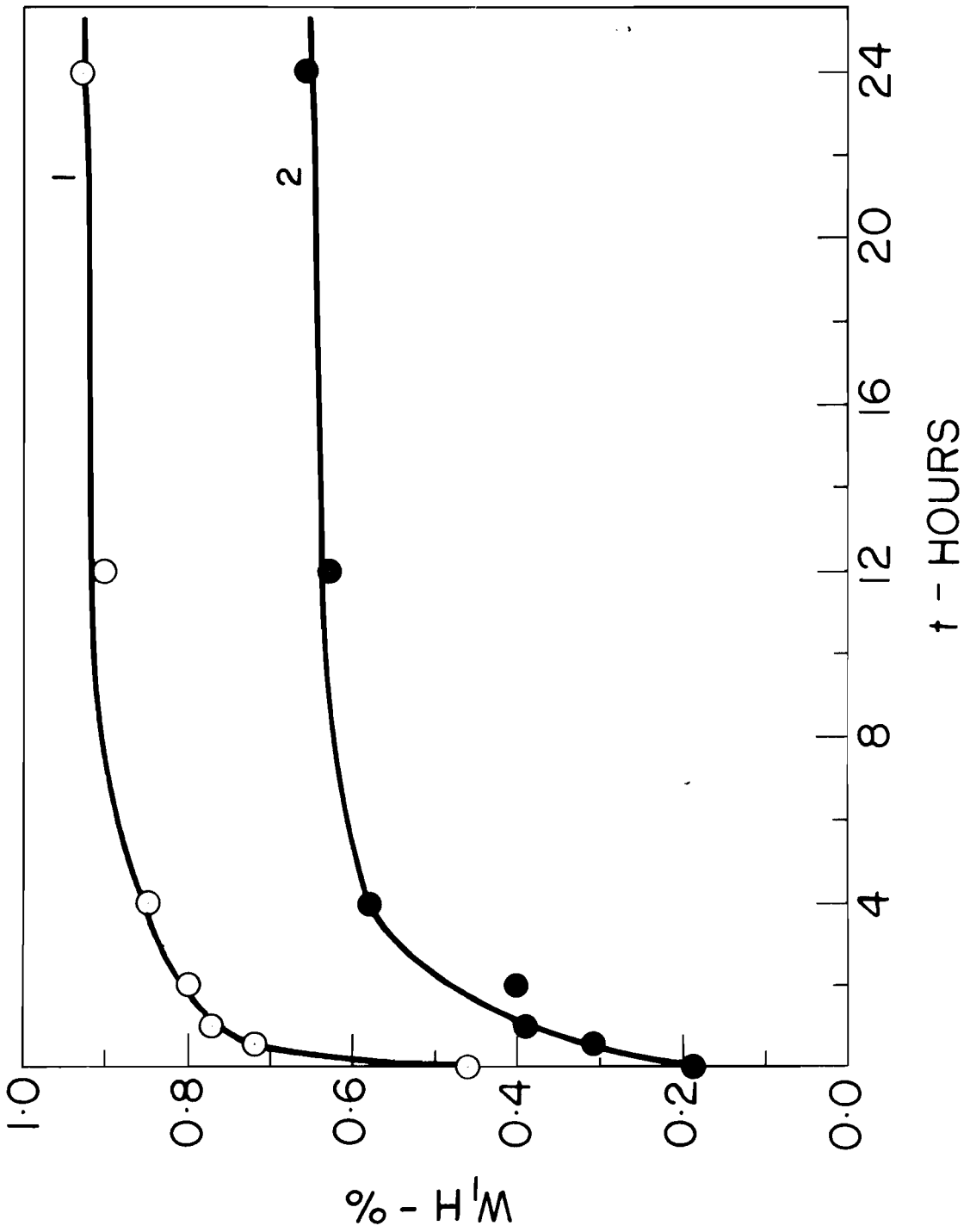
Sample Duration of Hydrolysis	Saponified Cellulose Triacetate		Cotton Linters	
	W_{1H}	$W_{1H} - W_1 = W_H = (W_{1H} - 0.46)$	W_{1H}	$W_{1H} - W_1 = W_{1H} = (W_{1H} - 0.19)$
	%	%	%	%
Blank	0.46	0	0.19	0
0.5	0.72	0.26	0.31	0.12
1	0.77	0.31	0.39	0.20
2	0.80	0.34	0.40	0.21
4	0.85	0.39	0.58	0.39
12	0.90	0.44	0.63	0.44
24	0.93	0.47	0.66	0.44

FIGURE 2

Variation of W_{1H} with time of hydrolysis.

Curve 1. Saponified cellulose triacetate

Curve 2. Cotton cellulose


1
2
1·0
0·8
0·6
0·4
0·2
0·0
W/H - %
4
8
12
16
20
24
t - HOURS

washing in distilled water (blank run) and corresponds to $\underline{W}_1$. The net amount of the tritium taken up during hydrolysis is also shown in Table II. It is quite obvious from the values that the tritium taken up is small. Even at the greatest extent of hydrolysis considered in these experiments, the maximum value of $\underline{W}_{1H}$ corresponds only to about 0.5 per cent exchange. It is interesting to note that the formation of inaccessible hydroxyl groups begins in the earliest stages of hydrolysis, and that when the depolymerization has progressed to the levelling-off DP stage, the amount of tritium taken up is the same for native and regenerated celluloses. However, the value of $\underline{W}_1$ for regenerated cellulose is much higher than that for native cellulose.

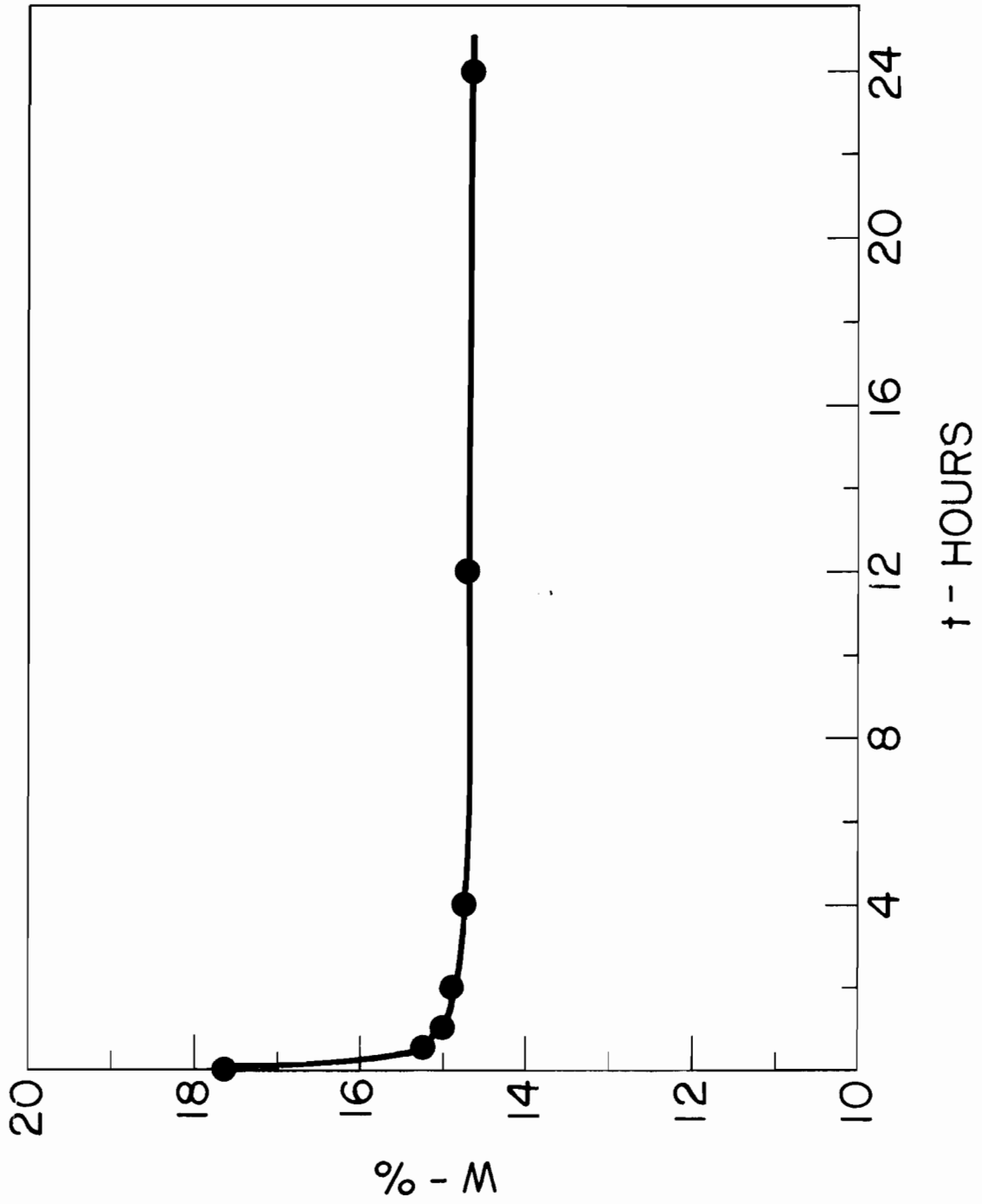
The results obtained in studying the rate of hydrolysis of completely tritiated inaccessible regions are presented in Fig. 3. After the tritiation of cellulose by wetting and drying in HTO and subsequent exposure to 75% relative humidity the amount of tritiated resistant hydroxyl groups corresponded to a value of $\underline{W}_n$ equal to about 40%. Thirty minutes of immersion of the sample caused rehydrogenation and the proportion of tritiated inaccessible hydroxyl groups fell to about 17.6 per cent. A longer period of immersion produced no further change. The effect of the hydrolysis is to produce a further reduction in the proportion of tritiated inaccessible hydroxyl groups by about three per cent.

(b) Oligosaccharides

The accessibility of the oligosaccharides was found to depend on the method of preparation and on the relative humidity of the tritiated water vapour. Specimens freeze-dried from aqueous solution were completely accessible, and as illustrated in Fig. 5a, the X-ray diagrams

FIGURE 3

Curve showing the effect of acid hydrolysis on tritiated inaccessible hydroxyl groups. W is the net amount of tritiated inaccessible hydroxyl groups present in the sample.


20
18
16
14
12
10
% - M
4
8
12
16
20
24
t - HOURS

showed a single diffuse halo. Thus oligosaccharides prepared in this way are amorphous.

The accessibilities of the oligosaccharides precipitated by slow evaporation from water-ethanol and water-ethylene chlorohydrin mixtures are given in Table III for various values of the relative humidity. Fig. 4 shows the plot of accessibility of oligosaccharides against relative humidity. The X-ray powder diagrams of specimens prepared in this way showed numerous sharply defined Debye-Scherrer rings, indicating a well-defined highly crystalline structure. An example is shown in Fig. 5(b).

From Table III it is seen that the accessibility of crystalline oligosaccharides depends markedly on the relative humidity of the tritiated water vapour. In all cases the accessibility rises sharply at low relative humidities (0-25% RH) and thereafter attains a value which remains practically constant at higher relative humidities. For cellobiose and cellotriose the accessibility shows a tendency to increase further at the highest RH values. This may be connected with their greater water solubility.

The asymptotic value of the accessibility may be taken to be characteristic of a given oligosaccharide. Thus when crystallized from water-ethanol solutions the accessibilities of cellobiose, cellotriose, cellotetraose and cellopentaose are 15, 60, 55 and 50 per cent respectively.

Cellotetraose was also crystallized from a water-ethylene chlorohydrin solution. Under these conditions the crystallization was observed to take place considerably more slowly. The characteristic accessibility was then 45 per cent. This is appreciably lower than the value obtained for the same oligosaccharide crystallized from water-

TABLE III

ACCESSIBILITY OF OLIGOSACCHARIDES

	SAMPLE	ACCESSIBILITY (%) Relative Humidity (%)					
		5	25	50	75	85	95
Cellopentaose	Crystallized from water and ethanol	36	50	49	49	50	-
Cellotetraose	Crystallized from water and ethanol	26	51	54	55	58	59
	Crystallized from water and 2-chloroethanol	29	35	41	45	45	46
	Freeze-dried	-	-	-	100	-	-
Cellotriose	Crystallized from water and ethanol	43	56	58	63	67	-
	Freeze-dried	-	-	-	100	-	-
Cellobiose	Commercially crystallized	7	9	14	22	24	29

FIGURE 4

Accessibility of oligosaccharides at various relative humidities.

1. Cellobiose (commercially crystallized)
2. Cellotriose (crystallized from water and ethanol)
3. Cellotetraose (crystallized from water and ethanol)
4. Cellotetraose (crystallized from water and 2-chloroethanol)
5. Cellopentaose (crystallized from water and ethanol).

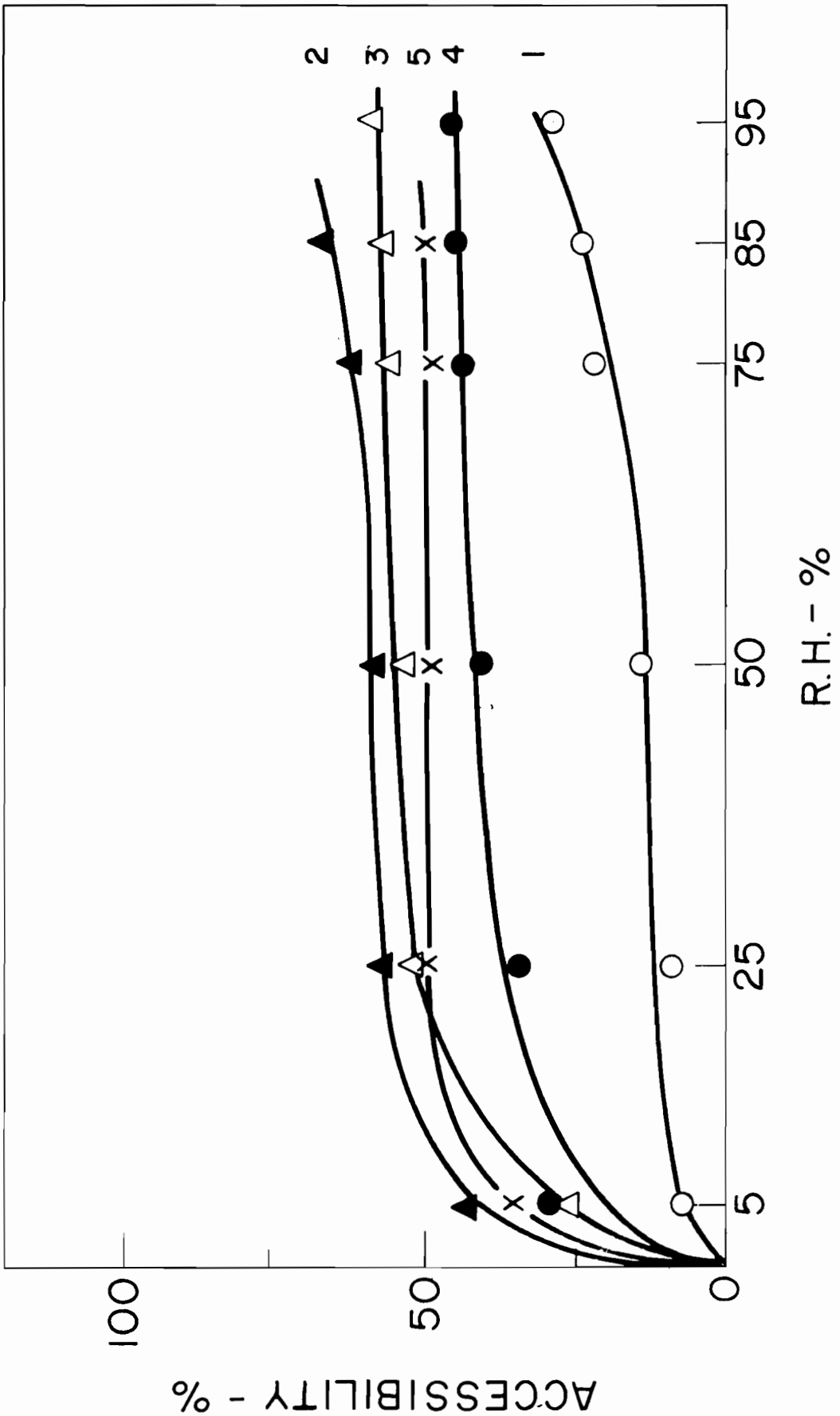
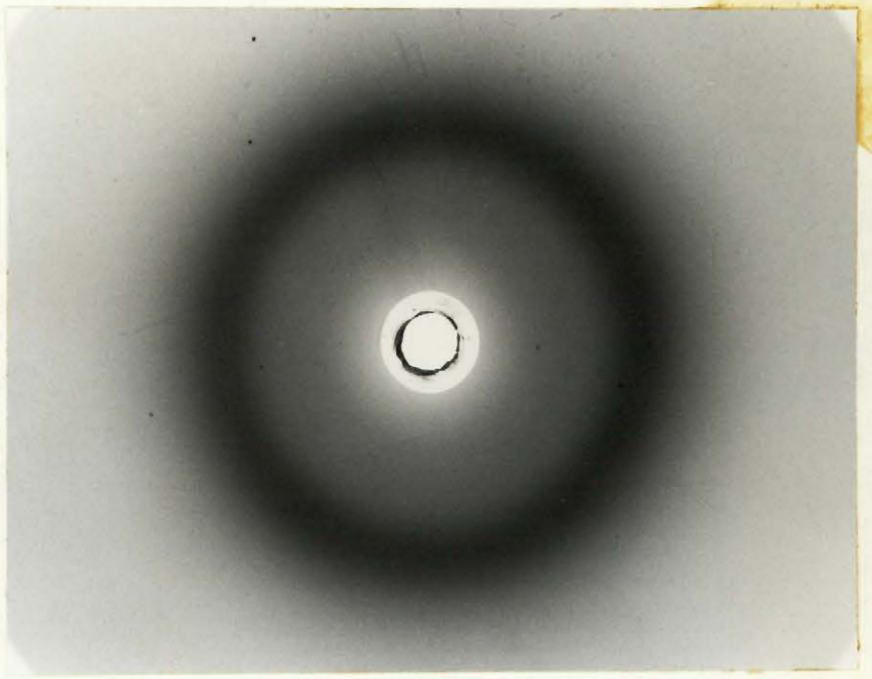

ACCESSIBILITY - %
100
50
0
5
25
50
75
85
95
R.H. - %
1
2
3
4
5

FIGURE 5

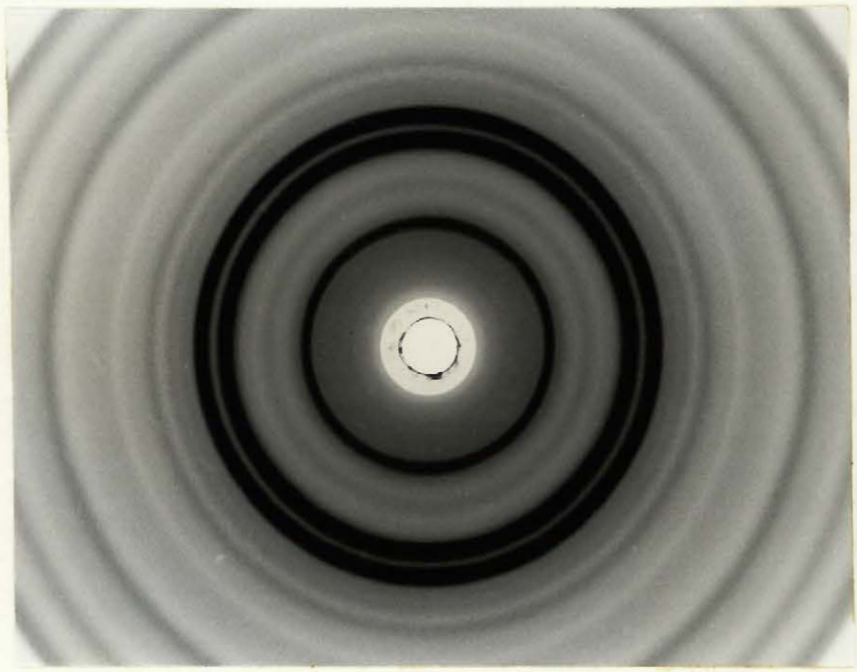
X-ray diffraction diagram of cellotetraose

(a) freeze-dried

(b) crystallized from water and ethanol



(a)



(b)

ethanol solutions. A clue to the origin of this difference in accessibility was obtained by an examination of the crystalline precipitates of cellotetraose in the optical microscope. The precipitates consisted of spherulites which in polarized light (Fig. 6) revealed the characteristic dark maltese cross with arms corresponding to the vibration directions of the polaroids. In Fig. 7 a comparison is made of the spherulites crystallized from water-ethanol and water-ethylene chlorohydrin solutions. The spherulites obtained from the former solutions are smaller and more poorly formed than in the latter case. It thus appears that the rate of crystallization influences the perfection of the crystals and thereby their accessibility.

Fig. 8(a) compares the X-ray crystallinities of the cellotetraose crystallized in the two ways described above. The intensity of the strong reflection at $2\theta = 22$ is plotted against the intensity of the (111) nickel reflection. The slope of the line, which gives a measure of relative crystallinity, is higher for the slowly crystallized sample, i.e., from water-ethylene chlorohydrin. This is in accord with expectations from the accessibility measurements. Similar observations have been made in the comparison of the crystallinities of different oligosaccharides (Fig. 8(b) shows the comparison of X-ray crystallinities of cellotetraose and cellotriose, both crystallized from water and ethanol solvent mixture). Thus, as with cellulose, a low accessibility corresponds to a high crystallinity.

FIGURE 6

Spherulites of cellotetraose in polarized light.

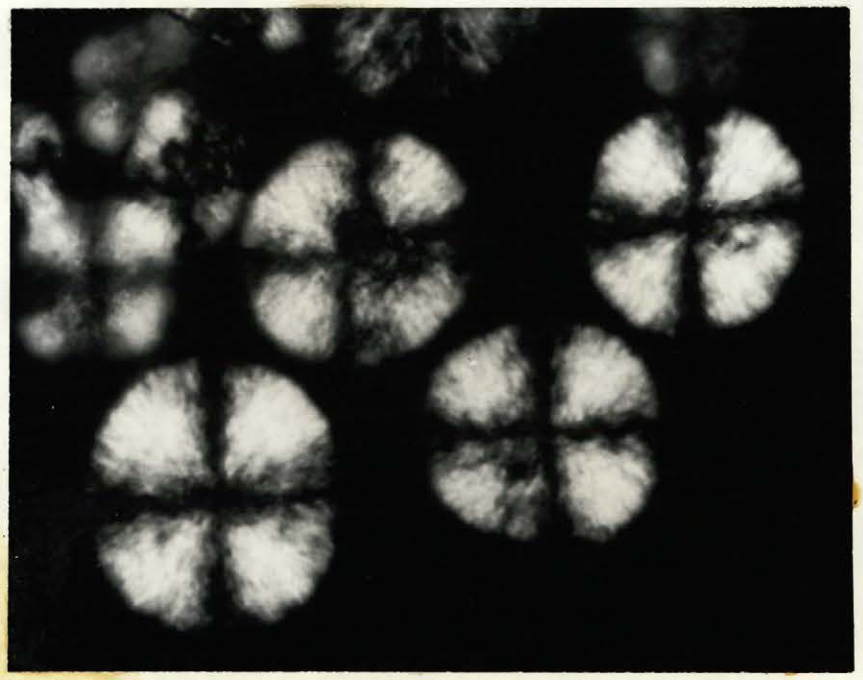
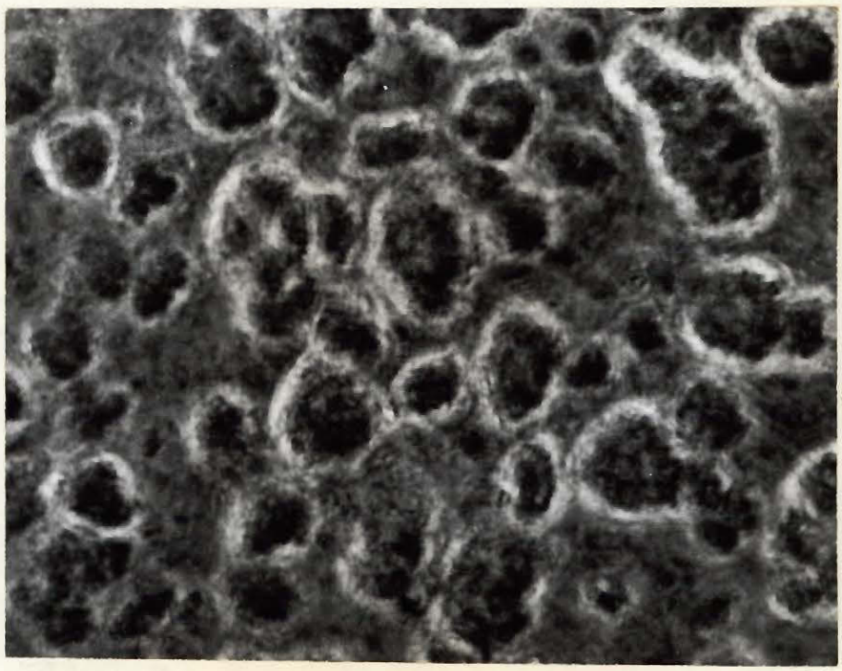


FIGURE 7

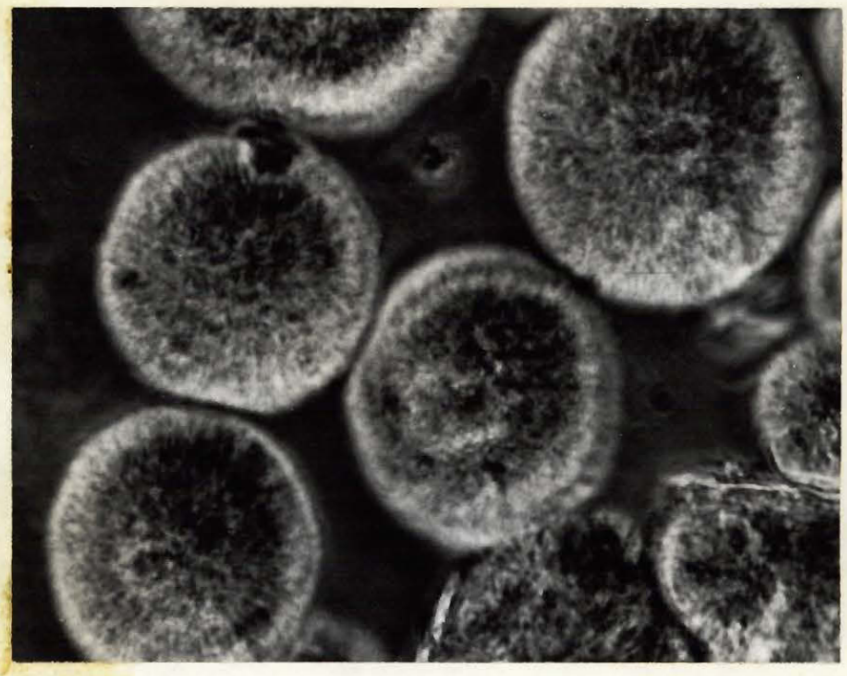
Comparison of spherulites of cellotetraose:

(a) crystallized from water and ethanol

(b) crystallized from water and ethylene chlorohydrin



(a)



(b)

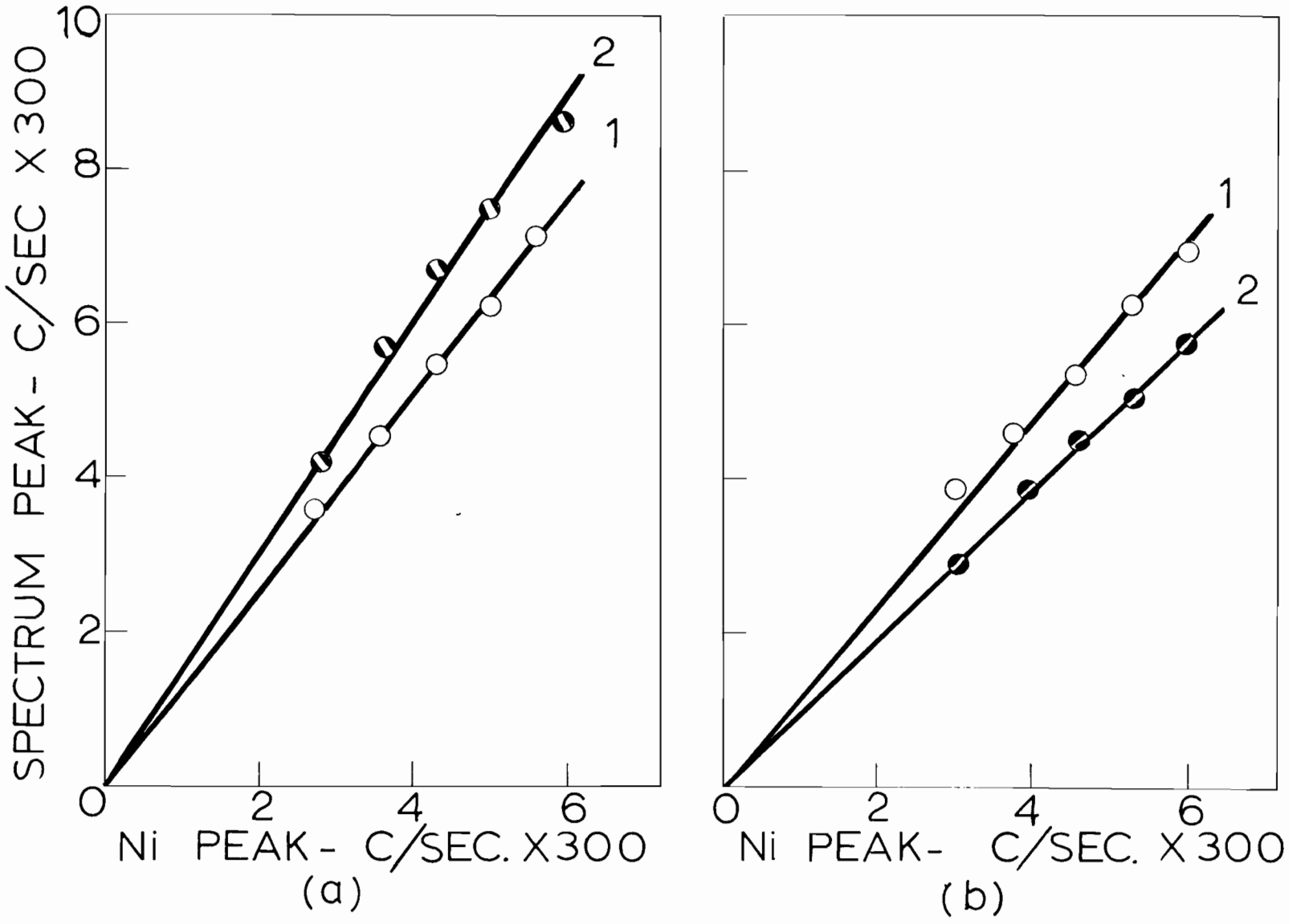
FIGURE 8

Plot of specimen peak height vs. nickel peak height

(Peak heights are plotted in terms of counts per second)

(a) Curves 1 for cellotetraose crystallized from water and ethanol and 2 for cellotetraose crystallized from water and ethylene chlorohydrin.

(b) Curves 1 for cellotetraose crystallized from water and ethanol and 2 for cellotriose crystallized from water and ethanol.


SPECTRUM PEAK - C/SEC X 300
0
2
4
6
8
10
Ni PEAK - C/SEC. X300
(a)
1
2
Ni PEAK - C/SEC. X300
(b)
1
2

DISCUSSION

The experimental observations presented above indicate that there is no change in the accessibility of cellulose during heterogeneous acid hydrolysis. The degree of crystallinity of the samples studied has been stated to be of the order of 40-70 per cent[30-32]. Accordingly if it is assumed that accessibility gives a measure of amorphous content and that hydrolysis involves chain cleavage exclusively in the amorphous regions a substantial decrease in accessibility would be expected. The constancy of the accessibility during hydrolysis is thus at variance with the generally accepted fringed micelle model for the fine structure of cellulose and an interpretation on some other basis is required.

A rigorous interpretation of the observed invariance of the accessibility during hydrolysis in terms of structure seems impossible at the present time. Consequently we can do no more than confine ourselves to suggesting some reasonable possibilities. It would seem possible to explain the observations satisfactorily on the basis of a model in which the cellulose is essentially completely crystalline. Of the many facts which lead to the two-phase "crystalline-amorphous" concept of cellulose structure, one of the most compelling is the simultaneous existence in the X-ray patterns of sharp interferences and diffuse scattering, the latter being assumed to originate from an amorphous phase. In recent years, however, it has been realised that diffuse scattering in the X-ray diagrams of polymers need not necessarily arise from amorphous material. Lattice imperfections of various kinds and small particle size can produce diffuse scattering which is in no

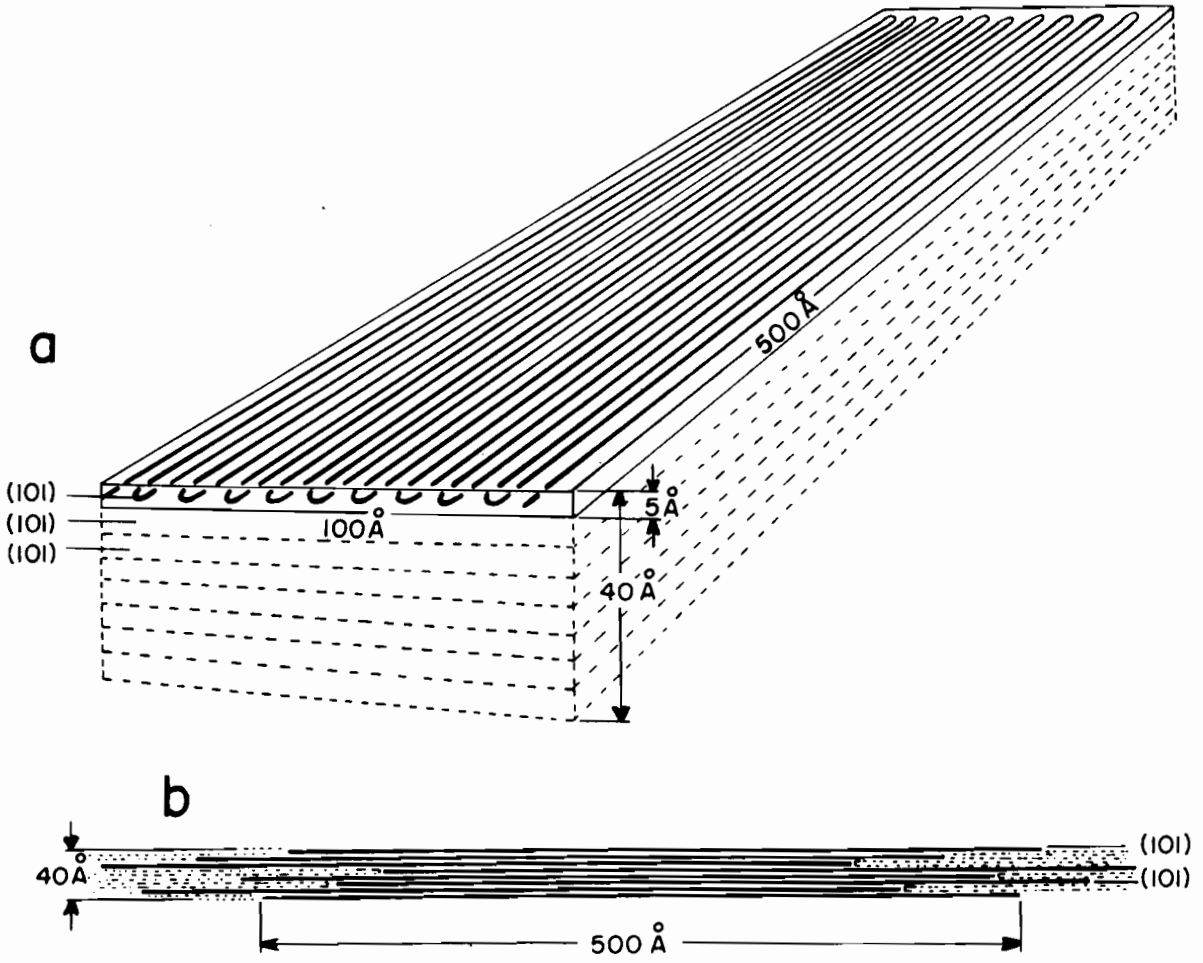
way related to an amorphous phase. In this context the work of Gjönnes et al.[33] is of interest. In a study of the state of order in cellulose the experimental intensity curves were compared with curves calculated on the basis of a Cauchy distribution. It was thus established that the intensity distribution of native celluloses could be completely explained without introducing the concept of an amorphous phase.

A possible approach to a consideration of cellulose in these terms is based upon the recent discovery of chain folding in polymer single crystals[34-39]. Such crystals are obtained by precipitation from dilute solution through supercooling. They have a plate-like habit and are composed of layers about 100Å in thickness. The chain molecules are perpendicular to the plane of the layers and assume folded configurations in order to be accommodated within the layers. The crystals thus consist of folded molecules packed in an ordered arrangement.

Tonneson and Ellefsen[40] have suggested that cellulose fibrils may be built up in an analogous manner. The chain folding model suggested by them is reproduced in Fig. 9 which represents a microfibril in which the cellulose chain molecules are assumed to fold in layers parallel to the (101) plane. The molecular chain axis is oriented in the direction of the fibre axis. The length of the molecule between folds is taken to be 500Å while the width and thickness of the fibril are 100Å and 40Å respectively. A single layer of this fibril, 5Å in thickness, would be able to accommodate a folded molecule with a total length of 10,000Å. As the length of an anhydroglucose residue is about 5Å, this would correspond to a degree of polymerization of 2000.

FIGURE 9

Reproduction of the chain folding model as proposed by Tonneson and Ellefsen[40)]


a
(101)
(101)
(101)
100Å
5Å
40Å
500Å
b
40Å
(101)
(101)
500Å

We turn now to a consideration of the behaviour of cellulose during heterogeneous acid hydrolysis in the light of such a model. Hydrolysis may be assumed to involve cleavage of the glycosidic bonds in the chain folds. A large decrease in the average chain length would be produced by the hydrolysis of a very small proportion of the glycosidic bonds. This would account for the initial rapid decrease in the degree of polymerization. The levelling-off degree of polymerization would be reached when all the folds have been severed.

It is known that the major part of the decrease in degree of polymerization occurs before an appreciable part of the sample goes into solution. Generally, the levelling-off DP is reached when only about 5 per cent of the material has been hydrolysed away. On the chain folding model this would be understandable, as the loss in weight would be derived from the dissolution of the severed chain segments at the folds. Finally, it is evident that as there is no amorphous phase, the accessibility and X-ray crystallinity would remain constant during hydrolysis.

In discussing the relevance of reactivity to structure in cellulose, it is pertinent to note some recent experiments on amylose single crystals[39]. It has been shown that amylose forms single crystals in which the lamella with folded chains is the basic structural element. The dilute acid hydrolysis of these crystals leads to DP-time curves showing a remarkable similarity to those obtained in the case of cellulose. For the amylose crystals it has been demonstrated that there is no separate amorphous phase. Consequently the course of the hydrolysis cannot be explained by a preferential acid attack on amorphous regions.

By analogy it seems possible that the behaviour of cellulose during hydrolysis does not necessarily indicate a two-phase structure.

So far we have been primarily concerned with the case of native celluloses. It is more difficult to visualize the structure of regenerated cellulose in terms of an all crystalline model. According to generally accepted views, in the concentrated solutions from which regenerated celluloses are made, the chain molecules are envisaged to be intimately entangled and a considerable amount of molecular mobility must be assumed if chain folding is to occur. Nevertheless, there is increasing evidence that even in the solid state polymer molecules have a surprising mobility. For example during the annealing of polyethylene single crystals the fold length has been shown to increase by as much as 300Å.[41] The process apparently takes place by a refolding of the chains. There is no reason to suppose the cellulose molecules do not exhibit similar mobility, and in fact recent experiments by Sepall and Mason[18] strongly suggest that molecular mobility plays an important role in changes in the accessibility of cellulose during drying.

It has been shown that during hydrolysis a small amount of tritium becomes incorporated into resistant or inaccessible regions which do not become rehydrogenated on exposure to water. The formation of these inaccessible regions during hydrolysis may be due to recrystallization as freedom of motion and rotation acquired by the severed chain segments permits them to become ordered. If the effect is in fact due to recrystallization it is rather surprising that it starts at the earliest stages of the hydrolysis when only a small proportion of the chains would have been broken. However, this is in accord with the

findings of Sharples[7)] who showed that recrystallization begins when approximately one-eighth of the inter-crystalline chain segments have been broken. The extent of recrystallization during hydrolysis has been investigated by other authors[7 and 42)] who give estimates of 2-5 per cent. These figures are, however, not directly comparable with that obtained (0.5 per cent) in the present investigation as different methods of measurements are used.

The small amount of tritium taken up during acid hydrolysis may be taken to substantiate the view that the fringed micelle theory is untenable. It is clear that if the figures for the accessibility of various celluloses really give an indication of amorphous content then the amount of the tritium incorporated during hydrolysis should have been much higher.

The loss in tritium radioactivity by rehydrogenation during hydrolysis was found to be small, amounting to only about 3 per cent re-exchange. This is in accord with the results on the effect of hydrolysis on the formation of inaccessible regions and again suggests that there are no extensive changes occurring at the molecular level during hydrolysis.

The tritiation of the "crystalline" saccharides has shown that the accessibility of all the sugars increases up to a characteristic constant value as the RH of the tritiated water vapour is increased. Only when the sugars are amorphous (e.g. when freeze-dried) are they fully accessible.

The extent to which the crystalline saccharides are accessible

is quite surprising. Except for cellobiose the constant characteristic accessibility at the higher RH values is of the order of 50%. The present concept of the significance of the term accessibility is that it gives a measure of the proportion of sample OH groups available for exchange. These exchangeable OH groups are widely believed to occur preferentially in amorphous regions.

In the case of the crystalline oligosaccharides the material is precipitated from solution as spherulites which are known to be aggregates of single crystals, and certainly do not contain amorphous material - at least as a separate phase. Their high crystallinity is substantiated by the X-ray diagrams. The results thus indicate that the oligosaccharide crystals are partially accessible for tritiation, which presumably takes place by a penetration of the crystal lattice by the HTO molecules. If this is true the question still remains why the crystal lattice is only partially accessible. A possible answer may be provided by the observation that the accessibility of a given oligosaccharide can be varied widely by simply altering the conditions of crystallization from solution. It is therefore suggested that the accessibility may be related not to crystalline-amorphous ratio but to the perfection of the crystal lattice. By analogy the accessibility of various celluloses would appear to be explicable on a similar basis.

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PART III

IRREVERSIBLE EXCHANGE OF HYDROGEN IN THE DRYING OF CELLULOSE AT HIGH TEMPERATURES

ABSTRACT

A study of the effect of temperature on the irreversible exchange of hydrogen during the drying of cellulose has been made. The number of drying and wetting cycles required for the complete exchange of hydroxyl hydrogens in cellulose has been shown to decrease rapidly with increasing temperature. At 190°C there is complete exchange in one cycle. The increased hydrogen exchange at elevated temperatures is believed to be caused by increased chain mobility which in turn is attributed to the co-operative effect of water acting as a plasticizer and of the molecular thermal energy derived from heating.

INTRODUCTION

This Part is concerned with hydrogen exchange involving inaccessible groups in cellulose during cyclic wetting and drying. Marrinan and Mann[1] have shown that the exchange of accessible hydroxyls with hydrogen isotopes is not completely reversible. Part of the cellulose becomes inaccessible during drying. It was suggested that the phenomenon might be partly due to a reduction in the number of reactive hydroxyls by drying.

Lang and Mason[2] studied the effect of repeated wetting and drying. Samples were immersed in HTO and dried and the reacted inaccessible hydroxyl groups were determined by measuring the residual tritium radioactivity after removing the labile tritium by soaking in water. The quantity $\underline{W}_n$ was defined as the net inaccessible hydroxyl groups reacted during $\underline{n}$ drying cycles in HTO. It was found that $\underline{W}_n$ increased with n. The effect was attributed to a partial interchange of accessible and inaccessible regions as a result of molecular rearrangements. It was also predicted that all the hydroxyl groups in cellulose would be reacted with water if a sample was repeatedly wetted and dried a sufficient number of times. In a continuation of these experiments Sepall and Mason[3] studied the irreversible exchange at temperatures up to 100°C and showed that all the inaccessible hydroxyls could indeed be exchanged, at least at higher temperatures. It also appeared that the higher the temperature the smaller the number of wetting-drying cycles required for complete exchange.

In the present investigation the aim was to extend the knowledge of the effect of temperature on the irreversible exchange of hydrogen during the drying of cellulose. In particular it was of interest to determine whether there might exist a temperature at which complete exchange could be achieved in a single wetting-drying cycle. The quantities

measured were $\underline{W}_n$ and $\underline{A}_n$. $\underline{W}_n$ has already been defined above, while $\underline{A}_n$ is the limiting extent of the exchange reaction with water at 75% relative humidity and 25°C after n drying cycles.

EXPERIMENTAL

Materials and Methods

The cellulose sample used was a softwood, acetate grade wood pulp

Cyclic wetting and drying experiments were conducted on sheets in inverted U tubes at 110 and 120°C. Three-quarters of a gram of the sample and 8 cc of tritiated water (HTO) with a total activity of 8 millicuries, large enough so that the dilution effects could be neglected, were placed in the tube with the sample held at one end. The air was then evacuated and the tubes sealed off. The wetting step was performed by tilting the tube and allowing the HTO to flow to the sample end. Two cycles were conducted per day: 4 hours of immersion was followed by 8 hours of drying.

The drying of the samples was conducted at 0 and 75% relative humidity as follows:

(a) drying at 0% RH; the arm containing HTO was placed in a freezing mixture (ice + sodium chloride), the other arm being maintained at room temperature (25°C).

(b) drying at 75% RH; here the two arms containing HTO and the sample were placed in thermostatically controlled baths maintained at 20.3 and 25°C respectively.

After the required number of cycles the tubes were opened and the samples exposed to water vapour at 75% RH to rehydrogenate the labile tritiated hydrogens. The residual tritium activity was measured in a counter as described earlier except that there were no accessories for tritiation. The fraction of tritiated hydroxyl groups, $\underline{W}_n$, was calculated by comparing the count rate with that of a standard sample whose count rate and accessibility were both known.

The effect upon $\underline{W}_n$ of prolonged soaking in tritiated water was

also studied. The procedure here was the same as that for a single cycle but with slight modifications, i.e., the immersion time was 30 days in duration.

$\underline{W}_1$ was measured by conducting a single cycle of drying and wetting (4 hours of immersion followed by 8 hours of drying) at 100, 125, 150 and 175°C, the rest of the procedure being the same as previously described.

RESULTS AND DISCUSSION

The results of the cyclic wetting and drying experiments at 110 and 120°C are given in Figs. 1 and 2 respectively. In both figures curves 1 and 2 correspond to data obtained when the sample was dried at 0 and 75 per cent relative humidity. It is seen that $\underline{W}_n$ increases rapidly to a limiting value which remains constant with further increases in the number of cycles. From the data in Figs. 1 and 2 it is clear that the number of cycles required for complete exchange of the hydroxyl hydrogens in cellulose decreases as the temperature of wetting is raised. As reported by Sepall and Mason[3] at 100°C about 100 cycles are required for complete exchange. In the present experiments at temperatures of 110 and 120°C the number of cycles required is 35 and 20 respectively.

As the number of cycles required for complete exchange decreases markedly as the temperature is raised higher than 100°C, only one cycle of drying and wetting was conducted in further experiments at still higher temperatures.

The results of the measurement of $\underline{W}_1$ at 100, 125, 150 and 175°C are given in Table I. There is a remarkable increase in the value of $\underline{W}_1$. Thus, it is seen that out of a total of 42 per cent inaccessible hydroxyl groups, 33 per cent could be exchanged in a single cycle at a temperature of 175°C. It would have been of interest to carry on the measurements of $\underline{W}_1$ at still higher temperatures, i.e., beyond 175°C, but because of experimental difficulties this was not possible. Nevertheless from a plot of $(\underline{A}_1 + \underline{W}_1)$ against temperature (Fig. 3) it appears that complete exchange would have been achieved in a single cycle at a temperature of about 190°C.

The question arises as to the possible interpretation of this observation. It is well known that polymeric materials exhibit a thermal transition (the glass transition temperature) which corresponds to the onset

FIGURE 1

$\underline{A}_n + \underline{W}_n$ for wood cellulose at 110°C

Curve 1. For dryings conducted at 0% RH

Curve 2. For dryings conducted at 75% RH

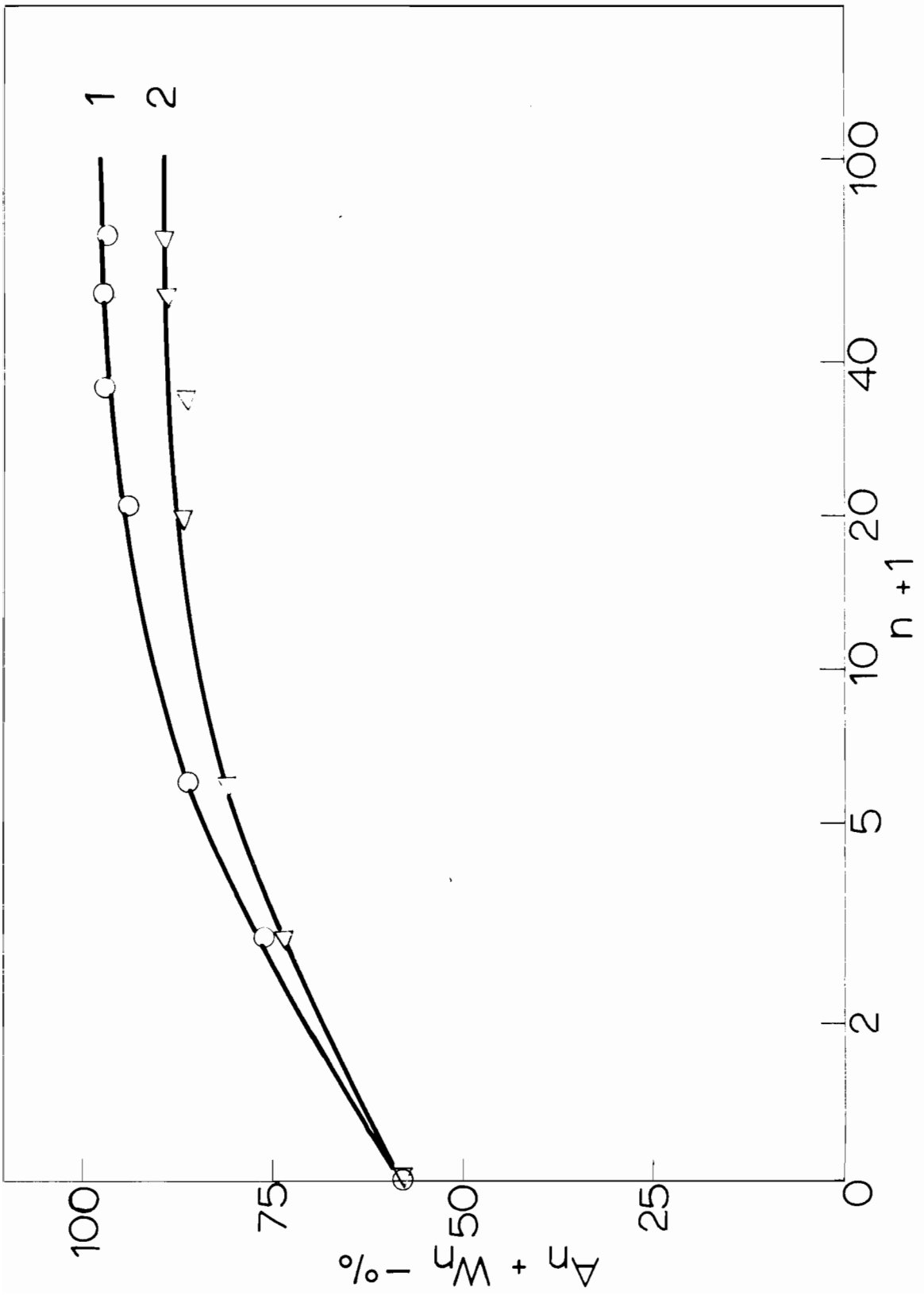

1
2
100
75
50
25
0
2
5
10
20
40
100
n + 1
$A_n + W_n$ — %

FIGURE 2

$\underline{A}_n + \underline{W}_n$ for wood cellulose at 120°C

Curve 1. For dryings conducted at 0% RH

Curve 2. For dryings conducted at 75% RH

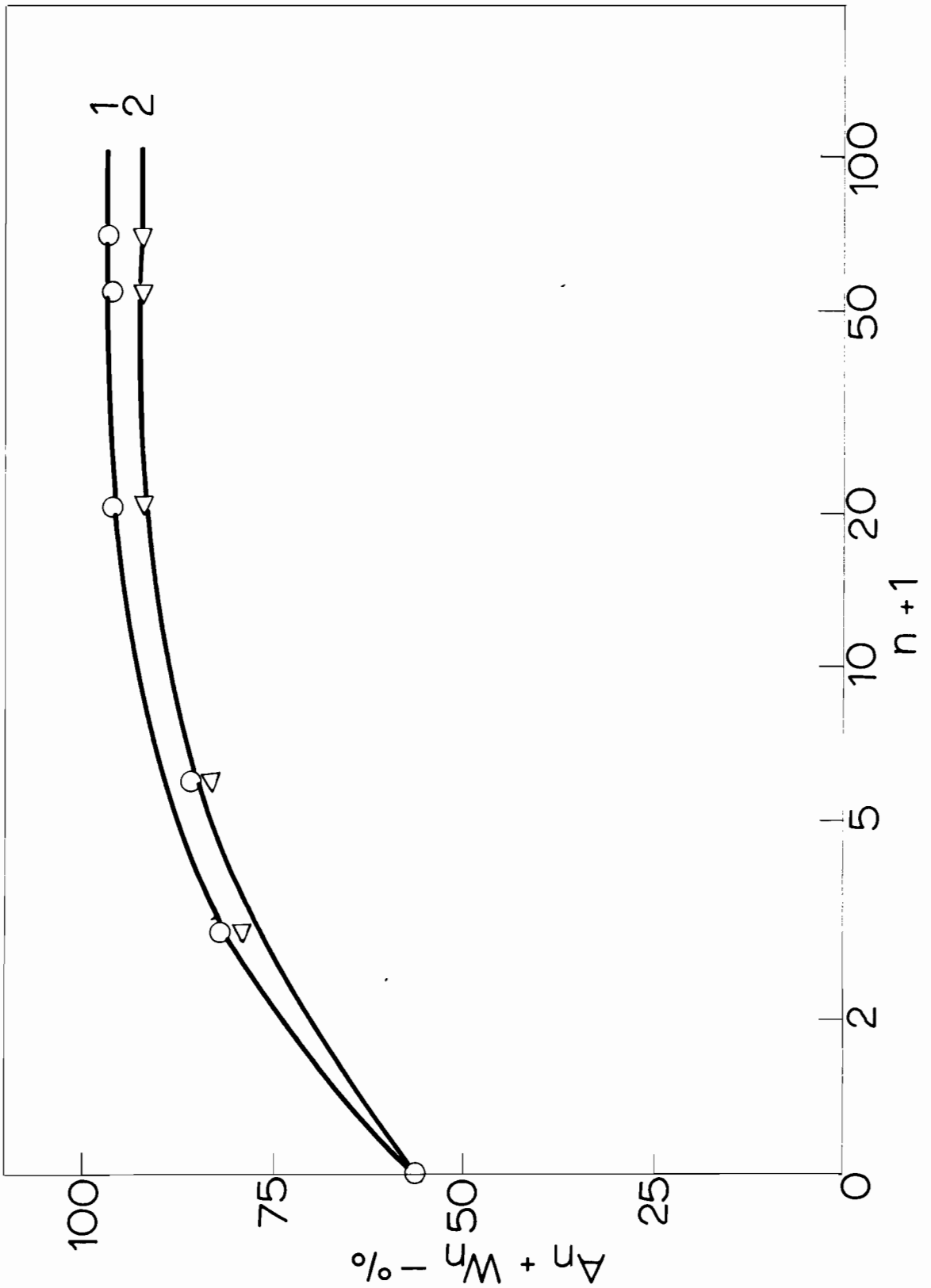

1
2
100
75
50
25
0
$A_n + W_n$ — %
2
5
10
20
50
100
n + 1

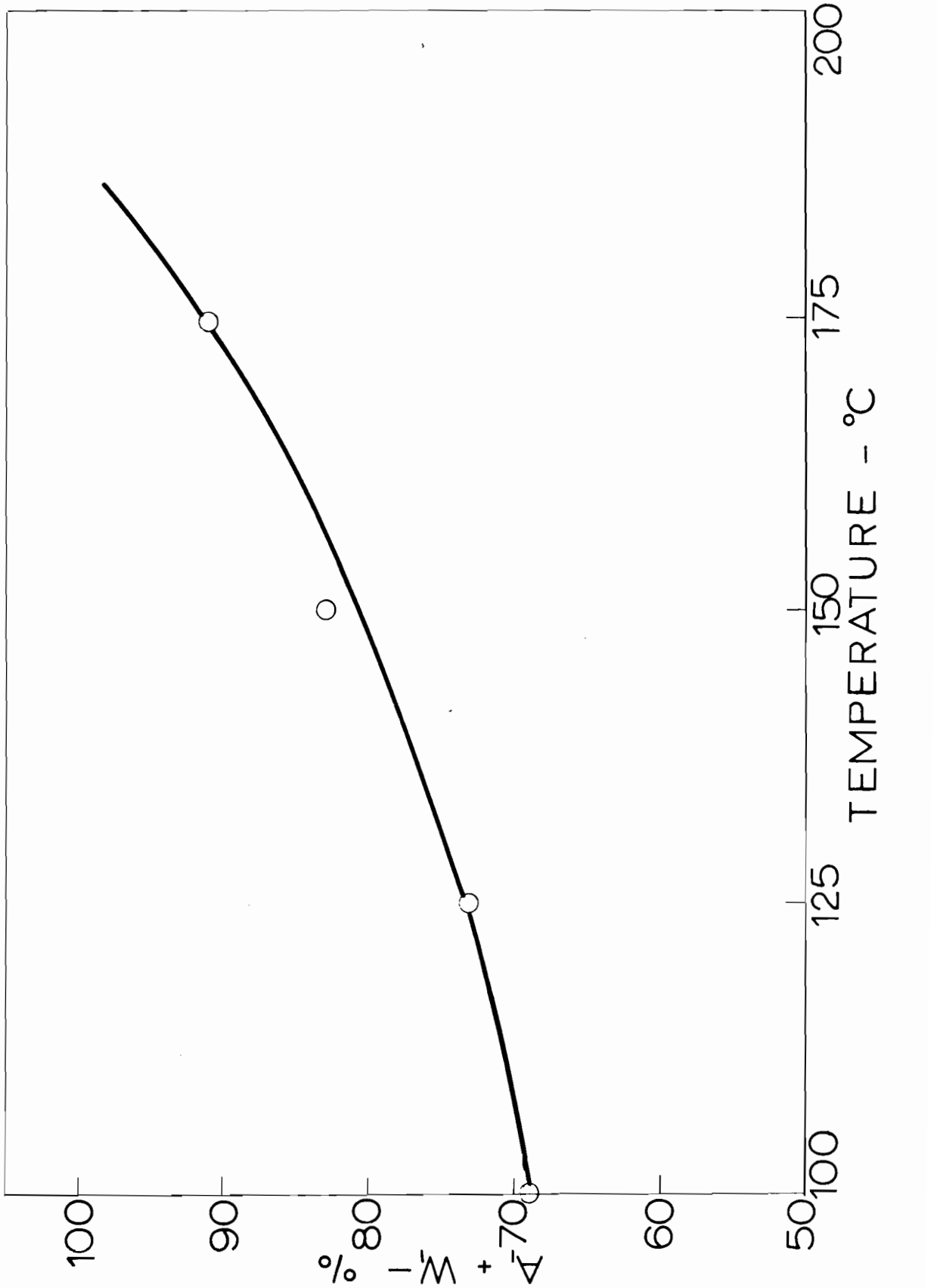
TABLE I

Measurements of W_1

Temperature	W_1	$(A_1 + W_1)\%$ $(A_1 = 58)$
100	11	69
125	15	73
150	25	83
175	33	91

FIGURE 3

Plot of $\underline{A}_1 + \underline{W}_1$ vs. temperature


100
90
80
70
60
50
100
125
150
175
200
TEMPERATURE - °C
$A_i + W_i -$ %

of large scale molecular motion. It may reasonably be supposed that in cellulose the increased molecular motion at temperatures at or above the glass transition would favour the inaccessible/accessible transformation. Recent measurements by Goring[4] and Kargin et al.[5] indicate that the glass transition temperature of cellulose may be in the range 220-250°C. It is therefore suggested that the observed complete accessibility at 190°C is related to the glass transition of cellulose which is lowered by the plasticizing action of the liquid water present.

The results described above are in substantial agreement with a recent study by Okajimo and Inoue[6]. They investigated the IR spectrographs of cellulose II film heat treated with liquid D_2O at 170-220°C in order to trace the increase in crystallinity of the film caused by the D_2O treatment. It was shown that deuteration and rehydrogenation reach the "crystalline regions" of cellulose II rather easily at these high temperatures. Practically all (92%) of the inaccessible hydroxyl groups were exchanged during heat treatment for about 5 minutes in liquid D_2O at 170°C.

In studying the effect of prolonged immersion on W_n, Sepall and Mason[3] showed that when n is large, the exchange of inaccessible hydroxyl groups during immersion constitutes the major part of W_n. In the present work these studies were extended to higher temperatures. The samples were immersed for times as long as 30 days - a period in which 60 consecutive cycles of wetting and drying were conducted and during the process of which both the curves 1 and 2 (Figs. 1 and 2) had attained their asymptotic values. The samples were then dried at 0% RH. It is interesting to observe that as much as 30 and 33 per cent inaccessible hydroxyl groups were exchanged at 110 and 120°C respectively. The same results were obtained when drying was conducted at 75 per cent RH at corresponding temperatures.

It may be noteworthy to mention that in similar experiments at 100°C, for a period of immersion of 64 days (corresponding to 192 cycles) Sepall and Mason[3] found $\underline{W}_1$ = 28.9 per cent. It is thus seen that increased temperature exerts a profound effect upon the exchange - a much reduced period of immersion suffices to attain the same degree of exchange at higher temperature.

The accessibility of wood cellulose, determined by the tritium exchange method at 25°C and 50 per cent RH, is known to be 58 per cent[7]. In Table II measurements of the accessibility at temperatures up to 100°C are presented. It is of interest to note that the accessibility remains constant over the entire temperature range studied and thus that none of the residual hydroxyl groups could be exchanged.

From the foregoing it is clear that an increasing proportion of the inaccessible hydroxyl groups in cellulose are exchangeable at elevated temperatures. It seems possible that this phenomenon can be understood in terms of the co-operative effect of water acting as a plasticizer and of the molecular thermal energy derived from heating. Both factors may serve to increase molecular mobility and thereby render inaccessible hydroxyl groups available for exchange.

The cellulose chain molecules and the hydrophilic hydroxyl groups are in a state of thermal vibration that will increase with increasing temperature. This can result in both the making and breaking of cross-linking hydrogen bonds, and the final effect will depend on the amount of water present.

Water may be considered to act as a plasticizer promoting micro-Brownian movement of the cellulose chain segments by cutting the weaker hydrogen bonds between them. The presence of water in excess accelerates hydration of hydroxyl groups and facilitates the formation of hydrogen

TABLE II

Accessibility of wood cellulose at higher temperatures

Measured by tritium exchange method at 50% R.H.

Temperature °C	Accessibility %
25	57
40	59
55	58
70	59
85	57
100	57

bonds between cellulose and water rather than between cellulose chains. On the other hand if the amount of water is too little its plasticizing action is weakened.

On this basis the constant accessibility of cellulose at 50 per cent RH and temperatures up to 100°C may result from the fact that there is not enough water present to act as a plasticizer. However, this does not rule out the possibility of the complete exchange of the hydroxyl groups at low relative humidity, but at very high temperatures where thermal energy alone may cause enough molecular motion to bring about the complete exchange of inaccessible hydroxyl groups.

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PART IV

CONCLUDING REMARKS AND SUGGESTIONS FOR FUTURE WORK

CONCLUDING REMARKS AND SUGGESTIONS FOR FUTURE WORK

In the present work an attempt has been made to obtain a better understanding of the fine structure of cellulose through the use of the hydrogen exchange reaction between cellulose and water.

Accessibility measurements made on native and regenerated celluloses after various times of hydrolysis showed that the accessibility remains unchanged. This is contrary to the "fringed micelle" theory, on the basis of which a sharp decrease in accessibility was expected. The results suggest that the present concepts of the fine structure of cellulose need revision, and that cellulose, at least in the native state, may be completely crystalline.

From studies on the crystalline oligosaccharides it was suggested that the accessibility parameter is related to the crystal lattice perfection and does not measure the amorphous/crystalline ratio as has previously been supposed.

As expected on the basis of an "all crystalline" model, the amount of recrystallization taking place during acid hydrolysis of cellulose is slight though significant. This is attributed to the preferential acid attack at lattice imperfections: the chain ends thus set free undergo crystallization.

Most of the molecular changes in cellulose take place in the early stages of hydrolysis. The hydrolysis of tritiated cellulose showed that crystallites obtained during hydrolysis are very resistant to further hydrolytic attack.

It is interesting to observe that appreciable crystallization of amorphous cellulose takes place only on immersion in water or exposure to very high relative humidities, i.e., around 100%. At lower relative

humidities, the amount of crystallization taking place is slight though significant. It is believed that water acts as a plasticizer and facilitates the movement of cellulose chains leading to alignment and crystallization.

Although cellulose is partially accessible yet all the hydroxyl hydrogens can be exchanged by cyclic wetting and drying. The number of cycles required for complete exchange decreases as the temperature of treatment is raised. At 175°C about 91% hydroxyl hydrogens in wood cellulose can be exchanged in a single cycle. Because of the plasticizing action of water and the increased thermal energy of the molecules at high temperatures, the mobility of the chains increases appreciably, thus resulting in the exchange of more and more inaccessible hydroxyl groups. However, at lower temperatures where there is not enough thermal energy in the molecules, simple immersion in water alone does not bring about enough molecular motion to effect an exchange of the inaccessible hydroxyl groups. It is suggested that swelling takes place at the site of lattice imperfections and the stresses produced by the swelling cause the creation of new imperfections in the lattice, thus allowing the exchange of more inaccessible hydroxyl groups. Drying brings the chains together again causing new imperfections to form and simultaneously amending the old ones. Thus by cyclic wetting and drying, all the hydroxyl hydrogens are exchanged.

No attempt has been made to propose a new model for the fine structure of cellulose but the possibility of chain folding in cellulose has been discussed.

Further study along these lines may prove fruitful especially with regard to the question of chain folding in cellulose. In particular electron microscopy of the fine structure of cellulose should be of great interest.

The following is a list of suggestions for further work.

1. Chain folding in nylon has been conclusively proved[1]. Accessibility measurements if carried out by tritium exchange (through the exchange of H in the -NH grouping) on nylon as such and after annealing at various temperatures (this will change the fold length) should give some information as to how accessibility is related to chain folding.
2. Electron microscope studies if conducted on microfibrils obtained from native and regenerated celluloses, by negative staining techniques should throw some light on the presence of chain folding, if any, in cellulose. This may also help in comparing the structure of native and regenerated celluloses which, although generally considered to be different are not fully understood.
3. Measurement of the accessibility of oligosaccharides higher than cellopentaose may give further information on the question of the significance of such measurements in case of cellulose.
4. Whelan et al.[2] have fractionated a series of linear dextrins up to maltoheptaose from the hydrolysates of potato amylose by column chromatography. Accessibility studies on them and on chain folded amylose may afford further insight into the significance of accessibility and its possible relation to the cellulose case.
5. Accessibility measurements on other crystalline substances containing exchangeable groups such as -COOH, $-NH_2$, -NH, and -OH, and the relation of accessibility to crystal structure.
6. Measurements of the accessibility of cellulose at high temperatures (beyond 100°C) but at low relative humidity (say 50%) should give further information on the behaviour of crystallites to hydrogen exchange.
7. Wetting and drying of tritiated cellulose in ordinary water to see

if the same number of cycles are required for rehydrogenation as for tritiation.

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CLAIMS TO ORIGINAL RESEARCH

1. The accessibility of cellulose remains constant even after prolonged heterogeneous acid hydrolysis.

2. During the hydrolysis of cellulose a slight yet significant amount of tritium is taken up.

3. The crystallites are quite resistant to hydrolytic attack and are only slightly affected.

4. The accessibility of oligosaccharides depends upon the relative humidity. It was also shown that the accessibility of crystalline oligosaccharides when measured under identical conditions of RH and temperature, depends upon the crystal lattice perfection.

5. Amorphous cellulose is completely accessible.

6. Whereas water brings about complete crystallization of amorphous cellulose, the alcohols have no effect at all.

7. The number of wetting-drying cycles required for complete exchange of the hydroxyl groups in cellulose was found to decrease at elevated temperatures. At 175°C, as much as 91% hydroxyl groups could be exchanged in a single cycle.

8. It was observed that at least up to a temperature of 100°C the accessibility of wood cellulose remains constant.

9. A new sample holder was designed to permit the measurement of the accessibility of powder samples by the tritium exchange method.

10. The body of the counter designed by Sepall and Mason[1)] was provided with a water jacket. This allows the tritiation reaction to be carried out at any desired relative humidity.

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APPENDIX I

THE RECRYSTALLIZATION OF AMORPHOUS CELLULOSE

ABSTRACT

Recrystallization of amorphous cellulose prepared by saponification of cellulose triacetate in non-aqueous medium was found to occur only at very high relative humidities i.e., near 100%. At lower relative humidities the amount of recrystallization occurring was slight though significant. Correspondingly, the accessibility decreased from 100% (for amorphous cellulose) to a minimum of 79%, the same as that for cellulose II. The mechanism of recrystallization has been discussed in terms of the surface tension, plasticizing action and the hydrogen bonding ability of the solvent used for bringing about the recrystallization.

INTRODUCTION

It is well known that cellulose can be obtained in an amorphous form by dry grinding in a vibratory ball mill[1,2] or by saponification of cellulose triacetate in a non-aqueous medium[3]. On immersion in water this amorphous cellulose crystallizes to cellulose II[4]. However, little attention has been paid to the details of the crystallization process.

In the present work an attempt was made to study more elaborately the recrystallization of cellulose by the use of the hydrogen exchange reaction between cellulose hydroxyl hydrogens and water and by X-ray diffraction. Although it was not possible to develop this work into a comprehensive investigation it is believed that the principal results as they stand are of sufficient interest to warrant a concise presentation as an appendix.

EXPERIMENTAL

The amorphous cellulose used was obtained by treating cellulose triacetate sheets with 1 per cent sodium methylate dissolved in anhydrous methanol. The saponification was allowed to proceed for about 12 hours. The regenerated cellulose thus obtained was neutralized with glacial acetic acid and washed with various alcohols until free of acetic acid. As a control, one sample was also washed in H_2O. Subsequently the samples were dried in a vacuum desiccator. The following alcohols were used: anhydrous methanol, ethanol, propanol and n- and iso-butanol.

In another series of experiments amorphous cellulose was exposed to water vapour at different relative humidities obtained through the use of various saturated salt solutions[5].

The accessibility of all samples was measured by the tritium exchange method, details of which were as previously described.

Kinetic studies of the recrystallization of amorphous cellulose were carried out by an X-ray diffraction method using a Geiger counter diffractometer (see Appendix II). The rate of development of the $10\bar{1}$ and 002 peaks was observed at various relative humidities.

The X-ray diffraction patterns were obtained in a flat-film camera using nickel-filtered CuKα radiation (see Appendix II).

RESULTS AND DISCUSSION

The accessibility of amorphous cellulose after treatment with water and various alcohols is presented in Table I. The corresponding X-ray diffraction patterns are shown in Fig. 1. It is seen that all alcohol treated samples show the same accessibility, i.e., 90%, whereas the water treated sample has the significantly lower accessibility of 79%. This is consistent with the results of the X-ray diffraction experiments. For amorphous cellulose treated with alcohols the pattern is dominated by a single broad diffuse halo in all cases. This indicates that alcohols do not cause recrystallization of the cellulose. On the other hand, the X-ray pattern of the water treated sample is well defined and corresponds to the structure of cellulose II. It was expected that amorphous cellulose would be completely accessible (i.e., 100%). The observed accessibility of 90% probably results from the fact that the exchange measurements were made at 50% relative humidity. This might have caused a small amount of recrystallization with a resultant lowering of the accessibility.

In order for the amorphous cellulose to crystallize, intermolecular forces must come into play. The amount of recrystallization will depend upon the extent to which the treatment permits the development of these intermolecular forces. Since the intermolecular forces in cellulose arise almost exclusively from hydrogen bonding[6] it is expected that the hydrogen bonding capacity of the liquid will play an important role in the recrystallization process. The liquid plasticizes cellulose permitting it to assume favourable orientations for hydrogen bonding.

Surface tension forces operating between cellulose chains during the final stages of drying would also be expected to play a role in the recrystallization.

TABLE I

ACCESSIBILITY OF SAPONIFIED CELLULOSE TRIACETATE

(Measured by Tritium Exchange Method)

Saponified Cellulose Triacetate Dried From:	Accessibility (%) (at 50% RH)
I. Methanol	91
II. Ethanol	90
III. Propanol	89
IV. n-Butanol	89
V. Iso-Butanol	89
VI. Water	79

FIGURE 1

X-ray diffraction diagrams of amorphous cellulose dried from:

a) Methanol

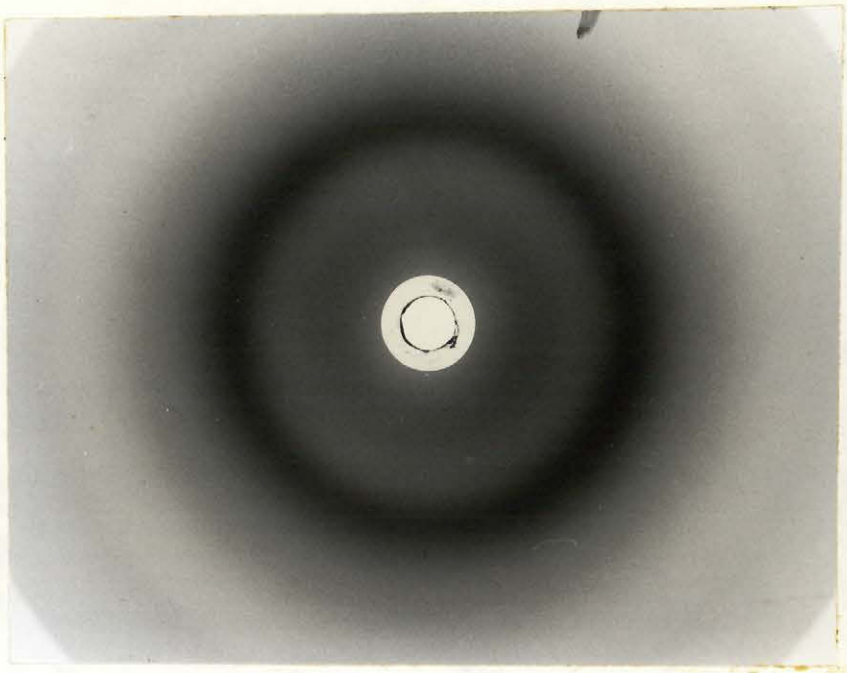
b) Ethanol

c) Propanol

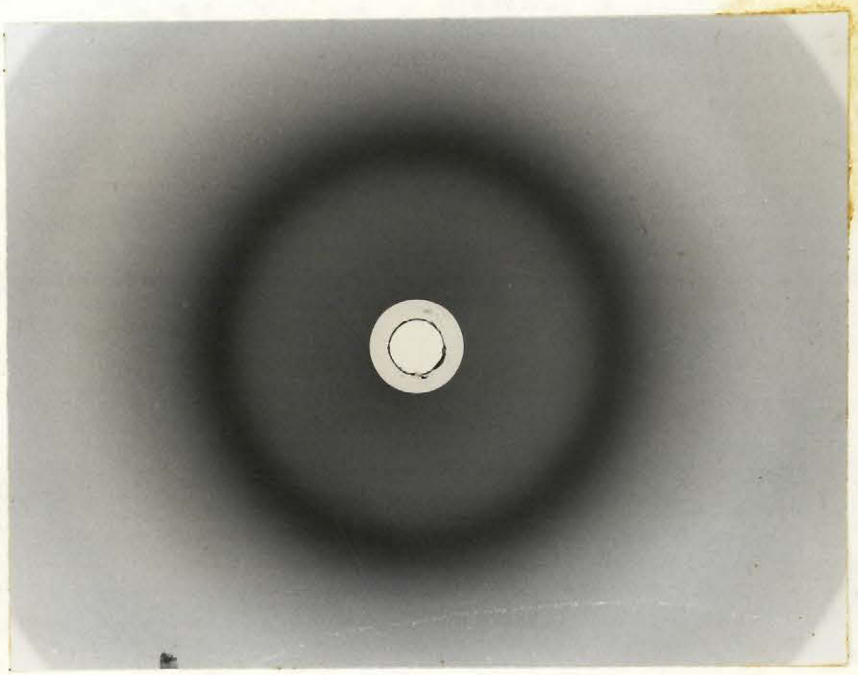
d) n-Butanol

e) iso-Butanol

f) Water



(a)



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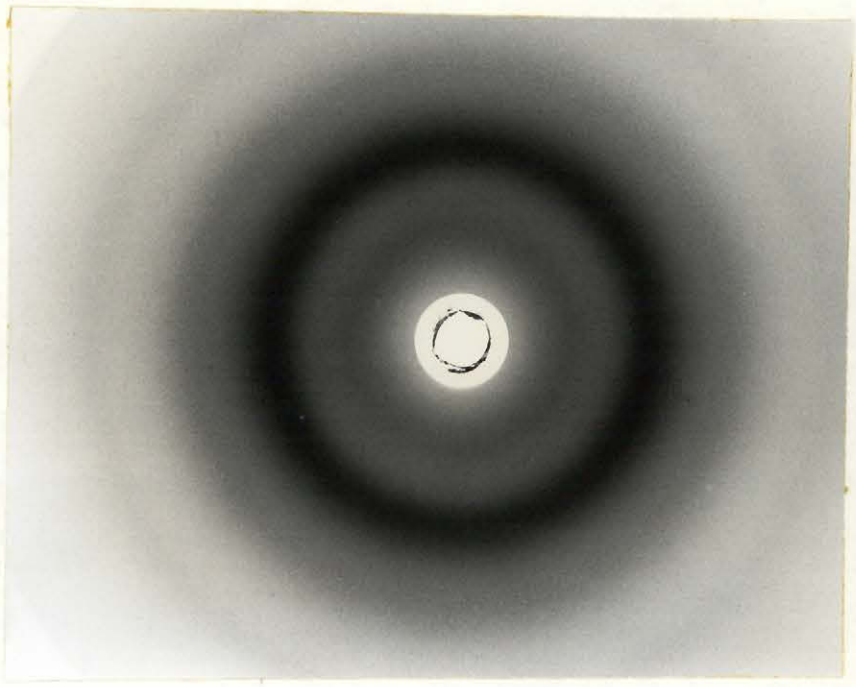

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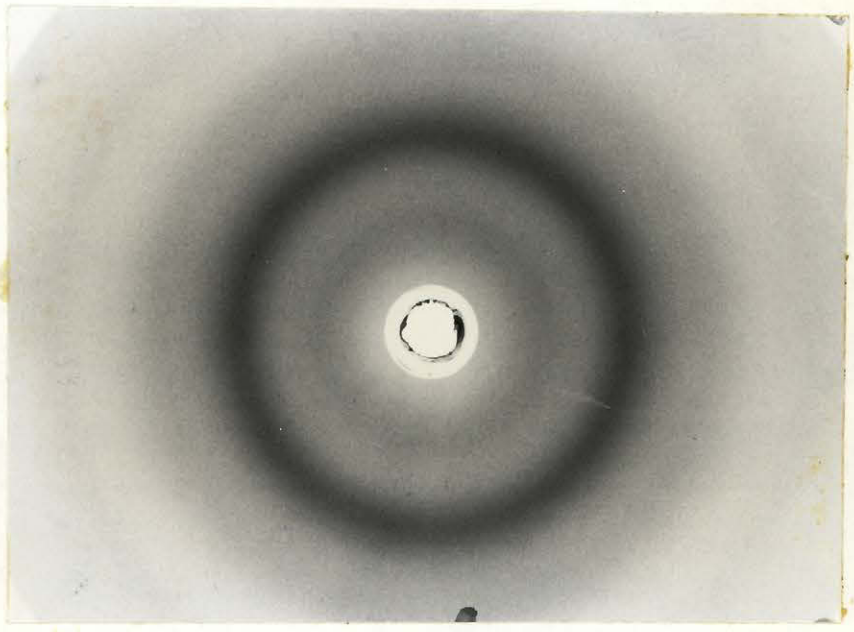

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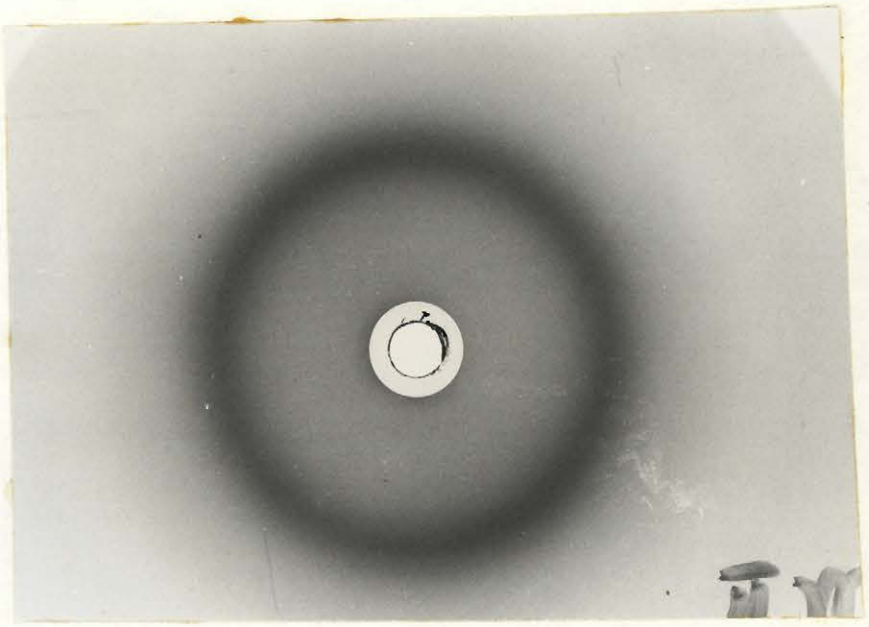

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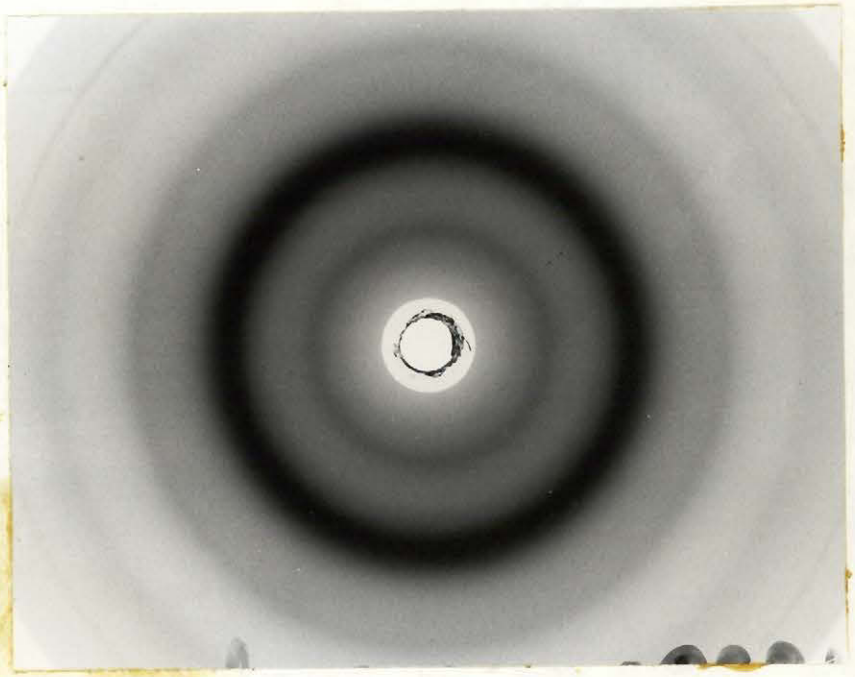


(f)

Thus the effectiveness of water as compared to the alcohols in promoting the recrystallization of amorphous cellulose would be related to its high surface tension and hydrogen bonding capacity. For the alcohols, both of these properties are low.

The recrystallization of amorphous cellulose also occurs on exposure to water vapour at high relative humidity. This can be seen in Table II which shows the accessibility of amorphous cellulose as a function of relative humidity. The accessibility decreases from 89 to 79 per cent as the relative humidity is increased from 0 to 100 per cent.

The effect of time on the recrystallization process is illustrated by the data in Fig. 2 which show a plot of the intensity of the $10\bar{1}$ peak against time of exposure for amorphous cellulose at 100% RH and a temperature of 25°C. It is seen that under these conditions the amorphous cellulose crystallizes quite rapidly initially and in about four hours reaches an asymptotic value after which further exposure produces only a very slight change. Similar experiments were also carried out at temperatures of 50 and 100°C. In both cases, the results were similar to those obtained at 25°C. Thus within this range temperature has no effect on the recrystallization. Unfortunately, such measurements could not be made above 100°C as another modification of cellulose (i.e., cellulose IV) was then formed.

At lower relative humidities the process of recrystallization was extremely slow. For example, after an exposure time as long as 30 days at 75% RH and 25°C only a very slight amount of recrystallization could be detected in the X-ray diffraction pattern which is shown in Fig. 3b. Also shown in Fig. 3 are the X-ray diffraction patterns of amorphous cellulose exposed at 0, 10, 20, 50 and 100% RH. It is seen that only a slight amount of recrystallization occurred in these cases also, except for the

one which had been exposed to 100% RH and shows complete crystallization (Fig. 3a).

TABLE II

ACCESSIBILITY OF AMORPHOUS CELLULOSE

(Tritium Exchange Method)

Relative Humidity (%)	Accessibility (%) (at 50% RH)
100	79
75	81
50	84
20	85
10	88
0	89

FIGURE 2

Kinetics of crystallization of amorphous cellulose exposed to 100% RH

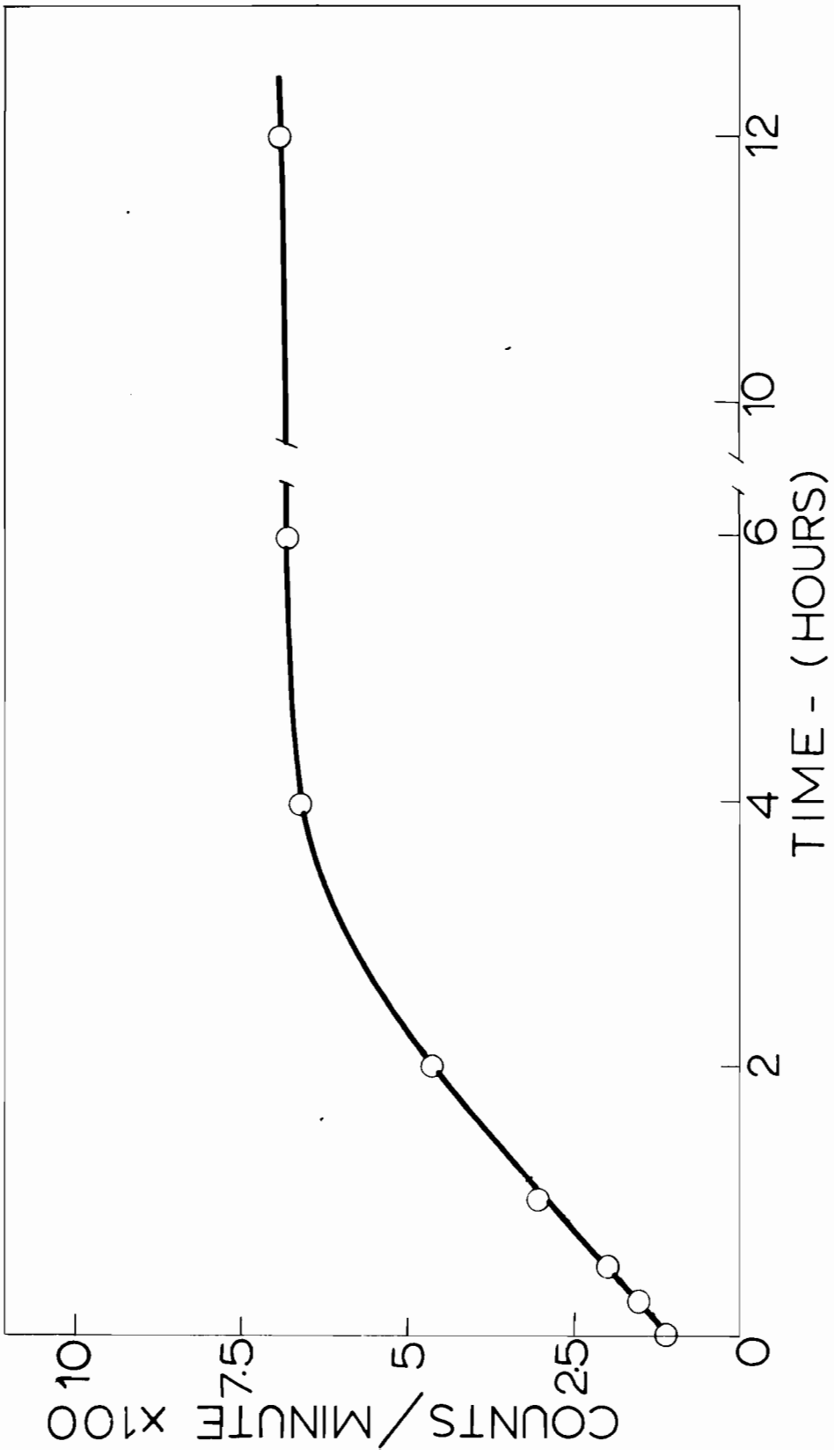

COUNTS / MINUTE x100
0
2.5
5
7.5
10
2
4
6
10
12
TIME - (HOURS)

FIGURE 3

X-ray diffraction pictures of amorphous cellulose exposed to various relative humidities

a) 100% RH

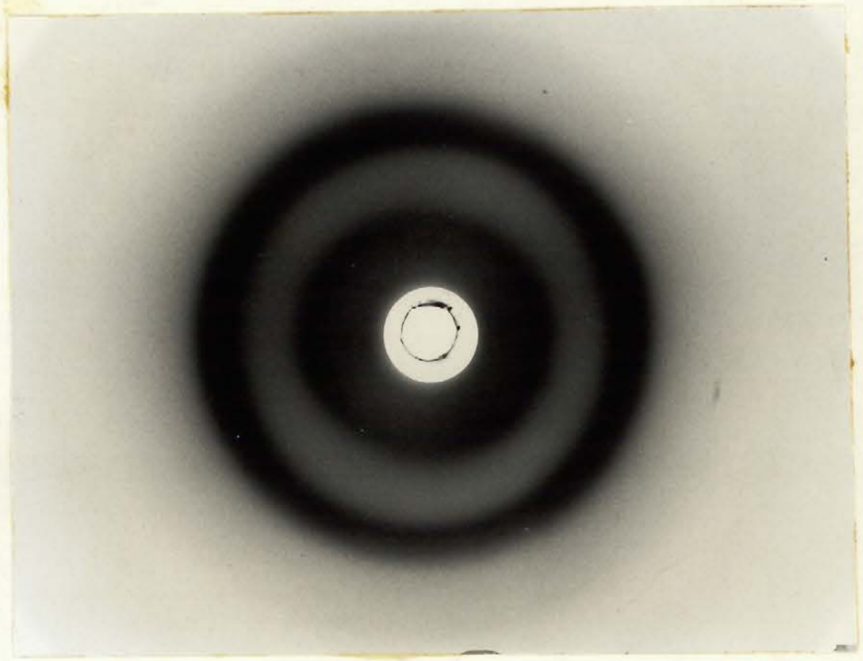
b) 75% RH

c) 50% RH

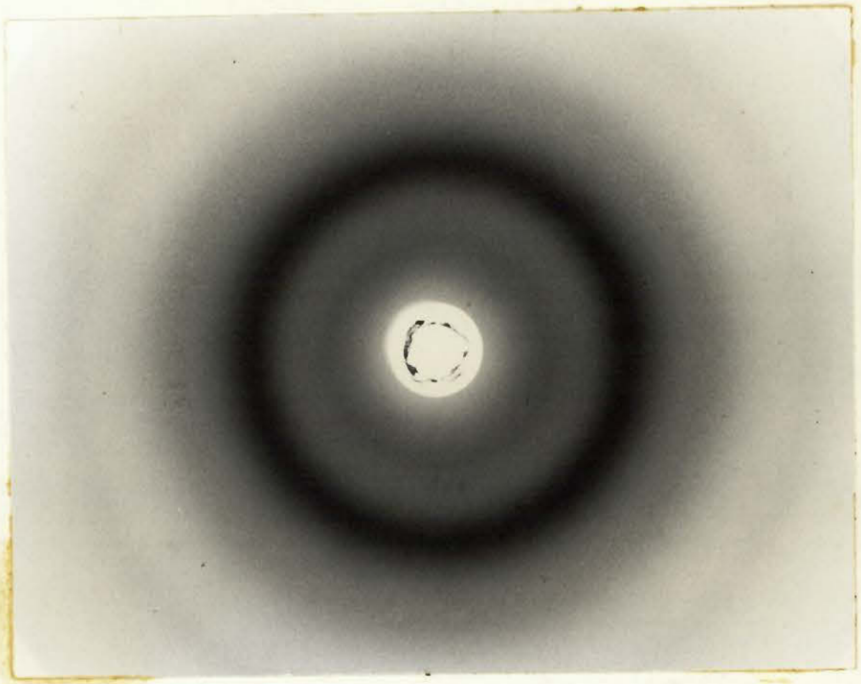
d) 20% RH

e) 10% RH

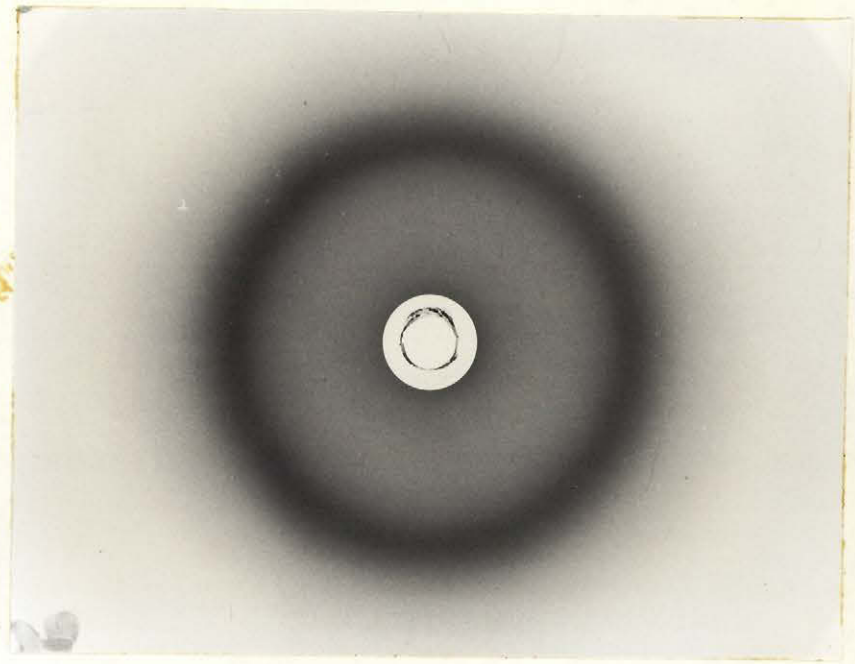
f) 0% RH



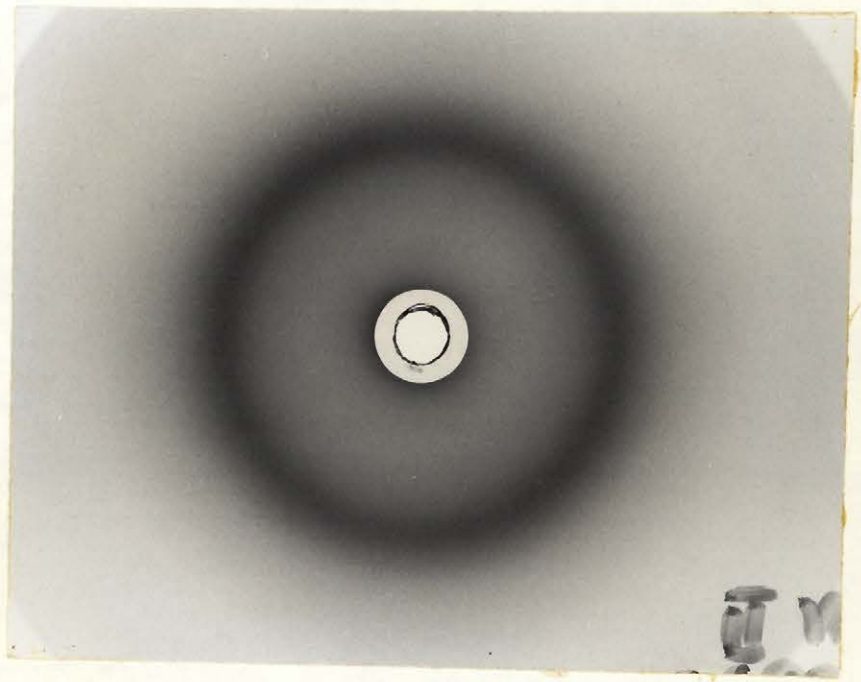
(a)



(b)



(c)



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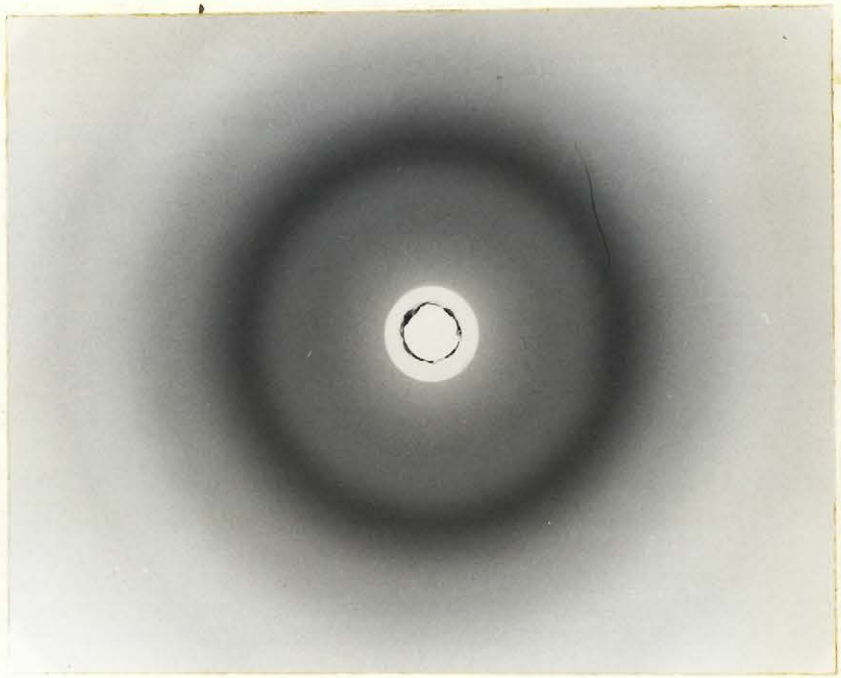

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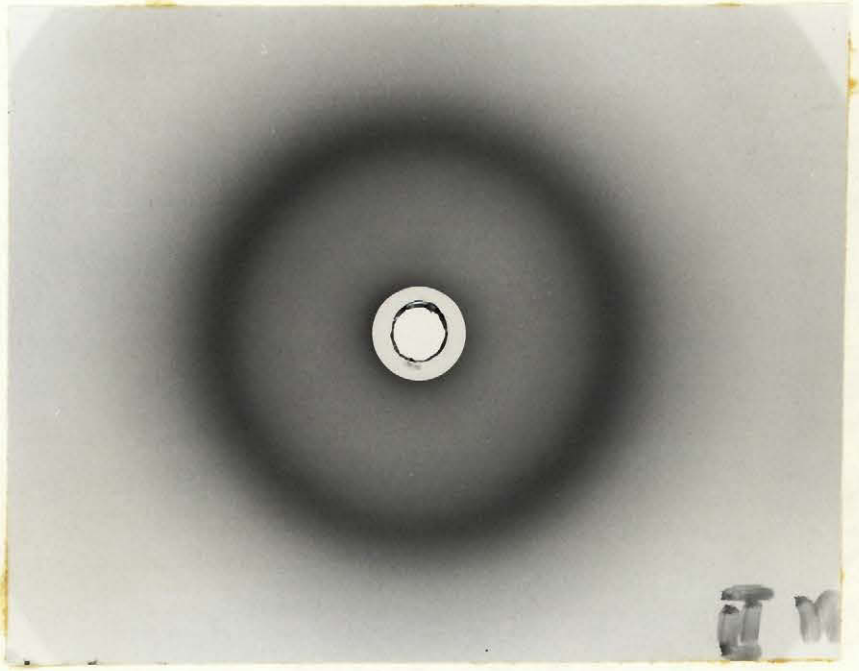

(f)

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APPENDIX II

SOME ADDITIONAL DETAILS OF THE EXPERIMENTAL TECHNIQUES

I. DEUTERIUM EXCHANGE: GRAVIMETRIC METHOD

The method involves determining the increase in mass of the samples following exchange with isotopically pure heavy water (D_2O) and vacuum drying.

The experimental technique is similar to that described by Sepall and Mason[1] and consists essentially of three steps, viz., (i) the drying of the sample, (ii) the deuteration and (iii) the evacuation of unreacted D_2O and determination of the increase in mass. A 100-ml. pyrex flask (G) (Fig. 1) with two outlets (A and E) was used for deuteration purposes. About 6 g. of the sample was introduced into the flask of tared weight $\underline{W}_1$ and dried by evacuating through outlet E for a period of four days at 75°C and 1μ Hg vacuum. The expanded section at D helped in forming a vacuum seal created by an O-ring. The vacuum system (not shown in the figure) consisted of a liquid air trap, a single stage oil diffusion pump and a mechanical backing pump. The flask was then sealed at the constriction F and weighed ($\underline{W}_2$). The break-seal (B) was opened by thrusting a steel rod through A and the deuteration allowed to take place for a period of 4 days. Dry air, obtained from the evaporation of liquid air in a Dewar flask (H) (Fig. 2) was bubbled through liquid D_2O in J and the D_2O vapour conducted into the sample contained in the flask L using a stainless steel tube K. By immersing tube J and flask L in thermostats maintained at specific temperatures, the deuteration can be carried out at any desired relative humidity. The unreacted D_2O was removed by evacuating the flask for 4 days at 75°C and 1μ Hg vacuum. The flask was weighed ($\underline{W}_3$) and the increase in mass ($\Delta\underline{w}$) was obtained from the difference in weights ($\underline{W}_3 - \underline{W}_2$), which had been corrected for changes in air buoyancy. Similarly the mass of the sample ($\underline{S}$) was obtained from the difference ($\underline{W}_2 - \underline{W}_1$).

FIGURE 1

Cell for gravimetric measurement of deuterium exchange (Sepall and Mason[1])

A - Outlet to fit Veeco Quick Vacuum Coupling (No. C-50)

B - Break-seal

C and F - Constriction for sealing

D - Expanded section for vacuum seal with O-ring

G - 100 ml. flask

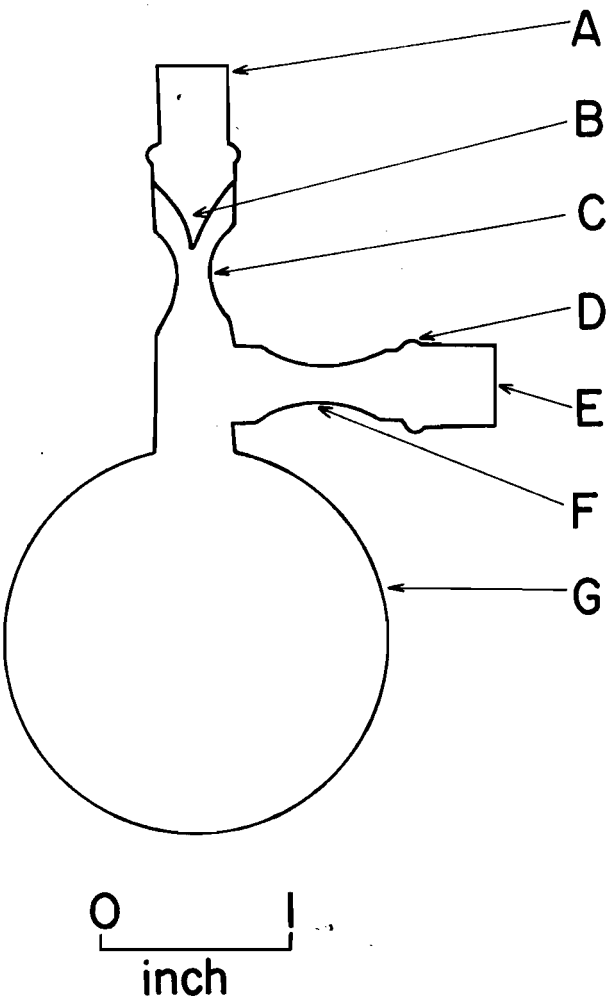

A
B
C
D
E
F
G
O
1
inch

FIGURE 2

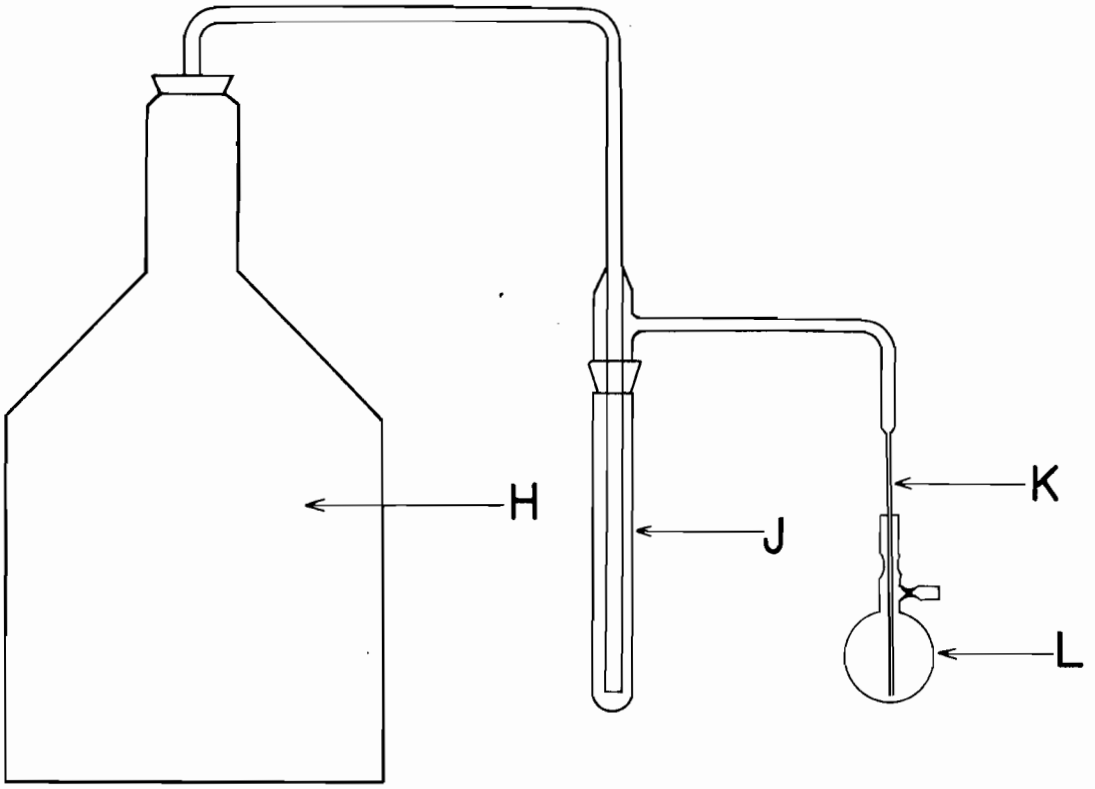
Apparatus for conducting exchange with D_2O
(Sepall and Mason[1])

H - Dewar flask with liquid air

J - Vessel containing D_2O liquid

K - Stainless steel tube

L - Vessel shown in Fig. 1


H
J
K
L

The percentage accessibility $\underline{A}_1$ was calculated from the relation

$$\underline{A}_1 = 100 (\Delta\omega) / F \times S$$

The factor F (for cellulose F = 0.0185) is the fractional increase in mass of the same substance after the hydroxyl hydrogens have been completely exchanged by deuterium.

Sample Calculations of $\underline{A}_1$

Data

Sample: Cotton linters

F = 0.0185

Volume of the flask $\underline{V}$ = 107.3 ml.

Tare weight of flask $\underline{W}$ = 56.2423 g.

Weight of flask + dry sample (at 774.4 mm Hg, 23-8°C and 29% RH) $\underline{W}_2$ = 62.7247 g.

Weight of flask + dry sample after deuteration (at 765.5 mm Hg, 24°C and 37% RH) $\underline{W}_3$ = 62.7750 g.

Calculations

The density of moist air was calculated from

$$\underline{D} = 1.2929 \left(\frac{273}{T} \quad \frac{(B - 0.3783e)}{760}\right)$$

where $\underline{D}$ is the density in g./l., T is the absolute temperature, B is the barometric pressure of moist air in mm Hg and e is the vapour pressure of moist air in mm Hg (Handbook of Chemistry and Physics).

The density $\underline{D}_1$ of air during weighing $\underline{W}_2$ was

$$\underline{D}_1 = 1.2929 \left(\frac{273}{296.8}\right) \left(\frac{774.4 - 0.3783 \times 0.29 \times 22.11}{760}\right) = 1.208 \text{ g./l.}$$

The density $\underline{D}_2$ of air during weighing $\underline{W}_3$ was

$$\underline{D}_2 = 1.1922 \text{ g./l.}$$

The dry weight $\underline{S}$ of the sample

$$= (\underline{W}_2 - \underline{W}_1) + (\underline{V}\ \underline{D}_1)$$

$$= 6.4824 + (107.3 \times 0.0001208)$$

$$= 6.6120 \text{ g.}$$

The increase in mass $\underline{\triangle w}$ due to exchange of hydrogen by deuterium

$$= (\underline{W}_3 - \underline{W}_2) + V(\underline{D}_2 - \underline{D}_1)$$

$$= 0.0503 + 107.3(0.00011922 - 0.0001208)$$

$$= 0.0486 \text{ g.}$$

The accessibility $\underline{A}_1$

$$= \frac{100\ (\triangle w)}{F \times S}$$

$$= \frac{100 \times 0.0486}{0.0185 \times 6.6120}$$

$$= 40 \text{ (to nearest 1\%).}$$

II. TRITIUM EXCHANGE METHOD

The method consists of three steps as follows: (a) tritiation of the sample for a definite period of time, (b) evacuation of unreacted and adsorbed tritium and (c) the measurement of the residual radioactivity in the sample by a counting technique. In general the method used was the same as described by Sepall, Lang and Mason[2], although a few modifications were introduced to enable the technique to be used for measurements of accessibility (a) at any desired relative humidity (b) of powdered specimens.

Fig. 3 shows the schematic drawing of the apparatus. The sample is placed inside the counter A, while reservoir C contains tritiated water (50 cc, total activity 75 millicuries). After the evacuation of air in the counter the valves F were opened, and the tritiation was allowed to take place through circulation of vapour by convection created by electrical heating of the tube E. The reaction was conducted at any desired relative humidity by maintaining reservoir C (by immersing it in a water bath) and counter A (by circulating water through the copper jacket L) at specific temperatures.

The time required for complete exchange of the available hydroxyl hydrogens in cellulose is about 8 hours[1]. However, the oligosaccharides required about 24 hours for the same process. The evidence of this is presented in Fig. 4, which shows a plot of time of tritiation vs. accessibility for a representative oligosaccharide (cellobiose). The curve can be seen to reach an asymptotic value after about 16 hours. The higher oligosaccharides also required the same time for the complete tritiation of the available hydroxyl groups.

After the desired time of reaction the valves F were closed and the unreacted HTO was evacuated through the valve G at a pressure of

FIGURE 3

Schematic drawing of apparatus

A. Counter (methane filled)

B. Electrical heater

C. Tritiated water reservoir

D. Copper tube

E. Electrical heater

F. High vacuum bellows-type valve

G. Vacuum connection

H. Pirani gauge

J. Methane inlet

K. Pressure gauge (0 to 100 mm.)

L. Copper jacket

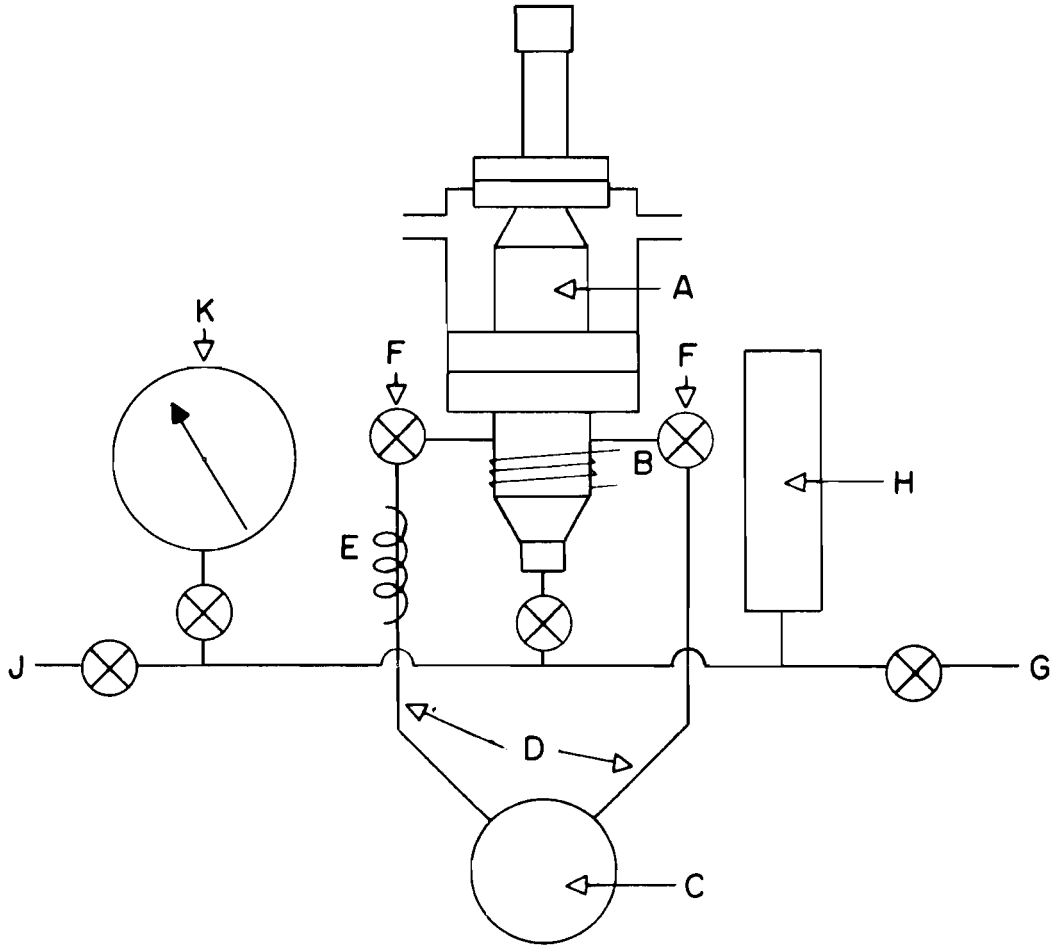
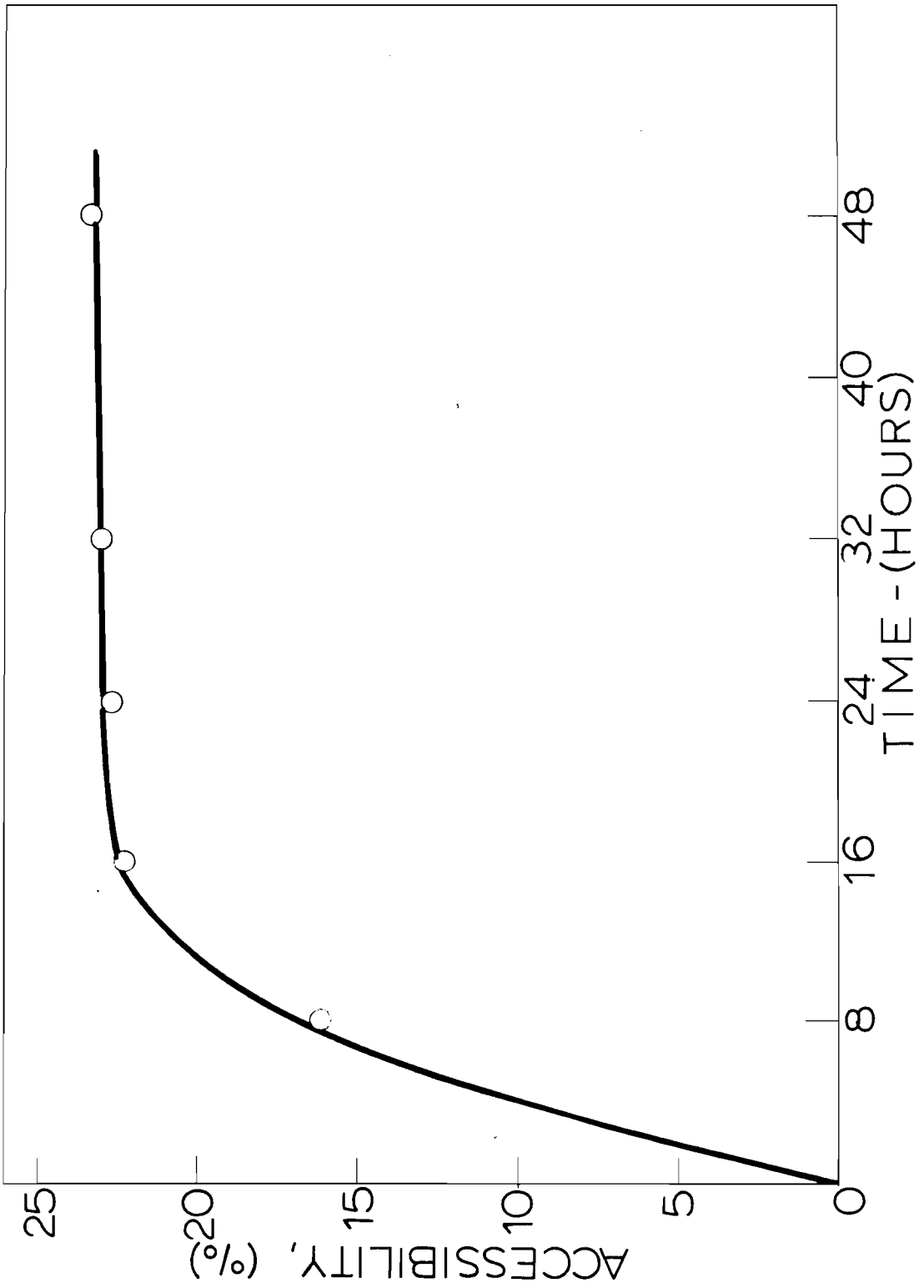

A
K
F
F
B
H
E
J
G
D
C

FIGURE 4

Plot of time of tritiation vs.

accessibility of cellobiose


ACCESSIBILITY, (%)
0
5
10
15
20
25
0
8
16
24
32
40
48
TIME - (HOURS)

1μ Hg and 75°C. The vacuum system (not shown in the figure) consisted of a liquid air trap, a single stage oil diffusion pump and a mechanical backing pump. The vacuum could be measured at any time during the evacuation by the Pirani gauge head H. A special type of sample holder was designed to allow measurements to be made on powders. This is shown in Fig. 5(D). Pellets could be formed directly in the sample holder by using the stainless steel die and anvil, B and G respectively. Copper disc F helps in limiting the electric field in the counter to the space above the sample holder. The holder fits into slots cut in the lower vacuum flange F (Fig. 3), and can be rotated from outside the counter with the aid of a magnet.

After an evacuation of about 14 hours, i.e. after the removal of unreacted HTO, pure methane gas was introduced into the counter from the valve J at a pressure of 90 mm (measured by the gauge K). A sectional view of the counter is shown in Fig. 6. It is made from copper bushing as this gives the lowest background count as compared to other metals[2)]. The standard amphenol plug A, which is soldered to the body of the counter E through the vacuum flange C, connects the tungsten collector electrode D to the pre-amplifier (not shown in the figure). The counter is connected to the vacuum line through the flange G. From the counting rates for the sample and the background (Ms), and the copper disc and the background (Md), the counting rate for the sample (S) only was calculated by the following relation

$$S = (Ms - 0.77 \times Md)$$

The factor 0.77 was determined from measurements of the background counting rate for the sample which was found to be 77% of the counting rate measured from the copper disc.

The method of calculation is illustrated in the following, using data obtained on cotton micelles:

FIGURE 5

Sectional view of the sample holder and the stainless steel die

B. Stainless steel die

C. Sample

D. Stainless steel sample holder

D. Supporting pins

F. Copper disc

G. Stainless steel anvil

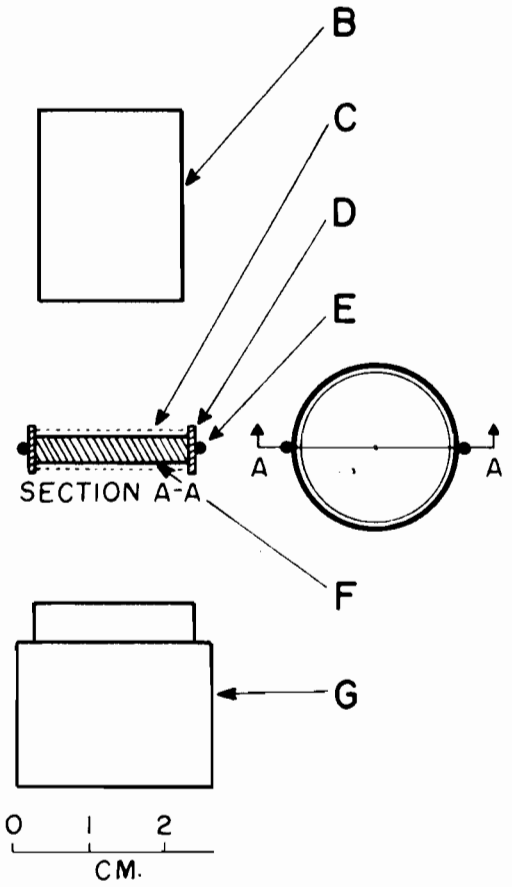

B
C
D
E
A
A
SECTION A-A
F
G
0
1
2
CM.

FIGURE 6

Sectional view of counter

A. Amphenol connector

B. Alloy-glass insulation (Stupakoff No. 95-0047)

C. Brass vacuum flange

D. Collector electrode

E. Copper bushing

F. Sample holder

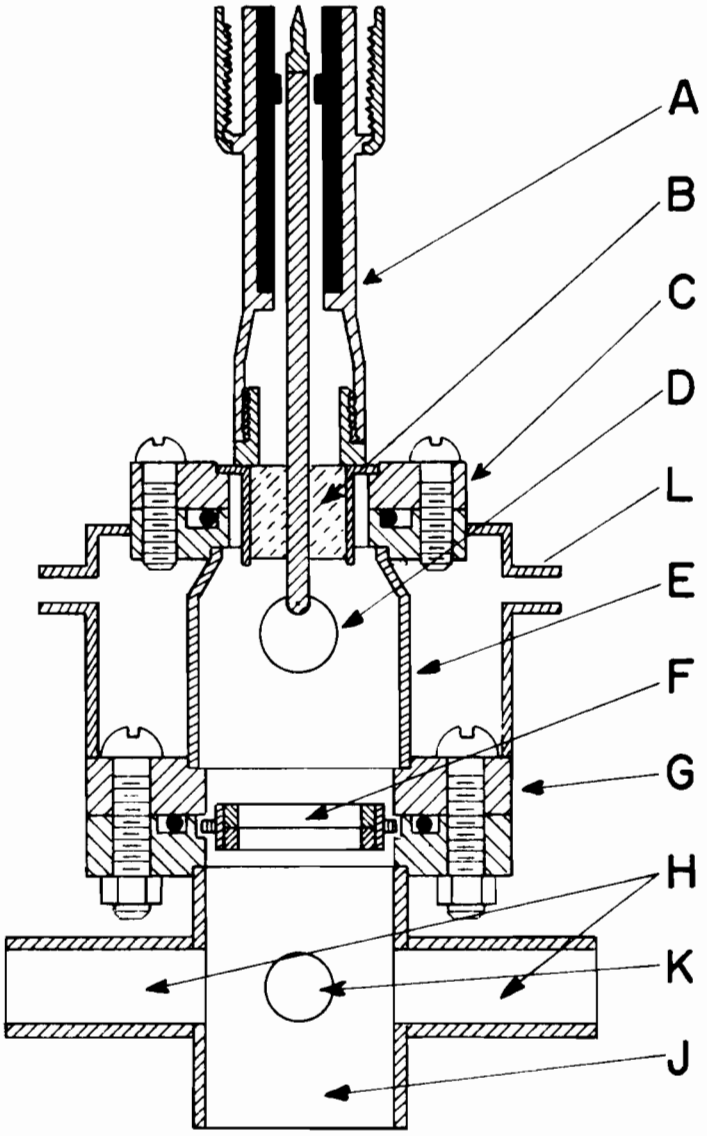
G. Vacuum flange

H. Vapour circulation line

K. Methane inlet

J. Outlet to vacuum system

L. Copper jacket


A
B
C
D
L
E
F
G
H
K
J
0 1 2 3
CM.

Data

Sample: Cotton micelles

Temperature: 25°C.

Counting rate R: 15670 counts/min.

HTO reservoir temperature: 25°C.

f - the ratio of isotopic concentration in vapour and liquid phases (after the reaction): 0.90

c - the activity of HTO (after the reaction): 75 millicuries

Standard sample: cellophane

Temperature: 25°C.

Counting rate R_o: 30210 counts/min.

f_o - the ratio of isotopic concentration in vapour and liquid phases (before the reaction): 0.90

c_o - the activity of HTO (before the reaction): 75 millicuries

$A_{1,o}$: 79%.

Calculations

$$\text{Accessibility } A_2 = \frac{R_f}{c} \times \frac{c_o}{R_o f_o} A_{1,o}$$

$$= \frac{15670 \times 0.90}{75} \times \frac{75}{30210 \times 0.90} \times 79$$

$$= 41\% \text{ (to nearest 1\%)}.$$

It may be pointed out here that the accessibility determined by the gravimetric deuterium exchange method has been found to be in good agreement with that obtained from the tritium exchange method.

III. PREPARATION OF OLIGOSACCHARIDES

Oligosaccharides (cellotriose to cellopentaose) were fractionated from hydrolysates of cellulose by column chromatography. The method used was similar to that described by Miller and co-workers[3]. Twenty grams of Whatman cellulose powder was wetted and dispersed in 200 ml. of concentrated hydrochloric acid (sp.gr. 1.19) at room temperature. This was followed by the addition of 200 ml. of ice-cold fuming hydrochloric acid (sp.gr. 1.21) (prepared by passing hydrogen chloride gas through ice-cold concentrated hydrochloric acid). The clear solution formed after stirring of about one minute was warmed to room temperature (25°C) and allowed to stand for three hours. The resulting mixture was then diluted to 1400 ml. with ice-cold water. About 440 g. of sodium bicarbonate was then added gradually and the pH adjusted to 4-5. Gelatinous materials were removed by centrifugation. The mixture was then fractionated for oligosaccharides by column chromatography. A stearic acid (1%) treated absorbant consisting of 800 g. each of charcoal Darco G-60 and Celite, was washed with about 8 l. of water, before the hydrolysates were introduced into the column. The column was again washed with another 8 l. of water. This removed salt and glucose. Oligosaccharides were then obtained by gradient elution using water and 60% ethanol (v/v). Fractions of about 25 ml. were collected with the help of a volume-type fraction collector. Every fifth tube was tested for carbohydrate content by a colorimetric method. The colouring reagent consisted of 0.2% orcinol in 70% (v/v) sulphuric acid. Aliquots of 0.05 ml. were added to 3 ml. of the colouring reagent and heated at 100°C for 20 minutes. After cooling, the absorbances were measured at 550 mμ in a Beckman spectrophotometer. Absorbances were plotted against tube number to obtain the distribution of oligosaccharides in the eluate fraction.

On the basis of these data the fractions were combined to give

separate pools of the different oligosaccharides, which were then concentrated in a vacuum evaporator and finally dried by freeze-drying. In all four fractions were obtained. The identity of the oligosaccharides was confirmed through paper chromatography and X-ray diffraction studies. The details of X-ray diffraction techniques appear in the following section while those for paper chromatography are as follows: aqueous solutions (about 50 mg./ml.) were made from each fraction and were run against a standard solution of cellobiose. The solutions were spotted on a pencil line drawn on Whatman No. 1 mm. paper strips (19 x 56 cm.). The paper strips were then suspended in tanks, the atmosphere within which was kept saturated with the solvent vapour. The solvent or the developer consisted of fresh solution of ethyl acetate, acetic acid and water in the ratios 18:7:8. The development time was about 96 hours. The spots on the paper were located by spraying with a solution of ortho aminodiphenyl (prepared by dissolving 15 g. in 500 ml. of acetic acid and 7 ml. of concentrated ortho phosphoric acid), and examined under ultraviolet light. The compound obtained in the first of the four fractions, believed to be cellobiose, moved at the same rate as standard cellobiose, thus confirming its identity, and establishing the sequence of subsequent fractions.

The oligosaccharides were crystallized as follows: about 0.5 g. each of the cellotriose, -tetraose and -pentaose were dissolved separately in 50 cc of water; an equal amount of ethanol was then added. Crystallization was effected by slow evaporation of the solvent mixture. The crystals were removed from the mother liquor by centrifugation, washed with small quantities of anhydrous ethanol, and dried at 50°C in a vacuum oven. In the same way cellotetraose was also crystallized from another solvent system, i.e., water and ethylene chlorohydrin 50:50 v/v.

IV. X-RAY MEASUREMENTS

(a) X-ray Diffraction Patterns - The X-ray diffraction pictures were obtained with a Seifert unit using a flat-film camera with Ni-filtered Cuk_{α} radiation. The camera is a simplified form of the one described by Ruck, Kouris and Manley[4]. The principle of the camera is illustrated in Fig. 7, the details of which are self-explanatory. The samples were mounted in a holder in the form of compressed pellets, care being taken to ensure isotropic packing in the plane of the holder. The X-ray unit was generally operated at 40 KV. and 20 mA and exposure times were then about 3 hours. Kodak-F type films were used throughout, and were processed as follows:

Developer: Kodak liquid X-ray developer;

Fixing solution: Kodak liquid X-ray fixer;

Temperature of developing and "fixing": 20°C;

Time of "developing": 5 minutes;

Time of "fixing": 5 minutes;

Washing: one hour in running water at 20°C;

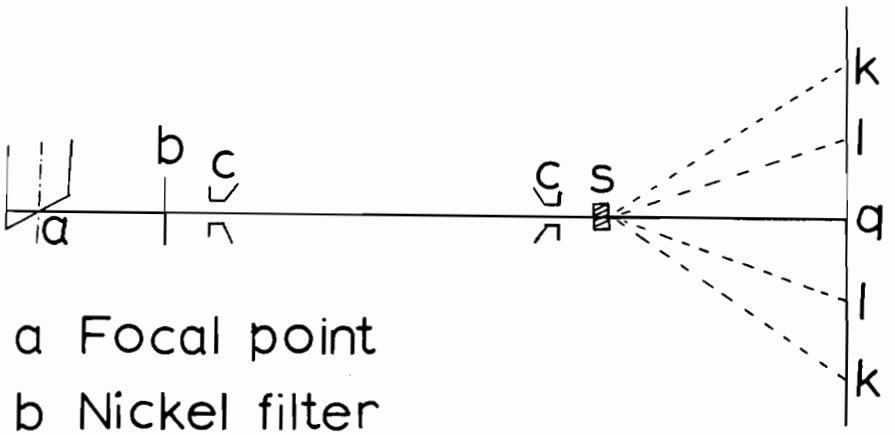
Drying: conducted at room temperature.

(b) Kinetics of Crystallization

Kinetic studies on crystallization of amorphous cellulose were carried out by the X-ray diffraction method using a Geiger counter diffractometer. Amorphous cellulose, used in the sheet form was prepared as follows: cellulose triacetate sheets were first prepared by deposition from a solution in methylene chloride and absolute methanol. The triacetate solution was spread on a polished chromium plated surface and the solvent allowed to evaporate slowly at room temperature. The cellulose triacetate sheets thus obtained were saponified in 0.5N sodium methylate in anhydrous methanol at room temperature for about 10 hours. Finally the saponified

FIGURE 7

Camera and collimating system



a Focal point
b Nickel filter
c Pin hole aperture
s Sample
aq Path of primary beam
kl Sample interferences

sheets were washed thoroughly with absolute methanol. Samples were exposed to different relative humidities and the rate of development of $10\bar{1}$ and 002 peaks was observed. The desired relative humidities were obtained from saturated solutions of inorganic salts[5] contained in dessicators. Nickel filtered Cuk_α radiation was used, and the intensity of the scattered radiation with the Geiger counter diffractometer.

(c) Comparison of X-ray Crystallinities

The method is based on that of Hermans[6], except that the scattering from a nickel foil was used as a reference for the incident X-ray intensity instead of the Goppel[7] camera. The ratio of the intensity of a given specimen interference I_{hkl} to the intensity of a reference interference from the nickel foil I_{ref} is a constant independent of the incident X-ray intensity. Thus

$$I_{hkl} / I_{ref} = K_{hkl}$$

For a series of specimens differing only in crystallinity K_{hkl} gives a measure of crystallinity provided the quantity of specimen irradiated is the same in each case. Ni-filtered Cuk_α radiation was used and the intensity of scattered radiation was measured with the help of a Geiger counter diffractometer. The (111) in Debye-Scherrer interference produced by the Ni-foil was used as a reference, the nickel foil being positioned between the specimen and Geiger tube. The specimens were mounted in a holder in the form of compressed pellets. The amount of specimen was adjusted so that the intensity of the nickel peak (111) was the same for each sample being compared. The measurements were then made at several levels of incident intensity (obtained by changing current while keeping voltage constant). I_{ref} was plotted against I_{hkl} and the values of K_{hkl} evaluated from the slope of the straight line thus obtained.

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APPENDIX III

THE EFFECT OF INTERFIBER BONDING ON THE ACCESSIBILITY OF CELLULOSE

The strength properties of paper are known to be increased by the process of wet beating[1,2]. It has been shown that the effects of beating are related to the degree of interfiber bonding in the paper sheet. A greater degree of beating results in increased interfiber bonding[1,2]

This appendix describes an investigation of the effect of interfiber bonding on the accessibility of cellulose sheets.

The sample used was cotton linters dewaxed by alcohol/benzene extraction. The specimen was beaten in a laboratory PFI mill in four separate batches of 20 g. each. The first two batches were beaten for 20,000 revolutions and the third and fourth for 40,000 and 60,000 revolutions respectively. The beaten fibers were then dispersed in water with the aid of a Waring blender.

The first batch (20,000 revolutions) was dried by freeze-drying. All other batches were transformed into hand sheets and dried at room temperature.

The accessibility of the samples was determined by the gravimetric deuterium exchange method at 100% RH and 25°C. Details of this method have already been described in Part II of this thesis.

The results are shown in Table I. It is seen that within the experimental error all the beaten samples show the same accessibility, whereas the unbeaten sample has a somewhat lower value.

The freeze-dried sample would be expected to have the minimum degree of interfiber bonding, while the sample beaten for 50,000 revolutions would represent the other extreme showing the highest degree of interfiber bonding. The results therefore indicate that the accessibility is independent of the degree of interfiber bonding.

TABLE I

ACCESSIBILITY OF PFI BEATEN

COTTON LINTERS

(Deuterium Exchange Method)

Sample	Accessibility (%)
1. Cotton linters (unbeaten)	40
2. Cotton linters beaten for 20,000 r. (Freeze-dried)	47
3. Cotton linters beaten for 20,000 r. (Sheet form)	46
4. Cotton linters beaten for 40,000 r. (Sheet form)	46
5. Cotton linters beaten for 60,000 r. (Sheet form)	45

It is interesting to note that beating causes an apparent increase in accessibility. However, it is not possible at present to give a convincing reason for this effect.

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