STARCH NITRATES: PREPARATION, PROPERTIES AND STRUCTURE

by

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A Thesis submitted to the Faculty of Graduate Studies and Research at McGill University, in Partial Fulfilment of the requirements for the Degree of Doctor of Philosophy.

Montreal, Canada April 1943.

ACKNOWLEDGMENTS

To Prof. Harold Hibbert, director of this investigation, the author expresses sincerest appreciation for patient guidance, for his genuine interest in the work and especially for the opportunity to share his inspirational zeal,

and to

Dr. L.M. Cooke and Dr. T.H. Evans for helpful advice and assistance in the earlier and later sections of this research, respectively.

The author also wishes to express his gratitude to the Canada Starch Co. for supplying the starch used in these investigations and to Mr. M.C. Fletcher, chief explosives chemist, Dep't of Mines and Resources, Ottawa, under whose direction the Bergmann-Junk tests reported herein were carried out by Dr. A. Gillies.

To the National Research Council of Canada, sincere thanks are extended for the award of a Studentship and a Fellowship.

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Starch Nitrates: Preparation, Properties and Structure

The preparation, properties and structure of nitrates from whole starch, amylose and amylopectin have been studied by the following methods: (a) fractional precipitation, (b) fractional dissolution and (c) denitration. The products obtained by these methods were characterized by the usual tests for such substances.

Certain pretreatments of the starch resulted in lower yields and lessened stability of the nitrated products than in the absence of such treatments. Nitration by use of nitric acid alone was found much more advantageous than employment of mixed acid.

Fractionation studies showed that (a) the branched chain portion of starch (amylopectin) yields a less stable nitrate than the linear type (amylose), (b) ethanol soluble nitrates of low molecular weight and nitrogen content are unstable and that this instability is uninfluenced by ethanol dissolution. This solvent confers increased stability on the ethanol-insoluble portion of the nitrate, (c) not only starch, but also the isolated amylose and amylopectin are heterogeneous products.

Studies on maltose octanitrate and cellobiose octanitrate (a new compound) indicate that cellulose nitrate should possess a greater stability on the basis of its β-1,4 glucosidic structure, and this is known to be the case.

Contribution to Knowledge

1. Corn starch has been separated into its two components, amylose and amylopectin by the Tanret-Pacsu procedure and the nitrates of each carefully examined by new methods, namely, (1) Fractionation by Precipitation and Dissolution Methods, (2) a Denitration procedure. The products isolated were subjected to the customary tests used for the characterization of such substances.

2. Certain pretreatments of the starch were shown to yield less satisfactory products than when whole starch was used, while nitration by nitric acid alone was found to give more satisfactory products in higher yield than when a "mixed acid" was used.

3. The fractionation studies on the nitrates from whole starch, amylose and amylopectin respectively have proven that the amylopectins, having a branched chain structure, yield nitrates much less stable than those obtained from amylose, the latter possessing a linear chain structure.

Low molecular weight nitrated polymers from either whole starch, amylopectin or amylose have a low viscosity and stability; those of low nitrogen content are also unstable. The instability remains unchanged in all cases on precipitation from a mixture of acetone and ethanol.

Starch is shown to be a mixture of heterogeneous polymers and the isolated amylopectin and amylose were also found to be non-homogeneous.

4. The stabilization of starch nitrate by ethanol is shown to be the result of a dual action, namely, (a) removal of highly unstable material of low molecular weight and nitrogen content, and, (b) the conferring of increased stability by some, as yet unknown, mechanism on the insoluble portion.

5. Evidence has been obtained to show that the instability of nitrated starch is not due to the presence of free aldehyde groups formed in the nitration process, but is primarily due to some factor associated with the branched chain structure of amylopectin.

6. Based on the stabilities of maltose and cellobiose octanitrates, it is shown that cellulose nitrate should possess greater inherent stability than does starch nitrate and this has long been recognized to be the case.

7. Cellobiose octanitrate has been characterized for the first time.

8. A more satisfactory type of Abel test heating bath than that ordinarily used is described.

INTRODUCTION: THE STRUCTURE OF STARCH

In order to obtain an adequate background for a study on the preparation, structure and properties of nitrated starches, the subject of the author's thesis, it is necessary to review the earlier investigations, biochemical, chemical and physical, pertaining to this field.

Starch is present in (practically) all plants in the form of amorphous colorless granules and functions as the plant reserve material which after transformation into carbohydrate serves the purpose of providing the energy necessary for plant life and growth. Similarly in the form of food-stuffs it forms one of the most important constituents of human diets.

On the industrial side starch forms the basic material used for the manufacture of sugar, alcohols, beverages, explosives, textiles etc.

Earlier Investigations

It was recognized early that, dependent on the plant source (corn, wheat, rice, potato etc.) from which the starch was prepared, certain marked difference in properties were observable especially those associated with the "gel" forms.

Hydrolysis with dilute acids gave, with all starches, a practically quantitative yield of glucose, so that, corresponding to its

empirical formula $C_6H_{10}O_5$, it could be regarded as made up of glucose anhydride units though the manner in which these are combined in the starch aggregate is still uncertain.

One feature differentiates starch very sharply from cellulose,- a polysaccharide also derived from anhydroglucose units - namely the very much greater ease by which it undergoes degradation to glucose in presence of acids.

The second important difference between these two polysaccharides is the remarkable behavior shown by starch towards the enzyme, diastase, by which it is degraded, with particular ease, into the disaccharide, maltose, the latter in turn being readily hydrolysed to glucose by dilute acids. While concurrent studies on cellulose and starch during the last twenty years have led to a solution of the problem of the structure of cellulose in very large measure, that of starch remains still unknown.

Cellulose has been shown by both chemical, physico-chemical and x-ray studies to consist of large linear chains of anhydroglucose units linked through the 1:4 positions, the cyclic glucose structure being of the pyranose type and the stereochemical configuration represented by linkage of the β-type.

In the case of starch the problem is much less simple, the difficulty arising from the fact that there are two types of polysaccharide present in all starches, namely amylopectin and amylose.

Although this was recognized prior to the commencement of the

numerous experimental investigations carried out on starch by various workers (5,18,48) the opinion was apparently held that the differences presumably were concerned with nothing more than variations in chain length of the same linear type building unit.

For this reason much of the earlier work is losing any present day significance in that there is now abundant evidence to show that the two constituents, amylopectin and amylose are structurally different polymers of anhydroglucose, linked in both cases stereochemically in the alpha and not in the beta form.

General Structure of the Starch Aggregate

As in the case of cellulose, this empirical formula of starch is $(C_6H_{10}O_5)_x$, the value of x being still unknown. Due to the presence of three hydroxyl groups per $C_6H_{10}O_5$ unit it can form esters and ethers, both simple and mixed. For the greater part, the hydroxyl groups consist of two secondary and one primary alcohol per $C_6H_{10}O_5$ unit, the evidence resting on methylation studies on "native starches" *.

I. Modern Views of the Structure of Starch

A. The Component Sugar and the Nature of its Linkage

The earliest workers in the carbohydrate field discovered that starch may be converted almost quantitatively to glucose by

* Under the term "native starch" is meant a starch in which both the original amylose and amylopectin are present.

acid or enzymic hydrolysis, under conditions much less drastic than in the case of cellulose. In the year 1819, de Saussaure isolated crystalline maltose from the syrup produced in the hydrolysis of starch by malt diastase. Shortly after, other investigators obtained a yield of over 90% of maltose by enzymic hydrolysis of starch (1). Karrer (2) proved that this maltose did not arise as a result of enzyme synthesis by hydrolyzing starch with acetyl bromide to acetobromomaltose (80% yield). By debromination of the latter a yield of 22-27% crystalline heptaacetyl maltose was obtained. Since maltose, when subjected to the same treatment, yielded 30% heptaacetyl maltose, it was inferred that starch was probably composed entirely of maltose anhydride units. Definite evidence that maltose was not a secondary reaction product from the hydrolysis of starch was provided by Haworth (3) who found that trimethyl starch, on hydrolysis with acetyl bromide, yielded a bromacetylhexamethyldiglucoside the parent sugar of which was maltose.

In 1930, Freudenberg hydrolyzed starch with acetyl bromide, and isolated small amounts of non-crystalline material which he considered to be tri- and tetra-glucosides containing α-linkages only (4). After methylation of these acetolysis residues he was able to isolate crystalline undecamethylmaltotriose and decamethylmaltotetraose which compounds contained α-linkages only (5). The colligative nature of the observed rotations throughout the series, involving methylated derivatives of maltose, of the tri- and tetra-

α-polyoses and finally of methylated starch may be considered as additional evidence for the presence of only α-linkages in starch. Freudenberg, from his studies of the kinetics of acid hydrolysis of starch, concluded that not more than one link in thirty anhydro-glucose residues could be other than α (6).

Thus from a survey of the literature of starch, it can be seen that most workers are in agreement with the hypothesis that starch is formed from glucose-anhydride units joined by α-1,4 linkages.

B. Physical Structure of the Starch Granule

Starch always occurs in the green plant in the form of definite granules (starch grains) which vary in size from macroscopic to almost ultramicroscopic. The structure of the starch grain is characteristic in that each consists of numerous layers or lamellae which are laid down either concentrically or eccentrically around one or more morphological centres usually designated as the hilum, nucleus or locus of the grain.

The cause of the layered or lamellar structure of the starch grain has been the subject of many theories. The layers consist, according to Nägeli (7) and Meyer (8), of elongated crystalline micelles, arranged radially, as in spherulites. The radial structure is revealed by the double refraction of the grains. X-ray diagrams also show this lattice-like arrangement. The interference lines of starch are rather broad and show that the crystallites are

small, probably not exceeding 100 Å. units in size (9). The difficulty in interpreting this data is shown by the fact that despite years of investigation by Katz and co-workers (10), (11) nothing more has been established beyond the fact that starch exists in various modifications having a limited degree of crystallinity. X-ray diagrams of completely dehydrated starch indicate an amorphous condition (12). Therefore, water must be associated with the glucose molecule in starch in order to produce crystallinity. According to Frey-Wyssling (13) the double refraction of the grains amounts to 0.0155 which is about one-fourth that of cellulose. The planes of the glucose rings therefore cannot have the same arrangement as in cellulose where they lie parallel to the long axis of the crystallite; instead they must lie at a definite angle to the axis. A screw-like arrangement of glucose residues as in the case of cellulose is consistent with some of the properties of the starch grain and provides space to accommodate water molecules between the chains. On the other hand it is inconsistent with the evidence of double refraction, for the uniaxial birefringence is not negative as would be the case if the screw axes lay radially.

Sponsler (14) in x-ray studies of the structure of starch found that the natural regularity of atoms in the starch grain is destroyed when the grain is crushed. This result is considered evidence showing that the regularity is not that of a crystalline structure, but is only indicative of the fact that the starch grain is built up of concentric layers of units and that the structure

is neither amorphous nor crystalline in the ordinary sense of the terms.

Regarding the actual nature and mode of union of the starch building units, x-ray analysis has been helpful, but here again the results are not as conclusive as with cellulose. Herzog and Jancke (182) believe that cereal starch crystallites have axial ratios of 0.7252:1:0.5509 with orthogonal axes. Sponsler (14) concludes that the unit cells in starch are tetragonal and of the following dimensions, namely $a_o = b_o = 5.94\text{Å}$, $c_o = 5.05\text{Å}$. According to Bear and French (183) this cell does not account for several of the innermost rings found in the starch diagram. The latter workers also have criticized the work of Ott (184) who found the number of glucose residues per cell in starch to be two. The results of Náray-Szabó (185) were likewise criticized as indicating too great symmetry within the starch molecule, which was pictured as a tetragonal cell with $a_o = b_o = 16\text{Å}$, $c_o = 9.8\text{Å}$, containing sixteen glucose units. Bear and French (183) have conducted a systematic study of the original Katz' (10) (11) three types of starch x-ray diffraction patterns. The unit cells for the A and B modifications have the following dimensions respectively,

A type	$a_o = 15.4\text{Å}$	$b_o = 8.87$	$c_o = 6.18$
B type	$a_o = 16.1\text{Å}$	$b_o = 9.11$	$c_o = 6.34$

and there is evidence for a ring for ring correspondence between

these extreme (A and B) types of pattern.

These values for the cell dimensions suggest, according to the authors, a number of glucose residues per cell equal to four, assuming 1.50 for the density and a water content of 18%. This, and other evidence, is taken by the same authors to indicate that the diffractions are those of a single major component of the starch granule and that starch granule crystallites are built on triclinic lattices whose axes are very nearly orthogonal. Cell dimensions for the v-type of powder pattern (Katz's Verkleisterung) have not been determined but it is indicated that there are several varieties of this type (186).

C. Non-carbohydrate Constituents of Native Starch

Grains of unmodified natural starch consist of 80-85% of true carbohydrate material, 20-15% of water and variable amounts of organic and inorganic substances including fats, proteins, tannins, phosphates and other products. (31,32).

1. Phosphorus

M. Samec has perhaps done more work than any other investigator on the phosphorus content of starches and on the influence of phosphorus on its colloidal properties. He submits evidence to show that phosphorus forms an integral part of the starch molecule (15). According to Samec amylopectin contains 0.185% and amylose, 0.007% phosphorus (calculated as P_2O_5). Samec claims to have made a separation of amylose and amylopectin by electrolysis, the separation being based on their different phosphorus contents. Meyer disputes this (16), finding evidence that it is not the phosphoric acid content but the particle size which is important in anodic separation. Large carbohydrate particles separate even if they are phosphorus-free and the phosphorus-containing amylopectin of potato starch may remain in solution for a long time, even on electrodialysis, if it has previously been converted into a water-soluble form by treatment with chloral hydrate (17).

In contrast with Samec's views that amylose is practically free from phosphorus, Hirst (18) and co-workers have prepared a soluble

amylose the full phosphorus content of the original starch having been retained. The amylopectin portion, which has the same phosphorus content, differs completely in solubility and paste-forming properties. These workers disagree with Samec that the properties of amylopectin are due to its phosphorus content.

Samec has compared the phosphorus contents of different starches (19). In potato starch phosphorus is probably bound directly to the glucose residue and is believed to be responsible for the fundamental colloid properties. In wheat starch, the amylopectin portion contains phosphorus and nitrogen in a 1:2 ratio together with fatty acids in the form of phosphatides. On the basis of later work Samec divides starches into the potato group and the grass-like group (wheat, corn, rice) (20). In the starches of the former, phosphorus is present as ortho-phosphoric acid esterified with the starch polysaccharide. These starches have a high electro-conductivity and a high hydrogen ion concentration. Starches from the grass-like sources have a low conductivity and a low hydrogen ion concentration. Their phosphorus content, while probably present as phosphate, is masked, i.e. not easily freed, and appears to be linked with a nitrogenous compound (protein) as an organic amino phosphate. The potato starch group shows good correlation between phosphorus content and conductivity or pH. This correlation is absent in the wheat group.

Lampitt and co-workers (21) in studies on the aging of solutions of various starch fractions produced by grinding and dissolution

conclude that the behavior of these fractions is connected with their phosphorus contents. Thus the presence of a considerable proportion of bound (non-dialyzable) phosphorus is correlated with a greater tendency to retrograde from solution on aging.

Dahl (22) has shown that the abilities of various amylopectins extracted by electrodialysis and possessing different phosphorus contents to bind inorganic phosphorus in the presence of muscle extracts show no proportionality to the phosphorus content. Amyloses took up only 33-35% of the phosphorus bound by the corresponding amylopectins.

Thus it can be seen from these experimental observations that while the presence of phosphorus in starch is quite definite its exact role is still obscure. Certain older theories in which the phosphorus content was held responsible for the physical properties are no longer considered to possess much significance. It is possible, however, that the bound phosphorus is present as a product left behind in the process of the enzymic synthesis of starch.

2. Nitrogen

Little is known concerning the nitrogen content of starches. The amount present is always small and the nitrogen appears to be chemically bound to the phosphorus (23), (24). In accordance with his views that the impurities in starch determine its properties Samec (25) produces evidence to show that the nitrogen content

exerts a definite influence on the characteristics of starch.

3. Fatty Acids

The fatty acid content of starch was investigated principally by Taylor and co-workers. Early work showed that although the major portion of the fatty material present in starch cannot be removed by solvents prior to hydrolysis (26), following this, the fatty acid content of corn starch is solvent-extractable, the principal product being palmitic acid which is liberated when hydrolysis has reached the erythrodextrin stage. The palmitic acid apparently is attached indirectly to the carbohydrate, but directly to an unsaturated component which is present with it. In later work by Taylor and Sherman (27) the unsaturated component was identified as oleic and linoleic acids. These are liberated by acid, alkaline or enzymic hydrolysis. It has been shown, too, that these fatty acids are associated with the amylopectin portion of the starch, the amylose being present as pure carbohydrate.

The fatty acid content of various starches has been investigated (28),(29). Cereal starches have a higher content than potato starch. Samec (24), in agreement with Taylor, believes that the fatty acids are present as esters of the polysaccharide but Schoch (29) has been able to remove completely these materials by extraction with such solvents as aqueous dioxane (80%) and methanol without changing the properties of the starch. Lehrmann (30) disagrees with Schoch's conclusion that the fat is distributed

extraneously as an impurity in the starch granules.

D. The Basic Unit of the Starch Molecule

While glucose has been almost universally accepted as the elementary building block of starch there has been considerable controversy concerning the existence therein, in whole or in part, of supposedly larger basic units possessing an independent structure. Such a concept is the basis of what Hanes (33) chooses to call the low molecular "elementary unit" hypothesis. The foundation of this hypothesis was that the true molecular building units of polysaccharides were relatively simple "elementary units" considered to be anhydrosaccharides of crystalloidal dimensions. Such "elementary units" were assumed to be capable of associating into larger aggregates as a result of the interaction of residual, auxiliary or co-ordinate valence forces.

While the "elementary unit" hypothesis is no longer considered tenable it is interesting to note the various types that have been proposed.

1. Glucosan - an anhydride of glucose; Bergmann and Knehe (34).

2. Maltosan - an anhydride of maltose; Karrer and Nägeli (35).

3. Dihexosan for the amylose fraction and trihexosan for the amylopectin fraction; Pringsheim (36).

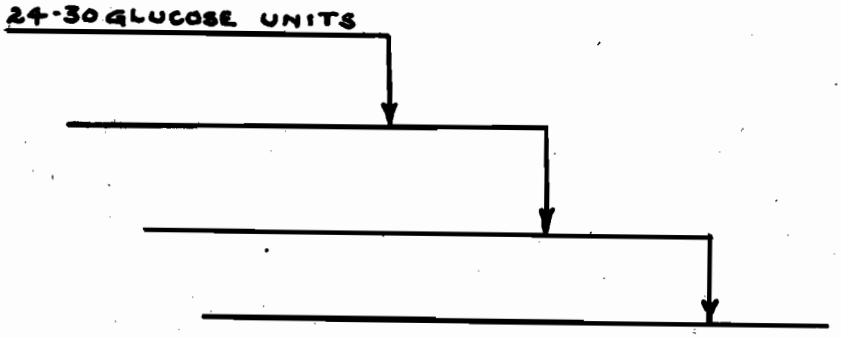
4. Anhydride of a hexasaccharide; Ling and Nanji (37), Irvine (38).

The evidence for the "elementary unit" hypothesis was based primarily upon cryoscopic molecular weight determinations of acetylated derivatives dissolved in organic solvents. The misleading results obtained were due to the fact that this method is not applicable to such substances.

The opposing view on the question of the fundamental structure of the starch polysaccharide molecule was that of the saccharide linear chain hypothesis. According to the view originally held by Emil Fischer the polysaccharide molecule consists of a chain structure made up of a limited number of monosaccharide units joined together by ordinary valence forces.

Haworth and co-workers (39, 40, 41, 42, 43, 44, 45, 46) took a middle course in marshalling their evidence in an attempt to show that while a saccharide chain structure was indicated there was also reason to believe that a basic chain unit was involved in the starch molecule. The method of end group assay which was developed originally in connection with the determination of the chain length of cellulose was applied to whole starches from natural sources and in every case approximately the same proportion of end group was found. This proportion indicated the presence of one non-reducing terminal group in 24 to 30 glucose residues. Peat (47) made it clear that the Haworth school does not believe that this chain of 24 to 30 glucose residues constitutes the whole molecule of starch, but in fact regards it as a repeating unit. Haworth and Hirst pictured the ramified starch chains as follows, that is a

series of single chains grouped in parallel arrangement and held to one another through an individual chemical union. They referred



to the link between the units, indicated by the arrow in the diagram, as a polymeric bond (48).

Thus Haworth's work did not prove the existence of a basic unit in starch so much as it provided evidence for the possible presence of branched chains. The conception of a single basic building unit is no longer held, the controversy now centering around the nature of the branching chains and the types of bonds involved. A more detailed account of the work on chain branching and the methods of determining chain length is given later. (pages 15-19, 32).

E. The Molecular Weight of the Starch Molecule

The general picture presented thus far indicates that starch consists of α-glucopyranose units linked together in chain form. This chain may exhibit branching, the mode of which is not yet clear. At this point it is interesting to consider the ultimate molecular weight and size of the molecule of starch. Measurement

of the molecular weight of a highly complex substance such as starch is a difficult matter and the ordinary methods do not apply. In addition the behaviour of starch molecules in solution must be considered, for the phenomenon of association, which is dominant in the case of starch, exercises an appreciable influence on molecular weight measurements.

1. Osmotic Pressure Measurements

The use of osmotic pressure measurements for the determination of the molecular weight of starch has received consideration. It is important to note that all physical measurements on liquids should be limited to those homogeneous solutions which do not undergo changes with time. With starches and their derivatives, however, alterations in solution on standing are very frequent. Aqueous solutions of starches and their derivatives are particularly unstable, exhibiting a series of phenomena, known as "aging" which results in the production of super-molecular aggregates. As a result of this "aging" phenomenon it is apparent that the measurement of the osmotic pressure of aqueous starch or amylose solutions will give the pressure only for a certain stage in the association or aging process and that the molecular weight of the parent molecule cannot be obtained from this. On the other hand, osmotic pressure measurements can be applied to degraded starches in which the association phenomenon has been greatly reduced. Osmometric measurements can be made on starch esters in such solvents as

tetrachlorethane and benzyl alcohol where association is reduced to a minimum. Even with such solutions, however, care is necessary in the interpretation of results since polymeric substances with chain-like structures show considerable deviations from van't Hoff's law even at very low concentrations. The osmotic pressure is not proportional to the concentration, but increases faster than concentration and conforms for the most part with the relationship

$$p = ac + bc^2$$

or $$p/c = a + bc$$

p = osmotic pressure
c = concentration
a + b = constants

Thus, the osmotic pressure must be measured at different concentrations and the reduced osmotic pressure p/c extrapolated, graphically, to zero concentration, where the van't Hoff relationship holds.

Staudinger and Husemann (49) found osmometrically that starch dissolved in formamide had a molecular weight of 286,000. However, the reliability of this value is questionable as starch in formamide solution is unstable (50), (51). The molecular weight of about 200,000 which was calculated by Samec (52) from osmotic pressure measurements, using aqueous solutions of amylose, is also open to question due to the discrepancies caused by the aging process. Measurements of osmotic pressure in hydrazine hydrate or in ethylene-diamine, where the phenomenon of aging is not apparent, have not yet been carried out. Meyer and co-workers have used

osmotic pressure methods to determine the molecular weights of starch esters and ethers since, when dissolved in suitable solvents, they do not exhibit aging. Thus the osmotic pressure of triacetylamylose in tetrachloroethane indicated a molecular weight of about 78,000 (corresponding to a molecular weight of 45,000 for the parent amylose) (53), while a methylated amylose, which forms stable aqueous solutions at room temperature, gave a molecular weight of 50,000 (17).

2. Viscometric Determination of Molecular Weight

According to Staudinger's well-known equation the limiting viscosity of homologous chain polymers should be proportional to the molecular weight

$$\lim_{c \to o} \left[\frac{\eta_{sp}}{c} \right] = K_m M$$

η_{sp} = specific viscosity

where M is the molecular weight and Km is a constant whose value must be determined for each new polyhomologous series. However, after a critical survey, Meyer (54) observed that the only definite conclusion which could be drawn was that the limiting viscosity increases continuously with the molecular weight as do density and boiling point in a homologous series.

Applied to starch and its derivatives, the method according to Meyer (9) is valid only for the unbranched portion of the molecule (amylose) and then only if products of comparable molecular size are considered. Most of the reported viscometric determinat-

ions on starch have been made without regard to the two possible molecular configurations (branched and unbranched chains) found in native starch. In general, the limiting viscosity in the case of isomers is less for branched than for normal isomers. Therefore, of two substances in solution, the one with the smaller limiting viscosity can be considered to have a branched chain structure if the substances are of the same molecular weight and of similar constitution. Thus amylose and its derivatives exhibit higher viscosities than amylopectin of equal molecular weight (55). Staudinger (56) compared the viscosities of cellulose acetates and starch acetates of equal molecular weights as determined by other methods and concluded that the latter were branched.

In viscometric measurements the phenomenon of aging is an important factor. Aqueous solutions are particularly troublesome in this respect. On the other hand, strongly hydrolytic solvents cannot be used since in these cases chain fission occurs as well as disruption of secondary valence linkages. Hydrazine hydrate and ethylenediamine hydrate have been used successfully as solvents for viscometric determinations (9).

3. The Ultra-Centrifuge and Molecular Weight Determination

The ultra-centrifuge has been used by several workers in the starch field. Here again care must be taken to correct for variations found with changes in concentration by determining the sedimentation values at several concentrations and extrapolating to zero

concentration. Lamm (57) has studied the molecular weights of starch using the ultra-centrifuge method and has obtained values ranging from 100,000 to 1,000,000. This worker used an aqueous solution prepared by dissolving starch in concentrated aqueous zinc chloride solution, diluting and then removing the zinc chloride by dialysis. Other workers (9) claim that these values do not represent molecular weights, but the weights of molecular aggregates.formed by an indefinite degree of aging.

4. The End-group Method of Molecular Size Determination

The method of end-group analysis was developed originally by Haworth and co-workers (42) in connection with the determination of the chain length of cellulose. The method involves complete methylation followed by hydrolysis, the details of which will be described in a later section. The method has been applied by Haworth and co-workers to whole starches from many natural sources, and in every case approximately the same proportion of end group has been found. Recent examples are furnished by the assay of rice starch (58), banana starch (59), wheat starch and chestnut starch (60). In all cases a chain of 24 to 30 glucose residues linked in one continuous chain is indicated. Hess (61), using a modified method for the estimation of the terminal tetramethylglucose molecule, found a chain length of 52 glucoseanhydride units. Freudenberg's results show values ranging from 25 to 100 units depending, apparently, on the method used for the isolation

of the tetramethylglucose (62).

Other methods of chain length determination show marked variation from the end group method. Richardson (63) from a study on reducing values decided that the chain length varied from 460 to 1470 units.

Samec (24) from osmotic pressure measurements concluded that the starch chain consisted of 1230 units, while Svedberg (64) from his work on the sedimentation velocity in the ultra-centrifuge obtained a value of 5550.

5. Molecular Size by use of the Mercaptalation Method

An independent method of end-group analysis, due to Wolfrom (65), consists in the hydrolysis of methylated starch with concentrated hydrochloric acid in the presence of ethyl mercaptan , the latter compound reacting with the reducing groups as they are released by hydrolysis. The resulting mixtures of mercaptalated hydrolyzed products were isolated at various time intervals during the hydrolysis and subjected to sulphur analyses. The degree of polymerization was calculated from the sulphur content. Results indicated an initial average degree of polymerization not greater than 150 glucose units.

II. The Colloidal Nature of Starch in Solution

A. Physical Manifestations of the Colloidal Nature of Starch

As with most substances which are essential to plant or animal life, starch in solution exhibits colloidal properties. This was recognized at an early date in the chemical study of starch.

1. Swelling in Solution

Starches in aqueous solution have a definite temperature at which swelling occurs and also at which the granules burst. These characteristic temperatures for some common starches are as follows:

Starch	Swelling Temp.	Bursting Temp.
Potato	46.2	62.5
Rye	45.0	55.0
Corn	50.0	62.5
Wheat	50.0	67.5
Rice	53.7	61.2
Arrowroot	66.2	70.0
Acorn	57.5	87.5

Various substances such as chloral hydrate, sodium trichloracetate and thiourea in concentrated aqueous solution act as swelling agents. Their function seems to be the breaking of secondary valence bonds (cross-linkages) which hold the molecules together (51). The starch grains exhibit a limited swelling in not water. They absorb from thirty to fifty times their volume of water and

form an elastic jelly. According to Meyer (9) such limited swelling is characteristic of chain molecules which are held together by cross-linkages to form loose three-dimensional, giant molecules.

The paste-forming properties of starch have been associated with the amylopectin fraction. Samec believes that the properties of amylopectin are due to the phosphorus and nitrogen contents (66) Meyer (9), on the other hand, suggests a purely physical interpretation of the phenomenon of swelling in which the typical properties of paste formation are bound up with the morphological structure of the swollen grains. Thus a starch recovered by precipitation after complete dissolution (destruction of the grain structure) is incapable of forming a paste due to the fact that the linkages forming the micellar crystallites into a network have been destroyed by solvent action. On standing, however, a solution of such a starch undergoes the so-called "aging" process by which the crystaline micelles are reformed with consequent return of the paste forming properties.

Thus the behaviour of starch in the presence of a solubilizing agent can be visualized as follows (9). The main mass consists of a loose, three-dimensional net composed of amylopectin molecules. The looseness of this net depends upon the strength of the solubilizing agent and upon the temperature. Heterogeneously mixed with the amylopectin network are the amylose molecules which are either dissolved or remain as a disconnected part of the network. The amylose molecules will gradually diffuse out of the mass to form a

colloidal solution. Increase of temperature causes more and more destruction of the linkages joining the crystalline micelles until there is complete destruction of the grain. These changes are reversible; hence the occurrence of retrogradation which is merely the reforming of crystalline micelles with the expulsion of water from the vicinity of the glucose chains.

The most recent measurement of the heat of swelling was made by Küntzel and Dolhner (67), who obtained a value of 36.75 cal. per gram. This heat comprises the heats of fusion and of solution of the "molten" polysaccharide. On melting, 39.35 gm. cal. per gm. is absorbed and on dissolution with the accompaniment of hydration, 2.6 cal. per gm. is evolved. The heat of fusion of roughly 40 calories is about equal to that of α-glucose.

As indicated above, the changes observed in paste formation of starch are reversible. On removal of water or on cooling, a starch paste will gel. X-ray investigation shows that this solidification is traceable to the formation of crystallized regions. Different starches crystallize at different rates, just as different starches swell at different temperatures.

B. Chemical Manifestations of the Colloidal Nature of Starch

The chemical aspects of the colloidal nature of starch have been investigated by Samec (68) who suggested that the paste-forming properties of starch and the peculiar properties of amylopectin

were due to the electrochemical character of the starch components which in turn depended upon the chemically-bound phosphorus. This theory is no longer considered significant, especially since Posternak (69) showed that the amylopectin of cereal starches does not contain any chemically-bound phosphorus. Phosphorus may be detected in these components, but it is readily removed by solvent extraction or precipitation.

Koets and Kruyt (70) have made comparative observations on solutions of amylose, amylophosphoric acid and cellulose. According to these workers the zeta-potential on amylose arises in part from the dissociation of hydrogen ions from the hydroxyl groups (repressed at pH 3.7) and in part from the thickness of the double ionic layer which decreases in high alkali concentration. Conversion of amylose into amylophosphoric acid greatly raises its negative potential until it is comparable to that of agar and gum arabic.

III. Previous Studies on the Constitution of Starch

Numerous experimental procedures have been employed in attempting to determine the structure of starch. They are included here, because while many of them have not found general acceptance, still they either have contributed or may contribute later in some way to our knowledge of starch.

A. Pyrolysis of Starch

Pictet and Sarasin (71), (187) distilled cellulose, starch and dextrins at a pressure of 12-15 mm. and obtained a crystalline substance with the composition $C_6H_{10}O_5$ and showed it to be a glucose anhydride, namely, laevo-glucosan. Later they modified their procedures by heating the starch with glycerol at 200° to obtain a trihexosan (72) $(C_6H_{10}O_5)_3$. This result was interpreted as evidence that starch is an aggregate composed of three linked hexose anhydride groups rather than a polymer of an anhydride of maltose. By interrupting the heating at a certain stage (red coloration with iodine) they claimed (73) to have obtained a mixture of tri- and hexahexosans. The identification of these products was based upon cryoscopic measurements, which later were proved to be valueless when applied to starch products. By continued heating of starch with glycerol at 200° the substance is depolymerized into soluble starch, hexahexosan, trihexosan and finally into crystalline laevo-

glucosan (74). In a later communication (75) Pictet reconsidered his previous assertion that he had isolated the structural unit of starch. By a modification of the degradation procedure in glycerol, as reaction medium, an isotrihexosan was obtained which was thought to be a structural unit of starch because it showed a color with iodine and had a tendency to polymerize. Properties of this substance which are tabulated include its cryoscopic molecular weight (472) and its tendency to polymerize to an isopolyhexosan, a substance with starch-like properties having a molecular weight of 3325. Karrer (76) regarded these substances as merely partially-hydrolyzed starch degradation products.

The pyrolysis of starch, while not leading to any clear conception of the structure of starch, did provide a mode of synthesis of a new class of compounds, namely, the anhydro sugars.

B. Work of Pringsheim

Previous mention has been made of the work of Pringsheim on the postulated "elementary unit" of the starch molecule (36). He was primarily concerned with (a) the crystalline dextrin-like carbohydrate obtained from starch by means of B. macerans, and (b) "polyamyloses", the chemically depolymerized products of these dextrins. These amyloses are not to be confused with the soluble portion of native starch variously designated as amyloamylose, amylose and α-amylose. In a series of papers published from 1912 to

1932, Pringsheim endeavoured to prove that "dihexosan" was the basic unit of amylose and that "trihexosan" was the basic unit of amylopectin. Cryoscopic methods were used to determine molecular weights and therein lay this worker's greatest error, as it has since been shown that these methods are not applicable to polysaccharides. Therefore the nature of the structures of the products designated by Pringsheim and others as "dihexosan" and "trihexosan" must be considered unknown.

C. Chemical Degradation of Starch

1. Acid Hydrolysis

The action of acids on starches was one of the first degradation reactions to be studied in connection with this polysaccharide. In general the stepwise degradation of starch by treatment with acids can be followed by the iodine color or by the reducing value. For example, Friedrichs (77) hydrolyzed potato starch with 0.05 N oxalic acid for one hour under a pressure of 2-3 atmospheres and isolated a glucose β-glucoside in addition to three "achrodextrins", two "erythrodextrins" and one "amylodextrin". Similar results were obtained by Tychowski (78) who studied the effect of temperature on the hydrolysis of starch pastes at pH 5.25. The presence of chalk was found to inhibit the hydrolysis reaction. Potato starch in the presence of 7.5% hydrochloric acid at room temperature for thirty days was converted into soluble starch containing unchanged

amounts of phosphoric acid. With 15% hydrochloric acid marked hydrolysis and loss of phosphoric acid occurs with the production of a substance resembling "amylohemicellulose". Studies on the degradation of starch by heat alone with and without the presence of calcium carbonate (78) led to the conclusion that phosphoric acid is chemically bound in potato starch and is not present merely as an adsorption complex.

The action of hydrofluoric acid on starch has been investigated (79). Starch was treated in the dry state with hydrogen fluoride to obtain "amylan" which had the empirical formula $(C_6H_{10}O_5)_x$.

Finally, the hydrolysis of starch by means of acids provided the first evidence that the starch molecule was made up of glucose units and that maltose might be involved in the constitution of this polysaccharide.

2. Alcoholysis

Mention was made earlier (p. 25) of the work of Pictet on the degradation of starch in the presence of glycerol. Further investigations have been carried out (80) which indicate that this degradation is the result of an alcoholysis reaction. Complete degradation of starch by glycerol yielded a mixture of α- and β-glyceryl d-glucosides. The nature of the products depended on the temperature of alcoholysis. Vogel (81) quite recently has defended the existence of Pictet's isohexosan which is produced as a result of the action of glycerol on starch.

3. Acetolysis of Starch

Acetolysis of starch was first investigated by Böeseken (82) and by Bergman (83). The latter was searching for a cleavage reagent which would so react that by its hydrolytic action the original point of union would be definitely fixed due to the introduction of a characteristic substituent. The mechanism of the acetolysis of starch has played a part in the development of the "elementary unit" hypothesis. Sutra (84) by acetolysis of starch isolated "maltosose" and considered it to be the recurring "unit cell" in starch.

Freudenberg, in his intensive studies on the constitution of starch, investigated the kinetics of the hydrolytic and acetolytic degradation of starch (85) and concluded that the polysaccharide behaved as the α analog of cellulose.

Mention was made previously concerning Karrer's work on acetolysis (2). Freudenberg (86) repeated these experiments on both octaacetylmaltose and starch with acetyl bromide and was able to isolate 57-58% heptaacetylmaltose from the former and 37% acetobrommaltose plus 22% heptaacetylmaltose from the latter. Freudenberg, as did Karrer , inferred that starch was composed of maltose anhydride units, united in continuous chains.

Haworth (3) using the acetolysis reaction claimed to have obtained proof of the presence in starch of continuous chains of α-glucopyranose units linked in the 1,4 position. That maltose is not a secondary reaction product in the hydrolysis of starch was

shown by hydrolysis of trimethyl starch with acetyl bromide which yielded a bromacetyl hexamethyldiglucoside, the parent sugar of which was maltose.

Recently Peat (87) has suggested the acetolysis of methylated starch as an expeditious method for the detection of end-groups. It is shown that the acetolysis of methylated starch by means of acetyl chloride in cold chloroform proceeds with great rapidity and that a selective removal of end-groups is observable, the whole of the tetramethyl-glucose being separated within five minutes after commencement of the reaction.

4. Oxidation of Starch

Hönig (88) investigated the oxidative decomposition of starch by an alkaline solution of bromine and found that maltobionic acid was formed. This was considered evidence that starch is derived from a basic diglucohexosan molecule. Samec (89) digested corn starch with alkaline hypochlorite solution containing 1.5% active chlorine in about 0.001 N sodium hydroxide and obtained a product resembling soluble starch. Oxidation of this type was believed to introduce carboxyl groups into the molecule.

Some of the most important work relative to the structure of starch by the oxidative method has involved the use of periodic acid and periodates. Jackson and Hudson (90) found that periodic acid rapidly oxidizes corn starch at room temperature with the

utilization of one mole of oxidant per $C_6H_{10}O_5$ unit. The structure of the oxidized starch unit was established by the isolation of D-erythrose and glyoxal. This provided evidence of cleavage of the carbon chain of each glucose molecule between carbon atoms 2 and 3. Caldwell and Hixon (91) found that the consumption of periodic acid by starches varied inversely with the total fat and phosphorus contents of the samples. The isolation of glyoxal after hydrolysis of the oxidized starches indicates cleavage of the non-terminal glucose units between C-2 and C-3. On the basis of the reducing power of the products and the amount of CH_2O produced, the authors concluded that starches must have a greater average chain length than the Haworth figure (24-30). By the action of periodate on starch , Purves and co-workers (92) isolated a crystalline substance in small yields (0.7-0.9%) with an empirical formula $C_{13}H_{16}O_8(OMe)_4$. They regarded it as the methyl acetal of an aldehyde or ketone.

Recently Nazarov (93) has studied the kinetics of the oxidation of suspensions of potato starch by means of 0.01N $KMnO_4$ and H_2SO_4 at 20°C. A unimolecular reaction was indicated. The reaction proceeded on the surface as well as inside the starch grains.

There has been much experimentation on the production of the so-called "thin-boiling" starches by oxidative means. These investigations were usually carried out with a view towards producing more suitable sizes and coatings in the paper and textile industries (94). By treatment with such reagents as potassium

dichromate, hypochlorites and sulphuric acid more suitable starch products are obtained.

D. Methylation Studies and Terminal Group Analysis

1. Origin and Purpose of Methylation

The first investigations on the methylation of starch were carried out by Karrer (95) (2), Schmidt (96), Irvine (97) and Freudenberg (4). Using either methyl iodide (95), dimethyl sulfate (2) or diazomethane (96), they obtained only methylated starches of low methoxyl content (17-35%). Irvine (97) using dimethyl sulfate increased the methoxyl content to 43.75% after twenty-four methylations. He was able to isolate a di- and a tri-methylmethyl glucoside after hydrolysis of the methylated product. Freudenberg (4) was one of the first to apply the technique later used with success by Haworth, namely, the methylation of a pre-acetylated starch.

The first successful application of methylation to the problem of starch structure was made by Haworth, Hirst and Webb (39). These workers methylated the product obtained by acetylation of an ethanol-precipitated starch. The methylation carried out with dimethyl sulfate and alkali in acetone solution was repeated as many as six times, in order to produce what was believed to be a fully-methylated product.

The structure of starch as assigned by the Haworth school is based upon a determination of the quantity of terminal glucose

units. The so-called "end-group" analysis is based upon the quantitative separation by careful fractionation of 2,3,4,6 tetramethyl methyl glucoside from the mixture of di,-tri-and tetramethyl methyl glucosides obtained on the methanolysis of fully-methylated starch (42). A slightly modified method (98) has been applied by Hirst (44) to a number of different starches (58) (60). In addition, many other investigators have introduced changes and improvements in the Haworth fractionation technique (99) (62) (18) (60) (100).

2. Modifications in the Methylation Technique

While it was claimed by Haworth that his method (39) gave complete methylation, it was soon found that even after many repeated methylations, the methoxyl content did not exceed 42 to 43% whereas the theoretical value is 45.6%.

Complete methylation, however, has been attained in several ways. Freudenberg (62) added metallic sodium and methyl iodide to a suspension of well-dried starch in liquid ammonia and after several such treatments a methoxyl content of 45.5% was obtained. A concurrent decrease in chain length as shown by viscosity measurements (101), however, limited the extension of this technique until Hess (101) successfully applied it to anisole solutions of premethylated (dimethyl sulfate) starch.

E. Enzymic Degradation of Starch

1. Introductory Section

Studies on the enzymic hydrolysis of starch have been many and varied and results of these investigations have led to numerous conceptions of the structure of starch, many of which are no longer considered significant. An attempt will be made here to evaluate, in a general way, the results of enzymic degradation of starch and to indicate methods by which this work is carried out.

(a) Isolation and Properties of Amylases

(1) The Amylase Systems

The most important enzymes which attack starch are the amylases which are obtained from cereals, particularly barley. The work done on these hydrolytic agents has shown that malt amylase consists of at least three components which are variously designated in the literature as follows:

(i) The saccharogenic amylase or β-amylase: This enzyme is destroyed by heating an aqueous extract for 20 minutes at 70°, (103) and is relatively stable to acids.

(ii) The dextrinogenic amylase or α-amylase: In contrast to the β-enzyme this is much more thermostable but is inactivated by acids (below pH of 3.6).

(iii) The existence of a specific type of liquefying amylase, assumed to be present in ungerminated grain, has been confirmed by the isolation of the so-called "amylophosphatase" by Waldschmidt-

Leitz and Mayer (104).

(2) The α and β-glucosidases

These enzymes have been used to determine the glucosidic link in the side chains of residual dextrins (9).

(3) Phosphorylase

In the presence of inorganic phosphates, starch is converted by phosphorylase into glucose-1-phosphoric acid (104).

(4) Separation of the Enzymes

The differential inactivation separation methods developed by Ohlsson (106) are considered impractical since a single enzyme might be substantially altered as a result of the relatively drastic conditions employed. However, β-amylase can be prepared pure by permitting an aqueous ungerminated grain extract containing α and β-amylase to stand for several days at a pH of 3.6 (107). In malt amylase, the β variety is destroyed by heating the aqueous extract at 70° for 20 minutes (107) (103).

Van Klinkenberg (108), on the basis of earlier work by Wysman, has devised a separation method which takes advantage of the fact that β-amylase diffuses through a stiff gelatin gel more rapidly than does α-amylase.

A third general method of separation of the amylases consists of selective adsorption of the two substances from malt extract by alumina. At pH 3.8, the β-amylse is adsorbed together with a small amount of α-amylase leaving only β-amylase in solution. (109)

Holmbergh (110) demonstrated a selective adsorption of α-amylase from 40% alcoholic solutions of the mixed amylases by a number of different adsorbents. Particularly effective were native starch grains, rice and potato.

(i) The Glucosidases

Emulsin (β-glucosidase) is widely distributed in the plant kingdom. The term is usually applied to a definite enzyme preparation such as yeast extract and designates a mixture of enzymes rather than a single enzyme. A method of preparation is given by Waksman and Davison (111). α-Glucosidase is obtainable from yeast.

(ii) Phosphorylase

Hanes (105) isolated phosphorylase from the juices of peas and potatoes.

(5) A fourth variety of starch hydrolyzing enzyme is that responsible for the activity of certain bacteria. For example, upon hydrolyzing starch with Bacillus macerans, Schardinger (112) obtained crystalline dextrins the so-called α- and β-dextrins. Tilden and Hudson (113), found that similar dextrins were obtained by use of a cell-free enzyme prepared from Bacillus macerans.

(b) Criteria of Starch Degradation

(1) One of the most apparent results of the enzymic degradation of starch is a reduction in viscosity. Accordingly, the rate

of enzymic degradation can be followed by viscometric measurements.

(2) Iodine Coloration

As is well known, undegraded starch in aqueous solution gives a blue coloration with iodine. When starch is degraded by enzymic action the coloration changes from blue through violet, red and orange to the so-called achroic stage. Thus iodine coloration can be used as a measure of the degree of degradation.

(3) Reducing Power

During degradation there is a progressive increase in reducing power. Since maltose is not the only degradation product of starch (some glucose is formed, Hanes (33), Myrbäck (114)) it is not possible to calculate the amount of maltose formed in an enzymic reaction merely on the basis of optical rotation or from the reducing power calculated for maltose.

To determine the breakdown products of enzymic hydrolysis completely, the solution must be divided into four aliquots (9). In one sample the reducing power is determined; in the second sample the glucose is destroyed (33) by the yeast Monilia krusei or, according to Somogyi (115), by use of baker's yeast at pH 8; any maltose or dextrins present are not attacked and the reducing power is determined by titration. In the third aliquot, both glucose and maltose are removed by action of Monilia tropicalis or baker's yeast. Its reducing power is then determined. The fourth portion is treated likewise, except that, after removal of the glucose and maltose, the carbohydrate present is hydrolyzed with

acid and the final reducing power determined. Sample 1 minus sample 2 is a measure of the reducing power due to glucose; likewise sample 2 minus sample 3 gives the maltose content. Sample 4 gives the dextrin content and sample 3 the reducing power of the residual dextrin.

(4) Optical Rotation

As in the last mentioned method the measurement of optical rotation does not suffice to detect and determine the maltose produced in any enzymic degradation. Change in optical rotation can be used, however, to measure diastatic activity (116).

The above methods when applied to known quantities of carbohydrate material can be used to measure diastatic activity of enzyme preparations. Wohlgemuth (117) has developed a method based upon the disappearance of starch as indicated by use of iodine solutions.

(c) Early Enzymic Studies

(1) Hydrolysis by α-amylase

(i) Characteristics of the Reaction

The action of α-amylase is characterized by deep-seated destruction of the starch molecule so that the iodine coloring property of the carbohydrate is lost. A further property is that, with sufficient enzyme, a "rate of hydrolysis" curve shows two distinct phases namely; an initial one in which the rate is rapid

followed by a prolonged phase in which there is a very slow increase in reducing power. An increase in enzyme concentration leads to an increase in the initial velocity.

A consideration of the available information suggests that with pure α-malt-amylase the termination of the initial rapid phase occurs at about 28 to 30% apparent conversion and that the limit of hydrolysis would not exceed a value of approximately 50% degradation to maltose (110).

During the initial stages of hydrolysis by α-amylase, the principal products are dextrins. Hanes (33) reports the results of numerous early researches which support this point of view. Similar conclusions were reached at a later date by Ohlsson (103) (106) and by Freeman and Hopkins (118).

(ii) Mode of Attack by α-amylase

Hanes (33) concludes that α-amylase is capable of removing from the main body of the starch molecule fragments of about six glucose units. Products investigated have properties which indicate a mean chain length of about this value. The second slow stage in the hydrolytic curve is explained on the basis of a decreased ability of the enzyme to sever these shorter chains oftener than about one linkage in each dextrin molecule.

(iii) Relation of α-amylase Activity to Starch Structure

Hanes accepted the early Haworth picture of starch in which the polysaccharide was believed to be unbranched and to consist of

24 to 30 glucopyranose units and on the basis of the action of α-amylase postulated a spiral configuration for the long glucopyranose chains. These were assumed to be arranged in such a way that points susceptible to attack by the enzyme were close together, thus allowing for the removal of a unit of six glucose residues as indicated above. He assumed that two distinct parts of the enzyme reacted with different groupings in the substrate. This coiled arrangement, postulated for the undegraded starch, presumably is necessary, as assumed by Freudenberg (62), for formation of the iodine color and thus the postulation of other types of linkages was unnecessary to account for the iodine coloration. This is in harmony with the early theory of Haworth.

(2) Hydrolysis of Starch by β-amylase

(i) Characteristics of the Reaction

This enzyme hydrolyzes starch to give β-maltose. The optimum pH for the reaction appears to be 4.7-4.8. The reaction was investigated by numerous early workers e.g. Baker (119), Ling and Nanji (120), Syniewski (121), who reported the limit of hydrolysis to be a conversion to maltose of 60-67%. The reaction apparently proceeds rapidly until the reducing power reaches a value corresponding to 50-55% of the theoretical maltose value, after which there is a gradual decrease in the rate. Later Blom (122) showed that the pH value determined the limit of hydrolysis; at pH 3.4 the reaction ceased sharply at 53% conversion, whereas at pH 4.6

hydrolysis continued until 61% conversion was reached. However, it is conceivable that the so-called "liquefying enzyme" of Waldschmidt-Leitz (104) also may play a role in addition to that exerted by the pH value. It appears that the limit of hydrolysis is not affected by the enzyme concentration and that starches from various sources are degraded to about the same extent (33).

The products of hydrolysis are maltose (the only low-molecular weight product) and dextrins of varying degrees of complexity which give iodine colorations varying from blue to violet. These latter products are non-reducing (119) (121) (118) (122). The limiting dextrin produced has been variously termed "the residual dextrin", "α-amylodextrin" and erythrogranulose. Normally at this stage, the dextrin resists further attack, but if it is dissolved in a suitable solvent, precipitated with alcohol, and re-suspended in water further enzymic degradation will take place. Likewise, after autoclaving in neutral solution for 30 minutes at 120°, further hydrolysis takes place and a new "resting stage" is reached. Apparently, as a result of these treatments, new points of enzymic attack are opened up. It would appear, therefore, that there are constitutional restrictions to further enzymic action at these various "resting stages".

(ii) Mode of Attack by β-amylase

Direct evidence indicating the mode of attack of this enzyme is provided from ~~two~~ sources. (1) Maltose constitutes the

sole low-molecular weight product and is formed continuously from the beginning to the end of the reaction; and (2) the similarity of the dextrins in most of their properties to undegraded starch. Ohlsson (106) produced further evidence by osmotic pressure measurements, and on the basis of these results he advanced the view that successive molecules are detached from each starch molecule in such a manner as to leave at each stage a single residual molecule of dextrin. Furthermore, since the dextrins are normally almost non-reducing it was postulated that cleavage of the maltose units proceeded from the non-aldehydic end of the starch molecule. More direct evidence of this was obtained by Brown and Miller (123) who conducted investigations on the enzymic degradation of a carboxylated dextrin. The products were maltose and a carboxylated dextrin of lower molecular weight.

Hanes (33) postulates a combination of the enzyme with the terminal glucose unit in a "lock and key" arrangement. The enzyme is regarded as consisting of two main functional parts. One of these combines with some grouping in the terminal glucose unit and this enables the second part of the enzyme to approach and cleave the linkage between the second and third glucose residues.

(iii) Relation to Starch Structure

The above considerations on enzymic degradation of starch seem to favor a chain length of about 30 units (33). They provide no explanation for the cessation of β-amylose activity after about

60% starch conversion to a structure (α-amylodextrin) which resists further degradation (unless the dextrin is solvent treated or autoclaved). There are two possible explanations for this cessation of activity. Either (1) it is associated with some structural alteration in the glucose chain which is not open to attack by the enzyme; or (2) it is connected with an association into macromolecules. Supporting evidence for the latter suggestion in the case of amylose will be given later.

2. Recent Developments in Enzymic Hydrolysis

The more important contemporary workers in the enzyme field of starch chemistry include Samec (124), Myrbäck (125), Meyer (9), Somogyi (115), Hanes (105), and Hassid and McCready (126). Myrbäck has concerned himself with the reaction products of enzymic action - the so-called stable dextrins. In agreement with earlier workers, these are shown to be primary reaction products and to be only very slowly (if at all) attacked by the enzymes. It is concluded that the amylases preferably attack only some specific linkages in starch, the structure of which is not as simple as that originally pictured by Haworth. In accordance with experimental evidence the following hypothesis was presented by Myrbäck (125).

(1) Maltose is formed from those parts of the starch molecule which are formed in accordance with the Haworth concept.

(2) Either linkages other than α-1,4 glucosidic are present or certain hydroxyls are phosphorylated.

(3) Possibly normal amylases are unable to attack chains shorter than those of a certain critical length.

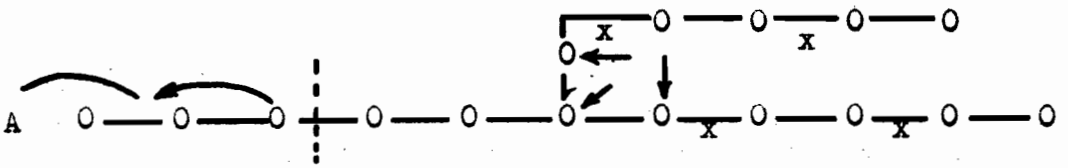
Since stable dextrins have been found to have a reducing power corresponding to one aldehyde group per molecule, anhydrides or rings cannot be present.

Recent work has confirmed earlier observation that β-amylase attacks at the non-reducing end of the starch molecule and splits off one maltose molecule at a time. The limiting value depends on the pre-treatment of the starch (103) (33) (114).

In the case of α-amylase, the end products of the hydrolysis are low-molecular weight dextrins, maltose and a small amount of glucose. It is concluded that the action of α-amylase is unrelated to chain length or distance of the glucosidic linkage from the chain ends.

Meyer et al (9) have investigated the degradation of amylase and of amylopectin. The former can be completely hydrolyzed by β-amylase. However, if "aging" of an aqueous amylase solution has taken place, hydrolysis stops due to the presence of coagulated particles which appear to be resistant to enzymic attack. This would suggest the influence of a purely physical phenomenon. Amylopectin is attacked by β-amylase with formation of a residual substance of high molecular weight termed "residual dextrin I" or

erythrogranulose. The other product of the hydrolysis is maltose. This residual dextrin I can be made vulnerable to β-amylase hydrolysis by treatment with α-amylase, α-glucosidase or super-heated water, "residual dextrin II" being formed. The enzymic degradation of amylopectin according to Meyer (9) may be represented as follows:



O - Glucose anhydride unit

x - points of attack by β-amylase

→ branch points prevent further attack by β-amylase

⊥--- cleavage point by α-amylase or super-heated water

↷ subsequent attack by β-amylase

A - aldehyde end of chain

Probably the most interesting and significant of the current experimental investigations on enzymic treatment of starch are those of Hanes. He has been able to synthesize starch in vitro from glucose-1-phosphate in the presence of a phosphorylase from such plants as peas and potatoes (105) (128). The reaction is reversible, the synthetic product resembling native starch except that it is completely converted to maltose by β-amylase, whereas natural starch is degraded only to the extent of about 60%. On methylation of the synthetic product and determination of end groups (127) a very small proportion of the latter was found; the

number corresponding to a chain length of 80-90 glucose residues. Other workers (126) have failed to detect any end group in this synthetic product. Astbury, Bell and Hanes (188) have conducted an x-ray examination of the synthetic product and have found that native potato starch granules and granules of the synthetic polysaccharide give the same x-ray diffraction pattern. Bear and Cori (129) demonstrated that the polysaccharide synthesized by the action of muscle phosphorylase on glucose-1-phosphate gives an x-ray diffraction pattern very similar in structure to that shown by natural starches.

The structures of the crystalline dextrins first obtained by Schardinger (112) by the action of the enzymes present in Bacillus macerans appear to have been settled by Freudenberg and co-workers (85) (130) (131) who have shown that the α- and β-dextrins probably consist of closed rings of 5 or 6 glucose units joined together by maltose-type linkages. The x-ray study of Schardinger α-dextrin supports this conclusion (132).

IV. Amylose and Amylopectin

A. Nomenclature

The terms "amylose" and "amylopectin" as introduced by Maquenne (133), designated the malt-hydrolyzable and the malt non-hydrolyzable portion of starch respectively. Amylose has been variously termed "β-amylose" and "amylo amylose" in the literature while amylopectin is referred to as "α-amylose"

and "amylocellulose". In this review the term amylose will be reserved for the unbranched chain form of starch and amylopectin for the branched chains (9).

B. Fractionation of Starch into Amylose and Amylopectin

(1) Treatment with Super-Heated Water

On treatment with super-heated water at 120°, starch undergoes gradual dissolution (134) (68). However, it appears that this method destroys some glucosidic linkages as well as certain secondary valence bonds which hold the molecules together in the starch grain.

(2) Treatment with Water at 80°C.

Meyer et al (9) treat starch with water at 80°C thereby causing the amylose to diffuse out in a water-soluble form. This method does not destroy glucosidic linkages.

(3) Centrifugation

This method was used by Sherman (135) and Karrer (136). According to the former, dispersions of starch in water can be separated into a heavier very viscous opalescent layer containing more than 90% of the more-abundant, less soluble component of starch (amylopectin) and a lighter, limpid solution containing more than 90% of the more-soluble amylose. This method does not give a quantitative separation.

(4) The Freezing Technique of Ling and Nanji

This method was developed by Ling and Nanji (134) for the separation of amylose and amylopectin while Baldwin's method (137) is a slight modification of same. According to the original procedure, when starch paste is kept at 0° for 10-12 hours, the starch is precipitated as a fibrous mass. It is then kept at a temperature a little below that at which gelatinization occurs (around 45-50°) causing the amylose to pass into solution and leaving the insoluble amylopectin. The amylose solution can be concentrated and precipitated with ethanol. The amylopectin is obtained pure by leaching the precipitate with water repeatedly until the filtrate no longer gives a blue color with iodine.

(5) Electrodecantation (Dialysis)

The method of electrodecantation has been used by Samec (138), Taylor (139) and Freudenberg (130). Samec combined electrodialysis with a preliminary treatment using super-heated water at 120° for 30 minutes. The latter treatment removed most of the amylose. The undissolved material was subjected to electrodialysis causing the particles of amylopectin to migrate to the anode. By repeating this treatment seven times a satisfactory separation was effected. Taylor gave his starches a preliminary treatment with alcoholic hydrogen chloride and then ruptured the starch granules by treatment with an aqueous solution of ammonium thiocyanate. Electrodialysis effected a separation of the two components. Freudenberg

isolated amylopectin from potato starch by allowing it to swell in potassium rhodanide solution, followed by dialysis and electrodialysis.

(6) Ultrafiltration

Taylor (139) has used ultrafiltration as an alternative method to that of electrodialysis for separating amylose from amylopectin following the disintegration of the granules by a swelling agent. Collodion membranes were used; the amylose passed into the filtrate.

(7) Selective Adsorption

The above methods employ drastic swelling agents (super-heated water, ammonium thiocyanate) which bring about hydrolysis of the glucosidic linkages so that the products isolated are not the true constituents of native starch. A recent method (140) based upon an earlier discovery of Tanret (189) avoids the use of such reagents and employs activated carbon, Fuller's earth or cotton cellulose for the preferential adsorption of the amylose. The last-mentioned was found to be the most effective. The cotton-amylose adsorbate is formed instantaneously when a cold corn starch paste (1%) is brought into contact with cotton, and can be washed free of amylopectin by cold water. The adsorbate is then readily decomposed by boiling water to give a clear solution of pure amylose. The dissolved amylose can be converted to the solid form by concentration of the solution followed by precipitation with alcohol. Retrogradation of amylose solutions is prevented by the addition of pyridine.

C. Structure and Properties of Amylose

As early as 1858 Nageli came to the conclusion that the starch grain was not homogeneous (141). Meyer (142a) in 1895 stated that the starch granule consists of two parts, α-amylose and β-amylose. Maquenne and Roux (142) showed that amylose, which comprises 20-25% of the starch granule, is completely converted into maltose in the presence of malt diastase.

Hirst and co-workers (18) concluded from methylation studies that amylose and amylopectin had the same chemical structure, the differences in properties being due to differences in micellar formation. End-group analysis of both amylose and amylopectin gave a chain length of 23-24 glucose units corresponding to a molecular weight of 5000. Samec (68) expressed the view that amylose contains no phosphorus and that the insolubility and paste-forming properties of amylopectin are due to its phosphorus content. The latter view is no longer considered tenable.

Taylor (143) assumed that retrograded amylose consisted of highly-associated chains so that sheaf-like bundles were formed.

On the basis of kinetics of hydrolysis Freudenberg (130) concluded that there was little difference between amylose and amylopectin.

Proof that actual differences do exist has been obtained primarily through enzymic studies. Samec (144) showed that amylose and amylopectin can be distinguished by their different reactions

with enzymes. Thus while amylases of animal and vegetable origin cleave amylose to maltose, amylopectin is hydrolyzed to maltose only to the extent of 70%.

The recent work on amylose, including his own researches, has been summarized by Meyer (9), and is reviewed below.

(1) Behaviour in Aqueous Solution

(a) Amylose can be separated into fractions having different solubilities (50), by precipitation from water solution. The finely divided precipitates, on standing, become water-insoluble due to a slow increase in crystallite size.

(b) The greater the purity of the fractions, the greater the effect of crystallite size on the solubility.

(c) Aqueous solutions of amylose undergo "aging" or "retrogradation", a phenomenon indicated by (1) increased resistance towards the action of β-amylase and (2) precipitability of the retrograded fraction. Pure amylose solutions will retrograde in a few hours.

(2) Chemical Properties

(a) With alkalies, amylose forms salt-like compounds which are sparingly soluble in water.

(b) Amylose has an acidic dissociation constant of about 5×10^{-12}.

(c) It yields an unstable addition compound with chloral hydrate.

(d) Amylose can be esterified and etherified. Triacetylamylose and trimethylamylose form solid elastic films on evaporation from solvents.

(e) Amylose is completely hydrolyzed by β-amylase.

(3) Structure of Amylose

Methylation studies on amylose (145) (17) (146) show that hydrolysis of the fully-methylated products yields 0.4% of 2,3,4,6-tetramethylmethyl glucoside. The principal product was 2,3,6-trimethylmethyl glucoside indicating that the glucose residues are combined by 1,4-linkages. The molecular weight (end group analysis) was 50,000, corresponding to about 250 glucose units. Since there was only 0.4% tetramethylmethyl glucoside the chain of the amylose molecule cannot be branched.

Crude amylose from corn starch was separated into fractions ranging in molecular weight from 10,000 to 60,000 (50). From optical rotation measurements amylose was shown to consist of a chain of glucose residues with α-1,4-linkages. Free aldehyde groups were determined (145), one aldehyde group being present in 200 glucose residues.

D. Structure and Properties of Amylopectin

(1) Properties

(a) Amylopectin has long been known to differ from amylose in solubility and paste-forming properties, these differences now

being regarded only as quantitative in character.

(b) Amylopectin constitutes the bulk of the starch (80-90%).

(c) Amylopectin is hydrolyzed by β-amylase only to the extent of 55-65%.

(d) Amylopectin may be resolved into fractions by dissolution with aqueous choral hydrate (33%).

(e) The solubility of amylopectin in water depends on the degree of aggregation of the branched molecules. Fairly stable solutions are obtainable in dilute alkali.

(f) Cereal starch amylopectins do not contain chemically-bound phosphorus in contrast with the reserve starch amylopectins (69).

(2) Structure

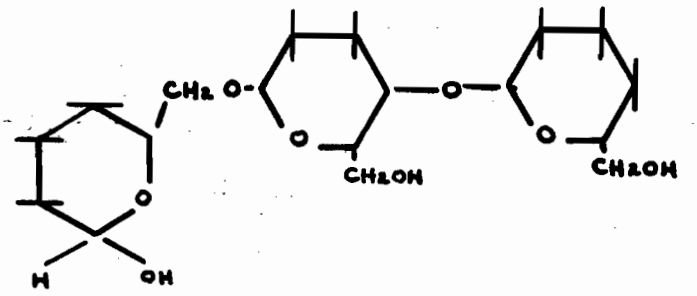
Haworth (39) showed that at least part of the starch molecule consisted of branched chains from the results of his end-group analyses which indicated the presence of 4-5% tetramethylglucose residues. Meyer et al (145) found with corn amylopectin a value of 3.8%. A branched structure hypothesis was proposed by Staudinger (190) in an attempt to reconcile his macromolecular conception of starch structure with Haworth's results indicating a much shorter chain structure (147) (49). He suggested that in the starch molecule a side chain of 20 glucose groups is combined with every second glucose residue of the main chain in such a way that this side chain is connected alternately by ether linkages to

carbon atoms in the 3 and 6 positions of the respective glucose groups. Freudenberg (62) has also accumulated evidence from end-group measurements indicating a branched chain structure for at least part of the starch molecule. He showed later that these branched portions are linked through the C-6 position.

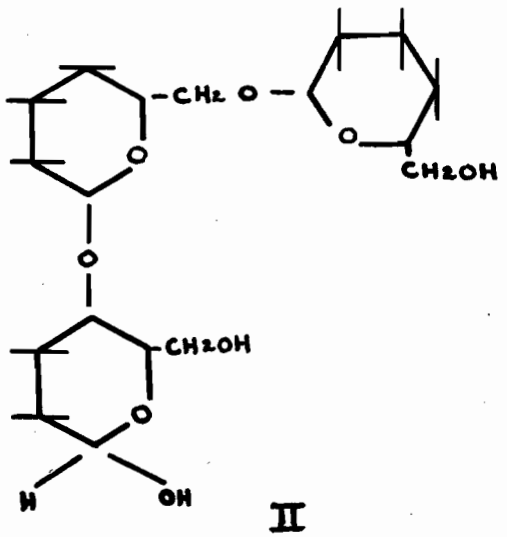
Studies have been made on the nature of the bonds which link the branched chains in the starch molecule (148) (149). On the basis of the reaction velocity and activation energy of the dis-aggregation process involved in hydrolytic degradation, the linkage is regarded as a normal covalent bond. Union through the C-6 position is shown by the isolation of 2,3-dimethyl glucose (3%).

Meyer et al (9) claim that this accumulated evidence of Haworth, Hirst, Staudinger and Freudenberg indicates a branched structure for amylopectin.

Recent enzymic investigations on amylopectin also emphasize this branched nature (150). Among the products of decomposition using malt amylase a trisaccharide was isolated in which one of the hydroxyl groups was attached to the C-6 carbon atom. The isolated trisaccharide was assumed to have either the structure I or II.



I



II

In another series of investigations (9) amylopectin was degraded by β-amylase with production of a high molecular weight residual substance (45%). This product termed "residual dextrin I" or "erythrogranulose" contained tetrahydroxyl end-groups (9%) indicating that no destruction of branched positions by the enzyme had occurred. Since residual dextrin I is attacked by α-glucosidase but not by β-glucosidase, apparently the branching link is an α-bond. Removal of the branching groups in this manner yielded residual dextrin II which is non-resistant to hydrolysis by β-amylase. The latter splits off maltose by the action of β-amylase and produces residual dextrin III. These results may be compared with those obtained by Hanes (33).

Amylopectin is oxidized only slightly by alkaline silver or copper solutions indicating the presence of one aldehyde group to about 1500 to 3000 glucose units.

Hess (146) applied the Haworth methylation-hydrolysis method of end-group assay to both amylose and amylopectin, the relative degrees of polymerization for the two substances being 283 and 23 glucose units respectively. The value for amylose agrees with

that obtained by other workers using different methods e.g. aldehyde determination and methylation.

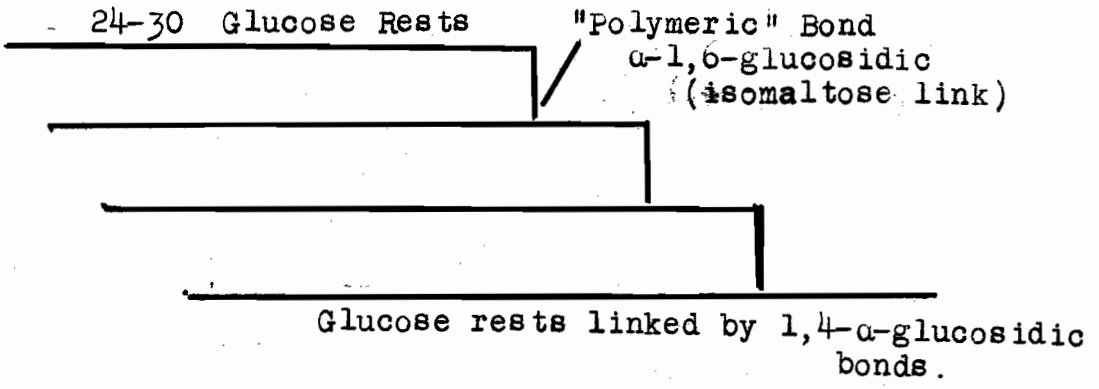
Beckmann and Coles (151) have attempted to show diagrammatically the constitution of amylopectin based on their work on the molecular weight of α-amylodextrin. They use the Haworth postulation of 30 glucose residues as constituting the fundamental unit of the starch molecule.

V. The Structure of Starch: Summary of Present Conceptions

A. Haworth School

Haworth has never attempted to define the individual structures of amylose and amylopectin. He has assumed (18) that there is no difference, chemically, between them. More interest has been shown recently by workers in the field on the problem of the structural differences between the two fractions.

Without attempting to structurally distinguish between amylose and amylopectin, Haworth visualizes the starch aggregate as follows. The so-called "polymeric link", at first unspecified, is now designated as α-1,6-glucosidic in character.



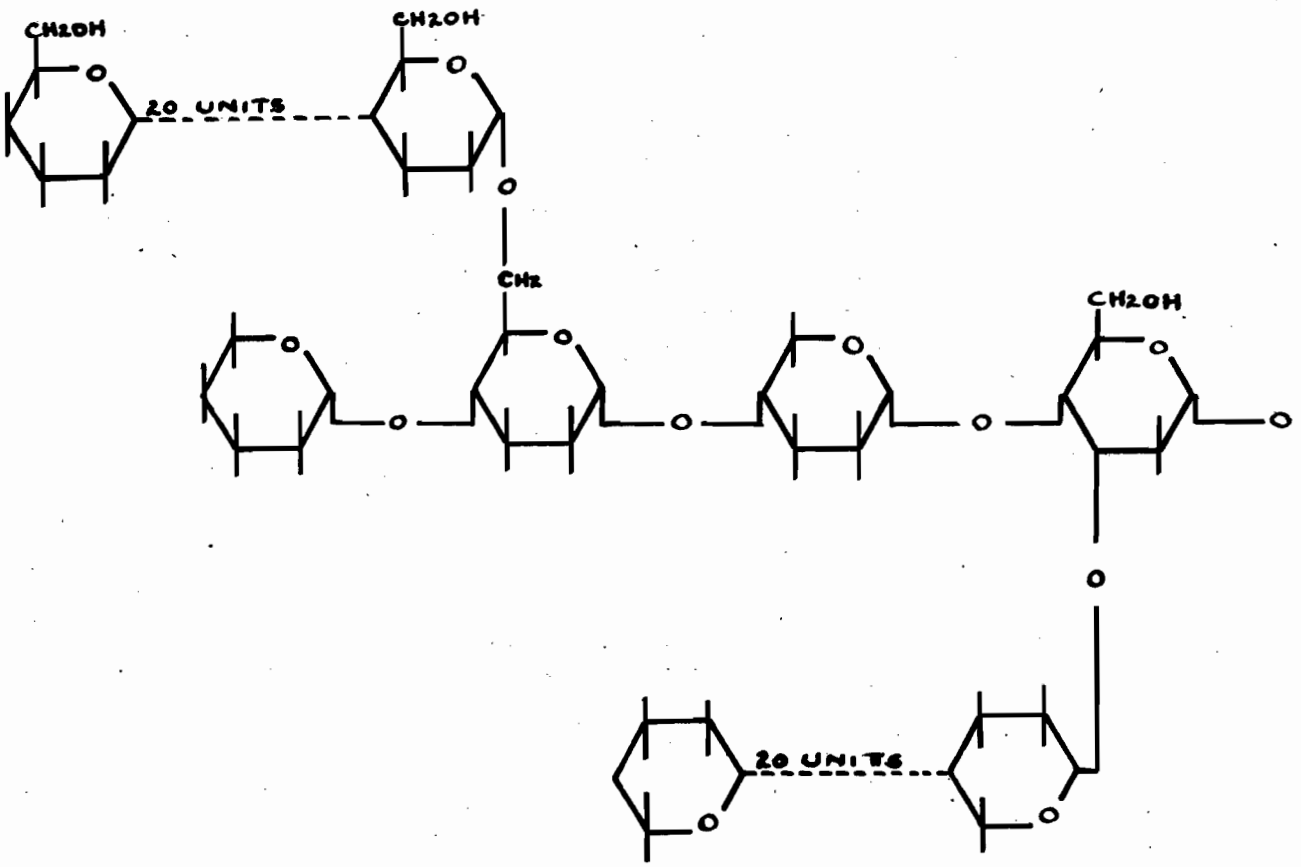
He believes there is not sufficient evidence to provide a clearer representation of the starch molecule. However, the investigator apparently has failed to evaluate the recent results on enzymic degradation which, in the opinion of other workers (9) (125), indicate the existence of a branched chain structure.

B. Hess' Investigations

While Hess has put forward no concrete model for the starch molecule as a result of his methylation studies with various co-workers, he envisages an average chain length of some 52 units as compared with Haworth's 24-30. In solution these units are considered to be united by secondary valence forces in contrast to Haworth's union through a "polymeric" bond. In more recent work, Hess finds the degrees of polymerization of amylose and amylopectin to be 238 and 23 respectively. This result is assumed to be indicative of a distinct difference in constitution between the two components.

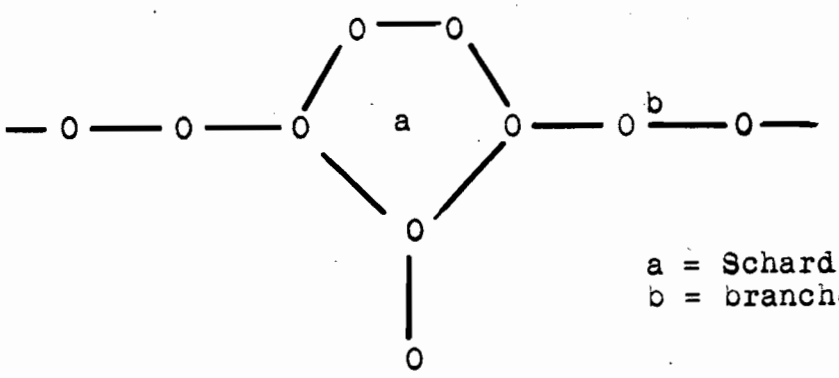
C. Staudinger's Views

Staudinger (190) has put forth much evidence in favor of his macromolecular theory of starch structure in an attempt to reconcile the long chain conception with that of Haworth's shorter one. His proposed structure can be represented diagrammatically as follows:



D. Freudenberg's Theories

In earlier investigations (4) (5) Freudenberg was a proponent of a straight chain structure for starch. More recently (62), as a result of his work on methylation studies and end-group analyses, he has accepted Staudinger's views of a branched chain, the branching taking place at the C-6 atom. Freudenberg (191) considers that Schardinger's dextrins are combined chemically with the true starch chains in the following manner:



a = Schardinger dextrin
b = branched glucose chains

O = glucose residue
Schardinger ring with branched chains

This view of the constitution of starch explains many of the phenomena observable when starch is attacked by enzymes, but there is scant chemical evidence to support it.

E. Speculations of Caesar and Cushing (152)

Founded entirely on physical speculations based upon atomic models these authors have suggested a structure for amylose which reconciles the Haworth short-chain picture with other views indicating much greater complexity. They suggest that amylose chains exist as a type of helical "spring". These are twisted around one another to form a sort of "rope" micelle, the latter possibly forming the building block of the granular package known as starch. Retrogradation of amylose is pictured as a dehydration process. Water present between the springs of the amylose aggregate is squeezed out following which the forces R-O$\overset{H}{}$----$\underset{O-R}{H}$ have free play.

F. Coles and Beckmann Theories (151)

These workers have constructed a model of starch which satisfies the data obtained by centrifugal studies of α-amylodextrin and is also in agreement with the results of end-group analyses. It is based on the following considerations:

1. The Haworth unit of 30 glucose residues is a fundamental unit of the starch molecule.

2. The evidence of Freudenberg and Meyer indicates that these units are linked by 1,6-α-glucosidic bonds.

3. The experimental evidence supplied by Hanes and others indicates that the fission of maltose under the influence of β-amylase starts at the 2,3,4,6-tetrahydroxyglucose end of the chain.

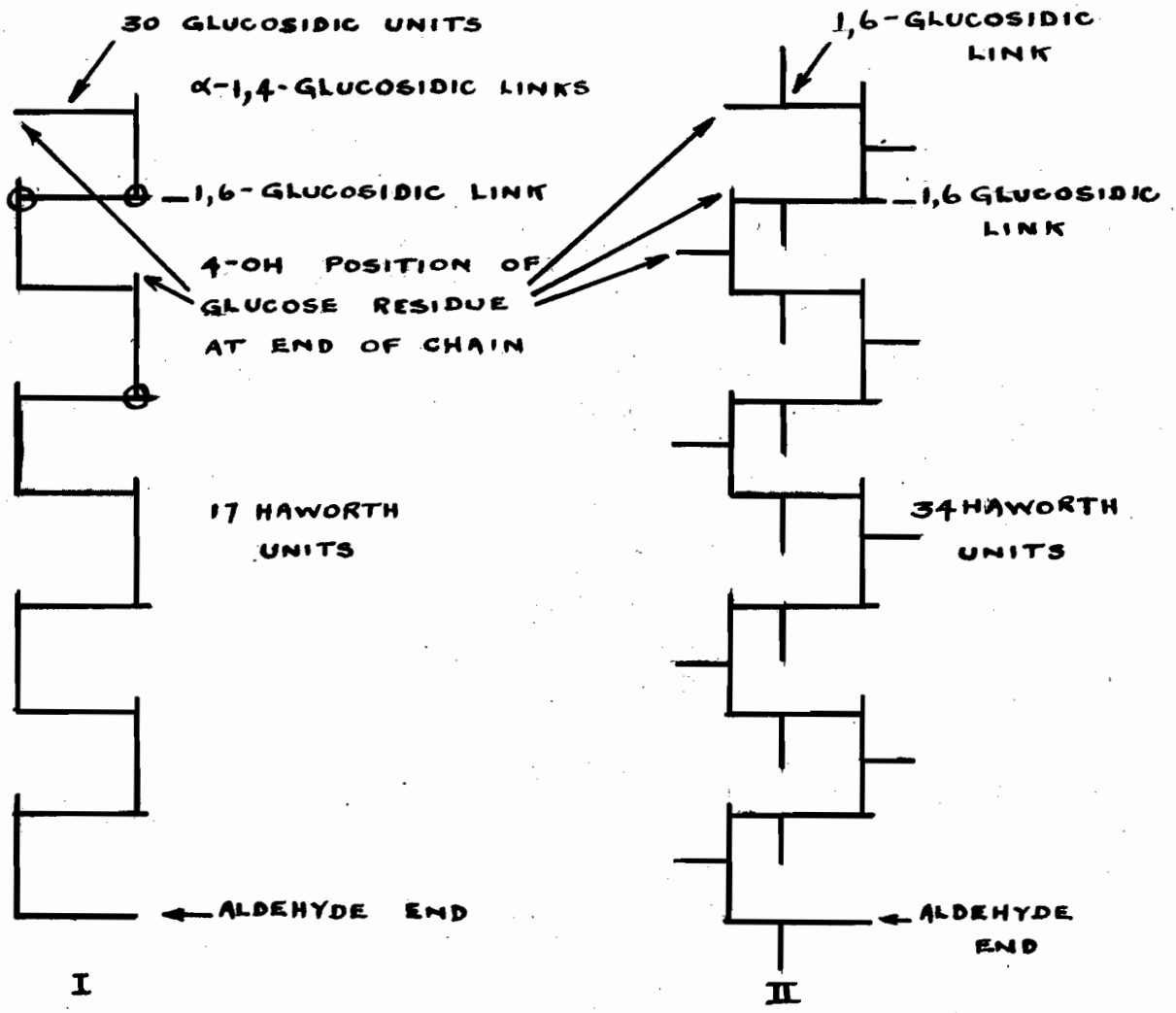
4. It is assumed that during the process each molecule is degraded to the same extent.

5. The physical properties of starch are explained more satisfactorily by a disc-like rather than by a chain-like structure.

The α-amylodextrin is pictured as a Haworth unit with two branched chains. Each of these is in turn again doubly branched, giving finally a structure of 16-17 Haworth units. If one branch of each pair is situated near the end of the preceding branch the molecule would not be hydrolyzed further by β-amylase since only 1,6-linkages and an aldehydic end-group are exposed. By continuing the branching process one step further there is obtained a model structure for the lower molecular weight component (measured by

the ultracentrifuge) of potato starch having 34 Haworth building units.

The model for α-amylodextrin is shown in Fig. I and that for the second lower molecular weight component in Fig. II.

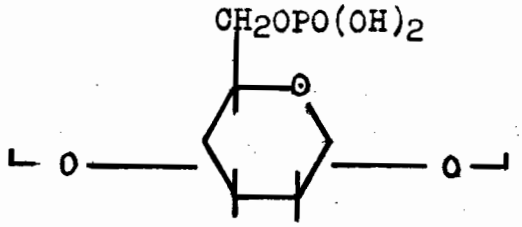


G. K. H. Meyer's Views

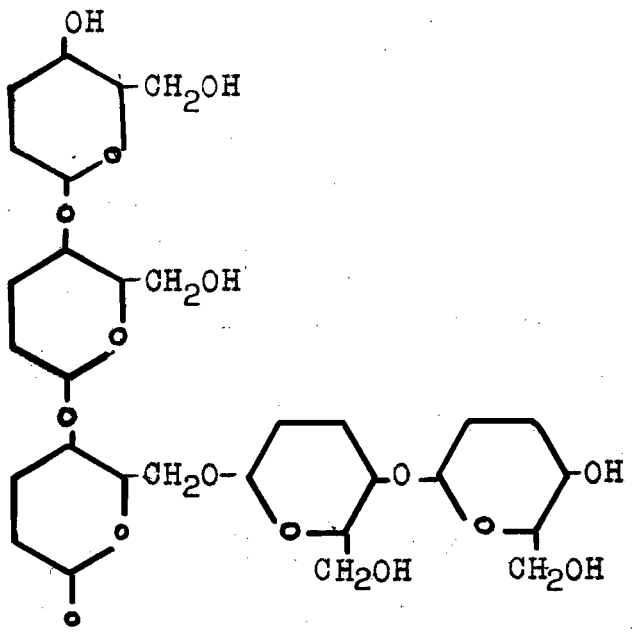
A branched-chain structure for starch was proposed as early as 1930 (153) by Meyer and Mark. In recent work (9) Meyer has

amplified the original conception principally as a result of the recent work on enzyme hydrolysis. Amylose and amylopectin are considered to be separate and distinct structural entities. The amyloses are unbranched and have molecular weights ranging from 10,000 to 100,000. They contain free tetrahydroxyl glucose groups (0.4%) per chain of 250 glucose units, so that branching is impossible. The amyloses are hydrolyzed completely by β-amylase. Triacetylamylose and trimethylamylose resemble the corresponding cellulose derivatives.

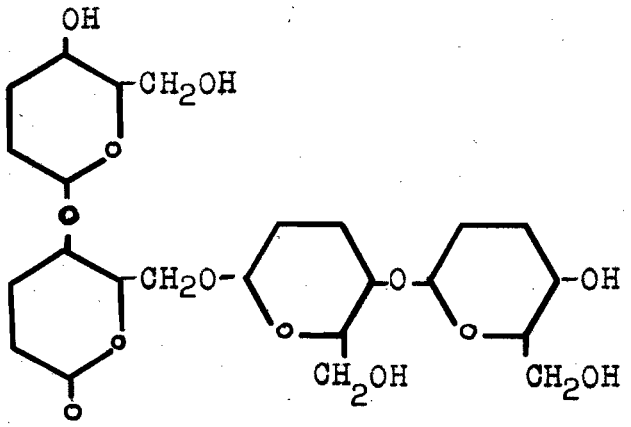
Amylopectin must possess a branched-chain structure. Posternak's (69) isolation of glucose-6-phosphoric acid, from potato starch by enzymic hydrolysis, indicates the presence of the following unit in the amylopectin.



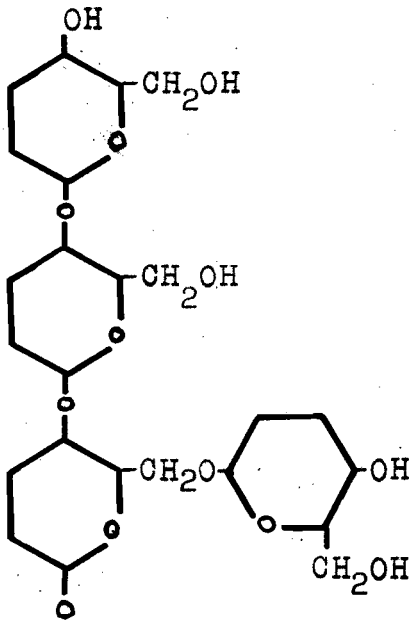
Meyer postulates the presence of the following groups as branching points in his residual dextrin I (erythrogranulose) obtained from amylopectin by the action of β-amylase: (page 63)



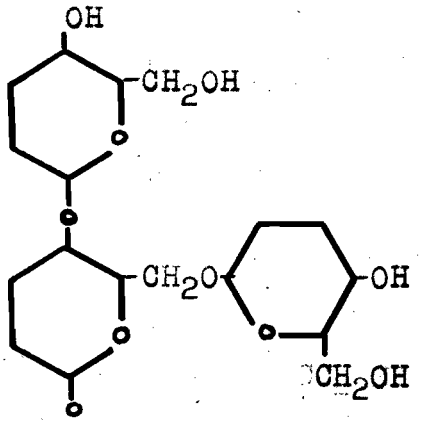
I.



II.



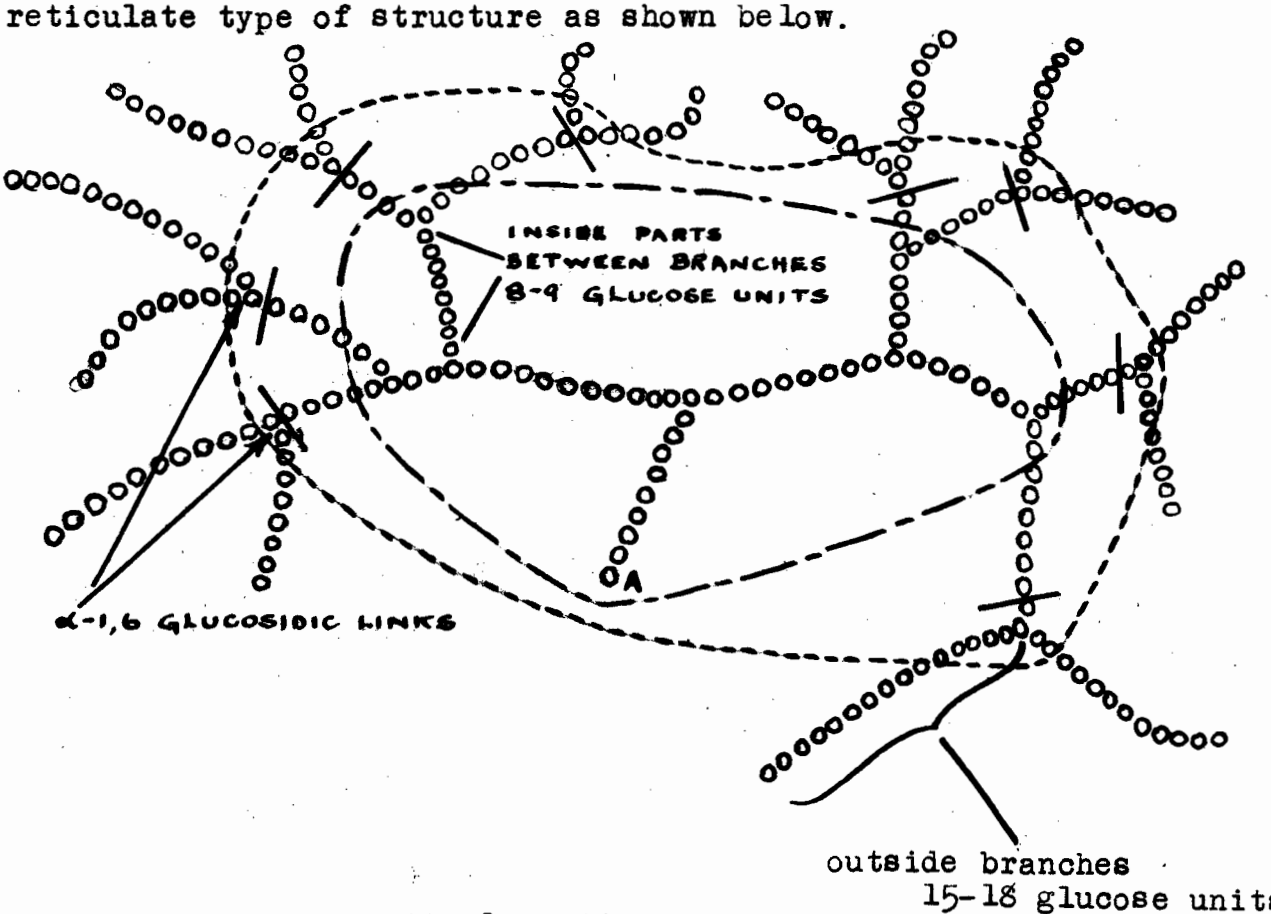
III.



IV.

The α-1,6-glucosidic links in these aggregates are hydrolyzed by α-glucosidase giving residual dextrin II. Further action is then possible with β-amylase (the interrupting 1,6 linkages having been removed) with the production of residual dextrin III.

According to Meyer these results can be explained only by a reticulate type of structure as shown below.



Amylopectin

Successive stages of enzyme hydrolysis
O = glucose rest A = aldehydic end-group
------- end of initial degradation by β-amylase yielding residual dextrin I
—— hydrolysis continued by α-glucosidase yielding residual dextrin II, hydrolyzable by β-amylase
——·—— end of further attack by β-amylase yielding residual dextrin III.

The amylopectins thus are assumed to be branched; their molecular weights exceed 300,000, and they have about 4% free tetrahydroxyl glucose end content. Their ethers and esters form brittle inextensible films.

H. Hanes' Synthesis of Starch

Details will be given in the succeeding section of Hanes' method of enzymic synthesis of a starch-like substance from glucose-1-phosphate. This material is composed of glucose units united by α-1,4-glucosidic linkages resembling in this respect native starch. From end group analysis results there appear to be 80-90 glucose residues in the chain. The low end-group content indicates a non-branched structure as is the case with amylose. The question is still undecided as to whether this synthetic product actually occurs in natural starch or whether it is only a laboratory synthetic product.

VI. The Bacterial and Enzymic Synthesis of Carbohydrates

A. Bacterial Synthesis of Polysaccharides

1. Bacterial Cellulose

Bacteria have long been known to possess the power of synthesizing a variety of polysaccharides. Early workers concerned themselves chiefly with the task of determining the optimum conditions for the process and identifying the products with known natural substances. Hibbert and Tarr (169) determined the optimum conditions for the preparation of bacterial polysaccharide membranes synthesized by the action of Acetobacter xylinum on such simple substances as glucose, fructose, glycerol, sucrose, mannitol or galactose, and later Hibbert and Barsha (170) showed that these membranes were composed in each case of highly-hydrated cellulose chemically identical with cotton cellulose. The specific agency at work in these syntheses was not identified, but there was reason to believe that the formation of these polysaccharides was brought about by enzymic action.

2. The Levans

A second group of polysaccharides produced by bacterial synthesis and having apparently the same basic structure are the levans, investigated by Harrison, Tarr and Hibbert (171). Two levans, later found to be identical (172), (173) are formed by the action of Bacillus subtilis and Bacillus mesentericus. It was shown that the

essential condition for the formation of the polysaccharide is a terminal fructofuranose grouping in the substrate molecule. Such a group is present in both sucrose and raffinose from which the levans were synthesized. Hibbert and Tarr also showed that levan-like substances (171) could be synthesized by a bacteria-free enzyme product prepared from Bacillus mesentericus. The chemical constitution of these bacterial synthesized levans has been investigated by Hibbert and Tarr, and by Haworth (179) and found to consist of fructofuranose units linked through the 2 and 6 hydroxyl groups. End-group analyses indicate that the polysaccharide consists of about 10-12 units of fructose. More recently, Stacey (180) has prepared levans from B. megaterium, Bact. pruni and Bact. prunicola and has shown them to be polyfructoses represented by a chain of 10-12 contiguous fructofuranose units linked through positions 2 and 6 as in the levan formed by B. subtilis.

3. Dextrans

The formation of the polysaccharide dextran obtained by the action of Leuconostoc mesenteroides on sucrose was studied by Tarr and Hibbert (174). Polysaccharide formation apparently took place in appreciable amounts only in sucrose solutions although, in the case of two strains of bacteria, small amounts were observed in solutions of glucose. More recently, Hehre (175) has obtained a similar polysaccharide from sucrose by the use of a completely sterile bacterial extract prepared from cultures of Leuconostoc mesenteroides. Under

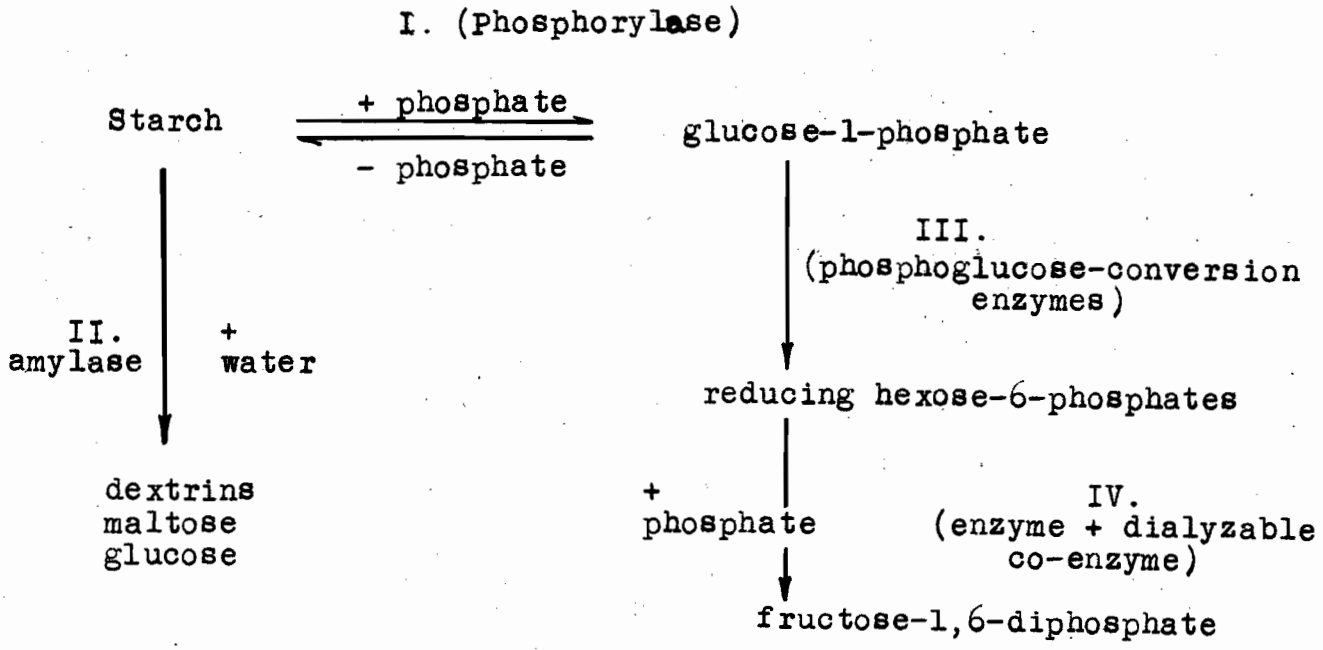
these conditions the living micro-organisms could have played no part in the reaction, the effect being entirely that brought about by enzymic effect. The product obtained was similar in both chemical and serological properties to the dextran formed by cultures of the living bacteria. The chemical structure of dextran has been investigated by Hibbert (178) and found to consist entirely of glucose anhydride units. Hehre (175) has suggested that x molecules of sucrose are converted into x molecules of fructose plus a dextran polymer of x glucose anhydride units, the mechanism suggested being similar to that proposed for the living bacteria.

This work on bacterial synthesis is interesting in relation to the formation of carbohydrates in plants, since, according to the most recent conception of photosynthesis (181) enzymes are assigned a share in this complex reaction. Bacterial synthesis provides proof of the specificity of their action and the existence of enzymes, capable of producing interconversion of the sugars, is now indicated (176), (177).

B. The Enzymic Synthesis of Starch

The most interesting development in the synthesis of carbohydrates by enzymes is that described by Hanes (105) who has synthesized starch enzymatically by means of a phosphorylase extracted from potatoes. He showed that extracts from ungerminated peas contain an enzymic system, which, in the presence of inorganic

phosphate, catalyzes the formation of hexosephosphates from starch or various carbohydrates of the starch-maltose series. The first stage in this transformation (catalyzed by the enzyme phosphorylase) consists in the conversion of starch to glucose-1-phosphate; by a reversible type of reaction. Both starch and glucose-1-phosphate, however, take part in alternate reactions catalyzed by separate enzymes, as indicated in the following diagram.



The reversible reaction I. was carefully investigated. The enzyme phosphorylase which catalyzes the reaction has been found to occur in a number of higher plants, particularly potatoes. It can be obtained free from the enzymes which catalyze reactions II. and III.

The reversibility of the transformations, starch + inorganic phosphate $\rightleftharpoons$ glucose-1-phosphate, is shown by the fact that the

reaction proceeds in either direction until the ratio of inorganic orthophosphate to glucose-1-phosphate attains a value which is not significantly altered by wide variations in the concentrations of the reactants or the enzyme, but which does vary considerably with alterations in the concentration of hydrogen ions. The reaction velocity is greatly increased by small additions of starch or maltose.

In certain characteristics (e.g. nitrogen and phosphorus content, reducing power, rotatory power) the synthetic product is indistinguishable from natural starch. However, it is less soluble in water and retrogrades more easily than natural starch. The blue color produced with iodine has greater brilliancy and intensity than that formed with natural starch. The synthetic product is completely hydrolyzed by β-amylose while natural whole starch is only 60% converted. Thus the synthetic product resembles β-amylose rather than whole starch.

The results of this enzymic synthesis of starch have an important bearing on the question of the metabolism of carbohydrates in the plant. Glucose-1-phosphate is considered by Hanes to occupy a position of central metabolic importance. It is the direct precursor of starch and it forms the initial reactant in the sequence of interconversions leading to the formation of fructose-diphosphate. Also, starch may be considered to constitute an accessory supply of hexose phosphate.

Some interesting observations have been made recently by Bois, Chubb and Nadeau on the possibility of an enzymic interconversion of the three disaccharides maltose, cellobiose and sucrose (176), (177). These authors claim that cellobiose and sucrose are produced by the enzymic hydrolysis of starch by sugar maple sap and birch sap, and it is inferred that these disaccharides exist preformed in starch. However, the only reasonable conclusion would seem to be that the hydrolytic products of starch depend upon the enzyme employed and that configurational rearrangement is apparently possible.

The starch content of the roots of the sugar maple has been investigated (176). In winter, starch grains are present in the vessels of the roots, but when the sap starts to run in the spring, these starch grains disappear. This transformation is probably the result of enzymic action. The sugars produced as a result of this transformation are sucrose and cellobiose. Thus the sap of the sugar maple contains "sucrogenic" and "cellobiogenic" amylases. Similarly birch sap (177) may contain a "cellobiogenic" amylase and probably a "glucogenic" amylase.

VII. Starch Nitrates: Preparation, Properties, Structure, and Stabilization

A. General Introduction

Most of the early work, (from 1833-) on starch nitrates, is concerned with methods of preparation, stabilization and use in explosives. Numerous experiments were conducted to determine the optimum yield of maximum nitrogen content, the variables investigated being choice of nitrating agent, duration of the reaction and reaction temperature. The nitrating agents used include nitric acid, mixtures of nitric and sulphuric acids in various concentrations, mixtures of nitric and phosphoric acids, and nitrogen pentoxide.

Numerous pre-treatments of the starch have been tried, including oxidation, removal of combined phosphorus etc., together with various degradation methods in order to obtain a more easily nitrated product. None of these has been found effective for the synthesis of a highly-nitrated stable product. While numerous methods of stabilization have been described and patented, in general, only temporary stabilization is effected and the cause of the instability still remains unknown.

Chemical and physical methods have not been applied hitherto to a study of the nitrates of starch as fully as they have to cellulose nitrates. The use of x-ray analysis has not proven of

much value in the case of starch nitrate and difficulty has been experienced in interpreting viscometric and osmotic pressure results. However, newer methods of fractionation are expected to result in a greater knowledge of the structure of starch and better x-ray technique to give a more exact knowledge of the structure, properties and constitution of the nitrates.

B. Summary of Methods of Preparation

1. Modifications of the Starch prior to Nitration

In 1917, Sadtler (154) claimed to have produced a stable starch nitrate containing 12-13.3% nitrogen as a result of a special pre-treatment of the starch. This consisted in treating starch in finely-powdered form for two to four hours at room temperature with a dilute solution of sodium hydroxide to remove fat and proteins and to swell the granules. The product was then washed with water, treated with calcium hypochlorite solution (2%), washed thoroughly, dried and nitrated. The nitrated product was claimed to possess the desirable properties of a good commercial explosive.

Grard (155) used a pre-treatment which undoubtedly resulted in degradation by hydrolysis. Starch was moistened with 35% of its weight of water and heated in an autoclave at 150° for 15 minutes, to yield a plastic mass which could be extruded through a 0.2mm. die as long fine threads. These hardened on exposure to air or on

washing with alcohol. After drying they were easily powdered prior to nitration.

These two methods of pre-treatment are merely representative of the various methods employed earlier. Further modifications will be discussed in the survey of the patent literature.

2. Nitrating Agents

Most of the investigators used aqueous mixtures of nitric and sulphuric acids. Different proportions of these three constituents have been recommended, but in all cases nitric acid is present in largest quantity, usually 65 to 75%. Some of the early workers, notably Will (156), used nitric acid alone as a nitrating agent, the nitrated product being precipitated either with water or concentrated sulphuric acid. He obtained in good yields products, containing 13.90 to 14.04 % nitrogen, and claimed marked stability for his product. Berl and Butler (157) used a mixture of nitric and fuming sulphuric acids and obtained nitrates containing 12.86 to 13.85% nitrogen. Nitrating conditions were investigated thoroughly by Hackel and Urbanski and reported in a series of papers (158). From their results on the nitration of potato starch using only nitric acid the following conclusions were drawn:

(1) An increase in the acid concentration (as compared with the weight of starch used) causes an increase in the nitrogen content and viscosity of the nitrate.

(2) Starch nitrates precipitated by water show a higher

chemical stability than those precipitated by sulphuric acid.

In nitrating starch with mixed acids a maximum nitrogen content is obtained when water and sulphuric acid are in equimolecular proportions, provided the sulphuric acid content is not too low. On the other hand too high a concentration of sulphuric acid results in the formation of esters of sulphuric acid. With greater than 20% water present, nitration is irregular and the yields are low.

Nitric-phosphoric acid mixtures have been used (159) to produce a nitrate containing 13.0% nitrogen, and as with cellulose, the use of phosphoric acid gave a product which was less degraded viscometrically than that obtained with sulphuric acid. Centola (160) used various mixed acid concentrations and nitration periods, the nitrates being characterized by x-ray analysis.

Finally, it is interesting to note the use of nitrogen pentoxide as a nitrating agent (161). Cellulose is nitratable by this reagent to the extent of 14% nitrogen content and starch to 13.85%.

Starch thus can be nitrated by the customary methods and the same considerations are applicable to both cellulose and starch. In general starch is more difficult to nitrate than cellulose and the process involves a greater degree of degradation. A complete review of methods of nitration has been published by Marshall (162).

3. Other Factors Involved in Nitration

Two important factors are time and temperature of the reaction. Increase of the temperature above 0°C causes a lowering of the

nitrogen content, the yield and the viscosity of the nitrates; and an increase in ethanol solubility (158). The changes are probably due to secondary oxidation reactions induced by the rise in temperature. With increasing reaction time the nitrogen content of the product rises rapidly, initially, the increase becoming later progressively smaller. Increase in the nitration period also favors the secondary reaction mentioned above which results in a decrease of viscosity, an increase in solubility and a lowering of the yield.

4. Purification of the Nitrated Starch

This usually consists first of all in the removal of the excess of nitrating acids by a series of extractions generally with hot water alone, but sometimes also involving dilute alkali. Prolonged boiling is necessary, as in the case of cellulose nitrate, in order to remove all of the nitrating acids and to decompose the sulfate esters. According to Hackel and Urbanski (158) such stabilizing treatments have a detrimental effect, resulting in denitration and an increase in alcohol solubility. Even washing the product with cold water containing 1% sodium carbonate gives products of lowered stability according to these investigators. Any treatment subsequent to the treatment with boiling water is considered to be a special stabilization. Substances used for this purpose will be considered in a later section.

C. Survey of the Patent Literature

A complete survey of the patent literature is unnecessary for this review because it does not aid materially in a solution of the present problem, namely the cause of starch nitrate instability. The theoretical principle involved in the various patented methods of nitration and stabilization is usually unknown so that the methods given are purely empirical. The following important factors are covered in the patent literature. (1) Pretreatment of starch (2) Nitrating methods (3) Nitrostarch explosives (4) Stabilization.

Most of the pre-treatments recommended involve marked degradation of the starch, as for example use of dilute alkali, or oxidation. The nitrating methods used are typical for polysaccharides as described in the literature, and involve various concentrations of mixed acid. Usually some special type of nitrating apparatus is employed.

Numerous explosive mixtures of nitrostarch and other products have been described, a typical one containing the following components: nitrostarch 60-70%, ammonium nitrate 19-9%, calcium nitrate 4-8% and water 17-13% (162). In many of the patents the starch nitrate is mixed with certain stabilizers for the purpose of increasing the stability e.g. cyanamide and its salts, diphenylamine, ammonium salts of phosphoric, chromic, acetic, propionic and tartaric acids; urea and substituted ureas, substit-

uted anilines and many other slightly alkaline substances. Ethanol was recently described as a stabilizing agent for starch nitrate (192).

D. Chemical and Physical Properties of the Nitrates

1. Solubility

Starch nitrate is soluble in most of the solvents which dissolve cellulose nitrate, in both cases, a sol and not a true solution being formed. Among the best solvents for both of these nitrates are acetone, fatty acid esters and glycol monoethyl ether. Ether-ethanol mixtures make fair solvents especially if the nitrate is a somewhat degraded product.

The solvent action of any particular liquid seems to depend mainly upon the chain length and nitrogen content of the nitrate. For example, ethanol will dissolve starch nitrates of relatively short chain length and low nitrogen contents. The solubilizing effect of the solvent decreases with increase in chain length and nitrogen content. Acetone is one of the best solvents for a starch nitrate of high nitrogen content and chain length. It does not dissolve fractions of lower nitrogen content or lower chain length. These fractions, however, are soluble in acetone-water mixtures.

2. Hygroscopicity

The hygroscopicity of a starch nitrate is in some way connected with stability, possibly as a function of nitrogen content.

Products of lower nitrogen content are usually hygroscopic, as is the case with starch itself.

3. Explosive Properties

The explosive properties of twelve samples of starch nitrate varying in nitrogen content from 13.43 to 6.49% were determined by Hackel and Urbanski (158) and the following properties found to have a direct relationship to the nitrogen content; (a) rate of detonation, (b) lead block expansion test, (c) copper cylinder compression and (d) sensitivity. Samples having less than 9% nitrogen were found to have only minor explosive properties.

A stable nitrostarch containing 13.2% nitrogen has been claimed recently (163). The product has a high density and considerable explosive power; on a volume basis its power is equal to that of T.N.T., but on a weight basis only 85-95% of the power. When freshly-prepared it had an ignition temperature of 400°F. (T.N.T. 870°F.) and like T.N.T. was insensitive to the impact of a rifle bullet.

4. Viscosity

The viscosity of starch nitrates has probably been investigated more than any other physical property. Staudinger (56) showed viscometrically that starch fractions suffered degradation during nitration. Berl and Butler compared the viscosities of cellulose and starch nitrates (157). These latter workers found that a 5% solution of cellulose nitrate in acetone had more than 9000 times

the viscosity of a corresponding starch nitrate solution, the nitrogen content being the same in both cases. That the viscosity is dependent upon the conditions of nitration used was shown by Hackel and Urbanski (158). In nitrating with nitric-phosphoric acid mixtures (159) the viscosity showed a flat maximum after a nitration period of twelve hours. They (164) found that starch preparations having different viscosities gave nitrostarches having almost the same viscosities.

5. Fractionation of starch nitrate

Although much work has been done on the fractionation of cellulose nitrate (165), little attention has been paid to that of starch. Berl and Kunze (159) using a freezing technique separated starch nitrate into two components which, they claimed, were amylose and amylopectin nitrates respectively. The amylose nitrate contained 11.6% nitrogen and the amylopectin 12.1%. In agreement with the work of Meyer (9), amylopectin nitrate showed a lower viscosity than the corresponding compound of amylose. Centola (160) has separated amylose and amylopectin nitrates on the basis of their solubilities in methanol. Nitrated amylose is said to be soluble in methanol whereas amylopectin nitrate is not. Starch nitrate containing 12.45% nitrogen gave a nitroamylose containing 12.50% nitrogen and an amylopectin nitrate containing 12.34% nitrogen.

Snelling (166) has shown that starch nitrate can be separated into three fractions on the basis of their solubilities in methanol.

These were isolated by extraction of the crude starch nitrate with methanol at about -15 to -20°C., leaving an undissolved portion (Fraction I). On warming the alcoholic extract, part of the dissolved starch nitrate is precipitated (Fraction II) leaving the third and largest fraction (III) in solution. Ethanol may be substituted for methanol.

6. X-Ray Studies

Unlike cellulose nitrate, starch nitrates have not been characterized to any great extent by means of x-ray technique. Berl and Kunze (159) found the number of x-ray interference rings was decreased from six to three on nitrating potato starch; on de-nitration of the nitrate, however, the regenerated starch showed six rings.

The nitrogen content of the starch nitrate determines the type of x-ray diagram produced (160). Nitrates with 12.4% nitrogen have a fairly regular structure, both the positions and intensities of the interference lines resembling those of unstable cellulose nitrate containing 12.0% nitrogen. With decrease in the nitrogen content, the diffraction lines became less distinct; apparently the degree of crystallinity decreases with decrease in the nitrogen content. These results led the author to postulate that the lower nitrogen-containing products were composed of starch molecules with their hydroxyl groups incompletely esterified, instead of mixtures of unaltered starch molecules and completely esterified molecules.

Apparently starch esterifies more rapidly than cellulose, as is evident from the fact that x-ray analysis of the product formed by a very short nitration treatment of starch indicates a definite lattice structure; in the case of cellulose the time interval for the lattice appearance is much longer.

In contrast with the above author, Kolaczkowska and Urbanski (167) believe that nitrated potato starch and soluble starch give the same principal interference rings.

E. The Problem of the Stability of Nitrated Starch

The properties of nitrated starch indicate its much greater instability as compared with cellulose nitrate. It would appear that the cause of this phenomenon lies in the structural differences between the two parent materials. Cellulose exists in the form of long linear chains of glucose anhydride residues which are connected through 1,4-β-linkages. There is no tendency toward the formation of branched chains as in starch. In the case of cellulose these long chains are united laterally by secondary valence bonds and Van der Waal's forces to produce the crystalline micelle. Starch, on the other hand, has a much more heterogeneous composition. Significant evidence exists indicating the presence of branched and unbranched chains (amylopectin and amylose in starch, the latter showing certain resemblances to cellulose). Furthermore, starch differs from cellulose in having its glucose units joined through 1,4 α-linkages which are more susceptible to rupture than

the β-linkages. These differences in chemical structure lead to a variety of different physical manifestations in the two substances.

Cellulose nitrate is essentially stable, once the excess of nitrating acids, shorter chains, partially nitrated fractions and foreign esters have been removed. This is not the case with the nitrates of starch. Careful purification by the methods used for cellulose nitrate fails to yield a stable product.

There have been many suggestions made as to the possible causes for the instability of nitrated starch (108). Some of these concern particle size, the presence of carboxyl groups, of short chain molecules, partial nitration of certain hydroxyl groups, different types of nitrogen linkages and the effects due to a mixture of amylose and amylopectin nitrate. The purpose of this study was to investigate the causes responsible for this instability.

These were isolated by extraction of the crude starch nitrate with methanol at about -15 to -20°C., leaving an undissolved portion (Fraction I). On warming the alcoholic extract, part of the dissolved starch nitrate is precipitated (Fraction II) leaving the third and largest fraction (III) in solution. Ethanol may be substituted for methanol.

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Addendum to Introduction

Since the preceding review was written, a paper has been published (210) dealing with the degradation of starch by *B. macerans* to yield so-called Schardinger dextrins (p. 46). Evidence is produced to indicate that these substances are synthesized by the enzyme from those components of starch which are most readily modified by acid treatment.

1. Both Schardinger dextrins are resistant to barley diastase and therefore cannot be true units of amylose.

2. The Schardinger dextrins are not related to the "limit dextrins" produced from whole starch by barley diastase, because the latter do not yield Schardinger dextrins on treatment with *B. macerans*.

3. Acid-degraded starches yield little or no Schardinger dextrins.

The mercaptalation method (p. 20) has recently (211) been employed to determine the degree of polymerization of a starch-like polysaccharide prepared by enzymic synthesis (105, p. 68) and indicates the presence of 32 ± 1 glucose units in this synthetic polysaccharide chain.

DISCUSSION OF RESULTS

I. The Nature and Objectives of the Investigation

From the data contained in the Historical Introduction, it is readily seen that the chemistry of starch is at an interesting stage of evolutionary development, and that its fundamental structure is a matter of marked uncertainty. The methods employed in starch chemistry have not been as exact as those in other chemical fields and physico-chemical methods have not been employed with as much success as in the case of cellulose. The following concept of the structure of starch was assumed as a basis for the experimental work: 1. Starch is a polysaccharide of plant origin composed of two principal constituents, namely, amylose and amylopectin in a proportion of about 1:4. These possess markedly different physical properties and chemical structures. Despite this fact, much of the earlier research on starch was carried out with "whole" starch, without any serious attempt being made to isolate these two products. 2. Each of these components is a glucose anhydride polymer with the glucose anhydride units in the principal chains linked through the 1,4 positions; cross linkages occur at the 1,6-positions. 3. Non-carbohydrate constituents such as nitrogen, phosphorus and fatty acids are present in starch in small but variable amounts. 4. Finally, it may be assumed that starch is a heterogeneous substance. Different starches are found to vary in the proportions of the two components mentioned above. Further, each component is, in turn, not a homogeneous product.

A review of the literature on the nitrates of starch, shows that here also many contradictions exist and that while there is much information concerning their preparation, and dealing with their stabilization, little attempt has been made to base the latter on fundamental principles in order to determine its cause. Such studies as x-ray analysis and viscosity measurements which have provided valuable information in the case of cellulose nitrate have, either not as yet been applied to starch nitrate, or have proven thus far, to be of little value. This is probably due to the fact that these studies were carried out on nitrates prepared from non-fractionated starches; indeed, no efficient fractionation of starch nitrate was attempted.

Available information concerning starch nitrates suggested that the first task to be accomplished was the development of suitable methods for the investigation of their properties. It appeared that, by a systematic fractionation of whole starch nitrate, the extent of its heterogeneity could be reduced very markedly and hence a means provided for determining the cause of its instability. This was carried out and the physical and chemical characteristics of the fractions determined by the customary analytical methods. A second technique developed for studying the properties of the nitrate was that of denitration. Finally, fractionation of starch itself, before nitration, was undertaken in an attempt to obtain more homogeneous initial products. In the course of the work, the effect of various stabilizers was investigated, although this phase of the work was considered to be of

secondary importance.

The second objective of this research was an investigation of the instability of starch nitrate and its relationship to the chemical structure of starch. It was necessary, therefore, to characterize starch nitrates,obtained by various methods, so as to permit the evaluation of factors responsible for their instability.

Thirdly, it was part of the assignment to determine if starch nitrate could be used as an explosive.

In the Experimental section, a description is given of the methods employed in the attempt to reach these objectives.

II. Methods of Enquiry into the Properties of Nitrated Starches

In this section are discussed the general usefulness and validity of certain methods applied to the study of starch and its nitrates.

A. The Nitration Technique

At the outset,it was necessary to choose a satisfactory method of nitration which could be used in all experiments, and thus provide a basis for logical comparison. As indicated in the Introduction (p. 74), many methods of nitration were available. The one which gave the best results not only in yields and nitrogen content but was also characterized by simplicity of application was that of Will and Lenz (156). Mixed acid nitration was less satisfactory than use of nitric acid alone followed by precipitation of the ester with concentrated sulfuric acid. Also, there is less

opportunity for the formation of starch sulfates in this case.

B. Analytical Methods

1. Viscometric Measurements

A survey of the general applications of the viscometric method was given in the Introduction (p. 17). This method does not give absolute molecular weights in the case of such a heterogeneous substance as starch, exhibiting as it does various degrees of chain branching. However, the viscometric results obtained indicate the heterogeneity of starch nitrate, i.e. the presence of various molecular-weight molecules, and also show that a high degree of fractionation was accomplished by the methods used.

2. Determination of Stability

Many tests have been devised to measure the stability of nitrated polysaccharides of the nitrocellulose type of explosive (162). In most of these the heat stability of the product under examination is measured. In the present investigation, the two most widely-used tests, namely the Abel Heat test and the Bergmann-Junk test, have been employed.

(a) The Abel Heat Test

This test is simple in theory and easy to perform on almost any explosive which decomposes with formation of nitrogen peroxide. It consists of heating a weighed sample in a special test tube, placed in a definite position in a standard heating bath maintained at a constant temperature, and then measuring the time taken for the nitrogen peroxide evolved to react sufficiently on a specially-

prepared test-paper, suspended in a prescribed manner in a specific position relative to the sample, to give a color equivalent in depth and shade to that shown by a standard tint paper. The test paper prepared by impregnating with starch and potassium iodide is, just before use, treated in a certain manner with a non-drying solution of glycerol and water. The color indication is given at the interface of the dry and wet sections of the test-paper. It is generally assumed that the Abel test indicates the physico-chemical condition of an explosive, from the point of view of the ease of decomposition which may exist due to the presence of minute amounts of foreign substances.

The main objection to the Abel heat test is its extreme sensitiveness. In cases where no interfering factors exist, it has been calculated that the standard tint can be produced by as little as 0.000135 mg. of nitrogen peroxide. Many precautions must be taken, as for example non-exposure of samples to direct sunlight. Variations in results of the heat test may be due to internal as well as external factors, e.g., gases other than nitrogen peroxide, notably ozone, apparently lower stability. On the other hand, mercuric chloride in very low concentrations in the explosive produces a masking effect and leads to a false interpretation of stability, since it retards the effect of nitrogen peroxide formation and thus its effect on the paper. Similarly, the presence of ethyl acetate or ethanol in gelatinized explosives gives rise to abnormally high apparent stabilities. Moisture in the explosive sample must be regulated carefully for the same reason.

For example, a cellulose nitrate which is too dry will give a high Abel test due to the fact that the test paper will lose moisture to a certain extent. On the other hand, a product of high moisture content will likewise give a high test due to absorption of nitrogen peroxide in the condensed vapours on the sides of the tube.

As indicated in the Experimental section (p. I33) the technique of the Abel test was simplified, and all precautions taken. The Abel Heat test values obtained are therefore considered to represent the amount of impurity present in the sample at the time of the test and to indicate the initial stages of decomposition during the time of the test.

(b) The Bergmann-Junk Test

The Bergmann-Junk test is used primarily for explosives of the cellulose nitrate type and is a measure of the degree of decomposition which occurs when a definite weight of sample is maintained at a temperature of 132 $\pm$ 0.2°C. for a period of two hours. In contrast with the Abel test, the Bergmann-Junk is quantitative and is not affected to nearly the same extent by added adulterants. Furthermore, it indicates the stability that may be expected over a long period of storage, since the products of decomposition are left in contact with the nitrate during the entire heating period. This test is also dependent on the moisture content of the samples and is influenced by the presence of certain impurities such as urea.

The Bergmann-Junk test as applied to starch nitrates was modified in one respect only, namely, the size of sample used. Instead of the customary 2.0 g. sample, amounts weighing 0.5 g. were used and compared with a similar quantity of cellulose nitrate.

C. Fractionation Methods for Starch Nitrates

There are three fractionation methods applicable to a high polymeric substance such as starch nitrate, namely, (1) fractional dissolution, (2) fractional precipitation and (3) solvent fractionation, the last being a special technique developed by Lovell and Hibbert (196) for use with lignin. These methods depend upon the different solubility characteristics exhibited by fractions of varying molecular weight and chemical constitution. Such fractions are usually present in high polymeric substances, especially those of natural occurrence, for example, starch, the heterogeneity of which has been indicated by many workers (9, 125, 21, 56).

Each of these fractionation methods was applied, in turn, to whole starch nitrate in an attempt to obtain products having some degree of homogeneity. The first two methods were also applied to the nitrates of amylose and amylopectin.

1. Fractional Dissolution Methods

In order that solution of a solid substance may occur, solvation forces must overcome the two cohesions (van der Waal's forces) existing between molecules of solute and of solvent respectively. The solute cohesion in the case of a polymer is deter-

mined by the shape and length of the chain and is usually much smaller for branched chain polymers than for the linear chain type; the latter are, therefore, much less soluble. The extent of solvation is also determined by the chemical nature of the groups attached to the polymer chain, especially in relation to the chemical structure of the solvent.

The fractional dissolution method was fully developed and tested first with cellulose nitrate (11.4% N) by Rogovin and Glazman (201). These authors claim a number of important advantages for this technique over that of fractional precipitation (discussed later, p. 93). Lower members of the polymer homologous series are consistently adsorbed on the higher members when these are precipitated out first, whereas, in the dissolution method, these low members are removed first. Also the reverse effect (adsorption of high members on the lower) cannot occur. This must therefore lead to a more uniform fractionation with a more consistent reproducibility. Secondly, there is no uncertainty regarding "precipitation thresholds" (the concentration of non-solvent present when the dissolved polymer just begins to settle out) in the extraction method. Thirdly, results (201) indicate that some separation on the basis of chemical composition is possible.

A disadvantage of the dissolution method is that solute and solvent are not in complete and intimate contact, i.e. the process involves a heterogeneous system.

The solubility of a nitrated starch depends upon (1) its chain length, (2) its degree of association and the power of the

solvent to break associative bonds and (3) the nitrogen content. At the end of the nitration reaction, there is present a heterogeneous mass of varying chain lengths, degrees of association and nitrogen contents. Thus the use of fractional dissolution for the fractionation of starch nitrates permits a separation based on both physical and chemical properties. Shorter, non-associated chains, especially those of lower nitrogen content, are removed, leaving a product which may be visualized as a micellar mass formed of tightly-interlocking chains. The size of this residual product will depend upon the dissociating power of the solvent used.

2. Fractional Precipitation Methods

The fractional precipitation method has the advantage (as compared with dissolution methods) that only one homogeneous phase, a solution, is involved. Thus the precipitant has an equal chance of contacting all the molecules of the dissolved solute.

In its simplest form it involves two liquids, a solvent and non-solvent, the choices of which are of prime importance both in regard to the sensitivity of the method and the form of the precipitated phase. An unfortunate choice of solvent and non-solvent may result in failure to obtain a fractionation due to the fact that most of the solute separates at once (too high sensitivity).

One of the first problems encountered in applying the fractional precipitation technique is the determination of the "precipitation threshold" (p. 92). This is different for each sample of

material and must be determined in each case. The first attempt to solve this difficulty was made in 1928 by Kumichel (202) who discovered that the addition of a precipitant to a solution of a high polymer brings about an initial decrease in the solution viscosity to a constant value, which is not lowered by further addition of precipitant until the solute just begins to precipitate; a sharp break occurs in the viscosity-solution composition curve at this point.

In preliminary investigations on the fractional precipitation of starch nitrate, it was found that the most suitable solvent was a mixture of acetone and ethanol (1:1). The fractions separated from this solvent in a more manageable form than when other liquids were used. Likewise, it was found that water was the most suitable precipitant. A temperature gradient was used to cause more rapid precipitation and to increase the sensitivity.

By use of the fractional precipitation method, it was possible to separate starch nitrates into a number of fractions which differed appreciably in relative viscosity and nitrogen content, and the method thus made possible a more detailed study of these products.

3. Solvent Fractionation

This method of fractionation, as applied to lignins (196), depends upon the fact that substances of different chemical structure are distributed in different amounts between two non-miscible solvents. The simplest case in this type of fractionation

is one involving two immiscible solvents for the substance to be fractionated. In order to secure two layers, it is necessary in most cases to incorporate water into the system since all common organic solvents are mutually miscible in all proportions. In the case of lignins the best solvent system is the combination chloroform, aqueous methanol, carbon tetrachloride.

Various organic solvents and non-solvents for starch nitrate were first investigated; qualitative data are reported below(p.96).

The more promising of these solvents were used in three-liquid combinations, i.e. aqueous solvent plus some other liquid of lesser solvent power. Subsequently, four liquid combinations were tried. The difficulty encountered in all of these systems was that the distribution of the "good" solvent between the two liquid layers was always very one-sided with the result that extraction by the aqueous layer was almost negligible, so that only small amounts of starch nitrate could be used. Other difficulties arose in the formation of emulsions and colloidal suspensions which destroyed the possibilities of any system in which they occurred.

After much experimentation not reported in detail in this thesis the solvent fractionation of starch was considered impractical.

Solubility of Starch Nitrate

Solvent	Solubility of solvent in water	Solubility of starch nitrate
Tetrachloroethane	insol.	sl. sol., suspension formed
Butylene glycol	insol.	sl. sol., viscous suspension
Cyclohexanone	insol.	very sol.
Pentachloroethane	insol.	sl. sol., suspension formed
Benzaldehyde	0.33/100 cc.	very sol.
Nitrobenzene	0.19/100 cc.	good solvent
Anisole	insol.	fair solvent
Aniline	insol.	slightly sol.
Butyl acetate	0.5/100 cc.	good solvent
Nitromethane	sl. sol.	good solvent
Glacial acetic acid	completely sol.	fair solvent
Pyridine	completely sol.	fair solvent
Acetone	completely sol.	good solvent
Ethylene glycol mono-ethyl ether	sol.	good solvent

D. The Denitration of Nitrated Starches

1. Purposes of Denitration

The denitration of cellulose nitrate is one of the final steps in a process which was important for many years in the preparation of one variety of rayon silk. However, little work has

been done on the denitration of starch nitrate; certainly none of a quantitative nature. The process offers a method of following the chemical decomposition of starch nitrate and thus is a measure of the chemical stability of the substance, the subject of this research. The stability as measured by denitration was compared with that indicated by the heat tests.

Denitration studies of the ethanol-soluble (unstable) fraction (p.I58) were used in an attempt to explain its relative instability. It was hoped that the denitration results on the nitrated amylose and amylopectin fraction might provide an explanation of the differences between the amylose and amylopectin derived from "native" starch.

2. Factors Involved in Denitration

(a) Denitration Reagent

Many reagents have been used for the denitration of cellulose nitrate, for example, alkali hydrosulfides, mixtures of nitric and sulfuric acids; ammonium sulfide and ammonium hydrosulfide. The action of alkali hydrosulfides on starch nitrate proved to be too severe and ammonium hydrosulfide was finally selected as most suitable.

(b) Temperature of Denitration

This is the second most important factor. Nadai (197) found that an increase of 10° in reaction temperature doubled the rate of denitration of cellulose nitrate by sodium hydrosulfide. A similar effect was found in the case of starch nitrate using

temperatures of 25°, 30°, 35° and 40°. The temperature finally selected for denitration experiments was 35°.

3. Preliminary Denitration Experiments

(a) The Denitration Blank

A blank run was made with ammonium hydrosulfide to determine its behaviour during eight hours at 35°, that is under the experimental conditions to be used in the denitration studies. The results are shown graphically in Fig. I. Considerable decomposition occurs as the temperature is raised from room temperature to 35°; when equilibrium is reached, there is relatively little change over a period of eight hours.

(b) Denitration of Starch and Cellulose Nitrates

These were denitrated under identical conditions. Comparative rate curves (Fig. II) indicate that starch nitrate denitrates faster than cellulose nitrate.

(c) Reproducibility of the Denitration Curves

When the experimental conditions (p.I42) are followed closely, excellent reproducibility is possible (Fig. V).

E. Fractionation of Starch

1. General Discussion

The earlier methods for the fractionation of starch have been reviewed (p. 47). Two more recent methods are that of Schoch (199) and a modification (of same) by Kerr (203). The former is based

on the selective precipitating action of n-butanol toward starch sols, two products of markedly different physical and chemical characteristics being obtained. The "butanol-precipitated" fraction averages 22% of native corn starch; no value has been given for the "non-precipitated" fraction. The method of Kerr (203) is similar except that the precipitant consists of a mixture of n-butanol and methanol which is added to a corn starch paste. The less-soluble portion, about 45% of the total, was shown to differ from the more soluble portion in having a higher conversion limit when treated with β-amylase (203) and also in its higher alkali lability (p.II2).

In the author's inveatigations, starch was fractionated by the method of Tanret (189) and of Pacsu (140), (with the modifications noted below (p.I48)), into cotton-adsorbable and non-adsorbable fractions designated as "amylose" and "amylopectin" respectively. The purpose of this fractionation was to obtain, if possible, homogeneous products suitable for nitration and the characteristics of the nitrates indicate that this was accomplished to a considerable degree. However, this method of separation cannot be taken as evidence that starch consists of only two components, since, on the basis of previous work employing fractionation techniques, it would seem there are present at least three major components (204).

2. The Components of Starch as Revealed by Fractionation Studies

There are two major concepts regarding the number of constit-

uents in starch, namely the multiple amylose concept (203, 204) and the two component concept (9, 199, 205). In support of the former view, Kerr claims to have divided potato starch into three constituent amyloses. With corn starch, which, in addition contains "gamma-amylose" (206) not found in potato starch, there are four amyloses present. A further difference between corn and potato starch is the apparently larger proportion of the more highly branched fraction present in the latter.

Evidence for the two-component concept is to be found in Schoch's (199) fractionation of starch into two components by a single precipitation, and secondly in the work of Bates, French and Rundle (205), who have shown that starch probably consists of only two components which show a distinct difference in their reactions with iodine to form complexes. Heterogeneity in degree of branching of starch molecules is, therefore, considered to be discontinuous; that is, there is a limited number of components each with a fair uniformity in degree of branching, and separated from other components by a significant difference in this property.

Further evidence is provided by the butanol precipitation method of fractionation in that starches from different sources vary in the number of components. For example, waxy maize starch contains no butanol-precipitable fraction (amylose). Other starches, namely waxy barley and waxy rice have been shown by iodine-complex formation (205) to consist mostly of amylopectin.

The method of starch fractionation involving adsorption of one constituent on cotton cellulose seems to indicate the presence

of two or more constituents. Although the adsorbed and non-adsorbed fractions have different properties and yield derivatives having different properties, (Table X, Fig. VII-XI), this does not entirely preclude the possibility that there are molecules of a nature intermediate between amylose and amylopectin as the adsorption method does not yield the theoretical amount of amylopectin. There is a portion of the starch granule, apparently strongly adsorbed on cotton cellulose, that is not removed by either hot or cold water (Fig. XV).

III. Early Experiments on Nitration

A. Use of Starch Swollen with Pyridine

Pyridine and pyridine-water solutions have been used as swelling agents for starch in the quantitative preparation of such compounds as tritosyl-, ditosyl-6-iodo- and tribenzylstarch (195).

Starch was treated with pyridine by the writer to obtain a swollen starch of maximum nitratability. Apparently hydrolysis during nitration was more pronounced, the yield of nitrated starch being lower than with untreated starch (Table I, p.I4I). As its stability was very low, as measured by the Abel test, the method was abandoned.

B. Nitration of Starch in Formic Acid Solution

The object was to obtain a solution of dissociated starch molecules which would permit of intimate contact of nitric acid

with the hydroxyl groups in the starch aggregate. Formic acid reacts upon starch under these conditions to give a monoformate and oxidation of this formate ester with periodic acid indicates that the formyl group is located primarily, but not exclusively, on the carbon atom in the 6-position (207).

Nitration of this formic-acid-treated product gave a low yield of nitrate (95%) having a low nitrogen content (Table I).

The nitrated product, presumably a mixed ester, was obtained in such low yield and with such a low nitrogen content that this method of pretreatment of the starch was discontinued.

C. Renitration of Starch Nitrate

Starch nitrate of nitrogen content 12.6% was renitrated in acetone solution (Table I). The results were unsatisfactory, the low yields indicating that considerable hydrolysis had occurred with no increase in nitrogen content. These results together with the low stability led to the abandonment of the method.

D. Nitration of Starch Swollen According to the Haworth Procedure

Haworth (39) prepared starch for acetylation by allowing it to swell in boiling water for thirty minutes and then precipitating the swollen product by addition of ethanol. In this way he obtains a starch giving an almost quantitative yield of a tri-acetate. In this investigation, corn starch was prepared for nitration by a similar method with the results shown in Table I. Inasmuch as the yield and atability were low, the method was not investigated further.

E. Conclusions Regarding Nitration

From the results of the experiments just outlined, it was concluded that pre-treatment of the starch resulted in degradation with no increase in stability or nitrogen content, and, therefore, the method of nitration described below (p.129) was used in all subsequent experiments and found to give satisfactory results (6, 7 Table I).

IV. Results of Fractionation: Characteristics of the Fractionated Products

A. Fractional Dissolution

1. Fractional Dissolution of Whole Starch Nitrate

(a) Introduction

Fractional dissolution in these experiments involved extraction with cold ethanol and in some cases further treatment with hot ethanol, acetone-ethanol, and ether (Fig. XVI, XVII). With ethanol, the dissolved material amounted to 8-10% of the crude nitrate and in the further extractions with other solvents to 20-25%. The characteristics of the ethanol-soluble fractions and ethanol-insoluble fractions are given in Tables IV, V, VI and shown graphically in Fig. IV, VI, XVI, XVII .

(b) Nitrogen Content

The nitrogen content of the readily extractable fractions was, in all cases, lower than that of the residual products (Tables IV, V). It varied from about 6 to 9% in the various ethanol-

soluble fractions.

(c) Viscosities of Ethanol-Soluble Fractions

Viscosity determinations on the ethanol-soluble fractions (Table IV) showed considerable variation in molecular complexity. They had a lower relative viscosity (Table IV) which is not surprising since shorter chains show greater solubility in ethanol and in solvents generally.

(d) Stability Tests

In all cases, the ethanol-soluble portion of starch nitrate was found to have a lower stability as judged by the Abel test, while the residual products possessed greatly increased stabilities as indicated by both the Abel and the Bergmann-Junk tests (Tables V, VI). A residual product obtained by ethanol extraction (Fig. XVII, No. 3) on examination by the latter test was found to evolve 2.97 mg. of nitrogen per gram sample. This value apparently represented the maximum degree of stability attainable by ethanol extraction, as the best value obtained with other products treated similarly was 2.27 mg. of nitrogen per gram. Samples of unextracted starch nitrate were very unstable under the conditions of this test. The maximum instability allowed commercially in the case of cellulose nitrate is that corresponding to an evolution of 1.25 mg. of nitrogen per gram of sample.

Thus, in the case of whole starch nitrate, ethanolic extraction with consequent removal of low-molecular weight material results in an appreciable increase in stability.

THE INCREASE IN NITROGEN CONTENT OF THE RESIDUAL NITRATED STARCHES ISOLATED BY REPEATED ETHANOL EXTRACTION UNDER VARYING CONDITIONS RUNS PARALLEL TO AN INCREASE IN THEIR VISCOSITY AND STABILITY.

(e) Results of Denitration

The denitration curve (Fig. VI) of the ethanol-soluble fraction from whole starch nitrate shows three well-defined rates of denitration, each separated by a flat portion of the curve where the rate is relatively constant. This change of rate was observable also (Fig. IV, Curve I) with the most soluble (that is, least precipitable and most unstable) fractions in all precipitation procedures investigated.

The cause of these differences in the rate of denitration is not known. Inasmuch as whole starch nitrate consists of various micellar sizes and different degrees of molecular aggregation resulting from chain branching, presumably differences will exist in the ease of accessibility of the various nitrate groups to the denitration reagent. It is also possible that structural dissimilarities in the two starch components, amylose and amylopectin, may play an important role in this connection. The differences in rates of denitration may also be associated with the relative ease of removal of nitrate groups from secondary and primary hydroxyl positions respectively. In any case, these differences are found only in the case of the unstable, more soluble fractions of nitrated

starch having lower nitrogen content and viscosity.

Denitration of the ethanol-soluble fraction of starch nitrate was stopped prior to completion (Fig. XVII) and the reaction products isolated as indicated in Fig. XIV and examined for viscosity, nitrogen content, stability and iodine coloration (Fig. XVII).

The soluble starch (Fig. XVII, 6) isolated from the denitration liquors was a white amorphous powder, readily soluble in cold water even after drying. It gave a purple color with iodine in water solution indicating its probable structure to be of the amylopectin rather than the amylose type. Degradation during the denitration process was indicated since the viscosity of this soluble fraction was much lower than that of the original ethanol-soluble nitrate. That this recovered starch (Fig. XVII, 6) was completely denitrated was shown in the total absence of nitrogen. It is significant that this nitrogen-free soluble amylopectin-like material constituted the greater part of the less stable ethanol-soluble fraction of whole starch nitrate.

The residual partially-denitrated starch nitrate (Fig. XVII, 4, 5) was divided into aqueous-acetone soluble and insoluble fractions differing markedly both in nitrogen content and viscosity. The insoluble fraction, in fact, is seen to possess a higher reduced viscosity than the original ethanol-soluble material from which it was derived. It was rendered water-soluble by treatment with warm dilute alkali (2%) whereby complete denitration was effected. The neutralized starch solution gave a bright blue color

with iodine indicating its amylose structure. This denitrated product represented only a small part (8%) of the original, unstable, ethanol-soluble fraction of whole starch nitrate.

The nitrate (Fig. XVII, 4) from which the water-soluble denitrated product was derived, possessed a remarkable stability as determined by the Abel test (40 min.).

THESE RESULTS INDICATE THAT THE INSTABILITY OF STARCH NITRATE IS ASSOCIATED PRIMARILY WITH THE AMYLOPECTIN CONSTITUENT OF THE STARCH MOLECULE.

(f) Explosive Properties of the Residual "Ethanol-insoluble" Fraction of Whole Starch Nitrate (Fig. XVI, Table VI)

Tests* carried out included the Ignition test, the Machine Impact test and the Trauzl Lead Block test. In the Ignition test, brown fumes appeared at a lower temperature (about 170°) than in the case of cellulose nitrate (182°). Also, the actual ignition temperature was about five degrees lower. The Machine Impact test indicated a sensitivity some two to three times greater than that of trinitrotoluene.

The Trauzl Lead Block test is a measure of the power of an explosive. Briefly, it is based upon the volume of the cavity formed when a given weight of explosive is fired in a bore-hole made in a block of pure lead. With starch nitrate, the increase in volume was 358 cc. as compared with Trinitrotoluene 254 cc.,

* Carried out through the courtesy of Mr. M.C. Fletcher, Dep't. of Mines and Resources, Ottawa.

Nitroglycerine 540 cc., Tetranitromethylaniline 375 cc., Dynamite 300 cc., and Cellulose nitrate 290 cc. (208) .

2. Fractional Dissolution of Amylose and Amylopectin Nitrates

The amylose and amylopectin were obtained by the fractionation of starch (Fig. XV) and nitrated by the standard procedure (p.I29). The amylopectin nitrate was 86% ethanol-soluble, whereas only 20% of the amylose nitrate was soluble, (Table II), a result of marked significance in view of the previous findings (p.I06) that the ethanol-soluble (unstable) fraction (Fig. XVII, Tables IV, V) from whole starch nitrate was essentially amylopectin in type.

The ethanol-soluble and insoluble fractions obtained from amylopectin and amylose respectively, (Table II), were analyzed for nitrogen content and stability (Table VII). The ethanol-soluble portions contained slightly less nitrogen than the insoluble fractions.

A high stability (Abel test) was found with both the soluble and insoluble amylose nitrate fractions, those of the corresponding amylopectin nitrates being less stable. Prior to testing, all traces of ethanol were carefully removed by repeated boiling in distilled water, drying and extraction with petroleum ether.

The Bergmann-Junk test confirmed these Abel Heat test results in that the amylose nitrates had a higher stability than the amylopectin nitrates. It is of interest that the treatment of crude amylopectin and amylose nitrates with ethanol in the fractionation process actually brought about an increase in the stability of both

the alcohol-soluble and alcohol-insoluble fractions.

B. Fractional Precipitation Methods

1. Fractional Precipitation of Whole Starch Nitrate

(a) Introduction

Fractional precipitation of starch nitrate was carried out using an acetone-ethanol mixture as solvent and increasingly larger amounts of water as the precipitant (p.152). The procedure yielded a number of fractions of varying nitrogen contents and molecular weights (indicated by reduced viscosities (Tables III, VIII)).

(b) Nitrogen Contents (Table VIII)

The more readily precipitable fractions are seen to have the higher nitrogen contents, while the last fraction to precipitate (most soluble) was always of appreciably lower nitrogen content.

(c) Viscosity (Table VIII)

Fractions 1-6 are seen to have approximately similar molecular sizes as judged by their reduced viscosity values. The less readily precipitable products (8, 9) (the most soluble) have the lowest viscosities and nitrogen content indicating a definite correlation between these two properties.

(d) Stability of the Precipitated Fractions

As indicated (Table IX) the most soluble (that is, least precipitable) starch nitrate fraction obtained by any fractional precipitation procedure, is the least stable. Furthermore it. seems to be a general rule that the fractions of maximum stability

appear midway in the series, rather than initially.

It is probable that the bulk of the extraneous products present in the crude nitrate and responsible for lowering the stability is precipitated along with the first fraction.

(e) Denitration Studies

Denitration studies were made upon certain of the fractions obtained by the fractional precipitation procedure (Tables III, IX). Smooth denitration curves were found (Fig. III) with all fractions except the last to precipitate (most soluble) which exhibited rate changes (Fig. IV) similar to those observed with an ethanol-soluble fraction (Fig. VI, XVII). The curves for the most stable fraction (Table IX, 6) and the least stable (Table IX, 7) are shown in Fig. III. The differences in the rates of denitration are quite remarkable. In Fig. IV the curves for the denitration of a final fraction of maximum solubility and of an ethanol-soluble fraction are shown and it is evident they show a close parallelism.

In general, with each of the precipitated fractions (Table IX) a fair correlation was observed between stability and rate of denitration, those fractions of low stability having a high rate of denitration.

2. Fractional Precipitation of Amylose and Amylopectin Nitrates

(a) Nitrogen Content, Viscosity and Stability

Fractional precipitation (Table III) of amylose and amylopectin nitrates prepared from the isolated amylose and amylopectin (Fig. XV) yielded in each case eight fractions varying in nitrogen

content and reduced viscosity (Table X). The same relative general trends are observable in the nitrogen content and viscosities of the various fractions found for the similar fractions of whole starch: 1. The nitrogen content of the first six fractions shows little variation, the last fraction (most soluble) is considerably lower; 2. The viscosity of the initially precipitable fractions is much higher.

It is important to note the consistently lower viscosity of the amylopectin nitrate fractions as compared with the corresponding amylose products, a result in conformity with the theory of a branched-chain structure for amylopectin as compared with the long chain linear type postulated for amylose. The results also point to the effectiveness of the cotton-adsorption process used by the writer for the separation of the two starch components (Fig. XV).

Results obtained by the Abel and Bergmann-Junk tests (Table X) show: 1. Unfractionated amylose nitrate is more stable than unfractionated amylopectin, 2. Each fractionally precipitated product from the former was more stable than the corresponding fraction from the latter. Also the same general stability trends are evident here as found for whole starch nitrate (Table IX) in that the final precipitates (most soluble) are least stable, while maximum stability is found in the products isolated midway.

(b) Denitration Experiments

The greater rate of denitration of the fractions of amylopectin nitrate compared with the corresponding fractions of amylose

nitrate (Fig. VIII-XI) indicate the greater relative ease of hydrolysis of the former, this presumably in turn being associated with the relative stabilities.

The reason for the marked difference in behaviour of the two nitrated starch components is unknown, but presumably has its origin in the marked difference in structure of a branched chain polymer as compared with one of the linear type. In the former there is a less complete chain association, so that the activation energy required for reaction of nitrate groups is less than with the linear type of polymer.

Moreover, it is also probable that secondary intramolecular effects due to closer spatial proximity of reactive groups in the nitrated "repeating" units of the branched chain molecule are more pronounced than in the corresponding linear type amylose structure.

(c) Action of Alkali on the Nitrates of Starch, Amylose and Amylopectin

The effect exerted by alkali on starches has been defined by Taylor (198) and Schoch (199) as "Alkali-Lability" and according to the latter author represents the amount of 0.1 N sodium hydroxide consumed by one gram of starch when heated in this solution for one hour at 100°. Starches which have not been subjected to hydrolysis have a negligible or low "alkali-lability" value (198). Schoch believes that this alkali consumption results from the sole interaction of the alkali with free terminal aldehyde groups and he has shown (199) that the α-1,4- and the β-1,4-glucosidic

linkages in maltose and cellobiose respectively are stable to alkali.

The consumption of alkali on heating amylose and amylopectin respectively for fifteen minutes was negligible in both cases, while with the nitrated products it amounts to 3.81 and 2.81 milli-equivalents of 0.3982 N sodium hydroxide respectively, indicating considerable hydrolysis during the nitration procedure. Presumably, this involved liberation of additional terminal free aldehyde groups (199) to an equal extent in both cases since the basic chain structure of amylose and amylopectin is the same (α-1,4-glucosidic linkages). Thus the higher alkali consumption of amylose nitrate is associated with a greater stability of this substance (Table X, XII) as compared with the lower alkali lability of the less stable amylopectin nitrate. THE INSTABILITY OF THESE POLYSACCHARIDE NITRATES CANNOT, THEN, BE ASSOCIATED WITH THE PRESENCE OF FREE ALDEHYDE GROUPS, A CONCLUSION BORNE OUT BY THE FACT THAT THE MORE STABLE CELLULOSE NITRATE IS MORE READILY ATTACKED BY ALKALI THAN EITHER AMYLOSE NITRATE OR AMYLOPECTIN NITRATE. (Table XII)

Whole starch nitrate, when treated with sodium hydroxide (1.6%), was decomposed to the extent of 60%, whereas cellulose nitrate was totally destroyed under the same conditions. With one-half per cent alkali, 20% of starch nitrate was decomposed. The result of this alkali treatment, apparently, is the removal of material of low molecular weight and nitrogen content, since the residual product

had suffered no denitration (Table XI). However, the stability of the starch nitrate (Table XI) was not increased to the same extent as that effected by ethanol extraction.

When the individual fractions of amylose and amylopectin nitrate (Table III) were treated with alkali (Table XII) they consumed varying amounts of sodium hydroxide, the amounts increasing fairly uniformly from the first to the last fraction (lowest nitrogen content and viscosity), a behaviour especially noticeable in the case of amylopectin. The amylose fractions consumed more alkali than the corresponding amylopectin fractions.

No satisfactory explanation of these results can be given, but they emphasize further the heterogeneity of the components of starch and its nitrates. The fact that greater alkali consumption is found with the low-molecular weight material supports the belief that alkali reacts only with terminal groups. A structural similarity of amylose and cellulose is indicated since nitrates of both consume more alkali than the supposedly branched amylopectin fraction.

V. Use of Ethanol as a Stabilizing Agent for Starch Nitrates

Fractional dissolution (Fig. XVI, XVII; Tables II, IV) of starch nitrate using ethanol was shown to yield products of greater stability and possessing good explosive characteristics (Table VI). The ethanol presumably functions in a dual capacity: 1. As an

extraction reagent for certain unstable portions of starch nitrate and 2. as a stabilizer. However, treatment with ethanol apparently does not stabilize all fractions (Table V) as those portions of starch nitrate extractable by cold ethanol remain unstable.

Fractional dissolution with ethanol increases the stability of both amylose and amylopectin nitrates as discussed previously (p. 108)(Table VII).

VI. A Study of Maltose and Cellobiose Octanitrates

A. Purpose of Study

The nitrates of maltose and cellobiose were prepared and studied for the purpose of comparing the properties, particularly stabilities, of the fundamental building units of starch and cellulose respectively. Cellobiose octanitrate has not been characterized previously.

The two major chemical differences between starch and cellulose are (a) the type of glycosidic linkage (α- and β- respectively) and (b) the presence of branched chains in the former. It seemed that any differences due to glycosidic linkages (α- or β-) might be made apparent from a study of these disaccharides, and that these would provide a possible explanation of the relative instability of starch nitrate.

B. Preparation and Purification

The nitrates of maltose and cellobiose were prepared as white amorphous solids in high yield by the usual nitration method (p. 129).

Like the corresponding polysaccharides they were insoluble in water and were precipitated from their solution in concentrated nitric acid by the addition of concentrated sulfuric acid.

The fact that cellobiose nitrate was obtained in higher yields (191%) than maltose octanitrate (183%) indicated that during nitration hydrolysis took place to a somewhat greater extent in the case of the latter, a result to be expected in view of the presence of the α-linkage in maltose (209).

The products were purified by an initial treatment with charcoal and anhydrous sodium sulfate in methanol solution. The maltose derivative was then recrystallized from diethyl ether and the cellobiose derivative from ethanol. The crude nitrates were much more soluble in methanol, ethanol, diethyl ether etc., than when more highly purified, this being especially true of cellobiose octanitrate. Both substances showed a marked tendency to separate from solution in the amorphous state, but crystallization could be induced by very slow concentration of these solutions at room temperature. The crystalline structure of both compounds appeared to be the same, namely needles arranged in the form of tiny rosettes.

The melting point of the crude products was very indefinite (70-90°) due to the presence of glucose pentanitrate (m.p. 10°). The purified maltose octanitrate melted at 163-164° and the cellobiose octanitrate at 154-155°.

C. Properties of the Nitrates

Their analyses (Table XIV) corresponded to the pure octanitrates.

The Abel test indicated that the cellobiose nitrate was the more stable while neither compound was of sufficient stability to withstand the Bergmann-Junk test for the usual period (2 hours).

Denitration experiments (Fig. XII) indicated that the initial rate of decomposition of maltose nitrate was greater than that for cellobiose.

On alkali treatment (p.164)(Table XIV) cellobiose octanitrate consumed more alkali than did the maltose compound; 7.97 and 13.82 milliequivalents of sodium hydroxide (0.0567 N) respectively. This result is to be compared with previous experiments (Table XI) where cellulose nitrate consumed more alkali than starch nitrate.

ON THE BASIS OF THESE RESULTS IT WAS CONCLUDED THAT CELLOBIOSE OCTANITRATE POSSESSES SLIGHTLY GREATER STABILITY THAN MALTOSE OCTANITRATE AND THAT, IF THIS DIFFERENCE IS CUMULATIVE, THEN CELLULOSE NITRATE SHOULD BE INHERENTLY MORE STABLE THAN STARCH NITRATE.

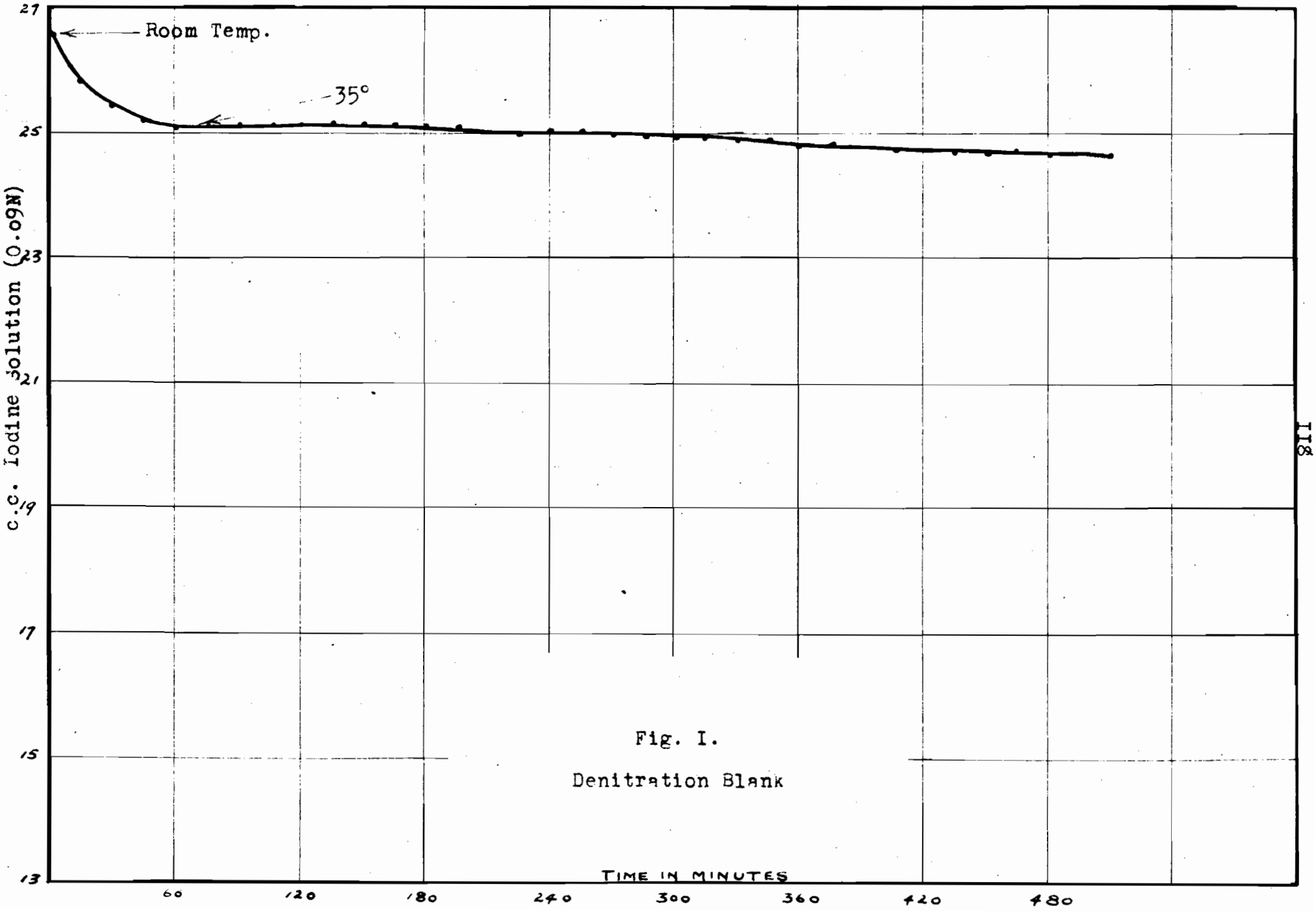
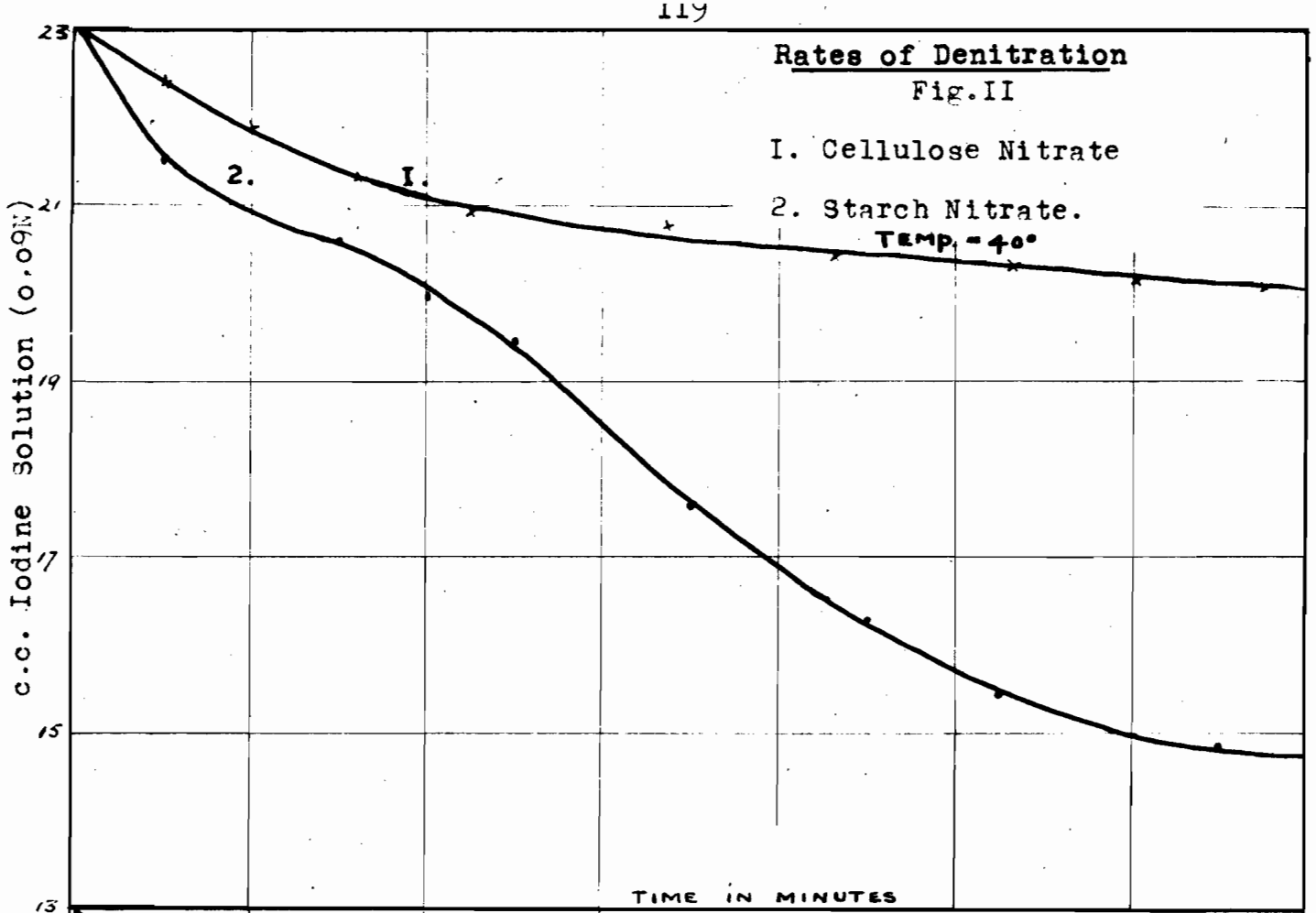
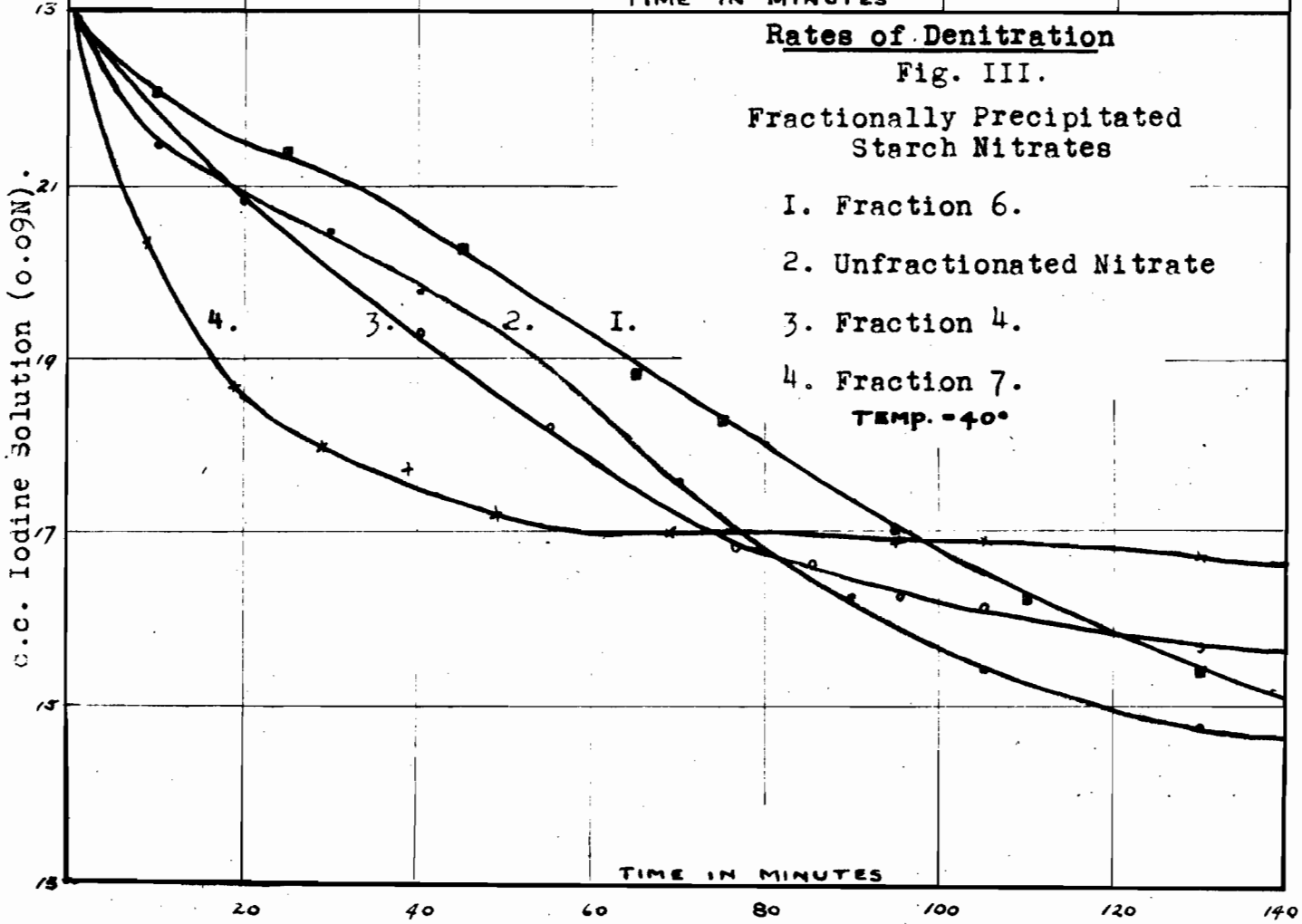


Fig. I.

Denitration Blank


Rates of Denitration
Fig. II
I. Cellulose Nitrate
2. Starch Nitrate.
TEMP. = 40°
c.c. Iodine Solution (0.09N)
TIME IN MINUTES


Rates of Denitration
Fig. III.
Fractionally Precipitated Starch Nitrates
I. Fraction 6.
2. Unfractionated Nitrate
3. Fraction 4.
4. Fraction 7.
TEMP. = 40°
c.c. Iodine Solution (0.09N).
TIME IN MINUTES

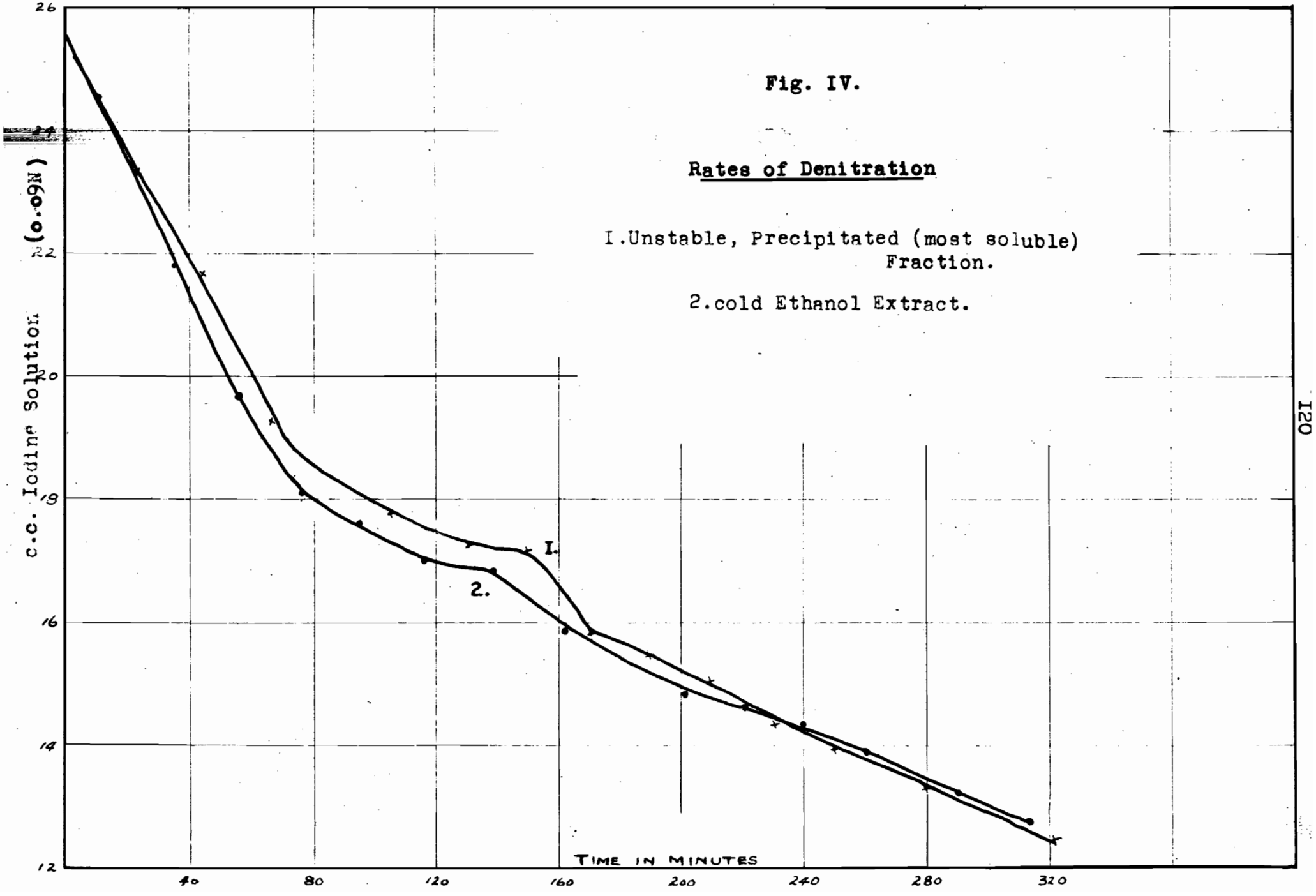


Fig. IV.
Rates of Denitration
I. Unstable, Precipitated (most soluble) Fraction.
2. cold Ethanol Extract.
c.c. Iodine Solution (0.09N)
26
24
22
20
18
16
14
12
I.
2.
TIME IN MINUTES
40
80
120
160
200
240
280
320
120

Fig. V.

Rates of Denitration.

Denitration of a precipitated Fraction.

Reproducibility.

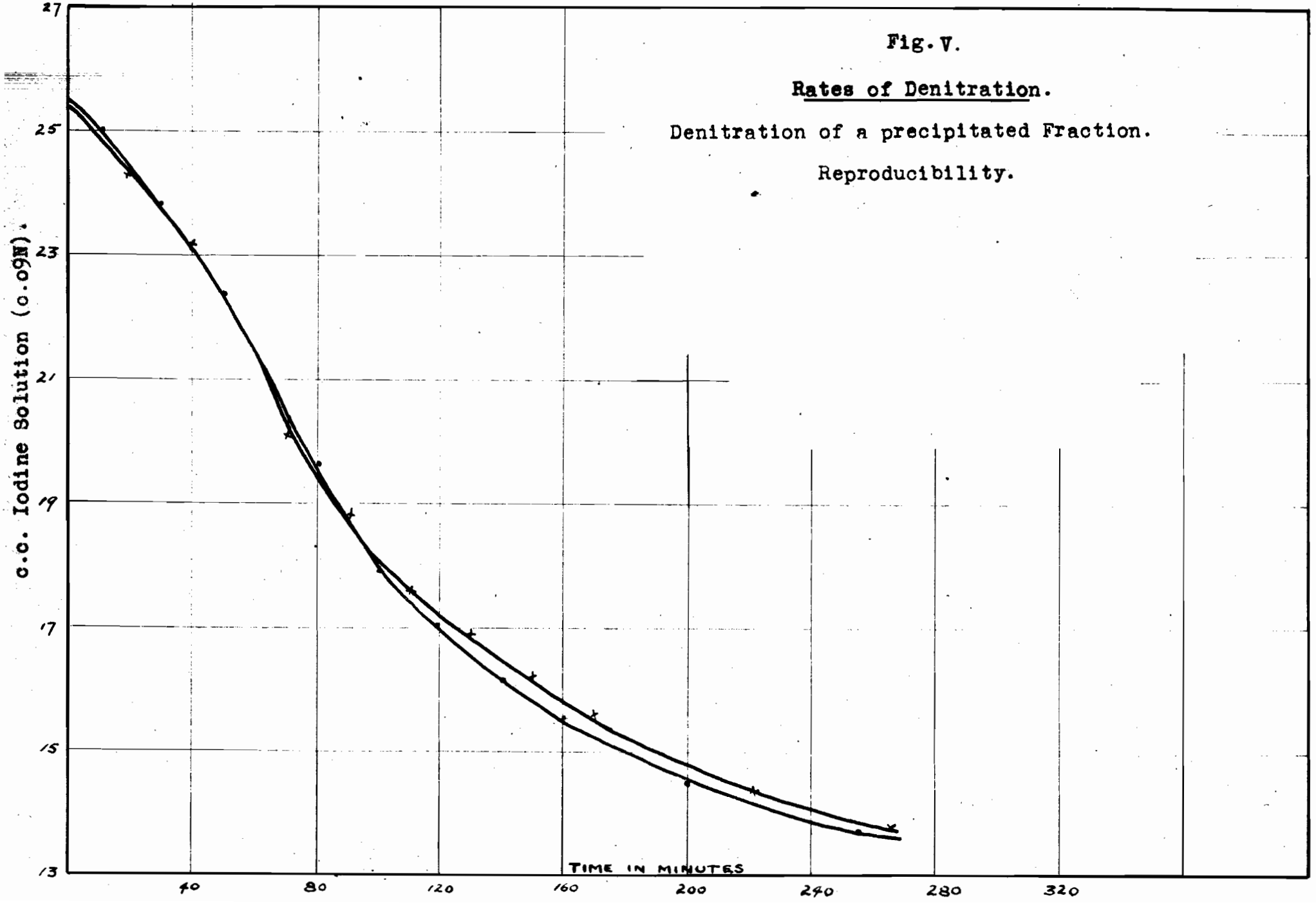
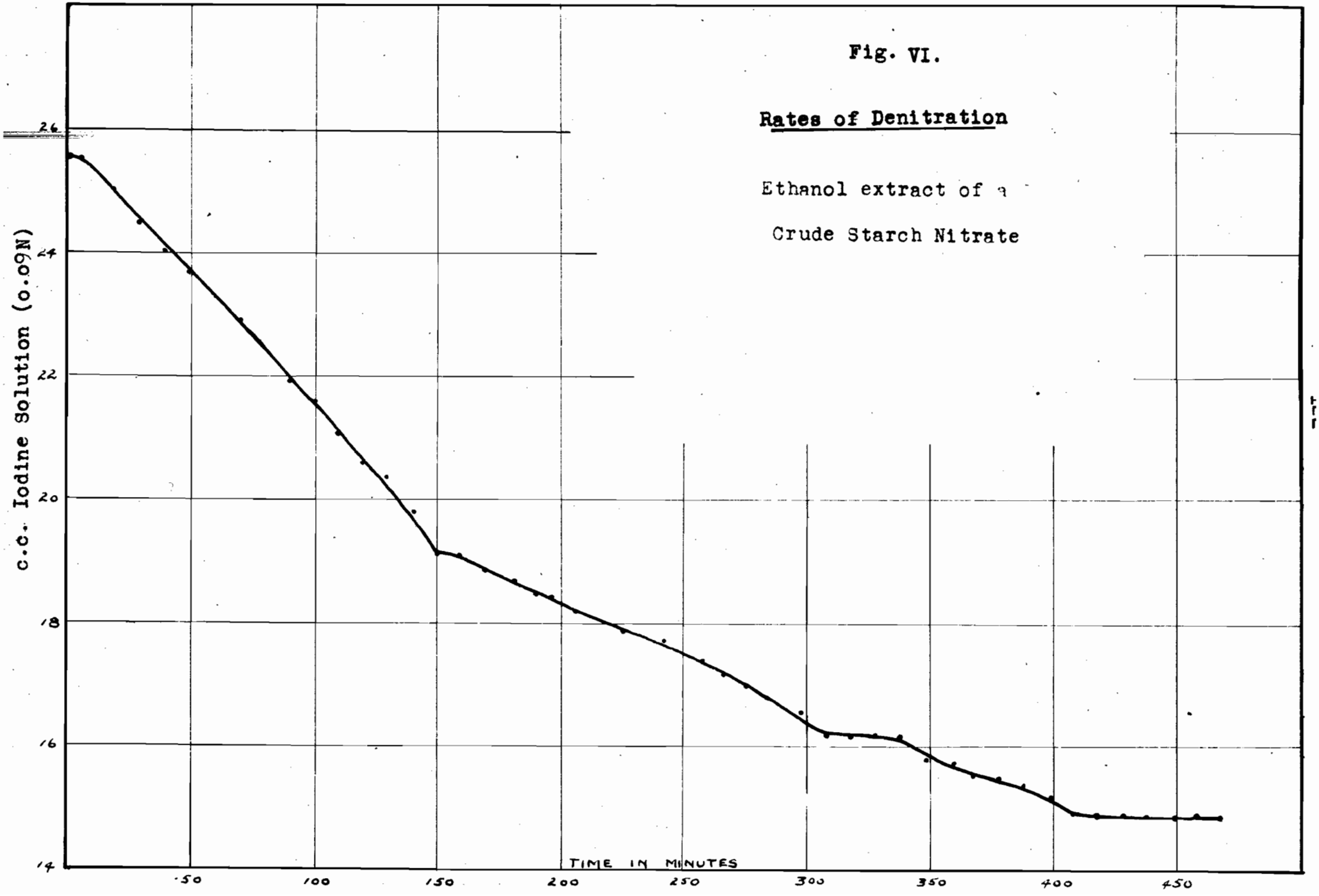


Fig. VI.

Rates of Denitration

Ethanol extract of a

Crude Starch Nitrate



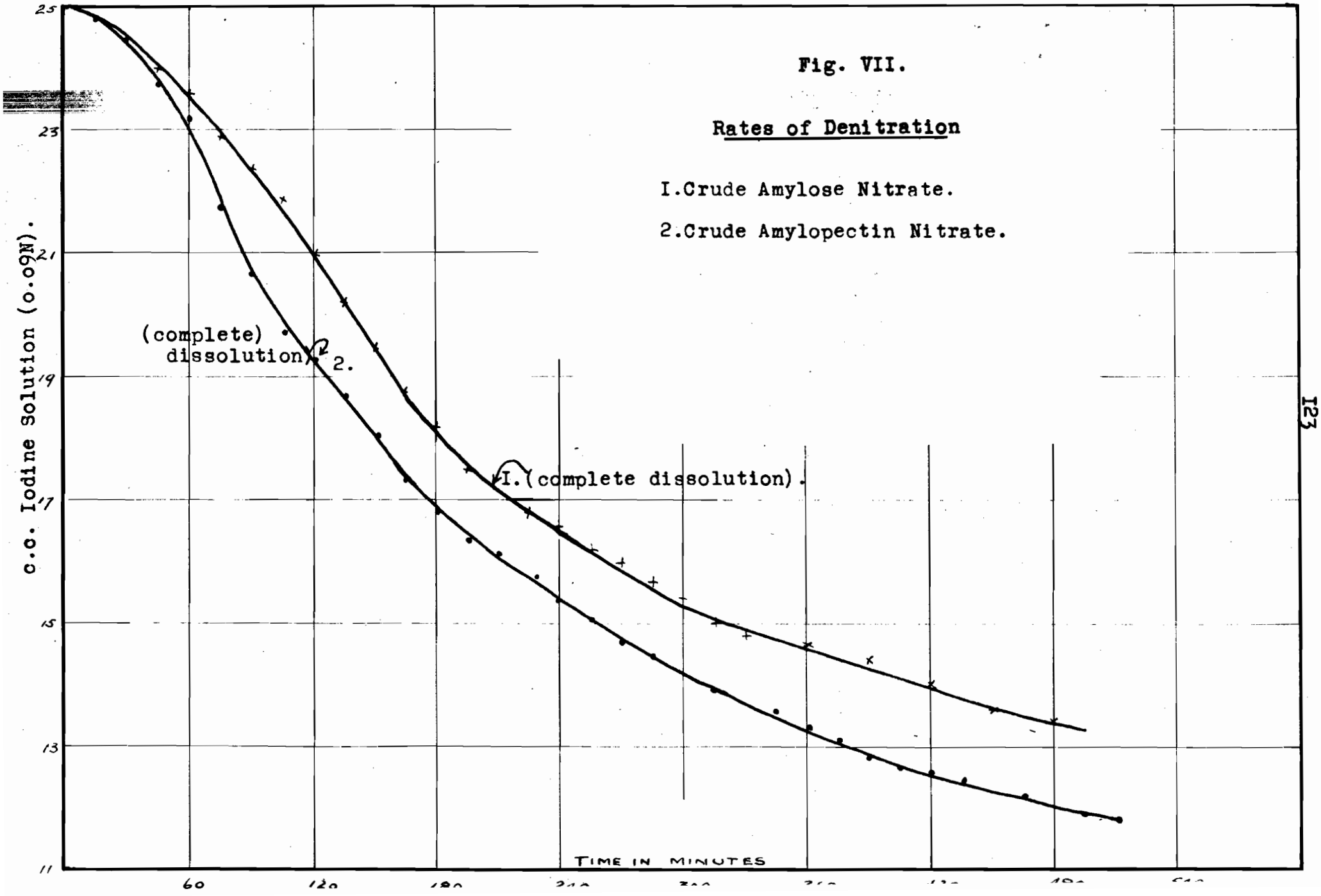


Fig. VII.
Rates of Denitration
I.Crude Amylose Nitrate.
2.Crude Amylopectin Nitrate.
c.c. Iodine Solution (o.o9N).
25
23
21
19
17
15
13
11
(complete) dissolution
2.
I.(complete dissolution).
TIME IN MINUTES
60
120

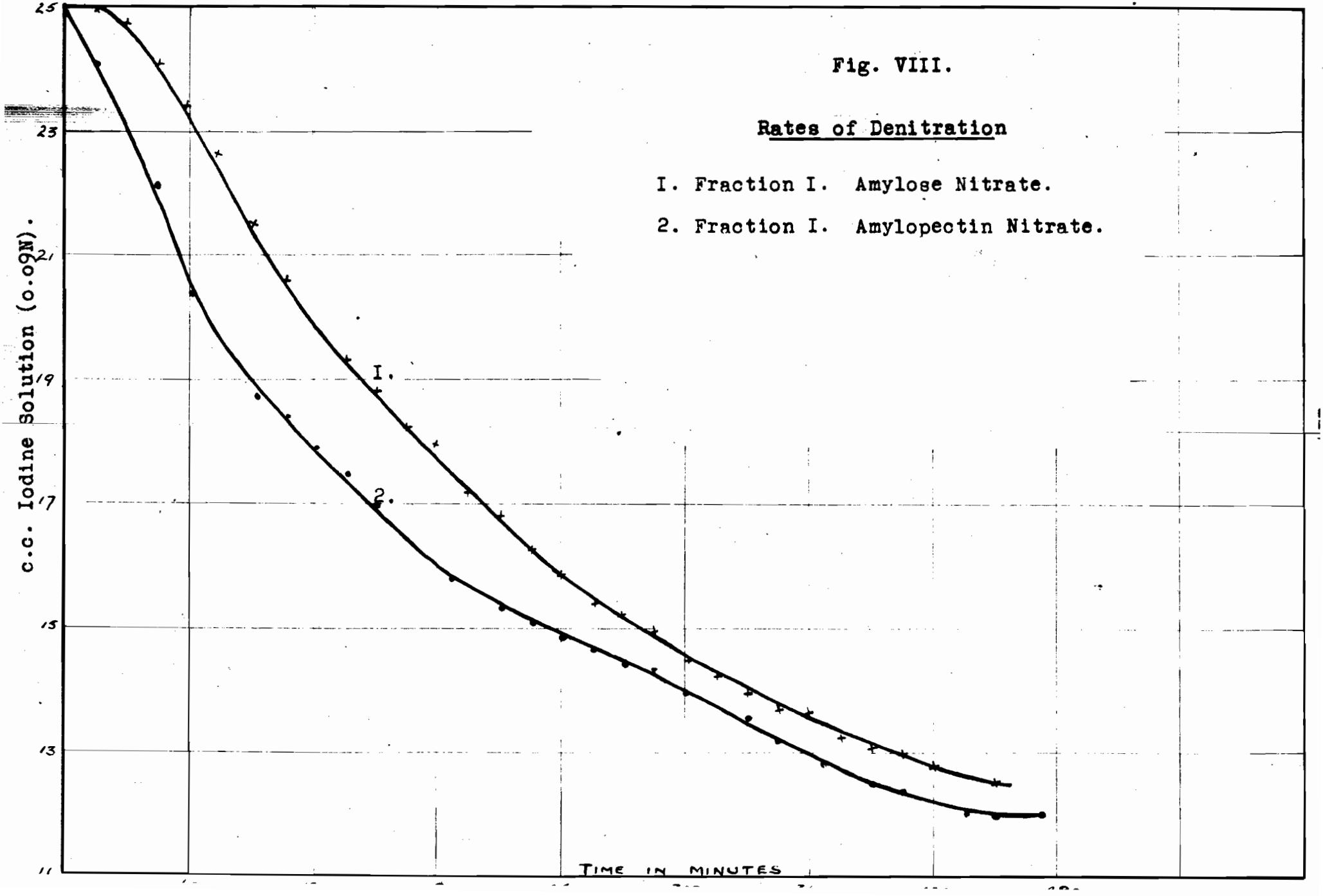


Fig. VIII.
Rates of Denitration
I. Fraction I. Amylose Nitrate.
2. Fraction I. Amylopectin Nitrate.
c.c. Iodine Solution (o.o9N).
25
23
21
19
17
15
13
11
I.
2.
TIME IN MINUTES

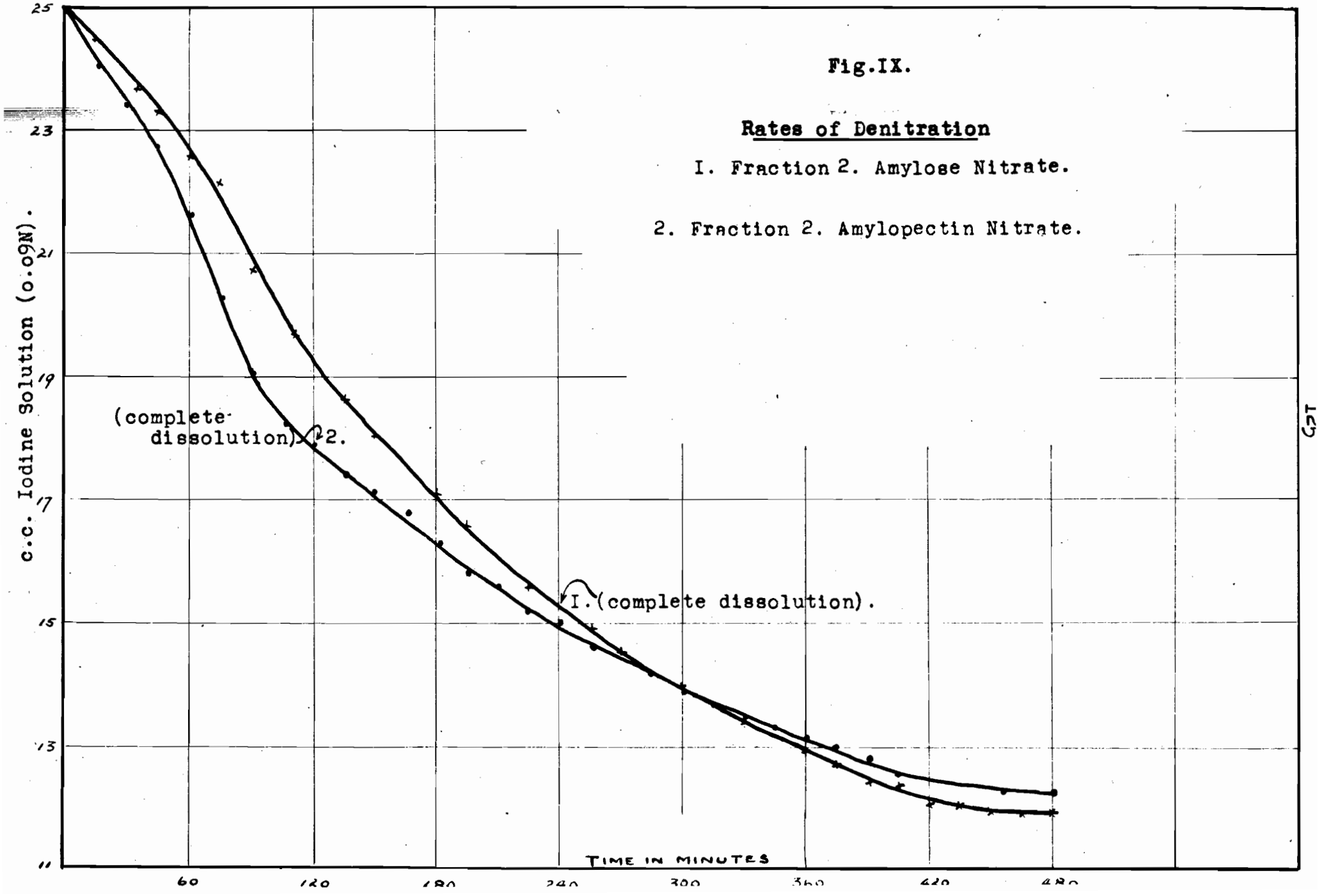


Fig.IX.
Rates of Denitration
I. Fraction 2. Amylose Nitrate.
2. Fraction 2. Amylopectin Nitrate.
c.c. Iodine Solution (o.o9N).
25
23
21
19
17
15
13
11
(complete dissolution) 2.
I. (complete dissolution).
TIME IN MINUTES
60
120
180
240
300
360
420
480

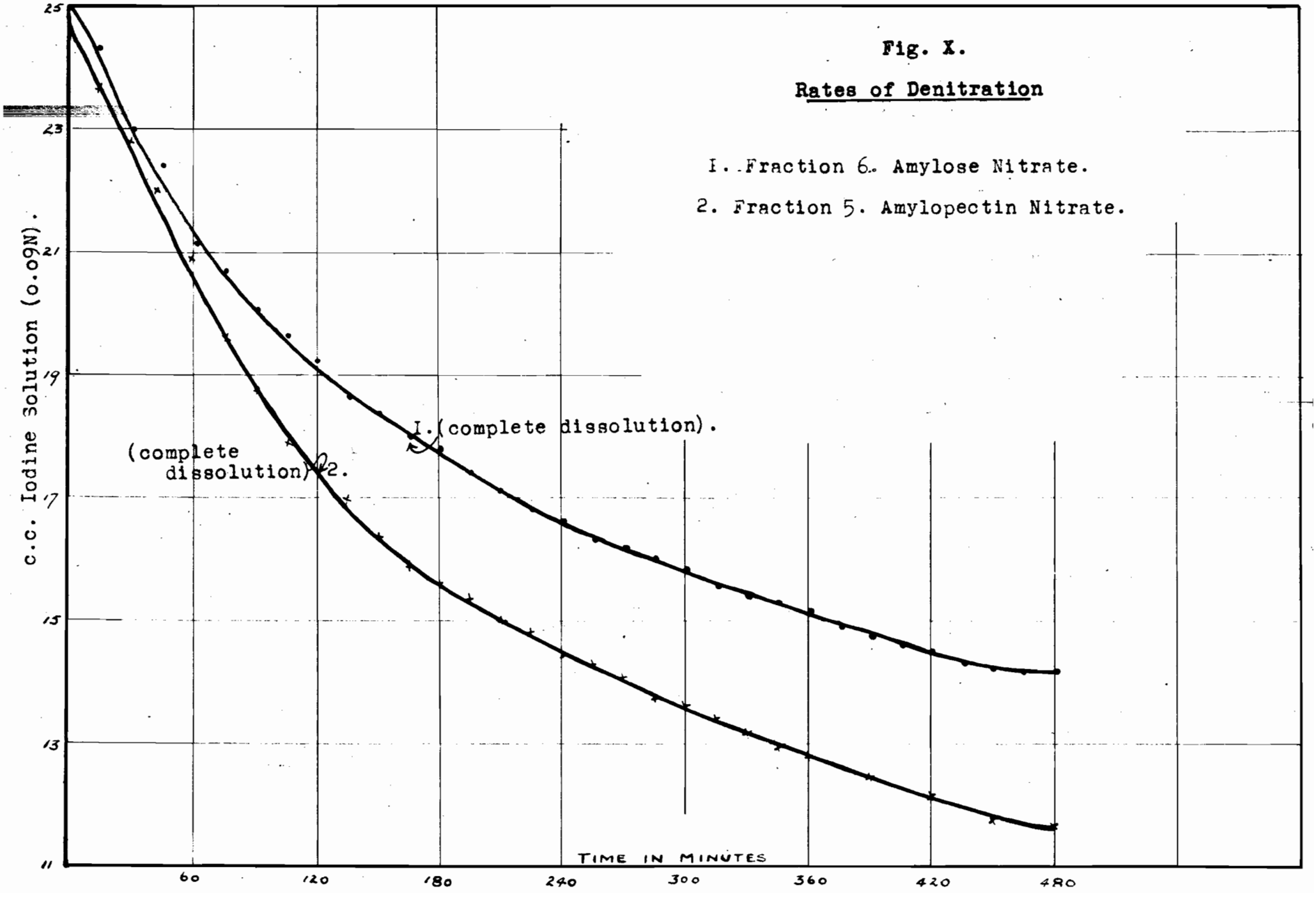


Fig. X.
Rates of Denitration
1. Fraction 6. Amylose Nitrate.
2. Fraction 5. Amylopectin Nitrate.
c.c. Iodine Solution (0.09N).
25
23
21
19
17
15
13
11
1. (complete dissolution).
(complete dissolution) 2.
TIME IN MINUTES
60
120
180
240
300
360
420
480

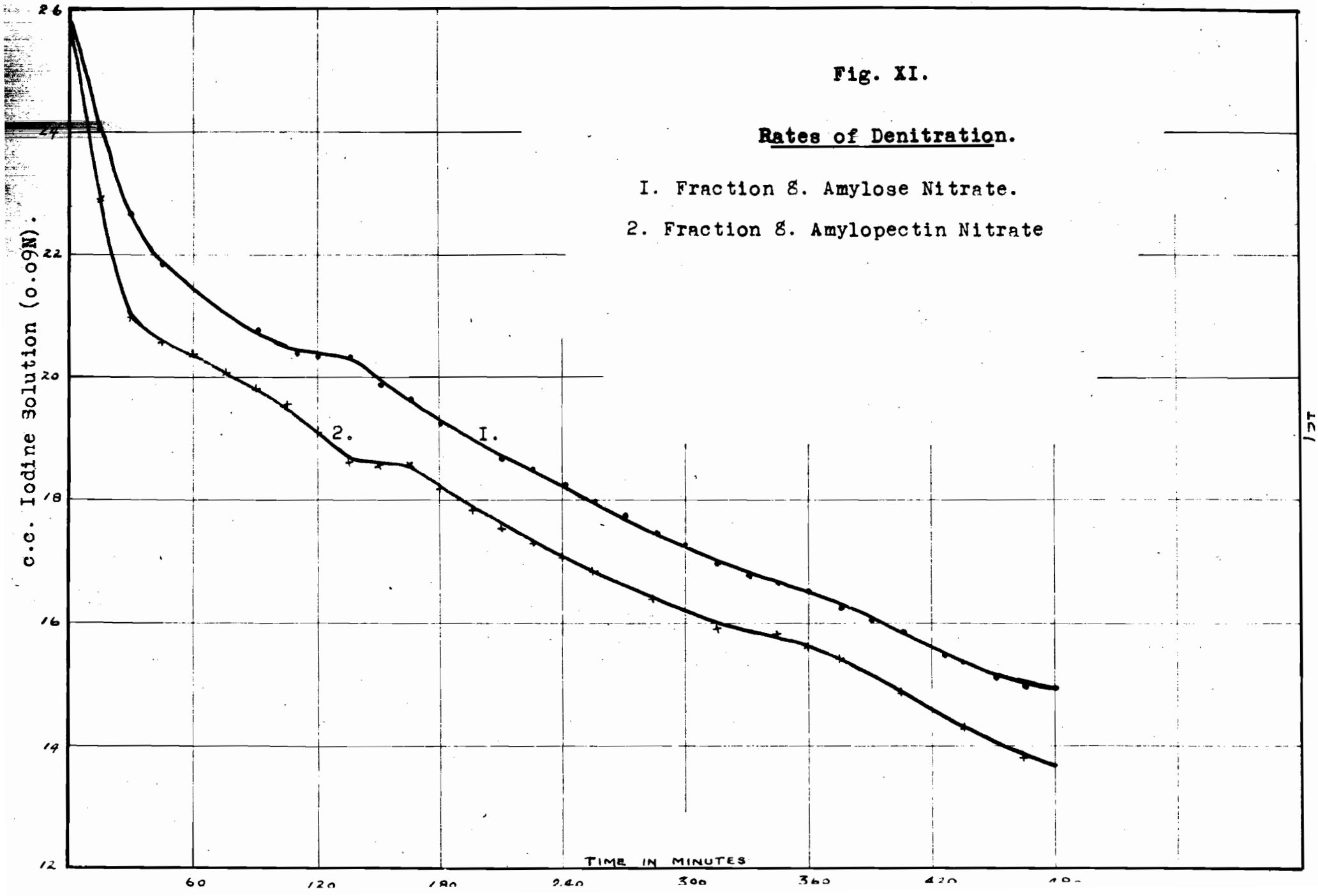


Fig. XI.
Rates of Denitration.
I. Fraction 8. Amylose Nitrate.
2. Fraction 8. Amylopectin Nitrate
c.c. Iodine Solution (0.09N).
26
24
22
20
18
16
14
12
2.
I.
TIME IN MINUTES
60
120
180
240
300
360
420

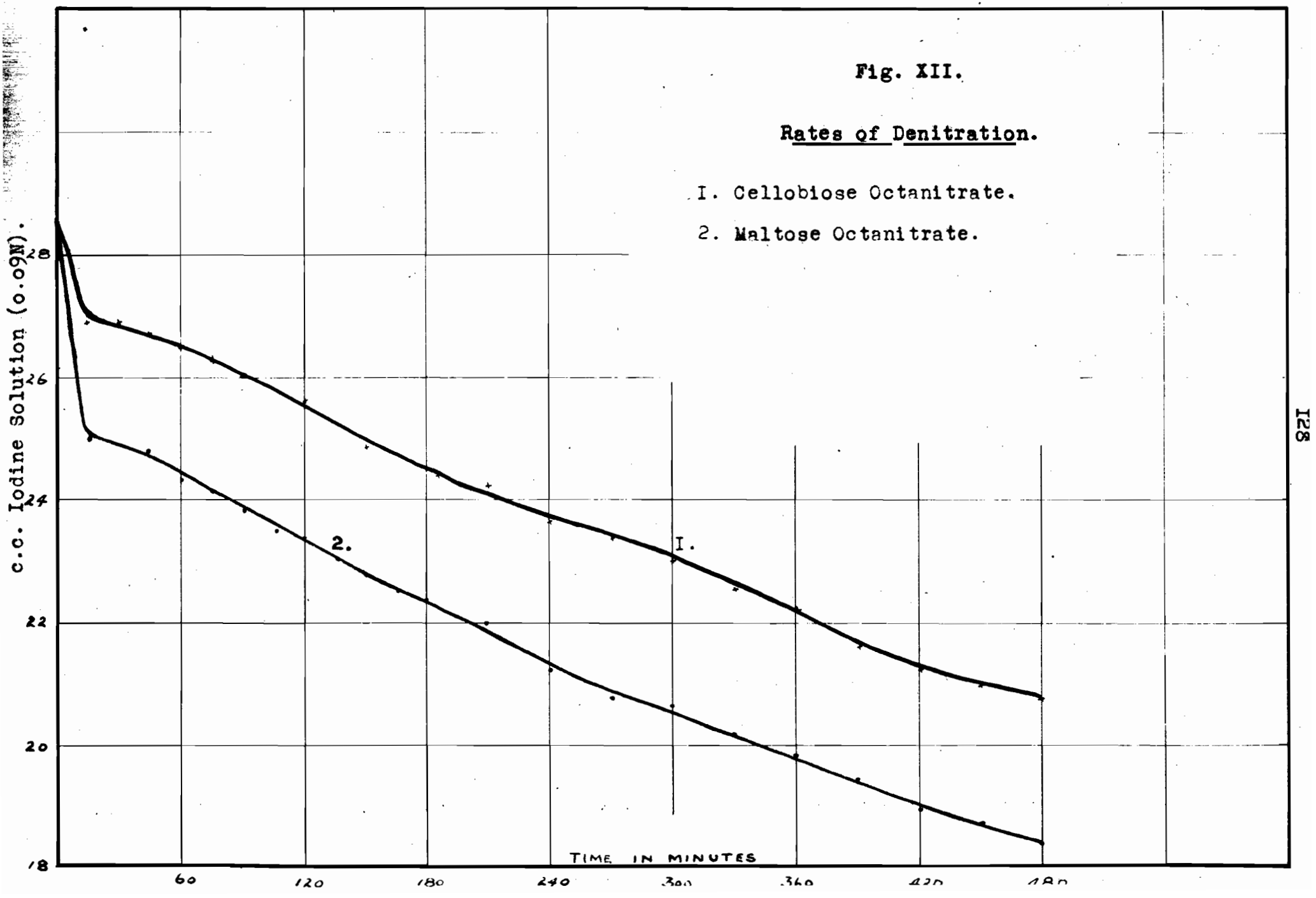


Fig. XII.
Rates of Denitration.
I. Cellobiose Octanitrate.
2. Maltose Octanitrate.
c.c. Iodine Solution (0.09N).
28
26
24
22
20
18
I.
2.
60
120
180
240
300
360
420
480
TIME IN MINUTES

Experimental Methods and Results

I. The Nitration Technique Employed

A. Preparation of Nitric Acid

Colorless nitric acid (98.6%) was prepared as required by distillation (60-70°, 20-30 mm.) from a 1:1 mixture of nitric (1.42 sp. gr., or commercial fuming nitric) and concentrated sulphuric acids (1.84 sp. gr.) in an all-ground-glass set-up.

B. Preparation of the Sample

An adequate supply of the best quality corn starch was generously provided by the Canada Starch Co. of Montreal and this was used throughout these experiments.

The starch to be nitrated was ground in a mortar, dried for three hours in a current of warm air at a temperature of 100° and stored in a vacuum desiccator. Starch dried in this manner contained 5-8% moisture.

C. Nitration Procedure

Nitrations were carried out according to the Will and Lenz method (156) which involves the use of concentrated nitric acid as the nitrating agent and concentrated sulfuric acid as the precipitant. A typical example of the procedure is as follows: Concentrated nitric acid (sp. gr. 1.5, 1200 cc.) in a three litre, three-necked flask, fitted with a stirring motor, was placed in a brine bath at a temperature of -7°C. Corn starch (120 g.), suspended in chloroform or other inert liquid, was added to the nitric acid solution from a separatory funnel over a period of

forty-five minutes during which time the mixture was stirred vigorously, and the temperature maintained at -7°. Nitration was completed by continuing the stirring in nitric acid for a period of six hours at the same temperature and then adding concentrated sulfuric acid (600 cc.) over a period of one and one-half hours. The nitrated starch was isolated by pouring the nitration mixture on to vigorously-stirred finely-chopped ice and the product separated by filtration. It was washed on the filter with large quantities of cold distilled water until the filtrate was no longer acid to litmus. The white, finely-granular product was placed in five litres of distilled water in a three-necked flask fitted with a reflux condenser, and boiled for at least one hundred fifty hours using two changes of water, one after fifty and the second after one hundred hours, respectively. At the end of this period the product was filtered and an aliquot portion cautiously dried and weighed. (Yield, 204 g. or 170%*) (Table I p.141)

This nitration technique was modified slightly in the case of isolated amylose and amylopectin respectively by decreasing the length of time of the reaction from six to two hours, all other conditions being the same. In all other cases, the above procedure was strictly followed.

* % yields are based upon the theoretical value for starch nitrate (182.7 g. from 100 g. starch)

II. Analytical Methods Employed for the Characterization of the Nitration Products

A. Determination of Nitrogen Content

Determinations of nitrogen content were made by two methods (a) The Bowman-Scott method (193) using certain modifications and (b) the Dupont nitrometer metnod (194).

The Bowman-Scott method was carried out as follows:

1. Preparation of Reagents

Standard nitric acid was prepared of such strength that one cc. of the solution contained approximately tne same quantity of nitrogen as a sample of starch nitrate weighing 0.05 g. and containing 12.5-13.0% nitrogen. Standard ferrous sulfate ($FeSO_4 . 7H_2O$) solution was prepared of such strength that about 5.0 cc. of the solution was equivalent to one cc. of the above standard nitric acid solution when used according to the conditions of the Bowman-Scott method. The nitric acid and ferrous sulfate solutions usually employed were 0.45 N and 0.25 M respectively. The strength of the ferrous sulfate solution was determined in terms of grams of nitrogen per cc., before each set of analyses, by titration against a standard nitric acid solution in the following manner: A one cc. sample of standard nitric acid was added slowly and with cooling to 25 cc. concentrated sulfuric acid. The samples were then placed in an ice-bath and, in turn, titrated to a permanent end point (colorless to pinkish-brown) with standard ferrous sulfate solution. The equivalence of one

cc. of ferrous sulfate solution in grams of nitrogen is given by the expression.

$$\frac{1}{1000} \times \frac{\text{Normality } HNO_3 \times 14}{\text{cc's } FeSO_4 \text{ used}}$$

2. Determination of nitrogen in Starch Nitrates (Bowman-Scott method)

The samples were dried at 63° for a period of three hours in an Abderhalden. Duplicate samples of about 0.05 g. were weighed out and dissolved in 25 cc. of concentrated sulfuric acid at room temperature. The samples were then titrated as described above in the standardization of the ferrous sulfate (p. 131).

Determinations of nitrogen with the Dupont Nitrometer were carried out according to the usual procedure (194).

B. Determination of Viscosities

1. Solvents employed

Pure acetone was generally employed as a solvent for all starch nitrates. The solvent was prepared in a dry and dust-free state by distilling from potassium permanganate, drying over solid potassium carbonate and redistilling in an all-ground glass apparatus. Ethylene-glycol monoethyl ether was employed as solvent for certain starch nitrates. This solvent was purified by distillation of the stock material at atmospheric pressure in an all-glass apparatus.

Hydrazine hydrate was used as a solvent to determine the relative viscosities of starch, amylose and amylopectin. This was purified by distillation of the stock solution at atmospheric pressure.

2. Method employed

The relative viscosities were determined by the capillary flow method using an Ostwald Viscometer in a thermostatically-controlled constant temperature bath (temperature control of $\pm$ 0.05°C).

Relative viscosities (ηr) were calculated from the expression

$$\frac{\text{time of flow for solution}}{\text{time of flow for solvent}} \times \frac{\text{density (solution)}}{\text{density (solvent)}}$$

Viscosities of starch nitrate solutions are expressed as Specific Viscosities (ηsp) and as Reduced Viscosities ($\eta reduced = \eta sp/conc.$) where $\eta sp = \eta r - 1$ and $\eta reduced = \eta sp/conc.$, with the concentration (conc.) expressed as "basic mols per litre". (A "basic molecular weight" in the case of starch nitrate is 296 g.).

C. Determination of Stabilities

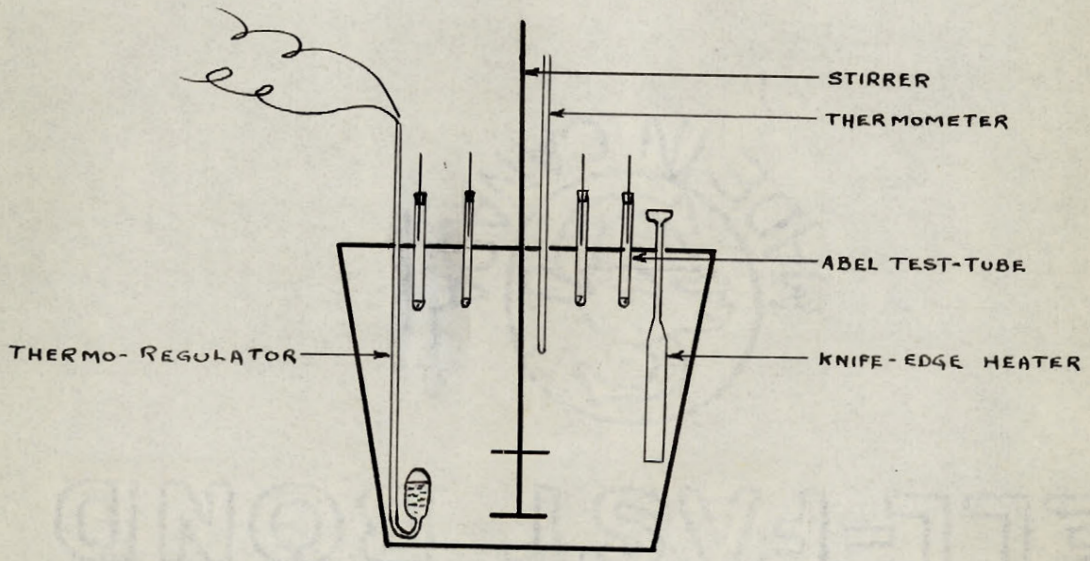
The stabilities of the starch nitrate samples were determined by two methods, namely the Abel Heat test and the Bergmann-Junk test (162).

1. The Abel Heat Test

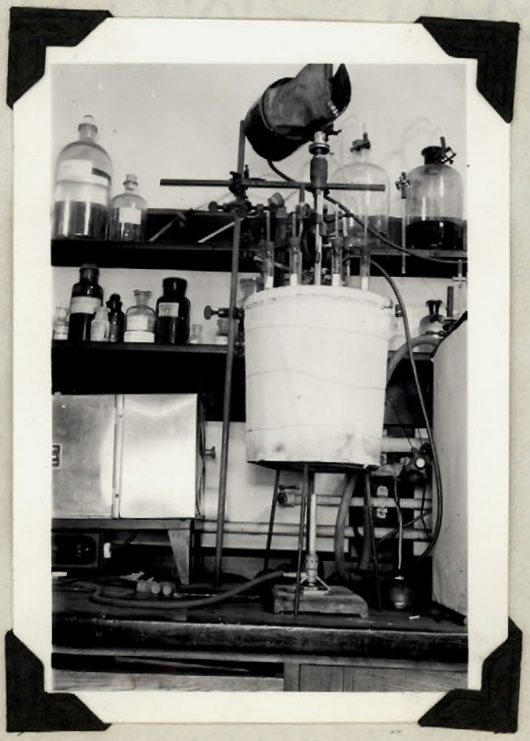
The Abel Heat test was modified in some details for the purposes of this investigation, but the essential features were not changed. The most important modification was in the type of bath employed to maintain the samples at the required temperature of 76.7°C (170°F). The standard Abel Heat bath usually employed is not thermostatically-controlled and is difficult to maintain at the required temperature. The bath used (Fig. XIIIp 134) consisted of a metal container about twelve inches deep and ten

inches in diameter with a tightly-fitting, removable lid. The outside surface of this vessel was surrounded by several layers of asbestos. Through separate orifices in the lid there were fitted a strong mechanical stirrer, a thermo-regulator and a knife-edge heater. These fixtures were held in place by tightly-

Fig. XIII



Heating Bath for Abel Test



fitting rubber stoppers so that the vessel, when sealed, was steam-tight. In addition, the lid was provided with six openings each fitted with a one-holed rubber stopper of such size as to accommodate the standard Abel test tube. The water in the bath was raised to a temperature of about 75°C by means of a Bunsen burner and then increased to the required 76.7°C by means of the knife-edge heater, and maintained at this value by means of the thermo-regulator which was of the usual type (filled with mercury and carbon tetrachloride).

Tests were carried out in duplicate using 0.5 g. samples. These were first dried at 63° in an Abderhalden for one hour, then allowed to stand in an open dish at room temperature at a relative humidity of 55-65%. This insured a moisture content of about 1%. The starch-iodide test papers were standardized by testing a known sample of cellulose nitrate. The starch-iodide test paper was held in position in the Abel test-tube by means of a hooked glass rod inserted through a rubber stopper. After use, these glass hooks and rubber stoppers were boiled for a few minutes in a dilute solution of sodium hydroxide (0.5%) and then in distilled water. They were then dried at 50° on a piece of clean filter paper. The remainder of the procedure was that officially specified for the Abel test (162).

2. The Bergmann-Junk Test

The nitrated starches were tested for stability by means of the Bergmann-Junk test using 0.5 g. samples instead of the 2.0 g. samples prescribed in the official test. Cellulose nitrate samples

(0.5 g.) were used as a standard. These Bergmann-Junk tests were carried out by the Department of Mines and Resources, Explosives Division, Ottawa, Canada, under the direction of Mr. M.C. Fletcher.

D. The C.I.L. Explosion Test

The C.I.L. explosion test is designed to measure the temperature at which a substance explodes under the influence of heat, and is therefore a measure of the heat-stability of the substance. To carry out the test an oil-bath fitted with a mechanical stirrer was used. The bath was heated by means of a gas burner so that, from a temperature of 100°, the temperature increased at a uniform rate of about 2° per minute. A loosely-fitting cover was provided with openings through which a four-inch Pyrex test tube was fitted, the lower portion of the test-tube being immersed in the oil-bath to a depth of one inch.

The procedure was as follows: Duplicate samples of 0.03 g. were weighed out and transferred to four-inch Pyrex test tubes. These were closed with loosely-fitting corks and transferred to the oil-bath. The temperature at which brown fumes appeared was noted and also that at which the corks were blown from the test-tubes. Duplicate samples should agree within 2°.

E. Miscellaneous Tests

In addition to the tests enumerated above, the following tests, used to characterize explosives, were kindly carried out on certain starch nitrates for the writer by the Department of

Mines and Resources, Explosives Division, Ottawa, Canada

1. Ignition temperature test
2. Machine Impact test
3. Trauzl Lead Block test

III. Nitration Experiments Involving various Pretreatments of the Starch

In the early part of this investigation, various miscellaneous experiments were carried out on the nitration of starches which had previously been subjected to the action of various chemical reagents, which pretreatment, it was hoped, might increase the ease of nitration. These experiments are described in the following section.

A. Starch Swollen with Pyridine

Corn starch (30 g.) was added to one litre of cold distilled water with stirring, the mixture heated to the boiling-point, and maintained there for thirty minutes. Pyridine (300 cc.) was added and the mixture distilled, until most of the pyridine-water azeotrope had been removed. The starch was precipitated from the paste by means of ethanol, and recovered by centrifuging. The precipitated starch was washed with ethanol and ether and dried in a vacuum desiccator.

The pyridine-treated corn starch was now nitrated according to the method previously described (p. 129). The product was stabilized as usual by boiling with distilled water and examined

for nitrogen content and stability. The results are given in Table I, (p. 141)*.

B. Nitration of Starch in Formic Acid

Dried corn starch (10 g.) was dissolved in 95% formic acid (180 cc.). The starch was added in small amounts to minimize formation of lumps. The solution was very viscous at first, but became less so on standing for about one-half hour. The starch-formic acid solution was nitrated by the usual procedure (p.129). The nitrated product, on precipitation into ice-water, was a white flocculent powder. It differed from other nitrated starches in that it formed a gummy mass when placed in hot water. This gum reverted to its original flocculent powdery form, however, when it was again placed in cold water. Accordingly, the product was washed with cold distilled water and dried at 55° (Table I , p.141).

C. Nitration with Mixed Acids

The same procedure was followed when mixed acids, instead of nitric acid alone, were used for nitration. The nitrating mixture analyzed as follows: nitric acid, 76.2%; sulfuric acid, 22.4%; water, 1.4%, (Table I, p.141).

D. Effect of Renitration

The sample chosen for renitration contained 12.55% nitrogen and had been prepared by the mixed acid method. The nitrate (5 g.)

* All products described in this section were examined for their nitrogen content and stability.

was dissolved in acetone (50 cc.) and added to conc. nitric acid (sp. gr. 1.5, 35 cc.) with vigorous stirring, the temperature being maintained at -7° by means of a brine bath. Nitration in nitric acid alone was allowed to proceed for one hour. Sulfuric acid (96.5%, 35 cc.) was then added slowly to the mixture with vigorous stirring. The precipitated product was poured on to finely-chopped ice, filtered, and washed with hot distilled water until free of excess nitrating acids, and finally subjected to two stabilizing water boils, each of fifty hours' duration. (Table I p. 141).

E. Nitration of Starch Swollen According to the Haworth Method

Starch was prepared for nitration, according to the method of Haworth (39). Corn starch (30 g.) was added to cold distilled water (500 cc.) with stirring and the mixture heated to the boiling-point and maintained there for thirty minutes with continued stirring. The starch was precipitated from its paste by the addition of ethanol (250 cc.) to the cold solution. The ethanolic solution was decanted, as far as possible, and the remainder separated by centrifuging. The precipitated starch was washed with ethanol and ether and dried in a vacuum desiccator.

This swollen product was nitrated with nitric acid according to the procedure previously described (p. 129), the time of nitration being reduced in this case to one and one half hours. It was noted that, in this case, the starch dissolved in the nitric acid, whereas this did not occur in previous experiments with un-

treated starch (Table I, p. 141).

F. Nitration of Amylose and of Amylopectin

Purified amylose and amylopectin prepared from corn starch by the adsorption method (p. 140) were nitrated respectively in the manner previously described for whole starch (p. 129) except that the nitration period was reduced to two hours. Both starch fractions were added to the nitration mixture in the dry state and each dissolved almost immediately (Table I, p. 141).

IV. The Denitration of Nitrated Starches

In order to characterize further the various starch nitrates, their fractionated products, and the nitrates of the isolated amylose and amylopectin, the following procedure was developed.

A. Development of the Denitration Technique

1. Reagent Employed

The reagent adopted was a solution of ammonium hydrosulfide (about 4.5%) containing a small amount of ethanol. Alkali hydrosulfides proved to be unsatisfactory. The reagent was prepared in the following manner: Ammonium hydroxide (5N, 450 cc.) was saturated at 0°C. with washed hydrogen sulfide gas. The cold saturated solution was filtered and to the saturated filtrate 50 cc. absolute ethanol added and the mixture diluted to 2500 cc. with water. This reagent was stored in rubber-stoppered flasks in a refrigerator until used. Its strength, in terms of cc. of iodine, was determined before each denitration.

TABLE I.

Nitration Experiments

Treatment received by starch	Yield (%) on nitration	Characteristics of Nitrated Product: Physical	%N	Stability (Abel)	Stability (B&J)*
1. Starch swollen with pyridine	153	faint yellow product, burns poorly, large residue	11.1%	0.5 min.	decomposed, 2-3 min.
2. Nitration of starch in formic acid	95	faint yellow powder, burns poorly large residue	4.5	15 min.	4.14
3. Mixed acid nitration	125	faint yellow product	12.6	2 min.	10.51
4. Renitration of a nitrate obtained by mixed acid nitration of starch at -7°	79	white product	12.0 (before) 12.4 (after)	15 min.	3.81
5. Starch swollen by Haworth method	154	white product	12.67	10 min.	8.97 (33min. in bath)
6. Starch fractionated into		white products:			
(a) amylose	167	fine powder	13.15	11 min.	11.17
(b) amylopectin	165	finely granular	13.3	6 min.	15.20
7. Crude dried corn starch	170	fine, white granular product	13.96	30 min. (EtOH-treated)	----

* Bergmann-Junk: mg. of N_2 evolved per 1.0g.sample of nitrate

2. Procedure

All denitrations were carried out at a constant temperature (35°) in the usual type of thermostatically-controlled water bath. One half gram samples were added to 50 cc. of the above denitration solution, and the mixture placed in a three-necked flask fitted with a motor-driven mercury-seal stirrer. The flask was kept tightly sealed except when the samples for titration were being withdrawn (every fifteen minutes). Stirring was stopped in each case one minute before removal of the sample to allow the starch nitrate to settle. These one cc. samples were withdrawn by means of a graduated one cc. pipette, a piece of filter paper being fastened over the end of the pipette by means of a cotton thread to prevent the removal of any starch nitrate. By following this sampling procedure no difficulty was experienced in obtaining reproducible results serving to show the course of the denitration process. The one cc. portions were run into 200 cc. cold distilled water and immediately titrated with standard iodine solution. Denitration curves were obtained by plotting cc. of standard iodine solution as ordinates against time in minutes.

B. Denitration Experiments and Reproducibility of the Curves

1. The Denitration Blank

A denitration blank was run on the reagent in order to determine any changes taking place at 35° during the usual period prescribed for the denitration experiments. The curve for this blank run is shown in Fig. I .

2. Action of the Denitrating Agent on Corn Starch

The action of the denitrating agent on unmodified corn starch was determined under the conditions of denitration described above. It was found that over a period of eight hours the consumption of the reagent by the starch was negligible.

3. Comparison of Starch and Cellulose

Nitrates of starch and cellulose were denitrated under similar conditions. It was found that the rate for starch was greater than that for cellulose. The two curves are shown in Fig. II .

4. Reproducibility of the Denitration Curves

When the conditions specified above (p. 142) for denitration were followed, an excellent reproducibility was obtainable as shown for a precipitated fraction of starch nitrate in Fig. V . Denitration curves for various other fractions of starch nitrate are given in the appropriate sections.

C. Recovery of the Products of Denitration

In order to study completely the results of denitration of the starch fractions, it was necessary to separate and to purify the denitrated products. The following procedure was developed, the results indicating that it was satisfactory (Fig. XIV).

The denitration mixture was removed from the bath and placed in a brine bath (-7°C°) for a few minutes. This caused the un-

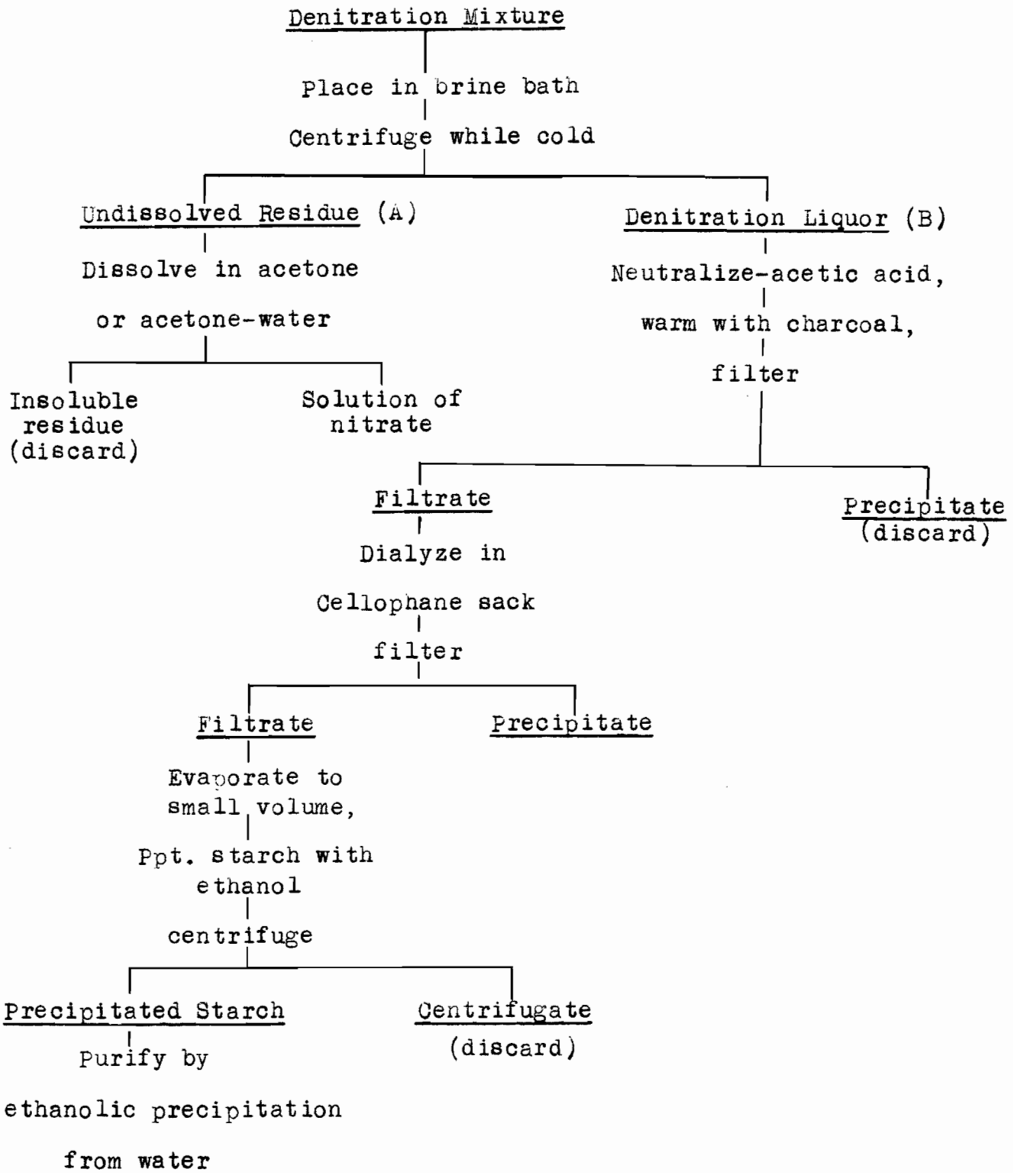
dissolved material to settle out and also inhibited further denitration. The cold supernatant liquor was removed, and the residue separated from the remaining denitration liquor by centrifuging while still cold. The undissolved material was thus recovered practically free from sulfur. The supernatant liquor recovered after centrifuging was combined with the main portion of the denitration liquor.

Colloidal and difficultly-filterable sulfur were removed by neutralization of the denitration liquor with acetic acid, and warming with charcoal for a few minutes. The filtrate was dialyzed in a Cellophane sack at a temperature of 40°. Dialysis for about ten hours was sufficient to remove inorganic ions. The dialysate was filtered and concentrated in vacuo to a small volume, from which the soluble starch precipitated on the addition of two volumes of ethanol. It was purified by two re-precipitations from water solution by means of ethanol.

The undissolved residue (A,Fig. XIV) was freed from admixed sulphur by dissolving in acetone or an acetone-water mixture.

Fig. XIV.

Flow Sheet: Recovery of the Denitration Products



V. Separation of Amylose and Amylopectin from Corn Starch by the Adsorption of Amylose on Cotton Cellulose

To study the component parts of starch, a fractionation of a sample of corn starch was carried out by the method which consists in adsorbing the amylose on purified cotton cellulose, (139) (140) leaving the amylopectin in solution.

The fractionation was carried out in the following manner: (Fig. XV) Corn starch (100 g.) was added to five litres of distilled water and the mixture boiled for two hours to insure complete rupture of the granules. Five pounds of pure absorbent cotton were introduced into the cotton solution and allowed to stand for one and one-half hours at room temperature. The paste-soaked cotton was then placed in a large earthenware filter and the liquid remaining from the five litres of starch solution removed by suction filtration. The filtrate (Liquid A) was reserved for future work. The cotton was washed with five, four-litre portions of cold distilled water, and the wash waters added to the original filtrate. The cotton was washed with further portions of cold distilled water until the filtrate gave no color with iodine, indicating absence of amylopectin. The cotton was then subjected to live steam while still on the filter, and suction applied. The hot moist cotton while still hot was treated repeatedly with boiling water, using a total of about 15 litres, in order to remove the adsorbed amylose. The filtrate (Liquid B)

gave a brignt blue color witn iodine. The absorbent cotton can be used repeatedly.

Treatment of Liquid "B" (Amylose fraction)

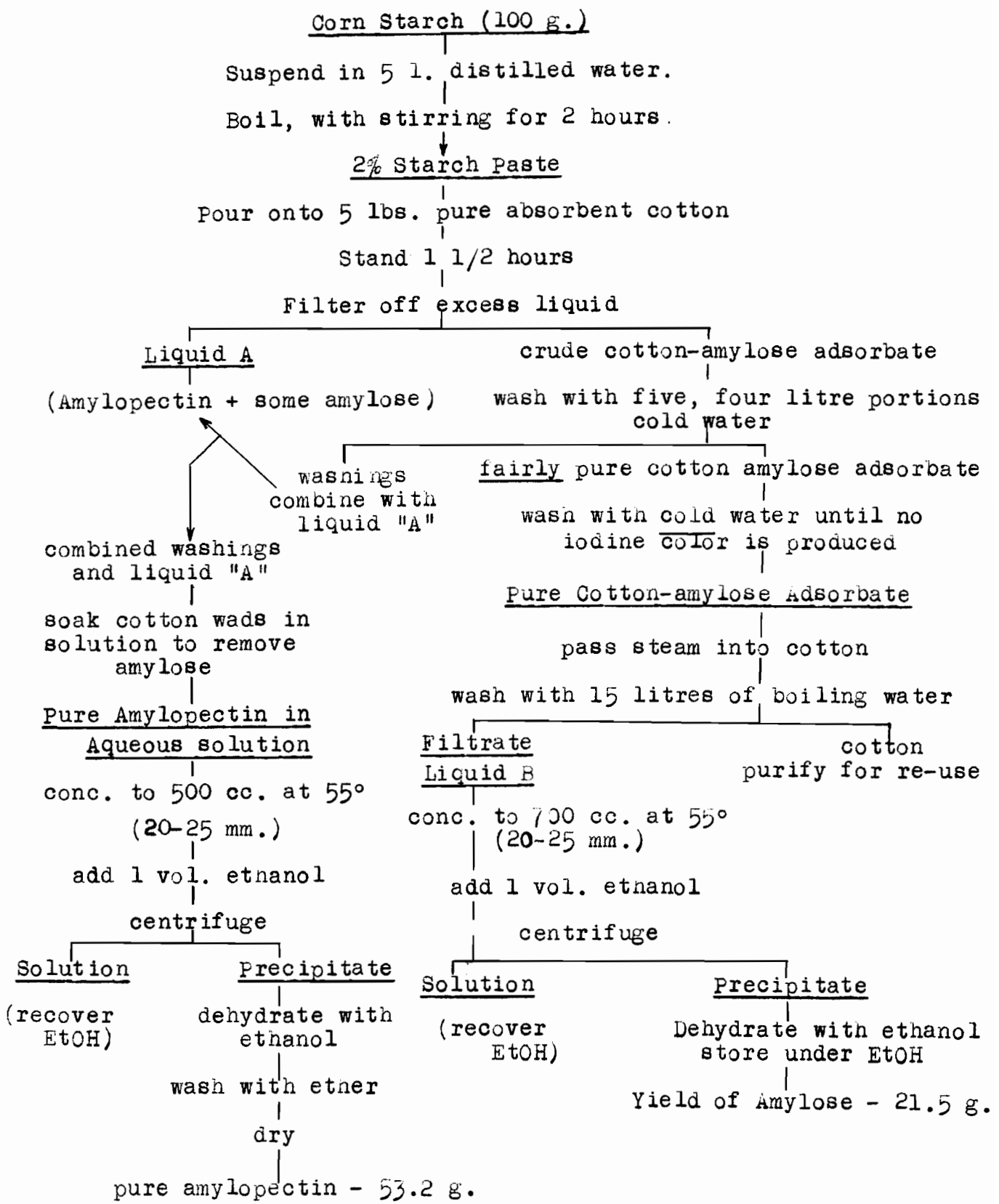
As amylose retrogrades easily, care must be taken to concentrate tne amylose solution as rapidly as possible. It was, therefore, concentrated to about 700-800 cc. at a bath temperature not exceeding 55°, and the amylose precipitated quantitatively by the addition of an equal volume of etnanol. The product was separated by centrifuging as a fine, white precipitate and was readily dehydrated by the use of 80% etnanol followed by a further centrifuging and by grinding under absolute ethanol. The final product, a white powder, was stored in ethanol to prevent drying and consequent retrogradation. Yield of dry product (calculated by drying an aliquot portion) was 21.5 g. or 21.5% of the starch granule.

Treatment of the Amylopectin fraction (A)

Liquid "A" was freed from amylose by soaking wads of cotton in the solution until such time as a cotton wad after wringing out and washing thoroughly with cold water gave no iodine color on hot water elution. The twenty litres of solution were then concentrated at 50-55° (20-25 mm.) to 500 cc. The addition of an equal volume of ethanol caused precipitation of a flocculent, sticky mass of amylopectin. The product was centrifuged and dehydrated with ethanol, washed with ether and dried in a desiccator. Yield: 53.2 g.

Fig. XV .

Fractionation of Starch by the Adsorption Process



VI. Fractionation Methods Applied to Starch Nitrates

The following methods of fractionation were applied to starch nitrate in order to obtain more homogeneous products characterizable with a greater degree of exactitude.

A. Fractional Dissolution

1. Fractionation of Whole Starch Nitrate

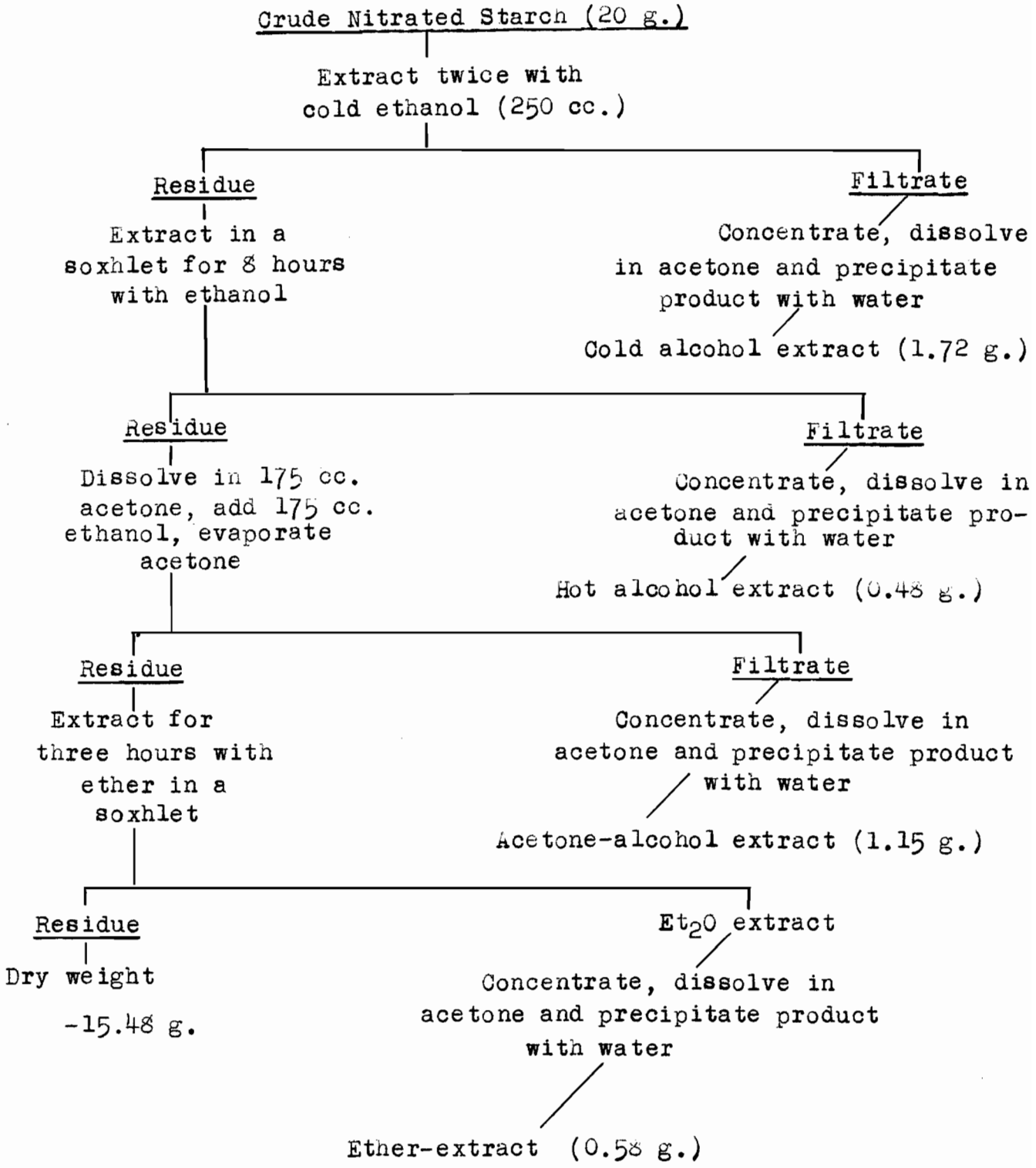
Ethanol (95-98%) was used as the solvent in fractional dissolution studies. The nitrated starch was dried (at 55°) prior to treatment with the ethanol, as the presence of more than 5% water greatly reduces the solubility of nitrated starch in ethanol. Ethanolic extraction was carried out by suspending the starch nitrate (20 g.) in ethanol (250 cc.) and stirring the mixture vigorously at room temperature for three hours, the process being twice repeated. In most cases, it was necessary to grind the nitrate in ethanol in a mortar in order to remove lumps. The undissolved residue was separated by filtration.

The ethanolic extract was concentrated to small volume (50°, 20-25 mm.) until the dissolved nitrate precipitated, and this was completed by the addition of petroleum ether. The nitrate was separated by filtration and petroleum ether removed by drying at 50°. The product was then boiled with water (2 litres) under reflux for fifteen to twenty hours to insure removal of the ethanol. The insoluble residue was treated in a similar manner. About 8-10% of the total nitrate (Fig. XVII) was removed in this way.

The extraction of starch nitrates with cold ethanol was extended in certain cases to include furtner extractions as recommended by Will (156). The procedure was as follows: Nitrated starch (20 g.) was extracted by stirring at room temperature with two portions of ethanol (250 cc.) (98%) for periods of three hours. The undissolved residue was removed by centrifuging and then extracted for a period of eight hours in a Soxhlet, using ethanol as the solvent. The residue left after this treatment was dissolved in acetone and an equal volume of ethanol (98%) added to the mixture. When the acetone was removed under reduced pressure, the nitrated starch precipitated from the remaining ethanol. The solution and residue were separated by centrifugation. The dried product remaining from the acetone-ethanol treatment was then extracted for three hours with diethyl ether in a Soxhlet. The residue was placed in three litres of distilled water and boiled at reflux temperature for twenty hours to remove traces of solvent. A small aliquot portion was dried (55°) and the remainder left in the wet condition until needed.

The various ethanolic extracts obtained by cold ethanol extraction, hot ethanol extraction and by acetone-ethanol treatment were each concentrated at 50° (20-25 mm.) to small volume. Each precipitated nitrate was dissolved in a small quantity of acetone and precipitated by the addition of water. The products were washed by boiling with water at reflux temperature. The results of such a fractionation are shown in the flow sheet (Fig.XVI, p. 151).

Fig. XVI. Fractional Dissolution of Whole Starch Nitrate



2. Fractionation of Amylose and of Amylopectin Nitrates

Sixty grams of each nitrate were placed in a litre of ethanol (98-99%) and stirred for a period of ten hours at room temperature, and the residue separated by centrifuging and filtration. The ethanolic extract in each case was evaporated to small volume (30-35°, 20-25 mm.). In the case of the amylopectin extract, the nitrate was precipitated by the addition of petroleum ether (30-50°) and in the case of the amylose extract by the addition of water. The extracted nitrates were placed in two litres of distilled water and the solution boiled under a reflux condenser for twelve hours in order to replace the solvent. In similar manner, the residues left undissolved by ethanol were treated by boiling in water for twelve hours to remove ethanol. Results are shown in Table II, (p. 153).

B. Fractional Precipitation

This was effected by use of a 1:1 mixture of acetone and ethanol as solvent, water as precipitant and use of a temperature gradient to insure more rapid and definite precipitation. The same method was used for both starch nitrates and amylose and amylopectin nitrates. Starch nitrate (20 g.) was dissolved in acetone (170 cc.), an equal volume of ethanol (98%) added, and the mixture filtered to remove impurities. Distilled water was added from a burette to the solution with vigorous mechanical stirring until a slight turbidity developed, the solution being

TABLE II.

Fractional Dissolution of Amylose and Amylopectin Nitrates

(1) Amylose Nitrate

Product	Weight (g.)	% EtOH soluble
Amylose nitrate	60.0	
Residue		
EtOH-insoluble	46.0	$\frac{12.3}{60} \times 100 = 20.5\%$
Extract		
EtOH-soluble	12.3	

(2) Amylopectin Nitrate

Amylopectin nitrate	60.0	
Residue		
EtOH-insoluble	6.9	$\frac{52}{60} \times 100 = 86.7\%$
Extract		
EtOH-soluble	52.0	

placed in a brine bath (-7°). After fifteen to thirty minutes, a precipitate settled out as a sticky mass. (This stickiness was due to the fact that the nitrate molecules were solvated with acetone and ethanol.) The greater part of the supernatant liquor was poured off and the precipitated fraction obtained by centrifuging while cold as a clear, sticky mass.

The fraction was transferred to a shallow, tared crystallizing dish and concentrated at 40-50° to a thick consistency. Petroleum ether (30-50), was added to precipitate the nitrate, which was filtered, dried and subsequently extracted with petroleum ether to remove acetone and ethanol.

A similar procedure was used for each fraction. The final product remaining in solution after addition of about three volumes of distilled water was recovered by concentrating (40-50°, 20-25 mm.) the acetone-ethanol-water solution to small volume and then proceeding as above. Table III gives the results with crude starch nitrate, amylose and amylopectin nitrates.

TABLE III.

Fractional Precipitation of Crude Starch Nitrate

Amylose Nitrate and Amylopectin Nitrate

Starch Nitrate		Amylose Nitrate		Amylopectin Nitrate	
Fraction	Wt. (g.)	Fraction	Wt.(g.)	Fraction	Wt.(g.)
crude product	20	crude product	60	crude product	60
1	3.95	1	4.45	1	2.26
2	6.91	2	12.5	2	4.07
3	4.10	3	4.45	3	6.40
4	1.17	4	5.28	4	9.48
5	1.62	5	9.25	5	12.53
6	1.00	6	6.65	6	1.67
7	0.89	7	3.73	7	9.99
Total	19.64	8	2.51	8	0.95
Recovery	98.1%	Total	48.82	Total	47.35
		Recovery	97.6%	Recovery	94.7%

VII. Characteristics of Fractionated Products

A. Starch Nitrates obtained by Fractional Dissolution

The relationship of the nitrogen contents to the viscosities of the various fractions of a starch nitrate obtained by fractional dissolution with ethanol is shown in Table IV and the Abel stabilities of a similar series of products obtained as shown in Fig. XVI (p. 151) are given in Table V.

TABLE IV.

Characteristics of Products obtained from Whole Starch Nitrate by Fractional Dissolution

Fraction (see Fig II)	%N Content	η_{sp} Specific viscosity	Abel Stability
Exp. No. I			
Cold ethanol extract	9.1	0.1910	—
Hot ethanol extract	8.81	0.5777	—
Acetone-ethanol fraction	10.55	0.8129	—
Residual product	13.43		55 min.
Ignition point - 183° Brown fumes - 165°			
Exp. No. 2			
Cold ethanol extract	8.99	0.0357	—
Acetone-ethanol extract	10.63	0.4050	—
Residual product	13.96	0.509	30 min.
Ignition point - 185° Brown fumes - 173°			

The unstable fraction of starch nitrate obtained by a cold ethanol extraction (p. 149) amounted to about 10% of the total product (Table V).

This ethanol-soluble, unstable product (5.7 g.) was denitrated by the method previously described (Fig. VI p. I22). The products

of denitration including the soluble recovered starch and the incompletely denitrated, undissolved residue were isolated by the method described earlier (p. 143). The origin of these various products is shown in Fig.XVII on which are inserted nitrogen contents, specific viscosities and Abel heat values.

TABLE V.

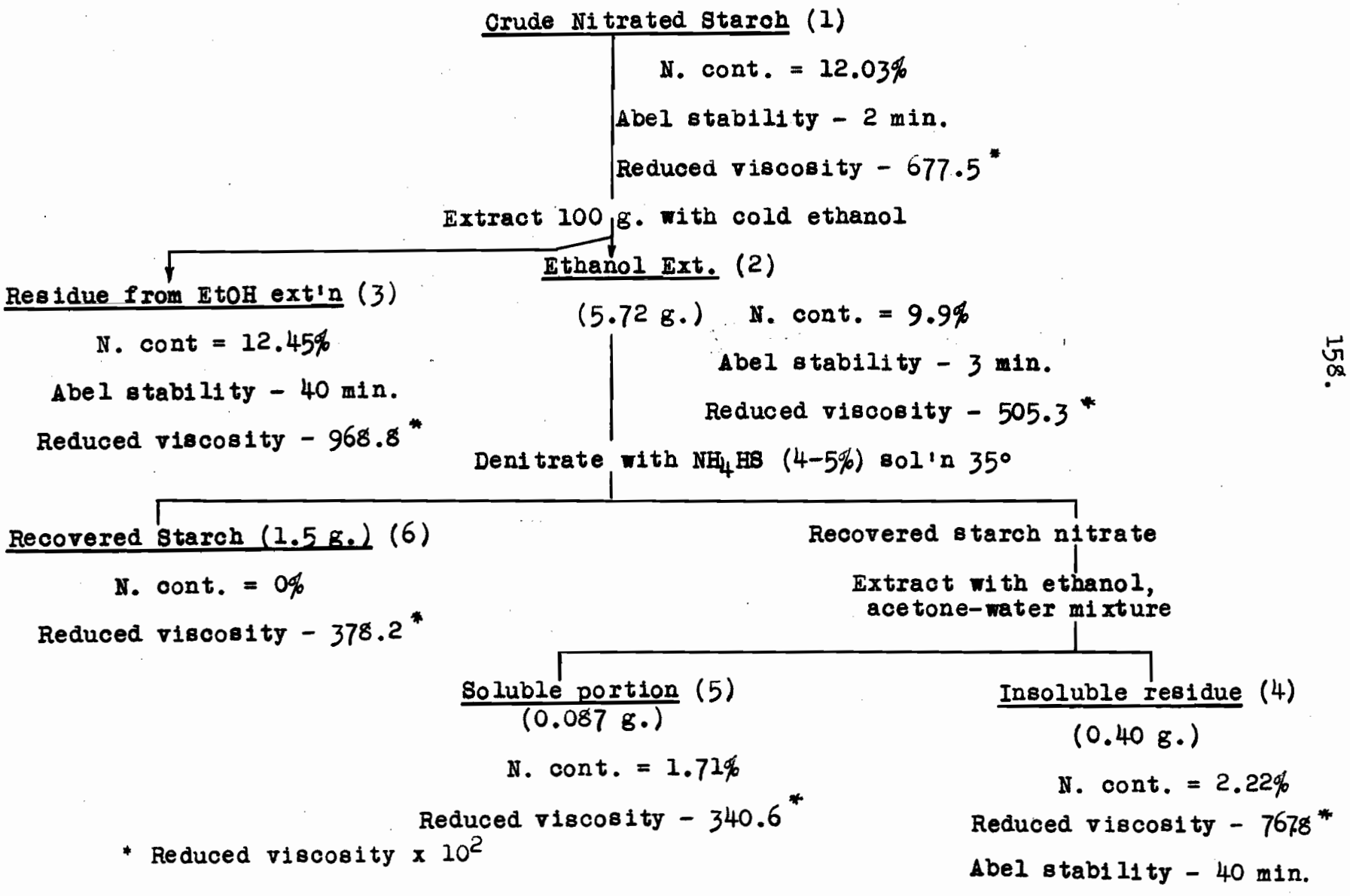
Stabilities of Products from Fractional Dissolution of Starch Nitrate

Fraction	Abel Stability (min.)
Unfractionated	3
1. Cold ethanol extract	3
2. Hot ethanol extract	7
3. Acetone-ethanol extract	25
4. Ether extract	20
5. Residual product	40

The "residual products" resulting from the ethanolic extraction (Table IV) were examined for certain explosive characteristics by the Explosives Division of the Department of Mines and Resources, Ottawa, and the results are shown in Table VI.

Fig. XVII

Ethanol-soluble Fraction from Starch Nitrate and Denitration Products



* Reduced viscosity x 10^2

TABLE VI.

Explosive Characteristics of EtOH-insoluble Starch Nitrate

Moisture	Ignition temperature		Machine Impact test: Hammer drop	Hammer drop Sensitivity		%N	Abel test
%	Ignites °C	Brown fumes °C	Cms	% TNT = 100%	Times TNT TNT = 1.0	Nitrometer	min.
Residual Product							
Exp. No. 1 (Table IV)							
0.68	183° with flame	167°	44	27.5	3.64	13.43	55
Residual Product							
Exp. No. 2 (Table IV)							
0.55	185° with flame	173°	58	36.2	2.76	13.96	30
Trauzl lead block test (50% of each product)							
Net expansion 358 cc.							
Net expansion, TNT standard, 256 cc.							

B. **Fractional Dissolution of Amylose and Amylopectin Nitrates**

In Table II above were shown the results of ethanol extraction of amylose and amylopectin nitrates. These products were characterized by nitrogen contents, Abel stabilities and stabilities as measured by the Bergmann-Junk test (Table VII).

TABLE VII.

Characteristics of Amylose and Amylopectin Nitrates

Product	% of Crude product	% N	Abel heat value	Bergmann-Junk* - Mg. N_2 -
Crude amylose nitrate	100	12.96	10	7.28
EtOH-insol. amylose nitrate	79.5	13.28	40	2.62
EtOH-sol. amylose nitrate	20.5	11.62	55	1.90
Crude amylopectin nitrate	100	12.25	4	15.20
EtOH-insol. amylopectin nitrate	13.3	12.58	16	3.74
EtOH-sol. amylopectin nitrate	86.7	11.82	13	2.23

* Mg. of N_2 evolved per 1.0 g. sample of nitrate

C. Fractional Precipitation of whole Starch Nitrate

Starch nitrate was fractionated by precipitation from ethanol-acetone solution using water as the precipitant in the manner previously described (p. 152). The following Table VIII shows the nitrogen contents and reduced viscosities of the products of a typical fractionation.

TABLE VIII.

Nitrogen Contents and Viscosities of Fractionated Starch Nitrate

Fraction	% N content	Reduced viscosity $\eta sp/c \times 10^2$
1	12.6	906.9
2	12.3	878.5
3	12.8	898.1
4	12.5	868.8
5	11.8	900.1
6	11.95	844.3
7	10.50	632.2
8	9.35	384.2
9	5.83	95.0

Table IX shows the relative stabilities of a similar series of precipitated products (previously described , Table III).

TABLE IX.

Stability of Fractionally Precipitated Nitrates

Fraction	Abel value
unfractionated	3
1	5
2	5
3	5
4	15
5	6
6	16
7	3

Denitration curves are shown in Fig. III .

D. Fractional Precipitation of Amylose and Amylopectin Nitrates

The method used to fractionate amylose and amylopectin nitrates by precipitation from acetone-ethanol solution has already been described (p. 152) and the results given in Table III. These products were examined for their nitrogen contents, viscosities and stabilities. Certain of them were denitrated by the method described earlier (p. 142). Values for the former set of characteristics are given in Table X. Denitration curves are shown in Figs. VII-XI.

TABLE X.

Characteristics of Fractions of Amylose and Amylopectin Nitrates

Amylose Nitrate

Fraction	% N	($\eta_{reduced} \times 10$) Reduced Viscosity	Abel Value (min)	Bergmann-Junk (Mg N_2)*
Crude	12.78	63.1	3.5	11.17
1	12.45	163.6	14	—
2	12.49	113.3	13	2.24
3	12.61	51.3	19	1.46
4	12.76	60.9	28	—
5	13.28	51.2	20	2.03
6	11.31	65.5	21	1.22
7	9.60	42.6	20	0.65
8	7.78	12.8	11	unstable

Amylopectin Nitrate

Crude	13.31	10.09	3.5	15.20
1	11.2	20.3	2	—
2	13.18	13.1	7	2.53
3	12.9	9.89	8	2.60
4	13.6	11.75	8	2.94
5	13.31	9.45	15	2.36
6	13.02	7.90	9	—
7	12.59	6.99	7	2.73
8	7.76	6.95	3	unstable

* Mg. of N_2 evolved per 1.0 g. sample of nitrate

VIII. The Action of Alkali on the Nitrates of Starch

A. General Procedure

Certain starch nitrates were examined for their stability towards hot dilute alkali. This was measured as follows: A suspension of starch nitrate (0.5 g.) in standard sodium hydroxide solution (25 cc.) was refluxed for fifteen minutes with intermittent shaking of the flask. Cold distilled water (50 cc.) was then added through the condenser and the solution titrated at 15° with standard nitric acid using phenolphthalein as indicator.

Two concentrations of sodium hydroxide (0.3982 N and 0.1132 N) were used in different experiments.

Larger amounts of crude starch nitrate than those above were treated with sodium hydroxide solutions to determine the amount of nitrate consumed. The residual product from the alkali treatment was freed from alkali by boiling under reflux with distilled water for periods of fifty hours.

B. Miscellaneous Experiments on the Alkali Treatment of Starch Nitrates

1. Crude Starch Nitrate

Crude starch nitrate (5 g.) was treated with alkali as described above (p. 164). The residual starch nitrate was white in color, while the alkaline extract was deep reddish brown. The dried undigested residue weighed 2 g. Nitrogen content and stability are given in Table XI.

Crude starch nitrate was also treated with 0.5% alkali and the stability of the resulting product determined (Table XI).

2. Cellulose Nitrate

For purposes of comparison cellulose nitrate (0.5 g.) was treated with sodium hydroxide (0.3982 N) in the manner previously applied (p.164) to starch nitrate. The effect of 0.5% alkali on cellulose nitrate was also investigated (Table XI).

TABLE XI.

Treatment of Starch Nitrates with Alkali

Treatment	Effect on	
	Starch Nitrate	Cellulose Nitrate
5 g. refluxed for 30 min. with 1.6% NaOH	partial digestion, dissolving 60% of nitrate % N before - 12.83% % N after - 13.32% Abel value for residual product - 25 min. Bergmann-Junk* - 10.53 mg. N_2 Ash - 0.31%	0.5 g. sample, 25 cc. alkali, complete digestion to dark red solution cc. alkali consumed = 18.65
as above with 0.5% NaOH	partial digestion, dissolving 20% of product Bergmann-Junk*- 8.12 mg N_2	practically complete dissolution with formation of dark red liquid

* Mg. of N_2 evolved per 1.0 g. sample of nitrate.

3. Amylose, Amylopectin and their Derivatives

Amylose and amylopectin, their nitrates and various portions of their nitrates were treated with sodium hydroxide solution as described above (p. [redacted] T[redacted]lts are shown in Table XII.

TABLE XII.

Alkali Treatment of Amylose, Amylopectin and their Nitrates

Product	Treatment	Result
Amylose	250 mg. sample refluxed in 25 cc. NaOH (0.3982 N) for 15 min.	completely soluble in solution NaOH consumed—negligible
Amylopectin	as above	flocculent globules remained undissolved NaOH consumed—negligible
Unfractionated amylose nitrate	0.5 g. sample refluxed in 25 cc. NaOH (0.3982 N) for 15min.	clear, amber-colored filtrate cc. alkali consumed - 9.55 N cont. before - 13.15% N cont. after - 12.95%
Unfractionated amylopectin nitrate	as for amylose nitrate	clear, amber-colored filtrate cc. alkali consumed - 7.05 N cont. before - 13.25% N cont. after - 13.02%
Fractionated amylose nitrate product described in Tables III, X.		(No. of milli-equivalents of NaOH consumed per g. of nitrate)
Fraction 1	250 mg. samples refluxed with 25 cc. NaOH (0.1132 N) for one hour	10.8
2	"	6.04
3	"	15.15

(continued)

TABLE XII. (cont.)

Product	Treatment	Result
4	250 mg. samples refluxed with 25 cc. NaOH (0.1132 N) for one hour	7.72
5	"	12.09
6	"	50.8
7	"	71.5
8	"	61.1
Fractionated amylopectin nitrate (see Tables III, X.)		
Fraction 1	"	2.94
2	"	2.73
3	"	4.26
4	"	4.54
5	"	4.77
6	"	12.8
7	"	15.88
8	"	81.1
Cellulose nitrate	conditions as above	110.1

IX. The use of Alcohols as Stabilizing Agents for Starch Nitrates

(a) Ethanol

The use of ethanol as a stabilizer for starch nitrates was discussed in an earlier section (p. 114) with respect to previous experiments (Tables IV, V, VII, IX).

(b) n-Butanol and Cyclohexanol

Starch nitrate was extracted with each of these alcohols by the same procedure used with ethanol. Extractions were carried out at 50° for three hours. The residues were treated with petroleum ether to remove the solvent and examined for stability by the Bergmann-Junk test (Table XIII).

TABLE XIII.

Effect of "Stabilizers" on Starch Nitrates

Stabilizer	Bergmann-Junk Test*
EtOH	2.97 mg. N_2
n-Butanol	3.88 mg. N_2
Cyclohexanol	4.14 mg. N_2

* mg. of N_2 evolved by 1.0 g. sample of nitrate

X. A Study of Maltose and Cellobiose Nitrates

A. Preparation

The cellobiose used was prepared from cellobiose octaacetate by hydrolysis with sodium methylate in absolute methanol (200).

Cellobiose (13 g.) was nitrated, according to the procedure used for whole starch (p. 129), with nitric acid (95 cc. sp. g.1.5) and sulfuric acid (110 cc., 96.5%). The cellobiose dissolved immediately in the nitric acid and the nitration was allowed to proceed for one hour. Concentrated sulfuric acid precipitated the nitrate as a gummy mass, which was ground in a mortar with ice water to yield a pulverulent mass. The product was filtered and washed until acid-free.

Maltose (Kahlbaum) was nitrated in a manner identical with that used for cellobiose.

Both nitrates were white amorphous solids, insoluble in water. The crude products were unstable, especially the maltose preparation, which decomposed slowly at 50-55°.

The yields of crude products were: Maltose nitrate 183.2%, cellobiose nitrate 191.0% (theoretical, 205.3%).

B. Purification

1. Maltose Octanitrate

A methanolic solution of crude maltose octanitrate was treated with charcoal at 40-50°, then dried with anhydrous sodium sulfate. The removal of methanol under reduced pressure at room

temperature caused the nitrate to separate in crystalline form (needles in rosette formation) (m.p. 95°). The crystals were treated with diethyl ether to remove glucose pentanitrate, leaving an insoluble residue of maltose octanitrate (m.p. 163-4° with decomposition). The ether-soluble portion of the crude crystalline product was allowed to stand at room temperature, and, on evaporation of the solvent, deposited a good yield of crystalline maltose octanitrate (m.p. 163-4°). Reported value, 163-4° (156).

2. Cellobiose Octanitrate

The crude product was treated with methanol, charcoal and anhydrous sodium sulfate as was the maltose octanitrate. The first crop of crude crystals had an indefinite melting point of 85-95°. These crystals were recrystallized from ethanol and gave a product melting at 149-50°. Recrystallization from ether-methanol solution (2:1) raised the melting point to (154-5°) which did not change on subsequent recrystallizations.

C. Properties of the Nitrates

The nitrates were examined for their nitrogen contents, molecular weight, stability and their alkali lability with the results shown in Table XIV.

D. Denitration Studies

The nitrates of maltose and cellobiose were denitrated by the usual procedure (p. 142) with the results shown in Fig. XII.

TABLE XIV.

Properties of Maltose and Cellobiose Octanitrates

Property	Maltose octanitrate	Cellobiose octanitrate
Nitrogen content (%)	15.87 (theoretical - 15.95)	15.91
Molecular weight	699 (calculated - 702)	698
Abel test (min.)	18	32
Bergmann-Junk (mg. of N_2 per g.)	Both products too unstable to stand the test	
Alkali - lability*	7.97	13.82

* Milliequivalents of NaOH (.0567 N) consumed during 30 min. refluxing.

Summary and Conclusions

1. Corn starch has been separated into its individual components, amylose and amylopectin, by means of preferential adsorption on cellulose. (Tanret -- Pacsu method). Each of the three products (whole starch and its two constituents:—non-branched and branched chains respectively) have been nitrated by the use of concentrated nitric acid (98.6%). The nitrates from amylose and amylopectin possess markedly different properties, the former being much more stable. The experimental results (viscosity, alkali consumption, denitration) are in accordance with the theory of a branched chain structure for amylopectin and the linear type for amylose.

2. The greater relative instability of amylopectin nitrate is shown by the Bergmann-Junk test and is not due to the presence of free aldehyde groups; the alkali consumption is much greater for amylose nitrates (and also for cellulose nitrate) than for amylopectin nitrates.

3. Fractional precipitation methods have been applied to each of the crude products and fractions isolated having a varying nitrogen content, molecular complexity and stability, thus permitting of a more detailed study of starch structure and of the fundamental causes of the instability of the nitrated products. The results proved the heterogeneous nature of the nitrates and showed that the fractions of low-molecular weight and low nitrogen content, particularly of the branched chain type, are characterized by marked instability.

4. Fractional dissolution of the same three crude nitrates by use of ethanol resulted in the removal of certain fractions largely responsible for the instability of the original products. The ethanol-soluble material from whole starch nitrate is probably largely amylopectin nitrate as judged by denitration studies, solubility experiments and stability tests. In all cases, the non-branched chain starch constituents gave the most stable nitrates.

5. The ethanol-soluble fractions from whole starch nitrate are not stabilized during their isolation by use of ethanol although the residual fractions possess increased stability. Both the soluble and insoluble fractions of amylose nitrate are stabilizable by ethanol; those from amylopectin to a less marked degree.

6. The rate of denitration of whole starch nitrate of amylose and of amylopectin nitrates by ammonium hydrosulfide was investigated. The method serves to differentiate nitrate fractions having varying degrees of stability.

7. Pretreatment of whole starch by (a) swelling in pyridine (b) dissolution in formic acid (c) swelling by water and (d) renitration in acetone gave products which when nitrated were characterized by much greater instability and lower yield than when whole, untreated starch was used.

8. Nitration employing nitric acid alone was found more useful and advantageous than that using mixed acids in that higher yields of products with an over-all greater stability were obtained.

9. Cellobiose octanitrate is more stable than maltose octanitrate as measured by the Abel test and both are less stable than cellulose and starch nitrates. Neither of the octanitrates is sufficiently stable to permit of the application of the Bergmann-Junk test. If the stability of the cellulose-nitrate building unit, namely, cellobiose octanitrate, carries over into its polymeric form, it would be expected and it is actually a well-known fact -- that cellulose nitrate (characterized by 1,4-β glucosidic linkages) should be inherently more stable than starch nitrate (α-1,4-glucosidic linkages).

Bibliography

1. Barbier, Bull. trimus. assoc. éléves école sup. brasserie univ. Louvain, 27: 161 (1927) C.A. 21: 2572 (1927)

2. (a) Karrer, Nägeli, Hurwitz and Walti Helv. Chim. Acta., 4: 678 (1921)

   (b) Karrer and Nägeli, ibid., 4: 263 (1921)

3. Haworth and Percival, J. Chem. Soc., 1342 (1931)

4. Freudenberg and Friedrich, Naturwissenschaften, 18: 1114 (1930)

5. (a) Freudenberg, Friedrich and Bumann, Ann., 494: 41 (1932)

   (b) Freudenberg, Kuhn, Dürr, Bolz and Steinbrunn, Ber., 63: 1510 (1930)

6. Freudenberg, Blomquist, Ewald and Soff, Ber., 69: 1258 (1936)

7. Nägeli, C., "Die Stärkekorner. Morphologische, physiologische, chemische-physicalische und systematische-botanische" Monographie, Zurich p. 625 (1858)

8. Meyer, A., Bot. Ztg., 39: 841, 856 (1881)

9. Meyer, K.H., "Advances in Colloid Science" Interscience Publishing Co. New York, p. 166 (1942)

10. Katz and Derksen, Z. physik. Chem. A150: 100 (1930)

11. (a) Katz and v. Itallie, Z. physik. Chem. A155: 199 (1931)

    (b) Katz and v. Itallie, ibid. A166: 27 (1933)

12. Meyer, K.H., Hopff and Mark, Ber., 62: 1103 (1929)

13. Frey-Wyssling, Naturwissenschaften, 28: 78 (1940)

14. (a) Sponsler, Am. J. Bot., 9: 471 (1922)

    (b) Sponsler, J. Gen. Physiol., 5: 757 (1923)

15. Samec., Kolloidchem. Beihefte 6: 23 (1914)

16. Meyer K.H., and Bernfeld, Helv. Chim. Acta 23: 875 (1940)

17. Meyer K.H., Wertheim and Bernfeld, ibid. 24: 378 (1941)

18. Hirst, Plant and Wilkinson, J. Chem. Soc., 2375 (1932)

19. Samec, Kolloid Z. 85: 247 (1938)

20. Samec, ibid. 92: 1, (1940)

21. Lampitt, Fuller and Goldenberg. J. Soc. Chem. Ind., 60: 301 (1941)

22. Dahl, Z. physiol. Chem. 263: 39 (1940)

23. Samec and Beniger, Kolloidchem. Beihefte 33: 95 (1931)

24. Samec, Trans. Faraday Soc., 31: 395 (1935)

25. Samec, Helv. Chim. Acta., 15: 43 (1932)

26. (a) Taylor and Nelson, J. Am. Chem. Soc. 42: 1726 (1920)

(b) Taylor and Nelson, ibid. 49: 1584 (1927)

27. Taylor and Sherman, ibid. 55: 258 (1933)

28. Taylor and Schoch, ibid. 55: 4248 (1933)

29. Schoch, ibid. 60: 2824 (1938)

30. Lehrmann, ibid. 61: 212 (1939)

31. Schryver and Thomas, , Biochem J. 17: 497 (1923)

32. Ling and Nanji, M. Chem. Soc. 125: 652 (1925)

33. Hanes, New. Phytologist 36: 101 (1937)

34. Bergman and Knehe, Ann. 452: 141 (1927)

35. Karrer and Nägelli, Helv. Chim. Acta., 4: 263 (1921)

36. Pringsheim, Ber. 57: 158 (1924)

37. Ling and Nanji, J. Chem. Soc. 125: 636 (1925)

38. Irvine, "Comprehensive Survey of Starch Chemistry" p. 20 R.P. Walton, New York (1928)

39. Haworth, Hirst and Webb, J. Chem. Soc. 2681 (1928)

40. Haworth and Percival, ibid. 1342 (1931)

41. Haworth, Hirst and Ant. Wuorinen. ibid. 2368 (1932)

(c) Freudenberg and Boppel, Naturwissenschaften 26: 123 (1938)

(d) Freudenberg and Boppel, ibid. 28: 264 (1940)

63. Richardson, Higginbotham and Farrow, J. Text. Inst. 27: 131T (1936)

64. Svedberg, Ber., 67A: 117 (1934)

65. (a) Wolfrom, Meyers and Lassettre, J. Am. Chem. Soc., 61: 2172 (1939)

(b) Wolfrom, Meyers and Lassettre, ibid. 63: 1336 (1941)

66. Samec, Kolloid Z. 85: 247 (1938)

67. Küntzel and Dolhner, ibid. 88: 209 (1939)

68. (a) Samec and Blinc, Kolloidchem. Beihefte 47: 371 (1938)

(b) Samec and Blinc, ibid. 49: 75 (1939)

(c) Samec and Blinc, ibid. 52: 57 (1940)

(d) Samec, ibid. 51: 359 (1940)

69. Posternak, Helv. Chim. Acta, 18: 1351 (1935)

70. Koets and Kruyt, Kolloidchem. Beihefte, 47: 100 (1937)

71. Pictet and Sarasin, Helv. Chim. Acta I: 78 (1918)

72. Pictet and Jahn, ibid. 5: 640 (1922)

73. Pictet and Stricker, ibid. 7: 932 (1924)

74. Pictet and Salzmann, ibid. 10: 276 (1927)

75. Pictet and Vogel, ibid. 12: 700 (1929)

76. Karrer and Krauss, ibid. 13: 1071 (1930)

77. Friedrichs, Ark. Kemi. Min. Geol. 5: No. 2 (1913) C.A. 8: 2876 (1914)

78. (a) Tychowski and Masior, Biochem. Z. 291: 399 (1937)

(b) Tychowski and Masior, ibid. 292: 141 (1937)

(c) Tychowski and Masior, ibid. 292: 218 (1937)

79. Helferich, Starker and Peters, Ann. 482: 183 (1930)

80. Berner and Melhus, Ber., 66B: 1333 (1933)

81. Vogel, ibid. 72B: 2052 (1939)

82. Böeseken, Berg and Kirstjens, Rec. trav. Chim. 35: 320 (1916) C.A. 11: 39 (1917)

83. Bergman and Beck, Ber. 54: 1574 (1921)

84. Sutra, Compt. rend., 195: 1282 (1932)

85. Freudenberg, Blomquist, Ewald and Soff, Ber., 69B: 1258 (1936)

86. Freudenberg and Soff, ibid. 69B: 1252 (1936)

87. Peat and Wheatstone, J. Chem. Soc. 276 (1940)

88. Hönig and Ruziczka, Biochem Z. 218: 397 (1930)

89. Samec and Blinc, Kolloidchem. Beihefte 38: 40 (1933)

90. (a) Jackson and Hudson, J. Am. Chem. Soc., 59: 2049 (1937)

(b) Jackson and Hudson, ibid. 60: 989 (1938)

91. Caldwell and Hixon, J. Biol. Chem. 123: 596 (1938)

92. Grangaard, Michel and Purves, J.Am. Chem. Soc. 61: 1290 (1939)

93. Nazarov, Colloid J. (U.S.S.R.) 6: 245 (1940) C.A. 35: 2400 (1941)

94. (a) Jambuserwala, J. Text. Inst. 29: T149 (1938)

(b) Jambuserwala, ibid. 30: T85 (1939)

(c) Jambuserwala, ibid. 31: TI (1940)

95. Karrer and Nägeli, Helv. Chim. Acta 4: 185 (1921)

96. (a) Schmid, Ber., 58: 1963 (1925)

(b) Schmid and Zentner, Monatsh 49: 111 (1928)

97. Irvine and Macdonald, J. Chem. Soc. 1502 (1926)

98. Hirst and Young, ibid. 1247 (1938)

99. (a) Hess and Neuman, Ber., 70B: 710 (1937)

(b) Hess, Papier fabrik 36: 333 (1938)

100. Freudenberg and Jacob, Ber., 74: 162 (1941)

101. (a) Hess and Lung, Ber., 70: 1259 (1937)

(b) Hess and Lung, ibid. 71: 815 (1938)

102. Haworth, Hirst and Isherwood, J. Chem. Soc. 577 (1937)

103. Ohlsson, Z. physiol. Chem. 189: 17 (1930)

104. Waldschmidt-Leitz and Mayer, Hoppe-Seyl. Z. 236: 168 (1935)

105. (a) Hanes, Proc. Roy. Soc. London, B128: 421 (1940)

(b) Hanes, ibid. B129: 174 (1940)

106. Ohlsson, C.R. lab. Carlsberg 16 No. 7 (1926)

107. Blom, Bak and Braae, Z. physiol. Chem. 241: 273 (1903)

108. van Klinkenberg, Proc. Acad. Sci. Amst. 34: 893 (1931)

109. Waldschmidt-Leitz, Reichel and Purr, Naturwissenschaften 20: 254 (1932)

110. Holmbergh, Biochem Z. 266: 203 (1933)

111. Waksman and Davison, "Enzymes" p. 186, William Wilkins Co. Baltimore (1926)

112. Schardinger, Centr. Bakt. Parasitenk II 29: 188 C.A. 5: 2575 (1911)

113. Tilden and Hudson, J. Am. Chem. Soc. 61: 2900 (1939)

114. (a) Myrbäck, Biochem Z. 307: 132 (1941)

(b) Myrbäck and Ahlborg, ibid. 307: 69 (1940)

115. Somogyi, J. Biol. Chem. 134: 301 (1940)

116. Gore, J. Am. Chem. Soc. 47: 281 (1925)

117. Wohlgemuth, Biochem. Z. 9: I (1908)

118. Freeman and Hopkins, Biochem. J. 30: 442 (1936)

119. Baker, J. Chem. Soc. 81: 1177 (1902)

120. Ling and Nanji, ibid. 123: 2666 (1923)

121. Syniewski, Ann. 441: 285 (1925)

122. Blom, Bak and Braae, Hoppe-Seyl Z. 241: 273 (1936)

123. Brown and Miller, J. Chem. Soc. 75: 286 (1899)

124. Samec Z. physiol, Chem. 259: 204 (1939)

125. Myrbäck, Ann. Rev. Biochem. p.69, Annual Reviews Inc. Stanford Univ. (1939)

126. Hassid and McCready, J. Am. Chem. Soc. 63: 2171 (1941)

127. Haworth, Heath and Peat, J. Chem. Soc. 55 (1942)

128. Hanes, Nature 145: 348 (1940)

129. Bear and Cori, J. Biol, Chem. 140: 111 (1941)

130. Freudenberg and Rapp, Ber. 69: 2041 (1936)

131. Freudenberg and Meyer-Delius ibid. 71: 1596 (1938)

132. Kratky and Schneidmesser, ibid. 71: 1413 (1938)

133. Maquenne and Roux, Compt. rend 140: 1303 (1905)

134. Ling and Nanji, J. Chem. Soc. 123: 2666 (1923)

135. Sherman and Baker, J. Am. Chem. Soc. 38: 1885 (1916)

136. Karrer, Helv. Chem. Acta 15: 48 (1932)

137. Baldwin, J. Am. Chem. Soc. 52: 2907 (1930)

138. Samec, Compt. rend. 172: 1079 (1921)

139. Taylor and Iddles, Ind. Eng. Chem. 18: 713 (1926)

140. Pacsu and Mullen, J. Am. Chem. Soc. 63: 1168 (1941)

141. Nägeli, Die Stärkekörner, Zurich 1858

142. Maquenne and Roux, Ann. Chim. Phys. (8) 2: 109 (1904)

142. (a) Meyer A., Untersuchungen Uber die Stärkekörner, Jena (1895)

143. Taylor and Morris, J. Am. Chem. Soc. 57: 1070 (1935)

144. Samec and Waldschmidt-Leitz, Z. physiol Chem. 203: 16 (1931)

145. Meyer, Wertheim and Bernfeld, Helv. Chim Acta. 23: 864 (1940)

146. Hess and Krajnc, Ber. 73: 976 (1940)

147. Staudinger, ibid. 70: 1451 (1937)

148. Bawn, Hirst and Young, Trans. Faraday Soc. 36: 880 (1940)

149. Barker, Hirst and Young, Nature 147: 3723 (1941)

150. Myrbäck, Ortenblad and Ahlborg, Biochem. Z. 307: 53 (1940)

151. Beckmann and Coles, Chem. and Ind. 60: 750 (1941)

152. Caesar and Cushing, J. phys. Chem. 45: 776 (1941)

153. Meyer, K.H., and Mark, Der Aufbau der hochpolymeren org. Naturstoffe, Akadem. Verlagsges, Leipzig (1930)

154. Sadtler, Met. Chem. Eng. 16: 361 (1917) U.S. pat. 1,211,761 Jan. 9 (1917)

155. Grard, Compt. rend. 200: 410 (1935)

156. Will and Lenz, Ber. 31: 68 (1898)

157. Berl and Butler, Zeit. ges. Schiess-Sprengstoff. 5: 82 (1910)

158. (a) Hackel and Urbanski, Roczniki Chem. 12: 276 (1932)

(b) Hackel and Urbanski, ibid. 13: 221 (1933)

(c) Hackel and Urbanski, Z. ges. Schiess-Sprengstoff. 28: 306, 350, 378 (1933)

(d) Hackel and Urbanski, ibid. 29: 14 (1934)

159. Berl and Kunze, Ann. 520: 270 (1935)

160. Centola, Gazz. Chim. ital. 66: 8 (1936)

161. Urbanski and Janiszewski, Roczniki Chem. 17: 349 (1937)

162. Marshall, "Explosives", J. and A. Churchill, London, p.644, (1917)

163. Young, The Military Engineer 31: 11 (1939)

164. Hackel and Urbanski, Chem. Zentr. I: 1938 (1940)

165. Spurlin, J. Ind. Eng. Chem. 30: 538 (1938)

166. Snelling and Rees, U.S. pat. 2,271,877 Feb. 3 (1942)

167. Kolaczkowska and Urbanski, Roczniki Chem. 15: 339 (1935)

168. MacInnes, Report to the National Research Council of Canada, Sept. 8 (1941)

169. Hibbert and Tarr, Can. J. Research, 4: 372 (1931)

170. Hibbert and Barsha, ibid. 5: 580 (1931) and 10: 170 (1934)

171. Hibbert, Harrison and Tarr, ibid. 3: 449 (1930)

172. Brauns and Hibbert, ibid. 4: 596 (1931)

173. Hibbert, Tipson and Brauns, ibid. 4: 221 (1931)

174. Tarr and Hibbert, ibid. 5: 414 (1931) 15B: 487 (1937)

175. Hehre, Science 93: 237 (1941)

176. Bois and Nadeau, Can. J. Research 16: 114 (1938)

177. Bois and Chubb, ibid. 20: 114 (1942)

178. Fowler, Buckland, Brauns and Hibbert, ibid. 15: 487 (1937)

179. Challinor, Haworth and Hirst, J. Chem. Soc. 676 (1934)

180. Lyne, Peat and Stacey, ibid. 237 (1940)

181. Manning, J. phys. Chem. 42: 815 (1938)

182. Herzog and Jancke, Ber. 53B: 2162 (1920)

183. Bear and French, J. Am. Chem. Soc. 63: 2298 (1941)

184. Ott, Physik. Z. 27: 174 (1926)

185. Náray-Szabó, Ann. 465: 299 (1928)

186. Bear, J. Am. Chem. Soc. 64: 1388 (1942)

187. Pictet and Sarasin, Compt. rend. 166: 38 (1918)

188. Astbury, Bell and Hanes, Nature 146: 558 (1940)

189. Tanret, Compt. rend. 158: 1353

190. Staudinger and Husemann, Ann. 527: 195 (1937)

191. Freudenberg, Ann. Rev. of Biochem p. 81 (1939) Annual Reviews Inc. Stanford Univ. Calif.

192. Hungarian Patent 121,180 Aug. I (1939)

193. Bowman and Scott, J. Ind. Eng. Chem. 7: 766 (1915)

194. Lawrie, "Glycerol and the Glycols" (A.C.S. Monograph series No. 44) p. 401 (1928)

195. (a) Hess, Pfleger and Trogus, Ber. 66: 1505 (1933)

(b) Hess and Pfleger, Ann. 507: 48 (1933)

(c) Pacsu and Mullen, J. Am. Chem. Soc. 63: 1487 (1941)

196. Lovell and Hibbert, ibid. 63: 2070(1941)

197. Nadai, Z. physik. Chem. 136: 289 (1928)

198. (a) Taylor, Ind. Eng. Chem. Anal. Ed. 7: 321 (1935)

(b) Taylor and Salzmann, J. Am. Chem. Soc. 55: 264 (1933)

199. (a) Schoch and Jensen, Ind. Eng. Chem. Anal. Ed. 12: 531 (1940)

(b) Schoch, Wilson and Hudson, J. Am. Chem. Soc. 64: 2871(1942)

(c) Schoch, ibid. 64: 2957 (1942)

200. Braun, "Organic Syntheses" XVII p. 34 J. Wiley and Son Inc. New York (1937)

201. Rogovin and Glazman, Kolloid Z. 76: 210 (1936)

202. Kumichel, Kolloidchem. Beihefte 26: 161 (1928)

203. Kerr, Trubel and Severson, Cereal Chem. 19: 64 (1942)

204. Kerr and Severson, J. Am. Chem. Soc. 65: 193 (1943)

205. Bates, French and Rundle, ibid. 65: 142 (1943)

206. Kerr and Trubel, Cereal Chem. 18: 530 (1941)

207. Gottlieb, Caldwell and Hixon, J. Am. Chem. Soc. 62: 3342 (1940)

208. Barnett, "Explosives" p. 181, Bailliere, Tindal and Co., London (1919)

209. Freudenberg, Dürr, Hochstetter, Ber. 61: 1735 (1928)

210. Kerr, J. Am. Chem. Soc. 65: 188 (1943)

211. Wolfrom, Smith and Brown, ibid. 65: 255 (1943)