THE EFFECT OF OXIDATION ON THE 2- AND 3-POSITIONS ON THE DECARBOXYLATION OF CERTAIN POLYANHYDROURONIC ACIDS

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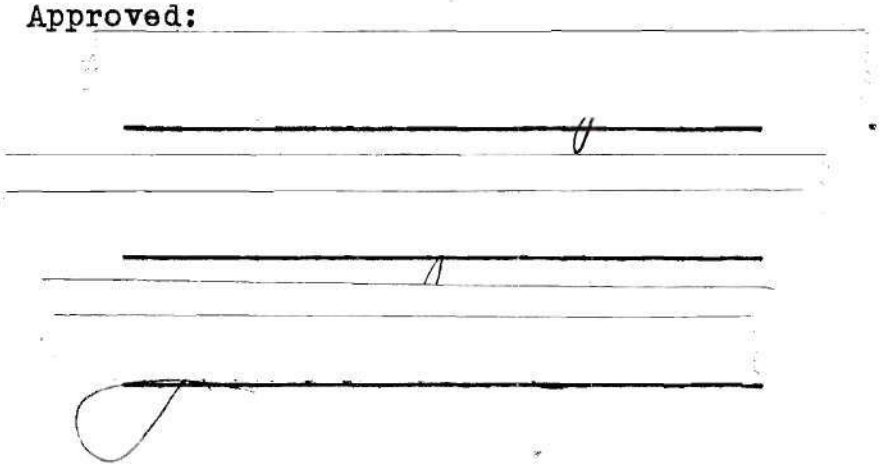
Approved:



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ABSTRACT

The Problem

The problem was to find whether the Lefèvre and Tollens method of uronic acid analysis could be applied to certain polyanhydrouronic acids which had been oxidized at the 2- and 3-positions of the anhydrouronic acid units. To know this it was necessary to find the behaviour of carboxyl groups on the 2- and 3-carbon atoms under the conditions of the analysis and to find whether decarboxylation of the carboxyl group on the 6-carbon atom was interfered with by oxidation at the 2- and 3-positions.

The Method of Attack

The method of attack was to prepare suitably oxidized materials and examine them under the conditions of the analysis. Five oxidized celluloses were prepared and examined. Three samples of pectic acid were oxidized, but all attempts to recover the oxidized material in pure form from the reaction mixture were unsuccessful. The materials were examined by subjecting them to the action of boiling twelve per cent hydrochloric acid and measuring the

amounts of carbon dioxide evolved. Corroborative determinations of total carboxyl content were made by the calcium acetate method.

Results

Under the conditions of the Lefèvre and Tollens method of uronic acid analysis, decarboxylation of the carboxyl groups on the 6-carbon atom of galacturonic acid, polyanhydrogalacturonic acid, glucuronic acid, and certain other uronic acids proceeds exponentially and is quantitatively complete is eight hours. In the case of polyanhydromannuronic acid and polyanhydroglucuronic acid, evolution is not complete in fifteen hours. The applicability of the method to every uronic acid is questioned.

Decarboxylation of the carboxyl groups on the 2- and 3-carbon atoms of oxidized cellulose proceeds linearly at a slow rate. Under the conditions of the analysis these carboxyl groups decarboxylate at the rate of approximately one-third of one per cent per hour.

Attempts to prepare a compound oxidized on the 2-, 3-, and 6-carbon atoms were unsuccessful. Other considerations indicate that such a substance would decarboxylate linearly at a slow rate.

INTRODUCTION

The oxidation of cellulose is a subject of importance because it is of widespread occurence in industry and in nature. In the viscose rayon industry one of the first steps of the process makes use of the oxidative degradation of cellulose in strongly alkaline solutions. When cotton is bleached, the bleaching agents oxidize not only the colored impurities but also to some extent the cellulose itself. Whenever cellulosic material is exposed to the action of sun and weather, oxidation of the cellulose takes place.

Despite its importance and the fact that a great deal of work has been done on the subject, the mechanism of the oxidative attack on cellulose by most reagents is not clearly understood. The reasons for this are the complexity of the process and the fact that suitable analytical procedures do not exist[84] *.

The complexity of the process is due to the fact that cellulose may be attacked in a variety of ways by oxidizing agents. On each anhydroglucose

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*References are given on pages 98 through 107.

unit of the cellulose chain there are three free hydroxyl groups occupying positions on the 2-, 3-, and 6-carbon atoms*. The hydroxyl group on the 6-carbon atom is a primary hydroxyl and may be oxidized either to aldehyde or to carboxyl. The hydroxyl groups at the 2- and 3-positions are secondary and may be oxidized to ketone, aldehyde, or carboxyl groups[84]. There are twenty-three conceivable products of the oxidation of these three free hydroxyl groups. In addition there is a free hydroxyl group in the 4-position at one end of each chain and an aldehydic group in the 1-position at the other end. These groups also are subject to oxidation.

Oxidation may cause cleavage of the 1,4-glucosidic linkages[85]. Each such cleavage produces two additional reactive groups, which are in some cases subject to further oxidation. The whole process may result finally in the complete breakdown of the cellulose into carbon dioxide and water.

A further complication arises from the lack of homogeneity of most reactions of cellulose. These

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*Structural formulae are shown in the appendix, pages 109 and 110.

reactions are generally of a topochemical nature and proceed slowly from the exposed outer portion of the fiber towards the interior[85]. At some point in the course of the reaction the surface chains of the fiber may have suffered rather complete oxidation while the interior of the fiber is relatively unreacted. The result is that most oxycelluloses are mixtures of unoxidized celluloses and several of its possible oxidation products.

To characterize the products of the oxidation of cellulose, analytical methods must be found which are of sufficient sensitivity and specificity to determine accurately the type and number of groups formed and to allocate them to definite positions on the oxycellulose molecule. Of the existing analytical methods, the Lefèvre and Tollens estimation of uronic acids[4] is the only one that applies to a specific group in a specific position. In this determination, carboxyl groups on the 6-carbon atoms of celluronic or other uronic acids are quantitatively converted to carbon dioxide by boiling twelve per cent hydrochloric acid. The method is applicable to an oxycellulose oxidized only on the 6-carbon atom.

The purpose of this work was to determine whether

the Lefèvre and Tollens method for estimating oxidation of the 6-carbon atom is interfered with by other oxidation. It was desired to know the behavior of carboxyl groups at the 2- and 3-positions of the glucose residue under the conditions of the analysis, and in particular whether carboxyl groups at these positions interfered with the determination of carboxyl at the 6-position.

REVIEW OF THE LITERATURE

The Carbon Dioxide Evolution Method of Uronic Acid Analysis

The basic reaction

$$C_6H_8O_6 \xrightarrow{12\ \%\ HCl} C_5H_4O_2 + 2H_2O + CO_2 \qquad (I)$$

underlying the carbon dioxide evolution method of uronic acid analysis was discovered by Mann and Tollens[2] in 1895. They noted that very nearly twenty-five per cent by weight of carbon dioxide was given off by glucuronic acid anhydride when boiled in twelve per cent hydrochloric acid, and gave the above equation. The carbon dioxide was measured by bubbling through an ammoniacal barium chloride solution and weighing the barium carbonate formed.

Léo Vignon[3] in 1898 noted the evolution of carbon dioxide under similar conditions from boiled cellulose, oxycellulose, and hydrocellulose.

In 1907 Lefèvre and Tollens[4] further studied Reaction I and based on it the method of analysis which bears their names. The substance to be examined was placed in a flask with 100 ml. of hydrochloric acid of density 1.06 (twelve per cent), boiling chips,

and copper filings. The reaction flask was fitted with a reflux condenser. From the reflux condenser gases passed through two Peligot tubes filled with water, through a calcium chloride tube, and to a potash weighing bulb where the carbon dioxide was measured. A calcium chloride guard tube followed the potash weighing bulb. Air was sucked through the apparatus, having first been bubbled through a potassium hydroxide solution. The material in the reaction flask was brought to boiling and the run continued for four hours or less. At the end the potash bulb was weighed to determine its increase in weight. Glucuronic acid, euxanthinic acid, the magnesium salt of euxanthinic acid, and the sodium salt of urochloralic acid were examined and very close to theoretical yields of carbon dioxide were obtained in all cases. Accordingly, Lefèvre and Tollens stated that the method was suitable as a quantitative determination for glucuronic acid.

Since 1907 many investigations have been carried on using modifications of the Lefèvre and Tollens method. (References 5 through 37) The numerous modifications involve mainly methods of purifying the entering gas stream, acids used in the reaction vessel, methods of purifying the gas stream after it leaves the reaction

vessel and before taking out the carbon dioxide, and methods of trapping and measuring the carbon dioxide. Different methods of purifying the entering gas stream involve the use of potassium hydroxide solutions, barium hydroxide solutions, soda lime, and ascarite. Some investigators did not purify the entering gas stream at all. Various concentrations of hydrochloric acid or sulfuric acid, with or without the addition of other substances as catalysts, have been used in the reaction vessel. The gas from the reaction vessel has been purified by passing through water, calcium chloride, phloroglucinol, silver nitrate, silver sulfate, aniline, granulated zinc, anhydrous copper sulfate, hydroxylamine hydrochloride, sulfuric acid, dehydrite, and phosphorus pentoxide. The carbon dioxide has been collected and measured by passing through barium chloride solution and weighing the barium carbonate formed, by passing through barium chloride solution and titrating, and by collecting in potash or ascarite and weighing.

Previous to 1940 all investigators using the Lefèvre and Tollens method measured only the total amount of carbon dioxide evolved in runs varying from two to ten hours. Whistler, Martin, and Harris[27] were the first to make a study of the rate, and factors

affecting the rate, of carbon dioxide evolution from uronic acids and other substances. Galacturonic acid, glucose, pectin, and cotton were studied. Harris and his coworkers showed that whereas carbon dioxide was evolved from glucose and purified cotton at a constant slow rate for runs up to eight hours duration, carbon dioxide evolution from galacturonic acid and pectin proceeded at a rapid rate for the first few hours of the run and then diminished, the evolution of carbon dioxide being complete in several hours. This rate difference permitted estimation of the individual amounts of carbon dioxide evolved from uronic and cellulosic materials, respectively, in mixtures of the two.

The work of Whistler, Martin, and Harris has been followed by several investigations using their method. Taylor, Fowler, McGee, and Kenyon[36] investigated cellulose oxidized by nitrogen dioxide and concluded that nitrogen dioxide converted anhydroglucose units of cellulose into anhydroglucuronic acid units. T. P. Nevell[37] examined celluloses oxidized by potassium dichromate, sodium metaperiodate, sodium hypobromite, and sodium metaperiodate and chlorous acid, and concluded that the method fails to provide a means for the exact determination of uronic acid groups in oxycelluloses,

but that it may nevertheless be used to obtain a rough estimate of the proportion of such groups present.

Other work that has been done on the decarboxylation method of uronic acid analysis includes: the development of micro methods by Kemmerer and Hallett[10], R. W. Buston[18], and Burkhart, Baur, and Link[21]; studies of the mechanism of the decarboxylation reaction by C. M. Conrad[16], H. Franken[17, 19], Seisha Machida[31, 32], and Ogata, Kometani, Tsunemitsu, and Oda[33]; investigations of the use of sulfuric acid instead of hydrochloric acid as the decarboxylating agent by Link and Niemann[14], C. M. Conrad[15], and T. P. Nevell[37]; and investigations of the use of ferrous chloride as a catalyst to promote the decarboxylation reaction by R. F. Nickerson[29] and C. C. Conrad and A. G. Scroggie[34].

A partial list of materials which have been investigated by the carbon dioxide evolution method appears in Table I.

TABLE I

Materials Which Have Been Investigated By the Carbon Dioxide Evolution Method of Uronic Acid Analysis

MATERIAL	REFERENCE
Oxalic Acid	36
Glyoxylic Acid	36
Ascorbic Acid	36
Tartaric Acid	36, 37
Potassium Acid Saccharate	36
Mucic Acid	37
Dehydro Mucic Acid	31
5-Formyl Mucic Acid	32
Furan Carboxylic Acid	31
5-Methyl Furan Carboxylic Acid	31
5-(Hydroxy methyl) Furan Carboxylic Acid	31
5-Formyl Furan Carboxylic Acid	31
Euxanthinic Acid	4
Magnesium salt of Euxanthinic Acid	4
Sodium salt of Urochloralic Acid	4
Mannose	23
Maltose	36
Xylose	23, 36
Rhamnose	23

TABLE I, (Continued)

Materials Which Have Been Investigated By the Carbon Dioxide Evolution Method of Uronic Acid Analysis

MATERIAL	REFERENCE
Arabinose	23
Glucose	6, 23, 25, 27, 29, 34, 36, 37
2,3,6 Trimethyl Glucose	23
D-Gluconic Acid	36, 37
D-Glucuno-γ-lactone	36
Glucuronic Acid	2, 4, 6, 13, 14, 17
Cellobiose	36
Galactose	23
Galacturonic Acid	9, 11, 12, 13, 14, 15, 27
Barium salt of Galacturonic Acid	13, 16, 19
Digalacturonic Acid	13
Sucrose	23, 25, 37
Fructose	25, 29, 37
Mannitol	23
Inulin	23
Araban	8
Pectin	8, 9, 13, 18, 27, 35, 37
Pectic Acid	36
Calcium Pectate	15

TABLE I, (Continued)

Materials Which Have Been Investigated By the Carbon Dioxide Evolution Method of Uronic Acid Analysis

MATERIAL	REFERENCE
Alginic Acid	35, 36, 37
Sodium Alginate	37
Starches	25, 29, 36
Oxidized Starches	36
Cotton	6, 27, 28, 29, 37
Cellulose	3, 23, 34, 36
Hydrocelluloses	3, 6
Cellulose Oxidized by Nitrogen Dioxide	36
Cellulose Oxidized by Permanganate	6
Cellulose Oxidized by Chromate	6
Cellulose Oxidized by Dichromate	37
Cellulose Oxidized by Hypobromite	37
Cellulose Oxidized by Chlorate	6
Cellulose Oxidized by Periodate	37
Cellulose Oxidized by Periodate and Chlorite	37
Cellulose Oxidized by Nitric Acid	6
Agar-Agar	8
Pjuri	4
Grasses and Hemps	25

The Calcium Acetate Determination of Total Carboxyl Content

In this method the carboxyl content of a cellulosic material is obtained from the amount of acid liberated by cation exchange when the cation-free material is treated with a solution of calcium acetate. The liberated acid is determined by alkali titration of the solution after equilibrium has been reached, and it is assumed that the acid so determined is equivalent to the carboxyl groups present in the cellulosic material.

The method was introduced by Ludtke[38, 39, 40] in 1934 and was subsequently modified by Yackel and Kenyon[30] and by Meesook and Purves[45]. Critical comparisons of the calcium acetate with other methods of carboxyl determination have been made by L. Brissaud[42] and by Davidson and Nevell[48]. Although the methylene blue absorption method[90] is considered by Davidson and Nevell to be the most generally applicable, the calcium acetate method and the silver absorption method[91] give satisfactory results. The alkali titration method of Neale and Stringfellow[92] was found to give high and fictitious results for the carboxyl content of reducing oxycelluloses. The calcium acetate method was used in this investigation because of its relative simplicity.

Oxidation with Nitrogen Dioxide

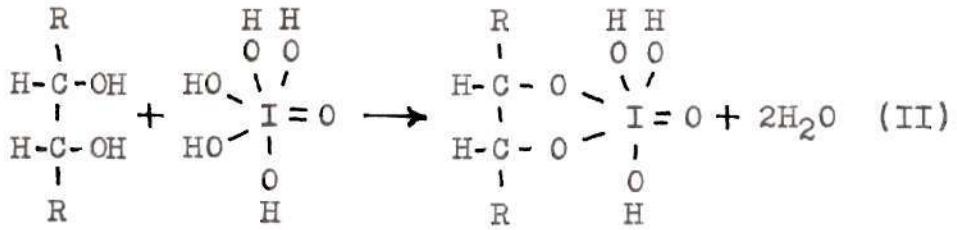
Nitrogen dioxide is specific in its oxidation of the 6-carbon atom of cellulose to a carboxyl group. The first mention of this oxidation occurs in a patent of Yackel and Kenyon[49]. In a subsequent article[51] they give a gaseous method of performing this oxidation and describe the resulting oxycelluloses. Unruh and Kenyon[52] prepared a series of nitrogen dioxide oxycelluloses up to about twenty-five per cent carboxyl by weight. The calculated value for polyanhydroglucuronic acid is 25.5 per cent. These oxycelluloses were examined by the carbon dioxide evolution method and the conclusion reached that nitrogen dioxide preferentially attacks the primary hydroxyl group. Maurer and Drefahl[53] performed the oxidation on galactose using nitrogen dioxide dissolved in chloroform and obtained 75 per cent of mucic acid. Menchand and Degering[55] oxidized starch by nitrogen dioxide dissolved in carbon tetrachloride. By decarboxylating a series of celluloses oxidized by nitrogen dioxide in carbon tetrachloride, Taylor, Fowler, McGee, and Kenyon[36] provided additional evidence that the oxycellulose produced consisted of β-D-glucuronic acid units and unchanged D-glucose units. McGee, Fowler, Taylor, Unruh, and Kenyon[56] studied the mechanism of the reaction in carbon tetrachloride and gave sufficient data

so that a cellulose of an approximately given carboxyl content can be preparad. They propose that the cellulose is first nitrated by nitric acid formed from nitrogen dioxide and small amounts of water present, and that the nitrate ester is then converted to a carboxyl group, nitric acid catalysing. There is, however, some evidence[86], that nitrous acid, not nitric, is the true oxidant and that it is the nitrous acid ester of the primary hydroxyl group that is deesterified with nitric acid as a catalyst to form the carboxyl group.

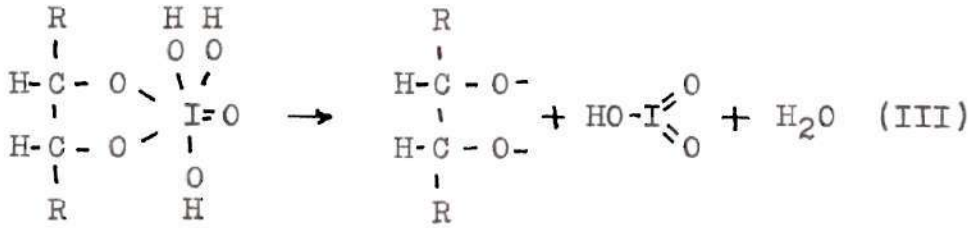
Oxidation with Periodic Acid

The action of periodic acid upon compounds containing adjacent hydroxyl groups was first applied by Malaprade[59] and the reaction is sometimes referred to by his name. The reaction is applicable to compounds having hydroxyl groups or a hydroxyl group and an amino group attached to adjacent carbon atoms[87]. The mechanism of the reaction involves the formation of an ester with the glycol (Equation II). The ester then decomposes, liberating the oxidant in its lower valence state (Equation III), and the remaining free radicals

of the glycol rearrange to form a dialdehyde (Equation IV) [61].



(II)



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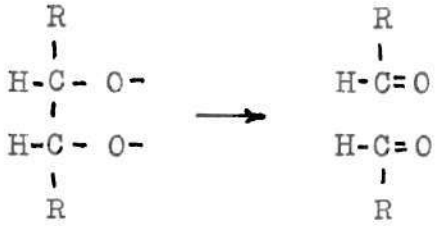


(IV)

In a chain of carbon atoms the reaction will continue until a carbon atom is reached which does not carry an unsubstituted hydroxyl, a carbonyl, or an amino group. This is illustrated by Khouvine and Arragon's[69] oxidation of glucose to obtain five moles of formic acid and one mole of formaldehyde and by

Jackson and Hudson's[63] oxidation of a methyl glucoside to obtain a dialdehyde with one less carbon atom than the original methyl glucoside.

Cellulose, starch, alginic acid, and pectic acid each contain only one pair of adjacent unsubstituted hydroxyl groups per monomeric residue. These occur on the 2- and 3-carbon atoms, and the carbon chain is broken only between these carbon atoms. Levene and Kreider[64] established this by hydrolysing the periodic acid oxidation products of a polygalacturonide methyl ester to obtain levo-tartaric acid. Jackson and Hudson[66] provided additional evidence by hydrolysing the periodic acid oxidation products of cornstarch and cotton cellulose and obtaining glyoxal and d-erythrose. Other studies of the periodic acid oxidation of cellulose have been made by G. F. Davidson[68], by Rutherford, Minor, Martin, and Harris[71], and by Goldfinger, Mark, and Siggia[72]. The reaction with alginic acid has been carried out by Lucas and Stewart[67].

Oxidation with Chlorous Acid

Chlorous acid has been shown by Jeans and Isbell[75] to be specific in its oxidation of aldehyde groups in carbohydrate materials to carboxyl groups. Aldoses are

oxidized to the corresponding aldonic acids by the following reaction

$$RCHO + 3\ HClO_2 \longrightarrow RCOOH + 2\ ClO_2 + HCl + H_2O \quad (V)$$

Aldonic acids, ketoses, glycosides, and polyhydric alcohols are noticeably attacked by chlorous acid only after many days treatment.

Titration for Periodates in the Presence of Iodates

The volumetric determination of periodates in the presence of iodates is based on the fact that whereas in acid solution both iodates and periodates are reduced by iodides to iodine(Equations VI and VII)[76]

$$IO_4^- + 7\ I^- + 8\ H^+ \longrightarrow 4\ I_2 + 4\ H_2O \quad (VI)$$
$$IO_3^- + 5\ I^- + 6\ H^+ \longrightarrow 3\ I_2 + 3\ H_2O \quad (VII)$$

in neutral or slightly alkaline solution periodates are reduced by iodides to iodates only (Equation VIII)[82]

$$IO_4^- + 2\ I^- + H_2O \longrightarrow 2\ OH^- + IO_3^- + I_2 \quad (VIII)$$

The liberated iodine may be titrated with sodium arsenite[82] (but not sodium thiosulfate), or sodium

arsenite may be added in excess and the excess back titrated with iodine solution (Equation IX)[79].

$$NaAsO_2 + I_2 + 3NaHCO_3 \longrightarrow Na_2HAsO_4 + 2NaI + 3\ CO_2 + H_2O \quad (IX)$$

The solution is buffered with sodium bicarbonate to prevent reaction between iodate and iodide (Equation VII) which occurs in even slightly acid solution[80] and between iodine and hydroxyl (Equation X) which occurs in alkaline solution[81].

$$I_2 + 2\ OH^- \longrightarrow I^- + IO^- + H_2O \quad (X)$$

EXPERIMENTAL

The experimental procedure involved the preparation of oxidized materials and their subsequent examination by the carbon dioxide evolution method of analysis and by the calcium acetate method for the determination of total carboxyl content. The starting materials are given below. The preparation of five oxidized celluloses is described on pages 23 through 27, and three unsuccessful attempts to obtain a pure sample of oxidized pectic acid are outlined on pages 27 through 57. Following this is a description of the methods of analysis used.

Materials

Glucose

The d-glucose used was Eimer and Amend C. P. grade. Its moisture content was 0.08 per cent as determined by drying at 110° C. for ten hours. Before use it was dried in vacuo over phosphorus pentoxide.

Pectic Acid

The pectic acid used was Eastman Kodak technical grade. The moisture content determination showed 13.0 per cent when dried for six hours at 105° C. and 15.6

per cent when dried for ten hours at 110° C.

Standard Cellulose

The standard cellulose used was prepared from cotton according to the procedure of Worner and Mease[88]. Its moisture content was 5.6 per cent as determined by drying for ten hours at 110° C.

Preparation of Oxidized Celluloses

Oxycellulose I

Dry standard cellulose, 34.4 grams, was placed in a five liter glass-stoppered bottle with 1152 grams of carbon tetrachloride in which had been dissolved 260 grams of nitrogen dioxide. The reaction was allowed to take place for 25 hours at room temperature. The nitrogen dioxide to carbon tetrachloride weight ration was 0.226 and the nitrogen dioxide to cellulose weight ration was 8.02. From the data of McGee, Fowler, Taylor, Unruh, and Kenyon[56] an oxycellulose of approximately 13 per cent carboxyl should have been produced. That is, about half of the anhydroglucose units should have been attacked. After the reaction the oxycellulose was washed repeatedly with distilled water. After each washing the oxycellulose and water were allowed to stand

several hours or overnight with occasional shaking. Washing was continued until the pH of the wash water became constant. Eleven washings were required. The oxycellulose was then washed once with 50 per cent ethyl alcohol, twice with 95 per cent ethyl alcohol, and once with anhydrous ether. The product was dried 12 hours in a vacuum oven at 50° C. and stored in a vacuum dessicator over phosphorus pentoxide. The yield was 35 grams.

Oxycellulose II

Standard cellulose, 23.77 grams with a moisture content of 5.6 per cent, was submerged in 1800 ml. of a 0.125 molar sodium periodate solution. The reaction was allowed to proceed for 54 hours at 25° C. and was followed by titrating for periodate. The titration results were converted into millimoles of aldehyde formed per mole of sample by the formula given on page 167. A plot of the course of the reaction appears in Figure 12. The product was washed with water in the same manner as Oxycellulose I and divided into two approximately equal parts.

The first part was further washed with alcohol and ether and dried. The yield was 13.6 grams of an

oxicellulose oxidized by periodate only. This product was never used because it was later decided to concentrate on materials oxidized to the carboxyl stage. The second part was reacted for one hour at room temperature with a solution prepared by dissolving 36.2 grams of sodium chlorite in 350 ml. of distilled water and acidifying to pH 2.5 with glacial acetic acid. The product was washed with water, alcohol, and ether and dried, all after the manner of Oxycellulose I. The yield was 8.1 grams of Oxycellulose II. It was stored in a vacuum dessicator over phosphorus pentoxide.

Oxycellulose III

This material was prepared by reacting 15 grams of standard cellulose for 115 hours with 1500 ml. of a 0.1 molar sodium periodate solution. It was subsequently oxidized with sodium chlorite and washed. The product was stored in a vacuum dessicator over phosphorus pentoxide.

Oxycellulose IV

A portion of Oxycellulose III, 2.54 grams, was placed in a one liter glass-stoppered bottle. The bottle was evacuated with an aspirator and gaseous nitrogen dioxide was admitted. The sample was left in

contact with the gas for 17.5 hours and was then washed and dried in the same manner as Oxycellulose I. The yield was 2.67 grams. The weight of nitrogen dioxide admitted to the flask was calculated to be approximately 1.9 grams so that the nitrogen dioxide to cellulose weight ratio was 0.78. From the data of Yackel and Kenyon[51] an oxycellulose of approximately 10 per cent by weight of uronic carboxyl should have been formed. Oxycellulose IV was stored in a vacuum dessicator over phosphorus pentoxide.

Oxycellulose V

A portion of Oxycellulose I, 22.62 grams, was submerged in 1800 ml. of a 0.125 molar sodium periodate solution. The reaction was allowed to proceed for 54 hours at 25° C. The reaction was followed by titrating for periodate and converting the results into millimoles of aldehyde formed per mole of sample. A plot of the course of the reaction appears in Figure 12. The product was washed with water in the same manner as Oxycellulose I and divided into two approximately equal parts.

The first part was further washed with alcohol and ether and dried. The yield was 4.8 grams of an oxycellulose oxidized by nitrogen dioxide and periodate

only. This product was not used.

The second part was reacted for one hour at room temperature with a solution prepared by dissolving 36.2 grams of sodium chlorite in 350 ml. of distilled water and acidifying to pH 2.5 with glacial acetic acid. This product was then washed with water, alcohol, and ether and dried, all after the manner of Oxycellulose I. The yield was 1.44 grams of Oxycellulose V. It was stored in a vacuum dessicator over phosphorus pentoxide.

Preparation of Oxidized Pectic Acids

Oxidation of Pectic Acid to Oxidized Pectic Acid I.

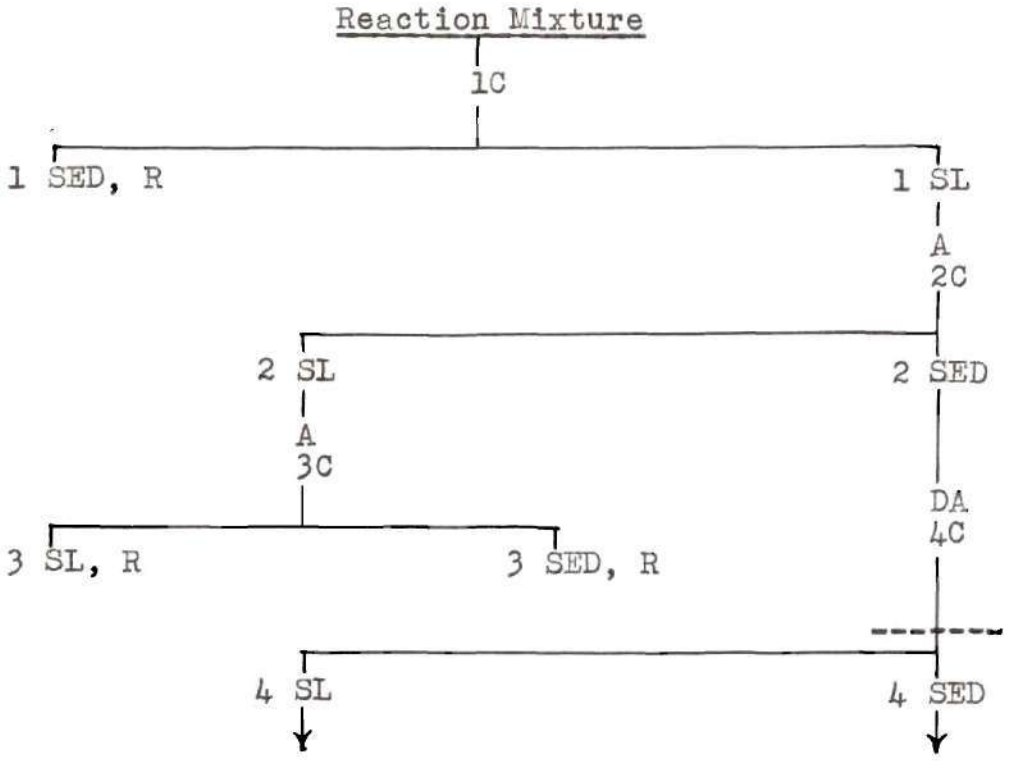
To 12 grams of technical pectic acid with a moisture content of 13 per cent was added 20 ml. of distilled water to wet the material thoroughly. Half of a solution prepared by dissolving 5.05 grams of sodium periodate in 360 ml. of water was then added. The amount of pectic acid was 0.059 moles as anhydrogalacturonic acid, and the added amount of sodium periodate was 0.0117 moles so that the amount of sodium periodate used was sufficient to oxidize twenty per cent of the anhydrogalacturonic acid units. The reaction was allowed to proceed for three and one-half

hours at 5 °C. and was followed by titrating for periodate. A plot of the course of the reaction appears in Figure 13.

Recovery of Oxidized Pectic Acid I from the Reaction Mixture.

The attempt to recover the oxidized product from the reaction mixture is outlined in Figure 1. The reaction mixture was centrifuged (1C). The sediment (1 SED), a granular material of dark color with an odor of iodine, was rejected. To the supernatant liquor (1 SL), 167 ml. of a clear straw colored solution, was added 100 ml. of tertiary butyl alcohol. An apparently large amount of flocculent precipitate was thrown down. This mixture was centrifuged(2C). To the supernatant liquor (2 SL) was added an additional 100 ml. of tertiary butyl alcohol to test for completeness of precipitation. The result was a slight cloudiness which did not disappear on centrifuging (3C) although a thin film of material (3 SED) deposited on the bottom of the jar. This material was rejected. The sediment (2 SED) from the second centrifuging (2C) was dissolved in 150 ml. of water to form a clear solution. To this was added ethyl alcohol to reprecipitate, 400 ml. being required. The mixture was then

centrifuged (4C). To the supernatant liquor (4 SL) was added an additional 100 ml. of ethyl alcohol to test for completeness of precipitation. The result was again the appearance of a cloudiness which on standing, recentrifuging (5C), and drying yielded 0.77 grams of a green, hard, lumpy material (5 SED). The sediment from the fourth centrifuging (4 SED) was washed with ethyl alcohol and ether. To it was added 110 ml. of water. The material partially peptized to form a cloudy dispersion. The addition of 100 ml. of tertiary butyl alcohol caused an apparently large precipitate. The mixture was centrifuged (6C). To the supernatant liquor (6 SL) was added an additional 100 ml. of tertiary butyl alcohol. The result was the appearance of a cloudiness which on standing, recentrifuging (7C), and drying yielded 0.91 grams of a yellow, hard, lumpy material (7 SED). The sediment from the sixth centrifuging (6 SED) was treated as before, and the process was repeated until the supernatant liquors failed to become cloudy on testing for completeness of precipitation. The final sediment (16 SED) was washed with ethyl alcohol, ether, and dried yielding 2.16 grams of a fine straw colored powder.



Abbreviations

C, Centrifuge

SL, Supernatant liquor from centrifuging

SED, Sediment from centrifuging

R indicates that the material was rejected

D indicates addition of water to dissolve

A indicates addition of alcohol to cause precipitation

Weights of materials retained are given in grams.

FIGURE 1: RECOVERY OF OXIDIZED PECTIC ACID I FROM REACTION MIXTURE.

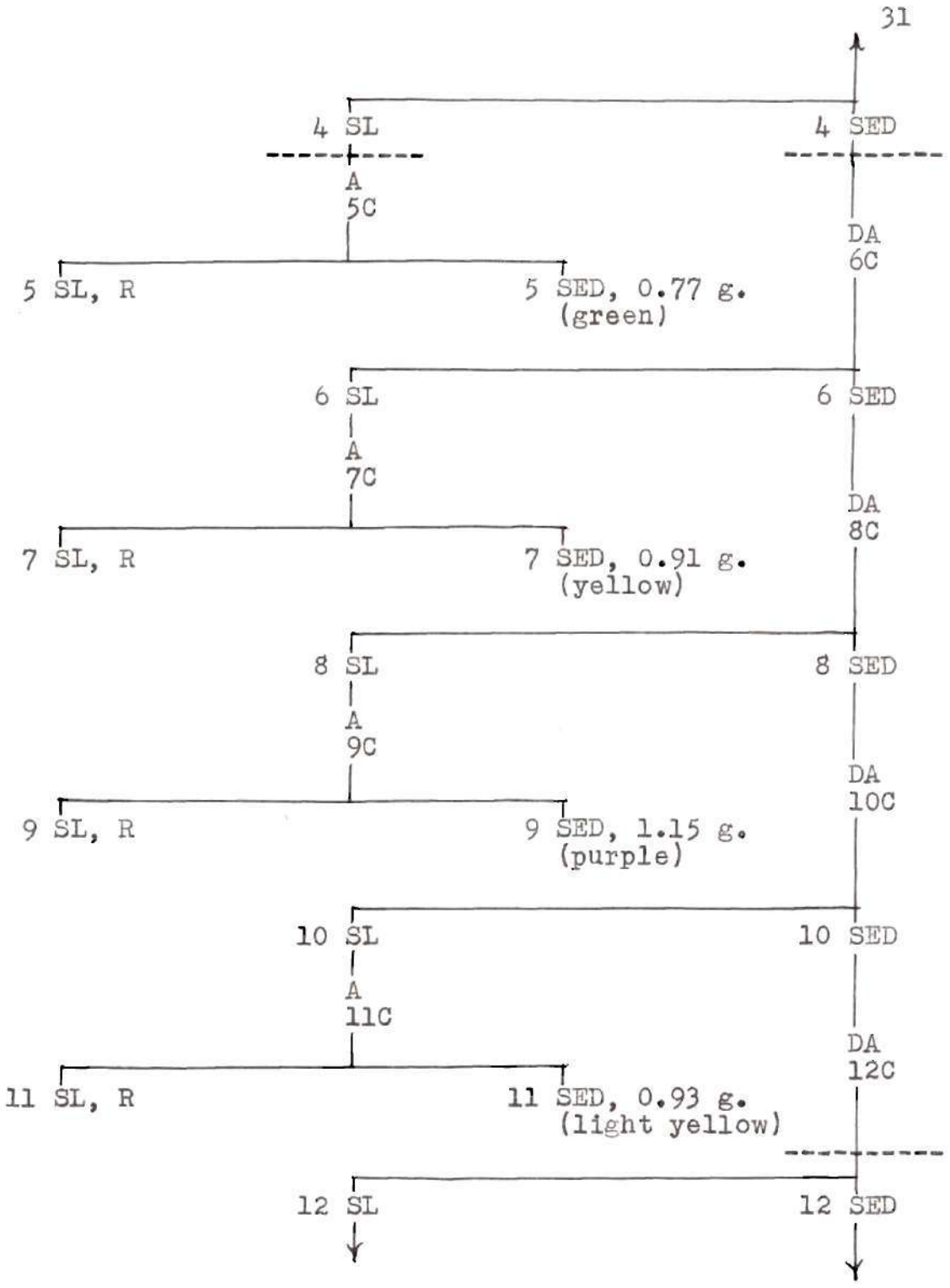
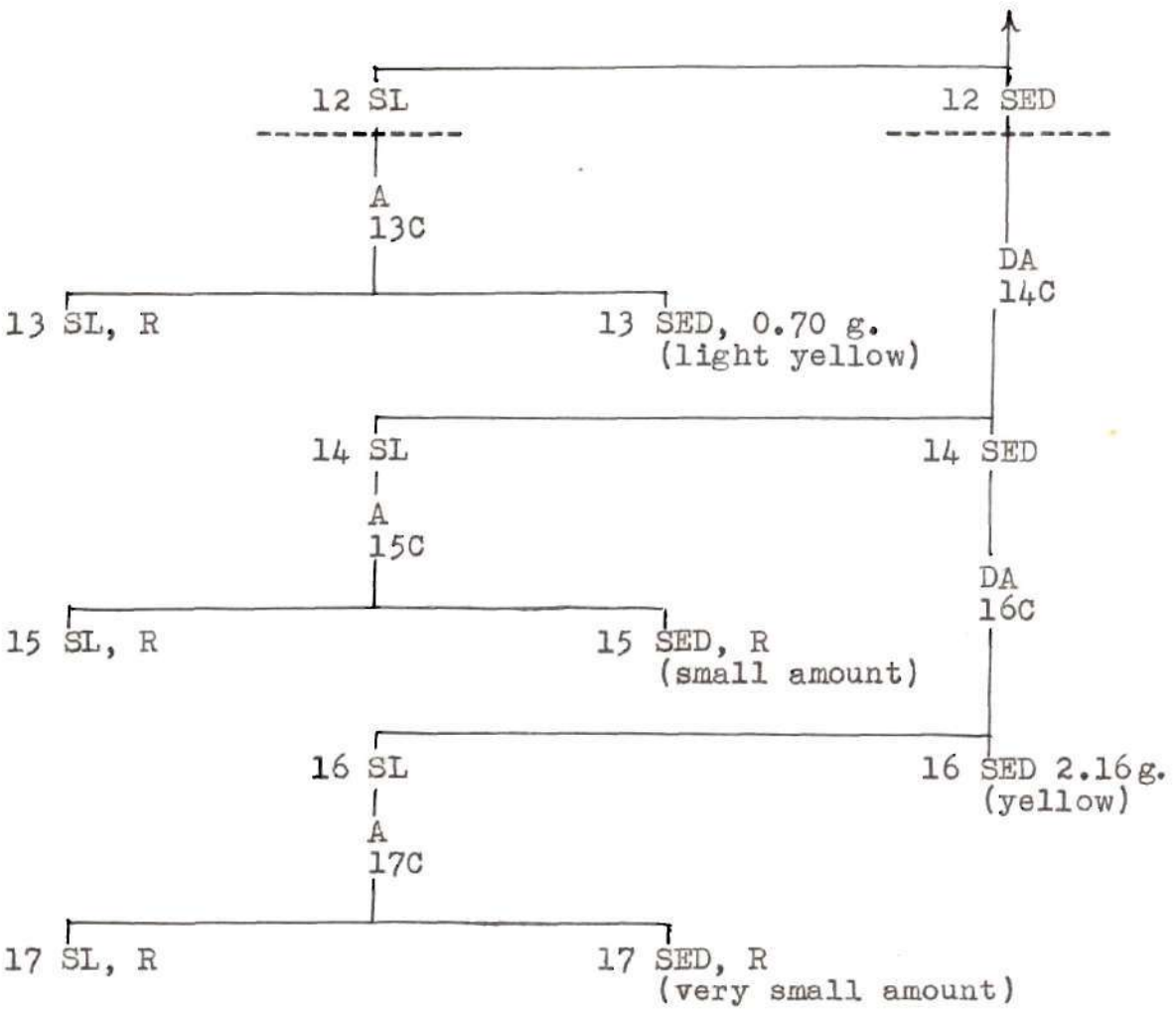


FIGURE 1: Continued.



Total of 5, 7, 9, 11, and 13 SED	4.46 grams
16 SED	2.16 grams
Total Material Recovered	6.62 grams

FIGURE 1: Concluded.

Inasmuch as only 20 per cent of the anhydrogalacturonic acid units of the original pectic acid were oxidized and the oxidized units have a greater solubility in water than the unoxidized units, it is probable that the 2.16 grams of final residue (16 SED) was part of the 80 per cent of the pectic acid that was not oxidized and that the 20 per cent that was oxidized was either lost in solution or was present in the materials recovered from the supernatant liquors (5, 7, 9, 11, and 13 SED).

Reprecipitation of Oxidized Pectic Acid I.

The small amounts of material and its obvious impurity made calcium acetate determinations or decarboxylation runs on any of the fractions of Oxidized Pectic Acid I unfeasible. The best that could be done was to use the material available to try to find a better method of precipitating it. Accordingly, the materials from the fifth, seventh, and ninth centrifugings (5 SED, 7 SED, and 9 SED), a total of 2.83 grams, were lumped together and dissolved in 100 ml. of water. Fifty milliliters more water was added. To this solution was then added 100 ml. of isopropyl alcohol with no apparent effect. Upon the addition of 10 ml. of 0.3 N hydrochloric acid,

there appeared an immediate precipitate. The addition of 35 ml. more of 0.3 N hydrochloric acid caused the precipitate to redissolve. Large amounts of alcohol added to small quantities of this solution in test tubes failed to cause reprecipitation.

From this it appears that small amounts of acid aid in precipitating oxidized pectic acid from alcohol-water solutions. Too much acid causes hydrolysis.

Oxidation of Pectic Acid to Oxidized Pectic Acid II.

The plan of attack for Oxidized Pectic Acid II was altered from that used for Oxidized Pectic Acid I in three respects. First, it was decided to oxidize 50 per cent of the anhydrogalacturonic acid units so that in the event of a good yield there would be less probability of its being the unoxidized portion of the pectic acid. Second, the sodium chlorite-acetic acid solution was to be added directly to the periodate reaction mixture without attempting to recover the intermediate dialdehyde product. An inspection of the oxidation-reduction potentials of all materials concerned indicated that there would be no interference with the chlorite oxidation by other inorganic materials present. Third, the sodium periodate solution was to be acidified to pH 2.5 at the beginning of the oxidation to eliminate

any possibility of alkaline degradation and to insure that the chlorite oxidation occured at a pH of 2.5.

Accordingly, 16 grams of sodium periodate were dissolved in 500 milliliters of water. The pH of the solution was 4.3. Twenty milliliters of glacial acetic acid were added to bring the pH to 2.5. Samples of the solution were titrated for periodate immediately before, immediately after, and two hours after the addition of the acetic acid, as shown in Table XVI. There was negligible change in periodate concentration indicating no reaction between the acetic acid and the sodium periodate solution. Thirty grams of pectic acid with a moisture content of 13 per cent was added to this periodate solution. The reaction was allowed to proceed for four hours at 5 ^{o}C. At the end of this time the pH of the mixture was 2.2.

The solution contained 0.074 moles of sodium periodate, and the amount of pectic acid was 0.148 moles as anhydrogalacturonic acid so that the amount of the periodate was sufficient to oxidize 50 per cent of the anhydrogalacturonic acid units.

A sodium chlorite solution was prepared by dissolving 60 grams of sodium chlorite in 500 milliliters of distilled water. The pH of this solution was 9.7.

Five hundred milliliters of glacial acetic acid were added to bring the pH to 2.5.

After the periodate oxidation had been allowed to proceed for four hours, the chlorous acid solution was added directly to the periodate oxidation reaction mixture. Immediately upon addition an intense yellow color appeared. After an hour the mixture was a cloudy suspension with only a small amount of sediment present. The addition of 50 milliliters of concentrated hydrochloric acid produced a clear solution.

An attempt was made to precipitate the product by addition of tertiary butyl alcohol. Three liters were added without success. The solution was then evaporated under vacuum almost to a paste. The temperature at no time exceeded 35 °C. The paste was washed with 95 per cent alcohol. It was peptized in 200 ml. of water and filtered. The addition of 400 ml. of 95 per cent ethyl alcohol caused ready precipitation. The precipitate was washed with methyl alcohol and ether and air dried yielding 7.5 grams of a white powder.

Solubility of Oxidized Pectic Acid II in Organic Solvents

An attempt to find an organic solvent for oxidized pectic acid II was made as follows in the hope that some method of purification by solvent extraction might be

found. Small amounts of Oxidized Pectic Acid II, just enough to be easily seen, were put in test tubes. Fifteen milliliters of solvent were added and the tubes were allowed to stand for 24 hours with occasional shaking. After 24 hours the tubes were heated to boiling and again allowed to stand. The following solvents were tried: methyl cellosolve, petroleum ether, carbitol, methyl alcohol, butyl lactate, trichloroethylene, butyl acetate, nitromethane, diacetone alcohol, tetrahydronaphthalene, dioxane, chloroform, carbon disulfide, acetone, toluene, water, and benzene. In no case except water was there any evidence of solution. Water dissolved the material immediately. In the case of tetrahydronaphthalene the supernatant liquor and the material in the bottom of the test tube turned dark after 24 hours.

Solubilities of the Inorganic Salts Present in the Oxidation Reaction Mixtures.

Having failed to find a solvent for Oxidized Pectic Acid II and attempt was made to find a solvent other than water for the inorganic salts present in the reaction mixture. Possible salts present include iodates and unreacted periodates from the periodate oxidation reaction (Equation III, page 18) and chlorides

and unreacted chlorites from the chlorous acid oxidation reaction (Equation V, page 20). Chlorates may be present due to the reactions[75]

$$2\ ClO_2 + H_2O \longrightarrow HClO_2 + HClO_3 \quad (XI)$$

$$3\ HClO_2 \longrightarrow HCl + 2\ HClO_3 \quad (XII)$$

The acetic acid used adds acetates.

The solubilities of these salts in various solvents were tried as shown in Table II. The procedure was as follows. Small amounts of the salt, just enough to be easily seen, were put in test tubes and fifteen milliliters of the solvent added. The tube was allowed to stand for three hours at room temperature with occasional shaking and then heated to boiling. An s. indicates that the material dissolved completely. An i. indicates that no visible solution took place. A sl. s. indicates that a small amount of the salt appeared to have dissolved, but that it did not completely dissolve.

No satisfactory solvent for the iodate or chlorate was found.

TABLE II

Solubilities of Small Amounts of Certain Salts in Fifteen Milliliters of Various Solutions

Abbreviations: i. indicates insolubility
s. indicates solubility
sl. s. indicates slight solubility

Solution	Potassium iodate	Sodium periodate	Sodium chloride	Sodium chlorite	Potassium chlorate	Sodium acetate
Chloroform						
Cold	i.	i.		i.		i.
Hot	i.	i.		i.		i.
Acetone						
Cold	i.	i.		i.		i.
Hot	i.	i.				i.
Ethyl alcohol, 95 per cent Water, 5 per cent						
Cold	i.	i.		i.		i.
Hot	i.	i.		s.		s.

TABLE II, Continued.

Solubilities of Small Amounts of Certain Salts in Fifteen Milliliters of Various Solutions

Solution	Potassium iodate	Sodium periodate	Sodium chloride	Sodium chlorite	Potassium chlorate	Sodium acetate
Ethyl alcohol, 75 per cent Water, 25 per cent						
Cold	i.	i.		s.		s.
Hot	i.	i.		s.		s.
Methyl alcohol						
Cold	i.	i.	i.	i.	i.	s.
Hot	i.	i.	sl. s.	s.	sl. s.	s.
Methyl alcohol, 90 per cent Water, 10 per cent						
Cold	i.	sl. s.	s.	s.	i.	s.
Hot	i.	sl. s.	s.	s.	i.	s.

TABLE II, Continued.

Solubilities of Small Amounts of Certain Salts in Fifteen Milliliters of Various Solutions

Solution	Potassium iodate	Sodium periodate	Sodium chloride	Sodium chlorite	Potassium chlorate	Sodium acetate
Methyl alcohol, 90 per cent Acetic acid, 10 per cent						
Cold	i.	i.	i.	s.	i.	s.
Methyl alcohol, 80 per cent Acetic acid, 10 per cent Water, 10 per cent						
Cold	i.	i.	s.	s.	i.	s.
Hot	i.	sl. s.	s.	s.	sl. s.	s.
Glacial acetic acid						
Cold	i.	i.	i.	s.	i.	s.
Hot	i.	i.	i.	s.	i.	s.

Precipitation of Oxidized Pectic Acid II by Alcohol-Acetic Acid Mixtures.

Having failed to find a suitable solvent for either Oxidized Pectic Acid II or for the inorganic salts, the next attack was in the direction of finding some agent which would precipitate oxidized pectic acid from a water solution without precipitating the inorganic salts.

Solutions of 50 per cent of the concentration of a saturated solution were made up of the following salts: potassium iodate, sodium periodate, sodium chloride, potassium chlorate, and sodium acetate. To 5 ml. of each of these solutions was added 20 ml. of methyl alcohol. It was found that potassium iodate and potassium chlorate precipitated out, but that the other salts did not. Results using 95 per cent ethyl alcohol instead of methyl alcohol were identical.

From this, and from the results shown in Table II, it appeared that the iodates and the chlorates were the most insoluble of the salts present. In addition, the oxidation reaction mixtures contain higher concentrations of sodium iodate than of any of the other inorganic salts present. An agent, therefore, which would precipitate the oxidized pectic acid and leave the

sodium iodate in solution would leave the other inorganic salts present in solution also. For this reason it was decided to concentrate on iodates.

A solution of potassium iodate was made up by diluting a saturated solution to four times its volume. To 5 ml. of this solution were added various mixtures of methyl alcohol and acetic acid and 95 per cent ethyl alcohol and acetic acid as shown in Table III. The results indicated that one volume of 95 per cent ethyl alcohol plus two volumes of acetic acid added to one volume of the quarter saturated iodate solution barely produced precipitation of the iodate. No iodate would be precipitated from an iodate solution of lower concentration.

Using Oxidized Pectic Acid II it was impossible to parallel the procedure used with potassium iodate. Although Oxidized Pectic Acid II would eventually dissolve in water to form a clear solution, there was no sharp point at which solution obviously was complete. It would form a colloidal suspension which would become less and less turbid as more water was added. A solution of the highest possible concentration was made by adding sufficient water so that no individual particles could be seen although the "solution" remained cloudy. A

dilute solution of Oxidized Pectic Acid II was made by diluting to 32 times its volume one volume of the more concentrated solution.

To 5 ml. portions of this dilute solution of Oxidized Pectic Acid II were added various mixtures of methyl alcohol and acetic acid and of 95 per cent ethyl alcohol and acetic acid, as shown in Table IV. The results indicated that one volume of 95 per cent ethyl alcohol plus two volumes of glacial acetic acid added to one volume of dilute Oxidized Pectic Acid II solution caused precipitation of Oxidized Pectic Acid II.

In summary, one volume of 95 per cent ethyl alcohol plus two volumes of glacial acetic acid added to one volume of dilute Oxidized Pectic Acid II solution precipitates Oxidized Pectic Acid II. One volume of 95 per cent ethyl alcohol plus two volumes of glacial acetic acid added to one volume of a fairly concentrated solution of potassium iodate does not precipitate potassium iodate. Of the inorganic salts present in a periodate-chlorite oxidation reaction mixture the iodates are the least soluble and are present in the highest concentration. Therefore the addition of one volume of 95 per cent ethyl alcohol plus two volumes of glacial acetic acid to one volume of a solution of the

pectic acid-periodate-chlorite reaction products may be effective in precipitating the oxidized pectic acid without precipitating the inorganic salts present.

Reprecipitation of Oxidized Pectic Acid II

The above procedure was tried out on a sample of Oxidized Pectic Acid II. Five grams of Oxidized Pectic Acid II were dissolved in 25 ml. of water. The solution was diluted to 100 ml. and 200 ml. of glacial acetic acid and 100 ml. of 95 per cent ethyl alcohol were added. A white precipitate formed. The mixture was centrifuged and the supernatant liquor poured off and discarded. The sediment was redissolved in 100 ml. of water. Two hundred milliliters of glacial acetic acid and 100 ml. of 95 per cent ethyl alcohol were again added. A precipitate formed and the mixture was again centrifuged. The supernatant liquor was poured off and discarded. The sediment was washed with 100 ml. of 95 per cent ethyl alcohol. This was done five times to wash out the acetic acid. A final washing was made with anhydrous ether and the product was air dried. The yield was 2.3 grams of a very fine white powder.

An ash determination was made on this material and showed 4.6 per cent ash. Inasmuch as periodates, iodates, chlorates, and hypochlorites decompose on heating to high

TABLE III

Precipitation of Potassium Iodate by Alcohol-Acetic Acid Mixtures

Solution added to 5 ml. of the potassium iodate solution.	Result
Methyl alcohol, 5 ml.	Precipitate formed
Methyl alcohol, 5.ml. plus acetic acid, 1 ml.	Precipitate formed
Methyl alcohol, 5 ml. plus acetic acid, 2 ml.	Precipitate formed
Methyl alcohol, 5 ml. plus acetic acid, 3 ml.	Precipitate formed
Methyl alcohol, 5 ml. plus acetic acid, 5 ml.	Precipitate formed
Methyl alcohol, 5 ml. plus acetic acid, 10 ml.	Turbidity
Ethyl alcohol, 5 ml.	Precipitate formed
Ethyl alcohol, 5 ml. plus acetic acid, 1 ml.	Precipitate formed
Ethyl alcohol, 5 ml. plus acetic acid, 2 ml.	Precipitate formed
Ethyl alcohol, 5 ml. plus acetic acid, 3 ml.	Precipitate formed
Ethyl alcohol, 5 ml. plus acetic acid, 5 ml.	Precipitate formed
Ethyl alcohol, 5 ml. plus acetic acid, 10 ml.	Turbidity

TABLE IV

Precipitation of Oxidized Pectic Acid II by Alcohol-Acetic Acid Mixtures

Solution added to 5 ml. of the dilute Oxidized Pectic Acid II solution	Result
Methyl alcohol, 10 ml.	Clear solution
Methyl alcohol, 20 ml.	Clear solution
Methyl alcohol, 10 ml. plus acetic acid, 1 ml.	Clear solution
Methyl alcohol, 10 ml. plus acetic acid, 3 ml.	Clear solution
Ethyl alcohol, 10 ml.	Clear solution
Ethyl alcohol, 20 ml.	Precipitate formed
Ethyl alcohol, 10 ml. plus acetic acid, 1 ml.	Precipitate formed
Ethyl alcohol, 10 ml. plus acetic acid, 3 ml.	Precipitate formed
Methyl alcohol, 5 ml.	Clear solution
Methyl alcohol, 5 ml. plus acetic acid, 1 ml.	Clear solution
Methyl alcohol, 5 ml. plus acetic acid, 2 ml.	Clear solution
Methyl alcohol, 5 ml. plus acetic acid, 3 ml.	Clear solution
Methyl alcohol, 5 ml. plus acetic acid, 5 ml.	Clear solution
Methyl alcohol, 5 ml. plus acetic acid, 10 ml.	Precipitate formed

TABLE IV, Continued

Precipitation of Oxidized Pectic Acid II by Alcohol-Acetic Acid Mixtures

Solution added to 5 ml. of the dilute Oxidized Pectic Acid II solution.	Result
Ethyl alcohol, 5 ml.	Clear solution
Ethyl alcohol, 5 ml. plus acetic acid, 1 ml.	Clear solution
Ethyl alcohol, 5 ml. plus acetic acid, 2 ml.	Clear solution
Ethyl alcohol, 5 ml. plus acetic acid, 3 ml.	Clear solution
Ethyl alcohol, 5 ml. plus acetic acid, 5 ml.	Turbidity
Ethyl alcohol, 5 ml. plus acetic acid, 10 ml.	Precipitate formed

temperatures, the amount of inorganic material present was probably considerably higher than the 4.6 per cent shown by the ash determination.

Oxidation of Pectic Acid to Oxidized Pectic Acid III.

To 50 grams of pectic acid with a moisture content of 13 per cent was added a solution prepared by dissolving 56 grams of sodium periodate in 1500 ml. of water and adding 50 ml. of glacial acetic acid. The pH of the solution was 3. The solution was made up to contain a 10 per cent excess of sodium periodate so that 100 per cent of the anhydrogalacturonic acid units of the pectic acid would be oxidized. The reaction was carried on with constant stirring for five hours. The temperature of the mixture was 5° C. at the beginning of the reaction and was 20° C. after the five hours. After the reaction the mixture was divided into two equal parts.

The first part was evaporated under vacuum to a paste. The paste was peptized in the least possible quantity of distilled water. Two volumes of glacial acetic acid and one volume of 95 per cent ethyl alcohol were added in an attempt to reprecipitate. No precipitation took place. This procedure, which is effective in precipitating pectic acid which has been oxidized

to the carboxyl stage on the 2- and 3-carbon atoms, evidently is not effective in precipitating pectic acid which has been oxidized only to the aldehyde stage on the 2- and 3-carbon atoms. The mixture was again evaporated to a paste and again peptized in water. The addition of a large quantity of 95 per cent ethyl alcohol caused a partial precipitation. A large amount of material remained as a colloidal dispersion which would neither settle, centrifuge, nor filter. The precipitate was washed twice with 95 per cent ethyl alcohol and twice with anhydrous ethyl ether. The product was a powdery white material which after two hours air drying weighed 9 grams. This product was not used.

To the second part was added a solution prepared by dissolving 75 grams of sodium chlorite in 500 ml. of distilled water and acidifying to pH 2.7 with glacial acetic acid. The reaction was carried on at room temperature. The mixture was allowed to stand a day until the vacuum apparatus was clear. It was then evaporated to a paste. At no time did the temperature exceed 35 ^{o}C. During the evaporation large amounts of chlorine or chlorine dioxide were given off. The paste was then peptized in the least possible amount of water

and the dispersion diluted to four times its volume, about 500 ml. To this was added 500 ml. of 95 per cent ethyl alcohol and 1000 ml. of glacial acetic acid. A large amount of white precipitate formed leaving a dark brown supernatant liquor. The mixture was centrifuged, the sediment was again peptized in water, and the precipitation was carried out again in the same way. The second precipitation left a supernatant liquor which had the appearance of milk. The mixture was centrifuged and the supernatant liquor poured off and discarded. The sediment was washed five times with 95 per cent ethyl alcohol and twice with anhydrous ethyl ether. After two hours of air drying the yield was 20 grams of a fine white powder. This product was Oxidized Pectic Acid III.

Analysis of Oxidized Pectic Acid III.

Two determinations were made on Oxidized Pectic Acid III.

An ash determination showed 12.8 per cent ash. As mentioned before this indicates a much higher percentage of inorganic materials present before ignition.

Samples of Oxidized Pectic Acid III were weighed out and titrated to a phenolphthalein end point with 0.1343 N sodium hydroxide. The results, shown in

Table XLVI, averaged 44.8 ml. of sodium hydroxide solution per gram of sample. This is equivalent to 6.03 millimoles of carboxyl per gram of sample or 1240 millimoles of carboxyl per mole of sample if the molecular weight of the sample is considered to be 206. The molecular weight of the monomer of poly-(2,3 erithraric acid glyoxylic acid acetal) is 206.

Since the pure monomer of poly-(2,3 erithraric acid glyoxylic acid acetal) contains 3000 millimoles of carboxyl per mole, and Oxidized Pectic Acid III contains 1240 millimoles of carboxyl per mole, the purity of Oxidized Pectic Acid III is near 40 per cent. The assumption is made that no acids or bases are present among the impurities.

Precipitation of Oxidized Pectic Acid III by Various Cations.

An attempt was made to find a cation capable of separating oxidized pectic acid from its oxidation reaction mixture. The requirements of such a cation are three. It must precipitate the desired product. It must not precipitate any of the impurities. It must be easily removeable from the product after precipitation. Alternate requirements are that it must not precipitate the desired product and that it must precipitate one or more of the impurities.

Salts of barium, cobalt, aluminum, and magnesium were tried.

Barium gave a large gelatinous precipitate from a clear solution of Oxidized Pectic Acid III. This precipitate did not redissolve when acetic acid was added. Barium also precipitated iodates and periodates, but not chlorates, iodides, or acetates.

Cobalt caused a slight turbidity when added to a clear solution of Oxidized Pectic Acid III. This turbidity disappeared when acetic acid was added. Cobalt precipitated periodate, but not iodate, chlorate, iodide, or acetate.

Aluminum caused a large flocculent precipitate when added to a clear solution of Oxidized Pectic Acid III. The aluminum salt is apparently very insoluble. The precipitate did not redissolve when acetic acid was added. Aluminum did not precipitate periodate, iodate, chlorate, iodide, or acetate.

Magnesium did not precipitate Oxidized Pectic Acid III or periodate, iodate, iodide, or acetate.

Of the ions tried only the aluminum ion appears capable of effecting an efficient separation of the oxidized pectic acid from its oxidation reaction mixture. To use aluminum, methods must be devised either to

reconvert the aluminum salt to the acid, or to utilize the material as an aluminum salt. The use of strong mineral acids to reconvert the aluminum salt to the acid is not recommended because of the danger of hydrolytic degradation of the product (Cf. page 34).

Dialysis of Oxidized Pectic Acid III.

An attempt to purify Oxidized Pectic Acid III by dialysis was made as follows. About 15 grams of Oxidized Pectic Acid III were suspended in 100 ml. of distilled water. This suspension was placed inside a non-moisture proof cellophane bag which was surrounded with distilled water. If the molecules of the inorganic impurities could pass through the small pores of the cellophane and the oxidized pectic acid molecules could not, a purification would be achieved.

After 24 hours the amount of water inside the bag had increased noticeably. The water outside the bag was tested by adding an aluminum chloride solution to small samples of it. The aluminum ion caused the formation of a white precipitate. The pH of one of the samples withdrawn from outside of the cellophane bag was 4.3 before adding the aluminum chloride and 2.7 after adding aluminum chloride. The possibility that the precipitate was aluminum hydroxide is therefore

eliminated. Inasmuch as the aluminum ion caused precipitation from a clear solution of Oxidized Pectic Acid III and not from periodate, iodate, chlorate, iodide, or acetate solutions, the formation of a white precipitate in this case strongly indicates the presence of Oxidized Pectic Acid III outside the cellophane bag.

A further test made was evaporation to dryness of a sample of the water from outside of the cellophane bag. The result was a black deposit which appeared to be carbonaceous.

Summary of Oxidized Pectic Acids.

None of the samples of oxidized pectic acid obtained were considered of sufficient purity to make calcium acetate determinations or carbon dioxide runs on them worthwhile. Most of the material obtained was used in attempts to find better methods of recovering it from the oxidation reaction mixtures as explained in the foregoing pages. A summary of the oxidized pectic acids prepared appears in Table VI.

TABLE V

Summary of Oxidized Celluloses

	Starting material	Oxidant First	Oxidant Second	Yield, Grams.	Yield, Per cent.	Calcium acetate value, millimoles of carboxyl per mole of anhydro unit.
Oxycell. I	Std. Cell. 34.4 grams	NO_2	---	35	102	331
Oxycell. II	Std. Cell. 22.44 grams	IO_4, ClO_2	---	8.1*	72*	744
Oxycell. III	Std. Cell. 15 grams	IO_4, ClO_2	---	---	---	361
Oxycell. IV	Oxycell. III 2.54 grams	(IO_4) (ClO_2)	NO_2	2.67	105	602
Oxycell. V	Oxycell. I 22.62 grams	(NO_2)	IO_4, ClO_2	1.44*	13*	384

* This material was divided into two approximately equal parts after the first stage of the periodate-chlorite oxidation. Data is presented for the portion oxidized by both periodate and chlorite. Percentage yield is based on one-half of the amount of starting material.

TABLE VI

Summary of Oxidized Pectic Acids

	Amount of oxidation, Per cent.	Yield	Purity of the yield
Oxidized Pectic Acid I	20	Fractions totaling 6.62 grams	Very impure
Oxidized Pectic Acid II	50	7.5 grams	Impure
Oxidized Pectic Acid III	100	20 grams	40 per cent

Methods of Analysis

The Calcium Acetate Determination of Total Carboxyl Content.

The calcium acetate determinations of total carboxyl content were made according to the procedure of Meesook and Purves[45]. Chemically pure calcium acetate was dissolved in enough boiling water to make an 0.5 N solution, which was boiled for a few minutes before cooling, filtration, and storage in a stoppered bottle. Samples of 0.2 to 0.6 grams of oxycellulose were immersed in 60 ml. or 75 ml. of the calcium acetate solution. Blanks were run. After twenty-four hours the mixtures were filtered and 50 ml. aliquots of the filtrate together with 50 ml. volumes of the blanks were titrated to pH 8.3 with 0.015 N sodium hydroxide solution. The results of the titration were converted into millimoles of carboxyl per gram of sample by the formula given on page 173.

Titration for Periodate in the Presence of Iodate.

The procedure used in titrating for periodates in the presence of iodates (Cf. page 20) was as follows: A sample, usually 10 ml., of the mixture to be analyzed, or a blank, 10 ml. of distilled water, was pipetted into

an Erlenmeyer flask. The following were then added in the order named: (1) about 2 grams of solid sodium bicarbonate; (2) a volume of 0.1 N sodium arsenite solution about 10 ml. in excess of the amount equivalent to the maximum amount of periodate to be found in the sample, measured from a burette; (3) an excess of 20 per cent potassium iodide solution. The contents of the flask was allowed to stand for ten minutes with occasional stirring and was then titrated with 0.1 N iodine solution using starch as an indicator. The 0.1 N sodium arsenite solution, the 0.1 N iodine solution,and the starch solution were made up, and the iodine solution was standardized according to the procedures of Willard and Furman[82].

The Carbon Dioxide Evolution Method of Uronic Acid Analysis.

To measure the amounts of carbon dioxide evolved by various substances under the conditions of the Lefèvre and Tollens uronic acid analysis, a duplicate of the apparatus of Whistler, Martin, and Harris[27] was constructed. A diagram of the apparatus appears in Figure 2, and a photograph in Figure 3. Referring to Figure 2, nitrogen, used as a carrier gas for the evolved carbon dioxide, flows from cylinder N and enters

the apparatus through an empty safety bottle A. It next passes through an alkaline solution of pyrogallol in bottle B. The inlet tube in this bottle is drawn out to a small orifice which produces fine bubbles. From B the gas passes through two absorption towers C filled with soda lime, into a second safety bottle D which is provided with a mercury manometer E. It then enters a 500 ml. reaction flask F by way of a side arm whose outlet is 5 to 10 mm. above the surface of the liquid in the flask. From the reaction flask the gas passes through a 40 cm. reflux condenser G and into a bubbling tower H containing concentrated sulfuric acid. The sulfuric acid serves to remove the interfering decomposition products which are carried over from the reaction flask. The gas next passes through the U-tube I which is filled with anhydrous copper sulfate, through the tube J, which contains phosphorus pentoxide, and finally through the valve K into one of the carbon dioxide absorption weighing bottles L containing ascarite backed by phosphorus pentoxide. During the run the gas stream is switched from one weighing bottle to the other by means of the valves K and M. The weighing bottles are connected to the apparatus by means of short pieces of surgical tubing to facillitate removal for weighing.

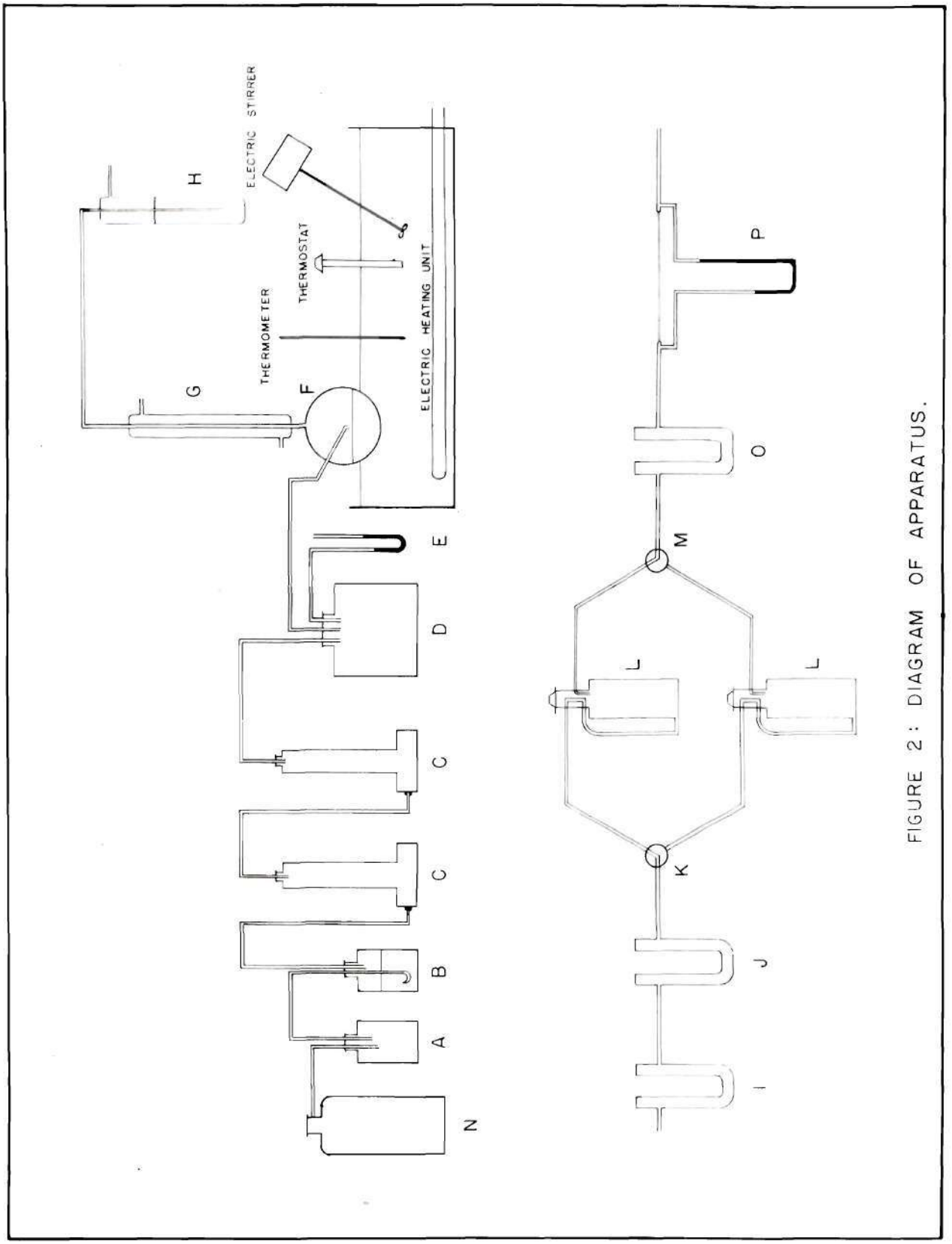


FIGURE 2: DIAGRAM OF APPARATUS.

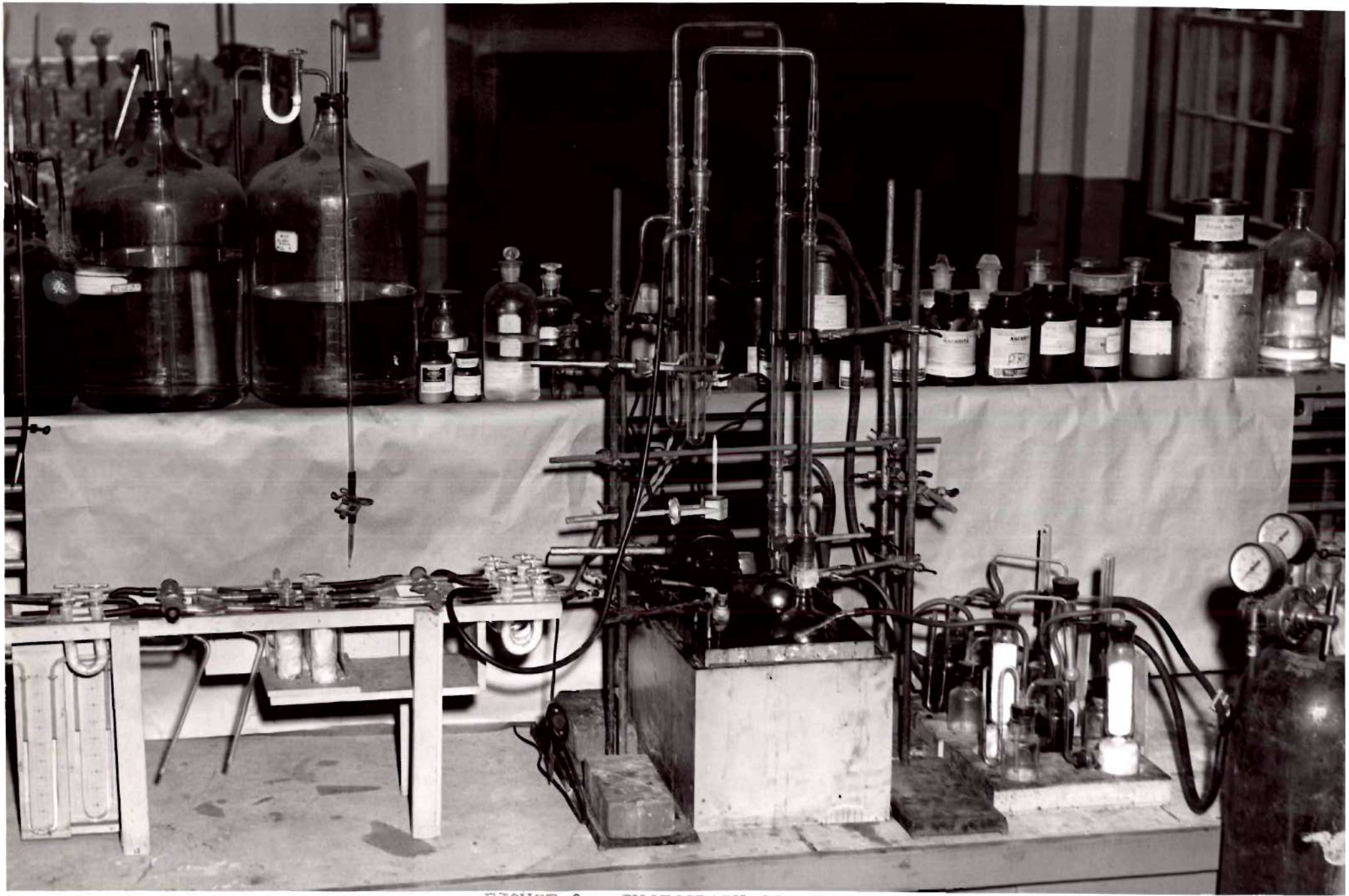


FIGURE 3: PHOTOGRAPH OF APPARATUS.

They are protected by a soda lime tube O which is followed by a calibrated flowmeter for estimating the rate of flow of nitrogen through the apparatus. The reaction flask is immersed in a peanut oil bath. Two electric immersion heaters, one of 500 and one of 1000 watts capacity and a thermostat maintain the operating temperature, 130 °C.

Two assemblies of the type described were employed simultaneously, the same source of carrier gas and the same oil bath being used for both. The sample to be analyzed was placed in the reaction flask with about 200 ml. of 3.288 ± 0.005 N hydrochloric acid. Since the rate of evolution of carbon dioxide is appreciably affected by variations in acid strength, the acid was carefully made up and standardized to be within 0.2 per cent of the standard of 3.290 N set by Whistler, Martin, and Harris. Varying the ratio of acid solution to sample within reasonable limits, however, did not affect the results. The optimum size of the sample depended upon the amount of carbon dioxide that it evolved. For materials which evolved small amounts of carbon dioxide, such as standard cellulose, at least 3 grams had to be used in order to obtain satisfactory results. The flask was placed in position

in the oil bath so that the oil level was 3 to 4 mm. lower than the liquid level inside the flask. This precaution was taken to prevent the baking of small bits of the sample splashed against the sides of the flask. In order to clear the apparatus of carbon dioxide, nitrogen at the rate of about 10 liters per hour was passed through the apparatus until the weighing bottles attained a constant weight. This operation took about 40 minutes. When the apparatus was free of carbon dioxide, the run was begun by turning on both heating units and bringing the temperature to 130 ^{o}C. For the first few hours weighings were made at half hourly intervals. The gas stream was switched from one weighing bottle to the other, and the bottle not in use was removed from the apparatus and weighed. After about four hours, weighings were made hourly, and later at longer intervals. The point of zero time was obtained from a plot of the data by extrapolation to zero of the amount of carbon dioxide evolved.

The main sources of error involved were as follows. High readings could be caused by improper absorption of moisture in the phosphorus pentoxide tube J resulting in moisture being absorbed in the weighing bottles and by dirt or dust adhering to the weighing

bottles. Low readings could be caused by improper absorption of carbon dioxide in the weighing bottles, due to channeling or by leaks in the gas train.

RESULTS AND DISCUSSION

Glucose

The results of the decarboxylation runs made on glucose are shown in Figure 4. The runs were made primarily to test the apparatus. Agreement with other investigators is as shown in Table VII.

TABLE VII

Carbon Dioxide Evolution from Glucose

Investigator	Data reported as	Mg. of CO_2 evolved per gram of glucose after five hours.
T. P. Nevell[37]	5.77 millimoles of CO_2 per 100 grams of glucose.	2.54
Whistler, Martin,[27] and Harris.	26.4 mg. of CO_2 per 10 grams of glucose.	2.64
This investigation	11.7 millimoles of CO_2 per mole of glucose.	2.87
Taylor, Fowler,[36] McGee, & Kenyon.	1.18 per cent by weight of CO_2 evolved after fifteen hours.	3.93

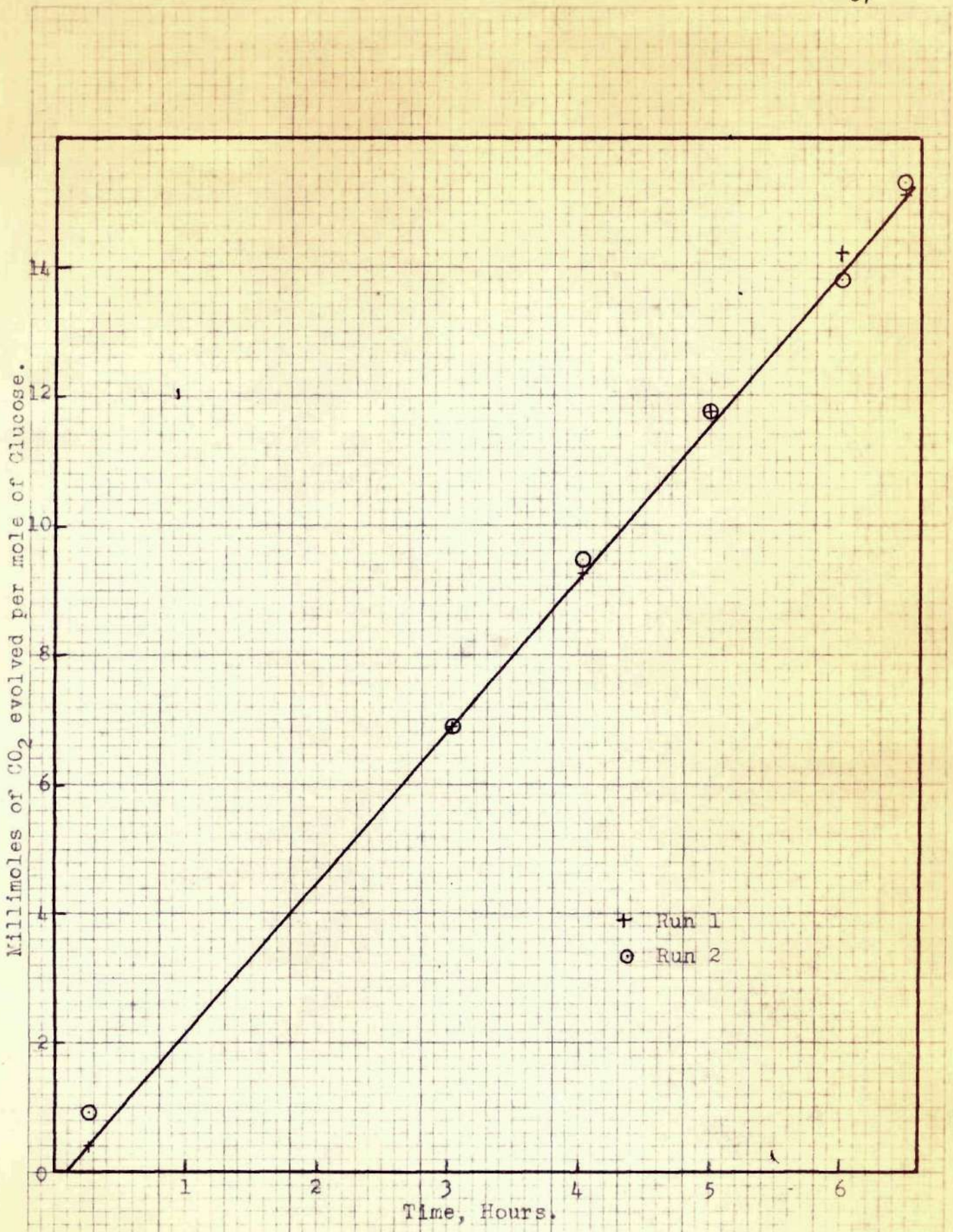


FIGURE 4: DECARBOXYLATION OF GLUCOSE.

Pectic Acid

The results of the decarboxylation runs on pectic acid are shown in Figure 5. The theoretical yield of carbon dioxide is 1000 millimoles per mole of anhydrogalacturonic acid, or, in other units, 250 milligrams of carbon dioxide per gram of polyanhydrogalacturonic acid. As shown in the figure, after seven hours only 852 millimoles of carbon dioxide per mole of anhydrogalacturonic acid, that is, 213 milligrams of carbon dioxide per gram of pectic acid were evolved. This agrees with the results of other investigators as is shown in Table VIII.

The decarboxylation runs on pectic acid show that carbon dioxide evolution does not cease after the initial period of rapid evolution of about seven hours. After seven hours carbon dioxide continues to be evolved at a constant rate of about 4 millimoles of carbon dioxide per mole of anhydrogalacturonic acid per hour. This rate is almost double the rate of carbon dioxide evolution from glucose.

TABLE VIII

Carbon Dioxide Evolution from Pectic Acid

Investigator	Data reported as	Mg. of CO_2 evolved per gram after seven hours.	Per cent of the theoretical evolution from polyanhydrogalacturonic acid.
T. P. Nevell[37]	270 millimoles of CO_2 per 100 grams of citrus pectin. (Value taken from a plot)	119	47.6
Whistler, Martin,[27] and Harris	208 milligrams of CO_2 per gram of pectin from cotton.	208	81.6
Taylor, Fowler,[36] McGee, & Kenyon	21 per cent by weight of CO_2 from pectic acid. (Value taken from a plot)	210	84.0
This investigation	852 millimoles of CO_2 per mole of anhydrogalacturonic acid.	213	85.2

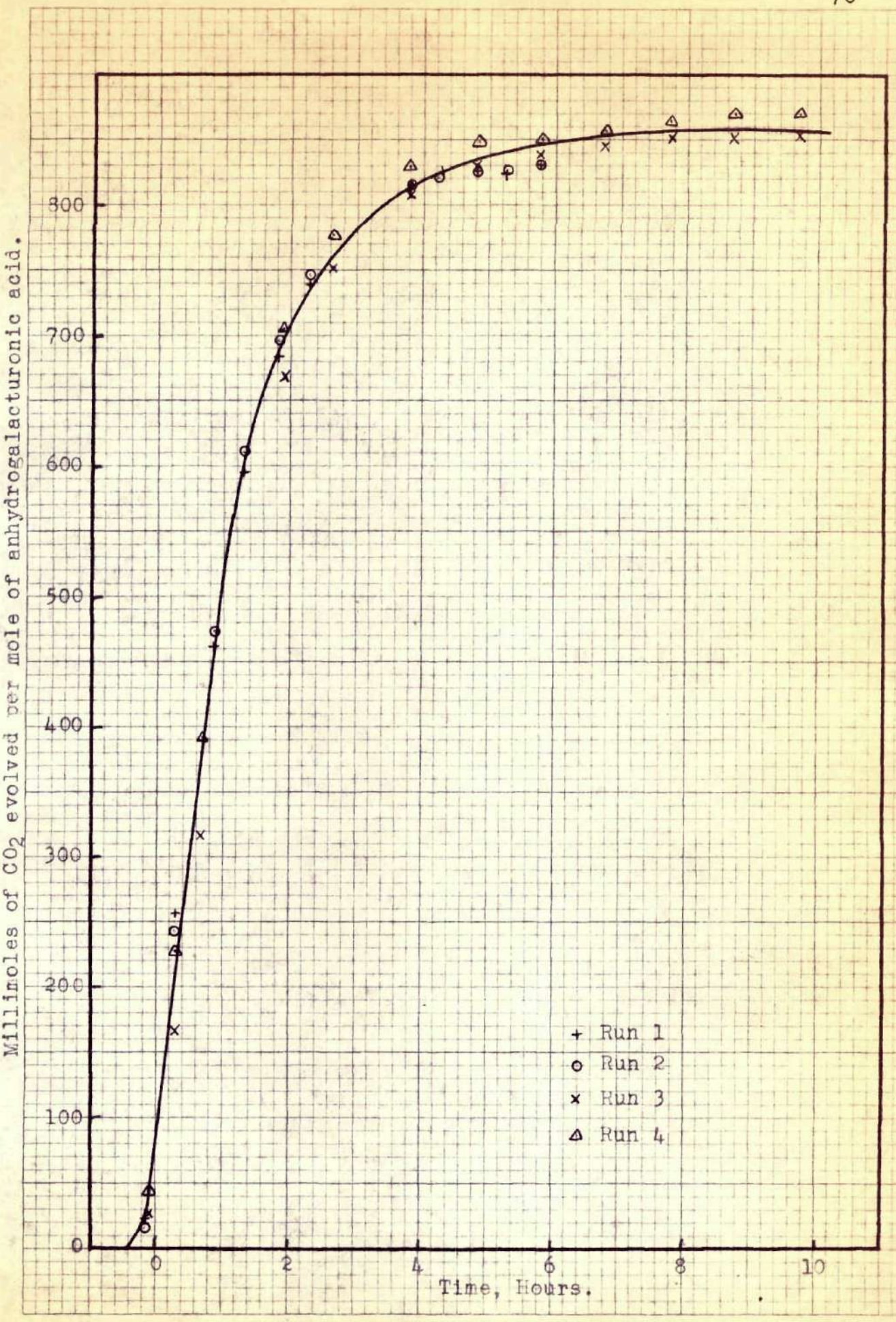


FIGURE 5: DECARBOXYLATION OF PECTIC ACID.

Standard Cellulose

The results of the decarboxylation runs on standard cellulose are given in Figure 6. For the initial period of 27 hours comparison with other investigators is as shown in Table IX.

TABLE IX

Carbon Dioxide Evolution from Cellulose

Investigator	Data reported as	Mg. of CO_2 evolved per gram of cellulose after ten hours.
T. P. Nevell[37]	3.45 millimoles of CO_2 per 100 grams of acid washed scoured cotton after 7 hours	2.17
This investigation	8.7 millimoles of CO_2 per mole of anhydroglucose after 10 hours	2.36
Whistler, Martin,[27] and Harris	1.9 milligrams of CO_2 per gram of purified cotton after 8 hours. (Value taken from plot)	2.38
Taylor, Fowler,[36] McGee, & Kenyon	0.81 per cent by weight of CO_2 from surgical gauze after 15 hours	5.4

After 27 hours there is a change in the rate of

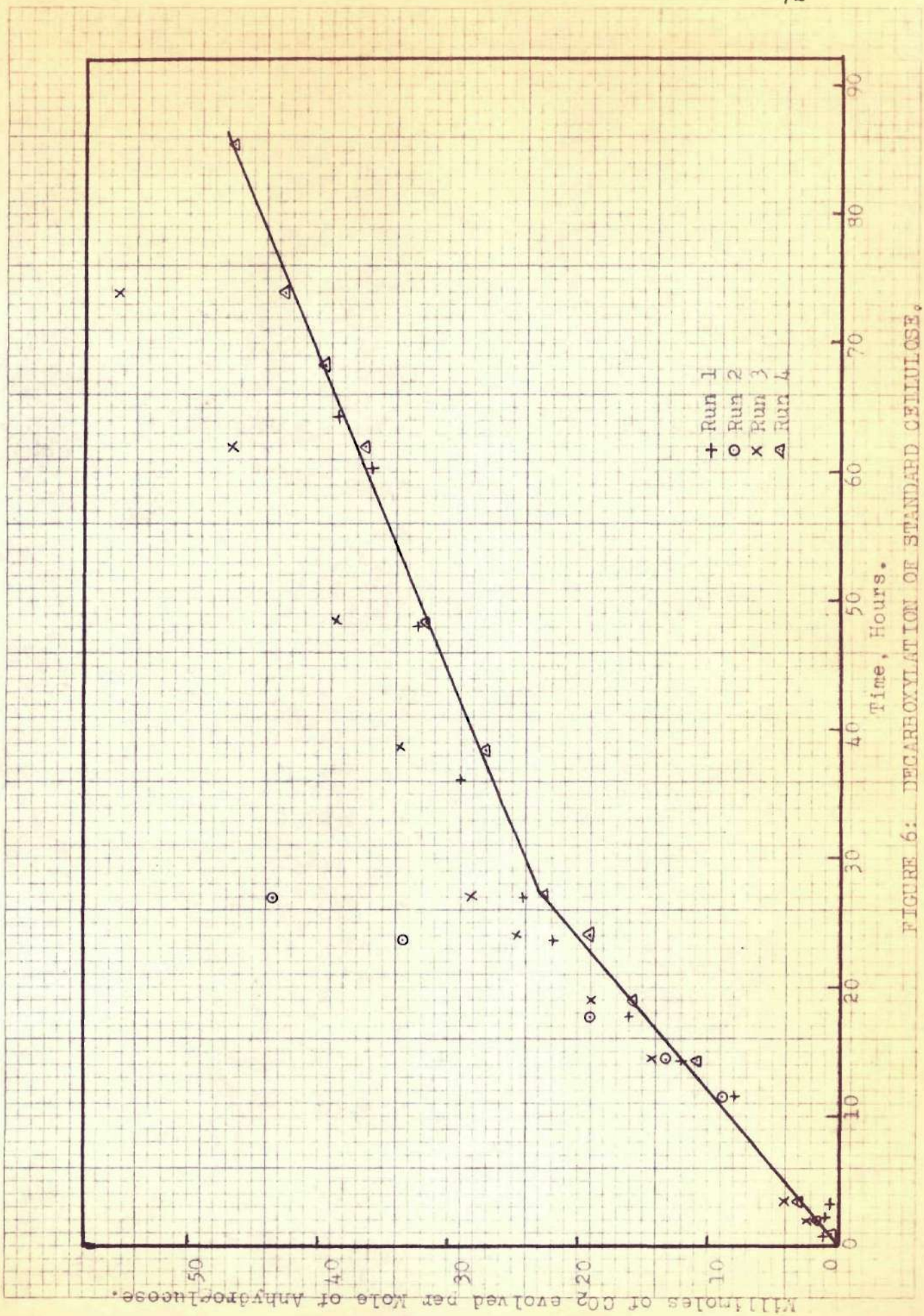


FIGURE 6: DECARBOXYLATION OF STANDARD CELLULOSE.

carbon dioxide evolution from approximately 0.87 millimoles per hour per mole of anhydroglucose to approximately 0.38 millimoles per mole of anhydroglucose per hour, a decrease of 56 per cent. (The rate of carbon dioxide evolution for glucose is 2.34 millimoles of carbon dioxide per mole of glucose per hour). This break in the curve occurs when approximately 24 millimoles of carbon dioxide have been evolved, that is, when one mole of carbon dioxide has been liberated for every 40 units of the cellulose chain. There are similar breaks in the carbon dioxide evolution curves of Oxycelluloses II and III which occur after approximately 30 and 20 millimoles of carbon dioxide per mole of anhydro unit respectively have been evolved.

Another break in the carbon dioxide evolution curve is noted by T. P. Nevell[37] and by Whistler, Martin, and Harris[27]. For a period of about three hours at the beginning of the run the carbon dioxide evolution rate is less than for the following 24 hours.

Oxidized Celluloses

Oxycellulose I.

Oxycellulose I was oxidized by nitrogen dioxide dissolved in carbon tetrachloride. The results of its decarboxylation are shown in Figure 7. The curve for Standard Cellulose is redrawn on Figure 7 for comparison. Of the two runs made Run 1 is considered to be the most accurate. The stepwise nature of the plot of Run 2 leads to the supposition that one of the weighing bottles was either leaking or not absorbing the carbon dioxide properly.

The carboxyl content of Oxycellulose I was estimated in three ways as given in Table X below.

TABLE X

Comparison of Estimates of the Carboxyl Content of Oxycellulose I

	Millimoles of carboxyl per mole of anhydro unit of Oxycellulose I
Estimated by the Calcium Acetate method.	331
Estimated from the conditions of oxidation according to the data of McGee, Fowler, Taylor, Unruh, and Kenyon[56].	approx. 500
Estimated from the decarboxylation run	300 - 450

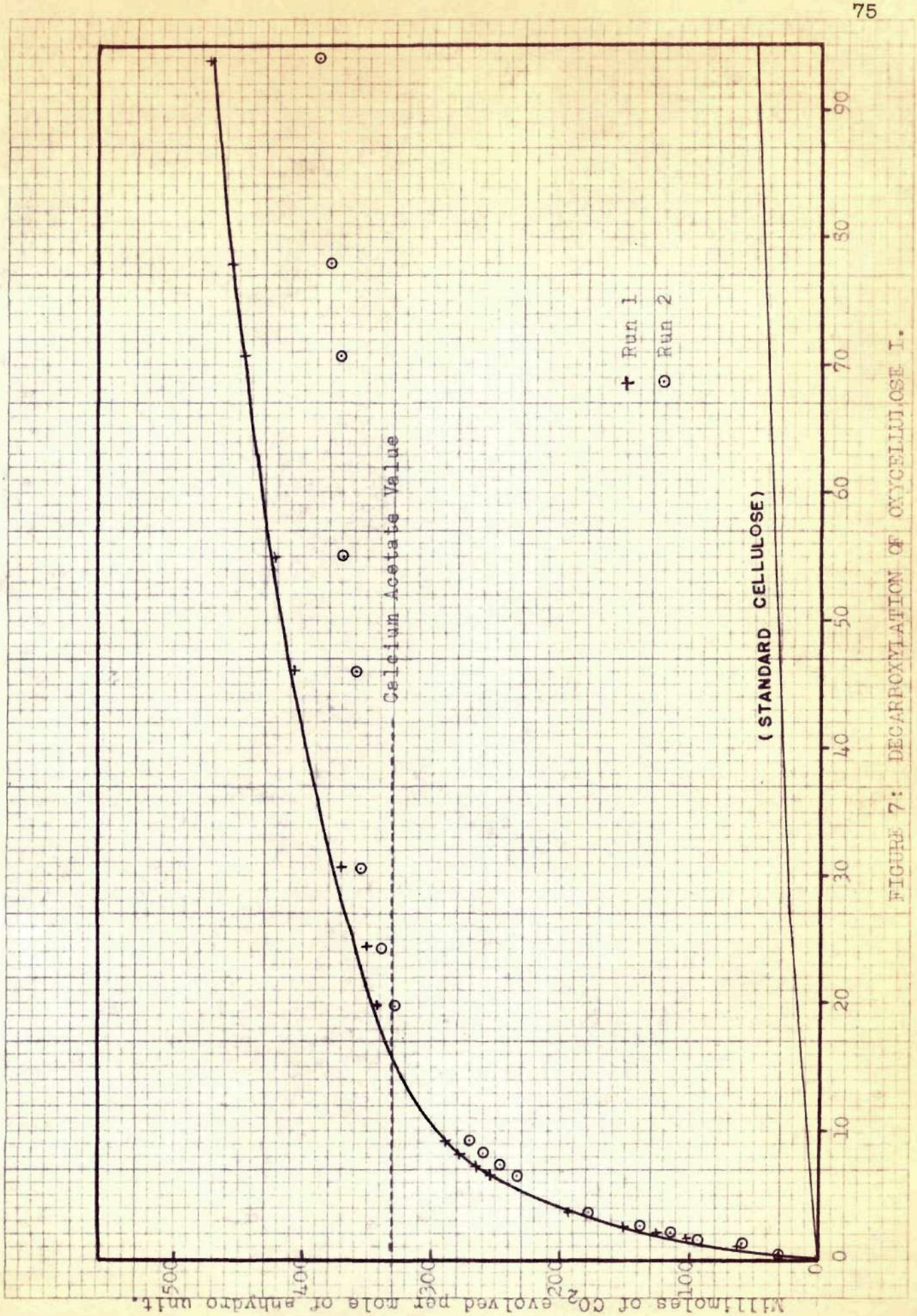


FIGURE 7: DECARBOXYLATION OF OXYCELLULOSE I.

The fact that the estimate from the conditions of oxidation is higher than either of the other two estimates can possibly be explained by the increased water solubility of the oxidized product. During the prolonged washing process the more oxidized product was preferentially leached out leaving a residue of lower net oxidation.

The indefiniteness of the estimate of the carboxyl content of Oxycellulose I obtained from the decarboxylation run illustrates a weakness of the Lefèvre and Tollens method. The plot shows a gradually changing curvature throughout the hundred odd hours of the run, there being no definite carbon dioxide evolution value after which the curve becomes straight. Alginic acid* gives decarboxylation results similar to Oxycellulose I in this respect. Inasmuch as the Lefèvre and Tollens method depends on finding a carbon dioxide evolution value after which the curve becomes relatively straight, a curve of the type obtained for Oxycellulose I does not give accurate results.

This raises a question as to whether the Lefèvre and Tollens method is applicable to all uronic acids.

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* See Table I, page 12, for references on all materials.

With glucuronic acid, for which the method was originally developed, with galacturonic acid, with ascorbic acid, and with polyanhydrogalacturonic acid (pectic acid) the results are definite. With polyanhydroglucuronic acid (e.g. Oxycellulose I, the oxycelluloses of Taylor, Fowler, McGee, & Kenyon[36]) and with polyanhydromannuronic acid (e.g. the alginic acids examined by T. P. Nevell[37] and Taylor, Fowler, McGee, and Kenyon[36]) the results are not definite.

Oxycellulose II and III.

Oxycelluloses II and III are both periodate-chlorite type oxycelluloses presumably oxidized to carboxyl only on the 2- and 3-carbon atoms. The results of the runs on these substances are given in Figures 8 and 9. The curves for Standard Cellulose and Oxycellulose III are redrawn on Figure 8 for comparison. The outstanding features of these plots are that they are much more nearly straight lines than the exponential type curves yielded by uronic acids and that the amount of carbon dioxide evolved is small. The amount of carbon dioxide evolved is significantly greater than that evolved by standard cellulose, however. T. P. Nevell[37] has observed similar results.

An attempt at correlating the rate of carbon

dioxide evolution with the total carboxyl content as given by the calcium acetate determination is as follows. For the first ten hours of the run on Oxycellulose II the rate of carbon dioxide evolution less the rate of carbon dioxide evolution given by standard cellulose was 0.35 per cent per hour of the total carboxyl present. For the second straight line portion of the curve of Oxycellulose III the corresponding figure is 0.33 per cent.

The results of T. P. Nevell[37] based on his methylene blue absorption determination of total carboxyl are not consistent with the above. Nevell's two periodate-chlorite oxycelluloses decarboxylated at rates (less the rate for standard cellulose) of 0.88 and 1.17 per cent per hour of the total carboxyl present.

Oxycellulose IV.

Oxycellulose IV is a sample of Oxycellulose III which was further oxidized by nitrogen dioxide gas. It therefore has carboxyl groups at the 2-, 3-, and 6-positions. The results of the runs made on Oxycellulose IV are given in Figure 10. The plot has the curvature to be expected from a celluronic acid.

There is a rough correspondence between the difference in the calcium acetate values of Oxycelluloses

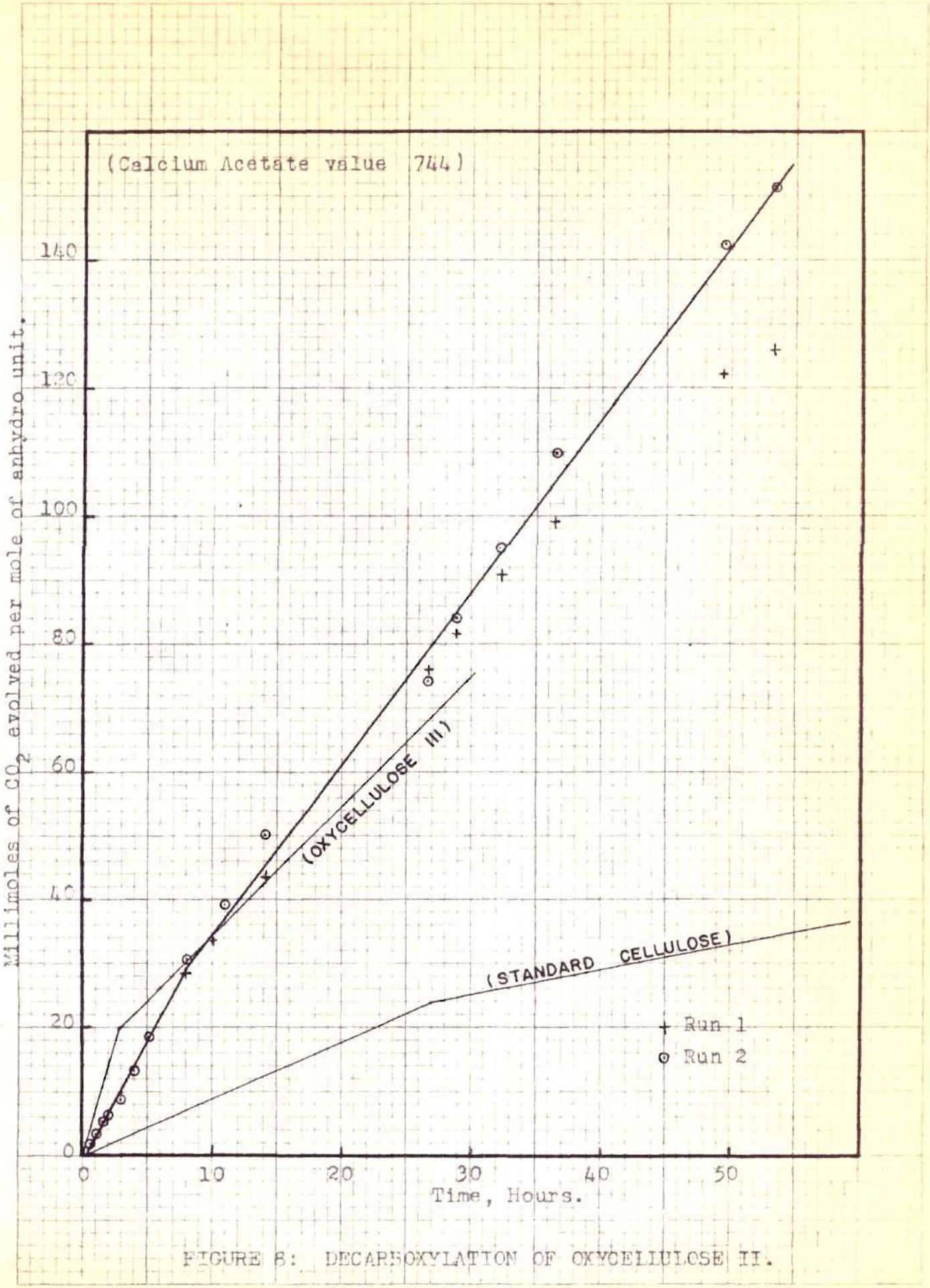

FIGURE 8: DECARBOXYLATION OF OXYCELLULOSE II.

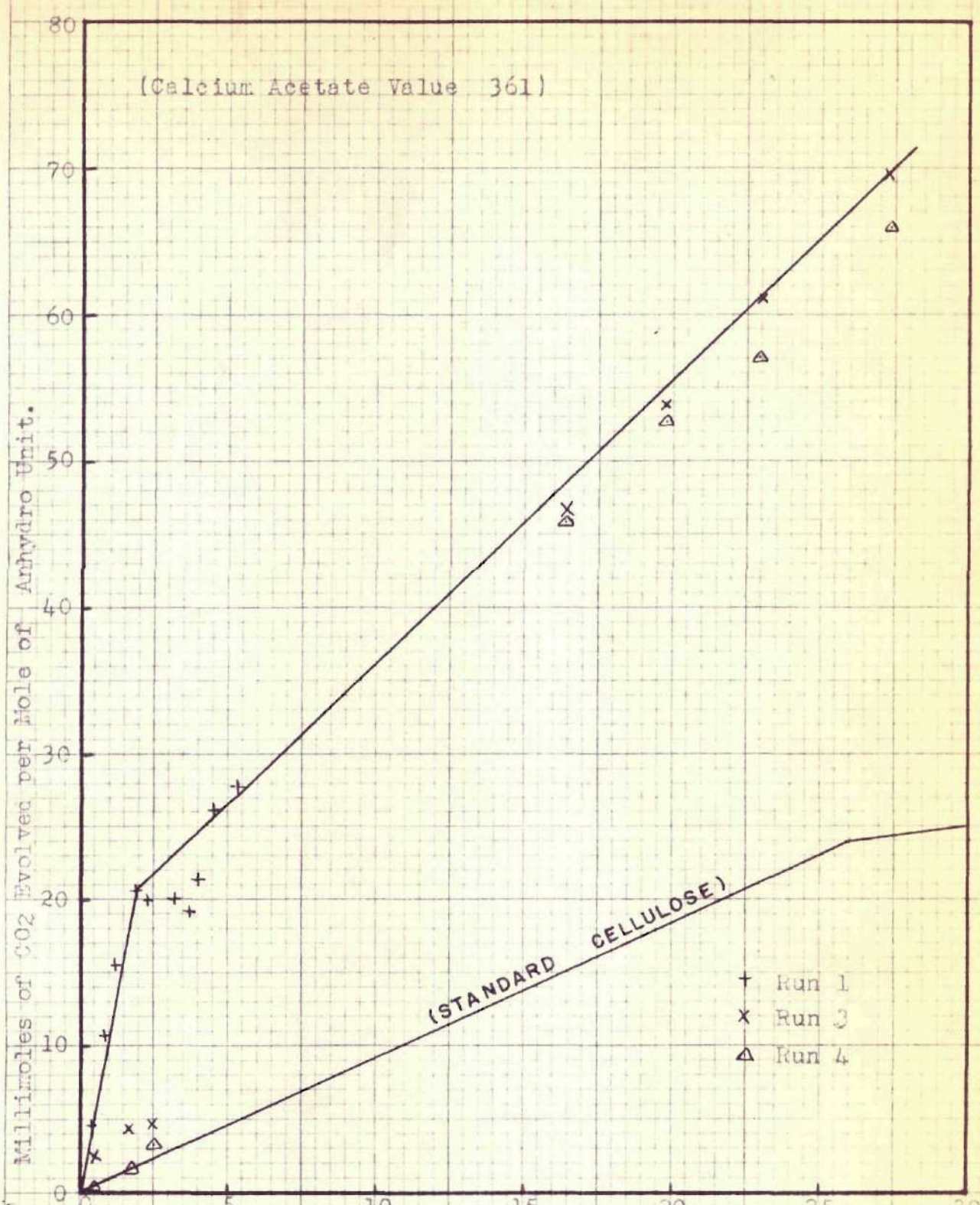

FIGURE 9: DECARBOXYLATION OF OXYCELLULOSE III

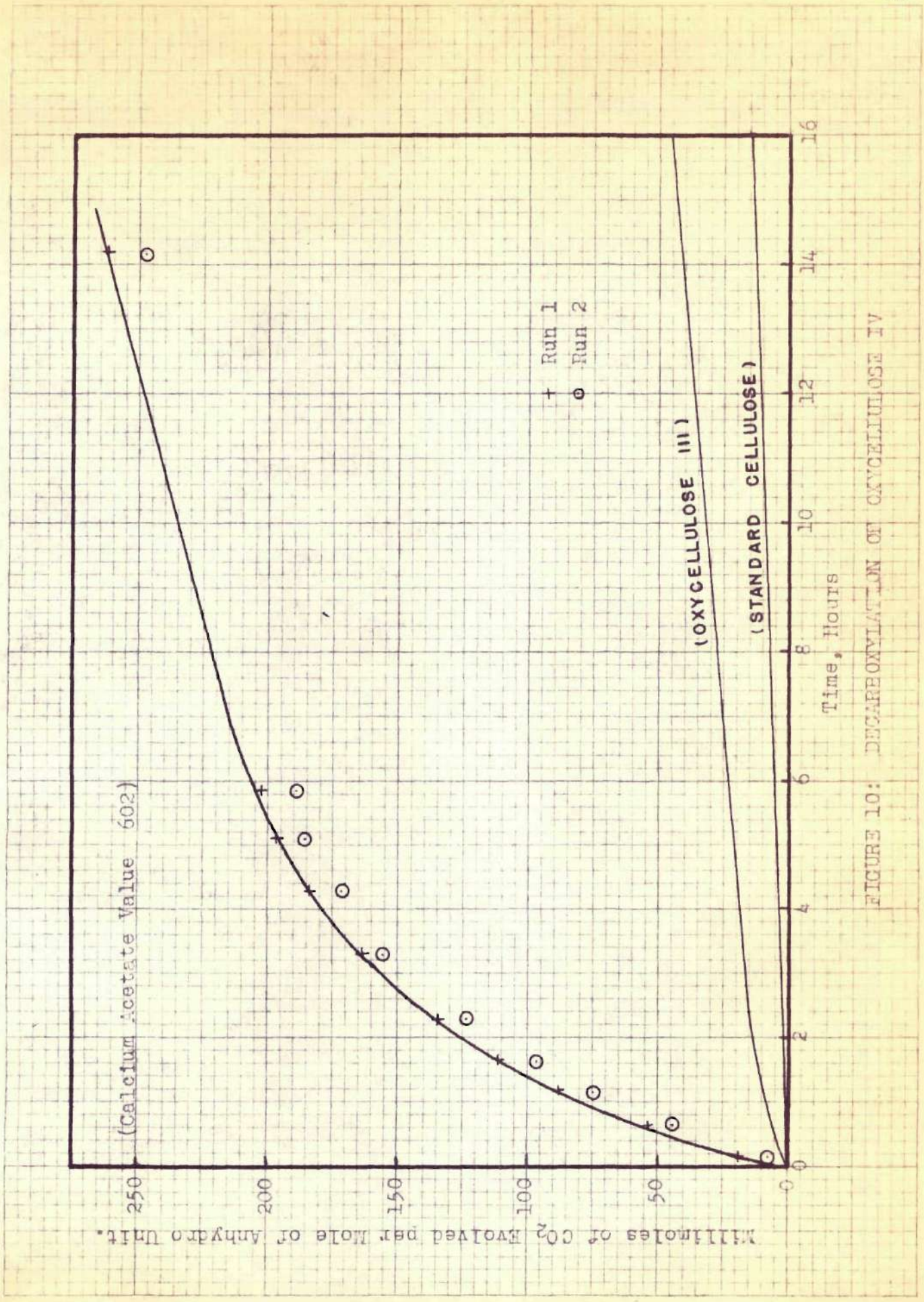

FIGURE 10: DECARBOXYLATION OF OXYCELLULOSE IV

III and IV and the difference in the carbon dioxide evolution values of Oxycelluloses III and IV. These differences, shown in Table XI, represent the amount of carboxyl added in the 6-position. These differences are not consistent with the approximately 10 per cent of uronic carboxyl (400 millimoles of carboxyl per mole of anhydro unit) which should have been formed by the nitrogen dioxide according to the data of Yackel and Kenyon[51]. The inconsistency may be due to selective leaching out of the more oxidized portions of the oxycellulose while washing it free of inorganic impurities.

Although Oxycellulose IV is a cellulose which has been oxidized at the 2- and 3-positions and at the 6-position, the results throw no new light on the possible interference with the decarboxylation of the carboxyl group at the 6-position by oxidation at the 2- and 3-positions. The reasons for this are as follows. Assuming that the calcium acetate determinations represent accurately the amounts of oxidation, Oxycellulose III was oxidized on less than one out of every five units of the anhydroglucose chain. The additional oxidation by nitrogen dioxide then attacked one out of every four units. If the attack by the oxidizing agents occurs

at random anywhere along the chain, one unit out of every 20 will then have been oxidized at all three positions. The distribution of oxidation of Oxycellulose IV is according to probability consideration,

1 unit out of 20 oxidized at the 2-, 3-, and 6-positions
3 units out of 20 oxidized at the 2- and 3-positions
4 units out of 20 oxidized at the 6-position
12 units out of 20 not oxidized.

The general characteristics of the decarboxylation of the three units oxidized at the 2- and 3-positions are already known from considerations of Oxycelluloses II and III and from other investigations. The general characteristics of the decarboxylation of the four units oxidized at the 6-position and the 12 units not oxidized are likewise already known. In Oxycellulose IV the unknown decarboxylation characteristic of the one unit oxidized at all three positions is completely masked.

If a cellulose were oxidized to the extent that every other unit was oxidized at the 2- and 3-positions and every other unit was oxidized at the 6-position, still only one-fourth of the units would have been oxidized at all three positions. In order, then, to obtain a substance which contains a sufficient amount of the tri-carboxy unit to determine the decarboxylation characteristics of the tri-carboxy unit, it is necessary to

TABLE XI

Correspondence Between Determinations on Oxycelluloses III & IV

	Calcium Acetate value, Millimoles of carboxyl per mole.	Carbon Dioxide evolution after 6 hours, Millimoles.	Carbon Dioxide evolution after 14 hours, Millimoles.
Oxycellulose IV	602	210	263
Oxycellulose III	361	28	44
	241	182	219

oxidize well over 50 per cent of the units by both periodate-chlorite and by nitrogen dioxide.

The above argument assumes that the oxidation of cellulose is a homogeneous reaction and that all anhydro units of the cellulose are equally accessible to attack by the oxidant in a perfectly random fashion. Such is not the case, some portions of the fiber being more accessible than others. This variation in accessibility would tend to increase the proportion of the units not oxidized at all and also increase the proportion of the units oxidized at all three positions.

Oxycellulose V.

Oxycellulose V was a sample of Oxycellulose I which was further oxidized by periodate and chlorite. The results of its decarboxylation are shown in Figure 11. The decarboxylation curve of Oxycellulose I is included in Figure 11 for comparison.

Oxycellulose I was oxidized by nitrogen dioxide to the extent that approximately one out of every three units of the chain had been attacked, based on its calcium acetate value of 331 millimoles of carboxyl per mole of anhydro unit. It was then oxidized by periodate and chlorite to the extent that approximately nine out of every ten units were attacked, thus adding 1800 milli-

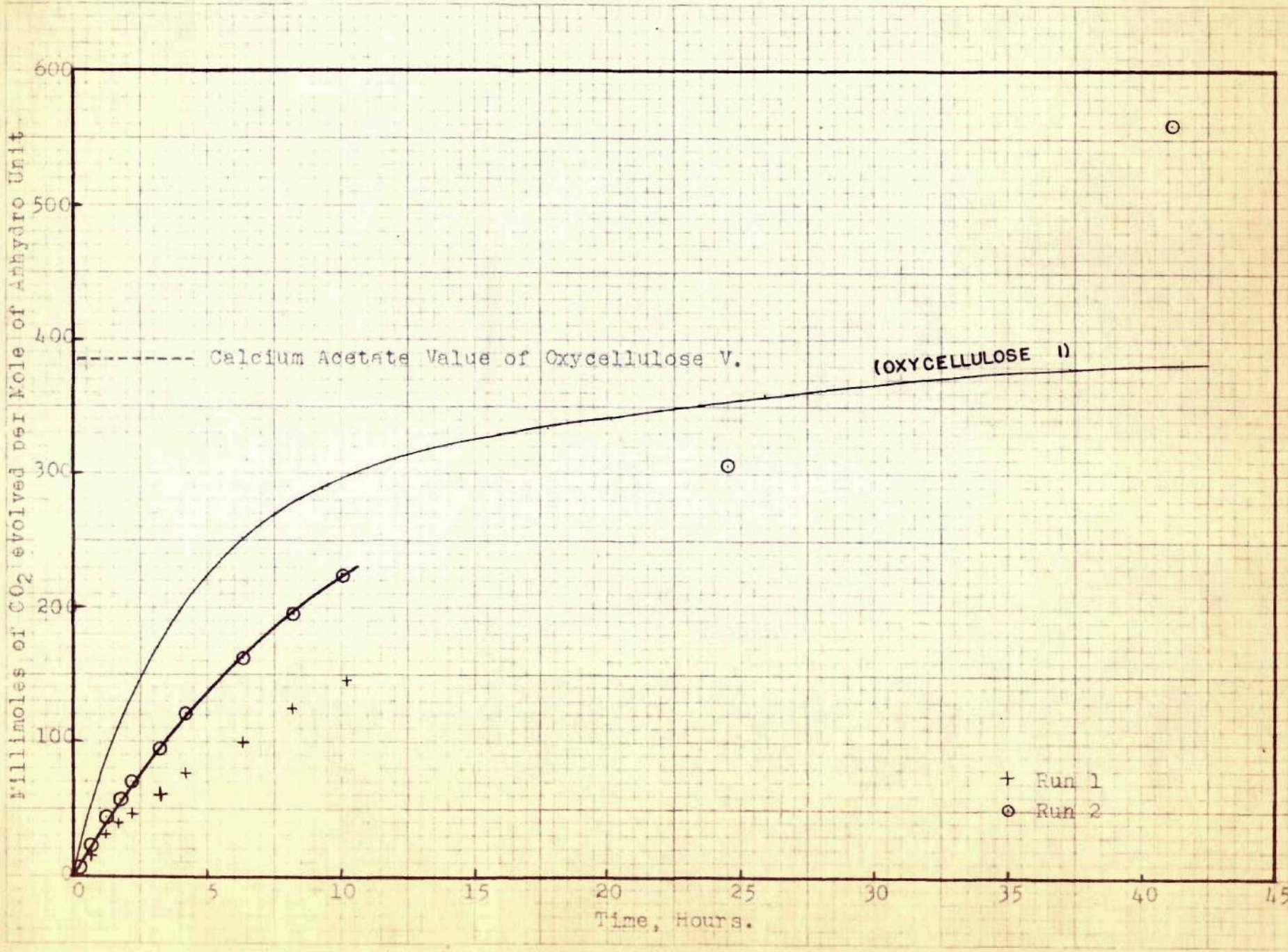

FIGURE 11: DECARBOXYLATION OF OXYCELLULOSE V.

moles of carboxyl per mole of anhydro unit. This is based on titration data of the periodate oxidation given in Figure 12. As a result of the periodate-chlorite oxidation, therefore, the calcium acetate value should have increased from 331 to 2131 millimoles of carboxyl per mole of anhydro unit. The actual increase was 53 millimoles of carboxyl per mole of anhydro unit as shown in Table XII. The amount of carbon dioxide evolved also should have increased. Instead of an increase there was a decrease as shown in Figure 11.

The explanation for the above discrepancy lies in the fact that as more carboxyl groups were added to the oxycellulose its water solubility increased. When Oxycellulose V was thoroughly washed with water to remove inorganic impurities, the oxidized product was also removed leaving behind that part of the cellulose which had been oxidized to a less extent. The fact that the cellulose was not uniformly oxidized is due to the topochemical nature of the reactions of cellulose.

TABLE XII

Correspondence Between Determinations

on Oxycelluloses I and V

	Calcium Acetate value, millimoles of carboxyl per mole.	Carbon Dioxide evolution after 6 hours, Millimoles.
Oxycellulose V	384	160
Oxycellulose I	331	255
Increase	53	- 95
Expected increase due to periodate-chlorite oxidation	1800	approximately 36 *

* This figure is one-third of one per cent per hour of the 1800 millimoles of carboxyl per mole of anhydro unit added by the periodate-chlorite oxidation. (Cf. discussion of Oxycellulose II and III)

Poly-(2,3 Erithraric Acid Glyoxylic Acid Acetal)*

After failing to prepare poly-(2,3 threaric acid glyoxylic acid acetal) from cellulose it was decided, rather than to continue with the cellulose, to use pectic acid as a starting material. Since pectic acid already has a carboxyl group in the 6-position, the nitrogen dioxide oxidation step is eliminated. The preparation of poly-(2,3 erithraric acid glyoxylic acid acetal) then consists of oxidizing pectic acid by periodate and chlorite and separating the oxidized product from the reaction mixture. The objective was to prepare a series of oxidized pectic acids oxidized on 20 per cent of the units, 50 per cent of the units, 80 per cent of the units, and 100 per cent of the units. From these it was hoped to obtain a family of decarboxylation curves showing progressively the effect of increased amounts of oxidation on the 2- and 3-carbon atoms. In all cases tried the oxidation proceeded as

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* Poly-(2,3 threaric acid glyoxylic acid acetal) is the substance which is obtained when the 2-, 3-, and 6-carbon atoms of cellulose are oxidized to carboxyl. Poly-(2,3 erithraric acid glyoxylic acid acetal) is the substance which is obtained when the 2-, and 3-carbon atoms of pectic acid are oxidized to carboxyl. See structural formulae on page 109.

expected and could be followed by titration, but in no case could a pure product be obtained from the reaction mixture.

Inasmuch as the material to be decarboxylated could not be purified, no results of decarboxylation studies were obtained from this part of the work. The results that were obtained consist of the experimental procedures devised which were unsuccessful or only partially successful in preparing the material and the ideas for other methods of attack which presented themselves. The experimental details will not be repeated here. Unpursued ideas for other methods of attack are given as suggestions for further work.

A conjecture at the decarboxylation characteristics of poly-(2,3 threaric acid glyoxylic acid acetal) can be made. Levene and Kreider[64] oxidized a polygalacturonide methyl ester with periodic acid and with bromine. They then hydrolyzed their product - poly-(2,3 threaric acid glyoxylic acid acetal) or its methyl ester - by refluxing with approximately 0.25 N sulfuric acid for thirteen hours and obtained levo-tartaric acid (threaric acid). Jackson and Hudson[66] hydrolyzed periodic acid oxidized cornstarch and cotton cellulose by heating to 99 ^{o} C. for sixteen hours with 0.1 N

hydrochloric acid and obtained glyoxal and d-erythrose. Since 0.25 N sulfuric acid at about 100 ° C. and 0.1 N hydrochloric acid at 99 ° C. were effective in hydrolyzing the acetal linkages in the above mentioned compounds, it is probable that 3.29 N (12 per cent) hydrochloric acid at 130 ° C. would quickly hydrolyze the acetal linkages in poly-(2,3 erithraric acid glyoxylic acid acetal) and poly-(2,3 threaric acid glyoxylic acid acetal). If this is so, poly-(2,3 threaric acid glyoxylic acid acetal) would be indistinguishable in a carbon dioxide evolution analysis from an equimolar mixture of threaric acid (levo-tartaric acid) and glyoxylic acid.

Tartaric acid gives a decarboxylation curve which is a straight line, the carbon dioxide being evolved at a rate of 0.70 millimoles of carbon dioxide per mole of tartaric acid per hour*. Glyoxylic acid gives a decarboxylation curve which is a straight line, the carbon dioxide being evolved at a rate of 1.34 millimoles of carbon dioxide per mole of glyoxylic acid per hour.** The carbon dioxide evolution curve of poly-(2,3

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* The data is reported as 0.44 weight per cent of carbon dioxide evolved in 21.5 hours from a tartaric acid of melting point 141 - 143 ° C. [36]

** The data is reported as 1.83 weight per cent of carbon dioxide evolved from glyoxylic acid in 23 hours[36].

threaric acid glyoxylic acid acetal) will then probably be found to be also a straight line, carbon dioxide being evolved at the rate of approximately 2.04 millimoles of carbon dioxide per mole of poly-(2,3 threaric acid glyoxylic acid acetal) per hour.

SUGGESTIONS FOR FURTHER WORK

Applicability of the Lefèvre and Tollens Method.

It has been shown on page 77 that whereas for galacturonic acid, glucuronic acid, polyanhydrogalacturonic acid, and ascorbic acid the Lefèvre and Tollens method of uronic acid estimation gives satisfactory results, for polyanhydroglucuronic and polyanhydromannuronic acids the results are inaccurate. The question arises as to whether the method gives accurate results for other uronic acids.

Decarboxylation of Glucose.

A prolonged carbon dioxide evolution run on glucose may show a change in rate of carbon dioxide evolution which can be correlated with the change in carbon dioxide evolution rate shown by cellulose after 27 hours.

Degradation of Cellulose by Boiling 12 per cent Hydrochloric acid.

A study of the rates of degradation of cellulose by boiling 12 per cent hydrochloric acid may yield information which can be related to the rates of carbon dioxide evolution from glucose. Such information would

be valuable in studying the mechanism of the cellulose decarboxylation reaction and might throw additional light on the structure of the cellulose fiber.

Decarboxylation of Periodate-Chlorite Oxycelluloses.

Further decarboxylation runs on periodate-chlorite oxycelluloses may yield a definite correlation between the rate of carbon dioxide evolution and the total carboxyl content.

Preparation of Oxycelluloses of High Degrees of Oxidation

In preparing oxycelluloses of high degrees of oxidation the water solubility of the product makes it impossible to remove inorganic impurities by washing with water. Some of the methods applied to or suggested for oxidized pectic acids may be applicable to the preparation of oxycelluloses of high degrees of oxidation.

Preparation of Poly-(2,3 Threaric Acid Glyoxylic Acid Acetal).

Methods of purifying pectic acid oxidized by periodate and chlorite which may be successful are:

Some metallic ion may be found which will selectively precipitate the oxidized pectic acid from the

oxidation reaction mixture and which may then be easily removed from the oxidized pectic acid.

Some combination of solvents may be found which selectively precipitates the oxidized pectic acid from the reaction mixture more efficiently than the alcohol-acetic acid mixture already tried. In this connection an investigation would be made of the optimum pH conditions for most efficient selective precipitation with least hydrolysis of the product.

In precipitating by adding organic solvents, sodium iodate appeared to be the most troublesome impurity since its solubility characteristics and the solubility characteristics of the oxidized pectic acid are similar. It may be possible to precipitate oxidized pectic acid in fairly pure form after first adding a reducing agent to reduce iodates and chlorates to iodides and chlorides.

Use of Impure Oxidized Pectic Acid.

Instead of attempting to obtain pure poly-(2,3 threaric acid glyoxylic acid acetal) useful information may be obtained by making decarboxylation runs on impure samples of this substance. Before doing this the impure poly-(2,3 threaric acid glyoxylic acid acetal) will have to be analyzed to determine the kind and amount of

impurities present, and decarboxylation runs will have to be made on substances of known decarboxylation characteristics to which known amounts of these impurities have been added in order to determine any possible effect that the impurities may have on the decarboxylation. The effect of impurities on other measurements of total carboxyl content necessary for correlation with decarboxylation results will also have to be considered.

Use of Salts of Oxidized Pectic Acid.

Experimental evidence indicates that salts of poly-(2,3 threaric acid glyoxylic acid acetal), particularly aluminum salts, may be precipitated from the oxidation reaction mixture without precipitation of inorganic impurities. Difficulties, however, are encountered in converting the salts back to the organic acid. There is a possibility that the substance may be used as the salt. To do this the effect of the metallic ion upon decarboxylation must be determined. Since existing methods for determining total carboxyl content are not applicable when the carboxyl group is present as a salt, a check method of determining total carboxyl content must be devised.

Use of Other Materials.

Other materials may be substituted for pectic acid, sodium periodate, and chlorous acid and similar results be obtained. Alginic acid might be used as a starting material. Lead tetraacetate, for instance, may be substituted for sodium periodate, and bromine for chlorous acid. By making some or all of these substitutions some of the difficulties encountered in the periodate-chlorite oxidation of pectic acid may be avoided.

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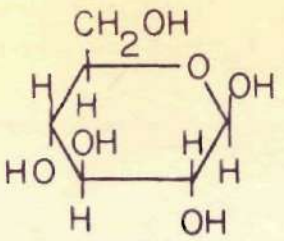
APPENDIX

STRUCTURAL FORMULAE



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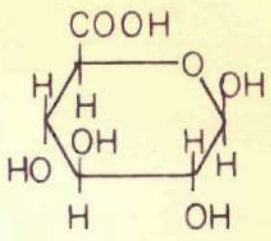

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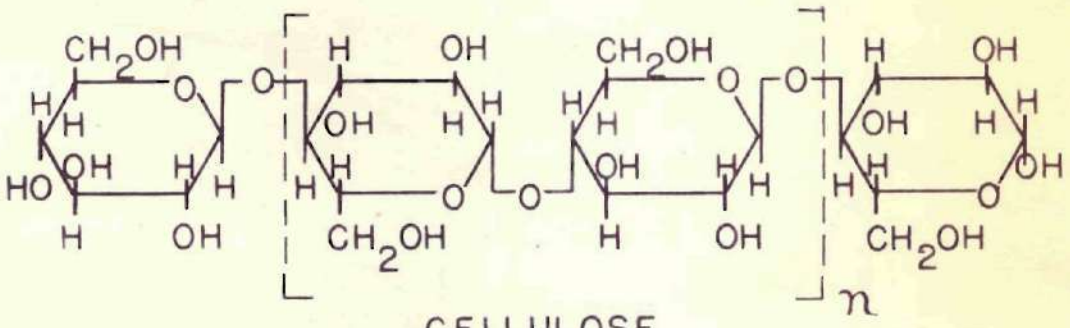

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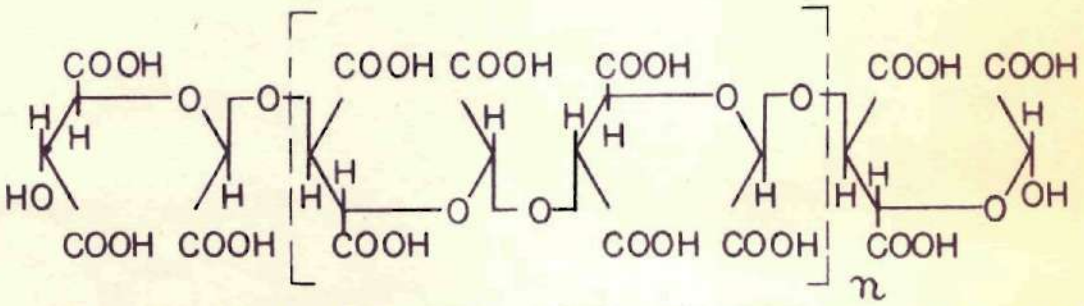

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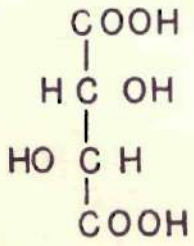
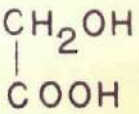


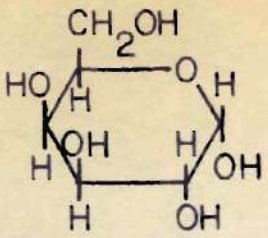
THREARIC ACID
(DEXTRO—TARTARIC ACID)



GLYOXYLIC ACID

ERITHRARIC ACID

STRUCTURAL FORMULAE (CONTINUED)



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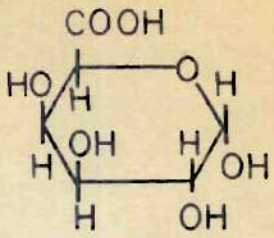

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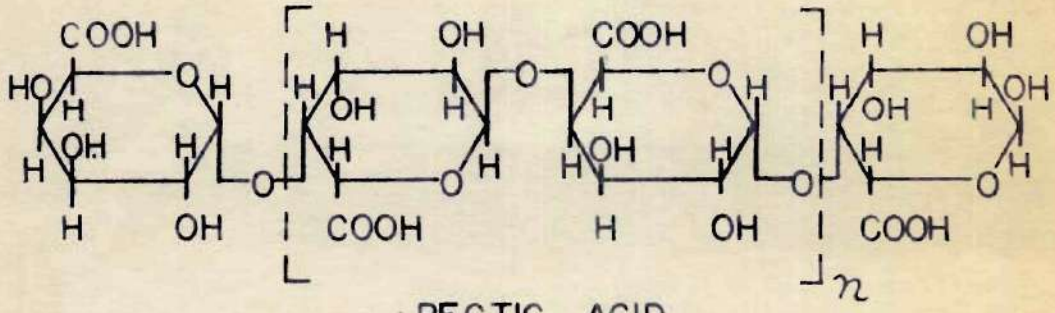
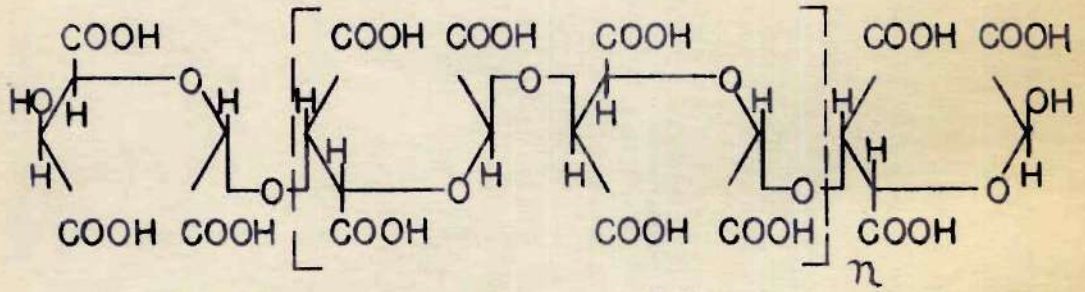
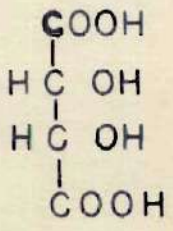


PECTIC ACID



POLY—(2,3 ERITHRARIC ACID GLYOXYLIC ACID ACETAL)



ERITHRARIC ACID
(MESO—TARTARIC ACID)

TABLE XIII

Titration Data, Periodate Oxidation of Standard Cellulose

Weight of cellulose, 23.77 grams.

Moisture content of cellulose, 5.6 per cent.

Volume of reaction mixture, 1800 ml.

Volume of samples withdrawn, 10 ml.

Normality of iodine solution, 0.1028 equivalents per liter.

Reaction Time, Hours.	Blank, Ml. of iodine solution	Titre I, Ml. of iodine solution	Titre II, Ml. of iodine solution	Fraction of anhydro-glucose units attacked. (Calculated)	Millimoles of aldehyde formed per mole of anhydroglucose. (Calculated)
0	37.90	15.69	15.67	0.000	0
1.5		16.48	16.48	0.054	107
4.5		17.22		0.103	206
12.0	34.22		15.39	0.226	453
25.0	34.25	17.20	16.80	0.318	636
50.0	34.32	20.82	20.66	0.578	1156
54.4		21.20	21.30	0.611	1222

TABLE XIV

Titration Data, Periodate Oxidation of Oxycellulose I

Weight of Oxycellulose I, 22.62 grams.

Moisture content of Oxycellulose I, dry.

Calculated molecular weight of anhydro unit of Oxycellulose I, 167.

Volume of reaction mixture, 1800 ml.

Volume of samples withdrawn, 10 ml.

Normality of iodine solution, 0.1028 equivalents per liter.

Reaction Time, Hours.	Blank, Ml. of iodine solution	Titre I, Ml. of iodine solution	Titre II, Ml. of iodine solution	Fraction of anhydro units of Oxycellulose I attacked. (Calculated)	Millimoles of aldehyde formed per mole of anhydro units of Oxycellulose I. (Calculated)
0	37.90	15.69	15.67	0.000	0
1.5		17.57	17.63	0.131	262
4.5		18.55		0.196	392
12.0	34.22	16.60	16.53	0.312	623
25.0	34.25	19.61	19.67	0.519	1038
50.0	34.32	26.90	27.52	1.010	2020
54.0		25.75	25.72	0.932	1864

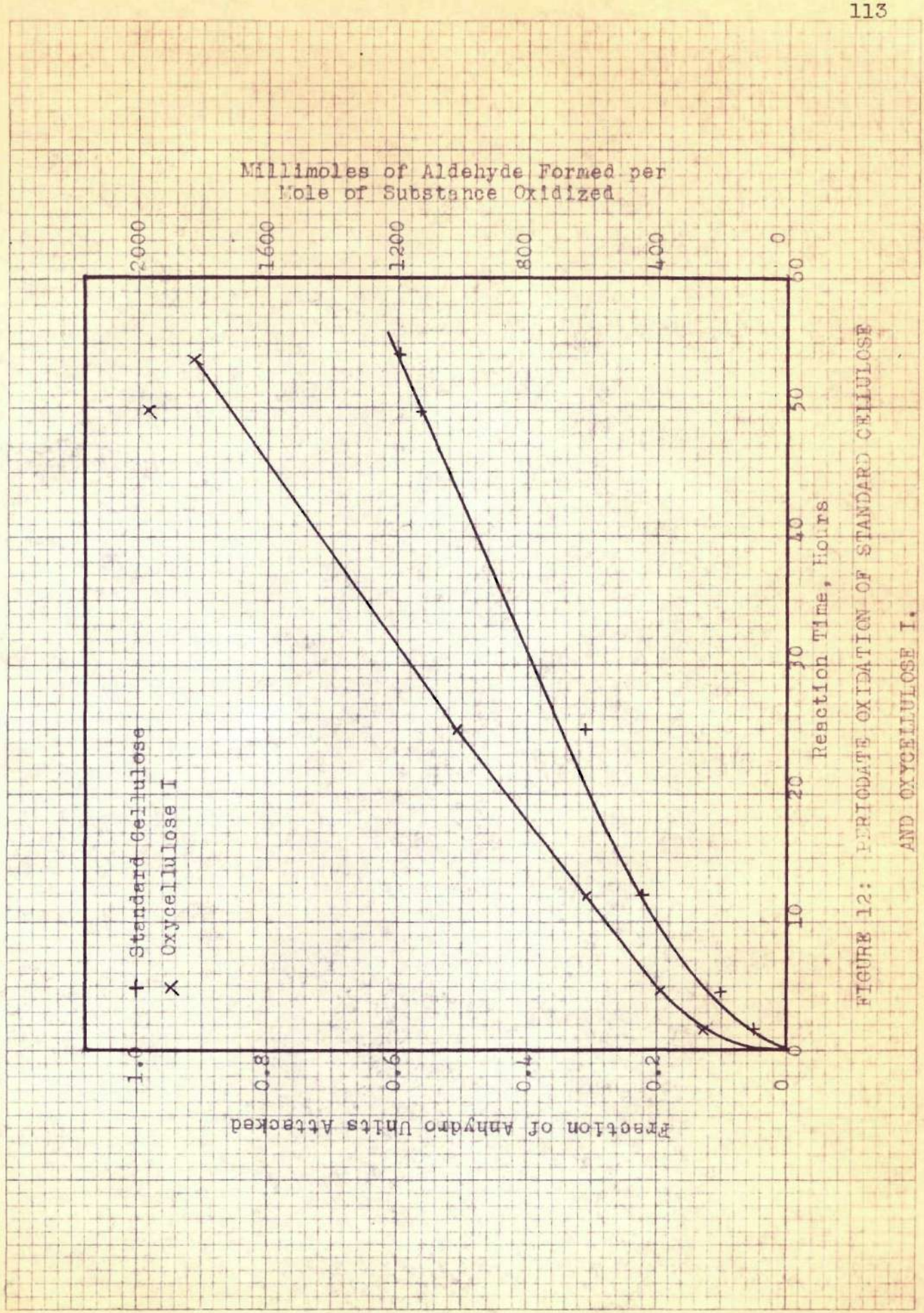


FIGURE 12: PERIODATE OXIDATION OF STANDARD CELLULOSE AND OXYCELLULOSE I.

TABLE XV

Titration Data, Periodate Oxidation of Pectic Acid, I.

Weight of Pectic Acid, 12 grams.

Moisture content of pectic acid, 13 per cent.

Volume of reaction mixture, 200 ml.

Volume of samples withdrawn, 5 ml.

Normality of iodine solution used, 0.102 equivalents per liter.

Reaction Time, Hours	Blank, Ml. of iodine solution	Titre, Ml. of iodine solution	Fraction of anhydrogalacturonic acid units attacked. (Calculated)	Millimoles of aldehyde formed per mole of anhydrogalacturonic acid. (Calculated)
0.0	10.30	4.35	0.000	0
0.1		7.35	0.103	206
0.5		8.70	0.150	299
1.5		10.05	0.196	392
2.5		10.15	0.200	399
3.5		10.35	0.205	413

TABLE XVI

Titration Data, Periodate Oxidation of Pectic Acid II.

Weight of pectic acid, 30 grams.

Moisture content of pectic acid, 13 per cent.

Volume of reaction mixture, 475 ml.

Volume of samples withdrawn, 10 ml.

Normality of iodine solution used, 0.098 equivalents per liter.

Time	Blank, Ml. of iodine solution.	Titre, Ml. of iodine solution.	Normality of periodate solution. (Calculated)	Fraction of anhydrogalacturonic acid units attacked. (Calculated)	Millimoles of aldehyde formed per mole of anhydrogalacturonic acid. (Calculated)
Before adding acetic acid	41.8	12.1	0.291		
Immediately after addition	43.0	13.1	0.293		
Two hours after addition	43.1	13.5	0.290		
Immediately before oxidation	42.2	13.0	0.286	0.000	0
Thirty minutes after oxidation begins	42.6	34.9	0.075	0.344	688

TABLE XVI, Continued

Titration Data, Periodate Oxidation of Pectic Acid II.

Time	Blank, Ml. of iodine solution.	Titre, Ml. of iodine solution.	Normality of periodate solution. (Calculated)	Fraction of anhydrogalacturonic acid units attacked. (Calculated)	Millimoles of aldehyde formed per mole of anhydrogalacturonic acid. (Calculated)
One hour after oxidation begins	42.6	41.0	0.014	0.465	930

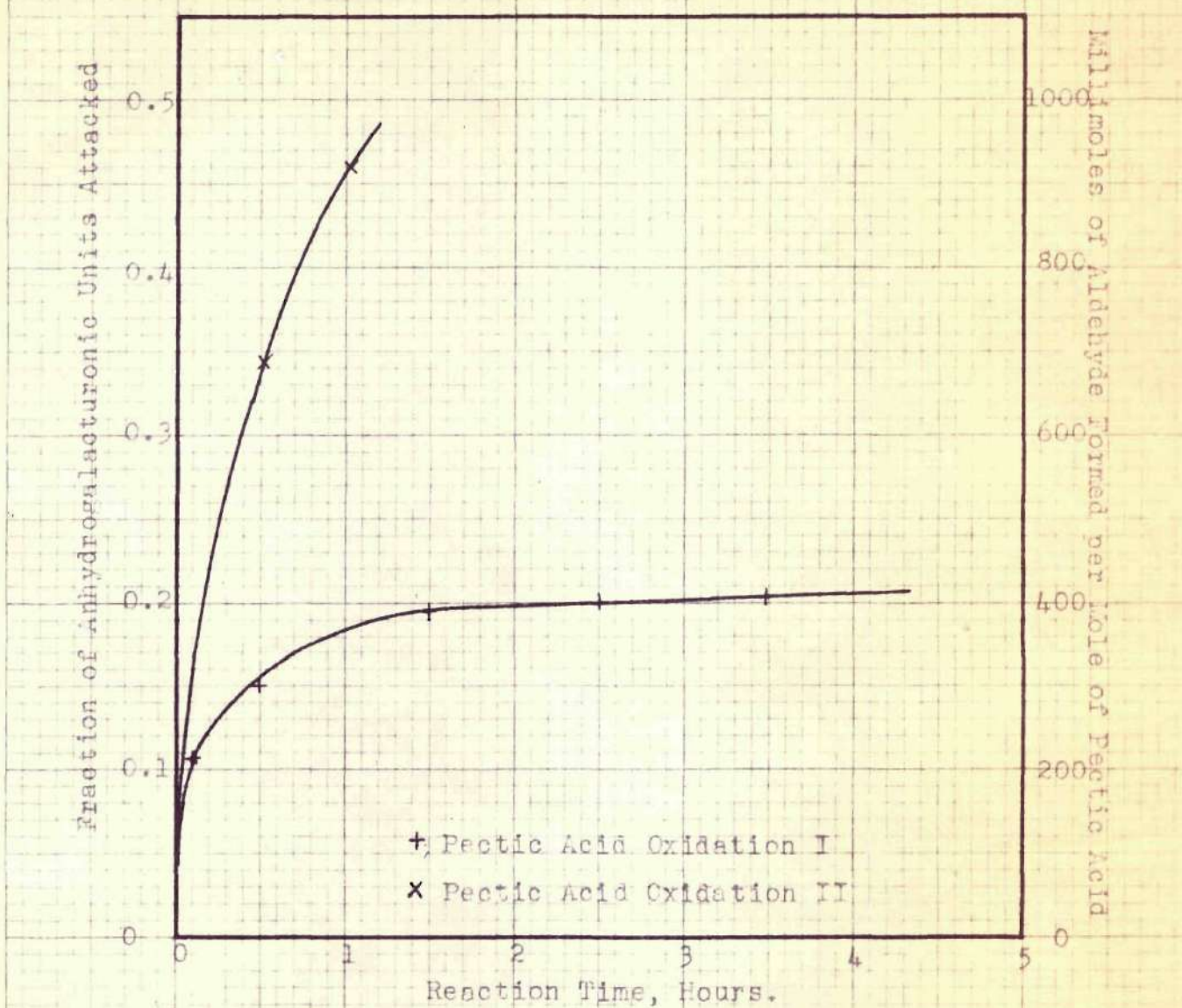


FIGURE 13: PERIODATE OXIDATION OF PECTIC ACID.

TABLE XVII

Carbon Dioxide Evolution Data, Glucose, Run 1.

Weight of glucose, 5.0472 grams.

Moisture content of glucose, 0.08 per cent.

Number of moles of glucose, 0.0280. (Calculated)

Clock Time	Run Time, Hours	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of glucose. (Calculated)
4:15 pm		65	(95.6532)#	102.4845		
4:55 pm		130	95.6575			
5:20 pm	0.00*	130			0.0	0.0
5:30 pm	0.17	130		102.4850	0.5	0.4
8:15 pm	2.95	130	95.6655		8.5	6.9
9:15 pm	3.95	130		102.4880	11.5	9.3
10:15 pm	4.95	130	95.6685		14.5	11.8
11:15 pm	5.95	130		102.4912	17.7	14.3
11:48 pm	6.47	130	95.6695		18.7	15.2

* Zero time was estimated by extrapolation to zero of the weight of carbon dioxide evolved.

\# Results of weighings made at the beginning of a run and not used in determining the amount of carbon dioxide evolved are enclosed in parenthesis.

TABLE XVIII

Carbon Dioxide Evolution Data, Glucose, Run 2.

Weight of glucose, 4.7992 grams.

Moisture content of glucose, 0.08 per cent.

Number of moles of glucose, 0.02665. (Calculated)

Clock Time	Run Time, Hours	Bath temp., °C.	Weight of weighing bottle 5, Grams.	Weight of weighing bottle 6, Grams.	Weight of CO_2 evolved Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of glucose. (Calculated)
4:15 pm		65	(92.0580)	101.2760		
4:55 pm		130	92.0705			
5:20 pm	0.00	130			0.0	0.0
5:30 pm	0.17	130		101.2770	1.0	0.9
8:15 pm	2.95	130	92.0776		8.1	6.9
9:15 pm	3.95	130		101.2800	11.1	9.5
10:15 pm	4.95	130	92.0803		13.8	11.8
11:15 pm	5.95	130		101.2825	16.3	13.9
11:48 pm	6.47	130	92.0820		18.0	15.4

TABLE XIX

Carbon Dioxide Evolution Data, Pectic Acid, Run 1.

Weight of pectic acid, 1.2050 grams.

Moisture content of pectic acid, 13 per cent.

Number of moles of anhydrogalacturonic acid, 0.00596. (Calculated)

Clock Time	Run Time, Hours	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydrogalacturonic acid. (Calculated)
3:05 pm		25	(95.6695)	(102.4912)		
3:30 pm		75	95.6697			
4:00 pm				102.4900		
4:30 pm		130	95.6755		5.8	22
4:40 pm	0.00	130				
5:00 pm	0.33	130		102.5518	67.6	258
5:30 pm	0.83	130	95.7295		121.6	463
6:00 pm	1.33	130		102.5875	157.3	599
6:30 pm	1.83	130	95.7532		181.0	689
7:00 pm	2.33	130		102.6025	196.0	747
8:30 pm	3.83	130	95.7728		215.6	821

TABLE XIX, Continued

Carbon Dioxide Evolution Data, Pectic Acid, Run 1.

Clock Time	Run Time, Hours	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydrogalacturonic acid. (Calculated)
9:00 pm	4.33	130		102.6048	217.9	830
9:30 pm	4.83	130	95.7730		218.1	831
10:00 pm	5.33	130		102.6058	219.1	834
10:30 pm	5.83	130	95.7742		220.3	838

TABLE XX

Carbon Dioxide Evolution Data, Pectic Acid, Run 2.

Weight of pectic acid, 1.2425 grams.

Moisture content of pectic acid, 13 per cent.

Number of moles of anhydrogalacturonic acid, 0.00614. (Calculated)

Clock Time	Run Time, Hours	Bath temp., ° C.	Weight of weighing bottle 5, Grams.	Weight of weighing bottle 6, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydrogalacturonic acid. (Calculated)
3:05 pm		25	(92.0820)	(101.2825)		
3:30 pm		75	92.0820			
4:00 pm				101.2825		
4:30 pm		130	92.0863		4.3	16
4:40 pm	0.00	130				
5:00 pm	0.33	130		101.3436	65.4	242
5:30 pm	0.83	130	92.1497		128.8	477
6:00 pm	1.33	130		101.3813	166.5	616
6:30 pm	1.83	130	92.1728		189.6	702
7:00 pm	2.33	130		101.3947	203.0	752

TABLE XX, Continued

Carbon Dioxide Evolution Data, Pectic Acid, Run 2.

Clock Time	Run Time, Hours	Bath temp., ° C.	Weight of weighing bottle 5, Grams.	Weight of weighing bottle 6, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydrogalacturonic acid. (Calculated)
8:30 pm	3.83	130	92.1917		221.9	821
9:00 pm	4.33	130		101.3968	224.0	829
9:30 pm	4.83	130	92.1925		224.8	832
10:00 pm	5.33	130		101.3979	225.9	836
10:30 pm	5.83	130	92.1933		226.7	838

TABLE XXI

Carbon Dioxide Evolution Data, Pectic Acid, Run 3.

Weight of pectic acid, 5.1205 grams.

Moisture content of pectic acid, 13 per cent.

Number of moles of anhydrogalacturonic acid, 0.0253. (Calculated)

Clock Time	Run Time, Hours	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydrogalacturonic acid. (Calculated)
7:00 pm		20	(95.7765)	(102.6080)		
7:40 pm		87	95.7755			
7:55 pm		117				
8:15 pm		130	95.8013		25.8	23
8:20 pm	0.00	130				
8:40 pm	0.33	130		102.7687	186.5	167
9:00 pm	0.67	130	95.9700		355.2	319
10:15 pm	1.92	130		103.1630	749.5	674
11:00 pm	2.67	130	96.0624		841.9	756
12:10 am	3.83	130		103.2301	909.0	817

TABLE XXI, Continued

Carbon Dioxide Evolution Data, Pectic Acid, Run 3.

Clock time	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydrogalacturonic acid. (Calculated)
1:10 am	4.83	130	96.0862		932.8	837
2:10 am	5:83	130		103.2398	942.5	847
3:10 am	6:83	130	96.0915		947.8	852
4:10 am	7.83	130		103.2442	952.2	856
5:10 am	8.83	130	96.0940		954.7	858
6:10 am	9.83	130		103.2458	956.3	859

TABLE XXII

Carbon Dioxide Evolution Data, Pectic Acid, Run 4.

Weight of pectic acid, 2.8166 grams.

Moisture content of pectic acid, 13 per cent.

Number of moles of anhydrogalacturonic acid, 0.0139. (Calculated)

Clock time	Run time, Hours	Bath temp., ° C.	Weight of weighing bottle 5, Grams.	Weight of weighing bottle 6, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydrogalacturonic acid. (Calculated)
7:00 pm		20	(92.1965)	(101.4020)		
7:40 pm		87	92.1958			
7:55 pm		117		101.4027		
8:15 pm		130	92.2225		26.7	44
8:20 pm	0.00	130				
8:40 pm	0.33	130		101.5160	140.0	229
9:00 pm	0.67	130	92.3235		241.0	394
10:15 pm	1.92	130		101.7102	435.2	711
11:00 pm	2.67	130	92.3690		480.7	785
12:10 am	3.83	130		101.7417	512.2	837

TABLE XXII, Continued

Carbon Dioxide Evolution Data, Pectic Acid, Run 4.

Weight of pectic acid, 2.8166 grams.

Moisture content of pectic acid, 13 per cent.

Number of moles of anhydrogalacturonic acid, 0.0139. (Calculated)

Clock time	Run time, Hours	Bath temp., °C.	Weight of weighing bottle 5, Grams.	Weight of weighing bottle 6, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydrogalacturonic acid. (Calculated)
1:10 am	4.83	130	92.3792		522.4	854
2:10 am	5.83	130		101.7442	524.9	858
3:10 am	6.83	130	92.3820		527.7	863
4:10 am	7.83	130		101.7488	532.3	870
5:10 am	8.83	130	92.3840		534.3	874
6:10 am	9.83	130		101.7490	534.5	875

TABLE XXIII

Carbon Dioxide Evolution Data, Standard Cellulose, Run 1.

Weight of standard cellulose, 3.9669 grams.

Moisture content of standard cellulose, 5.6 per cent.

Molecular weight of anhydroglucose, 162.

Number of moles of anhydroglucose, 0.0231. (Calculated)

Clock time	Run time, Hours	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydroglucose. (Calculated)
9:40 pm		(94.0460)	89.3042		
9:50 pm	0.00			0.0	0.00
10:20 pm	0.50	94.0424		0.0	0.00
10:45 pm	0.92		89.3055	1.3	1.28
12:00 m	2.17	94.0422		1.1	1.08
1:05 am	3.25		89.3053	0.9	0.89
9:20 am	11.50	94.0496		8.3	8.17
12:12 pm	14.37		89.3097	12.7	12.5
3:36 pm	17.77	94.0538		16.9	16.6

TABLE XXIII, Continued

Carbon Dioxide Evolution Data, Standard Cellulose, Run 1.

Clock time	Run time, Hours	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydroglucose. (Calculated)
9:24 pm	23.58		89.3157	22.9	22.5
12:48 am	26.97	94.0561		25.2	24.8
10:00 am	36.17		89.3207	30.2	29.7
10:15 pm	48.42	94.0594		33.5	33.0
10:30 am	60.67		89.3246	37.4	36.8
2:28 pm	64.63	94.0620		40.0	39.4
6:30 pm	68.67		89.3255	40.9	40.3

TABLE XXIV

Carbon Dioxide Evolution Data, Standard Cellulose, Run 2.

Weight of standard cellulose, 4.6772 grams.

Moisture content of standard cellulose, 5.6 per cent.

Molecular weight of anhydroglucose, 162.

Number of moles of anhydroglucose, 0.0273. (Calculated)

Clock time	Run time, Hours	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 4, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydroglucose. (Calculated)
9:40 pm		(95.7429)	93.4707		
9:50 pm	0.00				
10:20 pm	0.50	95.7415		0.0	0.0
10:45 pm	0.92		93.4715	0.8	0.7
12:00 m	2.17	95.7421		1.4	1.2
1:05 am	3.25		93.4724	2.3	1.9
9:20 am	11.50	95.7511		11.3	9.4
12:12 pm	14.37		93.4777	16.6	13.8
3:36 pm	17.77	95.7585		24.0	19.9
9:24 pm	23.58		93.4948	41.1	34.1

TABLE XXIV, Continued

Carbon Dioxide Evolution Data, Standard Cellulose, Run 2.

Clock time	Run time, Hours	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 4, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydroglucose. (Calculated)
12:48 am	26.97	95.7714		54.0	44.9
10:00 am	36.17		93.5588	118.0	98.1
10:15 pm	48.42	95.7914		138.0	114.8
10:30 am	60.67		93.6064	185.6	154.0
2:28 pm	64.63	95.8024		196.6	163.0
6:30 pm	68.67		93.6100	200.2	166.6

TABLE XXV

Carbon Dioxide Evolution Data, Standard Cellulose, Run 3.

Weight of standard cellulose, 5.5280 grams.

Moisture content of standard cellulose, 5.6 per cent.

Molecular weight of anhydroglucose, 162.

Number of moles of anhydroglucose, 0.0322 (Calculated)

Clock time	Run time, Hours	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydroglucose. (Calculated)
10:00 pm	0.0		113.9179	114.2504	0.0	0.0
10:40 pm	0.7	105	113.9182		0.3	0.2
11:00 pm	1.0	130		114.2519	1.3	1.3
12:00 m	2.0	130	113.9199		3.5	2.5
1:30 am	3.5	130		114.2542	5.8	4.1
12:30 pm	14.5	130	113.9348		20.7	14.6
5:00 pm	19.0	130		114.2607	27.2	19.2
10:00 pm	24.0	130	113.9434		35.8	25.2

TABLE XXV, Continued

Carbon Dioxide Evolution Data, Standard Cellulose, Run 3.

Clock time	Run time, Hours	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (calculated)	Millimoles of CO_2 evolved per mole of anhydroglucose. (Calculated)
1:00 am	27.0	130		114.2657	40.8	28.8
12:48 pm	38.8	130	113.9515		48.9	34.5
10:48 pm	48.6	130		114.2731	56.3	39.7
12:30 pm	62.5	130	113.9625		67.3	47.5
12:30 am	74.5	130		114.2858	80.0	56.4
12:06 pm	86.1	130	113.9735		91.0	64.2
8:06 pm	94.1	130		114.2938	99.0	69.8

TABLE XXVI

Carbon Dioxide Evolution Data, Standard Cellulose, Run 4.

Weight of standard cellulose, 7.2102 grams.

Moisture content of standard cellulose, 5.6 per cent.

Molecular weight of anhydroglucose, 162.

Number of moles of anhydroglucose, 0.0420. (Calculated)

Clock time	Run time, Hours	Bath temp., ° C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 4, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydroglucose. (Calculated)
10:00 pm	0.0		117.6837	111.3351	0.0	0.0
11:00 pm	1.0	130		111.3365	1.7	0.9
12:00 m	2.0	130	117.6857		3.4	1.8
1:30 am	3.5	130		111.3394	6.3	3.4
12:30 pm	14.5	130	117.7009		21.5	11.6
5:00 pm	19.0	130		111.3477	29.8	16.1
10:00 pm	24.0	130	117.7075		36.4	19.7
1:00 am	27.0	130		111.3549	43.6	23.6

TABLE XXVI, Continued

Carbon Dioxide Evolution Data, Standard Cellulose, Run 4.

Clock time	Run time, Hours	Bath temp., o C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 4, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydroglucose. (Calculated)
12:48 pm	38.8	130	117.7155		51.6	27.9
10:48 pm	48.6	130		111.3636	60.3	32.6
12:30 pm	62.5	130	107.3870#		69.0	37.3
12:30 am	74.5	130		111.3751	80.5	43.5
12:06 pm	86.1	130	107.3940		87.5	47.3
8:06 pm	94.1	130		111.3793	91.7	49.6

Bottle 6 of weight 107.3783 substituted for bottle 3 at 10:48 am.

TABLE XXVII

Carbon Dioxide Evolution Data, Oxycellulose I, Run 1.

Weight of oxycellulose I, 3.9457 grams.

Moisture content of oxycellulose I, dry.

Molecular weight of anhydro unit of oxycellulose I, 167. (Calculated)

Number of moles of anhydro unit of oxycellulose I, 0.02365. (Calculated)

Clock time	Run time, Hours	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose I. (Calculated)
2:30 pm		25	113.6768	113.9259		
3:00 pm		70		113.9265	0.6	1
3:27 pm	0.00					
3:30 pm	0.05	112	113.6784		2.2	2
4:00 pm	0.55	130		111.0415 #	31.9	31
4:30 pm	1.05	130	113.7140		67.5	65
5:05 pm	1.63	130		113.9928	105.0	101

At 3:30 pm weighing bottles number 2 and number 4 were placed in gas train in reversed positions.

TABLE XXVII, Continued

Carbon Dioxide Evolution Data, Oxycellulose I, Run 1.

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose I. (Calculated)
5:30 pm	2.05	130	113.7389		129.9	125
6:00 pm	2.55	130		114.0216	158.7	152
7:09 pm	3.70	130	113.7836		203.4	195
9:45 pm	6.30	130		114.0825	264.3	254
10:30 pm	7.05	130	113.7979		278.6	268
11:30 pm	8.05	130		114.0962	292.3	281
12:30 am	9.05	130	113.8085		302.9	291
11:18 am	19.85	130		114.1532	359.9	346
3:41 pm	24.23	130	113.8209		372.3	357
10:10 pm	30.72	130		114.1731	392.2	376
1:40 pm	46.22	130	113.8570		428.3	412
10:45 pm	55.30	130		114.1913	446.5	428

TABLE XXVII, Continued

Carbon Dioxide Evolution Data, Oxycellulose I, Run 1.

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose I. (Calculated)
2:45 pm	71.30	130	113.8815		471.0	452
9:54 pm	78.45	130		114.2026	482.3	462
1:36 pm	94.15	130	113.8976		498.4	479

TABLE XXVIII

Carbon Dioxide Evolution Data, Oxycellulose I, Run 2.

Weight of oxycellulose I, 3.9794 grams.

Moisture content of oxycellulose I, dry.

Molecular weight of anhydro unit of oxycellulose I, 167. (Calculated)

Number of moles of anhydro unit of oxycellulose I, 0.02385. (Calculated)

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 4, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose I. (Calculated)
2:30 pm		25	117.5229	111.0107		
3:00 pm		70		111.0118	1.1	1
3:27 pm	0.00					
3:30 pm	0.05	112	117.5241		2.3	2
4:00 pm	0.55	130		113.9553 #	31.1	30
4:30 pm	1.05	130	117.5560		63.0	60

At 3:30 pm weighing bottles number 2 and number 4 were placed in gas train in reversed positions.

TABLE XXVIII, Continued

Carbon Dioxide Evolution Data, Oxycellulose I, Run 2.

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 4, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose I. (Calculated)
5:05 pm	1.63	130		111.0760	97.5	93
5:30 pm	2.05	130	117.5788		120.2	114
6:00 pm	2.55	130		111.1019	146.2	139
7:09 pm	3.70	130	117.6213		188.7	179
9:45 pm	6.30	130		111.1622	249.0	237
10:30 pm	7.05	130	117.6346		262.3	249
11:30 pm	8.05	130		111.1761	276.2	262
12:30 am	9.05	130	117.6439		285.5	271
11:18 am	19.85	130		111.2404	349.8	332
3:41 pm	24.23	130	117.6538		359.7	342
10:10 pm	30.72	130		111.2595	378.8	360

TABLE XXVIII, Continued

Carbon Dioxide Evolution Data, Oxycellulose I, Run 2.

Clock time.	Run time, Hours.	Bath temp., ^{o} C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 4, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose I. (Calculated)
1:40 pm	46.22	130	117.6586		383.6	364
10:45 pm	55.30	130		111.2690	393.1	374
2:45 pm	71.30	130	117.6638		398.3	378
9:54 pm	78.45	130		111.2767	406.0	386
1:36 pm	94.15	130	117.6709		413.1	393

TABLE XXIX

Carbon Dioxide Evolution Data, Oxycellulose II, Run 1.

Weight of Oxycellulose II, 1.9289 grams.

Moisture content of Oxycellulose II, dry.

Molecular weight of anhydro unit of Oxycellulose II, 173. (Calculated)

Number of moles of anhydro unit of Oxycellulose II, 0.01115. (Calculated)

Clock time,	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Millimoles. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose II. (Calculated)
10:00 am	0.0		123.5734	(117.0044)	0.0	0.0
10:07 am	0.1	63		117.0043	0.0	0.0
10:30 am	0.5	104	123.5739		0.5	1.0
11:00 am	1.0	130		117.0052	1.4	2.9
11:30 am	1.5	130	123.5748		2.3	4.7
12:00 n	2.0	130		117.0060	3.1	6.3
1:00 pm	3.0	130	123.5763		4.6	9.4
2:00 pm	4.0	130		117.0080	6.6	13.4

TABLE XXIX, Continued

Carbon Dioxide Evolution Data, Oxycellulose II, Run 1.

Clock time.	Run time, Hours.	Bath temp., o C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2', Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose II. (Calculated)
3:00 pm	5.0	130	123.5786		8.9	18.1
6:00 pm	8.0	130		117.0131	14.0	28.5
8:55 pm	10.9	130	123.5820		17.4	35.4
12:12 am	14.2	130		117.0173	21.6	44.0
12:48 pm	26.8	130	123.5980		37.6	76.6
3:00 pm	29.0	130		117.0200	40.3	82.2
6:30 pm	32.5	130	123.6027		45.0	91.7
10:48 pm	36.8	130		117.0241	49.1	100.0
12:00 n	50.0	130	123.6142		60.6	123.3
4:00 pm	54.0	130		117.0255	62.0	126.2

TABLE XXX

Carbon Dioxide Evolution Data, Oxycellulose II, Run 2.

Weight of Oxycellulose II, 2.4274 grams.

Moisture content of Oxycellulose II, dry.

Molecular weight of anhydro unit of Oxycellulose II, 173. (Calculated)

Number of moles of anhydro unit of Oxycellulose II, 0.01404. (Calculated)

Clock time.	Run time, Hours.	Bath temp., o C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 4, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose II. (Calculated)
10:00 am	0.0		119.4010	117.5755	0.0	0.0
10:07 am	0.1	63		117.5757	0.2	0.3
10:30 am	0.5	104	119.4018		1.0	1.6
11:00 am	1.0	130		117.5768	2.1	3.4
11:30 am	1.5	130	119.4032		3.5	5.7
12:00 n	2.0	130		117.5770	3.7	6.0
1:00 pm	3.0	130	119.4049		5.4	8.7
2:00 pm	4.0	130		117.5799	8.3	13.4

TABLE XXX, Continued

Carbon Dioxide Evolution Data, Oxycellulose II, Run 2.

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 4, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose II. (Calculated)
3:00 pm	5.0	130	119.4078		11.2	18.1
6:00 pm	8.0	130		117.5875	18.8	30.4
8:55 pm	10.9	130	119.4132		24.2	39.2
12:12 am	14.2	130		117.5945	31.2	50.5
12:48 pm	26.8	130	119.4283		46.3	75.0
3:00 pm	29.0	130		117.6006	52.4	84.8
6:30 pm	32.5	130	119.4352		59.3	96.0
10:48 pm	36.8	130		117.6100	68.7	111.0
12:00 n	50.0	130	119.4557		89.2	144.2
4:00 pm	54.0	130		117.6155	94.7	153.0

TABLE XXXI

Carbon Dioxide Evolution Data, Oxycellulose III, Run 1.

Weight of Oxycellulose III, 1.3595 grams.

Moisture content of Oxycellulose III, dry.

Molecular weight of anhydro unit of Oxycellulose III, 167. (Calculated)

Number of moles of anhydro unit of Oxycellulose III, 0.00814. (Calculated)

Clock time.	Run time., Hours.	Bath temp., ° C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 5, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose III. (Calculated)
5:50 pm		25	(95.6633)	(93.4178)		
6:10 pm		75		(93.4192)		
6:30 pm			95.6635			
6:45 pm		130		93.4192		
7:00 pm	0.00	130				0.0
7:05 pm	0.08	130	95.6641		0.6	1.7
7:20 pm	0.33	130		93.4202	1.6	4.5
7:40 pm	0.67	130	95.6663		3.8	10.6

TABLE XXXI, Continued

Carbon Dioxide Evolution Data, Oxycellulose III, Run 1.

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 5, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose III. (Calculated)
8:12 pm	1.20	130		93.4220	5.6	15.6
8:48 pm	1.80	130	95.6681		7.4	20.6
9:18 pm	2.30	130		93.4218	7.2	20.1
10:18 pm	3.30	130	95.6681		7.2	20.1
10:40 pm	3.67	130		93.4215	6.9	19.2
11:00 pm	4.00	130	95.6689		7.7	21.5
11:30 pm	4.50	130		93.4232	9.4	26.2
12:30 am	5.33	130	95.6695		10.0	27.9

TABLE XXXII

Carbon Dioxide Evolution Data, Oxycellulose III, Run 2.

Weight of Oxycellulose III, 0.9058 grams.

Moisture content of Oxycellulose III, dry.

Molecular weight of anhydro unit of Oxycellulose III, 167. (Calculated)

Number of moles of anhydro unit of Oxycellulose III, 0.00574. (Calculated)

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose III. (Calculated)
5:50 pm		25	(93.9627)	(89.2476)		
6:10 pm		75		(89.2474)		
6:30 pm			93.9628			
6:45 pm		130		89.2470		
7:00 pm	0.00	130			0.0	
7:05 pm	0.08	130	93.9625		- 0.3	
7:20 pm	0.33	130		89.2471	- 0.2	
7:40 pm	0.67	130	93.9633		0.6	

TABLE XXXII, Continued

Carbon Dioxide Evolution Data, Oxycellulose III, Run 2.

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose III. (Calculated)
8:12 pm	1.20	130		89.2479	1.4	
8:48 pm	1.80	130	93.9626		0.7	
9:18 pm	2.30	130		89.2470	- 0.2	
10:18 pm	3.30	130	93.9620		- 0.8	
10:40 pm	3.67	130		89.2472	- 0.6	
11:00 pm	4.00	130	93.9623		- 0.3	
11:30 pm	4.50	130		89.2476	0.1	
12:20 pm	5.33	130	93.9624		0.2	0.8

TABLE XXXIII

Carbon Dioxide Evolution Data, Oxycellulose III, Run 3.

Weight of Oxycellulose III, 3.0668 grams.

Moisture content of Oxycellulose III, dry.

Molecular weight of anhydro unit of Oxycellulose III, 167. (Calculated)

Number of moles of anhydro unit of Oxycellulose III, 0.01840. (Calculated)

Clock time.	Run time, Hours.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose III (Calculated)
8:30 pm		94.0010	89.2870		
9:30 pm	0.00				0.0
10:00 pm	0.50		89.2891	2.1	2.6
11:15 pm	1.75	94.0024		3.5	4.3
12:00 m	2.50		89.2896	4.0	4.9
2:00 pm	16.50	94.0365		38.1	47.1
5:30 pm	20.00		89.2959	44.4	54.8
9:00 pm	23.50	94.0421		50.0	61.8
1:12 am	27.70		89.3029	57.0	70.4

TABLE XXXIV

Carbon Dioxide Evolution Data, Oxycellulose III, Run 4.

Weight of Oxycellulose III, 1.9902 grams.

Moisture content of Oxycellulose III, dry.

Molecular weight of anhydro unit of Oxycellulose III, 167. (Calculated)

Number of moles of anhydro unit of Oxycellulose III, 0.01191. (Calculated)

Clock time.	Run time, Hours.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 4, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose III (Calculated)
8:30 pm		95.7147	93.4609		
9:30 pm	0.00				0.0
10:00 pm	0.50		93.4612	0.3	0.6
11:15 pm	1.75	95.7153		0.9	1.7
12:00 m	2.50		93.4619	1.6	3.1
2:00 pm	16.50	95.7381		24.4	46.6
5:30 pm	20.00		93.4655	28.0	53.4
9:00 pm	23.50	95.7403		30.2	57.7
1:12 am	27.70		93.4699	34.6	66.0

TABLE XXXV

Carbon Dioxide Evolution Data, Oxycellulose IV, Run 1.

Weight of Oxycellulose IV, 0.8536 grams.

Moisture content of Oxycellulose IV, dry.

Molecular weight of anhydro unit of Oxycellulose IV, 171. (Calculated)

Number of moles of anhydro unit of Oxycellulose IV, 0.00498. (Calculated)

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose IV. (Calculated)
8:25 pm		93	93.9640	89.2518		
8:51 pm	0.00	125				0
9:00 pm	0.15	130		89.2557	3.9	18
9:30 pm	0.65	130	93.9722		12.1	55
10:00 pm	1.15	130		89.2630	19.4	88
10:36 pm	1.67	130	93.9778		25.0	114
11:12 pm	2.28	130		89.2680	30.0	137
12:12 am	3.28	130	93.9840		36.2	165

TABLE XXXV, Continued

Carbon Dioxide Evolution Data, Oxycellulose IV, Run 1.

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose IV. (Calculated)
1:12 am	4.28	130		89.2726	40.8	186
2:00 am	5.15	130	93.9866		43.4	198
2:42 am	5.85	130		89.2743	45.1	205
11:12 am	14.28	130	93.9997		58.2	265

TABLE XXXVI

Carbon Dioxide Evolution Data, Oxycellulose IV, Run 2.

Weight of Oxycellulose IV, 1.0092 grams.

Moisture content of Oxycellulose IV, dry.

Molecular weight of anhydro unit of Oxycellulose IV, 171. (Calculated)

Number of moles of anhydro unit of Oxycellulose IV, 0.00590. (Calculated)

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 5, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose IV. (Calculated)
8:25 pm		93	95.6702	93.4275		
8:51 pm	0.0	125				
9:00 pm	0.15	130		93.4297	2.2	8
9:30 pm	0.65	130	95.6798		11.8	45
10:00 pm	1.15	130		93.4375	19.6	76
10:36 pm	1.67	130	95.6860		25.8	99
11:12 pm	2.28	130		93.4443	32.6	126
12:12 am	3.28	130	95.6945		41.1	158

TABLE XXXVI, Continued

Carbon Dioxide Evolution Data, Oxycellulose IV, Run 2.

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 5, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose IV. (Calculated)
1:12 am	4.28	130		93.4486	45.4	175
2:00 am	5.15	130	95.6980		48.9	188
2:42 am	5.85	130		93.4496	49.9	192
11:12 am	14.28	130	95.7129		64.8	250

TABLE XXXVII

Carbon Dioxide Evolution Data, Oxycellulose V, Run 1.

Weight of Oxycellulose V, 0.3864 grams.

Moisture content of Oxycellulose V, dry.

Molecular weight of anhydro unit of Oxycellulose V, 170. (Calculated)

Number of moles of anhydro unit of Oxycellulose V, 0.002275. (Calculated)

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose V. (Calculated)
1:30 pm		25	(123.6294)	(117.0325)		
2:00 pm		45	123.6302			
2:30 pm		97		117.0337		
2:48 pm	0.00	130			0.0	0
3:00 pm	0.20	130	123.6310		0.8	8
3:30 pm	0.70	130		117.0347	1.8	18
4:00 pm	1.20	130	123.6322		3.0	30
4:30 pm	1.70	130		117.0356	3.9	39

TABLE XXXVII, Continued

Carbon Dioxide Evolution Data, Oxycellulose V, Run 1.

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 1, Grams.	Weight of weighing bottle 2, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose V. (Calculated)
5:00 pm	2.20	130	123.6330		4.7	47
6:00 pm	3.20	130		117.0370	6.1	61
7:00 pm	4.20	130	123.6347		7.8	78
9:02 pm	6.23	130		117.0393	10.1	101
11:00 pm	8.20	130	123.6373		12.7	127
1:00 am	10.20	130		117.0415	14.9	149

TABLE XXXVIII

Carbon Dioxide Evolution Data, Oxycellulose V, Run 2.

Weight of Oxycellulose V, 0.5340 grams.

Moisture content of Oxycellulose V, dry.

Molecular weight of anhydro unit of Oxycellulose V, 170. (Calculated)

Number of moles of anhydro unit of Oxycellulose V, 0.003140. (Calculated)

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 4, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose V. (Calculated)
1:30 pm		25	(119.4658)	(117.6153)		
2:00 pm		45	119.4666			
2:30 pm		97		117.6167		
2:48 pm	0.00	130			0.0	0
3:00 pm	0.20	130	119.4676		1.0	7
3:30 pm	0.70	130		117.6188	3.1	23
4:00 pm	1.20	130	119.4703		5.8	43
4:30 pm	1.70	130		117.6209	7.9	59

TABLE XXXVIII, Continued

Carbon Dioxide Evolution Data, Oxycellulose V, Run 2.

Clock time.	Run time, Hours.	Bath temp., ° C.	Weight of weighing bottle 3, Grams.	Weight of weighing bottle 4, Grams.	Weight of CO_2 evolved, Milligrams. (Calculated)	Millimoles of CO_2 evolved per mole of anhydro unit of Oxycellulose V. (Calculated)
5:00 pm	2.20	130	119.4720		9.6	71
6:00 pm	3.20	130		117.6244	13.1	95
7:00 pm	4.20	130	119.4752		16.3	121
9:02 pm	6.23	130		117.6304	22.3	165
11:00 pm	8.20	130	119.4797		26.8	199
1:00 am	10.20	130		117.6346	31.0	230
4:15 pm	25.45	130	119.4909		42.2	312
8:03 am	41.25	130		117.6692	76.8	569

TABLE XXXIX

Calcium Acetate Determination Data, Oxycellulose I

	Sample weight, Grams.	Volume of $Ca(C_2H_3O_2)_2$ solution used, Ml.	pH before titration.	Normality of NaOH solution.	Volume of NaOH solution titrated, Ml.	Millimoles of carboxyl per gram of sample. (Calculated)
Sample 1	0.3727	75.0	6.12	0.0149	35.05	2.035
Sample 2	0.5267	75.0	5.98	0.0149	49.23	2.005
Sample 3	0.5233	75.0	6.01	0.0149	45.78	1.908
Average blank	0.0000	75.0	7.65	0.0149	1.10	
					Average	1.98

Molecular weight of anhydro unit of Oxycellulose I, 167. (Calculated)

Millimoles of carboxyl per mole of anhydro unit of Oxycellulose I, 331. (Calculated)

TABLE XL

Calcium Acetate Determination Data, Oxycellulose II

	Sample weight, Grams.	Volume of $Ca(C_2H_3O_2)_2$ solution used, Ml.	pH before titration.	Normality of NaOH solution.	Volume of NaOH solution titrated, Ml.	Millimoles of carboxyl per gram of sample. (Calculated)
Sample 1	0.3618	75.0	5.82	0.0149	70.20	4.27
Sample 2	0.4638	75.0	5.70	0.0149	90.00	4.29
Sample 3	0.4462	75.0	5.70	0.0149	87.50	4.33
Average blank	0.0000	75.0	7.65	0.0149	1.10	
					Average	4.30

Molecular weight of anhydro unit of Oxycellulose II, 173. (Calculated)

Millimoles of carboxyl per mole of anhydro unit of Oxycellulose II, 744. (Calculated)

TABLE XLI

Calcium Acetate Determination Data, Oxycellulose III

	Sample weight, Grams.	Volume of $Ca(C_2H_3O_2)_2$ solution used, ml.	pH before titration.	Normality of NaOH solution.	Volume of NaOH solution titrated, Ml.	Millimoles of carboxyl per gram of sample. (Calculated)
Sample 1	0.2842	60.0	6.48	0.0155	34.48	2.15
Sample 2	0.3150	60.0	6.37	0.0155	39.30	2.22
Sample 3	0.3427	60.0	6.33	0.0155	40.76	2.12
Average blank	0.0000	60.0	7.59	0.0155	1.65	
					Average	2.16

Molecular weight of anhydro unit of Oxycellulose III, 167. (Calculated)

Millimoles of carboxyl per mole of anhydro unit of Oxycellulose III, 361. (Calculated)

TABLE XLII

Calcium Acetate Determination Data, Oxycellulose IV

	Sample weight, Grams.	Volume of $Ca(C_2H_3O_2)_2$ solution used, Ml.	pH before titration.	Normality of NaOH solution.	Volume of NaOH solution titrated, Ml.	Millimoles of carboxyl per gram of sample. (Calculated)
Sample 1	0.2305	60.0	6.30	0.0155	44.93	3.49
Sample 2	0.2668	60.0	6.28	0.0155	52.96	3.58
Sample 3	0.3097	60.0	6.20	0.0155	59.80	3.49
Average blank	0.0000	60.0	7.59	0.0155	1.65	
					Average	3.52

Molecular weight of anhydro unit of Oxycellulose IV, 171. (Calculated)

Millimoles of carboxyl per mole of anhydro unit of Oxycellulose IV, 602. (Calculated)

TABLE XLIII

Calcium Acetate Determination Data, Oxycellulose V

	Sample weight, Grams.	Volume of $Ca(C_2H_3O_2)_2$ solution used, Ml.	pH before titration.	Normality of NaOH solution.	Volume of NaOH solution titrated, Ml.	Millimoles of carboxyl per gram of sample. (Calculated)
Sample 1	0.2221	75.0	6.32	0.0149	23.65	2.26
Sample 2	0.2263	75.0	6.28	0.0149	23.95	2.26
Average blank	0.0000	75.0	7.65	0.0149	1.10	
					Average	2.26

Molecular weight of anhydro unit of Oxycellulose V, 170. (Calculated)

Millimoles of carboxyl per mole of anhydro unit of Oxycellulose V, 384. (Calculated)

TABLE XLIV

Moisture Content Determination Data

Substance	Drying temp., ° C.	Drying time, Hours.	Moisture content, per cent.
Glucose	110	10	0.08
Pectic Acid *	105	6	13.0
Pectic Acid *	110	10	15.6
Standard Cellulose	110	10	5.6
Alginic Acid	110	8	19.3

* On drying the pectic acid turned from a straw color to brown.

TABLE XLV

Ash Content Determination Data

Oxidized Pectic Acid II, reprecipitated	4.6 per cent
Oxidized Pectic Acid III	12.8 per cent

TABLE XLVI

Oxidized Pectic Acid III Titration Data.

Weight of sample, Grams.	Normality of NaOH.	Volume of NaOH used, Ml.	Ml. of NaOH per gram of sample. (Calculated)
0.5614	0.1343	26.0	46.3
1.0277	0.1343	47.3	46.1
0.4571	0.1343	19.2	42.0
		Average	44.8

Millimoles of carboxyl per gram, 6.03. (Calculated)

Millimoles of carboxyl per mole, 1240. (Calculated)

Calculations

Calculation of the Results of Periodate Titrations

The results of the titrations for periodate in the presence of iodate used in following the periodate oxidations were converted into millimoles of aldehyde formed per mole of the anhydro unit of the substance oxidized by use of the formula:

$$X = \frac{V_m \; M \; N}{V_s \; W} \left[(B_i - T_i) - (B - T) \right]$$

where X = millimoles of aldehyde formed per mole of anhydro unit of the substance oxidized

W = weight of the substance oxidized, grams

M = molecular weight of the anhydro unit of the substance oxidized

V_m = volume of the oxidation reaction mixture, ml.

V_s = volume of the sample of the oxidation reaction mixture, ml.

N = normality of the iodine solution, equivalents per liter

B_i = volume of iodine solution used in initial blank titration, ml.

B = volume of iodine solution used in other blank titrations, ml.

T_i = volume of iodine solution used in initial titration, ml.

T = volume of iodine solution used in other titrations, ml.

This formula was arrived at as follows. B is the amount

of iodine solution required to react with the sodium arsenite added, and T is the amount of iodine solution required to react with the sodium arsenite in excess of that consumed by the iodine liberated by the periodate in the sample. $(B - T)N$ is, therefore, the number of milliequivalents of iodine liberated by the periodate in the sample. It is also the number of milliequivalents of periodate in the sample. $(B_i - T_i)N$ is the number of milliequivalents initially in the sample so that

$$(B_i - T_i)N - (B - T)N$$

gives the number of milliequivalents of periodate consumed, and so also the number of milliequivalents of aldehyde formed. Since the equivalent weight and the molecular weight of an aldehyde group are the same, the above expression gives the number of millimoles of aldehyde formed in the sample withdrawn. Multiplying this by the ratio of the volume of the oxidation reaction mixture to the volume of the sample withdrawn, V_m/V_s, and dividing by the number of moles of the substance oxidized, W/M, the original expression is obtained.

If B always equals B_i, the expression reduces to

$$X = \frac{V_m \; M \; N}{V_s \; W} (T - T_i).$$

Since the aldehyde groups are formed in pairs, two on each unit that is oxidized, the number of units oxidized is half of the number of aldehyde groups formed.

Units Used to Express Total Carboxyl Contents and Amounts of Carbon Dioxide Evolved.

Total carboxyl contents as determined by the calcium acetate method and amounts of carbon dioxide evolved are expressed as millimoles of -COOH or CO_2 per mole of the anhydro unit of the substance in question. This method of expression, advocated by R. F. Nickerson,[29] has the following advantages. Comparisons of the extents of oxidation of a substance that is subjected to several oxidations are more obvious when expressed on a per mole basis than when expressed on a weight basis. Comparisons with the total possible amounts of oxidation are also more obvious. A cellulose completely oxidized at the 6-position, but nowhere else, will contain 1000 millimoles of -COOH and evolve 1000 millimoles of CO_2 per mole of the anhydro unit, in this case anhydroglucuronic acid. A substance oxidized completely at the 2-, 3-, and 6-position will contain 3000 millimoles of -COOH per mole of anhydro unit. The method of expression has the disadvantage that in a partially oxidized substance some of the anhydro units are oxidized and some of them are not. There is then no one molecular weight of the anhydro units of a partially oxidized material. A molecular weight used to determine the number of moles of a partially oxidized substance must necessarily then be an average molecular weight.

Other authors have expressed total carboxyl contents and amounts of carbon dioxide evolved as per cent by weight,

milligrams per gram, and millimoles per 100 grams. Conversion of these units into millimoles per mole can be accomplished as follows.

Per Cent by Weight and Milligrams per Gram: If P is the per cent by weight of carboxyl or carbon dioxide evolved from a substance,

$\frac{P}{100}$ = grams of CO_2 or -COOH per gram

10 P = milligrams of CO_2 or -COOH per gram

$\frac{10P}{44}$ = millimoles of CO_2 per gram

$\frac{10P}{45}$ = millimoles of -COOH per gram.

If M is the molecular weight of the anhydro unit,

$\frac{10PM}{44}$ = millimoles of CO_2 per mole of anhydro unit

$\frac{10PM}{45}$ = millimoles of -COOH per mole of anhydro unit.

Millimoles per 100 Grams: If C is the number of millimoles of carboxyl or carbon dioxide evolved per 100 grams,

$\frac{C}{100}$ = millimoles of -COOH or CO_2 per gram

$\frac{CM}{100}$ = millimoles of -COOH or CO_2 per mole of anhydro unit.

Estimation of Molecular Weights.

As explained above, the molecular weight of a partially oxidized substance must necessarily be an average molecular weight. The average molecular weights used were based on calcium

acetate determinations of the total carboxyl content and were arrived at as follows. A cellulose which has been oxidized to the extent that one carboxyl group has been produced on half of the glucose residues, the other half being unchanged, contains anhydro units of molecular weights 162 and 176. Since these anhydro units occur in equal numbers, the average molecular weight is 169. This substance contains 500 millimoles of carboxyl per mole of anhydro unit, that is, 500 millimoles of carboxyl per 169 grams. A calcium acetate determination would therefore show 2.96 millimoles of carboxyl per gram. It can be concluded then that a substance which shows 2.96 millimoles of carboxyl per gram by a calcium acetate determination has an average molecular weight of 169.

To avoid calculations a chart, Figure 14, was made showing total carboxyl content expressed in millimoles of carboxyl per gram plotted against average molecular weight. By this chart an average molecular weight could at once be obtained from the per gram basis calcium acetate determination. The chart was made by calculating several points as was done in the paragraph above.

A complication not mentioned above is that when the 2- and 3-carbon atoms are oxidized to carboxyl, there is a gain in molecular weight of 15 per carboxyl group, but when the number 6 carbon atom is oxidized to carboxyl, there is a gain in molecular weight of only 14. Since this difference in

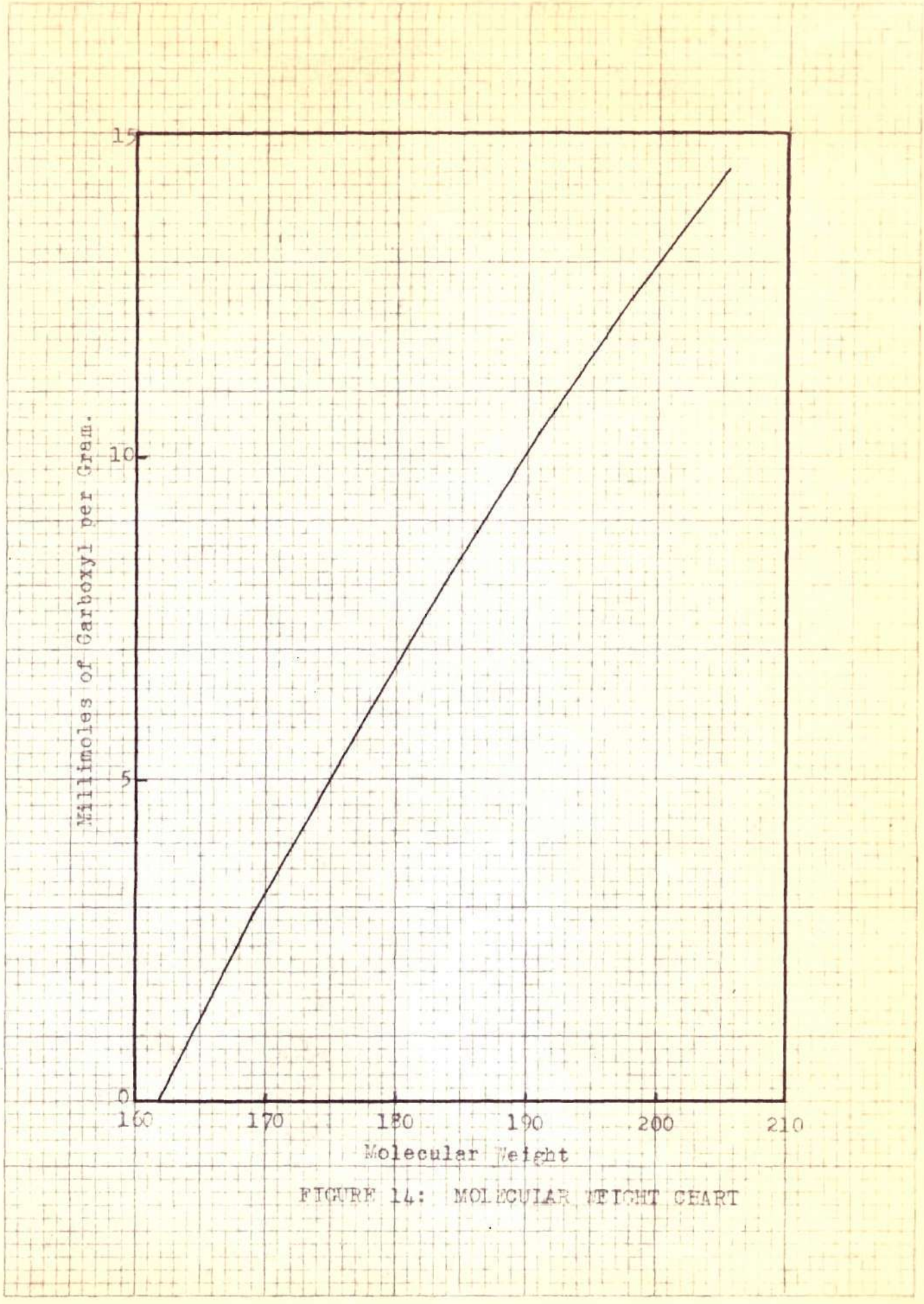


FIGURE 14: MOLECULAR WEIGHT CHART

molecular weight is small in comparison with errors involved in the calcium acetate determination, it could be distributed over the plot.

Calculations of the Results of Calcium Acetate Determination.

Let:

W = weight of sample being analysed, grams

V = total volume of 0.5 N calcium acetate solution used per sample, ml.

50 ml. = that portion of V titrated

T = volume of NaOH solution required to raise the pH of the 50 ml. portion of V to 8.3, ml.

B = volume of NaOH solution required to raise the pH of the blank to 8.3, ml.

N = normality of the NaOH solution, equivalents per liter.

Then,

$N(T - B)$ = milliequivalents of NaOH required to neutralize liberated acetic acid in the 50 ml. portion of V.

$\frac{VN(T - B)}{50}$ = milliequivalents of NaOH necessary to neutralize the total amount of liberated acetic acid

$\frac{VN(T - B)}{50}$ = millimoles of -COOH in the sample

$\frac{VN(T - B)}{50W}$ = millimoles of -COOH per gram of sample.

Calibration of Flow Meters

The flow meters were used to measure the amount of nitrogen gas passing through the apparatus so that the gas flow could be maintained at ten liters per hour. The meters were calibrated by passing gas through them at a constant rate and measuring the time required for the gas to displace the water from an upturned volumetric cylinder. The calibration curves are given in Figure 15.

Dispersion of Pectic Acid in Dilute Sodium Hydroxide Solutions

At one point the possibility of purifying the technical pectic acid used was considered. This was to be done by dissolving the pectic acid in a dilute sodium hydroxide solution and precipitating with hydrochloric acid. The idea was later discarded because there was danger of degradation and oxidative attack in the alkaline medium, because difficulties were anticipated in freeing the reprecipitated pectic acid from inorganic materials, and because not pectic acid itself, but oxidized pectic acid, was the primary object of the investigation. In the meantime, however, some data was collected on the solubility, or perhaps rather the dispersion, of pectic acid in dilute sodium hydroxide solutions.

The method of experimentation was to attempt to dissolve a known amount of pectic acid in a known amount of sodium hydroxide solution of a definite concentration. If an apparently homogeneous fluid was obtained, dispersion was assumed to

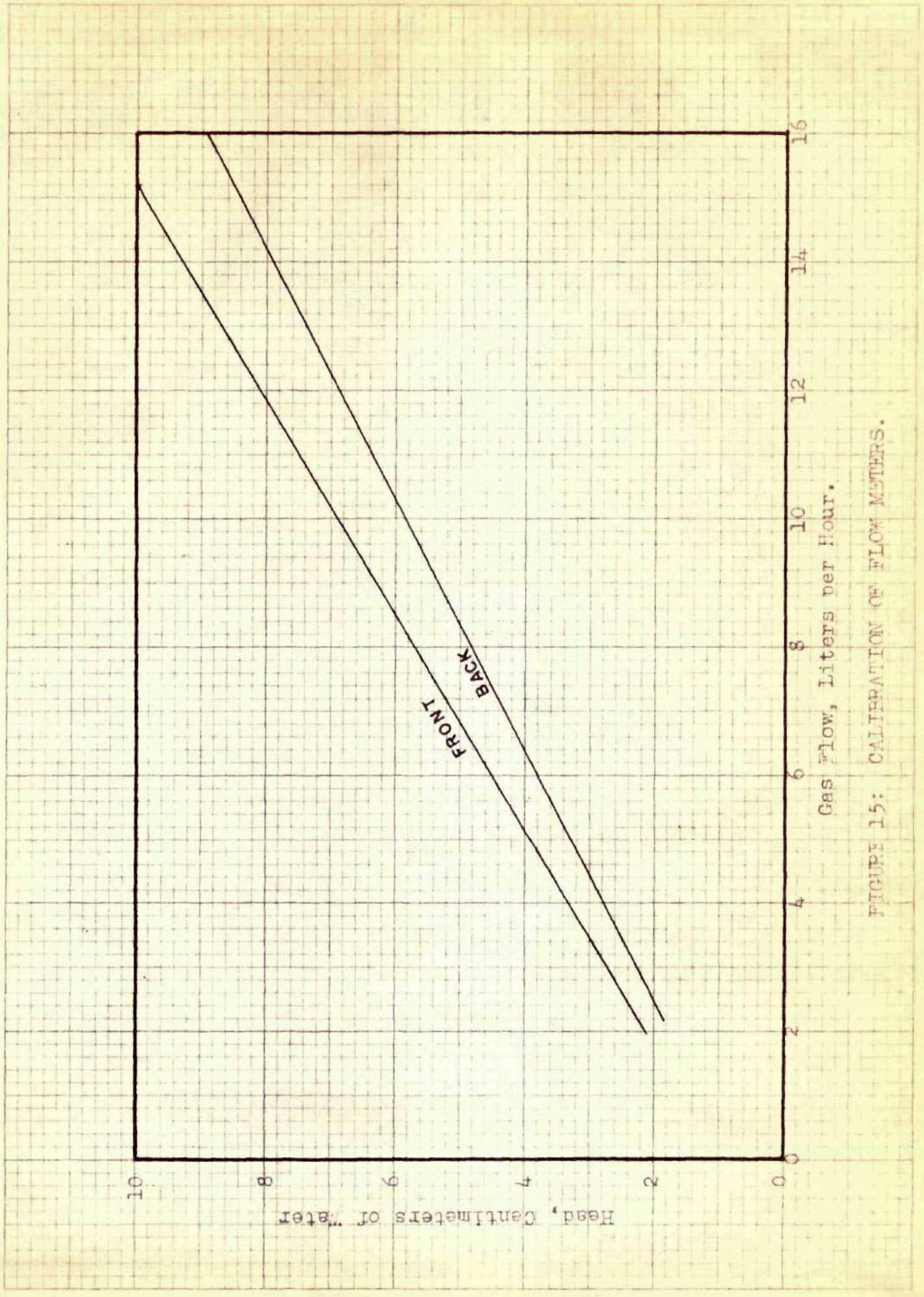


FIGURE 15: CALIBRATION OF FLOW METERS.

have occurred. No definite line could be drawn between dispersion and non-dispersion because there was a gradual change from the cases in which the pectic acid merely swelled, through the formation of very viscous suspensions, to the cases in which an apparently homogeneous though still viscous dispersion was obtained. The amount of sodium hydroxide necessary to cause dispersion of pectic acid is less than one fifth of the equivalent amount. The information obtained is presented in Figure 16.

The Original Prospectus of the Project

When cellulose is oxidized, there are a number of possibilities for the course of the oxidation. Attack at carbon atoms 2 or 3, or both, can produce ketone groups without fission of the carbon-carbon bond. Oxidation of the 2, 3-hydroxyl groups to aldehyde with fission, and further oxidation to carboxyl of one or both aldehyde groups may occur. Oxidation of the primary (6) hydroxyl group to aldehyde or carboxyl can also occur.

The broad problem involved is to be able to completely characterize the oxidized cellulose produced by a non-specific oxidant. Two types of specific oxidation are known. These are the periodate type and the nitrogen dioxide type. Periodate oxidation is specific for the 2, 3-hydroxyl groups, and oxidizes them to aldehyde, with fission of the carbon-carbon bond. The nitrogen dioxide type oxidizes the 6-carbon atom to carboxyl.

The nitrogen dioxide cellulose thus contains glucuronic

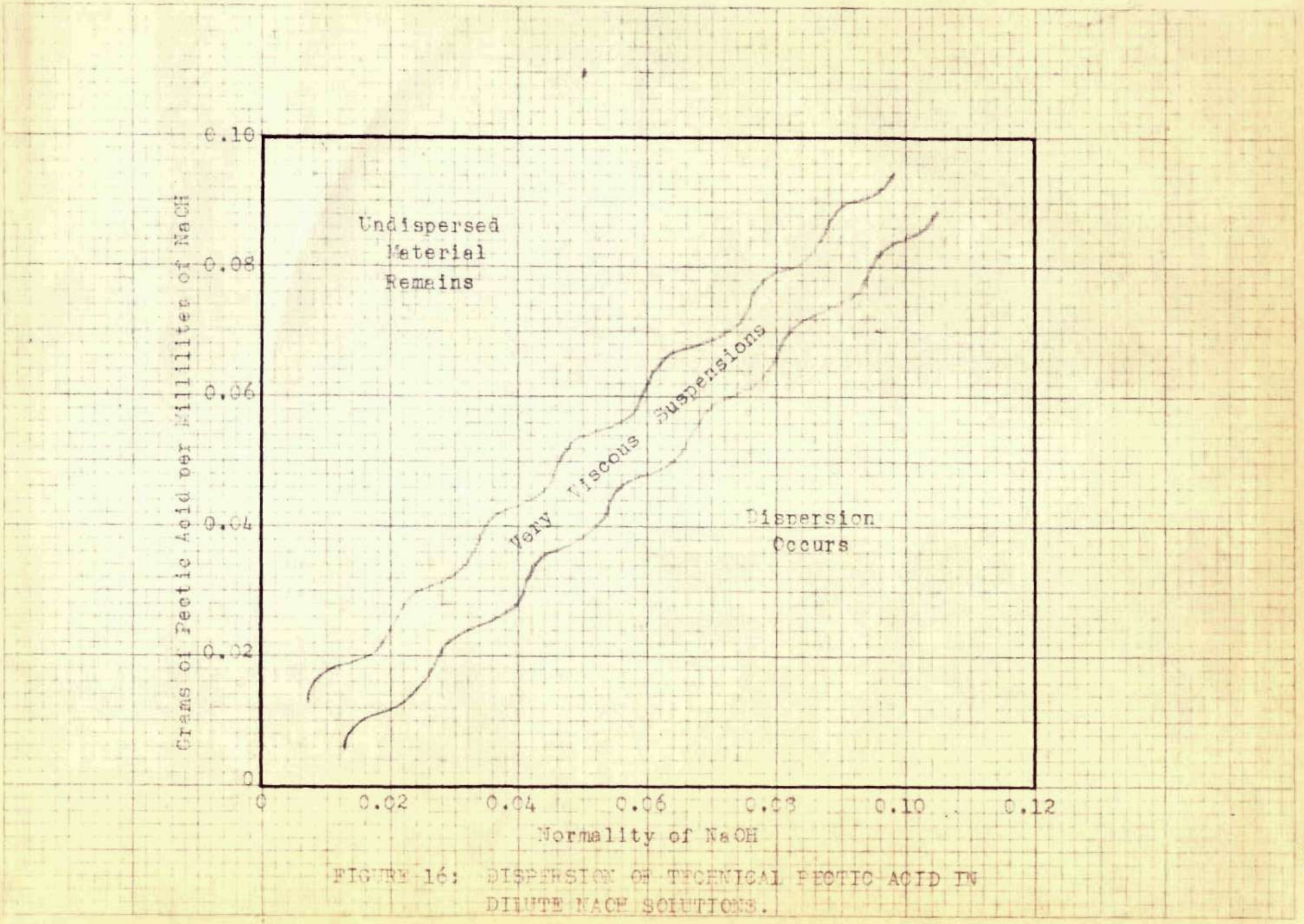


FIGURE 16: DISPERSION OF TECHNICAL PECTIC ACID IN DILUTE NACH SOLUTIONS.

acid groups, and these are determined by the carbon dioxide evolution method, which then is a measure of the carboxyl groups in the 6-position.

The question arises: If any other carboxyl groups are present on the glucose residue, would they interfere with the estimation of carbon dioxide from the 6-carbon atom. For instance, if the 2, 3-carbon bond were broken with oxidation to carboxyl, would these carboxyl groups resist the action of the boiling hydrochloric acid solution used in the analysis. If so, would rate studies make possible a differentiation between the positions of the carboxyl groups.

To answer these questions, the following methods of attack are suggested:

1. Study of carbon dioxide evolution of a nitrogen dioxide oxycellulose (celluronic acid)

2. Oxidize nitrogen dioxide cellulose further with periodate and sodium chlorite, and rerun carbon dioxide evolution

3. Study carbon dioxide evolution of carboxyl-containing compounds, such as tartaric acid and glycollic acid, and mixtures of the two.