CLXV.—*The Constitution of Polysaccharides. Part II. The Conversion of Cellulose into Glucose.*

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In the series of investigations on the constitution of polysaccharides with which we are engaged in this laboratory, a prominent place is naturally assigned to the more definite varieties of cellulose. The original literature on the reactions and constitution of cellulose is voluminous, but it cannot be claimed that views regarding even the fundamental nature of the complex are by any means established.

This obscurity is not surprising considering the special difficulties which surround constitutional studies of this type. Even if cellulose can be regarded as a chemical individual in the ordinary sense,

the customary methods of solving problems of structure are of little avail in view of the insolubility of the compound, the dubiety attending its molecular magnitude, its behaviour as a colloid, and the probability that a fibrous structure is not chemically homogeneous. As a result, many of the statements which find a place in the permanent literature are based on very insecure evidence, and are even contradictory. In reviewing briefly the present position of the subject, reference may at this stage be limited to recent papers which have a bearing on fundamental questions.

A normal cotton cellulose is represented by the formula $(C_6H_{10}O_5)_n$, and ideas as to the molecular structure have been formed largely on the evidence afforded by hydrolysis. It is important to note that in this particular case hydrolysis is not readily effected, and involves the use of somewhat drastic reagents, so that taking into account the unstable nature of the hydrolysis products, secondary reactions are inevitable. The fact that numerous and complex degradation compounds are formed has thus given rise to conflicting opinions on the chemical nature of cellulose, but it is unnecessary in the present paper to discuss these rival theories in detail.

Apparently the view which finds most acceptance is that cellulose, like starch, is essentially a polyglucose anhydride, and it may be well to state at once that this conception admits of a double interpretation. The complex may consist of the simple units $C_6H_{10}O_5$ (derived from a hexose by molecular loss of water) polymerised in unknown numbers. On the other hand, n molecules of a hexose may be directly connected together through the elimination of n molecules of water, or, where n is a large factor, of $n-1$ molecules. In either case the first point which must be settled is to ascertain beyond doubt if glucose is actually the hexose formed by the hydrolysis of cellulose, and, if so, to determine exactly the amount of sugar thus produced.

The inquiry becomes much more definite if it can be shown that, within the limits of reasonable experimental error, cellulose can be converted into glucose in terms of the equation:

$$(C_6H_{10}O_5)_n + nH_2O \longrightarrow nC_6H_{12}O_6.$$
$$100 \text{ parts} \longrightarrow 111{\cdot}11 \text{ parts.}$$

From time to time confident statements appear in the literature that practically quantitative yields of glucose have been obtained from cellulose, but the grounds upon which such claims are made are by no means convincing to workers in the sugar group. Practically speaking, the only experimental methods available for degrading cellulose depend upon the use of mineral acids, either alone

or in conjunction with acetic anhydride, and it is evident that any sugar thus liberated must undergo profound alteration when kept in contact with these reagents. This no doubt accounts for the fact that hitherto pure, crystalline glucose has never been obtained from this polysaccharide. Nevertheless, Flechsig (*Zeitsch. physiol. Chem.*, 1883, **7**, 523) claimed that a yield of 95—98 per cent. of the theoretical amount of glucose was formed by the action of sulphuric acid on cellulose, but the statement is based solely on the reducing power of a complex mixture and has little significance. Schwalbe and Schultz (*Ber.*, 1910, **43**, 913) supplemented Flechsig's experiments by isolating the products of hydrolysis and obtained a semi-crystalline sugar amounting only to 20 per cent. of the theoretical yield. Working on similar lines, Ost and Wilkening (*Chem. Zeit.*, 1910, **34**, 461) made the important claim that the yield of glucose was almost quantitative, but it may be remarked that they examined the products of hydrolysis polarimetrically and reported specific rotations ranging from $+29{\cdot}4°$ to $+44{\cdot}8°$, whereas the equilibrium value for glucose is $+52{\cdot}5°$. As a further means of estimating the amount of sugar formed, they adopted methods depending on the reduction of copper solutions, but, in this case also, irregularities were experienced, and the results indicated yields of glucose varying from 73·4 to 113·5 per cent. of the weight of cellulose treated.

The use of hydrochloric acid for degrading cellulose is even less satisfactory. As is well known, Willstätter and Zechmeister (*Ber.*, 1913, **46**, 2401) dissolved cotton-wool in 40—41 per cent. aqueous hydrochloric acid, and allowed the hydrolysis to proceed in the cold. The course of the reaction was followed polarimetrically, and, by data obtained from control experiments in which glucose was dissolved in the same acid medium, they calculated that the yield of the hexose formed from cellulose amounted to 96·3 per cent. of the theoretical value. This conclusion was apparently confirmed by the results of titrations which indicated the formation of 94 per cent. of the theoretical weight of glucose. The results appear convincing until they are considered in conjunction with the known effects of hydrochloric acid upon glucose. Willstätter and Zechmeister were of the opinion that, owing to the low concentration of sugar in the acid solution, no *iso*maltose was formed, but it has been shown (Davis, *J. Soc. Dyers and Col.*, 1914, **30**, 249) that the action of hydrochloric acid in promoting the auto-condensation of glucose extends to solutions containing as little as 1 per cent. of the sugar. In addition, few reagents effect more fundamental changes in reducing hexoses than hydrochloric acid in either dilute or concentrated solution. Thus, traces of the acid convert the butylene-oxide forms of glucose into the ethylene-oxide isomerides. In higher

concentrations of acid complex changes are therefore to be expected, so that when glucose is dissolved in 44·5 per cent. hydrochloric acid the specific rotation is +164·6°, and thus exceeds the maximum value for α-glucose by approximately 50°. That Willstätter's process has very little bearing on the primary constitution of cellulose is further shown (Cunningham, T., 1918, **113**, 173) by the fact that both cotton and esparto celluloses give practically identical rotation curves when hydrolysed with concentrated hydrochloric acid under the conditions described by him. In fact, the evidence of specific rotation and reducing power, even when apparently consistent, cannot be held to characterise an uncrystallisable syrup as a definite sugar.

A considerable advance on the use of mineral acids is marked by the conversion of cellulose into glucose acetates as elaborated in the exhaustive researches of Ost and his pupils. Recognisable crystalline products consisting of cellobiose octa-acetates and glucose penta-acetates are thus obtained, so that it is possible to ascribe trustworthy values to the yields. By using acetic anhydride containing approximately 10 per cent. of sulphuric acid as the hydrolytic reagent, Ost (*Chem. Zeit.*, 1912, **36**, 1099) isolated a mixture of solid acetates amounting to 60·6 per cent. of the theoretical quantity, the remaining products being uncrystallisable syrups. It is very doubtful if the latter can be included in calculating the total yield, or if polarimetric methods are admissible in estimating hexose acetates as many factors combine to render such a method uncertain (Hudson and Parker, *J. Amer. Chem. Soc.*, 1915, **37**, 1589; Hudson, *ibid.*, 1591; Hudson and Johnson, *ibid.*, 1916, **38**, 1223).

Up to the present time, in no research on the hydrolysis of cellulose, where a yield of glucose even approximating to the theoretical amount has been claimed, have the results been based on the quantity of the sugar, or of a characteristic derivative, actually isolated. In the work now described, we adhered to the principle that the yield of hexose should be ascertained from the weight of crystalline compounds, obtained in a condition of analytical purity, and in well-defined stereochemical forms. Adopting this standard, we have been able to show that, as a minimum, the yield of glucose obtained from cellulose is 85 per cent. of the theoretical amount.

The method used by us embodies the same principle as acetolysis in that it involved hydrolysis of cellulose and simultaneous condensation of the sugar liberated, so as to give a stable derivative which thereafter remained unaffected. In this way the glucose was protected from the destructive effect of the hydrolytic agents. The material employed was a normal cotton cellulose, for a supply of which we are indebted to Mr. Wm. Rintoul, of Nobel's Explosives

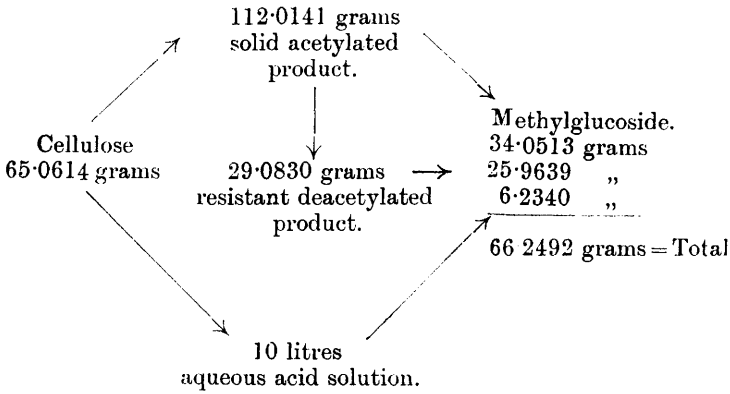
Co. This was treated, as described in the experimental part, with a large excess of acetic anhydride containing acetic and sulphuric acids. When the fibrous structure had disappeared, the product was poured into water and the precipitated solid separated. The filtrate then contained the lower acetylated glucoses, together with acetosulphates and other soluble degradation products, whilst the insoluble residue consisted of polysaccharide acetates. On heating the latter in an autoclave at 100° with methyl alcohol containing 0·5 per cent. of dry hydrogen chloride, the first effect was to remove the acetyl groups, which were converted into methyl acetate (Perkin, T., 1905, **87**, 107; Fenton and Berry, *Proc. Camb. Phil. Soc.*, 1920, **20**, 1, 16). Thereafter, simultaneous hydrolysis and condensation with the solvent ensued, the process then being parallel with the conversion of starch into methylglucoside (Fischer, *Ber.*, 1895, **28**, 1151). The main product of the reaction consisted of crystalline methylglucoside, but about 25 per cent. of the material persisted and remained practically unaffected on repeating the treatment with the acid alcohol. This amorphous residue was therefore hydrolysed by means of dilute aqueous hydrochloric acid, and the product again brought into reaction with acid methyl alcohol. In this way the total yield of methylglucoside from the fraction insoluble in water was ascertained.

Owing to the large volumes which had to be manipulated, the treatment of the products soluble in water was laborious. After removal of the free acids, the solvent was evaporated, and the sugar converted into methylglucoside in the usual way. In every case the methylglucoside was obtained as a colourless syrup which rapidly solidified to a hard mass of crystals. On hydrolysis with acid no difficulty was experienced in obtaining pure, crystalline glucose from the glucoside.

Statement of Yields Obtained.

Two methods of estimating the yields of methylglucoside were employed. The weights reported refer to pure, crystalline material dried in a vacuum oven until constant, and, as an additional check, the optical activity of each solution which yielded methylglucoside was determined. During the glucoside formation, heating with acid methyl alcohol was continued until equilibrium had been established between the α- and β-forms, and, under the conditions adopted, the proportion in which these modifications are present is respectively $\alpha = 77$, $\beta = 23$ per cent. (Jungius, *Proc. K. Akad. Wetensch. Amsterdam*, 1903, **6**, 99). The constant specific rotation attained when solutions of methylglucoside in acid methyl alcohol

are heated is thus of the order of $+114°$, so that we were able to check our gravimetric results polarimetrically. As we rely exclusively on our gravimetric data, this precaution may seem unnecessary, but its adoption secured that experimental conditions were used which precluded any possibility of the yields being affected by the formation of γ-methylglucoside. The combined results of one typical experiment are shown in the following chart, the weight of cellulose taken (70 grams) being corrected for the moisture and ash content.



Expressing the result in percentages:

Cellulose	→	Methylglucoside	→	Glucose
100 parts	gave	101·8257 parts	equivalent to	94·4775 parts.

If the cellulose molecule is composed entirely of glucose residues, 100 parts of the polysaccharide should give 111·11 parts of glucose, so that the operation of the above scheme gives a yield of methylglucoside (and therefore of glucose) of 85·03 per cent. of the theoretical amount. Although the manipulations were conducted with a standard of accuracy comparable with that employed in gravimetric analysis, it is obvious that the above result is a minimum value. The preparation of methylglucoside from glucose, although a smooth reaction, is not quantitative owing to inevitable experimental loss in isolating and crystallising the product. We do not propose, however, to introduce any correction in which allowance is made for experimental loss, and our future work will include an attempt to account for this divergence of 15 per cent. from the theoretical value.

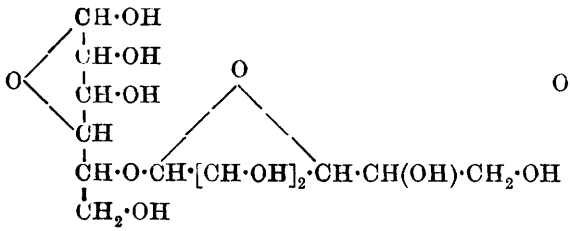
Discussion of Results.

Although the main object of the present research did not involve the detailed constitution of cellulose, some of the results obtained have a direct bearing on the problem, and may be discussed. As a rule it is an uncommon experience in the sugar group to obtain crystalline derivatives in yields which exceed 80 per cent., and the figures now submitted thus afford strong evidence that cotton cellulose is composed essentially of glucose residues condensed together. It is to be noted, however, that the processes adopted by us would not serve to isolate any ketose constituent, should such be present. In view of the fact that cellulose yields bromomethylfurfuraldehyde (Fenton and Gostling, T., 1901, **79**, 361), it is conceivable that the unexplained margin of 15 per cent. may be accounted for by the presence of a nucleus in the cellulose molecule which is resolved into a ketose on hydrolysis. It is possible, although somewhat improbable, that the hexose units in cellulose are symmetrically disposed as in inulin (Irvine and Steele, this vol., p. 1474), and the alternative has to be kept in view that two, three, or four hydroxyl groups of individual glucose molecules may be involved in the coupling. Should this be the case, the hydroxyl content of cellulose may still be regarded as three, but this would be an average value and would not imply that, as in inulin, three hydroxyl groups are present in every C_6 unit. Evidence in support of this second view has been contributed by Denham and Woodhouse (T., 1917, **111**, 244) from the study of trimethyl cellulose, and we hope, in consultation with these workers, to extend the investigation on methylated celluloses. Further evidence of the non-uniformity of the glucose linkages in cellulose is afforded by the remarkable variation in the ease with which the component parts of the molecule undergo acid hydrolysis. Another significant factor is that the yield of cellobiose obtained from cellulose, although varying greatly with the conditions of hydrolysis, has never exceeded the maximum quoted by Klein (*Zeitsch. angew. Chem.*, 1912, **25**, 1409). His figures are:

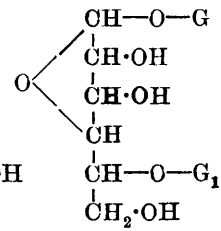
Cellulose	→	Cellobiose octa-acetate	→	Glucose
100 parts	gave	60 parts	equivalent to	31·9 parts.

From these results it would appear that at least one-third of the cellulose molecule contains the linkage characteristic of cellobiose. Now, cellobiose contains eight hydroxyl groups, one of which is a reducing group and therefore terminal. It follows that one of the remaining hydroxyl groups of the reducing component must be attached to the reducing group of the second glucose residue. The position of this linkage may be fixed from, among other factors,

the constitution assigned to trimethyl glucose, and formula I may thus be deduced for cellobiose:



(I.)



(II.)

The expanded structure II, in which G and G_1 represent glucose residues, can thus be deduced for a fragment of the cellulose complex. Of the two groups, G and G_1, the latter is the more stable to hydrolysts, and the system indicated in formula II evidently represents the most resistant portion of the cellulose molecule. It is significant that in our work we encountered the same progressive difficulty in eliminating the glucose residues from cellulose. The methylglucoside obtained was isolated from three groups of acetolysis products:

A. Soluble in water.

B. Insoluble in water and hydrolysed by acid methyl alcohol.

C. Insoluble in water and resistant to acid methyl alcohol.

Our results thus show that the cellulose molecule may be dissected into three portions, and the approximate ratio in which the groups A, B, and C are present is displayed below.

		Methylglucoside.		Glucose.		$C_6H_{10}O_5$.
Cellulose 100 parts	A gave	9·5817	equivalent to	8·9	equivalent to	8·0
	B ,,	52·3372	,,	48·6	,,	43·7
	C ,,	39·9068	,,	37·0	,,	33·3
				94·5		85·0

It will be seen that the proportion of C agrees approximately with the figure indicated by the maximum yield of cellobiose octaacetate obtained from cellulose, and it is our intention to continue the investigation by tracing the structural distinction between the units A, B, and C. In addition, we hope to ascertain whether the glucose belongs to the ethylene-oxide or butylene-oxide types, as the fundamental difference between cellulose and starch may depend on the nature of the oxidic linkage in the constituent hexose residues.

Experimental.

The material employed was a normal cotton cellulose supplied by the Research Department of Nobel's Explosives Co., Ardeer. So

far as the ultimate yields of methylglucoside are concerned, similar results were obtained with filter paper which was more readily disintegrated by the reagents. In quoting yields, allowance has been made for the moisture content (6·7 per cent.) and the ash left on ignition (0·355 per cent.).

The following is an account of a typical experiment. Seventy grams of the cellulose, cut into small pieces 2 cm. by 1 cm., were placed in an enamelled iron beaker surrounded by a bath cooled with running water. A mixture of 350 c.c. of acetic anhydride containing 6·25 per cent. of acetic acid and 20 c.c. of concentrated sulphuric acid was cooled to 15° and quickly added, the mass being rapidly agitated with a powerful stirrer. The best results were obtained when the maximum temperature did not exceed 75°, and in twenty minutes the fluid mixture was poured into ice-cold water with continuous stirring during the dilution. After twenty-four hours the white precipitate which had settled became brittle, and was filtered and washed until free from acid. The total volume of filtrate and washings was 10 litres, and the insoluble acetates, after drying at 40—50°/25 mm., weighed 112 grams. The material was separated into three portions according to the solubility in 95 per cent. alcohol: (1) soluble in the cold, (2) soluble only at the boiling point, (3) insoluble. As each fraction was convertible into methylglucoside, this separation was not carried out in the large-scale experiments.

Simultaneous Deacetylation, Hydrolysis, and Methylation of the Insoluble Acetates.

The mixed acetates were dissolved in methyl alcohol containing 0·5 per cent. of hydrogen chloride so as to give a 5 per cent. solution, and heated at 100° for seventy hours. On opening the autoclave, the odour of methyl acetate was noted, and a white, amorphous precipitate was found to have collected. This was filtered (Filtrate A), washed, and dried (Residue B).

Examination of Residue B.—The material, amounting to 29 grams, was a white, amorphous powder, insoluble in water, chloroform, ether, alcohol, or dilute hydrochloric acid, but readily soluble in dilute sodium hydroxide solution. It melted and decomposed at 237°, and reacted as a glucoside towards Fehling's solution, being hydrolysed on boiling with dilute acid. No chlorine was present, and a methoxyl determination gave a blank result (Found, C = 44·0; H = 6·24; OMe = 0. $C_6H_{10}O_5$ requires C = 44·4; H = 6·20; OMe = 0 per cent.).

Conversion of Residue B into Methylglucoside.

On boiling under a condenser with 3·75 per cent. aqueous hydrochloric acid, the white solid gradually dissolved with the exception of a small residue which, in a separate experiment, was hydrolysed by means of 8 per cent. acid. When the activity of the solutions was constant, indicating that hydrolysis was complete, the liquids were united, neutralised with barium carbonate, and evaporated to dryness under diminished pressure. A pale brown syrup remained, which was extracted five times with boiling methyl alcohol containing 0·5 per cent. of hydrogen chloride, and the solution was heated at 100° until the optical activity remained constant ($[\alpha]_D$ +109°, calculated on the weight of glucoside isolated). The acid was then neutralised by shaking the solution successively with lead and silver carbonates, after which the filtrate was boiled for some hours with charcoal. A colourless solution was thus obtained which, on concentration under diminished pressure, gave a clear syrup. This rapidly crystallised to a compact, hard mass on the addition of a nucleus of α-methylglucoside. The crystals were extracted five times with a large excess of boiling ethyl acetate and the pure glucoside isolated in one crop from the united liquors. The product consisted of the equilibrium mixture of α- and β-methylglucosides. Yield, 25·9 grams. (Found, C=43·20; H=7·41; OMe=15·31. Calc., C=43·30; H=7·22; OMe=15·98 per cent.)

The specific rotation in water (mean of two determinations) was +114·8°, in place of the calculated value +114·0°. The compound behaved sharply as a glucoside towards Fehling's solution, and showed the usual range of melting point (125—154°) for the mixed glucosides.

Examination of Filtrate A.—This solution contained the equilibrium mixture of α- and β-methylglucosides in acid methyl alcohol and showed $[\alpha]_D$ +113·8° (calculated on the weight of glucoside obtained). It was neutralised as described above with lead and silver carbonates, the further treatment involved in removing colloidal silver and in isolating the products being also identical. As before, no difficulty was experienced in obtaining the methylglucosides in the crystalline condition. The product was free from halogen or sulphur, and had no action on Fehling's solution until hydrolysed (m. p. 123—162°) (Found, C=43·30; H=7·15; OMe=15·56. Calc., C=43·30; H=7·22; OMe=15·98 per cent.).

The constant weight of pure glucoside was determined by heating at 50° under diminished pressure, a current of air dried over phosphoric oxide being led through the apparatus. A trap containing

the same dehydrating agent was placed between the receiver and the water-pump. Yield, 34 grams.

Preparation of Methylglucoside from Acetolysis Products Soluble in Water.

The aqueous acid filtrate obtained in removing the insoluble acetates was distinctly dextrorotatory and amounted with washings to 10 litres. This was subjected to distillation in steam, the volume being kept constant, to remove the excess of acetic acid, and the sulphuric acid present was then precipitated by shaking with barium carbonate. The filtered solution was evaporated to dryness under diminished pressure. A white, crystalline residue remained, together with a yellow syrup which was extracted five times with boiling methyl alcohol containing 0·5 per cent. of hydrogen chloride, the extraction process extending over ten hours. The united extracts (Solution C) were separated from the semi-crystalline, undissolved solid (Residue D).

Examination of Residue D.—Although largely inorganic, this residue contained some organic matter, derived either from soluble acetosulphates or from colloidal cellulose acetates. It was accordingly treated in exactly the same manner as Residue B and the hydrolysis product isolated as a syrup. This was extracted six times with boiling methyl alcohol containing 0·5 per cent. of hydrogen chloride and the solutions were united with Solution C.

Examination of Solution C.—This extract, together with that from Residue D, was heated at 100° until the rotation became constant, after which the equilibrium mixture of the methylglucosides was isolated in the usual manner. Somewhat greater difficulty was experienced in obtaining the product in a pure condition, and although the material behaved sharply as a glucoside towards Fehling's solution, the melting point showed a wider range than usual (105—140°). The specific rotation was also slightly low ($[\alpha]_D$ +111·5° in place of +114°). Yield, 6·2 grams (Found, C=43·17; H=7·16; OMe=15·22. Calc., C=43·30; H=7·22; OMe=15·98 per cent.).

Isolation of Crystalline Glucose from Cellulose.

The preparations of crystalline methylglucoside, obtained as described, were mixtures of the α- and β-forms in equilibrium. This was confirmed by crystallising a sample slowly from methyl alcohol. The β-form was then retained in solution, and the α-iso-

meride which separated, showed, after drying at 50°/25 mm., $[\alpha]_D$ +157·5° in water. This agrees exactly with the standard value (+157·6°). Moreover, the material melted at 165°, the melting point being unaffected by admixture with pure α-methylglucoside.

Further proof of the standard of purity attained was afforded by the hydrolysis of the mixed glucosides. A 5 per cent. solution in 4 per cent. hydrochloric acid was heated at 100°, polarimetric readings being taken every fifteen minutes. The permanent value observed, when calculated for the weight of glucose formed, was +53·0° in place of +52·5°. After neutralisation with barium carbonate and evaporation to dryness under diminished pressure, a syrup mixed with barium chloride remained. This was extracted with boiling absolute alcohol, the solution decolorised, filtered, and slowly concentrated at a low temperature. On nucleation and stirring, crystalline glucose readily separated. After a second crystallisation from absolute alcohol, the yield of dry sugar amounted to 60 per cent. of that required by theory. The glucose melted at 145° and gave glucosephenylosazone (m. p. 204—205° uncorr.) (Found, C=39·97; H=6·54; OMe=0. Calc., C=40·00; H=6·67; OMe=0 per cent.).

When dissolved in water the initial specific rotation of +100·8° was recorded, and this diminished to the constant value of +52·37°. The above result proves conclusively that the methylglucoside employed did not contain any isomeric methylhexoside, and that consequently no mannose or galactose residues are present in cellulose.

We desire to record our thanks to the Royal Commissioners for the 1851 Exhibition for a Research Scholarship held by one of us during the progress of the above research.

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[*Received, November 4th,* 1920.]