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# Amylose complexes with organic acids and alkyl halides

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# NOTE TO USERS

# AMYLOSE COMPLEXES WITH ORGANIC ACIDS AND ALKYL HALIDES

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TABLE OF CONTENTS

# INTRODUCTION

It has been known for a long time that appreciable amounts of fatty acids are associated with cereal starches. Several investigators have proposed hypotheses as to the nature of the interaction of the fatty acids with starch and the nature of the forces binding these acids to the starch. Among the earlier views presented were (1) formation of an ester linkage between the hydroxyl groups of the carbohydrate with the carboxyl group of the fatty acids, (2) combination of the fatty acid to non-carbohydrate material, such as the organic phosphate group, i.e., the formation of phospholipids, (3) distribution of the fatty acids throughout the starch granules as extraneous material and (4) adsorption. Not any of these seemed satisfactory.

Numerous attempts have been made to show that fatty acids are associated with a certain fraction of the starch. Since methods of fractionating starch reported in the earlier literature were very poor, conclusions based on them were also inaccurate. Recently it has been shown that starch can be separated into two distinct fractions. Investigators began immediately to characterize these two components. It has now been fairly well established that one, known as amylose, is essentially a linear chain of glucose residues with relatively few, if any, branch points while the other, amylopectin, is highly branched.

Although the two fractions have practically the same chemical groups available for reaction, their tendencies to form molecular complexes with various organic molecules and iodine differ greatly. Amylose is precipitated from a starch dispersion by alcohol and several organic compounds, whereas amylopectin remains dispersed. The well known blue

color of the starch indicator used in iodometric titrations is due to a complex formed between amylose and iodine. Iodine does not form a stable complex with amylopectin. It imparts a red color to amylopectin solutions. In the initial stages of this investigation of the associative forces binding the fatty acids to starch, it was of interest to know whether the fatty acids interacted with both or only one of the fractions of starch.

A broader aspect of this work was to study some of the other complexes between amylose and organic molecules, which would bring about a better understanding of the complex-forming tendency of amylose. Although essentially linear, amylose can take on a helical configuration while amylopectin, being highly branched, cannot. This helical concept was proposed about a decade ago. Although not universally accepted, it appears to be the most logical explanation for the complexing-ability of amylose. Many of the experiments included here were designed primarily to obtain further evidence for the helical concept of amylose, while others were designed to test critically the hypotheses presented by other investigators.

Furthermore, the textile and paper industries have utilized the interaction of soap, the sodium salts of fatty acids, in increasing the stability of starch suspensions and in obtaining a more complete utilization of starch in their manufacturing processes. A better understanding of this interaction might suggest improvements which would increase the efficiency of these processes.

## II. REVIEW OF LITERATURE

### A. Nature of Forces Binding Fatty Acids to Starch

Among the earlier investigations designed to determine the nature of the lipid material found in starch and the bonding between this material and the carbohydrate was that of Taylor and Nelson (51). They found that the greater portion of the fatty material could not be removed by solvents except after treatment with hydrolytic agents. The amount of "extraneous fat" obtained by successive extractions with anhydrous ether, petroleum ether and carbon tetrachloride amounted to about 0.1 per cent of the weight of the starch. The fatty acids liberated upon hydrolysis of the starch with a fairly concentrated solution of hydrochloric acid amounted to 0.6 per cent of the original weight of the corn starch. These fatty acids were liberated at any early stage of hydrolysis, the erythrodextrin stage. Diastatic hydrolysis released the fatty acids at a much slower rate; about a third was released at the erythrodextrin stage and another third upon prolonged treatment. This difference in the amount of lipid material removed by solvent extraction and by hydrolysis led Taylor and Nelson to conclude that the greater portion of the lipids were not just mixed with the carbohydrate but were held with forces of considerable magnitude.

Since acid and enzyme hydrolysis liberated the fatty material, Taylor and Nelson were curious to know whether cleavage of starch by Bacillus acetoethylicum in the presence of low hydrogen ion concentration would do the same. The results obtained with the bacilli were not as interesting as those obtained in the starch suspensions used as controls,

i.e., without Bacillus acetoethylicum. Upon standing, residues representing from 5 per cent to 8 per cent of the original starch were formed. Analysis of these residues revealed that they contained from 3.3 per cent to 5.5 per cent fatty material, or approximately half of the total lipids in the original starch. This observation, although very important, could not be interpreted with any degree of accuracy until much more work on the fractionation of starch and characterization of the fractions was done. Taylor and Nelson, however, felt that perhaps the "fat" might be associated with one fraction of starch.

Attempts to characterize the lipids completely have not been reported by these investigators. They knew that palmitic acid was one of the constituents along with an unsaturated portion which was called the "X" portion. They concluded that the palmitic acid was combined as an ester to the unsaturated portion which, in turn, was combined directly to the starch. The "X" portion served as a connecting link between the palmitic acid and the carbohydrate. No explanation of the nature of the bond between the unsaturated "X" portion and the starch was given.

A few years later, in 1925, Rask and Phelps (36) reported a method of removing the fat from the starch with no previous hydrolytic action. This was accomplished by extracting the starch with a mixture of ethyl alcohol, concentrated ammonia and water. They reported a yield of 0.54 per cent "lipoid" material. Since this value was very nearly that reported as "fat by hydrolysis" by Taylor and Nelson, the authors assumed that the greater portion of this "lipoid" material was "fat". This, however, was an incorrect assumption.

Taylor and Werntz (53) repeated the above method of extraction. Their experiments indicated that only a small portion of the "fat by hydrolysis" was removed from the corn $\alpha$-amylose. By modifying the

procedure so as to extract the starch eight successive times with the solvent, they freed nearly all of the fatty acids from corn starch. Preheating the starch aided in the removal of the fatty acids. As a result of this work, they concluded that the fatty acids were attached to a complex carbohydrate in corn $\alpha$-amylose.

Although the Rask and Phelps method was of little value as an analytical tool for determining the amount of lipids in cereal foods, it brought about renewed efforts of Taylor and his associates to study the nature of the linkage between the fatty acids and the carbohydrates. Taylor and Sherman (52) maintained that at least the greater part of the fatty material was combined chemically with the starch. In order to explain the fact that they were not able to prepare corn $\alpha$-amylose which contained all the fatty acid material in the starch, they postulated that all of the fatty acids were not combined in the same manner. Furthermore, since starch is a polyhydroxy compound, the fatty acid could be combined to the carbohydrate through an oxygen linkage at any one or more of the free hydroxyl groups. The groups were not all equivalent, hence the strength of the resulting bonds would not be the same.

Their method of attacking the problem was to study the ease with which the fatty acids were removed by hydrolysis. The effects of acid, alkali and enzymic hydrolytic reagents on corn $\alpha$-amylose were considered. By employing these different methods of hydrolysis upon the fatty acid derivatives of glucose with substituents in different positions, they expected to find that some of the linkages would be broken more easily than others.

Results in each case showed that the fatty acid groups combined with corn $\alpha$-amylose were preferentially liberated. The unsaturated fatty acids were removed more easily than the saturated. This they

explained as being due to a general lesser stability of compounds of carbohydrates with the unsaturated acids (oleic and linoleic) as compared with those of palmitic acid, or that these unsaturated acid residues are linked at positions different from that of saturated acids in the glucose residue. Neither one of the above explanations seemed entirely satisfactory on the basis of ester linkage between the carbohydrate and the fatty acid.

In 1938, Schoch (43) reported that the fatty acids in cereal starches, such as corn, wheat and rice, can be removed completely by Soxhlet extraction with certain water miscible organic solvents, particularly methanol, the cellosolves and 80 per cent dioxane. In a later work (45), he showed that defatted starches could be impregnated with fatty acids by heating the granular starch suspended in an alcohol-fatty acid solution on a water bath until the alcohol was evaporated. The fatty acids could not be removed by the common fat solvents, as the hydrocarbons and chlorinated hydrocarbons. He concluded that fatty acids were not combined chemically to the starch but that they were distributed throughout the granules as extraneous impurity. This work was definite proof that the binding was not due to primary valence bonds. To explain the selective extraction by some solvents, Schoch stated that the solvents with hydrophilic groups can penetrate into the granules, whereas the hydrocarbon type cannot. The latter merely washed the surface.

Lehrman (28) objected strenuously to the idea of the fatty acids being distributed throughout the starch granules as extraneous material. He suggested that there might conceivably be an adsorption complex, which would account for the selective solution by solvents used by Schoch. Furthermore, adsorption would explain the negative charge of the amylose

fraction which had fatty acid associated with it.

In some later work Lehrman (29) presented data which he interpreted as meaning that the fatty acids were adsorbed on the starch. Defatted potato, corn and rice starches were suspended in alcoholic solutions of palmitic acid of different concentrations. The amount of palmitic acid taken up was determined. A typical Freundlich isotherm was obtained when the amount of palmitic acid taken up by the starch was plotted against the concentration of the acid in the solvent. Potato starch, which originally had no fatty acids, took up a small amount. Corn starch took up considerably more, although less than the 0.6 per cent found in naturally occurring corn starch. Rice starch, on the other hand, took up more than that found in nature. Although the idea of adsorption met with greater approval than that of extraneous distribution, Lehrman admitted the difficulty of explaining the above mentioned variations in the amounts of palmitic acid taken up by the different defatted starches. The only explanation given was that these starches were different in their surface effects.

Whistler and Hilbert (56) reported that the fatty material difficult to extract from intact corn starch granules with methanol was easily removed after disintegration of the granules. Disintegration was but a mechanical process whereby the fatty acids were made more accessible to the solvent, hence the above would be expected. They concluded that the fatty acids were apparently bound by associative forces rather than by primary valence bonds.

More recently Mikus, Hixon and Rundle (34) showed that fatty acids form a complex with amylose similar to that of the amylose-iodine and the amylose-butanol complex. They suggested that the fatty acids in cereal starches not removable by common fat solvents are associated with amylose

as a complex. The low fatty acid content of waxy corn which is essentially amylopectin was attributed to its inability to form a complex with the fatty acids.

### B. Nature of the Fatty Acids in Cereal Starches

Taylor and Nelson (51) reported that palmitic acid constituted the main portion of the saturated fatty acids in corn starch. At the same time, they observed a portion which they called the "X" unsaturated portion. Later Taylor and Lehrman (50) reported that the fatty acids liberated upon hydrolysis of corn starch consisted of approximately 24 per cent palmitic, 40 per cent oleic and 36 per cent linoleic acids.

Evans and Briggs (8) made an exhaustive study of the lipids found in corn starch. Lipids liberated from starch by methanol extraction, acid hydrolysis and diastatic hydrolysis were analyzed. The mixtures of fatty acids obtained by saponification of the lipids extracted by methanol and those obtained from acid hydrolysis of the starch were very similar in composition. Diastatic hydrolysis did not appear to liberate all of the fatty acids from the starch. The acids liberated by hydrolysis consisted of palmitic 21.2 per cent, stearic 7.8 per cent, undetermined saturated acids 1.0 per cent, oleic 37.7 per cent, linoleic 31.1 per cent, and linolenic 1.2 per cent. No unsaturated acid having more than three double bonds was found.

Results showed that free fatty acids made up the major portion of the lipids obtained by methanol extraction and diastatic hydrolysis. A positive test for glycerol was reported for the methanol extract but the amount was too small for a quantitative determination. Evans and Briggs pointed out that methanol extraction would not be expected to hydrolyze

fatty acid esters. Conditions favoring hydrolysis would also favor the formation of methyl esters. A glyceride, olive oil, was subjected to the action of the malt extract in order to determine whether the extract contained any lipases that would split out fatty acids. Results showed very little, if any, hydrolysis of the glyceride.

Schoch (45) reported the extraction of the fatty acids in rice starch in the form of the sodium soaps. The starch used was prepared by the alkali steeping process, which, in all probability, accounted for the soap formation.

Several investigators in the field of starch chemistry have tried to find a relationship between the fatty acids and the phosphorus found in starch. Taylor and Iddles (49) were interested in determining whether the method which separated potato starch into a fatty acid-containing component would, at the same time, separate it into a phosphorus-bearing component. Results of their experiments showed that this was not the case.

Since, by exhaustive extraction of corn starch with methanol, he was able to decrease the phosphorus content from 0.017 per cent to only 0.015 per cent, Schoch (45) concluded that the lipids were present as free fatty acids. In the case of wheat starch, the phosphorus appeared to be present as phospholipids. On extraction, both were removed concurrently. The lipids, purified by solution in carbon tetrachloride, analyzed high in phosphorus. Lehrman (30) gave additional evidence that the wheat starch fatty acid material was in the form of phospholipids.

## C. The Starch-Soap Complex and Its Application in the Paper Industry

Richardson and Waite (37) were the first to recognize the reaction between soap and starch. They found that when raw starch was suspended in soap solution and heated, gelatinization was retarded and, when sufficient soap was present, gelatinization was even prevented. Small amounts of soap added to an already gelatinized starch paste retarded the normal aging changes and altered the viscosity considerably. In addition to recognizing that there was a definite interaction between the soap and the starch, Richardson and Waite observed that the addition of soap up to the minimum viscosity had a powerful stabilizing effect or checked the fall in viscosity during use. As a possible mechanism of the reaction, they suggested that the soap reacted with the starch in such a way as to prevent access of water to the points that were active when swelling occurred or to those susceptible to acid hydrolysis. The compound thus formed might be expected to have a branched structure in which a considerable number of chains did not fall in with the prevailing arrangement that determined the mechanical condition of the starch-water mixture.

Heald (17) showed that, in general, there was a slight minimum in the viscosity of starch pastes when approximately 1.5 per cent soap, based on the weight of the starch, was added; a maximum occurred in most systems when 12 per cent soap was added, while further addition of soap lowered the viscosity. A thorough study using various soaps on different starches and modifications of starches was made. The presence of soap exaggerated the difference in the viscosity between various starches and grades of starches considerably. This led to the conclusion

that the starch-soap reaction could be used as a method of following the different treatments and modification methods, and the viscosity increase on the addition of soap might be used as a method of evaluating starch.

The addition of soap to starch from which the "amylopectin gel fraction" had been removed by centrifugation or electrodecantation lowered its maximum viscosity. This indicated that viscosity changes in the starch-soap system were due to the amount and the physical condition of the amylopectin. Addition of sodium chloride, aluminum chloride, and calcium chloride lowered the maximum viscosity markedly and decreased the electrophoretic mobility, while the addition of the latter two not only lowered the viscosity but precipitated the starch and soap together as a complex. Viscosity increases were found to be proportional to the chain length of the soap used. Unsaturation in the chains tended to decrease the viscosity rise.

The various phenomena observed were attributed to special colloidal interaction between the starch and the soap in the following manner. The sodium oleate was adsorbed by the starch particles. The initial decrease in viscosity was due to decreasing the amount of water of hydration, which decreased the volume. As more soap was adsorbed, cohesive forces between soap molecules increased the viscosity. Beyond 12 per cent soap the negative charges on the soap-starch complex were discharged by the excess positively charged sodium ions. Also, the negative charge of the complex repelled more oleate ions. Thus, the changes in viscosity were due to an electroviscous effect from adsorption of negative charges and a structural viscosity effect because of gel formation, possibly as a result of cohesive forces between oleate

hydrocarbon chains.

Houts (19) showed that the strength characteristics of paper made by using starch were at a maximum when starch was cooked to and used at the point of maximum colloidality, as indicated by the starch-soap tests. There was a good correlation between the strength imparted by the starch in the condition of maximum colloidality and the height of the peak on a starch consistency-temperature curve which, in turn, was found to vary directly with the molecular weight of the starch.

Kesler and Black (26) confirmed, in general, most of the work of Heald. They obtained a maximum viscosity with a lower soap concentration than Heald. They confirmed the fact that modifications of starches reduced the effect of soap on viscosity, and that electrolytes such as sodium hydroxide reduced the effect caused by modifying the starch. They considered the starch-soap effect to be a measure of dispersion or hydration of the paste, since pastes cooked at 6 per cent and diluted to 3 per cent had a lower final viscosity than pastes cooked at 4 per cent and diluted to 3 per cent. (Final starch and soap concentrations and cooking were identical.)

Further work by Kesler and Black on the effects of electrolytes on viscosity showed that interaction of traces of minerals in the water or in the starch with the naturally occurring fatty acid content of starch changed the viscosity appreciably. Therefore they concluded that the use of starch-soap viscosity as a measure of quality of starches might be misleading.

The starch-soap complex was precipitated completely from suspensions of 0.1 per cent to 0.2 per cent starch with as little as 1 per cent soap, when conditions were optimum for precipitation. Absence of starch in

the supernatant liquid was determined by the starch iodine color test. This, as we shall see later, was not reliable. Other things being equal, the floc formed more rapidly at higher soap concentrations. Floc particles were fluffy and other properties depended on conditions of precipitation. The soap portion had to be made insoluble either by the addition of a metallic ion, such as calcium, which forms an insoluble soap, or by the addition of mineral acids, which cause the liberation of free fatty acids. Kesler and Black found that the former may be carried out at pH values up to 10. Addition of acid or alum to unmodified starch solutions containing 0.1 per cent to 0.2 per cent starch caused the precipitate to form and settle to the bottom, but the supernatant liquid was hazy or milky and gave a heavy starch test. The particles formed were smaller. Sulphonated fatty alcohols, which had little or no effect on viscosity of the starch paste, were quite effective as precipitating agents. Since the nature and quantity of the precipitate formed varied with modifications of the starch, these investigators suggested that the precipitating qualities could be used as an analytical measure in evaluating starch.

As a possible mechanism for the reaction, Kesler and Black believed that there was an adsorption of long chain fatty acid molecules through association of their active groups with those of starch, possibly by means of the hydrogen bond. They thought that viscosity effects might be due to structural configuration, as suggested by Richardson and Waite (37), or cohesive attraction between adsorbed hydrocarbon chains, as suggested by Heald (17). In regard to the precipitation with metallic ions forming insoluble soaps and acids, they said:

> If the hydrophilic balance of the entire complex is changed, such as by the substitution of calcium or hydrogen for sodium in the adsorbed long chain, etc., the entire

complex will be caused to become insoluble and so precipitate. The complex may apparently be formed by adsorption of material already relatively insoluble (precipitated) and the resulting complex may be soluble or not, depending on the hydrophilic loading of the final material.

Kesler (25) developed a method of using the starch-soap complex in the sizing of paper. When the complex was introduced into the pulp, it was retained in the web as voluminous flecks which disintegrated and dispersed along the fiber, with the result that it was more effective in binding fiber to fiber. Since dispersion took place at low temperatures, high machine speed and low weight paper could be used. Larger quantities of starch could be introduced by this method and less starch wasted. Furthermore, the sheets were more resistant to water than sheets containing the same amount of ordinary cooked starch.

### D. Crystalline Products formed by the Interaction of Starch and Organic Compounds

As early as 1893, Linter and Dull (31) reported that they obtained clusters of crystals (spherocrystals) on the addition of alcohol to an aqueous solution of amylodextrins obtained in the early stages of malt diastase hydrolysis. This crystalline material was insoluble in cold water but very soluble in hot water. Dehydration of a concentrated aqueous solution by evaporation in air yielded a glassy mass which was no longer soluble in hot water. The spherocrystals gave a deep blue color with iodine and had a specific rotation of 196°.

Tanret (48), in 1909, attempted to fractionate starch with alcohol. He used soluble starch prepared by treating starch with very dilute acid

at elevated temperatures for a short period of time, according to the method of Wolff and Fernbach (59). Obviously, the starch was degraded to some extent. After keeping the starch suspension at 100° to 110° for an hour, Tanret added fifty times the amount of 25 per cent boiling alcohol and filtered while hot. Upon addition of sufficient 95 per cent alcohol, enough to make a 50 per cent solution, he obtained a product which, when dried with absolute alcohol and over sulfuric acid in a desiccator, was slightly soluble in boiling water. This fraction showed an optical rotation, $[\alpha]_D$ = 208-210°, and gave an insoluble blue product with iodine. Potassium hydroxide dissolved it without the formation of a paste. These properties were very similar to those of the butanol-precipitated fraction reported in 1941 by Schoch (44).

Tanret further separated the alcoholic filtrate into two fractions. The first had a specific rotation, $[\alpha]_D$ = 180°, and gave a red violet iodine color; the second had a specific rotation, $[\alpha]_D$ = 173°, and gave a red iodine color. From the above, apparently both fractions were richer in the branched component, amylopectin, than the original starch.

Van de Sande-Bakhuyzen (54) reported the preparation of a crystalline form of starch by alcoholic precipitation. He prepared an amylose solution by leaching the amylose out of ground starch granules with water at room temperature. To a part of the solution thus obtained, he added two parts of 96 per cent alcohol and allowed the precipitate and supernatant liquid to stand for about three weeks. In the second week he added an equal volume of 96 per cent alcohol to a portion of the above, which gave an alcoholic concentration of 80 per cent.

After three weeks he examined the precipitate microscopically and found many spherocrystals which consisted of separate, very refractive radial needles attached to a central point or nucleus. The needles were

about 1 μ thick and up to 25 μ long. The spherocrystals found in the precipitate from the 80 per cent alcohol were more numerous and more perfectly shaped than those found in the precipitate from the 64 per cent alcohol. Van de Sande-Bakhuyzen suggested that the slow formation of the crystals was a continued dehydration process, the same as that which he described earlier for the formation of the refractive radial needles in starch granules (55).

A similar preparation of crystalline starch products was reported by Beijerinck (6). This work was verified and extended to include starches from various sources by Alsberg and Griffing (1). In both cases, the properties of the spherocrystals were identical to those reported by earlier investigators.

McCulloch (32) described starch-like crystals which formed in cultures of Bacillus marginatum when starch was a constituent of the medium. These were composed of needle-like parts radiating from a central point and were faintly luminous in polarized light. The large forms showed a dark cross with the lines intersecting at right angles in the center. It is very likely that the crystallization of the starch was due to some substance produced by the bacilli.

While studying the adsorption of starch on different surfaces, Horowicz (18) used, among other substances, amyl alcohol. After shaking a starch dispersion with the alcohol and allowing it to stand, he observed that starch was associated with the alcohol at the interface, which he interpreted as adsorption. Jirgensons (20) observed the effects of the lower alcohols on the coagulation of starch solutions. His interest seemed to have been more in conditions needed for coagulation than in the nature of the product obtained.

Schoch (44) reported that on cooling an autoclaved solution of starch to which an excess of butanol had been added, a precipitate formed slowly at the water-alcohol interface. When corn starch was used, six segmented spherulites formed; when potato starch was used, well-formed, six petalled rosettes formed. These precipitates exhibited optical and X-ray properties showing that they were definitely crystalline in nature.

Kerr and Severson (24) applied Schoch's butanol precipitation to the components of starch extractable by hot water. The precipitate formed was shown to consist of amylose molecules of shorter chain length. The form of the crystals depended on the source of the starch, corn starch giving rectangular platelets, and potato, needles which formed star-like clusters.

When Wiegel (57, 58) refluxed potato starch with 30 per cent ethanol he obtained needle-like particles, which on closer observation, appeared to be very thin platelets. Upon removal of the ethanol from the extract by evaporation, and addition of butanol to the hot solution, the crystals took the shape of an hour glass. Aqueous isobutyl alcohol gave typical spherocrystals.

Since there was such a variety of crystalline forms of starch reported, it was of interest to know whether the variations were due to differences in the components of starch or to differences in the crystallization technique. With this in mind, Kerr (23) proceeded to fractionate the butanol-precipitated fraction of corn starch using an ethylenediamine-ether phase separation. Several crystalline forms were obtained. Qualitatively, he showed that the solution viscosity of identical forms was the same, regardless of the source of the starch component.

## E. Development of the Concept of the Helical Structure of Amylose

As early as 1929, Haworth (14) mentioned in a speculative manner that the union by $\alpha$-(1-4) linkages of six glucopyranose units of the Sachse configuration might be expected to give rise to a strainless hexagonal ring structure of remarkable symmetry. Moreover, a series of such models placed symmetrically in space would leave practically no interstices between molecular units.

Hanes (13) suggested that Haworth's concept might be applied to chains of more than six glucose residues. Such an extension would necessitate a helix. Hanes claimed that dextrin molecules containing about six glucose units formed in considerable amounts in the $\alpha$-amylase degradation of starch. Since, in starch having the helical configuration, linkages separated by six units were brought in lateral proximity, Hanes used this configuration to explain his observation. Using this hypothesis, he did not have to postulate a difference in types of linkages in the starch molecules. Although Hanes' explanation may be wrong, it has since been observed that the enzyme from *Bacillus macerans* does produce cyclic dextrins of six and seven glucose units (11).

Haworth *et al* (15) reported that dextrins of 8 and 12 glucose residues gave a red coloration. Since the product of starch degradation at the achroic point corresponded to a six glucose unit chain, Hanes (13) believed that one turn in a helix having six glucose residues per turn was necessary for iodine color.

More recently Cori, Swanson and Cori (7) have shown that dextrins having an average chain length of 5.8 or less glucose residues give no iodine color, while those from 6.9 to 9.8 give a red color. They observed

a red-purple color for a chain of 10 to 11 glucose residues, purple for those of 11 to 12 residues, and blue-purple for chains from 12.6 to 15.4 residues.

Freudenberg et al (12) adopted a helical model to explain the coloration of the starch-iodine complex. In their model, the interiors were essentially hydrocarbons presumed to supply the absorptive power for iodine, thus causing the blue color. The helices had sufficient space to admit molecules of iodine with their long axes coincident with the helix axis.

Recently Rundle and Baldwin (38) presented additional evidence for the helical configuration of the starch-iodine complex. They noted that starch-iodine solutions exhibited dichroism of flow. Light with its electric vector parallel to the flow lines was more strongly absorbed than light with its vector normal to the flow lines. This observation required that the long axis of the iodine molecule be parallel to the long axis of the starch-iodine complex. This requirement was fulfilled by the helical structure or by a structure in which the starch molecule was an extended chain with the associated iodine molecules parallel to the chain. In view of the above and other evidence, the former seemed to be the more logical structure.

Rundle, Foster and Baldwin (40) proposed a mechanism of the interaction of iodine with starch. On the basis of results of potentiometric and spectrophotometric titrations of starch, they showed that iodine, instead of forming a solid solution, formed a true compound with the amylose in which the iodine molecules were bound to the amylose through secondary chemical interaction. If insufficient iodine were added to an amylose solution, it filled as many helices completely as it could instead

of partially filling all. The latter would be expected if solid solutions were formed. From a comparison of the amylose chain length and iodine activity of the complex, they observed that the longer the filled helix, the lower the iodine activity. Also, a helix filled with iodine had a lower iodine activity than a partially filled helix.

Any proposed mechanism for this interaction must account for the above observation. After a consideration of the amylose helix Rundle, Foster and Baldwin pointed out that it was made of glucose units, each consisting of a number of dipoles. Each unit has a resultant dipole of its own. When these glucose residues were arranged in a helix, the resultant dipoles normal to the axis of the helix should oppose each other, leaving a negligible or no dipole moment normal to the axis. If, however, the resultant dipole of each glucose residue were not normal to the helix axis, the components along the axis would add together. For a long helix, the resultant dipole moment should be quite large.

An iodine molecule introduced into the dipolar helix with its long axis parallel to the axis of the helix should acquire an induced dipole. Succeeding molecules introduced into the helix should acquire parallel dipoles so that there should be an attraction between the induced dipole of the iodine molecule and the dipolar amylose helix. The magnitude of the induced dipole moment should increase with the number of iodine molecules arranged in parallel, and with the strength of the dipole moment of the amylose which should increase with length.

Katz and Derksen (22) observed that starch which had been gelatinized and precipitated with alcohol yielded a product giving a different X-ray pattern, corresponding to a new crystalline modification of the starch. He called this the "V" modification. Bear (4) noted that this "V"

pattern resembled the X-ray diffraction pattern of the starch-iodine complex, and suggested that these patterns might be due to helical structures such as those proposed by Hanes and Freudenberg.

Kerr's "crystalline amylose" (24) appeared to consist of very small amylose crystals which produced an excellent "V" pattern when dry. The optical properties were reported by Rundle and French (41) (42). These minute crystals seemed to be psuedo-uniaxial in optical character. The index of refraction of these platelets was greatest for light with its electric vector in the plane of the platelets. When stained with iodine, they became very dichroic, but apparently remained psuedo-uniaxial. Polarized light, with its electric vector normal to the plane of the platelets, was very strongly absorbed. To explain these optical properties, the iodine molecules had to lie normal to the plane of the platelets. If the complex contained extended chains, the birefringence would have indicated that the chains lay in the plane of the platelets. In other words, the long axis of the iodine molecule would be normal to the long axis of the amylose. From dichroism of flow experiments mentioned above, this was not the case.

Rundle and his co-workers have investigated the X-ray diffraction pattern for the butanol and iodine precipitated amylose. Rundle and French (42) reported the dimensions of a unit cell for the starch-iodine complex which were in good agreement with the dimensions of a space-filling model of a helix with six glucose residues per turn. They also noted that the starch-iodine complex could be prepared in the absence of water or iodide, provided the starch had been put into the "V" configuration. Starch in the "A" or "B" configuration did not take up any appreciable quantity of iodine.

The pattern for the dried butanol precipitated amylose was indexed on the basis of a hexagonal unit $A_o$ = 27.4 Å, $C_o$ = 8.05 Å, or an orthorhombic unit $a_o$ = 13.7 Å, $b_o$ = 24.8 Å, and $c_o$ = 8.05 Å (42). A comparison of the space required for a turn of the helix in the dry butanol precipitate and in the starch-iodine complex provided additional evidence for the helical configuration. The distance between helices was 13.7 Å in the dry butanol precipitate, 13.0 Å in the iodine complex; a turn in the helix was 8.05 Å in the former and 7.9 Å in the latter.

The dry butanol precipitated starch fraction absorbed 26 per cent of its own weight in iodine and, in so doing its volume actually contracted. This would have been very unlikely if the molecules occupied a space outside the helices. A similar situation existed in the wet and the dry butanol precipitates. The wet precipitate had a period of 7.8 Å along the helix, whereas the dry had 8.0 Å. On the basis of this apparent tightening of the helix in the presence of butanol and on the basis of the excellent crystalline properties of the wet butanol precipitate, Rundle suggested that the starch formed, with the butanol, a complex in which the butanol was present within the helix.

Bear (5) reported that the X-ray diffraction patterns obtained from starch solids by precipitating with a linear alcohol, such as normal propyl, butyl and amyl alcohols, were identical. Those obtained from solids precipitated with branched alcohols, such as tertiary butyl and tertiary amyl alcohols, and the secondary alcohols, were identical in themselves but different from the first group; the dried precipitates of the second group had diffraction patterns extending to lower angles than the first group. An amylose helix having six glucose units per turn would have sufficient space on the interior to hold the iodine and the

normal alcohols, provided these molecules were arranged with their long axes parallel to the axis of the helix. From a consideration of the inside diameter of the helix and of the size of the branched alcohols, one was able to see that more space was needed. This enlargement could be obtained by increasing the number of glucose units per turn. The unit cell became correspondingly larger, as seen from the displacement of the diffractions to lower diffraction angles, i.e., larger spacings. This shift made the pattern of the branched alcohol complexes definitely non-hexagonal, which made it difficult to determine the change in the helix dimensions quantitatively from powder patterns alone. Since there were two distinct patterns from the two types of alcohols, Bear suggested that there must be a definite number of glucose residues per turn of the helix, i.e., either six or seven.

# III. EXPERIMENTAL

## A. Preparation of Materials

### Starch Fractions.

Fractions were prepared from the respective starches by the butanol precipitation method developed by Schoch (46). The potato starch was obtained from Stein Hall Company; the lily bulb and corn starches were milled in this laboratory. The amylose was further purified by re-crystallising three to five times with butanol. The apparent purity was determined by the potentiometric iodine titration developed by Bates, French and Rundle (3), using Kerr's "crystalline amylose" (24) as a reference standard. The "purity" of different preparations of the amylose fraction was uniformly about 90 per cent. The amylopectin fraction was concentrated *in vacuo* to about one-sixth volume, precipitated with methanol, filtered and dried.

### Amylose Complexes by Precipitation.

The butanol was removed from the amylose solutions by distilling two to four hours after the odor of butanol could no longer be detected. Although slight traces of butanol may have remained, the amount was insufficient to cause precipitation of the amylose in the "V" configuration. This was shown by the fact that on standing the amylose would retrograde. After drying, this material would not take up appreciable quantities of iodine vapor.

After removal of the butanol, the concentration of the solution was adjusted so that the amylose content was approximately one per cent. The complexes were formed by adding somewhat more than enough complexing agent to saturate the hot amylose solution. The hot solution was allowed to cool slowly. Complex formation was allowed to continue for several days at room temperature. The precipitated complexes were removed by centrifuging and dried in a vacuum desiccator. Excess complexing agent was removed from the dry powdered material by extraction with ether in a Soxhlet extractor for twenty-four hours.

The following complexing agents were used: lauric, oleic, palmitic and stearic acids; the potassium and sodium salts of palmitic and oleic acids; adipic, pimelic, suberic, azelaic and sebacic acids; isoamyl, n-amyl and n-butyl iodides; n-butyl bromide; tertiary amyl alcohol, ethyl adipate (33) and hexamethylene glycol (27).

In the case of fatty acids, the precipitates formed in one to five days depending on the solubility and the amount of fatty acids added. With soaps, the time required for complete precipitation was considerably longer, perhaps due to the higher pH of the solutions. When buffers were used to keep the solutions neutral, precipitation was speeded up considerably. All samples used for X-ray diffraction work were prepared without the use of buffers.

X-Ray Samples.

Samples of the wet fatty acid complexes were obtained directly from the centrifuge tube after decanting the supernatant liquid. Samples were also taken at two stages of drying: (1) samples were dried at 70° C. over phosphorus pentoxide in an Abderhalden drying tube evacuated by a water

aspirator, and (2) samples were dried at 100° C. over phosphorus pentoxide in an Abderhalden drying tube at a pressure below one mm. Hg.

Samples of other complexes studied were dried at 70° C. as in (1). The powder samples were sealed in thin walled glass capillaries of about 0.5 mm. diameter.

### B. X-Ray Methods

All X-ray diagrams were made using nickel filtered $CuK\alpha$ radiation and exposures of a few hours to several days at 15 m.a. and 40 K.V.P. All recorded spacings were obtained using cylindrical cameras of large radius (5.65 cm. or 10 cm.).

From the standpoint of economy of time, it was found expedient not to examine all samples prepared in the three different ways in the large cameras. Identification diagrams of all samples were made using fairly thick samples and a flat film with a sample-to-film distance of 5 cm. These diagrams could be made in a few hours and proved sufficient to reveal all but subtle changes in the X-ray patterns.

### C. Impregnation of Starch Fractions with Complexing Agents

Impregnation of Amylose and Amylopectin with Palmitic Acid from Methanol Solutions.

A modification of Lehrman's method (29) was used to introduce palmitic acid into the starch fractions. Dry samples of butanol-precipitated amylose in the "V" configuration, retrograded amylose in the "B" configuration, and alcohol-precipitated amylopectin were used. One gram samples of each of the above were suspended in 10 ml. of methanol

solutions containing varying concentrations of palmitic acid. The flasks containing the reaction mixtures were fitted with condensers for refluxing. These were kept at a temperature of 50° to 55° C. for seven hours by means of a water bath. Upon cooling, the residues were washed with carbon tetrachloride and allowed to dry, then extracted with carbon tetrachloride in a Soxhlet extractor for six hours. The amount of palmitic acid bound by the starch fractions was determined by the method outlined in the Analytical section.

In an effort to prepare samples having the same surface area, all samples of the material used were ground to a very fine powder in an agate mortar prior to treatment. Examined microscopically, the average size of the particles in each case seemed to be about the same. When examined for birefringence, the retrograded amylose and the amylopectin appeared amorphous. On the other hand, the "V" or helical amylose was obviously crystalline and quite birefringent. As far as could be observed, short of some elaborate method such as gas adsorption, all evidence indicated that the retrograded amylose and the amylopectin had as large a surface as amylose in the "V" configuration.

Impregnation of Amylose with Palmitic Acid from Carbon Tetrachloride Solutions.

Carbon tetrachloride was substituted for the methanol in the procedure of the preceding section for introducing plamitic acid into amylose in the "V" configuration. One gram of amylose was refluxed with 0.04 of a gram of palmitic acid in 20 ml. of carbon tetrachloride for two weeks.

Impregnation of Amylose with Alkyl Iodides.

Butanol-precipitated amylose, which had been dried in the vacuum oven at 60° C. for 12 hours, was suspended in isoamyl iodide at 76° C. for 24 hours. After removal from the tube, the amylose was washed with ether, followed by Soxhlet extraction with ether for 24 hours. Analysis showed that 5 per cent of its weight was isoamyl iodide. Similar treatment with n-butyl iodide yielded amylose containing 1.7 per cent to 2.9 per cent of the halide. A sample treated at 100° C. yielded amylose containing 5.7 per cent n-butyl iodide. Prolonged treatment at the elevated temperatures degraded the amylose into water soluble products.

Dry tertiary amyl alcohol-precipitated amylose was similarly treated with isoamyl iodide. The isoamyl iodide content was 4.48 per cent.

## D. Analytical Methods

Fatty Acids.

The dried samples (10-20 grams) were hydrolysed by boiling in 250 ml. of a 20 per cent hydrochloric acid solution for 20 minutes. The solutions, cooled to about 60° C., were filtered. The residues and filter papers were washed until the presence of the chloride could no longer be detected in the wash water. These were then dried in the oven at 50° C. for an hour. The fatty acid was extracted from the filter papers in a Soxhlet extractor. The residues, left upon evaporation of the ether, were dried for an hour at 50° C. and were weighed.

Composition of Dicarboxylic Acid-Amylose Complexes.

The sebacic acid-amylose complex was formed using a weighed amount of acid and amylose followed by the extraction of the excess acid with ether. The difference between the amount of acid used and that extracted was considered bound acid. When the extraction was carried out while the complex was still wet, i.e., both the wet complex and the water in which it was formed were extracted, the recovery of the acid was nearly 100 per cent.

Since ether was able to remove the acid from the wet complex, the method was modified so that the complex was dried before the ether extraction. Sebacic acid-amylose complex was prepared by treating a solution of 510 mg. of amylose in 40 ml. of water with about 100 mg. of acid for 48 hours at 50° C. A heavy precipitate formed. The flasks were opened to the air and kept at 50° C. until the water evaporated and the complex was dry. Each sample was extracted with several 15 ml. to 25 ml. portions of ethyl ether over a period of 30 hours. The extracted acid was dried and weighed.

A more direct method of determining the dicarboxylic acids in the complex was desired. Quantitative precipitation of the dicarboxylic acids as salts of heavy metals was attempted. Before this could be done the complex had to be disrupted and the carbohydrate portion made soluble. Weighed amounts, about 100 mg. of the sebacic acid complex, were added to 100 ml. of water. The amylose was hydrolyzed by adding 10 ml. of concentrated nitric acid and boiling for 20 minutes. Since the high hydrogen ion concentration completely suppressed the ionization of the weak sebacic acid, the acid was neutralized with ammonium hydroxide. The excess ammonia was removed by boiling. A solution containing 100 mg. of lead

acetate was added to each sample. The solution containing the lead sebacate precipitate was boiled for 15 minutes, cooled to room temperature, and filtered through a weighed sintered glass filter. The precipitate was washed six times with water, dried in an oven at 110° C. for an hour and a half, cooled in a desiccator and weighed.

Difficulties were encountered in attempting to apply the lead acetate method to the determination of adipic acid in the complex. Lead adipate is quite soluble in water. No precipitate formed at 100° C. but on cooling a crystalline precipitate, which filtered very easily, formed. The precipitate, however, did not contain all of the adipic acid which was dissolved in solution. The salt could be precipitated quantitatively from a 50 per cent methanol solution.

Alkyl Halides.

The halogen (iodine or bromine) in the complexes was determined by the micro pearl tube method as given by Johns (21).

Iodine Titration of Mixtures of Amylose and Fatty Acids.

The potentiometric titration method of Bates, French and Rundle (3) was adapted to the determination of the relationship between the amount of fatty acid available for complex formation and the amount of iodine bound by the amylose.

The standard procedure was used except that the fatty acids were introduced into the amylose by adding weighed amounts of them to the 0.5 N potassium hydroxide solution used for dispersing the amylose. Stock solutions were prepared and aliquots were used as needed. Ten milligram samples of amylose were used. It is likely that complexes were formed with the free fatty acids after neutralization with hydrochloric acid

rather than with the soaps, since strongly alkaline solutions do not favor complex formation.

In another series of experiments, amylose which had been impregnated with various amounts of palmitic acid was dispersed in alkali, neutralized and titrated in the standard manner. The data of this experiment are presented in Table VII.

The presence of free fatty acids in the solution during a titration interfered with the operation of the platinum electrode by forming a film on it. This film was removed prior to each determination by ignition in an alcohol flame.

### E. Reaction of Iodine Vapor with Palmitic Acid-Amylose Complex

A sample of palmitic acid-amylose complex containing 8.3 per cent acid was dried to constant weight in an evacuated Abderhalden drying tube at 78° C. The dry weight was 2.817 g. The sample was kept at this temperature in the presence of iodine vapors for eight days. The gain in weight, 0.644 g., determined at the end of the time, represented the weight of iodine taken up. The palmitic acid, no longer tightly bound, was removed by rinsing with carbon tetrachloride. The weight of the acid removed was 0.176 g. and that obtained upon acid hydrolysis of the complex was 0.049 g.

### F. Influence of Temperature on Complex Formation

Five-tenths of a gram of the various dicarboxylic acid was added to 100 ml. of an aqueous solution containing 1.12 grams of amylose. A solution of each was kept at 25°, 50°, 80° and 100° C. for 48 hours. The precipitate was removed by centrifuging. Precautions were taken to prevent temperature changes during the centrifuging. The acids were removed from

the supernatant liquid and the complexes by extraction with ether. The residues were dried and weighed. The data are presented in Table XVI.

### G. Separation of Adipic Acid from Sebacic Acid by means of Complex Formation

Mixtures of the two acids, consisting of 120 mg. each, were added to 500 ml. of solutions containing one gram of amylose. These were kept at 60° C. for six days. The precipitate which formed was removed by centrifuging and the supernatant liquid extracted continuously with ether for 48 hours to remove the unused portion of the acids. Since the only nonvolatile material removed by the ether was the dicarboxylic acids, the most convenient method of determining the relative amounts of the two was by neutral equivalents.

### H. Fractionation of Starch with Potassium Oleate

Ten gram samples of defatted corn starch were dispersed in a liter of water by autoclaving for one hour at 15 pounds pressure and, while still hot, were treated with various amounts of potassium oleate. On standing and allowing to cool over a period of 36 hours, a precipitate similar to the butanol-precipitated starch obtained by Schoch's method (46) was formed. It was slightly more gelatinous and somewhat darker in color. This was removed by centrifuging and the starch in the supernatant liquid precipitated with methanol. Both fractions were dried and Soxhlet extracted with methanol for 24 hours. After thorough drying, the per cent amylose in each fraction was determined by the iodine titration.

## I. Precipitation of Partially Dispersed Starch with Fractionating Agents

One per cent corn starch pastes were prepared by heating the aqueous suspensions at 85° C. for 40 minutes. The naturally occurring fatty acid content of the starch was 0.60 per cent of its weight. The pastes were treated with various amounts of potassium oleate, as shown in Table XI. After thorough stirring of the soap into the paste, the calcium and lead ions (calcium chloride and lead acetate solutions) were introduced in the respective pastes in amounts equivalent to the amount of soap added. In each case, one sample was left untreated. The starch settled out as a flocculent precipitate which was removed by centrifuging, leaving a relatively clear supernatant liquid. Although the iodine coloration of this liquid meant relatively little as a measure of the starch present, a small portion of the liquid was treated with iodine, as a comparison with the work of Kesler and Black (26). The test of the presence of starch in the supernatant liquid was the formation of turbidity or a precipitate on the addition of an equal volume of methanol.

Butanol, thymol (16) and hexamethylene glycol were added to starch pastes prepared in the same manner as above. The amounts of the precipitating agents used were in slight excess of that needed for saturation. After 24 hours the pastes were centrifuged. The completeness of precipitation of starch was determined by adding 3 volumes of methanol to the supernatant liquid and observing the amount of starch precipitated.

# IV. DISCUSSION OF RESULTS

## A. X-Ray Structure of the Fatty Acid Complexes

The complex of amylose with butanol, which has been analyzed fairly successfully from the structural point of view, has a microscopic crystalline structure. The complexes of amylose with fatty acids have now been shown to have a similar micro-crystalline form. Preliminary X-ray diffraction diagrams indicated a close degree of relationship between the structures of the butanol- and fatty acid-amylose complexes. In view of the close similarity of the two crystalline complexes, a detailed study and analysis of the X-ray diffraction patterns seemed appropriate.

### The Effect of Variables on the X-Ray Diagrams.

In order to note the effect of the chain length and unsaturation of the fatty acids upon the structure of the complex, lauric, palmitic, stearic and oleic acids have been used as precipitating agents. The identification diagrams revealed no differences between amylose complexes with the different fatty acids which had been prepared similarly.

It was soon discovered, however, that a significant and interesting change in the X-ray diagrams was caused by drying the samples under different conditions. The two methods of drying were therefore given careful study. Diagrams of samples dried in a relatively poor vacuum were scarcely distinguishable from samples taken directly from the centrifuge, while those given more thorough drying produced diagrams

shifted to larger scattering angles and with some maxima considerably altered in intensity.

Several palmitic acid precipitates were studied in the 10 cm. camera. The $\sin^2\theta$ values reported in the tables below are averages. In general, the variations in $\sin^2\theta$ values in similarly treated palmitic acid precipitates were as great as the variations from fatty acid to fatty acid.

The X-Ray Structures.

The maxima of the wet fatty acid amylose complexes and of samples dried at aspirator pressure are given in Table I. For comparison, the maxima of the dried butanol-precipitated amylose, as reported by Rundle and Edwards (39), are included. The variations in positions of the maxima of the dried fatty acid precipitates are thought to be within the reproducibility of the samples and measurements. The relative intensities of maxima of wet and of dried samples appear to be identical.

The chief difference between the diagrams of the fatty acid-amyloses dried at 70° C. and the dried butanol-precipitated amylose appears to be in the relative intensities of certain of the weak maxima, particularly those with indices (h,k,l) with $l \neq 0$. The fatty acid complex produced more diffuse maxima of this form. In this respect, it is more like the amylose-iodine complex, where maxima with $l \neq 0$ are barely visible. This can be attributed to random arrangement of the fatty acids or iodine molecules parallel to the C axis.

Since the positions of the maxima for the X-ray diagrams of the fatty acid and dried butanol complexes of amylose are very nearly identical, the unit cells of the two are also identical. The unit cell

Table I

X-Ray Data for Amylose-Fatty Acid Precipitates

| Indices | Intensity[b] I | Intensity[b] II | $\sin^2\theta$ (observed)[a] Dried Butanol Ppt. | Oleic Amylose (Dry)[c] | Palmitic Amylose (Dry)[c] | Palmitic Amylose (Wet) | Lauric Amylose (Wet) |
|---|---|---|---|---|---|---|---|
| (110),(020) | M | M | .0042 | .0043 | .0042 | .0042 | .0040 |
| (011) | VW | VW | .0103 | .0106 | .0105 | - | - |
| (200),(130) | S | VS | .0126 | .0126 | .0126 | .0124 | .0124 |
| (210) | MW | M | .0137 | not resolved | .0135 | - | .0134 |
| (040),(220) | VVW | VW | .0166 | .0166 | .0164 | .0162 | .0159 |
| (131),(201) | VW | W | .0216 | .0216 | .0219 | .0215 | - |
| (221),(041) | W | W | .0262 | .0258 | .0261 | .0256 | .0255 |
| (310),(150) | VS | VS | .0295 | .0295 | .0297 | .0290 | .0289 |
| (320) | W | S | .0326 | - | .0320 | - | - |
| (330),(060) | M | MS | .0379 | .0380 | .0378 | .0370 | .0374 |
| (321) | MW | VW | .0420 | - | .0433 | - | - |
| (331),(061) | W | W | .0469 | .0467 | .0469 | - | .0460 |
| (410),(070) | - | VW | .0515 | - | - | - | - |
| (420),(170) | VW | W | .0548 | - | .0547 | - | .0537 |

[a] $\sin^2\theta$ values for $CuK^{\alpha}$ radiation.

[b] Intensity notation: S, strong; M, medium; W, weak; V, very.

[c] Dry in this table refers to samples dried over phosphorus pentoxide at 70° C. in an Abderhalden drying tube evacuated with an aspirator.

I Fatty acid complexes. II Dried butanol precipitate.

Table II

X-Ray Data for Amylose-Fatty Acid Precipitates (Dry)[a]

| Indices | Intensity[c] | $Sin^2\theta$ (Observed)[b] Lauric Amylose | Palmitic Amylose | Stearic Amylose | Amylose Iodine Complex[e] | $Sin^2\theta$ (Calculated)[d] |
|---|---|---|---|---|---|---|
| (110) | M | .0046 | .0046 | .0046 | .0047 | .0046 |
| (011) | VVW | - | .0109 | - | - | .0102 |
| (200) | S | .0137 | .0137 | .0138 | .0140 | .0138 |
| (040) | VVW | .0177 | - | .0179 | .0188 | .0179 |
| (131) | VW | .0234 | .0229 | .0226 | .0236 | .0226 |
| (221) | W | .0275 | .0275 | - | .0274 | .0274 |
| (310) | VS | .0324 | .0323 | .0323 | .0328 | .0323 |
| (330) | VW & diffuse | - | .0404 | .0412 | .0422 | .0412 |
| (331) | W | - | .0503 | .0503 | .0519 | .0503 |

[a]Dried at 100° C. over phosphorus pentoxide at a pressure below 1 mm.

[b]$Sin^2\theta$ values are for $CuK\alpha$ radiation.

[c]Intensity notation: S, strong; M, medium; W, weak; V, very.

[d]Calculated $sin^2\theta$ values are for the fatty acid precipitates, based on an orthorhombic unit, $a_o$ = 13.0 Å, $b_o$ = 25.0 Å, $c_o$ = 8.05 Å.

[e]Ref. (42)

for butanol-precipitated amylose has been reported to be orthorhombic, with dimensions $a_o$ = 13.7 Å, $b_o$ = 23.8 Å, and $c_o$ = 8.05 Å (39).

The maxima for three different samples of more thoroughly dried fatty acid-amylose are given in Table II. These were portions of the same fatty acid complex preparation whose maxima were reported in Table I, except that these were dried at 100° C. and at a much lower pressure. The X-ray lines have definitely shifted to larger angles. The X-ray diagram is now very similar to that of the amylose-iodine complex shown

in the sixth column of the same table (42). Though the maxima can no longer be indexed on a hexagonal basis, since the axial ratio $b_o/a_o$ is 1.77 instead of 1.75 as required for an ortho-hexagonal unit, the positions of the lines are such as to make easy correlation with the maxima from the iodine complex and the intensities and positions are still related to the complexes listed in Table I. On the basis of this relationship, a very similar orthorhombic unit has been chosen for the dry fatty acid-amyloses with dimensions $a_o$ = 13.0 Å, $b_o$ = 23.0 Å, and $c_o$ = 8.05 Å. It should be noted that the corresponding lines have the same indices for all these complexes. The X-ray diagrams clearly indicate a structure for the fatty acid-amylose complex very similar to that of the butanol and of the iodine complexes.

The amylose helices must be packed in the unit cells of the fatty acid-amylose complexes in a manner quite like that reported for the iodine complex and the butanol complex by Rundle and French (41) (42), and Rundle and Edwards (39). In the pseudo-hexagonal cell of the dried fatty acid-amylose complexes, there are four helices, two in the orthorhombic unit. The nearly circular tubes are close packed. In the wet and in the partially dried samples, the amylose helix has a diameter of 13.7 Å. In the more thoroughly dried complex, the helix has a diameter of 13.0 Å, equal to that found for the helix in the amylose-iodine complex (39).

## B. The Influence of Fatty Acids on the Formation of the Amylose-Iodine Complex

Schoch and Williams (47) noted that small amounts of fatty acids repress the iodine binding power of corn starch. This repression was shown to increase as the fatty acid content of the starch increased. A 10 per cent fatty acid content was sufficient to totally repress the iodine binding power of amylose. An investigation of the stoichiometry involved in this interaction and the factors which favor or hinder this repression would certainly be useful in interpreting the nature of the fatty acid-amylose complex.

The results of iodine titrations of amylose solutions to which varying amounts of oleic, lauric, palmitic and stearic acids were added are given in Tables III, IV, V and VI and are shown graphically in Figures 1, 2, 3 and 4 respectively. Results of iodine titrations of amylose samples which had been impregnated with different amounts of palmitic acid are given in Table VII. A summary showing the relationship between the palmitic, oleic and lauric acid content of amylose and the iodine taken up by the amylose is given in Table VIII and is shown graphically in Figure 5.

Butyric acid had no effect on the titrations while caproic acid had only a very slight effect. The presence of an unsaturated bond in oleic acid had no apparent effect on the potentiometric measurements.

Effect of Different Acids on Amylose-Iodine Complex Formation.

An inspection of the figures shows that not all acids produce the same effect. For the same weight of acid, the effect for either lauric or oleic acid was twice that of palmitic acid and several times that of stearic acid. Apparently there is no direct relation between the length

Table III

Titration of Amylose to which Varying Amounts of Oleic Acid Have Been Added

| Iodine Added[1] | E.M.F.[2] Oleic Acid Added[3] 0.00% | 1.73% | 3.46% | 5.19% | 6.92% |
|---|---|---|---|---|---|
| 0 | 0.1890 | 0.1877 | 0.1890 | 0.1874 | 0.1854 |
| 1 | 0.1905 | 0.1906 | 0.1893 | 0.1899 | 0.1880 |
| 2 | 0.1938 | 0.1925 | 0.1923 | 0.1928 | 0.1983 |
| 3 | 0.1950 | 0.1934 | 0.1945 | 0.1957 | 0.2070 |
| 4 | 0.1956 | 0.1944 | 0.1969 | 0.2000 | 0.2124 |
| 5 | 0.1962 | 0.1948 | 0.1988 | 0.2059 | 0.2165 |
| 6 | 0.1966 | 0.1962 | 0.2008 | 0.2123 | 0.2194 |
| 7 | 0.1968 | 0.1972 | 0.2036 | 0.2169 | 0.2216 |
| 8 | 0.1972 | 0.1980 | 0.2076 | 0.2202 | 0.2238 |
| 9 | 0.1975 | 0.1994 | 0.2147 | 0.2230 | 0.2261 |
| 10 | 0.1981 | 0.2014 | 0.2197 | 0.2254 | 0.2278 |
| 11 | 0.1985 | 0.2040 | 0.2235 | -- | 0.2294 |
| 12 | 0.1998 | 0.2099 | 0.2267 | 0.2296 | 0.2313 |
| 13 | 0.2009 | 0.2171 | -- | -- | -- |
| 14 | 0.2023 | 0.2229 | 0.2316 | 0.2333 | 0.2344 |
| 15 | 0.2046 | 0.2271 | -- | -- | -- |
| 16 | 0.2080 | 0.2303 | 0.2352 | 0.2362 | 0.2374 |
| 17 | 0.2148 | -- | -- | -- | -- |
| 17.5 | -- | 0.2342 | -- | -- | -- |
| 18 | 0.2215 | -- | 0.2384 | 0.2392 | 0.2398 |
| 19 | 0.2272 | 0.2371 | -- | -- | -- |
| 20 | 0.2314 | -- | 0.2410 | 0.2414 | 0.2422 |
| 20.5 | -- | 0.2416 | -- | -- | -- |

[1] Milliliters of 0.001 N solution

[2] In volts, referred to the normal calomel electrode

[3] Percentage based on weight of amylose

Table IV

Titration of Amylose to Which Varying Amounts of Lauric Acid Have Been Added

| Iodine Added[1] | E.M.F.[2] Lauric Acid Added[3] | | | | |
|---|---|---|---|---|---|
| | 0.00% | 1.73% | 3.46% | 5.19% | 6.92% |
| 0 | 0.1890 | 0.1903 | 0.1885 | 0.1885 | 0.1880 |
| 1 | 0.1906 | 0.1911 | 0.1891 | 0.1913 | 0.1917 |
| 2 | 0.1938 | 0.1919 | 0.1907 | 0.1990 | 0.1984 |
| 3 | 0.1950 | 0.1928 | 0.1919 | 0.2051 | 0.2045 |
| 4 | 0.1956 | 0.1936 | 0.1932 | 0.2098 | 0.2100 |
| 5 | 0.1962 | 0.1940 | 0.1944 | 0.2135 | 0.2125 |
| 6 | 0.1966 | 0.1944 | 0.1968 | 0.2165 | 0.2160 |
| 7 | 0.1968 | 0.1949 | 0.1990 | 0.2197 | 0.2188 |
| 8 | 0.1972 | 0.1957 | 0.2019 | 0.2222 | 0.2216 |
| 9 | 0.1975 | 0.1970 | 0.2064 | 0.2246 | 0.2241 |
| 10 | 0.1981 | 0.1967 | 0.2110 | 0.2267 | 0.2263 |
| 11 | 0.1985 | 0.2009 | 0.2150 | — | — |
| 12 | 0.1998 | 0.2054 | 0.2187 | — | — |
| 13 | 0.2009 | 0.2122 | 0.2219 | — | — |
| 14 | 0.2023 | 0.2172 | 0.2251 | — | — |
| 15 | 0.2046 | 0.2216 | 0.2280 | — | — |
| 16 | 0.2080 | 0.2247 | — | — | — |
| 17 | 0.2149 | — | — | — | — |
| 18 | 0.2215 | — | — | — | — |
| 19 | 0.2272 | — | — | — | — |
| 20 | 0.2314 | — | — | — | — |

[1] Milliliters of 0.001 N solution

[2] In volts, referred to the normal calomel electrode

[3] Percentage based on weight of amylose

Table V

Titration of Amylose to Which Varying Amounts of Palmitic Acid Have Been Added

| Iodine Added[1] | E.M.F.[2] | | | | |
|---|---|---|---|---|---|
| | Palmitic Acid Added[3] | | | | |
| | 0.00% | 3.46% | 6.92% | 10.38% | 13.84% |
| 0 | 0.1890 | 0.1888 | 0.1891 | 0.1880 | 0.1880 |
| 1 | 0.1905 | 0.1924 | 0.1924 | 0.1913 | 0.1917 |
| 2 | 0.1938 | 0.1942 | 0.1940 | 0.1934 | 0.1972 |
| 3 | 0.1950 | 0.1954 | 0.1954 | 0.1961 | 0.2035 |
| 4 | 0.1956 | 0.1958 | 0.1964 | 0.1994 | 0.2106 |
| 5 | 0.1962 | 0.1967 | 0.1970 | 0.2041 | 0.2158 |
| 6 | 0.1966 | 0.1971 | 0.1994 | 0.2099 | 0.2177 |
| 7 | 0.1968 | 0.1978 | 0.2017 | 0.2165 | 0.2205 |
| 8 | 0.1972 | 0.1992 | 0.2048 | 0.2210 | 0.2229 |
| 9 | 0.1975 | 0.2004 | 0.2094 | 0.2238 | 0.2246 |
| 10 | 0.1981 | 0.2024 | 0.2165 | — | — |
| 11 | 0.1985 | 0.2051 | 0.2228 | — | — |
| 12 | 0.1998 | 0.2094 | 0.2268 | — | — |
| 13 | 0.2009 | 0.2177 | 0.2299 | — | — |
| 14 | 0.2023 | 0.2239 | — | — | — |
| 15 | 0.2046 | 0.2283 | — | — | — |
| 16 | 0.2080 | 0.2318 | — | — | — |
| 17 | 0.2149 | — | — | — | — |
| 18 | 0.2215 | — | — | — | — |
| 19 | 0.2272 | — | — | — | — |
| 20 | 0.2314 | — | — | — | — |

[1] Milliliters of 0.001 N solution

[2] In volts, referred to the normal calomel electrode

[3] Percentage based on weight of amylose

Table VI

Titration of Amylose to Which Varying Amounts of Stearic Acid Have Been Added

| Iodine Added[1] | E.M.F.[2] Stearic Acid Added[3] | | |
|---|---|---|---|
| | 0.00% | 3.46% | 6.92% |
| 0 | 0.1890 | 0.1875 | 0.1827 |
| 1 | 0.1905 | 0.1915 | 0.1898 |
| 2 | 0.1938 | 0.1938 | 0.1925 |
| 3 | 0.1950 | 0.1952 | 0.1940 |
| 4 | 0.1956 | 0.1959 | 0.1954 |
| 5 | 0.1962 | 0.1964 | 0.1961 |
| 6 | 0.1966 | 0.1967 | 0.1969 |
| 7 | 0.1968 | 0.1974 | 0.1978 |
| 8 | 0.1972 | 0.1983 | 0.1989 |
| 9 | 0.1975 | 0.1989 | 0.2000 |
| 10 | 0.1981 | 0.1997 | 0.2015 |
| 11 | 0.1985 | 0.2008 | 0.2033 |
| 12 | 0.1998 | 0.2024 | 0.2061 |
| 13 | 0.2009 | 0.2044 | 0.2109 |
| 14 | 0.2023 | 0.2069 | 0.2182 |
| 15 | 0.2046 | 0.2120 | 0.2238 |
| 16 | 0.2080 | 0.2195 | 0.2281 |
| 17 | 0.2149 | 0.2248 | 0.2313 |
| 18 | 0.2215 | 0.2292 | 0.2342 |
| 19 | 0.2272 | 0.2328 | 0.2365 |
| 20 | 0.2314 | — | 0.2384 |

[1] Milliliters of 0.001 N solution

[2] In volts, referred to the normal calomel electrode

[3] Percentage based on weight of amylose

Table VII

Titration of Amylose-Palmitic Acid Complexes

| Iodine Added[1] | E.M.F.[2] | | | |
|---|---|---|---|---|
| | Palmitic Acid Content[3] | | | |
| | 2.0% | 2.9% | 4.0% | 4.9% |
| 0 | 0.1887 | 0.1893 | 0.1885 | 0.1869 |
| 1 | 0.1904 | 0.1927 | 0.1916 | 0.1908 |
| 2 | 0.1928 | 0.1936 | 0.1929 | 0.1922 |
| 3 | 0.1932 | 0.1948 | 0.1946 | 0.1968 |
| 4 | 0.1940 | 0.1957 | 0.1967 | 0.2040 |
| 5 | 0.1947 | 0.1969 | 0.1995 | 0.2091 |
| 6 | 0.1959 | 0.1981 | 0.2061 | 0.2127 |
| 7 | 0.1970 | 0.1995 | 0.2133 | 0.2158 |
| 8 | 0.1981 | 0.2026 | 0.2182 | |
| 9 | 0.1992 | 0.2067 | 0.2219 | |
| 10 | 0.2017 | 0.2127 | | |
| 11 | 0.2048 | 0.2166 | | |
| 12 | 0.2102 | 0.2201 | | |
| 13 | 0.2165 | 0.2229 | | |
| 14 | 0.2225 | 0.2248 | | |
| 15 | 0.2070 | | | |
| 16 | 0.2301 | | | |
| 17 | 0.2330 | | | |

[1] Milliliters of 0.001 N solution

[2] In volts, referred to the normal calomel electrode

[3] Percentage based on weight of amylose

Table VIII

Relationship between Fatty Acid Content and Iodine Taken up by Amylose

(Percentages based on weight of amylose)

| % Fatty Acid | % Iodine Taken Up | | |
|---|---|---|---|
| | Palmitic[1] | Oleic[2] | Lauric[2] |
| 1.73 | — | 12.9 | 11.9 |
| 2.0 | 12.7 | — | — |
| 2.9 | 10.1 | — | — |
| 3.46 | — | 8.7 | 7.5 |
| 4.0 | 6.6 | — | — |
| 4.9 | 3.9 | — | — |
| 5.19 | — | 4.5 | 2.8 |

[1]Palmitic acid introduced by impregnation of dry amylose, data of Table VII.

[2]Oleic and lauric acids introduced in alkali dispersion of amylose, data of Tables III and IV.

of the carbon chain in the acid and the interference with the amylose-iodine reaction.

Since the only variable in the titrations was the use of different fatty acids, one could assume that the change in conditions in the systems was due to one or more properties of the acids. The results of titrations of amylose into which varying amounts of palmitic acid were introduced by refluxing with methanol solutions of the acid are included in Figure 5. From these results, it is noted that palmitic acid will cause the same hindrance to the amylose-iodine complex formation as oleic and lauric acids, provided it has sufficient opportunity to interact with the amylose. As the solubility of the higher fatty acids decreased,

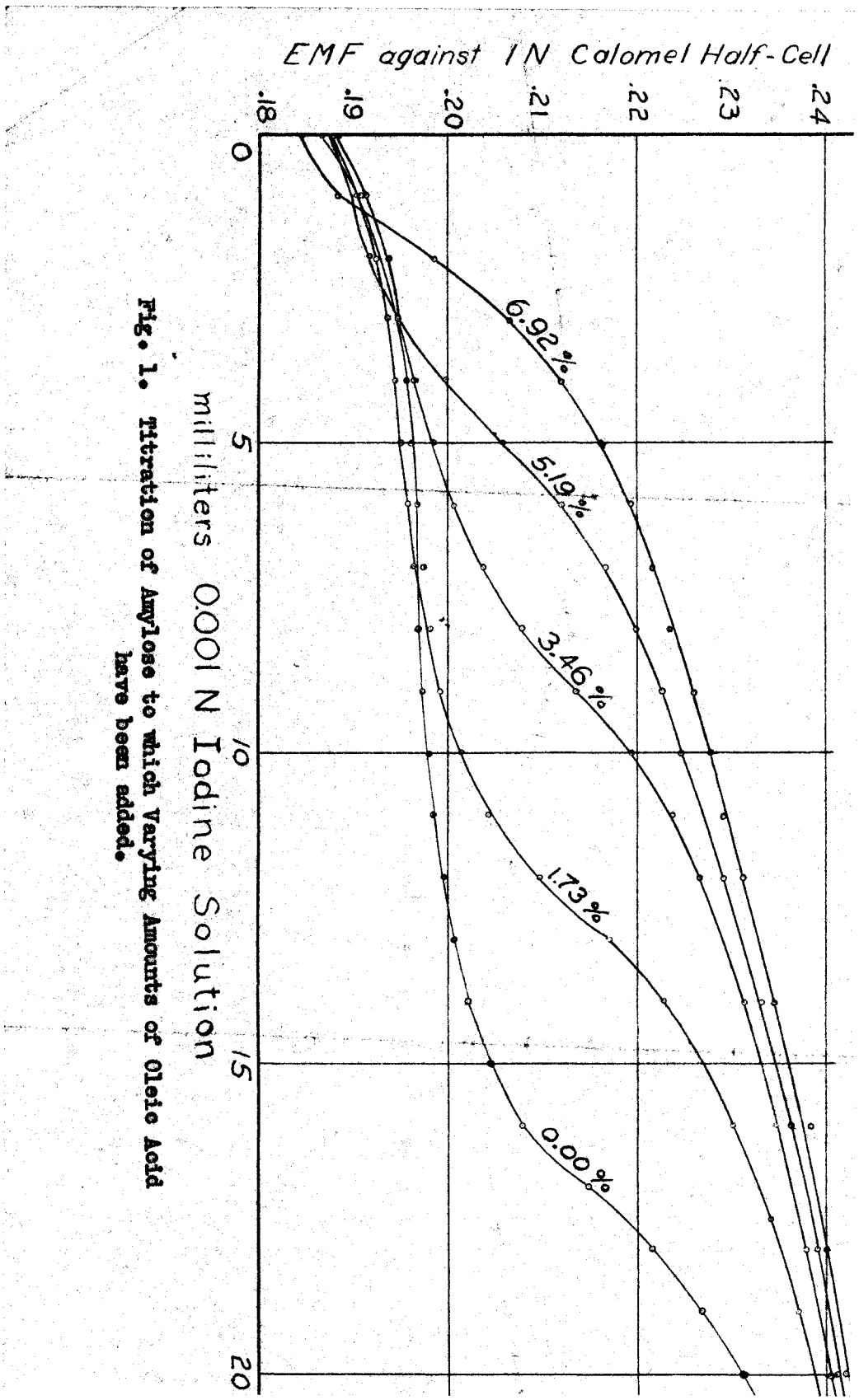


**Fig. 1. Titration of Amylose to which Varying Amounts of Oleic Acid have been added.**

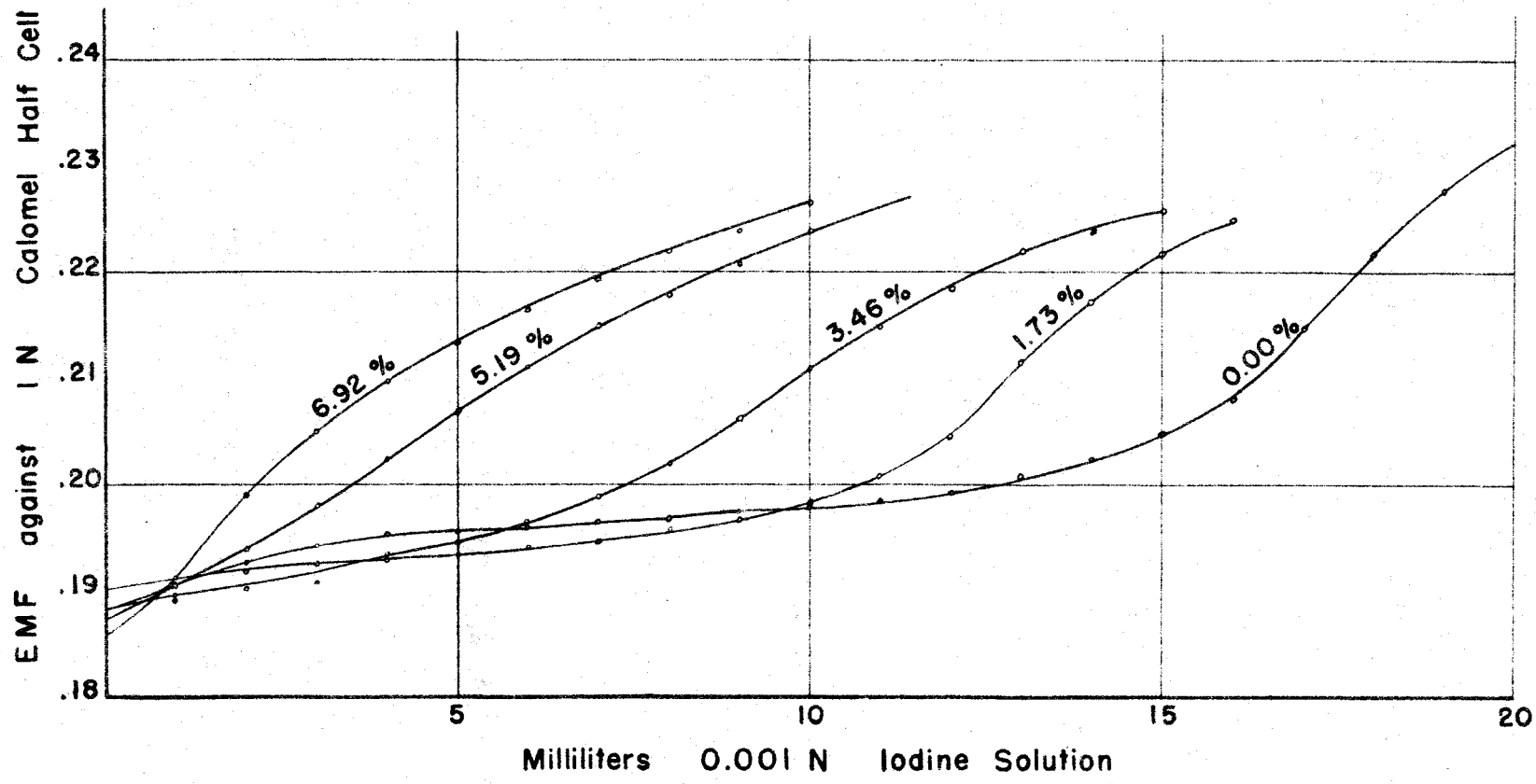


**Fig. 2. Titration of Amylose to which Varying Amounts of Lauric Acid have been added.**

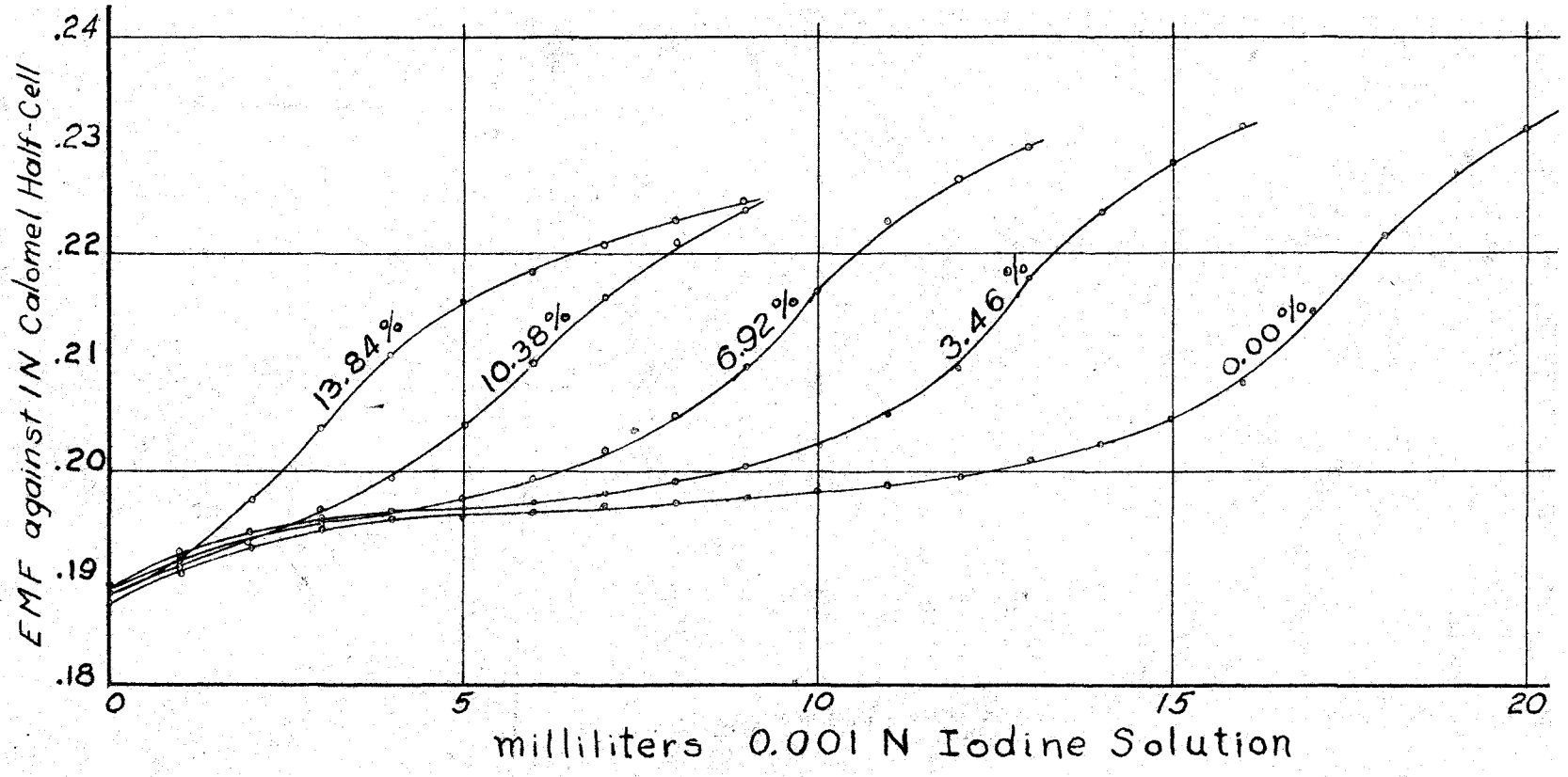


**Fig. 3. Titration of Amylose to which Varying Amounts of Palmitic Acid have been added.**

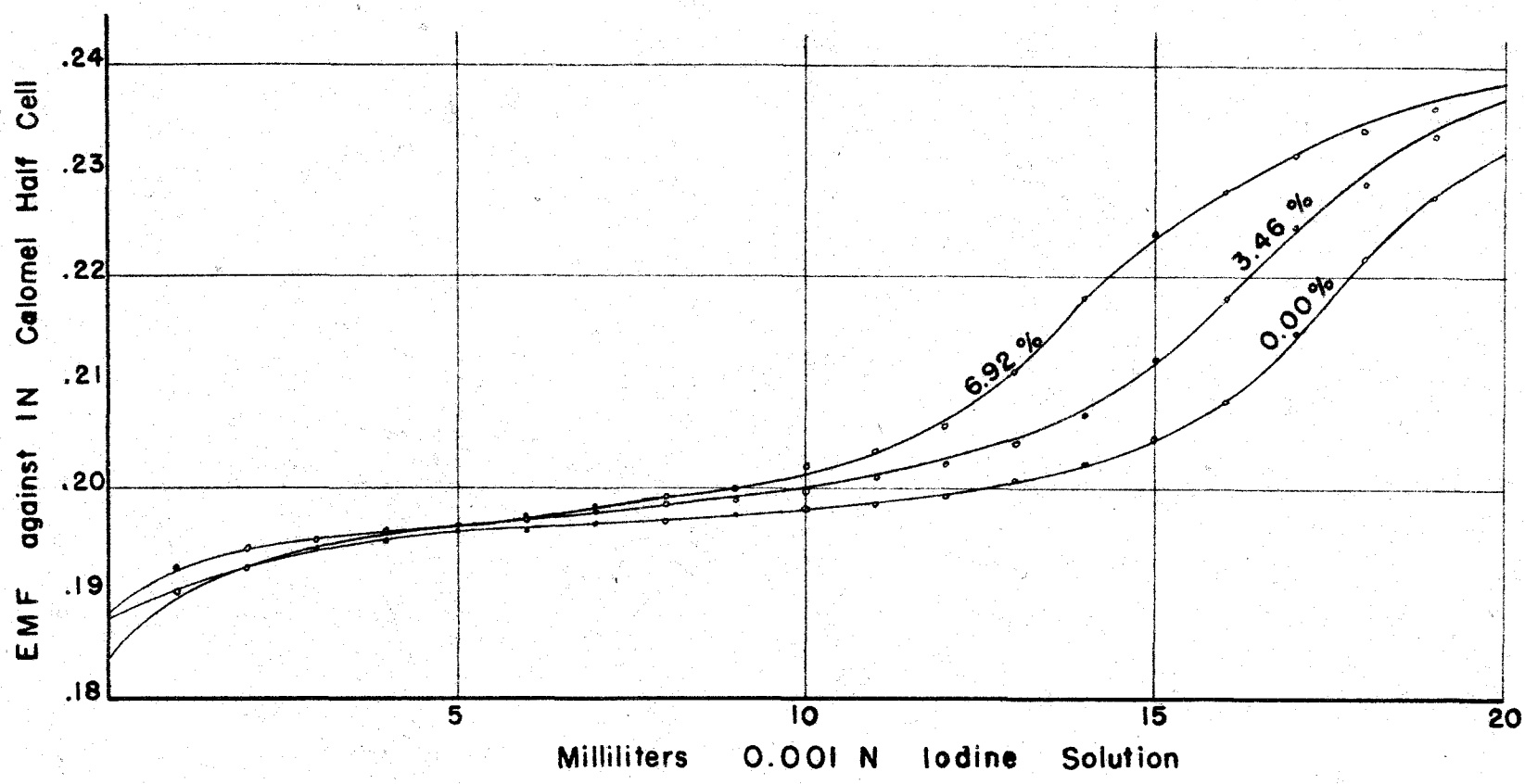


Fig. 4. Titration of Amylose to which Varying Amounts of Stearic Acid have been added.

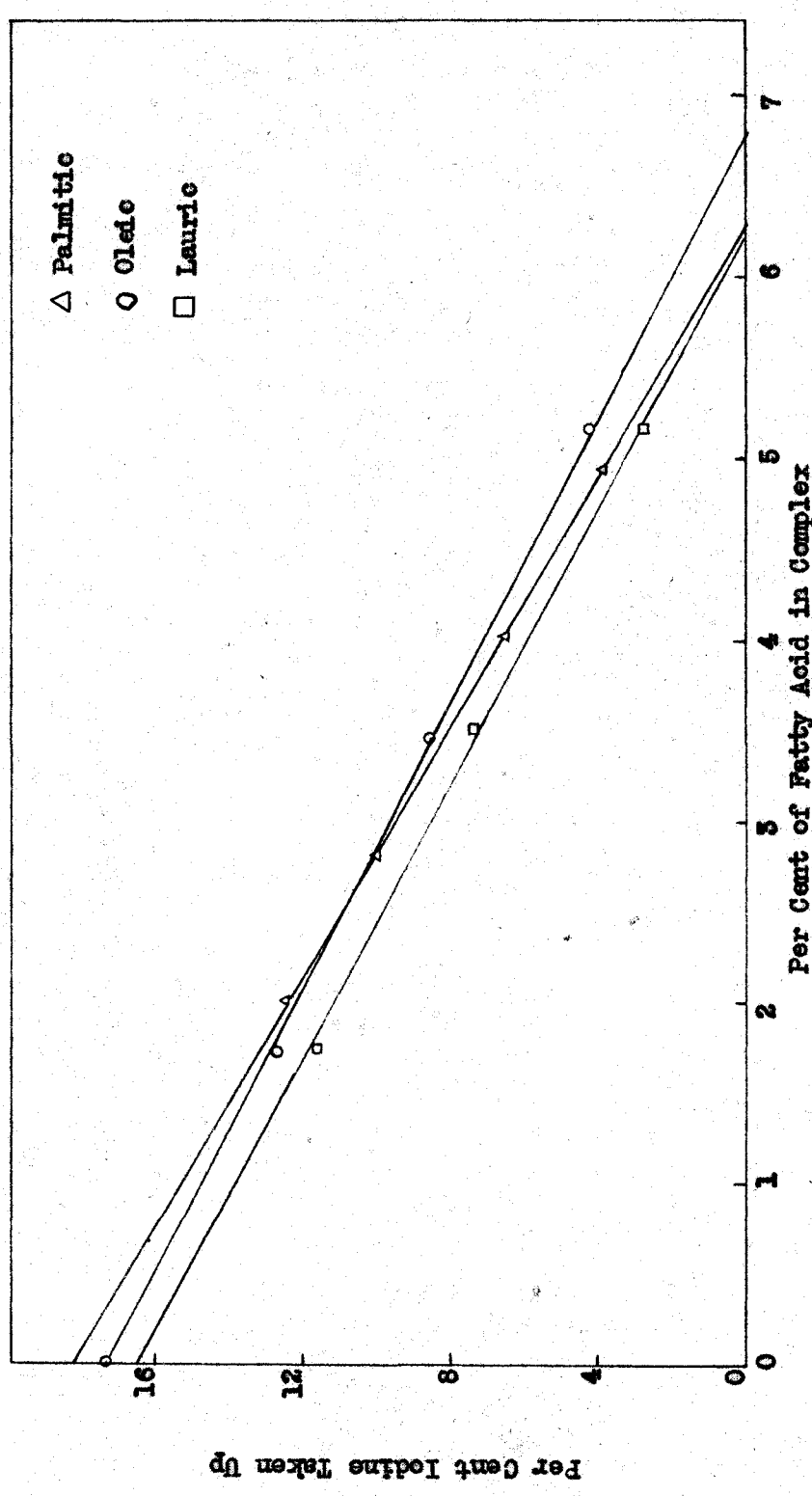


Fig. 5. Binding of Iodine by Amylose-Fatty Acid Complexes Containing Varying Amounts of Fatty Acid.

the effect on the iodine-binding power of amylose decreased. Stearic acid, which was the most insoluble, had the least effect.

Nature of Interaction and Its Significance.

The stoichiometry of the interaction between fatty acids and amylose is not a simple one. The linear nature of the relation between the per cent by weight of fatty acid in the amylose complex and the amount of iodine taken up by the amylose is clearly seen in Figure 5. The intercepts on the abscissa give the amounts of fatty acids needed for complete interaction with the amylose.

From the fact that the presence of higher fatty acids in starch solutions or suspensions prevents the formation of the amylose-iodine complex, it seems reasonable to believe that the position of the fatty acids and iodine relative to the amylose in their respective complexes may be the same. If the fatty acid molecule is within the amylose helix as already indicated from X-ray data of the preceding section, there should be a direct relation between the length of the fatty acid molecule and the length of the amylose helix associated with each acid molecule. This relationship, based on the data given in Figure 5, is shown in Table IX.

The distances and angles used in calculating the length of the fully extended fatty acid molecules were those reported by Pauling (35). The apparent length of helix associated with each acid molecule was found by multiplying the number of glucose residues per acid molecule by 8 Å (the length of a turn in the helix) and dividing by six (the number of glucose residues per turn).

The longer the acid molecule the more amylose it binds. From the correlation between the apparent length of the helix and the length of

Table IX

Relationship between Length of Acid Molecules and Amylose Helices Associated with Them

| | | Lauric | Palmitic | Oleic |
|---|---|---|---|---|
| 1. | Length of extended acid molecule[a] | 19.0 Å | 24.0 Å | 27.0 Å |
| 2. | Glucose residues per acid molecule | 17.6 | 22.6 | 25.0 |
| 3. | Length of amylose helix per acid molecule | 23.5 Å | 30.0 Å | 33.0 Å |
| 4. | Ratio (3)/(1) | 1.24 | 1.26 | 1.22 |
| 5. | Corrected ratio (3)/(1)[b] | 1.12 | 1.13 | 1.10 |

[a]Including Van der Waal's distances

[b]Corrected for "impurity" in amylose

the acid molecules, the most logical spatial arrangement would be one in which the acid molecules lie within the helix with their long axes parallel to the long axis of the amylose helix. The experimental binding power is from 88 per cent to 91 per cent of the fatty acid capacity calculated on the basis of a helical structure. The close agreement of the three values obtained adds to the evidence for the correctness of the hypothesis.

## C. Certain Aspects of the Interaction between Amylose and the Fatty Acids and their Relationship to the Helical Hypothesis

It has frequently been argued by starch workers who do not favor the helical hypothesis that such a concept is unnecessary in explaining typical starch complex formation reactions. In this section are shown the results of certain experiments and their relative bearing on the helical hypothesis as opposed to adsorption in complex formation.

Displacement of Palmitic Acid from the Amylose Complex by Iodine.

In the previous section it was shown that the iodine-binding power of amylose decreases linearly with an increase in fatty acid content. By considering the effect of iodine vapor on dry palmitic acid-amylose complex, one can see that a parallel relationship holds, i.e., that iodine reacts with the fatty acid complex to liberate an amount of fatty acid which is equivalent (in the spatial or steric sense) to the amount of iodine which enters into the structure.

The weight gained by the palmitic acid-amylose complex on prolonged treatment with iodine vapor was equivalent to 20.8 per cent of the weight of the amylose in the sample or about 78 per cent of the theoretical amount of iodine the amylose could take up within its helices. Seventy-five per cent of the palmitic acid initially in the complex was removed by washing with carbon tetrachloride. The remainder was bound more tightly and was removed only after hydrolysis of the amylose. The bound acid amounted to 1.82 per cent of the original weight of the amylose or 21.8 per cent of the palmitic acid originally in the complex. Since carbon tetrachloride does not extract an appreciable amount of fatty acids from the amylose complexes even upon prolonged treatment, it is believed that the removal was caused by the iodine treatment.

The results given above show that 75 per cent of the palmitic acid in the complex was displaced by iodine and that the amount of iodine needed for this displacement was 78 per cent of the total capacity of the amylose for iodine. This proportionate displacement of fatty acids by iodine is further evidence that the iodine and fatty acids occupy the same position in their respective amylose complexes.

The reversibility of the reaction,

Amylose . $I_2$(s) + Fatty Acid $\rightleftharpoons$ Amylose . Fatty Acid(s) + $I_2$

is clearly demonstrated. In aqueous solutions the equilibrium lies far to the right, whereas with dry solids and iodine in the vapor phase the equilibrium may be shifted in the opposite direction. It is to be noted that the activity of the iodine in the vapor phase at 78° C. is considerably greater than the activity of the iodine in the solution used in the iodine titration. Since the iodine must displace the fatty acids before it can assume its position within the structure, one would expect the reaction to be fairly slow. This was found to be the case, for after eight days the amylose was still gaining weight.

Relative Importance of Crystalline Configuration and Surface.

If the reaction of fatty acids with starch depended upon a surface type of adsorption, one would expect that the amount of fatty acids with which a given starch sample would react might be (1) directly proportional to surface area, (2) independent of the submicroscopic crystalline structure and (3) independent of the degree of branching in the starch sample. Experiments which have been carried out in this work have shown that none of these expectations is fulfilled.

Reference to Table X indicates that the binding of fatty acids by amylopectin and amylose in the "B" configuration (22) is insignificant when compared with the binding of fatty acids by amylose in the "V" configuration.

The experimental errors in the results given in Table X seem to be rather large. These variations are possibly due to differences in degrees of crystallinity.

Table X

Binding of Palmitic Acid by Amylose and Amylopectin

| Grams of Palmitic Acid | Palmitic Acid Bound[a] | | | | |
|---|---|---|---|---|---|
| | Amylose in "V" Configuration | | Amylose in "B" Configuration | Amylopectin | |
| | Trial 1 | Trial 2 | | Trial 1 | Trial 2 |
| 0.2 | 0.84 | 1.09 | 0.23 | 0.10 | - |
| 0.5 | 1.53 | 1.84 | 0.23 | 0.15 | 0.07 |
| 1.0 | 1.94 | 2.14 | 0.29 | 0.07 | 0.07 |
| 1.5 | 2.95 | 3.26 | 0.22 | 0.21 | - |
| 2.0 | 2.90 | 3.55 | 0.19 | 0.05 | 0.12 |
| 3.0 | 4.05 | 3.80 | 0.18 | 0.15 | - |
| 4.0 | 4.02 | 4.03 | 0.18 | 0.21 | 0.21 |

[a]Expressed as per cent by weight of amylose.

The configuration in which dry amylose is found is seen to be the most important factor in determining whether or not it will bind fatty acids. Analyses of X-ray diagrams indicate that in the "V" configuration the amylose chains are helical, while retrograded ("B") amylose consists of extended chains. It is in this form that amylose separates from solution in the absence of complexing agents.

Were surface the important factor in influencing the amount of fatty acids taken up, it would most probably manifest itself as adsorption. Adsorption cannot be used satisfactorily to explain the variations found and reported in Table X. The surface exposed to the fatty acids was certainly very nearly the same. Amylose precipitated with butanol in a form twice as voluminous and, as a result, exposing considerably more surface, took up a like amount of palmitic acid under identical treatments. Had this been a surface phenomena, a marked difference

should have been noticed.

Since Schoch's observation that the bulk of the fatty acids associated with granular starch can be removed by certain polar solvents such as alcohols, dioxane and cellosolves (43), adsorption has been gaining favor as an explanation for the interaction between granular starch and the fatty acids. This explanation does not seem satisfactory. Lehrman (29) pointed out a relation between the surface of starch granules and the amount of fatty acids taken up from a methanol solution. For starch with large granules, the amount of surface needed is greater than that offered by the granules. A large part of the fatty acids must be within the granules.

The removal of fatty acids from helices leaves the helices undisturbed and capable of combining with fatty acids again. In the case of potato starch, which contains essentially no fatty acids, it is probable that under the influence of heat and the reagents used in the introduction of the fatty acids, very nearly free chains can be induced to alter their configuration. More drastic conditions should cause a greater number of these chains to take on a helical configuration in the presence of a complexing agent. Prolonged treatment of retrograded starch will cause it to increase the amount of fatty acids it will take up. The number of free chains in retrograded amylose is expected to be considerably smaller than in granular starch.

The small amount of fatty acids taken up by amylopectin and the small amount of fatty acids found in the granules of waxy corn starch (which is essentially amylopectin), can be explained very well by the helical hypothesis. Amylopectin, being highly branched, is not capable of forming helices; hence it is not capable of binding the fatty acids to any appreciable extent.

Effect of Solvent.

Carbon tetrachloride is considered to be an extremely poor solvent for the removal of fatty acids from starch complexes or for the reintroduction of fatty acids into defatted starch. However, it has been found in the present study that it is possible to introduce fatty acids into helical amylose from carbon tetrachloride solutions.

Amylose bound 1.2 per cent of its weight of palmitic acid from a carbon tetrachloride solution upon refluxing for two weeks. There was no indication that equilibrium was reached even after that time. Equilibrium is attained very slowly, as seen from the fact that, after brief treatments (12 hours or less), the amount bound was too small to measure with any degree of accuracy. It has been noted that approaching equilibrium from the other direction is even slower, since prolonged extraction of the fatty acid complex by carbon tetrachloride removes only traces of fatty acids. The slowness of this reaction is probably due to the poor wetting and slow penetration of amylose by carbon tetrachloride.

Methanol, on the other hand, is a good extractant. Relatively large concentrations of fatty acids are necessary for the reintroduction of fatty acids into the starch in helical form from methanol solutions, as shown in Table X. Even though carbon tetrachloride is nearly ineffective in the extraction of fatty acids, small fatty acid concentrations in the solvent permit the introduction of fatty acids into helical amylose.

In addition to being able to dissolve fatty acids, a solvent must be capable of displacing the acids from the complexes. Apparently methanol is capable of entering the helices of amylose and competing with the fatty acids, whereas the carbon tetrachloride is not. Methanol is known to form a complex with amylose (57). Because of the lack of

binding power, non-polar solvents offer little or no competition for the space within the helices.

As seen from Table X, the amount of fatty acid reintroduced is a function of the fatty acid concentration. If the methanol complex and the fatty acid complex are independent phases in the reaction,

Fatty Acid + Amylose . MeOH(s) $\rightleftharpoons$ Amylose . Fatty Acid (s) + MeOH

the equilibrium constant would be $K_{eq} = 1/(A)$ (Fatty Acid), and independent of the amount of methanol in the amylose complex. It seems very probable that there is but one complex phase over a wide range of fatty acid and methanol contents of the amylose.

Since carbon tetrachloride cannot enter the helix, the reaction between the fatty acid and the amylose in that solvent can be represented by the equation

Amylose(s) + Fatty Acid $\rightleftharpoons$ Amylose . Fatty Acid(s).

Assuming that amylose and amylose-fatty acid complex are two separate phases, the fatty acid activity in the complex should be independent of fatty acid content until saturation of the amylose is reached. Extraction experiments indicate that the activity of fatty acids in the complex is very low, even when the amylose is saturated with fatty acids.

## D. The Soap-Amylose Complex

Since soap has been used to precipitate starch from its aqueous suspension in the manufacture of paper (25) (26), it was of interest to study some of the conditions under which the precipitation was carried out and interpret the findings in the light of what has been learned about the fatty acid- and the soap-amylose complexes. It is known that fatty acids will form complexes with the amylose fraction but not with

the amylopectin, yet Kesler and Black (26) reported that soap, added to partially dispersed starch, did not precipitate only the amylose fraction as one might expect, but rather insolubilized and precipitated both fractions.

Table XI shows that a small amount of soap, 1.2 per cent of the weight of starch, can precipitate all of the starch from a starch paste prepared by heating the mixture at 85° C. for 40 minutes. It is to be noted that the addition of calcium ions has very little, if any, effect on this precipitation and that the addition of the lead ions actually decreases the amount precipitated. This is probably due to greater insolubility of the lead soap as compared with that of the calcium soap. It will be remembered that the more insoluble fatty acids do not form the amylose-fatty acid complex as easily as the more soluble ones. Apparently soaps and fatty acids must not be present in too insoluble a form for optimum complex formation.

The results in Table XII indicate that fractionation occurs when soap is added to completely dispersed starch solutions. The iodine complexing power of the two fractions is considerably different. Although a considerable amount of amylopectin is brought down with the soap-precipitated fraction, that amount is far less than the amount indicated in Table XI. The fact that the percentages of amylose in the total starch recovered amount to approximately the amylose content of the corn starch, indicates that the greater portion of the soap was removed prior to the iodine titrations.

A comparison of the results of Tables XI and XII shows the effect of dispersion on the precipitation of starches with potassium oleate. The lower the degree of disintegration of the starch granules the greater the

Table XI

Precipitation of Corn Starch Pastes

| % Potassium Oleate Added | Cation Added | pH of Solution | Supernatant Liquid: Iodine Coloration | Supernatant Liquid: Precipitation with Methanol 50% Solution |
|---|---|---|---|---|
| | none | 6.6 | slightly purple | slightly turbid |
| 0.20% | Ca | 6.3 | purple | very slightly turbid |
| | Pb | 6.4 | blue | milky |
| | none | 6.6 | purple | very slightly turbid |
| 0.60% | Ca | 6.6 | purple | very slightly turbid |
| | Pb | 6.1 | blue | turbid |
| | none | 6.7 | purple | clear |
| 1.20% | Ca | 6.5 | purple | clear |
| | Pb | 6.1 | blue | slightly turbid |

amount of starch removed from suspensions. The soap interacts with the free chains of amylose extending from the fragments of partially disrupted granules to form the complex. Only such amylopectin or small fragments of granules which are not held to some amylose free to interact with soap will remain in solution.

Further evidence for the above explanation was obtained when other substances known to form complexes with amylose and not with amylopectin were added to the partially dispersed starch pastes. In every case almost complete removal of the starch was achieved. This behavior also indicates that the amylose is distributed throughout the granules. Apparently the

Table XII

Fractionation of Starch by Soap Precipitation

| Grams Soap Added | Grams Recovered | | % Amylose | | Grams Amylose Recovered | % Amylose in Recovered Material |
|---|---|---|---|---|---|---|
| | I | II | I | II | | |
| 0.525 | 0.50 | 5.72 | 73 | 28.0 | 1.91 | 30.8 |
| 1.05 | 1.83 | 7.40 | 57 | 16.0 | 2.27 | 24.6 |
| 2.10 | 3.30 | 6.20 | 66 | 4.6 | 2.47 | 26.0 |
| 4.20 | 3.91 | 5.45 | 52 | 3.8 | 2.25 | 24.0 |

I Soap precipitated fraction
II Fraction recovered from supernatant liquid by methanol precipitation

attractive forces between the two components are not disrupted to any great extent by heating at 85° C. for short intervals of time.

## B. Complexes of Amylose with Symmetrical Molecules

Polarity and Complex Formation.

It has been reported (40) (5) that the common characteristic of the agents which form complexes with amylose is a fairly linear molecule with a sizeable dipole and/or polarizability. Those symmetrical molecules which have no net dipole would, according to this hypothesis, have no tendency to form complexes with amylose. Alpha, omega disubstituted hydrocarbons (the dibasic acids, ethyl adipate and hexamethylene glycol) are molecules which would have no electric moment parallel to the long axis of the molecule; hence, they were used to test the validity of the hypothesis.

Addition of the dicarboxylic acids, especially the higher ones such as azelaic and sebacic, to an amylose solution causes precipitation of the amylose quite readily. It is apparent that before any conclusions could be drawn, it was imperative to establish definitely whether or not a complex similar to that formed with iodine, alcohols, fatty acids and other complexing agents is formed in the case of the dicarboxylic acids.

The maxima of the X-ray diagrams produced by the dry amylose precipitated by the dicarboxylic acids, recorded in Table XIII, indicate a "V" configuration identical with that produced by the other complexes mentioned.

Table XIII

Dicarboxylic Acid-Amylose Precipitates

| Intensities[b] | $Sin^2\theta$ (Observed)[a] | | | |
|---|---|---|---|---|
| | Adipic | Pimelic | Azelaic | Sebacic |
| VS | .0127 | .0131 | .0123 | .0129 |
| W | .0219 | .0217 | .0217 | .0218 |
| W | .0251 | .0256 | .0257 | .0256 |
| VS | .0295 | .0296 | .0294 | .0298 |
| MS | .0385 | .0384 | .0372 | .0379 |
| W | .0462 | .0466 | .0466 | .0466 |

[a] $Sin^2\theta$ values for Cu radiations

[b] Intensity notations S, strong; M, medium; W, weak; V, very.

Ethyl adipate and hexamethylene glycol precipitated amylose from its solution about as readily as the dicarboxylic acids. Microscopic examination of the larger crystals showed that they were definitely birefringent. Identification X-ray patterns of the complexes showed a line for line correspondence with those of the dicarboxylic acid-amylose complexes.

Results of the anlysis of the sebacic acid in the precipitated amylose by two independent methods are shown in Tables XIV and XV. The average value found by the ether extraction method is 7.9 per cent and by the precipitation as the lead salt, 7.3 per cent. Results obtained by the ether extraction method are higher than those obtained by the lead acetate method. Any mechanical loss of acid in the former method causes high results.

Table XIV

Sebacic Acid in Sebacic-Amylose Complex

| | mg. Acid Used | mg. Acid Extracted | mg. Acid Bound | % Acid in Complex |
|---|---|---|---|---|
| 1 | 100.3 | 59.8 | 40.5 | 7.36 |
| 2 | 100.2 | 57.3 | 42.9 | 7.75 |
| 3 | 99.9 | 55.3 | 44.6 | 8.05 |
| 4 | 100.0 | 57.5 | 42.5 | 7.70 |
| 5 | 99.9 | 55.5 | 44.4 | 8.01 |
| 6 | 100.6 | 56.5 | 44.1 | 7.96 |
| 7 | 100.7 | 53.7 | 47.0 | 8.44 |
| 8 | 100.4 | 56.5 | 43.9 | 7.93 |
| 9 | 100.5 | 56.6 | 43.9 | 7.93 |
| 10 | 100.8 | 57.7 | 43.1 | 7.79 |

The average of the values found by the two methods is 7.6 per cent. This value corresponds very closely with the theoretical amount, 8.2 per cent, needed to fill the helices completely. Here again, as in the case of the fatty acids, the experimentally found value is slightly lower than the theoretical value.

Table XV

Per Cent Sebacic Acid in Complex

| | By Precipitation as Lead Sebacate | By Ether Extraction |
|---|---|---|
| 6 | 7.71 | 7.96 |
| 7 | 6.79 | 8.44 |
| 8 | 7.57 | 7.93 |
| 10 | 7.19 | 7.79 |

The above shows that dicarboxylic acids form complexes with amylose of the same nature as those formed by the other complexing agents reported. Polarity of the molecule, while possibly playing an important role, does not seem to be necessary for complex formation. In this connection it might be mentioned that in crystals of the iodine-potassium iodide complex of cyclohexaamylose $(C_6H_{10}O_5)_6 \cdot KI \cdot I_2$ alternate molecules are arranged with their electric moments opposing (10). The resulting moment within the structure would be zero.

Complex Formation and Thermal Stability.

Data showing the amounts of amylose precipitated as the complex by 0.5 of a gram of acid from a 1.15 per cent amylose solution at various temperatures are shown in Table XVI. The larger the acid molecule the higher the temperature at which the complex will form. This also represents the temperature to which a complex may be heated before disruption. From this it is apparent that there is a positive relationship between the length of the dicarboxylic acid and the magnitude of the forces holding the complex together.

Table XVI

Influence of Temperature on Complex Formation

| | Per Cent of Amylose Precipitated | | | |
|---|---|---|---|---|
| | 25° | 50° | 80° | 100° |
| Adipic | 67 | 0 | 0 | 0 |
| Pimelic | 72 | 0 | 0 | 0 |
| Suberic | 95 | 89 | 0 | 0 |
| Azelaic | 95.4 | 92 | 14 | 0 |
| Sebacic | 96 | 94 | 84 | 36 |

Separation of Sebacic Acid from Adipic Acid by means of Amylose Complex Formation.

Results of five trials at separating sebacic acid from adipic acid on the basis of the solubility of their respective amylose complexes are given in Table XVII. It is clearly seen that fractionation has been effected.

Table XVII

Separation of Sebacic Acid from Adipic Acid

| Trial | Per Cent Acids in Supernatant Liquid | |
|---|---|---|
| | Adipic | Sebacic |
| 1 | 68.9 | 31.1 |
| 2 | 61.3 | 38.7 |
| 3 | 66.0 | 34.0 |
| 4 | 62.5 | 37.5 |
| 5 | 67.2 | 32.8 |

If the amylose bound only sebacic acid, the acids remaining in the supernatant liquid should consist of 25 per cent sebacic and 75 per cent adipic acids. The results show that some adipic acid was also removed. It would be very unlikely that all adipic acid molecules could escape being captured within the amylose helix during its formation. Once within the helix, it might be difficult to remove them. It is also to be expected that, under the conditions of the above experiments, some adipic acid would be adsorbed on the surface of the precipitated amylose.

Although there are other methods more effective and more convenient than the above for the separation, the fact that fractionation was achieved is interesting. It seems very probable that the ability of amylose to form complexes might be employed to make separations in cases where other methods fail.

## F. Periodicity and Hydrogen Bond Formation

The alkyl halide-amylose complexes were prepared with two purposes in mind, (1) to determine whether the periodicity of the spacing of the iodine atom could be observed from X-ray analysis and (2) to test further the validity of the idea of hydrogen bond formation between the complexing agent and amylose.

The complexes of the alkyl halides did not form very readily, hence, the danger of retrogradation of the amylose was always present. The n-amyl iodide complexes formed more easily than the n-butyl iodide. Samples which were prepared with excess of undissolved complexing agent and with vigorous stirring had the greatest amounts of the alkyl halides.

Isoamyl iodide complex contained 8.17 per cent iodine or the equivalent of 12.75 per cent isoamyl iodide. Two samples of n-amyl

iodide complex gave 7.31 per cent and 6.60 per cent for the halide analysis. The n-butyl iodide in one sample of the complex was 6.70 per cent of the weight of the complex.

X-ray identification diagrams were made of all the alkyl halide complexes prepared. Examination of these showed that the amylose was in the "V" configuration in all complexes containing the n-alkyl radical. Unfortunately the diagrams were not distinct enough to determine the presence of the periodicity of the iodine atoms.

A comparison of the diagrams of the n-butyl iodide complex with that of the isoamyl iodide complex proved quite interesting, in view of the differences observed by Bear in the n-butyl and isoamyl alcohol complexes of amylose. The same difference was noted for the corresponding alkyl halides, i.e., the isoamyl iodide complex produced diffraction patterns extending to lower angles than the normal hydrocarbon halide complexes.

Treatment of dry tertiary amyl alcohol-precipitated amylose with isoamyl iodide gave a product with an alkyl halide content of 4.48 per cent. The presence of the halide had no effect on the X-ray diffraction pattern. For comparison, the maxima by the three samples, t-amyl alcohol-precipitated amylose, the same amylose treated with iodide, and amylose precipitated by isoamyl iodide, are given in Table XVIII.

It has been noted previously that amylose and amylopectin have essentially the same number of hydroxyl groups which could form hydrogen bonds with complexing agents. The difference in the ability of amylose and amylopectin to form fatty acid complexes as shown in Table X cannot be explained on the basis of hydrogen bond formation; nor can the

Table XVIII

Isoamyl Iodide-Amylose Precipitates

| Intensities | $\sin^2\theta$ (Observed) | | |
|---|---|---|---|
| | t-amyl Alcohol Amylose | t-amyl Alcohol Precipitated Amylose Treated with Isoamyl Iodide | Isoamyl Iodide Amylose |
| S | .00362 | .00370 | .00362 |
| MS | .00563 | .00563 | .00563 |
| MS | .00736 | .00736 | .00736 |
| W | .0106 | .0106 | .0106 |
| M | .0128 | .0128 | .0132 |
| M | .0217 | .0220 | .0211 |
| VS | .0249 | .0249 | .0249 |

interaction between amylose and iodine be due to that effect. The fact that the alkyl halides, whose tendency to form hydrogen bonds is certainly negligibly small, form complexes with amylose is added evidence that the ability of a substance to form amylose complexes does not depend on its ability to form hydrogen bonds, but rather on its ability to induce amylose to take on a helical configuration.

## V. CONCLUSIONS

1. Fatty acids, dicarboxylic acids and alkyl halides form complexes with amylose similar in structure to the complexes which amylose forms with iodine and the alcohols. The structure consists of close packed helical amylose chains with the linear molecules of the complexing agent within the helices. The packing of amylose helices is not influenced by the complexing agents.

2. Inhibition of amylose-iodine complex formation is proportional to the fatty acid content of the amylose. This is attributed to a decrease in the capacity of the helices for iodine when the helices contain fatty acids. Evidence is presented that the amount of fatty acid bound by helical amylose correlates well with the calculated capacity of the interior of the amylose helix.

3. Fatty acids can be displaced from amylose-fatty acid complexes by iodine vapor. The amounts of fatty acids displaced and the iodine gained by the amylose are proportional in the spatial or steric sense.

4. Helical "V" configuration binds large amounts of fatty acids when treated with methanol-fatty acid solutions; retrograded "B" amylose and amylopectin, having essentially the same surface, do not bind significant amounts. This indicates that crystalline configuration is a more important factor than surface in determining whether amylose will bind fatty acids.

5. Soaps and other complexing agents will completely precipitate partially dispersed starch. The factors which determine the completeness of precipitation are (1) solubility of soap and (2) degree of dispersion

of the starch granules. Evidence is presented that in granular starch the amylose and amylopectin are mixed intimately throughout the granules.

6. Dicarboxylic acids, ethyl adipate and hexamethylene glycol, molecules which have no electric moment parallel to the long axis, form amylose complexes similar to those formed by iodine, butanol and fatty acids. Hence, it is concluded that dipolar molecules do not seem to be necessary for complex formation with amylose.

7. Thermal stability of the dicarboxylic acid complexes depends on the chain length of the acids. The longer the chain the greater the thermal stability.

8. Hydrogen bond formation cannot be used to explain the difference in complexing ability of amylose and amylopectin. Neither can hydrogen bond formation be used to explain the interaction between amylose and alkyl halides.

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# VIII. VITA

Felix F. Mikus was born in Bomarton, Texas, January 1, 1916, the fourth child of Mary (Hajek) and Robert Mikus. His early education was in the public schools in Yoakum, Texas. During the 1932-1933 session, he was enrolled at Victoria Junior College, Victoria, Texas. He entered Southwest Texas Teachers College January, 1934. From 1934 to 1938 he was a grade teacher at Lone Tree School in Yoakum, Texas. Meanwhile, his education was continued at Southwest Texas Teachers College during the summer quarters until August, 1938, when he received the degree of B.S. with majors in Chemistry and Education. From 1938 to 1940, he taught mathematics at Crescent High School in Wharton, Texas. In September, 1940, he entered the graduate school at Catholic University of America in Washington, D. C., and received the degree of M.S. in Chemistry in June, 1942. From 1942 to 1946, he was a graduate student at Iowa State College, except for the first six months of 1945 when he was employed as research chemist by U. S. Rubber Company. In 1945, he was married to Alice M. Runge of Houghton, Michigan.