STARCHES and RELATED POLYSACCHARIDES

in BARLEY and MALT

By

William McArthur, B. Sc.

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INTRODUCTION

Native starch is widely distributed in nature generally forming the reserve carbohydrate store of plants. In periods of active plant growth it is converted to soluble sugar whence it can be utilised for transformation into cellulose and other plant products. The fact that starch is hydrolysed in the human digestive tract with relative ease, makes it one of the main articles of diet. Both in the industrial field and in the home it finds many and varied applications.

Although great interest has been shown for many years by chemists and biochemists in elucidating the chemical structure of starch, progress has been slow. However, after 1925 when the ring structures of simple sugars such as glucose and maltose were established, many advances were made. Again, since 1939 when it was conclusively proved that starch granules contain at least two chemically distinct components the solution of many of the problems of its chemical configuration has been achieved, but many more remain to be solved. Indeed, although the broad outline of the starch structure has been well established, more investigation is required before the final picture can be fully accomplished.

The/
The object of this work is to determine the constitution of the starch from malted barley, and to compare the results with those of other starches already studied and in particular in the case of barley starch to determine any significant changes incurred during the malting process.
Starch usually occurs as granules which, on microscopic examination, exhibit characteristic differences in size and shape. Such variations, from oval to spherical or sometimes irregular in shape, help in determining the source of the starch. In addition to the carbohydrate content, the starch grains contain several constituents in small quantity, such as phosphorus (1), fatty acids (2), and protein matter (3), some of which are believed to have an integral function in the synthesis of the plant polysaccharide. It has been shown that the main constituent of the granules, namely, the starch itself, on hydrolysis yields glucose (4).

\[ \left( \frac{C_6H_{10}O_5}{x} \right)_x + (x) \text{H}_2\text{O} \rightarrow (x) \text{C}_6\text{H}_{12}\text{O}_6 \]

Starch Glucose

Although the fatty acid content was formerly considered to be combined with the starch (5), Schoch has indicated that it is strongly adsorbed on the carbohydrate and may be removed on extraction by water-soluble fat solvents such as methanol and dioxane. (6) Such solvents also remove the phosphorus impurities from all cereal starches, most workers ascribing no significance to this constituent. Samec and his associates (7), on the other—
other hand, are of the opinion that the combined phosphorus has an important function in the structure of the starch molecule. The fact that solvent extraction does not remove the phosphorus from root starches such as potato starch, indicates a chemical linkage between the phosphorus and the starch, and lends weight to this argument as far as root starches are concerned. The protein content of starch is generally very small, and may arise from the enzyme systems responsible for the synthesis of the carbohydrate. Except in a few cases the view is now generally held that such constituents are present not as an integral part of the starch molecule but solely as associated impurities whose function may have been protective or synthetic.

The only monosaccharide which has been conclusively shown to be present in starch is D-glucose (Fig. I) which has been isolated in crystalline form. The yield of glucose from acid hydrolysis is practically quantitative as estimated by polarimetric and reducing power methods.

From the controlled acid hydrolysis of starch it is possible to isolate the disaccharide maltose (Fig. II) which was also obtained by O'Sullivan (1872) (8) in his early experiments, as the principal yield (80%) from starch which was treated with \( \beta \)-amylase extracts/.
extracts, thus giving the first indication as to the glucose-glucose link in starch. It was still doubtful, however, whether the maltose was a breakdown product of the starch or a product of synthesis from the secondary action of the enzyme system. This latter possibility was disproved by Karrer(9) who, by treating starch with acetyl bromide, obtained hepta acetyl maltosido-1-bromide. This he converted into hepta acetyl maltose, thus proving that maltose could be obtained from starch.

The full significance of this result was not realised until 1926 when the structure of maltose (Fig. II) was proved to be 4-((\(\alpha\)-D-glucopyranosido)-D-glucopyranoside by Irvine and Black (10), and Cooper, Haworth, and Peat (11). It was therefore suggested that the majority of the glucose residues present in the starch molecule existed in the pyranose form and were mutually linked by \(\alpha\)-1:4-bonds as in maltose. This conclusion was supported by the work of Haworth, Hirst, and Webb(12) who methylated/

Fig. I. Glucose  
Fig. II. Maltose
methylated starch to give a trimethyl derivative, which on acid hydrolysis gave 2:3:6-tri-O-methyl-D-glucose in 90% yield (Fig. III). Haworth and Percival (13) obtained proof that maltose residues were present in the starch molecule. By the acetolysis of methylated starch at room temperature followed by oxidation, methylation, and separation of the products of acetolysis, they isolated methyl octamethyl maltobionate (Fig. IV). This ester on hydrolysis gave 2:3:4:6-tetra-O-methyl-D-glucopyranose, and 2:3:5:6-tetra-O-methyl-\(\gamma\)-gluconolactone, both products having been previously obtained from maltose (11).

Freudenberg's studies (14) on optical rotatory power of starch provided further evidence not only for the \(\alpha\)-linkage but also of their continuity within the starch chain. It was found that on correlating experimental results and theoretical considerations agreement could be reached on the assumption that at least 97% of the bonding in the glucose chains were of the \(\alpha\)-type.

From the above evidence it was apparent that in the starch molecule there were present chains of glucopyranose residues linked by \(\alpha-1:4\)-bonds, and if the chain were finite in length two end-glucose units should be distinguishable in the methylated derivative/
Fig. III. 2:3:4:6-Tetra-O-Methyl-D-glucose. 2:3:6-Tri-O-Methyl-D-glucose.

Fig. IV. (a) 2:3:4:6-Tetra-O-Methyl-D-glucose. (b) 2:3:5:6-Tetra-O-Methyl-Gluconolactone.
derivative. Firstly the "reducing" end-group (Fig. III) on hydrolysis will give 2:3:6-tri-O-methyl D-glucose since the methoxyl grouping on C₁ is glucosidic in character and removed by dilute acids. Secondly, the non-reducing end-group will yield 2:3:4:6-tetra-O-methyl-D-glucose (Fig. III) after acid hydrolysis. Therefore, by estimation of the proportion of non-reducing end-group, or in other words, the 2:3:4:6-tetra-O-methyl-D-glucose, the apparent chain-length of the starch molecule could be ascertained. Hirst, Plant and Wilkinson (15) used this method of end-group assay in their investigations of methylated potato starch, when the quantity of 2:3:4:6-tetra-O-methyl D-glucose was found to be 4%, corresponding to an apparent chain length of 25 units. Similar yields of non-reducing end-group were obtained from other methylated starches, these results being summarised below.

Table 1/
Table 1.

<table>
<thead>
<tr>
<th>Source of starch</th>
<th>Calculated chain length</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acorn</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>Banana</td>
<td>24</td>
<td>17</td>
</tr>
<tr>
<td>Canna</td>
<td>27</td>
<td>18</td>
</tr>
<tr>
<td>Horsechestnut</td>
<td>28</td>
<td>19</td>
</tr>
<tr>
<td>Maize</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Rice</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>Waxymaize</td>
<td>26–30</td>
<td>22</td>
</tr>
<tr>
<td>Wheat</td>
<td>24</td>
<td>19</td>
</tr>
</tbody>
</table>

From these observations it appeared that the starch molecule consisted of a straight chain of 24–30 units, corresponding to a molecular weight of 4,000–5,000: but from physical measurements of the size of the starch molecule (21, 23) using osmometric and centrifugal methods it was soon realised that the molecular weight was greatly in excess of such a figure. Again such a structure was not in agreement with the observed reducing power of starch indicating that the reducing hydroxyl group, which terminates each glucose chain, was involved in a type of linkage which blocked its reducing properties. Further in the hydrolysis products of the methylated starch the presence of di-<sup>2</sup>-methyl-D-glucose in 5% or greater yield indicated that starch contained some branched chains (Fig. V).
The suggestion of a branched chain molecule for starch was in conflict with the structure of Richardson (24) who proposed that the starch molecule was a chain of 1000 glucose units linked by \( \alpha-1:4 \) bonds. The chain length was greatly decreased on methylation or acetylation, the product yielding about 5% of non-reducing end-group (Fig. VI). On the other hand the enzymic results from the hydrolysis of starch by \( \beta \)-amylase could not be explained on such a structure. However the observations of Haworth and Hirst (25, 26) furnished the required explanation. These workers put forward the suggestion that the starch molecule was composed of a number of repeating units, each consisting of a straight/-
chain of 24-30 glucose residues, united by $\alpha$-1:4-linkages (Fig. VII). The terminal reducing glucose was linked by a primary valency to some point of an adjacent chain, thus producing a three dimensional 'laminated' structure which could explain most of the remaining anomalies concerning reducing-power, Fig. VII.

\[
\begin{align*}
G & \rightarrow [G]_m \rightarrow [G]_m \rightarrow [G]_m \rightarrow [G]_m \\
 & \rightarrow [G]_m \rightarrow [G]_m \rightarrow [G]_m \\
 & \rightarrow [G]_m \rightarrow [G]_m \rightarrow [G]_m \\
 & \rightarrow [G]_m \rightarrow [G]_m \rightarrow [G]_m \\
 & \rightarrow [G]_m \rightarrow [G]_m \rightarrow [G]_m \\
& \rightarrow [G]_m \rightarrow [G]_m \rightarrow [G]_m \\
& \rightarrow [G]_m \rightarrow [G]_m \rightarrow [G]_m \\
& \rightarrow [G]_m \rightarrow [G]_m \rightarrow [G]_m \\
\end{align*}
\]

The following chemistry demonstrates the degradation on methylation, enzymic action, and the production of almost equal amounts of di-$Q$-methyl and tetra-$Q$-methyl-$D$-glucose on methylation and acid hydrolysis.

Starch (after Haworth and Hirst)
The action of the amylases on starch

The classical experiments of Wijman (27) demonstrated the existence of two amylases which were able to degrade starch pastes. The first of these systems produced a rapid decrease in the viscosity of starch gels with the formation of reducing dextrins and destruction of the components giving colour with iodine, and these enzymes have been called liquefying-amylases or α-amylase. The other system had little effect on the viscosity of the paste but formed the reducing sugar maltose and a limit dextrin which gave a coloration with iodine and these have been called saccharifying or β-amylases. It has recently been shown that α-amylases from various sources have different actions on the same substrate (104). Both the α- and β-amylases have been obtained in a pure crystalline form (28, 29).

While the action of α-amylase on starch has given very little constructive information to the problem of the structure of the molecule, the study of the action of β-amylase on starch gels has proved invaluable in the elucidation of structure. The mode of action of β-amylase (30, 31) was believed to be that of a stepwise hydrolysis of the chains of α-1:4-linked glucose residues, the cleavage occurring at the alternate glucosidic links, liberating/−
liberating maltose. The hydrolysis started at the non-reducing end of the chain continuing until it encountered an obstruction which was regarded as strong evidence that an inter-unit linkage had been reached.

The dextrin which remained after the action of \( \beta \)-amylase has been isolated in 40–45% yield while the maltose was obtained in 55–60% yield. This dextrin was not further attacked by \( \beta \)-amylase unless it was first treated with acid, heat, or sensitised in some way (32, 33, 34). It was shown to be of high molecular weight and to have an apparent chain length of 11–12 units (35, 36).

**Recent knowledge of the chemical structure of starch**

Since all the physical and chemical properties of starch could not be explained on the basis of a single structure, it was suggested that starch was not homogeneous. While various workers reported the isolation of two components, Maquenne's contribution to the present knowledge remains outstanding (37). He isolated two components one which he named "amylose" could be completely hydrolysed to maltose by the action of \( \beta \)-amylase, and the other which he named "amylopectin" was not completely hydrolysed/-
hydrolysed by the enzyme to maltose but gave a $\beta$-limit dextrin. Recent investigations into these two components of starch have proved the accuracy of his work and conclusions. His definitions of amylase and amylopectin based on the enzymic experiments have now been accepted although Meyer (38) has specifically called the straight-chain molecules, amylase, and the branched chain ones amylopectin. The amylase component in solution gives a deep blue coloration with iodine while the amylopectin gives a reddish-purple coloration. Despite the fact that Maquenne's paper appeared in 1905, later workers ignored his findings with the result that progress for many years became very slow.

A fresh interest in the problem was aroused in 1940 by C. F. Hanes (39) who claimed to have prepared by the use of enzymes a synthetic amylase. Glucose-1-phosphate was treated with potato phosphorylase, the product which separated from solution as granules, giving an intense blue color with iodine, retrograding easily from solution and being completely converted to maltose by the action of $\beta$-amylase. It was realised that this synthetic polysaccharide was possibly a normal component of natural starch, a fact which urged many workers to search for methods whereby the components of starch could be obtained in/
in a reasonable degree of purity. Even if these attempts at fractionation have not been completely successful, they have established that starch is a heterogeneous polysaccharide, the two components amylose and amylopectin being isolated in a relatively pure state. It has not been fully ascertained, however, whether the amylose and amylopectin are completely independent of one another or mutually combined in the starch complex.

The fractionation of starch

It has now been generally accepted that starch is not a single polysaccharide, with the possible exception of waxy maize starch and similar varieties but a mixture of two components, amylose and amylopectin, the average proportion of amylose being about 20%. By the application of different techniques various workers have attempted to fractionate starch. The principal condition necessary for a successful fractionation is the complete dispersion of the starch granule in such a way as to prevent physical or chemical degradation. The ease with which starch tends to retrograde from solution makes such a state difficult to attain. The attempts of early workers did not fulfil this condition of dispersion but involved the retrogradation of the more unstable amylose from solution. The fractionation of starch by Maquenne was carried/–
carried out using this principle and, although his conclusions were substantially correct, his method was unsatisfactory and may be glanced over.

Samec (40) first obtained a satisfactory fractionation using potato starch by an electrophoretic method which was dependent on the organically bound phosphate which esterified the amylopectin, thus making it polar. Such a method would be impracticable in the case of cereal starches from which nearly all the phosphorus is removed by solvent extraction.

The procedure adopted by Baldwin (41) was of more general application, namely, leaching of the intact starch granules with hot water. The more soluble amylose fraction was taken up in the aqueous extract, while the amylopectin remained as the insoluble residue. Such a method involves repeated extractions and it is difficult to prevent the amylose retrograding from solution. Nevertheless, Meyer (42) has developed this technique and claims to have produced amylose and amylopectin fractions of high purity.

The discovery of Alsberg (43) that autoclaved starch solutions gave a crystalline precipitate with alcohol opened the way for Schoch (44) to develop this technique for obtaining pure starch fractions.
After he had dispersed his fat-free starch in water saturated with n-butanal by autoclaving at 120°, the starch solution was allowed to cool very slowly. The amylose settled out as a microcrystalline complex with butanal, but was found to contain amylopectin as impurity. The amylose fraction was further purified by a repetition of the foregoing procedure, the amylopectin remaining in solution. The amylopectin fraction was obtained by pouring the concentrated mother liquors into water-soluble alcohols.

It was soon realised that the amylose was capable of forming an insoluble complex with polar compounds, such as alcohols, which have the property of forming hydrogen bonds. The amylopectin either did not form a complex with the polar compound or formed one which was soluble. Among the polar compounds which have been employed by various workers (45-49) are primary alcohols (45, 47), pyridine (48, 49), nitro-paraffins (49), thymol (45), and carboxylic acids (49).

The above method of fractionation by precipitation with polar solvents is widely applied at present. However, much research has been directed to the method of "selective adsorption". When starch pastes were treated with neutral adsorbants such/
such as cotton (50, 51) the amylose was preferentially bound. Attractive as this process seemed since it involved no chemical breakdown, the very small yields of amylose were a great disadvantage.

Another adsorption technique in which the starch was adsorbed on aluminium hydroxide precipitated from an aqueous solution, was introduced by Bourne, Donnison, Peat and Whelan (52). On boiling this precipitate with water, the amylose dissolved but the amylopectin remained insoluble. Using less aluminium hydroxide these workers found that the amylopectin fraction was preferentially adsorbed while the amylose was left in solution. By this method the yields were again low, while the ash contents of the fractions was high. On the other hand when this method was combined with the thymol technique the fractionation of potato starch was carried out and relatively pure components isolated (53).

Not only was it now possible to separate starch into its two components in high purity but these fractions could also be subfractionated by the methods developed by Kerr (54, 55), Meyer (56), Higginbotham (57), and Schoch (58). Sufficiently pure fractions of amylose and amylopectin could therefore be obtained to carry out structural studies/-
studies and develop analytical methods.

The success of the fractionation procedure introduced by Schoch enabled Bates, French and Rundle (59) to develop a method by which the relative proportions of amylose and amylopectin in mixtures and in whole starches could be determined. This depended on the affinities of amylose and amylopectin for iodine - with the amylose the iodine was firmly bound in a blue complex while it appeared to be colloidally adsorbed on the amylopectin giving the characteristic reddish-purple coloration. In addition it was observed that the iodine was preferentially adsorbed by the amylose in the presence of amylopectin and hence the amount of iodine bound in the initial period was a function of the quantity of amylose present and could therefore be used for estimation. A potentiometric method was developed to estimate the bound iodine and therefore the amylose content. The purest sample of amylose was that fraction which, under standard conditions, bound the highest proportion of iodine. In order to ensure that the full adsorption of iodine on the starch is attained there should be no impurities present such as fatty acids which are capable of being adsorbed or associated with the starch (60).

Amylose/-
Amylose fractions with very high adsorptions have been isolated by Kerr and Severson (61) who combined the technique of butanol precipitation to the hot water extract of corn and tapioca starches. These amyloses took up 20.5% and 20.7% respectively of their own weight of iodine. Higginbotham and Morrison (62), using amylose components of various starches precipitated with pyridine and butanol, have shown that, under the standard conditions of Bates, French and Rundle (59), as modified by Hudson, Schoch and Wilson (63), the uptake of iodine by these fractions reached a constant value of 21.5% of their own weight after several precipitations. In assessing the amylose content of starches, this figure is normally assumed to represent the iodine uptake of pure amylose although it may depend on the molecular size of the individual amylose component. Schoch and co-workers have criticised such an assumption of a pure linear material with an iodine binding power of about 20.0%. Using the potentiometric method many starches have been examined and the amylose content found to be 15-25%.

Table 2/
Table 2

<table>
<thead>
<tr>
<th>Source of starch</th>
<th>Amylose content %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrowroot</td>
<td>20</td>
<td>64</td>
</tr>
<tr>
<td>Banana</td>
<td>21</td>
<td>64</td>
</tr>
<tr>
<td>Barley</td>
<td>19</td>
<td>65</td>
</tr>
<tr>
<td>Maize</td>
<td>23</td>
<td>64</td>
</tr>
<tr>
<td>Rice</td>
<td>14-15</td>
<td>64</td>
</tr>
<tr>
<td>Potato</td>
<td>18</td>
<td>64</td>
</tr>
<tr>
<td>Wheat</td>
<td>19</td>
<td>64</td>
</tr>
<tr>
<td>Wood</td>
<td>20</td>
<td>66</td>
</tr>
</tbody>
</table>

The differential potentiometric titration technique of Gilbert and Marriot (67) for the measurement of iodine uptake by starches has recently been developed by Anderson and Greenwood (68). A very sensitive system has been adopted whereby many α-1:4-glucosans have been studied and the difference between the binding power of normal glycogens and amyllopectins found sufficiently great to characterise the two structural types.

Certain starches, namely waxy maize and some protozoal starches, give a reddish-purple coloration with iodine, and contain less than 2% amylose (64, 59, 69, 70, 71). They may be considered as natural amyllopectins. On the other hand the starch from the "Steadfast" variety of wrinkled pea has been shown to contain only amylose (72). 50-60% amylose has been/-
been found in other varieties of wrinkled pea (73).

The reaction of starch with iodine has also been used by Hassid and McCready (74) in a colorimetric method of analysis. Under standard conditions laid down by these workers and modified by Bourne, Haworth, Macey and Peat (75), amylose gives a much more intense colour with iodine than amyllopectin. Mixtures of the two produce colorations which are intermediate in intensity between that of amylose and amyllopectin. The quantitative reading of the colour intensity of the carbohydrate-iodine complex measured by the Spekker photoelectric absorptiometer has been designated Blue Value. The Blue Values of the amylose fractions from different starches which absorbed the same proportions of iodine by potentiometric titration ranged from 1.26 to 1.48 while the Blue Values for the corresponding amyllopectins varied between 0.06 and 0.16 (76). The Blue Value determinations offer a simpler and much quicker though less accurate method for evaluating the ratio of amylose and amyllopectin.

The Chemistry of Amylose

The investigation of the chemical structure of amylose was accelerated by two developments, namely the fractionation techniques which enabled the isolation of pure amyloses, and the discovery of a synthetic/-
synthetic amylose by Hanes (39).

Hanes realised that the degradation of potato starch by potato phosphorylase (or Penzyme) could be reversed, and when glucose-1-phosphate was incubated with this enzyme, a polysaccharide product, which separated from solution in granular form, was obtained. The properties of this substance such as its optical rotation in solution were similar to normal starch but several marked differences were observed. It gave a deep blue colour with iodine, was less stable in aqueous solution, but most important it was completely converted to maltose by the action of $\beta$-amylase. Methylation studies and end-group assay were carried out by Haworth, Heath and Peat (77) who estimated that less than 1.5% of methyl 2:3:4:6-tetra-$\Omega$-methyl-D-glucoside was present in the methanolysis sugars, which was equivalent to one non-reducing terminal grouping per 80-100 glucose residues. Such a molecular weight was also indicated by "reducing-power" and viscosity measurements. Another "synthetic" starch was prepared by Hassid, Cori, and McCready (78) from glucose-1-phosphate and muscle phosphorylase. End-group assay in this instance revealed that not more than 0.5% of methyl 2:3:4:6-tetra-$\Omega$-methyl-D-glucoside was present, giving one non-reducing terminal group per 200-250 glucose units.
The amyloses isolated from whole starches by the fractionation methods exhibited similar properties to these synthetic starches which were therefore more precisely designated "synthetic amyloses".

The polysaccharide amylose was found to give an intense blue colour with iodine, to retrograde easily from aqueous solution, and to be quantitatively converted to maltose by $\beta$-amylase. Very small quantities of methyl 2:3:4:6-tetra-$\alpha$-methyl-D-glucoside have been estimated from the methanolysis products of methylated amyloses. Some results are given in Table 3.

Table 3

<table>
<thead>
<tr>
<th>Source of amylose</th>
<th>No. of glucose residues per non-reducing end-group</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>200-300</td>
<td>(79)</td>
</tr>
<tr>
<td>Potato</td>
<td>100-200</td>
<td>(64)</td>
</tr>
<tr>
<td>Potato</td>
<td>200-300</td>
<td>(60, 81, 82)</td>
</tr>
<tr>
<td>Sago</td>
<td>100-250</td>
<td>(64)</td>
</tr>
<tr>
<td>Barley</td>
<td>250-400</td>
<td>(65)</td>
</tr>
</tbody>
</table>

The above results must be considered as minimum values for the length of the amylose chains since there were traces of amylpectin as an impurity in the fractions under examination. However, figures of the same order have been obtained by Brown, Halsall, Hirst and Jones (64) for the proportion of terminal groups present in the amylose/
amylose fraction by the technique of periodate oxidation. A sample of potato amylose, containing 10% amylopectin for which allowance was made, was found to have one non-reducing terminal-group per 500 glucose residues.

Potter and Hassid (83, 84, 85) have employed the periodate oxidation method in their end-group determinations. The average unit chain lengths thus obtained have been compared with the values calculated from osmotic pressure measurements on the amylose acetates in chloroform solution.

Table 4

<table>
<thead>
<tr>
<th>Source of amylose</th>
<th>Chain length from periodate oxidation</th>
<th>Chain length from osmotic pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tapioca</td>
<td>960</td>
<td>1300</td>
</tr>
<tr>
<td>Potato</td>
<td>980</td>
<td>950</td>
</tr>
<tr>
<td>Wheat</td>
<td>540</td>
<td>860</td>
</tr>
<tr>
<td>Corn</td>
<td>490</td>
<td>300</td>
</tr>
<tr>
<td>Sago</td>
<td>420</td>
<td>740</td>
</tr>
<tr>
<td>Lily</td>
<td>640</td>
<td>620</td>
</tr>
<tr>
<td>Apple</td>
<td>630</td>
<td>500</td>
</tr>
</tbody>
</table>

Further support has been given to these results by the work of Meyer et alia (86) and Foster and Nixon (87) on the osmotic pressure measurements of acetylated corn and acetylated tapioca amyloses.
The degree of polymerisation of polysaccharides may also be determined by a method developed by Meyer, Noelting, and Bernfeld (88) which utilises the interaction of the reducing group of the carbohydrate with 3:5 dinitrosalicylic acid in alkaline solution. The nitrosalicylic acid is reduced to the corresponding amino compound which is yellow in colour. The colour intensity is measured photometrically and compared with standard curves using maltose. According to Meyer, the values obtained by this method show very good agreement with the results of the other techniques.

Amylose is thus seen to consist of long chains by the above results and the X-ray diffraction patterns obtained by Rundle and French (89) on the amylose-iodine complex, confirm this. They picture amylose as long chains arranged in the form of helices, each spiral consisting of six glucopyranose units inside which the iodine atoms are situated. It is the α-linkage which makes such an arrangement possible. The same idea had already been put forward by Freudenberg (90) who suggested that the hydrogen atoms at C₁ and C₄ on each side of the glucosidic bridge formed a hydrocarbon lining and iodine gave blue coloured solutions in hydrocarbons.

The X-ray studies of Rundle and Baldwin (91) are/
are in agreement with this structure since they observed that the amylose-iodine complex exhibited dichroism of flow, the absorption of light being strongest along the lines of flow and least at right angles to them. This is strong evidence that the iodine molecules have their axes parallel to the long axis of the complex.

Although these results indicate that amylose consists of long chains, no proof has yet been established whether these chains are slightly branched or not. Certain variations in physical properties among amyloses have led some workers to suggest that amylose possesses a slight degree of branching (62, 92, 93). If this were the case, a certain quantity of di-O-methyl-D-glucose would be present in the hydrolysis products of a fully methylated amylose. The quantity of di-O-methyl-D-glucose for slight branching would be very small, with the result that since no satisfactory method existed for the separation and identification of such small quantities of sugar for the earlier workers, the problem remained unsolved.

However, in 1944, Consden, Gordon and Martin (94) introduced a method whereby they separated and identified amino-acids from the hydrolysis of proteins. This procedure of partition chromatography required/-
required only very small quantities of material (ca 1 mg.) and was adapted by Partridge (95) for separation and identification of sugars. The development of quantitative methods of paper chromatography applied to different sugars and their methylated derivatives was carried out by Hirst, Jones and co-workers (96, 97, 98, 99). Not only was success achieved on the micro-scale but Hough, Jones and Wadman (100) have adapted the method in such a way that mixtures of sugars may also be separated on a much larger scale by the use of a column of powdered cellulose and recovered quantitatively. It thus looked as if the branching problem would be quickly solved.

Unfortunately it was possible that during the hydrolysis of the methylated amylase, demethylation could occur, whereby 2,3,6-tri-O-methyl-D-glucose produced di-O-methyl-D-glucose. In these cases where small quantities are being considered, this demethylation is small and may be allowed for in control experiments (101).

Although the separation of the mixtures of methylated sugars on a cellulose column is perhaps the most suitable method of separating the small amounts of end-group, columns of silica gel (102, 103) and alumina (64) have been utilised in estimating the "end-group" from the hydrolysis products of the methylated polysaccharide.

Summary/—
Summary

The picture of the amylose molecule from the results available at present is that it consists of very long chains of $\alpha-1:4$-linked D-glucopyranose units, the average chain length of the amyloses so far investigated being between 300 and 800 units. The question as to whether there is a slight degree of branching in the molecule still remains unsolved. The difficulty of isolating from starch an amylose completely free from amylopectin complicates this problem, but is evidence that the amylose is linked in some manner to the amylopectin component.
The Chemistry of Amylopectin

While the early investigations were conducted on the whole starch much of the resultant information can be applied to the amylopectin structure since it is the major component of the common starches. From the estimated chain length of 24-30 for the whole starch, the apparent chain length of the amylopectin fraction is deduced to be 20-25, allowing for the amylose proportion of the starch concerned. Methylation studies of potato and maize amylopectins (79, 80, 81) have confirmed the conclusion, the yield of 2:3:4:6-tetra-2-methyl-D-glucose being 4-5%. The methylated amylopectin must also account for the di-2-methyl glucose (5% or more) obtained in the hydrolysis products of the methylated starch since the amylose fraction contains little, if any, branching and demethylation of tri-2-methyl-D-glucose is negligible. Amylopectin must therefore have a much more complicated structure than amylose, and have a relatively short chain branched molecule.

Again the 13-limit dextrin isolated after the action of 13-amylase on whole starch must originate from the amylopectin because 13-amylase completely converts the amylose fraction to maltose. When an allowance for the amylose percentage is once more made, the amylopectin should be 50-55% converted to maltose, a conclusion which has been confirmed experimentally (32).
The first structure for starch which gave a satisfactory explanation of its known properties was proposed by Haworth, Hirst and Isherwood (25), and can now be applied to amylopectin. The polysaccharide was envisaged as a "laminated" structure consisting of a number of repeating units, each unit being a straight chain of about 24 glucose residues.

Before this structure was put forward it was realised that starch could be degraded by mild acid treatment giving products which, although they had the same optical rotation as the starting material, were of lower molecular weight (24). This indicated that there were weaker bonds in the whole starch connecting these particles of lower molecular weight.

Evidence supporting this suggestion was obtained from experiments on fully methylated rice starch by Hirst and Young (19). The starch was heated in a solution of oxalic acid in aqueous methanol, the process stopped at different times and samples removed and purified by precipitation from boiling water. The end-product of this "disaggregation" reaction was stable to further attack by the acid solution, it was homogeneous and had a molecular weight of around 20,000 determined by several physical methods. Very few of the $\alpha-1:4$-linkages had been ruptured since the percentage/...
percentage of 2:3:4:6-tetra-$\beta$-methyl-$\alpha$-glucose was almost identical with that of the whole methylated rice starch.

It was realised that several of the "inter-unit" bonds had been broken, but the molecular size which represented 4 or 5 repeating units could not be explained. However, the importance of the experiments lay in the constancy of the average chain length throughout, while the molecular size gradually became smaller.

The kinetics of the reaction were investigated by Bawn, Hirst and Young (105) and from the graph of the viscosity measurements (equivalent to the molecular weights) of the different fractions against the time, the rate of disaggregation could be followed. The reaction was found to be of the first order after the first 100 hours and the energy of activation to be 20,000 calories, a figure which is comparable with the corresponding one for the hydrolysis of a normal glycosidic linkage (106).

The problem of the nature of the bond between the glucose residue of one chain and the reducing-end of the next chain had now to be solved. In studies on the final product of the disaggregation reaction, Barker, Hirst and Young (107) found that in the hydrolysis products of this remethylated fraction there was a decrease in the proportion of di-$\beta$-methyl-$\alpha$-glucoses (1.9%) compared with that of...
of fully methylated whole starch (3.3%). A high percentage of this fraction was 2:3-di-α-methyl glucose indicating that the majority of the bonds were α-1:6 in nature. This result was confirmed by Freudenberg and Boppel (108) from their investigation of the di-α-methyl fraction from the hydrolysis of a fully methylated amylopectin.

Fig. VIII.

Since the di-α-methyl fraction was not wholly 2:3-di-α-methyl glucose, part of which could also have arisen from the demethylation of 2:3:6-tri-α-methyl-glucose there was no evidence that all the bonds were of the 1:6 type. The isolation of β-isomaltose octa acetate from the hydrolysis products of acetylated amylopectin under conditions allowing no formation of reversion products (109), provided the required evidence that the majority of the inter-unit linkages were 1:6-bonds.

Staudinger (110) and Hess (111) postulated structures containing one principal chain of glucose units with side chains attached by their reducing end-groups.

Fig. IX/
Closely related as these structures appeared to the Haworth-Hirst theory they did not explain the conclusions of the disaggregation experiments. The final product from such formulations would be of low molecular weight and not homogeneous, contrary to the experimental evidence. The yield of maltose (50-55%) from the action of \( \beta \)-amylase on amylopectin and the high molecular weight of the resulting dextrin were other factors which brought much criticism of these structures (32, 55), which necessitated higher yields of maltose and a dextrin of lower molecular weight.

In order to explain the results obtained by enzymolysis Meyer (32, 56, 112) put forward the multiple chain theory.
Amylopectin (after Meyer)

This structure and the laminated one are basically similar and may appear more realistic. On the one side the action of $\beta$-amylase according to the multiple chain theory should give a product with properties similar to the original amylopectin which is the case, whereas the dextrin on the "laminar" theory should be almost a straight chain. On the other hand the multiple branched structure does not explain the results of the disaggregation experiments. The laminated structure is more satisfactory in explaining most of the known properties but a structure intermediate between the two rival types seems to provide the solution.

Pacsu (113) has formulated another structure which/-
which proposes that there are present hemi-acetal bonds. In the disaggregation reaction, these bonds are ruptured, but there is no positive evidence for such acetal linkages.

Fig. XII

Amylopectin (after Pacsu)

Halsall, Hirst and Jones (64) have shown that the existence of such bonds is very improbable.

Summary

The amylopectin structure is branched and contains chains of α-1:4-linked D-glucopyranose residues whose average chain length ranges between 20 and 25 units. These chains are joined through the reducing-end-group of one to some other glucose residue of the adjacent chain. The majority of the bonds are of the 1:6-variety, and the glucose residue to which the reducing end-group of the adjacent/
adjacent chain is attached lies just over half way from its non reducing end. Only two of the suggested structures satisfy most of the known properties, namely, the laminated formulation of Haworth and Hirst, and the multiple branched theory of Meyer.

Periodate/-
Periodate oxidation of starch and its components

The technique of periodate oxidation has proved of great value in confirming the theories on the structure of amylose and amyllopectin. Although the degradative action of the periodate ion ($\text{IO}_4^-$) on $\alpha$-glycols had been applied for many years in carbohydrate chemistry it was only comparatively recently developed to the field of polysaccharides (64, 114, 115).

The action of the periodate ion is the selective oxidation of $\alpha$-glycols to form dialdehydes (116). The products from its oxidative attack on the grouping $-\text{CHOH-CHOH-CHOH}$ are a dialdehyde and one molecule of formic acid. The presence of a pyranose ring in $\alpha$- and $\beta$-methyl D-glucosides (Fig.XIII) was proved by Jackson and Hudson (117) using this reaction since it indicated that three contiguous hydroxyl groups were available for oxidation.

![Chemical Structure](image)

Fig.XIII. $\alpha$-Methyl-Glucoside

All the methyl hexapyranosides examined behaved in the same way, yielding one molecule of formic acid. Hence/-
Hence when applied to a polysaccharide consisting only of hexapyranose residues and with no appreciable amount of reducing end-group, the only residues which, on attack, will release one molecule of formic acid are the non-reducing end-group (linked through C₁) and those linked through C₁ and C₆ in the main chain.

Fig. XIV.

Fig. XV.

Much information on the structure of a given polysaccharide can therefore be obtained from the amount of formic acid released on periodate oxidation. The reaction must be strictly controlled to prevent "overoxidation" — the attack on groupings other than the selective α-glycol ones, a feature which restricts the scope of the method. Hirst, Jones and co-workers (64, 114) have studied the reaction/
reaction in detail and have found that, when the polysaccharide is oxidised in an aqueous solution by a suspension of potassium periodate, the possibility of "overoxidation" is at a minimum, since this salt is only slightly soluble in water. Ethylene glycol is added to destroy excess periodate and is itself oxidised to the neutral formaldehyde with the formation of the iodate ion which is also neutral. The formic acid can now be titrated with 0.01N alkali.

In the examination of a polysaccharide of the starch type with chains of $\alpha$-1:4-linked hexopyranose residues, the percentage of glucose residues from which formic acid is produced should be in agreement with the percentage of non-reducing terminal groups as determined by the methylation procedure. Otherwise there is evidence that in the main chains glucose residues linked through $C_1$ and $C_6$ are present since the excess formic acid provides a measure of such residues.

This investigation was carried out on the starches from banana, potato, sweet-potato and waxy maize (64), and the two sets of results were in close agreement. Hence it was concluded that, in all starches there are no glucose residues linked through $C_1$ and $C_6$ only. In addition the apparent chain length of all starch polysaccharides could now be determined by the use of periodate oxidation.
<table>
<thead>
<tr>
<th>Source of starch</th>
<th>Amylose content %</th>
<th>Average number of glucose residues per non-reducing end-group A by periodate oxidation</th>
<th>B by methylation</th>
<th>Calculated no of glucose residues per non-reducing end-group in the amylopectin fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana</td>
<td>21</td>
<td>27</td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>Potato</td>
<td>18</td>
<td>31</td>
<td>25,28</td>
<td>24-26</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>18</td>
<td>32</td>
<td>28,34</td>
<td>26</td>
</tr>
<tr>
<td>Waxy maize</td>
<td>0-1</td>
<td>20</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Potato amylopectin</td>
<td>0-1</td>
<td>24</td>
<td>-</td>
<td>24</td>
</tr>
</tbody>
</table>
Further, these results eliminated the structure proposed by Paesu (113) for starch involving hemiacetal linkages. If this were the true picture the production of formic acid would be much higher than one molecule per non-reducing end-group, since the hemiacetal bonding increases the number of contiguous hydroxyl groups.

In the amylpectin component, the proportion of non-reducing end-group may be determined from the liberated formic acid as in the case of the whole starch. The acid produced from the small proportion of reducing end-group may be ignored and it may be assumed also to originate from the non-reducing end-groups.

From the long-chain amylose fraction the reducing end-group may constitute half the end-group in the molecule and cannot be neglected. One molecule of formic acid will be released from the non-reducing end-group while the reducing end-group produces two molecules of formic acid and one of formaldehyde.

![Reducing end-group diagram]

Fig. XVI. **Reducing end-group**

The proportion of non-reducing terminal group
in the amylepectin fraction may be calculated from the results of the experiment on the whole starch. The formic acid liberated from the amyleose is neglected. This is permissible where the amyleose content is under 25% and the end-group in the amyleose is less than 1%. If the amyleose content is known the percentage of non-reducing terminal groups in the amylepectin can be determined.

Further information concerning the cross-linkages between the repeating chains of the amylepectin has been obtained from periodate oxidation studies. Assuming that the inter-unit bond

![Fig. XVII.](image)

is 1:6 in nature the bond between C₂ and C₅ will be severed on periodate oxidation. If the positions C₂ or C₅ are involved in the linkage there will be no periodate attack on this glucose residue. Hence, after periodate oxidation and hydrolysis and examination of the products no glucose should be found if all the links are 1:6. The presence of glucose should indicate that some of the inter-unit bonds involve C₂ or C₅. In practice, small
amounts of glucose (ca. 1%) have been recovered after periodate oxidation of potato, acorn, sago, and waxy maize starches (16, 114). The theoretical interpretation of these results is that more than 75% of the cross-linkages in amylopectin are of the 1:6-variety. Whether the remainder are 1:2 or 1:3 in nature has not so far been decided. The possibility also exists that the presence of glucose in the hydrolysate is due to "underoxidation" by the periodate ion.
The Synthesis and Degradation of Amylose and Amylopectin by enzymes

The discovery of an enzyme potato phosphorylase (39) which was able to catalyse the synthesis of a polysaccharide similar to amylose marked the beginning of the wide investigations into the enzyme systems of the starch components. Peat and his co-workers have covered a lot of ground on this complex problem in the last decade. They have isolated a very pure enzyme fraction, a phosphorylase or P-enzyme from the potato (118, 119) and from the broad bean (120). The P-enzyme was characterised as the factor responsible for the amylose synthesis since the product of its action on a substrate of glucose-1-phosphate was similar to the unbranched starch fraction in its iodine/-
iodine binding and its conversion to maltose by $\beta$-amylase. The average chain length from end-group assay was 80-90 units (121). Hassid and McGready also isolated a synthetic starch but found no end-group and concluded that it was either too long or looped (131).

On further investigation of the starch enzymic mechanisms another enzyme, Q-enzyme has been isolated in a highly purified form from the potato, broadbean, and wrinkled pea (119,120). This enzyme was found to work in conjunction with the P-enzyme in catalysing the synthesis of the branched amylopectin component (118,122) from glucose-1-phosphate. The properties of the product were similar to those of natural amylopectin namely, non-reducing, giving red-purple coloration with iodine, and being 46% converted to maltose by $\beta$-amylase. These conclusions have been verified by Hassid and Nussenbaum (123) and Cori and Illingworth (124). The difference between the synthetic and the natural amylopectins was found in the lower molecular weight of the former (ca 60,000 from osmotic pressure measurement).

Much work has been carried out in an endeavour to ascertain the precise functions of the two enzymes. The first postulate was that in the course of the synthesis of amylopectin by P- and Q-
enzymes an intermediate "pseudoamylose" of unbranched chains of 20-30 glucose residues was formed. The P-enzyme was responsible for this initial product and then the Q-enzyme formed the 1:6-linkages to produce the branched chain and in addition was able to break off by a cleavage of the $\alpha-1:4$-bonds any superfluous glucose units from the original unbranched chains. In a later paper from the Peat school, however, this hypothesis has been modified and simplified as the Q-enzyme did not have the ability to react with the short chain "pseudoamylose". The reaction was represented as follows (126).

\[ \text{P-enzyme} \quad \frac{\text{Glucose-1-phosphate}}{\text{Q-enzyme}} \quad \text{Amylose} \quad \rightarrow \quad \text{Amylopectin} \]

In support of this theory Hassid (126) showed that dextrins of average chain length 20 were not utilised by Q-enzyme in forming amylopectin but that dextrins of chain length 116 were converted.

Evidence has been obtained that polysaccharide-synthesising enzymes such as P-enzyme from both plants and animals are unable to make use of the substrate glucose-1-phosphate unless a preformed polysaccharide is present (127, 128, 129). In order to show priming activity this preformed chain was thought to have a minimum of three glucose units from which the enzyme has the ability to build up the polysaccharide by endwise attachment.
In a series of experiments using known mixtures of the P- and Q-enzymes (130) it was observed that with excess P-enzyme amylose was produced and with excess Q-enzyme the end-product was amylopectin. Polysaccharides intermediate between these components and some resembling glycogen were obtained using varying amounts of both enzymes. Examination of the amylose by end-group assay showed a chain length of 204, which was greater than when the P-enzyme was used alone, and the apparent chain length of the amylopectin was 21.

From his work on the Q-enzyme, Peat (132) observed that the enzymic action was non-phosphorolytic and was supported by Hassid who failed to find any isophosphorylase in potatoes. On the other hand Bernfeld and Meutemedian (133) claimed that the enzyme, isolated from potatoes and which catalysed the conversion of amylose to amylopectin, functioned by phosphorus transfer. Again Beckmann and Roger (134) have stated that the product from the interaction of amylose and Q-enzyme was not an amylopectin but merely an amylose-fatty acid complex. Hassid could not confirm this claim (123).

The rate of conversion of potato amylose to amylopectin by Q-enzyme has also been studied (135), and was found to increase markedly when maltosaccharides of short average chain length were added but those devoid of \( \alpha-1:4 \)-linkages do not have this effect.
Both the F- and Q-enzymes have also been shown to be present in the flagellate, Polytomella coeca (136, 137, 138). The gram negative coccus "Neisseria perflava" has the ability to form a polysaccharide with properties, such as iodine uptake and apparent chain length, intermediate between amylodextan and glycogen when in culture with sucrose (139, 140). The presence of an "amylosucrase" has been postulated. Another bacterium Cl butyricum display the same property, the iodophilic polysaccharide being of the poly-glucose amylopectin type (141).

Although much research has been carried out on the synthesis of starch and its components, the other side, namely, the hydrolytic action, has by no means been neglected. The isolation of a highly purified sample of $\beta$-amylase in a crystalline state from the sweet potato by Balls (24, 142) has enabled much structural work on the starch components to be accomplished.

$\beta$-Amylase ruptures only the second $\alpha$-1:4-glucosidic linkage from the non-reducing chain of both the amylose and amylodextan molecule, releasing one molecule of $\beta$-maltose. The attack is then continued on the penultimate link from the non-reducing end. In the case of amylose, it has been/—
been widely accepted that each chain is completely transformed before another is attacked (143). This hypothesis, the single chain mechanism has been criticised by Hopkins and co-workers (144) and by Bourne and Whelan (145) whose experimental results are consistent with a "multichain" theory whereby the attack is random and on chains of all lengths.

Peat has recently shown that the action of Balls' crystalline $\beta$-amylase differs from that of amorphous preparations of the enzyme in that the conversion of amylose to maltose is 70% and not 100% (146). Evidence has been put forward that the standard $\beta$-amylase preparations contain a debranching factor - the Z-enzyme. This new enzyme was prepared free from $\alpha$- and $\beta$-amylases from the soya bean and has been characterised as a $\beta$-glucosidase (147). This work has led to the conclusion that there appears to be a certain degree of branching in the amyloses examined, namely those from the potato, sago, tapioca and maize (146, 146). The type of branching is different from the $\alpha$-1:6-links in amylpectin and is specifically attacked by the Z-enzyme. Kerr and Cleveland (149) have supported these findings and claim that in potato amylose there is an average of 1/.
1 to 2 chains per molecule and 2 to 3 chains per molecule in tapioca amylose.

As in the amylose case, one molecule of $\beta$-maltose is removed after the other from the amyllopectin molecule until an obstacle is encountered in the substrate, i.e. a branching point. The conversion limit has been shown by various workers to vary between 50 and 62% (36, 35, 79, 82) depending on the origin of the amyllopectin. The $\beta$-amylase is unable to split the $\alpha$-1:4 bond on the glucose at the branching point. Hence the number of glucose residues from the non-reducing end-group of the dextrin to the branch point varies between 2 and 3 according as the number of glucose residues in the outer branch of the amyllopectin is even or odd (150).

![Diagram of amyllopectin and dextrin conversion](image-url)

Fig. XVIII.

Another/
Another enzymic system has been isolated in a pure state by Peat and his co-workers (151) who have designated it the R-enzyme. Its action is purely hydrolytic in that it is debranching with respect to the predominant inter-chain linkage in amylopectin namely the \( \alpha-1:6 \) bond. It does not break the main chain-forming \( \alpha-1:4 \) linkage and has no synthesising properties. Indeed its function appears to be the reversal of that of the Q-enzyme. By the use of this enzyme new evidence on the multiple branching in waxy maize starch has been acquired (152). The linear chains in amylopectin are represented by A, B and C types - "A" has only the \( G_1 \) group engaged in inter-bonding to another chain, a "B" chain \( G_1 \) and one or more of its \( G_6 \) groups, and "C" only \( G_6 \) groups. On \( \beta \)-amylolysis of this amylopectin the conversion limit was 50\%, all the "A" type chains being attacked, leaving short "stubs" of two or three glucose units while the "B" chains are completely unaffected.
The high molecular weight dextrin was then acted on with X-enzyme which attacked the \( \alpha-1,6 \)-bonds. Maltose and maltotriose were liberated by the enzyme and were shown to have come from the "A" chains and therefore the yield of these di- and tri-saccharides was a measure of the proportion of "A" chains. From the laminated type structure the theoretical yield would be 0.085 molar per cent, while the actual yield was greater. The conclusion is drawn that the proportion of "A" chains is higher and the authors favour the multiple branched model of Meyer.

However, Hirst and Manners (153) have recently used Peat's experimental results and shown that the ratio of "A" to "B" chains in the waxy maize/
maize starch is 1:4. Further the yield of maltose and maltotriose, after the action by the R-enzyme on the dextrin, from Meyer's multiple branched structure would be 12.5% approximately, much in excess of the experimental evidence. The waxy maize starch therefore contains only one chain in every five with more than one branch point. From these findings it is suggested that the amylopectin structure conforms more to the open, laminated model than the compact multiple branched theory as put forward by Meyer.

**Molecular weights of starch and its components**

Although many efforts have been made to measure the molecular weights of starches, there is considerable disagreement in the results of the various investigators, probably due to the difficulty of bringing the starch fractions into solution without degradation, and to the use of inhomogeneous fractions. Many workers have carried out studies using osmotic pressure and chemical methods which give a "number average", and intrinsic viscosity and light scattering where a "weight average" is produced. On the other hand the use of the ultracentrifuge which probably gives the most realistic picture, has shown that solutions of starch are extremely heterogeneous.

From osmotic pressure measurements a wide range
of values have been obtained. For amylepectin the figures are from 200,000 (154) to \(1 - 6 \times 10^5\) (85), while the range for amylose lies between 30,000 and 400,000.

Application of the viscometric method involves difficulties in the case of undegraded starches, because of the preparation of such starch solution and the tendency of the polysaccharide to retrograde from solution. Recently various workers (156, 157) have measured viscosities of amylose and amylepectin in caustic potash solution (N). Kerr et alia (157) have plotted the osmometrically determined degree of polymerisation against the intrinsic viscosity and claim to have determined a degree of polymerisation of 1400-1500 for corn amylepectin, and 1300-1400 for tapioca amylepectin.

Viscosity measurement has also been widely employed with acetylated and methylated starches in various solvents, such as chloroform, acetone, water, formamide and m-cresol. The values obtained were graphed against the osmometrically determined molecular weights in order to derive the constant \(K_m\) in the Staudinger equation (21, 79, 110, 158).

As already mentioned (p. 26) Meyer et alia have utilised the interaction of the reducing-group of the polysaccharide with 3,5-dinitrosalicylic acid to ascertain the degree of polymerisation (88).

Another/−
Another similar chemical method has been developed by Nussenbaum and Hassid (159) in which the oxidising agent is alkaline ferricyanide in the presence of cyanide. The authors report that both methods give molecular weights in close agreement with those determined by other means (160,159).
DISCUSSION

The major part of the present investigations is the elucidation of the structure of the starch and its components from the malted barley which is a mixture of the two varieties "Plumage Archer" and "Spratt Archer".

The initial isolation of a crude starch, which contained a little fibre, was accomplished by a purely mechanical process, the use of reagents likely to cause degradation being avoided. Chromatographic examination of the ethanol extract of the malted barley showed that glucose, maltose, probably isomaltose and a pentose were present in the grain after the action of the malting enzymes. On microscopic examination the starch granules were spheroidal in shape and small in size and many of them appeared ruptured, probably because of the malting process.

The whole starch was prepared by chloral hydrate extraction (32, 137) of the "crude" starch and was obtained as a fine white powder with low protein content (0.2%). To effect a thorough removal of fatty acids which interfere with the uptake of iodine by the polysaccharide, a further methanol extraction was given to the whole starch before potentiometric amylose determination. The amylose content determined by potentiometric iodine titration was 23.3% under the standard conditions of Bates/
Bates, French, and Rundle (59) as modified by Wilson, Schoch and Hudson (65) assuming that pure amylose has an iodine uptake of 21.5% (Technique A); it was 27.8% using the method adapted by Anderson and Greenwood (68) if the iodine uptake of pure amylose is 19.0% (Technique B). It is assumed that the conditions which hold for normal amylose and amylpectin will also be applicable to the amylose and amylpectin from the malt starch in these determinations even if these components have been degraded during malting. The Blue Value of the whole starch was 0.37 (74, 75). The specific rotation was +159° in sodium hydroxide solution (N) and +182° in perchloric acid (50%), values which are characteristic of most starches examined.

Glucose was the only sugar detected on the chromatogram after acid hydrolysis of the starch, the conversion being 97% of the theoretical as estimated by alkaline hypoiddite. It was hence concluded that the polysaccharide was composed solely of glucose residues.

The Fractionation of Crude Malt Starch

A separation of the amylose and amylpectin components of the starch was carried out by fractionation of the crude starch using several different methods but in no case was the fractionation complete. The dependence of a successful fractionation/-
fractionation on the complete dispersion of the starch has led to the variation of the many factors affecting it, namely concentration, temperature, viscosity, pH, and time of dispersion. The methods attempted (A-D) were carried out under the conditions which were found to be most successful for the fractionation of barley starch by MacWilliam and Percival (65). Method E involved the combination of adsorption on aluminium hydroxide with thymol precipitation in order to obtain a pure amyllose fraction (53).

Method A. Using the standard technique of Bourne Donnison, Haworth and Peat (45) with thymol as the precipitant and dispersing at 100° for three hours, the precipitated fraction had a Blue Value of 0.80 representing ca 57% amyllose = ca 50% of the total amyllose in the crude starch dispersed. The Blue Value of the "amylopectin" fraction was 0.134 representing 93% amyllopectin = ca 32% of the total amyllopectin dispersed.

Method B. With butanol as the precipitant (76) and the time of dispersion 2.5 hours the "amylose" precipitate had a Blue Value of 0.90 = 64% amyllose and represented 90% of the total amyllose. The Blue Value of the "amylopectin" was 0.134 = 93% amyllopectin and represented ca 52% of the total amyllopectin.

Method C/-
Method C. In this case using pyridine as the precipitating agent (76) the "amylose" fraction contained 67% amylose (Blue Value 0.94) representing ca 90% of the total amylose in the dispersion, whereas the non-precipitated fraction represented 90% amylopectin (Blue Value 0.140) but only 29% of the theoretical yield.

Method D (76, 65). The crude starch was dispersed for three hours with pyridine at 90°, given 5 minutes high speed stirring in an Ato-Mix Disperser before the addition of butanol. In this instance the precipitated fraction represented 32% amylose (Blue Value 0.45), and 12% of the total amylose while the "amylopectin" was equivalent to 97% amylopectin (Blue Value 0.078), and ca 70% of the total amylopectin dispersed.

Method E. In order to obtain a pure amylose fraction the technique of adsorption on aluminium hydroxide and precipitation with thymol (55) was attempted. The yield obtained was very low, 23% of the total amylose dispersed and the fraction had a Blue Value of 0.67.

In all the fractionation experiments (except D) the precipitated fraction contained 30-40% amylopectin as impurity but represented in B and C about 90% of the total amylose. On the other hand the non-precipitated fractions only contained 3-7% amylose but represented from 30 to 70% of the total amylopectin.
The percentages of each component in the various fractions are all calculated from the Blue Values obtained and cannot therefore be regarded as absolute, but serve as a good comparative method in these fractionations. The content of whole starch in the "crude" starch is assumed to be 80% from the yield on chloral hydrate extraction, the remainder being impurities such as husk and protein matter.

Two different methods were adopted in an attempt to increase the percentage purity in the precipitated fraction, namely reprecipitation with thymol and with butanol. In the former case there was no purification of the "thymol-amylose" but reprecipitation with butanol produced an increase in the "amylose" content of the precipitated fraction which was obtained in good yield.

Further treatment of the "amylopectin" fractions with the precipitants did not increase the purity of the component. However, when thymol amylopectin was fractionated with methanol (53) the Blue Value changed from 0.134 to 0.110 but the yield was only 50%. 

...
The Amylose Fraction

In the above fractionation experiments both the butanol and pyridine methods gave the same yield of total amylose from the crude starch but in the precipitation with pyridine the "amylose" fraction gave a higher Blue Value. Hence a quantity of crude starch was fractionated with pyridine, the product "pyridine-amylose" then being refractionated with butanol (76). The "pyridine-amylose" from the large-scale experiment had a lower Blue Value (0.84) but the initial yield of total amylose was the same. The refractionations with butanol were then carried out, two at 0.5% six at 0.2% as recommended by Higginbotham and Morrison (76) and four at 0.1%. Samples were removed after each precipitation in order to determine the Blue Value and iodine uptake. The final product had a Blue Value of 1.25, and an iodine uptake of 20.8% as determined by technique A and 19.0% by technique B. The yield of amylose was 55% of the total amylose in the crude starch and was considered to be in as pure a condition as could be realised.

After each precipitation the "butanol-amylose" complex was examined under the microscope in order to ascertain whether the crystalline product adopted a fixed structure. Various forms have been reported in/−
in the literature, six-segmented particles by Schoch (44) for corn amylose, rectangular platelets by Kerr (61) who has also found needle-shaped crystals for the tapioca amylose complex (55, 93). MacWilliam and Percival observed that as the reprecipitations with butanol were applied, the complex changed from six-segmented particles to rectangular platelets and finally appeared in the form of needles, which were not altered on further precipitation (65). It was therefore postulated by these workers that the amylose approached its purest state when it appeared as needle-shaped crystals. In the case of the malted barley amylose under consideration a spheroidal form predominated with the first butanol precipitation. On the second refractionation the orientation had changed to long rectangles and thereafter the crystals were needle-shaped.

The butanol-amylose complex dissolved readily in warm water but tended to retrograde from solution. Freeze-drying was adopted in the final isolation of the amylose from the complex. The product was more easily manipulated and went into solution quite readily. The specific rotation was $+200^\circ$ in water, $+143^\circ$ in sodium hydroxide solution (N) and $+205^\circ$ in perchloric acid (30%). On acid hydrolysis of the amylose, glucose was the only sugar detected on the chromatogram, and the yield was 95% of the theoretical as estimated by alkaline hypoiodite oxidation, a value
similar to that of the whole starch.

Further insight into the constitution of the amylose was obtained from the classical technique of methylation, hydrolysis and identification and estimation of the resultant fractions. In order to minimise any initial degradation which may arise on dispersing the polysaccharide in alkaline solution (161) before addition of the normal methylating agents, it was decided to methylate the amylose fraction using diazomethane by the method of Hough and Jones (162). The diazomethane concentration in ether was maintained for a period of 34 weeks at 0°C, the reaction being heterogeneous in nature since the polysaccharide was not in solution. Using diazomethane for this purpose there is the possibility of formation of methylene cross-linkages between adjacent glucose units. However, on acid hydrolysis of a sample of the partially methylated amylose, no formaldehyde could be detected (163). The methoxyl content was raised to 20.2% by this means. Three additional methylations were carried out with sodium hydroxide and dimethyl sulphate, to give a product whose methoxyl content (44.2%) was not raised on further treatment. The methylated amylose was obtained in 72% yield and was fractionated by dissolution in light petroleum-chloroform mixtures. The main fraction (B) had \([\alpha]^{15}_D + 205\) in chloroform/-
chloroform: OCH₃, 44.8%, and ηsp/o 0.89 in m-cresol (η = 0.83).

On hydrolysis of a small portion of fraction B and examination of the products by paper chromatography the presence of 2:3:6-tri-β-methyl-D-glucose in quantity and a trace of di-β-methyl-D-glucose was detected. A quantity of fraction B was hydrolysed in methanolic hydrogen chloride solution to glycosides and in aqueous hydrogen chloride solution to the corresponding methylated sugars which were separated on a cellulose column (100) using n-butanol (30%)-light petroleum (70%), saturated with water, as eluant. When the "end-group" had been removed, the proportion of the eluant components was altered to 50:50. Three fractions were obtained thus and a fourth in trace quantity from the butanol and water extractions of the column. This fraction showed the presence of a monomethyl glucose and glucose on the chromatogram but was not examined further. These sugars were considered to have arisen because of undermethylation of the amylose or demethylation during hydrolysis, and to have no structural significance.

Although fraction (1) showed the presence of 2:3:4:6-tetra-β-methyl-D-glucose alone on the chromatogram, quantitative estimation by alkaline hypoiodite oxidation (97, 164) proved that it was only 47%.
47% pure, indicating that it contained methyl 2:3:6-tri-\(\alpha\)-methyl glucoside as impurity. This was confirmed on hydrolysis of a portion of the syrup and chromatographic examination. The whole fraction was therefore hydrolysed and separated on Whatman 3MM paper, using benzene-ethanol-water (149:45:5, v/v) as solvent, to give fraction (1a) 2:3:4:6-tetra-\(\alpha\)-methyl-D-glucose and fraction (1b) 2:3:6-tri-\(\alpha\)-methyl-D-glucose. The 2:3:4:6-tetra-\(\alpha\)-methyl-D-glucose was obtained crystalline and identified by its melting point and mixed melting point.

Fraction (lb) (2:3:6-tri-\(\alpha\)-methyl-D-glucose), on the other hand failed to crystallise but the presence of 2:3:6-tri-\(\alpha\)-methyl-D-glucose was indicated by its \(R_f\) value on the chromatogram.

Fraction (2) crystallised on standing overnight and had properties (specific rotation in water, melting point and mixed melting point) identical with 2:3:6-tri-\(\alpha\)-methyl-D-glucose. It was also identified by its inversion of optical rotation in cold methanolic hydrogen chloride solution (165,166).

From chromatographic examination of fraction (3) the presence of two spots indicated that 2:3-di-\(\alpha\)-methyl-D-glucose, and 2:6-or 3:6-di-\(\alpha\)-methyl-D-glucose alone or a mixture of the two. Quantitative estimation by paper chromatography and hypoiodite oxidation showed that there was 7.5% of 2:3-di-\(\alpha\)-methyl-D-glucose present in the syrup. Inversion of optical/
optical rotation in methanolic hydrogen chloride solution was shown by the syrup, indicating that position 4 in the sugar was unsubstituted. The inversion is believed to arise from the formation of the glucosylfuransides. By using the method adopted by Bell (101) the amount of 2:6-di-O-methyl-D-glucose in the fraction was estimated. This method involves the attack of the periodate ion on the methylglucopyranosides, derived from heating the methanolic hydrogen chloride solution of the di-O-methyl-D-glucoses for several hours at 100°C. In the resultant glycosides, only the 2:6-isomer possesses contiguous hydroxyl groupings (C₃ and C₄) which are attacked by the periodate ion.

The molar uptake of periodate per methyl di-O-methyl glucoside unit is therefore an estimation of the amount of 2:6-di-O-methyl-D-glucose present. It was found that the di-O-methyl fraction under examination contained 79% of 2:6-di-O-methyl-α-glucose by this/.
this method.

After this estimation the periodate was destroyed by ethylene glycol and the solution evaporated to dryness. The residue was extracted with chloroform to obtain the remaining di-2-methyl glucosides which were hydrolysed and chromatographed. The presence of two sugars 2:3- and 3:6-di-2-methyl glucoses was indicated.

From this methylation study the molar percentages of the three fractions were:

2:3:4:6-tetra-2-methyl-D-glucose 0.32%
2:3:6-tri-2-methyl-D-glucose 98.09%

\[
\begin{align*}
2:3 & \quad \text{di-2-methyl-D-glucose} \quad 0.12 \\
2:3 & \quad \text{di-2-methyl-D-glucose} \quad 1.26 \\
3:6 & \quad \text{di-2-methyl-D-glucose} \quad 0.21
\end{align*}
\]

By Difference.

From the yield of "end-group" (2:3:4:6-tetra-2-methyl-D-glucose) the chain length of the malt amylose was 310±20 glucose units, assuming no branching points. Such a figure represents a molecular weight of ca 66,000, a figure which requires a constant \(K_m = 1.27 \times 10^{-5}\) in the Staudinger equation \([\eta] = K_m \times \text{Molecular Weight}\) from the viscosity measurements in m-cresol of fraction B, \([\eta] = 0.83\). At present there are no constants available whereby the specific or intrinsic viscosity of a methylated amylose in m-cresol can be employed to determine the apparent molecular weight./
weight weight of the polysaccharide.

Periodate oxidation studies were carried out on the amylose fraction in order to verify the chain length determined by end-group assay. This involves the estimation of the formic acid liberated from the end-groups by the periodate ion. Halsall, Hirst and Jones (155) have found that 150 hours is the time necessary for the complete oxidation of the structurally similar methyl β-maltoside by the periodate ion under specified conditions. By this method the estimated titre at 150 hours was equivalent to 1 mole of formic acid per $117+9$ glucose residues. Assuming that the amylose has a "straight" chain structure this value corresponds to $350+30$ glucose residues per molecule, which is in good agreement with the results from the methylation studies.

After hydrolysis of the periodate oxidised amylose and examination of the product by paper chromatography no glucose was found to be present. This indicates that there are no glucose residues in the amylose joined to each other through $G_1\theta_2$ and $G_1\theta_3$ but only through $G_1\theta_4$ and $G_1\theta_6$. Any branching point in the amylose can therefore occur only through the $G_6$ position. The presence of 2:6- and 3:6-di-$\alpha$-methyl-D-glucoses in the di-$\alpha$-methyl fraction from the methylation investigations must have/
have arisen because of undermethylation or demethylation during hydrolysis. It has been found (65) that the demethylation of 2:3:6-tri-O-methyl-D-glucose under the conditions employed for the hydrolysis of the methylated polysaccharide amounts to about 0.7%. This may therefore account for not more than 50% of the di-O-methyl fraction.

The 2:3-di-O-methyl-D-glucose could have originated from a 1:6 branching point. Whereas, for one branch point the molar ratio of 2:3:4:6-tetra-O-methyl-D-glucose to 2:3-di-O-methyl-D-glucose would be in the ratio 2:1, the yields obtained were 3:1. Hence the probability of a branch point through C₆ in the amylose exists.

On the basis of the above results it appears that the amylose from malted barley can be represented for the most part by a straight chain of about 300 glucose units joined by α-1,4-linkages. However the possibility of a structure with a slight degree of branching is not precluded.

The Amylopectin Fraction

In the fractionation experiments the amylopectin with the lowest Blue Value and in the highest yield was obtained by precipitation of the amylose with pyridine and butanol. The fractionation of thymol-amylpectin with methanol did effect some purification of the fraction but resulted in an appreciable loss.
A quantity of the crude starch was therefore fractionated by method D, the product being isolated and freeze-dried, and obtained in 60% yield of the total amyllopectin dispersed. Its Blue Value was 0.035. Potentiometric iodine titrations indicated that the amyllopectin contained less than 2% amylose by technique A and less than 1% by technique B. It had a specific rotation of +149° in sodium hydroxide solution (N) and +170° in perchloric acid. On acid hydrolysis of a sample and examination of the product by paper chromatography glucose alone was detected. The yield, as estimated by alkaline hypoiodite oxidation was 96% of the theoretical, similar to the values found for the amylose and the whole starch.

Methylation and end-group assay investigations were applied to the amyllopectin which was methylated by twelve treatments with dimethyl sulphate and sodium hydroxide in an atmosphere of nitrogen to give a product in 80% yield with OCH₃ 43%. When it was fractionated by dissolution in light-petroleum-chloroform mixtures, four fractions were isolated, the main one (B) being obtained in 80% yield of the total methylated amyllopectin. It had \([\alpha]_D^{15} + 200^\circ\) in chloroform, OCH₃ 43.2%, and \(\eta_s/c\) 2.21 in m-cresol.

Fraction/-
Fraction B was then methylated by Purdie's method (167) using methyl iodide and silver oxide. The properties of the product were very similar to those of the fraction before the Purdie methylation, and it was still homogeneous. A small portion of the fraction was hydrolysed and examined on the chromatogram. The presence of 2:3:4:6-tetra-0-methyl-D-glucose, 2:3:6-tri-O-methyl-D-glucose, 2:3-di-O-methyl-D-glucose, and 2:6 or 3:6-di-O-methyl-D-glucose or a mixture of both was indicated. Estimation by alkaline hypoiodite oxidation showed that the 2:3:4:6-tetra-O-methyl-D-glucose was present in 5.5% yield of the total sugars, 2:3:6-tri-O-methyl-D-glucose 86.1% and the di-O-methyl-D-glucoses 8.4%. These results corresponded to one non-reducing terminal group per 18 glucose residues in the amylopectin fraction.

A quantity of fraction B was hydrolysed and the resulting methylated sugars separated on a cellulose column as in the case of the methylated amylose. Five fractions were obtained. Even though fraction (1) showed the presence of 2:3:4:6-tetra-O-methyl-D-glucose alone, estimation by alkaline hypoiodite oxidation showed that it was 49% pure indicating the presence of methyl 2:3:6-tri-O-methyl glucoside. On hydrolysis of a small sample and chromatographic examination this was confirmed. The whole fraction was/-
was therefore hydrolysed and the sugars separated on Whatman 3MM papers using benzene-ethanol-water (149:45:15:v/v) as solvent, to give fraction (1a) 2:3:4:6-tetra-<n>Q</n>-methyl-D-glucose and (1b) 2:3:6-tri-<n>Q</n>-methyl-D-glucose both in high purity. Fraction (1a) was obtained in the form of long needle-shaped crystals which corresponded to 2:3:4:6-tetra-<n>Q</n>-methyl-D-glucose. The formation of the crystalline anilide confirmed this. Fraction (1b) also crystallised and was identical with 2:3:6-tri-<n>Q</n>-methyl-D-glucose.

Fraction (2) was obtained crystalline and was identified as 2:3:6-tri-<n>Q</n>-methyl-D-glucose by its melting point and mixed melting point, and by its inversion of optical rotation in cold methanolic hydrogen chloride solution.

On chromatographic examination fraction (3) gave two spots whose R<sub>G</sub> values corresponded to 2:3:6-tri-<n>Q</n>-methyl-D-glucose and 2:3-di-<n>Q</n>-methyl-D-glucose. The fraction was separated on Whatman 3MM paper using butanol-ethanol-water (4:1:5:v/v) as solvent to give fraction (3a) 2:3:6-tri-<n>Q</n>-methyl-D-glucose and (3b) 2:3-di-<n>Q</n>-methyl-D-glucose. Fraction (3a) was obtained crystalline and corresponded to authentic 2:3:6-tri-<n>Q</n>-methyl-D-glucose. Fraction (3b) on the other hand failed to crystallise but was identified as 2:3-di-<n>Q</n>-methyl-D-gluconophenylhydrazide.
The fraction (4) did not crystallise on standing in the cold for several weeks. The presence of 2:6- and 3:6-di-O-methyl-D-glucoses was indicated both by paper chromatography and by the inversion of optical rotation in cold methanolic hydrogen chloride solution. Estimation of the amount of the 2:6-isomer by Bell's method showed that it was present to the extent of 53%. The 3:6-isomer was isolated (see p. 68) the estimated content being 40% of the fraction.

On chromatographic examination of fraction (5) a trace of monomethyl glucose and glucose was indicated. This fraction was not examined further.

The following is a summary of the results from the methylation study of the amyllopectin; the weights of the sugars are given in molar percentages.

<table>
<thead>
<tr>
<th>Sugar Type</th>
<th>Molar Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:3:4:6-tetra-O-methyl-D-glucose</td>
<td>5.7%</td>
</tr>
<tr>
<td>2:3:6 - tri-O-methyl-D-glucose</td>
<td>87.4%</td>
</tr>
<tr>
<td>2:3 - di-O-methyl-D-glucose</td>
<td>4.3%</td>
</tr>
<tr>
<td>2:6 - di-O-methyl-D-glucose</td>
<td>1.5%</td>
</tr>
<tr>
<td>3:6 - di-O-methyl-D-glucose</td>
<td>1.1%</td>
</tr>
</tbody>
</table>

The yield of "end-group" from this methylation study of the amyllopectin corresponded to one non-reducing terminal group per 17-18 glucose residues, in close agreement with the result from the estimation of the methylated sugars by paper chromatography.

The viscosity measurements of fraction B gave $\eta_{sp/c}$ 2.21 (c. 0.4 in m-cresol). Assuming that the/
the viscosity/molecular weight relationship of Hirst and Young (21) for methylated starches is applicable to methylated amyllopectin this value corresponds to an apparent molecular weight of 320,000.

Hence the amyllopectin from malted barley is similar to other amyllopectins studied in that it possesses a highly branched structure.

**Controlled hydrolysis of methylated amyllopectin from malted barley in methanolic oxalic acid solution**

For many years it has been realised that there were present in the starch molecule easily ruptured bonds. The sensitising of a $\beta$-limit dextrin, whereby further $\beta$-amyolysis could be carried out, indicated the scission of some inter-chain bonding. Additional information was obtained from the controlled acid hydrolysis of methylated starches by Bawn, Hirst and Young (105).

These workers subjected the methylated starches to treatment with methanolic oxalic acid solution under conditions of controlled temperature. The molecular weight of the starch, ca 500,000 as determined from viscosity measurements, gradually became smaller until a value of ca 20,000 was reached. The mild acid conditions had no further effect on this unit of 4-5 chains. During the experiment no production of reducing oligosaccharides/-
ides could be detected, which showed that the predominant \( \alpha-1:4 \) -bonds in the chains were not ruptured. When the reaction had been carried out at two different temperatures, the activation energy was calculated to be ca 20,000 calories which is of the same order as the hydrolysis of a normal glycosidic linkage (106). The reaction at the lower temperature was found to obey first order kinetics after 100 hours, before which the reaction was thought to follow some complex pattern. At the higher temperature first order kinetics were obeyed throughout. On hydrolysis of the final remethylated disaggregation product and examination of the resultant glucosides, no increase in the percentage of non-reducing terminal group was found. This showed that the reaction involved the cleavage of inter-chain bonds.

Although these experiments were conducted on methylated whole starches the results were in close agreement with those obtained by Forsyth (168) on methylated waxy maize starch which may be considered as a naturally occurring amylopectin. In this case it was also shown that no less than 70\% of the di-\( Q \)-methyl-\( D \)-glucose fraction from the final disaggregation product consisted of the 2:3-isomer. This is strong evidence that the predominant inter-chain linkage in the amylopectin fraction, namely the 1:6 bond is not ruptured but that some other linkage...
or linkages are susceptible to the action of the acid.

The methylated amylopectin from the malted barley was treated with methanolic oxalic acid solution (pH 2.74) in two experiments, one at 44.3° and the other at 70.5° as in the above disaggregation experiments of Hirst and Young (21). The flask with the methanolic oxalic acid solution of methylated amylopectin was placed in a thermostatically controlled oil-bath at the required temperature and in an inert atmosphere of nitrogen. At various intervals samples were withdrawn, the polysaccharide isolated and the specific viscosity determined in an Ostwald viscometer using m-cresol as the solvent.

\[
\eta_{sp} = \frac{T_2 - T_1}{T_1} \quad T_1 - \text{time of flow of pure solvent in seconds.}
\]

\[
T_2 - \text{time of flow of solution in seconds.}
\]

The velocity constant of each reaction was calculated from the first order equation:

Velocity constant = \( \frac{1}{t} \ln \frac{\eta_{sp}/c_0}{\eta_{sp}/c_t} = \frac{\eta_{sp}/c_0}{\eta_{sp}/c_\infty} \)

where \( t = \) time in seconds during which the reaction has been progressing \( \eta_{sp}/c_t, c_\infty \) - specific viscosity/concentration at time \( t \) secs, \( c_\infty \) secs, and infinity.

From the average value of the velocity constants
of both experiments at the temperatures 44.3° and 70.5°, the activation energy E, and the collision number A of the reaction were calculated from the equation,

\[ k_1 = \frac{-E}{RT} \quad \text{or} \quad E = \frac{R \ln \frac{k_2}{k_1} T_1}{T_2 - T_1} \]

where

- A - collision number
- E - activation energy.
- R - gas constant.
- T_1 - absolute temperature of reaction
- k_1 - velocity constant of reaction at T_1.

The average figures for the velocity constants were as follows:

- for temperature 44.3°, \( k_1 = 3.67 \times 10^{-7} \text{ secs}^{-1} \)
- for temperature 70.5°, \( k_2 = 6.04 \times 10^{-6} \text{ secs}^{-1} \)

From these figures \( E = 20,010 \text{ cala} \),

\[ A = 10^{-7.31} \]

These results are of the same order as those derived by Bawn, Hirst and Young (105). Again the first order kinetics were obeyed throughout the experiment at 70.5° but only after the first 100 hours in the reaction at 44.3°. The calculated activation energy of the reaction is comparable with that for the hydrolysis of a normal glucosidic link, of a covalent bond whose susceptibility to hydrolysis is greater than that in the maltose unit (pyranose linkage) (106).
The specific viscosity in m-cresol of the sample at \( t = 0 \) was 0.089 \((c, 0.39)\), giving \( \eta_{sp/c} = 0.23 \), which corresponds to a molecular weight of 16,170,000 according to the Hirst and Young graph (21). This is equivalent to a molecule of 70-75 glucose residues or about 4 chains of 18 units. This fraction was remethylated with the Purdie reagents to block any hydroxyls freed by the acid attack, the product having the same viscosity and optical rotation as before. On hydrolysis and examination of the methylated sugars by chromatography the estimated "end-group" was equivalent to one non-reducing terminal group per 19 glucose residues. The di-O-methyl-D-glucoses appeared as two spots, the 2:3-di-O-methyl-D-glucose representing at least 50% of the fraction.

These disaggregation experiments indicate that in the amylopectin fraction of starch there are at least two types of inter-chain linkage, one of which is stable to, and the other of which is severed by mild acid attack. The fact that the limiting fraction was essentially homogeneous in nature and that the yield of polysaccharide from the experiment was over 90%, not only indicates that this breakdown is not a random hydrolysis but that the amylopectin molecular size lessens in a definite fashion to give amy-
an end-product of 4–5 chains with the predominant linkage of the 1:6-variety uniting the chains in this unit.

The conclusion that there are inter-chain bends other than the 1:6-type in the amylopectin molecule was also reached on examination of the periodate oxidised amylopectin after acid hydrolysis. Glucose was detected on the chromatogram. As has already been explained if all the inter-chain linkages were of the 1:6 variety, no intact glucose residues should remain on periodate oxidation. Although the yield of glucose (7.4 m g. from 1 g.) theoretically corresponded to the presence of 14% of the cross-linkages involving the 
\( \text{C}_2 \) or \( \text{C}_3 \) of the glucose residues concerned, the probability of under-oxidation cannot be overlooked. Hence it can only be stated that the majority of the inter-chain bonding is of the 1:6-type.

\section*{\$\beta$-Amylolytic of the malt amylopectin}

The malt amylopectin was incubated in solution at pH 4.6 with a sample of crystalline $\beta$-amylase which was free from maltase and $\alpha$-amylase and had no z-enzyme activity (147), kindly presented by Dr Manners. The conversion to maltose was 44% and remained constant after 4 hours. Assuming an apparent chain length of 17–18 glucose units, and a "stub" of 2.5 units beyond the cross-linkage of the amylopectin, this conversion corresponds to
an internal unit chain of 7-8 glucose residues.

A sample of β-amylase was prepared from the barley flour (from which the malt was obtained), according to Northcote (169), the enzyme being extracted by ethanolic fractionation of the resultant solution by the method of Halsall, Hirst, Hough and Jones (36). Using this enzyme the percentage conversion to maltose on prolonged incubation with potato starch was 55, indicating the absence of α-amylase. Maltose was the only sugar present in the digest on chromatographic examination, indicating that little, if any, maltase was present.

The β-amylase from barley flour was incubated at pH 4.6 with the malt amylopectin, a constant conversion to maltose of 47% being recorded after 48 hours. The β-limit dextrin was isolated. The apparent chain length of this dextrin from estimation of the formic acid liberated on periodate oxidation was 8.5, which gives an internal unit chain of 6 glucose residues.

The dextrin had $\left[\alpha\right]^{18}_D + 148$ in sodium hydroxide solution (N), and a negligible iodine uptake by technique B. It gave a reddish colour with iodine which was thought to be adsorbed on the polysaccharide.

The/-
The same procedure was carried out in isolating the dextrin from the amylepectin of the starch from Plumage Archer and Spratt Archer barley. In this case the number of glucose residues per non-reducing terminal group was 9.7, equivalent to an internal chain of ca. 7. The percentage conversion to maltose was 58-59, which corresponds to an internal chain of 8-9 glucose residues, assuming an apparent chain of 26 units in this amylepectin fraction as determined by estimation of formic acid liberated on periodate oxidation (see p. 69). The dextrin had a specific rotation of +155° in sodium hydroxide solution (N) and had no appreciable iodine uptake by technique B. It gave a reddish colour with iodine as did the dextrin from malt amylepectin.

From these results it appears that the external branch of the amylepectin from barley starch is shortened during the malting process while the internal chain remains essentially the same. Hence the possibility of the polysaccharide being resynthesised by the malting enzymes seems remote.
Determination of the apparent chain length of the starches and amyllopectins from barley and malted barley from the yield of formic acid on periodate oxidation.

The starches and amyllopectins investigated were the starch and amyllopectin from Plumage Archer and Spratt Archer barley and malted barley, and from Ymer barley and malted barley.

A crude starch was isolated in each instance in the manner described on p. 91 for that of Plumage Archer and Spratt Archer malted barley. In the case of this latter variety of barley the whole starch was isolated from the crude product by purely mechanical means. For the Ymer barley and malted barley starches chloral hydrate extraction was employed (32, 137).

The amyllopectins were all obtained by fractionation of the crude starches with butanol and pyridine.

The formic acid liberated on periodate oxidation was estimated with each starch and amyllopectin, the calculated apparent chain lengths of these fractions being summarised below:

Variety/-
Variety of polysaccharide | Calculated chain length of starch | Calculated chain length in amylopectin
--- | --- | ---
Plumage Archer and Spratt Archer
- malted barley starch | 21-22 | 16-17
- malted barley amylopectin | | 14
- barley starch | 30-32 | 25-24
- barley amylopectin | | 26
Ymer - malted barley starch | 27-28 | 20
- malted barley amylopectin | | 17-19
- barley starch | 29-32 | 22-25
- barley amylopectin | | 24-25

The percentages of amylose by technique B were:
22.6% for the starch from Plumage Archer and Spratt Archer - barley
27.8% - malt
0.7% - malt

0.7% for the amylopectin from Plumage Archer and Spratt Archer - barley
0.4 - 0.7% - malt

22.5% for the starch from Ymer - barley
26.8% - malt
0.40% for the amylopectin from Ymer - barley
4.19% for

The starches from the two varieties of malted barley seem to be degraded to different degrees. From these investigations there appear to be two essential differences between the components from the barley before and after malting, namely, the shortening/—
shortening of the amylopectin chain after malting and an increase in the amylose content in the malt starches.

In conclusion it may be stated that the general picture of the amylose fraction of malted barley starch is similar to that of barley amylose (65). From the above results the amylose may be represented by a "straight" chain of over 300 glucose residues linked by $\alpha-1:4$ bonds, with the possibility of a slight degree of branching.

In the case of the amylopectin fraction the general structure is indicated from the final stable disaggregation sample which consisted of 4 or 5 chains of 17-18 glucose residues, the predominant inter-chain linkages being of the 1:6 variety. The whole molecule appears to be built up of a series of these "laminated" units united to one another by their reducing "end-groups", these bonds being less stable than the inter-chain linkage in this unit structure.

With respect to the fine structure of the amylopectin the apparent chain length is 17-18 glucose residues. From the results of $\beta$-amylolysis using crystalline $\beta$-amylase the internal chain consists of 8-9 units while the external chain consists of 9-10 units.

There/
There is a decrease in the external chain length of the amylopectin after malting, and an increase in the amylose content of the malt starch compared with that of the barley.

Fig. Barley amylopectin before malting

Barley amylopectin after malting.

No marked difference could be detected between the two starches from the two varieties of barley, but the extent to which the starches from the malted barley varieties had been degraded, varied slightly.
EXPERIMENTAL RESULTS

Notes on General Reagents and Experimental Procedures.

Methoxyl Determination.

The microvolumetric technique was adopted in all cases. This procedure involved the interaction of the methylated compound under examination with hydrogen iodide to give methyl iodide which was absorbed in a sodium acetate/bromine solution. It was converted to methyl bromide and iodine bromide, the latter being oxidised to sodium iodate. The iodate was then estimated by titration with standard thiosulphate solution.

Determination of the Specific Viscosity of the Methylated Polysaccharides.

The viscosities were carried out in a standard Ostwald viscometer at a temperature of 20°, using 10 ml. of solution or solvent. In every case, the solvent was m-cresol, and the concentration of the solute was ca 0.4%. The specific viscosity was determined from the equation:

\[ \eta_{sp} = \frac{T_2 - T_1}{T_1} \]

where \( T_2 \) - time of flow of solution

\( T_1 \) - time of flow of solvent.

Solvent/
Solvent for Paper Chromatograms

Where the simple sugars such as glucose and maltose were under examination the solvent used was the upper phase resulting from a mixture of benzene, n-butanol, pyridine, and water (1:5:3:3, v/v). On the other hand with the methylated sugars, the upper phase from a mixture of n-butanol, ethanol, and water (4:1:5; v/v) was the solvent. In all cases the developing reagent was a saturated solution of aniline oxalate in water.

Drying Procedure

The samples used for quantitative determinations were dried under reduced pressure for 12 hours over phosphorus pentoxide at 40°.

Potentiometric Iodine Titration

Technique A (59, 63)

A known weight of dry sample (ca 40 mg.) was placed in the titration vessel and dispersed in potassium hydroxide solution (10 ml., 0.5N), five hours being allowed for complete dispersion. Hydrochloric acid (0.5N) was then added till the solution was just acid to methyl orange before the addition of potassium iodide solution (10 ml., 0.5N) followed by distilled water to a volume of 100 ml. The liquid was then 0.05N with respect to potassium iodide. The starch solution was titrated at room temperature/-
temperature with iodine solution (0.05N with respect to potassium iodide and 0.001N with respect to iodine). The iodine was added in 1 ml. portions, the additions being made with stirring and seven minutes allowed for equilibrium to be established before the E.M.F. of the solution was determined. A blank experiment was also carried out as above. For the method of calculation of the amyllose content of the sample see ref. (170).

Technique B. (68)

These potentiometric iodine determinations were kindly carried out by Mr D. M. W. Anderson and Dr C. T. Greenwood using the sensitive technique they have recently developed.

Determination of Blue Value (74, 75)

The dry starch sample (10 mg.) was introduced into a small tube and wetted with water (1 ml.) and one drop of ethanol. Sodium hydroxide solution (2 drops, 2N) was added, and the tube heated on the water bath to give a clear solution. A blank experiment was carried out under similar conditions. After two hours the tube was washed into a standard flask (10 ml.) and diluted to the mark. 5 ml. of this solution (5 mg.) were introduced into a standard flask (500 ml.) and the volume made up to ca. 100 ml. with distilled water. Hydrochloric acid (6 drops, 3N) was added, followed by iodine solution (5 ml/-
(5 ml. 0.02% iodine in 2% potassium iodide solution).
The solutions were diluted to the mark and examined in the Spakker absorbtiometer using 4 cm.
cells and red '508' filters.
Preparation of the Crude Starch

Malted barley (1\(\frac{1}{2}\) kg.) which was a mixture of the varieties Plumage Archer and Spratt Archer, was lightly pounded in a mortar in order to split open the husk without any undue fragmentation. The grain was then gently shaken in a very fine sieve, while a very powerful air jet was applied from below. The air jet was sufficient to remove the lighter husk but did not blow the heavier starch out of the sieve. Further husk was removed by sieving the residue through a mesh large enough to allow the starch through but small enough to trap the large pieces of husk remaining.

The product was heated under reflux with ethanol (85\%) for three hours to inactivate the enzymes present. The ethanol extract was concentrated, and examination by paper chromatography showed the presence of maltose, another disaccharide (Rg0.07) thought to be iso-maltose, fructose, glucose and a pentose (probably arabinose).

The inactivated malt starch which still contained a fairly high percentage of fibre, was dried and ground in a "Raymond" laboratory mill and suspended in alcohol with stirring in order to free the fibre from the starch, and sieved through a No. 40 mesh. This procedure resulted in some loss of starch but compensated by its much more efficient removal of fibre. After sieving, the starch was allowed/-
allowed to settle, the alcohol decanted, the starch dried, suspended in water, and stirred mechanically for three hours in order to remove any soluble degradation products not completely eliminated by the ethanol extraction. The aqueous extract was decanted and the crude starch dried by stirring several times with alcohol followed by a similar treatment with ether.

Examination of the aqueous extract after concentration showed the presence of glucose alone, indicating that all the pentoses, disaccharides, etc., had been removed in the ethanol extraction. The presence of glucose alone was probably a question of the large quantity present as free sugar in the original malt, an amount too great for the ethanol extraction to remove entirely.

In order to reduce the fat content of the product, it was heated under reflux for five hours with methanol (95%), washed with water and again dried as above.

Yield of crude starch 400 g.

The crude starch was examined under the microscope. The granules were small in size, ovoid to spheroidal in shape, and many of them appeared to be slightly ruptured.

Isolation of the Whole Starch/-
Isolation of the Whole Starch

A small sample of the crude starch (5 g.) was suspended in water (20 ml.) and slowly added to boiling water (1 l.) with vigorous mechanical stirring. Boiling and stirring were continued for two hours when the dispersion was centrifuged and a quantity of fibrous matter removed. When the cool centrifugate was poured into alcohol (2½ litre) the starch settled as a white precipitate which was removed by filtration. This gelatinisation of the crude starch proved troublesome in that the product invariably retrograded on drying, and that a trace of pentose was found in its hydrolysis products. Another method, namely chloral hydrate extraction was adopted.

Chloral Hydrate Extraction of the Crude Starch (32,137)

A small quantity of the crude starch (20 g.) was stirred with an aqueous solution of chloral hydrate (1500 ml., 33%) for one hour at 80°. When the paste was cool the insoluble residue was removed at the centrifuge and stirred with three fresh portions of warm chloral hydrate solution (200 ml., 33%). After the third treatment the residue consisted almost entirely of fibrous material and the supernatant liquid was quite clear. The combined supernatant liquid and extracts were slowly poured through a coarse sintered glass funnel and then into acetone (8 litre) with vigorous stirring, when the starch was deposited as a white floculent.
flocculent precipitate. The last traces of chloral hydrate were removed by extraction of the starch with acetone in a Soxhlet apparatus for two hours followed by extraction with ether for one hour. The starch was then dried in a vacuum desiccator over phosphorus pentoxide. All the above operations were completed in one day to minimise the period during which the starch was in contact with the chloral hydrate.

Yield of whole starch 14.5g.

Examination of the Whole Starch.

Optical Rotation

A) In N. sodium hydroxide solution.

\[ \alpha^16_D + 159^0 (c, 1.1) \]

B) In 30% perchloric acid solution.

\[ \alpha^16_D + 182^0 (c, 0.78) \]

Moisture content 13.5%

Protein content 0.2% (% age nitrogen x 6.25)

Hydrolysis with sulphuric acid (A).

A small sample of the starch (50 mg.) was hydrolysed by heating on a boiling water-bath with sulphuric acid (0.5 ml. 7%) for seven hours in a sealed tube. After neutralisation with barium carbonate the liquor was centrifuged and concentrated to a thick syrup before being run on a chromatogram together/-
together with a standard containing glucose, maltose and xylose. Glucose was the only sugar detected on the paper.

**Hydrolysis with sulphuric acid (B) and estimation of the glucose with alkaline hypoiodite.**

Dry starch (0.1170 g.) was hydrolysed by heating on a boiling water-bath with sulphuric acid (25 ml., 2%) for eight hours. The acid was neutralised with barium carbonate and the resulting barium sulphate was removed by filtration and washed. After the filtrate had been reduced in volume (to 20 ml.), iodine (20 ml., 0.1N) and excess of sodium hydroxide solution were added. A reagent blank was also prepared. The solutions were allowed to stand in the dark for one hour. Sulphuric acid (2N) was then added until the solution was acid, and the excess iodine was titrated with standard sodium thiosulphate solution.

Weight of glucose found 0.127 g.

This constituted 97.6% of the theoretical yield of glucose from the starch.

**Determination of the Amylose Content of the Malt Starch**

**A) Blue Value (74, 75 see p. 89)**

The Blue Value for the malt starch was found to be 0.368. Assuming that the Blue Value of pure amylose is 1.40, this is equivalent to 26% amylose.
B) Potentiometric iodine titration. Technique A (59, 63).

Before these estimations, the samples were further defatted by extraction for four hours with boiling methanol.

The results were as below:

Weight of starch sample = 40.77 mg.

Volume/-
POTENTIOMETRIC IODINE TITRATION OF MALT STARCH

Technique A

$\text{I}_2$ bound by 100 g starch

$\text{I}_2 \times 10^2$
<table>
<thead>
<tr>
<th>Volume of iodine (0.00086N.) added (ml.)</th>
<th>Blank e.m.f.</th>
<th>Titre e.m.f.</th>
<th>Diff. e.m.f.</th>
<th>$[I]_{g} \times 10^{-7}$</th>
<th>Iodine bound by 100g starch (g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>256.0</td>
<td>226.8</td>
<td>29.2</td>
<td>16.0</td>
<td>0.50</td>
</tr>
<tr>
<td>4</td>
<td>264.0</td>
<td>224.6</td>
<td>39.4</td>
<td>14.5</td>
<td>1.02</td>
</tr>
<tr>
<td>6</td>
<td>268.8</td>
<td>223.0</td>
<td>45.8</td>
<td>12.8</td>
<td>1.56</td>
</tr>
<tr>
<td>7</td>
<td>270.8</td>
<td>221.8</td>
<td>49.0</td>
<td>11.5</td>
<td>1.82</td>
</tr>
<tr>
<td>8</td>
<td>272.4</td>
<td>221.0</td>
<td>51.4</td>
<td>10.7</td>
<td>2.11</td>
</tr>
<tr>
<td>9</td>
<td>274.0</td>
<td>221.4</td>
<td>52.6</td>
<td>10.9</td>
<td>2.37</td>
</tr>
<tr>
<td>10</td>
<td>275.0</td>
<td>220.6</td>
<td>54.4</td>
<td>10.4</td>
<td>2.64</td>
</tr>
<tr>
<td>11</td>
<td>276.2</td>
<td>221.0</td>
<td>55.2</td>
<td>10.7</td>
<td>2.91</td>
</tr>
<tr>
<td>12</td>
<td>277.2</td>
<td>221.2</td>
<td>56.0</td>
<td>10.8</td>
<td>3.17</td>
</tr>
<tr>
<td>13</td>
<td>278.2</td>
<td>221.9</td>
<td>56.3</td>
<td>11.3</td>
<td>3.44</td>
</tr>
<tr>
<td>14</td>
<td>279.0</td>
<td>222.2</td>
<td>56.8</td>
<td>11.6</td>
<td>3.79</td>
</tr>
<tr>
<td>15</td>
<td>279.8</td>
<td>223.0</td>
<td>56.8</td>
<td>12.3</td>
<td>3.97</td>
</tr>
<tr>
<td>16</td>
<td>280.4</td>
<td>224.0</td>
<td>56.4</td>
<td>13.5</td>
<td>4.23</td>
</tr>
<tr>
<td>17</td>
<td>281.2</td>
<td>225.4</td>
<td>55.8</td>
<td>14.9</td>
<td>4.50</td>
</tr>
<tr>
<td>18</td>
<td>282.0</td>
<td>223.2</td>
<td>55.8</td>
<td>15.3</td>
<td>4.75</td>
</tr>
<tr>
<td>19</td>
<td>282.6</td>
<td>234.0</td>
<td>48.6</td>
<td>28.9</td>
<td>4.98</td>
</tr>
<tr>
<td>20</td>
<td>283.0</td>
<td>240.8</td>
<td>42.2</td>
<td>50.3</td>
<td>5.17</td>
</tr>
<tr>
<td>21</td>
<td>283.6</td>
<td>247.2</td>
<td>36.4</td>
<td>53.0</td>
<td>5.31</td>
</tr>
<tr>
<td>22</td>
<td>284.0</td>
<td>251.8</td>
<td>32.2</td>
<td>120.3</td>
<td>5.44</td>
</tr>
<tr>
<td>23</td>
<td>284.6</td>
<td>255.4</td>
<td>29.2</td>
<td>158.2</td>
<td>5.55</td>
</tr>
<tr>
<td>24</td>
<td>285.0</td>
<td>258.5</td>
<td>26.5</td>
<td>203.0</td>
<td>5.64</td>
</tr>
</tbody>
</table>

Plotting the concentration of free iodine against the amount bound by 100g starch and assuming that pure amylose takes up 21.5% of its own weight of iodine from graph % amylose = \( \frac{5.02}{0.215} \times 23.3\% \).
POTENTIOMETRIC IODINE TITRATION

OF MALT STARCH

TECHNIQUE B
Technique (E) (68)

From graph, amount of iodine bound by 100 g. starch = 5.28 g.

Assuming that pure amylose takes up 19% of its own weight of iodine under the conditions employed,

% amylose = 27.8%

Fractionation of the crude starch

Method A. By Thymol (45)

Crude dry starch (5 g.) suspended in cold water (20 ml.) was slowly added with continuous mechanical stirring to boiling water (150 ml.) containing sodium chloride (0.18 g.) to lower the viscosity of the system. After three hours the solution was cooled to 70°, centrifuged, the centrifugate removed, and the starch solution cooled to 50°, and powdered thymol (0.54 g.) added, and stirring continued for 10 minutes. The solution was then transferred to a Dewar flask and allowed to stand for 60 hours during which the insoluble amylose-thymol complex settled. This was removed by centrifuging, washed by stirring with water saturated with thymol (100 ml.) and again separated. This process was repeated twice. The precipitate was dehydrated and freed from thymol by triturations with alcohol and ether, and finally dried in vacuo over phosphorus pentoxide.

The amyllopectin fraction was precipitated by concentrating/—
concentrating the mother liquor (to 150 ml) and pouring into alcohol (1 litre). It was then washed with alcohol and ether and dried.

<table>
<thead>
<tr>
<th>Yield</th>
<th>Blue Value</th>
<th>%age Amylose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Amylose&quot;</td>
<td>1.8g.</td>
<td>0.80</td>
</tr>
<tr>
<td>&quot;Amylopectin&quot;</td>
<td>1.0g.</td>
<td>0.134</td>
</tr>
</tbody>
</table>

Both fractions on hydrolysis and examination by paper chromatography were found to be composed solely of glucose residues.

**Method B. By Butanol (73)**

Dry crude starch (5g.) was suspended in water (20 ml.) and added slowly with vigorous stirring to water (200 ml.) saturated with butanol (40 ml.) at 90°, the solution being maintained at this temperature for 2½ hours. After removing the fibrous material at the centrifuge, when the solution had cooled to 70°, the centrifugate was reheated to 90° and allowed to stand in a Dewar flask for 60 hours. The complex which formed was washed several times with butanol-saturated water, and dehydrated as in Method A.

The amylpectin was precipitated as in Method A.

<table>
<thead>
<tr>
<th>Yield</th>
<th>Blue Value</th>
<th>%age Amylose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Amylose&quot;</td>
<td>1.9g.</td>
<td>0.90</td>
</tr>
<tr>
<td>&quot;Amylopectin&quot;</td>
<td>1.7g.</td>
<td>0.134</td>
</tr>
</tbody>
</table>

Hydrolysis of these fractions and examination of the products by chromatography showed the presence of glucose alone.

**Method C/-**
Method C. By Pyridine (49)

Crude dry starch (5g.) was suspended in cold water (57 ml.) and slowly added with vigorous stirring to water (400 ml.) saturated with pyridine (75 ml.) at 90°. The solution was maintained at this temperature for 2½ hours. After cooling to 70° the solution was centrifuged at 2,000 r.p.m. for five minutes and the fibrous deposit removed. The solution was again heated to 90° and transferred to a Dewar flask and allowed to cool slowly for 60 hours during which the amylose-pyridine complex settled and was removed at the centrifuge. It was washed with water saturated with butanol and dried with alcohol and ether. After the mother liquor had been concentrated (to 150 ml.) it was poured into alcohol (1 litre) and the amylpectin fraction precipitated, washed with alcohol and dried with ether.

<table>
<thead>
<tr>
<th>Yield</th>
<th>Blue Value</th>
<th>Value of</th>
<th>Amylose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Amylose&quot;</td>
<td>1.7 g.</td>
<td>0.94</td>
<td>67</td>
</tr>
<tr>
<td>&quot;Amylopectin&quot;</td>
<td>0.8 g.</td>
<td>6.14</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Hydrolysis of these fractions and examination of the products by chromatography showed the presence of glucose alone.
Method D. By 20% aqueous pyridine and butanol (76)

Dry crude starch (5 g.) suspended in water (20 ml.) was added with vigorous stirring to water (160 ml.) containing pyridine (40 ml.). It was stirred for three hours at 90°, and then given five minutes high speed stirring in an Ato-Mix disperser. The paste was then cooled to 70° and centrifuged. The temperature was adjusted to 90° and sufficient butanol added to saturate the solution. The solution was allowed to cool slowly in a Dewar flask for 60 hours when the amyllose complex settled out.

The amyllopectin fraction was precipitated by concentrating the mother liquor to 150 ml. and pouring into alcohol (1 litre).

Both fractions were washed with butanol-saturated water and dehydrated by triturations with alcohol and ether.

<table>
<thead>
<tr>
<th>Yield</th>
<th>Blue Value</th>
<th>Zage Amylose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Amylose&quot;</td>
<td>1.2 g.</td>
<td>0.45</td>
</tr>
<tr>
<td>&quot;Amylopectin&quot;</td>
<td>2.1 g.</td>
<td>0.078</td>
</tr>
</tbody>
</table>

Hydrolysis of these fractions and chromatographic examination of the products showed the presence of glucose alone.

Method E/—
Method E. By aluminium hydroxide and thymol (53)

Dry crude starch (6 g.) was creamed with water and added to boiling 0.1% sodium chloride solution (200 ml.) with stirring. Boiling and stirring were continued for a further 45 minutes when the paste was cooled rapidly to 30°. Aluminium nitrate (Al(NO₃)₃·9H₂O, 2.5 g.), dissolved in the minimum quantity of water, was stirred into the paste and ammonia solution (d 0.88) added with mechanical stirring till the mixture was alkaline to phenolphthalein. The containing flask was stoppered and the solution allowed to "age" for three days at 30° before the aluminium hydroxide complex was removed at the centrifuge (2,500 r.p.m. for 30 minutes). The supernatant liquid was dialysed for two days.

Finely powdered thymol (0.13g./100 ml.) was added with shaking and the vessel stoppered and kept at 30° for 48 hours. The thymol complex was sedimented on the centrifuge and washed with thymol-saturated water, then alcohol twice and finally ether.

Yield of amyllose, 0.4g; Blue Value, 0.67; %age amyllose 49.

It must be realised that all the above fractionations have been carried out on the crude starch and not on the whole starch which was isolated from the crude starch by chemical treatment. No chemicals likely to cause the slightest degradation have come into contact with the starch before these fractionation experiments.

Summary/
### Summary of Fractionations

#### Conditions of Dispersion

<table>
<thead>
<tr>
<th>Conc. of Crude Starch %</th>
<th>Temp. °C.</th>
<th>Time (hrs.)</th>
<th>Solvent</th>
<th>Precipitant</th>
<th>&quot;Amylose&quot;</th>
<th>% Amylose</th>
<th>Yield g.</th>
<th>B.V.</th>
<th>% Amylopectin</th>
<th>Yield g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>100</td>
<td>3</td>
<td>water cont. Thymol NaCl</td>
<td>0.80</td>
<td>57</td>
<td>1.8</td>
<td>0.134</td>
<td>93.1</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>2.5</td>
<td>water sat. Butanol with butanol</td>
<td>0.90</td>
<td>64</td>
<td>1.9</td>
<td>0.134</td>
<td>93.1</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>90</td>
<td>2.5</td>
<td>water cont. Pyridine pyridine</td>
<td>0.94</td>
<td>67</td>
<td>1.7</td>
<td>0.140</td>
<td>92.7</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>3</td>
<td>water cont. Pyridine + butanol</td>
<td>0.45</td>
<td>32</td>
<td>1.2</td>
<td>0.078</td>
<td>97</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0.75</td>
<td>water cont. Aluminium NaCl hydroxide + thymol</td>
<td>0.67</td>
<td>49</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Five minutes high speed stirring in an Ato-Mix disperser.
Refractonation

"Amylose" — reprecipitation with thymol effected no purification.

— reprecipitation with butanol further increased the %age amylose in the precipitated fraction.

Further treatment of the amylpectin fractions with the precipitants did not yield any significant amounts of the amylose complex and did not increase the purity of the amylpectin.

Fractionation of thymol amylpectin with methanol(63)

Thymol amylpectin (5.5 g.) (prepared as in Method A) was moistened with ethanol and partly dispersed in water (400 ml.) by being heated to boiling with vigorous stirring and boiled for one minute. The hot dispersion was autoclaved at 30 lb. pressure for one hour and cooled to 15°. Sodium chloride (0.5 g.) was added and the solution diluted to 500 ml. Methanol (100 ml.) was added dropwise with rapid stirring and the precipitate which formed was removed on the centrifuge. To the supernatant liquid was added a further quantity of sodium chloride (0.5 g.) followed by absolute ethanol (2 volumes). The precipitate was allowed to coagulate and was removed on the centrifuge. It was dried by washing with ethanol and ether.

Amylopectin — Yield 1.7 g.  Blue Value 0.110.

The/-
The Amylose Fraction

Preparation of the Amylose from Crude Malt Starch

Since precipitation with pyridine in the above fractionation experiments yielded the "amylose" fraction with the highest Blue Value, and containing the greatest proportion of total amylose a larger quantity of the crude starch (130 g.) was fractionated by this method. This represents ca 100 g. whole starch and hence ca 20 g. amylose.

The product, pyridine-amylose was then reprecipitated several times with butanol at low amylose concentration.

Refractometric Technique - with amylose concentration 0.2%.

The complex was dissolved in butanol-saturated water (180 ml., 1500 ml.) and stirred at 90° for ½ hour. The clear solution was then cooled to 70° and filtered through a large sintered glass funnel (porosity G3). The temperature was adjusted to 90° and the solution poured into a mixture of butanol (720 ml.) and water (5½ litres) at 90° and the combined liquid stirred for 5 minutes at this temperature before being transferred to Dewar flasks, and allowed to cool slowly for 60 hours. Results/-
Results of the reprecipitations with butanol

<table>
<thead>
<tr>
<th>Amylose %age concentration</th>
<th>No. of reprecipitations</th>
<th>Iodine uptake by Technique A</th>
<th>Blue Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0</td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td>0.2</td>
<td>1</td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td>0.2</td>
<td>2</td>
<td></td>
<td>0.87</td>
</tr>
<tr>
<td>0.2</td>
<td>3</td>
<td></td>
<td>0.90</td>
</tr>
<tr>
<td>0.2</td>
<td>4</td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>0.2</td>
<td>5</td>
<td>15.6%</td>
<td>0.98</td>
</tr>
<tr>
<td>0.2</td>
<td>6</td>
<td></td>
<td>1.01</td>
</tr>
<tr>
<td>0.2</td>
<td>7</td>
<td>17.8%</td>
<td>1.01</td>
</tr>
<tr>
<td>0.2</td>
<td>8</td>
<td></td>
<td>1.02</td>
</tr>
<tr>
<td>0.1</td>
<td>9</td>
<td>19.0%</td>
<td>1.04</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>19.5%</td>
<td>1.08</td>
</tr>
<tr>
<td>0.1</td>
<td>11</td>
<td>20.8%</td>
<td>1.19</td>
</tr>
<tr>
<td>0.1</td>
<td>12</td>
<td>20.8%</td>
<td>1.25</td>
</tr>
</tbody>
</table>

After the 12th reprecipitation the amylose-butanol complex was dissolved in water (150 ml.) and freeze-dried.

Yield of amylose 11.1 g.

This represents ca. 55% of the amylose present in the original crude starch.

Freeze-drying

Freeze drying has been adopted in the final isolation of the amylose from the amylose-butanol complex and also in the case of the amylopectin. It has been found that a product is obtained which is more easily manipulated compared with that from the usual/—
usual procedure of washing with alcohol and drying with ether. The products show a tendency to go into solution more readily in solvents such as sodium hydroxide solution (N) and perchloric acid solution (30%) which were used to obtain optical rotations.

**Examination of the amyllose-butanol complex.**

After each precipitation the complex was examined under the microscope. After the first reprecipitation the general form of the crystalline complex was circular. On the second reprecipitation the crystals tended to have a long rectangular shape, and thereafter to the last fractionation they appeared as needle-shaped crystals.

**Examination of the amyllose.**

**Preparation of the amyllose for analysis from the amyllose-butanol complex.**

A quantity of the amyllose-butanol complex was dissolved in warm water and made up to a standard volume. An aliquot part of this solution was evaporated to dryness under reduced pressure and the weight of amyllose determined directly.

In making analytical determinations a volume of this solution, equivalent to the required weight, was taken.

The amyllose-butanol complex was found to dissolve easily in warm water, giving a clear solution which produced an intense blue colour on the/
the addition of one drop pf iodine solution (0.1N.)

After standing for three days a cloudiness developed in the solution and after eight days a precipitate of retrograded amylose was removed at the centrifuge.

**Optical rotations of the amylose**

A) In water – an aliquot part of the solution containing the amylose-butanol complex was used in this case.

\[ [\alpha]^1_\text{D} + 200^\circ \text{ (C, 0.5)} \]

In rotations (B) and (C) a known weight of the freeze dried amylose was dissolved in the respective solvents.

B) In sodium hydroxide solution (N).

\[ [\alpha]^1_\text{D} + 145^\circ \text{ (C, 0.5)} \]

C) In perchloric acid solution (30%)

\[ [\alpha]^1_\text{D} + 205^\circ \text{ (C, 0.5)} \]

**Moisture Content** 15.8%

**Protein Content** 0.18% (%age Nitrogen x6.25).

**Hydrolysis with sulphuric acid A).**

A small sample of the freeze dried amylose (50 mg.) was hydrolysed with sulphuric acid (2%) by heating on a boiling water-bath for seven hours in a sealed tube. After neutralisation with barium carbonate, the liquor was concentrated to a thick syrup and chromatographed along with a standard containing glucose, maltose, fructose, and xylose.

Glucose alone was detected on the paper.
Hydrolysis with sulphuric acid B) and estimation of the glucose with alkaline hypochlorite.

The procedure was the same as in the case of the whole starch.

Weight of freeze dried amylose 0.0927g.
Weight of glucose found 0.0978g.

This constitutes 95.0% of the theoretical yield of glucose from the amylose sample.

Amylose Determinations

Blue Value 1.25. Assuming that pure amylose has a Blue Value of 1.40, this sample was equivalent to 90% amylose.

Potentiometric iodine titration

Technique A (see p. 88)

A volume of the solution of the amylose-butanol complex equivalent to 8 mg. was employed.
<table>
<thead>
<tr>
<th>Volume of Blank (ml.)</th>
<th>Titre e.m.f.</th>
<th>Diff. e.m.f.</th>
<th>$[I]_g \times 10^{-7}$</th>
<th>Iodine bound by 100g. &quot;amylose&quot; (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>242.6</td>
<td>226.0</td>
<td>16.6</td>
<td>5.24</td>
</tr>
<tr>
<td>4</td>
<td>256.8</td>
<td>225.0</td>
<td>31.8</td>
<td>3.08</td>
</tr>
<tr>
<td>6</td>
<td>263.2</td>
<td>224.8</td>
<td>38.4</td>
<td>2.68</td>
</tr>
<tr>
<td>8</td>
<td>268.0</td>
<td>225.0</td>
<td>43.0</td>
<td>2.43</td>
</tr>
<tr>
<td>10</td>
<td>273.0</td>
<td>226.2</td>
<td>47.8</td>
<td>2.04</td>
</tr>
<tr>
<td>11</td>
<td>274.0</td>
<td>228.0</td>
<td>46.0</td>
<td>2.58</td>
</tr>
<tr>
<td>12</td>
<td>275.0</td>
<td>230.4</td>
<td>44.6</td>
<td>3.12</td>
</tr>
<tr>
<td>13</td>
<td>276.0</td>
<td>237.4</td>
<td>33.6</td>
<td>5.52</td>
</tr>
<tr>
<td>14</td>
<td>277.0</td>
<td>247.2</td>
<td>29.8</td>
<td>11.50</td>
</tr>
<tr>
<td>15</td>
<td>278.0</td>
<td>254.0</td>
<td>24.0</td>
<td>19.40</td>
</tr>
<tr>
<td>16</td>
<td>278.6</td>
<td>259.0</td>
<td>19.8</td>
<td>28.70</td>
</tr>
<tr>
<td>17</td>
<td>279.6</td>
<td>262.4</td>
<td>17.2</td>
<td>37.00</td>
</tr>
<tr>
<td>18</td>
<td>280.2</td>
<td>264.2</td>
<td>16.0</td>
<td>42.80</td>
</tr>
</tbody>
</table>

Plotting the concentration of free iodine against the amount bound by 100g. "amylose", from the graph the amylose takes up 20.8% of its own weight of iodine. Assuming that pure amylose takes up 21.5% of its own weight of iodine this sample has 97% purity.

Technique B. (68)

From graph amount of iodine bound by 100g. sample = 19g. Assuming that pure amylose takes up 19.8% under the conditions employed, % amylose = 98-99.

Methylation/-
POTENTIOMETRIC IODINE TITRATION

OF MALT AMYLOSE

TECHNIQUE B.
Methylation of the Amylose using Diazomethane (162)

Freeze dried amylose (7.5g.) was placed in a loosely stoppered flask (capacity 1 litre). Nitroso N-methyl urea powder in ether (not specially purified) (40g. in 400 ml.) was cooled in solid carbon dioxide–alcohol mixture, and sodium hydroxide solution (30 ml., 30%) added. The solution was taken out of the freezing mixture and allowed to warm up. The reaction was moderated by cooling in the cardice bath when necessary. When all the nitroso N-methyl urea had reacted the flask was cooled in the cardice–alcohol to remove water, etc., and the ethereal solution decanted on to the amylose. The mixture was left in the refrigerator loosely corked, until all the diazomethane had reacted, having been shaken at intervals. When colourless the ether was decanted and discarded and the process repeated.

After 10 weeks $\text{OCH}_3$ 10.5%
After 20 weeks $\text{OCH}_3$ 17.0%
After 34 weeks $\text{OCH}_3$ 20.2% Yield 7.1g.

Using this procedure there is a possibility of the formation of methylene cross linkages between adjacent glucose residues. A small sample of the partially methylated amylose (50 mg.) was therefore isolated, and hydrolysed with sulphuric acid (2%), and the resulting solution tested for the presence of formaldehyde as follows:

(Method/-
(Method by M. W. Rees)(163). To the solution in a boiling tube was added chromotropic acid (4 : 5 dihydroxy -2:7-naphthalene disulphonic acid) (0.5 ml.; 0.1M), followed by sulphuric acid (20 ml., conc. analar) very slowly, with cooling in a freezing mixture. The colour is developed by heating the solution for 10 minutes at 80°. No appreciable coloration was found compared with a blank and it was concluded that little, if any, formaldehyde was present and hence few methylene cross-linkages.

Methylation of partially methylated amyllose (c.f.16)

The partially methylated amyllose (7.1g, % OCH$_2$So, 2) was dispersed in water (100 ml.) and aqueous caustic soda (150 ml., 30%) added. More water (50 ml.) was added to disperse further the polysaccharide. When the containing flask was immersed in cold water the methylation was carried out with vigorous mechanical stirring in an atmosphere of nitrogen by the simultaneous dropwise addition of dimethyl sulphate (150 ml.) and aqueous caustic soda (200 ml., 30%) over a period of six hours. The stirring was continued overnight. A further two methylations were carried out in the above manner.

Acetone (300 ml.) was then added and the stirring continued for two hours, when the acetone was removed under reduced pressure and the insoluble/-
insoluble partially methylated amylose came to the surface. The liquid was decanted from the containing vessel and the polysaccharide dispersed in acetone (300 ml.) and water (100 ml.). The paste was methylated again by the simultaneous addition of aqueous caustic soda (250 ml., 30%) and dimethyl sulphate (120 ml.). This treatment was repeated.

The methylated product was isolated and purified by washing with boiling water until free from sulphate and by precipitation from chloroform solution by pouring into light petroleum (bp 40-60°).

Yield of methylated amylose 7.56 g. % OCH₃ 44.2.

This yield is 72% of the theoretical from the full methylation procedures.

Fractionation of methylated Amylose

The methylated amylose (7.56 g.) was fractionated by dissolution in light petroleum (b.p. 60-80°)-chloroform solutions. Increasing percentages of chloroform were employed as shown below and the solutions heated under reflux on a water-bath at 65°.

Percentage/
<table>
<thead>
<tr>
<th>Percentage Chloroform</th>
<th>Fraction</th>
<th>Weight (g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>A</td>
<td>0.83</td>
</tr>
<tr>
<td>10</td>
<td>B</td>
<td>0.17</td>
</tr>
<tr>
<td>15</td>
<td>C</td>
<td>0.39</td>
</tr>
<tr>
<td>20</td>
<td>D</td>
<td>0.13</td>
</tr>
</tbody>
</table>

The fractions had the following properties.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>%OCH₃</th>
<th>Specific rotation (°)</th>
<th>3°/°/c (0, 0.4 in mCresol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>44.4</td>
<td>+202</td>
<td>0.79</td>
</tr>
<tr>
<td>B</td>
<td>44.8</td>
<td>+206</td>
<td>0.82</td>
</tr>
<tr>
<td>C</td>
<td>42.3</td>
<td>+199</td>
<td>0.57</td>
</tr>
<tr>
<td>D</td>
<td>35.7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Hydrolysis/-
Hydrolysis of the Methylated Amylose on a small scale and examination of the products by paper chromatography.

Fraction B (50 mg.) was heated with methanolic hydrogen chloride solution (1 ml; 4%) in sealed tubes at 100° for six hours. After the hydrolysis the tubes were cooled and cautiously opened. After the removal of the solvent, the residual syrups were hydrolysed by heating at 100° for seven hours with hydrochloric acid (5 ml.; 4%), neutralised with silver carbonate and filtered. Silver was removed from the filtrate by hydrogen sulphide, and anions and cations by treatment with "Amberlite" resins IR-100 and IR-4B. The clear solution was then concentrated to a thin syrup at 40° under reduced pressure. The syrup was examined by paper chromatography.

The following sugars were present.

Rf found

<table>
<thead>
<tr>
<th>Rf</th>
<th>Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.85</td>
<td>2:3:6-tri-O-methyl-D-glucose (in quantity)</td>
</tr>
<tr>
<td>0.59</td>
<td>2:5</td>
</tr>
<tr>
<td></td>
<td>2:6</td>
</tr>
<tr>
<td></td>
<td>di-O-methyl-D-glucose</td>
</tr>
<tr>
<td></td>
<td>3:6</td>
</tr>
</tbody>
</table>

Hydrolysis/-
Hydrolysis of Methylated Amylose Fraction B

Methylated amylose (fraction B) (4.25 g.) was hydrolysed by heating with methanolic hydrogen chloride (250 ml., 1%) until the rotation was constant (7 hours). The solution was then cooled and neutralised with silver carbonate, the silver chloride being removed by filtration and washed well with hot dry methanol. Excess silver was removed by the passage of hydrogen sulphide through the solution, and the insoluble silver sulphide by filtration. The filtrate was evaporated to a fine syrup. Yield 4.53 g. (93% of the theoretical).

The syrupy glycosides were hydrolysed by heating at 100° with hydrochloric acid (180 ml., 2%) until the rotation was constant (8 hours). The solution was cooled and neutralised with silver carbonate and excess silver removed as above, before being shaken with "Amberlite" resins IR-100 and IR-4B for two hours to complete the removal of ions. The solution was evaporated to a clear syrup under reduced pressure. Yield 4.21 g. (91% of the theoretical yield).

Separation of the Methylated Glucoses on a Cellulose Column.

A column of powdered cellulose (65 x 3.5 cm.) was prepared and tested as described by Hough, Jones and Wadman (99). The mixture of methylated sugars obtained above (4.21 g.) was dissolved in the minimum amount/
amount of solvent used initially for eluting the column, namely, a mixture of light petroleum (b.p. 100-120°) (70%) and n-butanol (30%) saturated with water. This solution was added slowly, dropwise to the centre of the column, each drop being allowed to soak in before the next was added. After allowing the column to stand for two hours, the reservoir was filled and the elution process begun. Weight of methylated sugars transferred - 4.14g.

The eluate was collected in small tubes placed in a turntable, which automatically changed the receiving tube every twelve minutes.

The solution from every tenth tube was concentrated in a small basin over a water-bath at 80° and then examined by paper chromatography in the usual way. The residues in the basins were dissolved in acetone and transferred quantitatively back to their respective tubes.

1300 tubes were obtained each containing 7-8 ml. of solution. Tubes 140-200 were found to contain a sugar corresponding to 2:3:4:6-tetra-O-methyl-D-glucose. The presence of no other sugar was indicated in this fraction.

After all the 2:3:4:6-tetra-O-methyl-D-glucose had been collected from the column, the proportion of the solvents in the eluant was altered to light petroleum (b.p. 100-120°) (50%) -n-butanol (50%) saturated/-
saturated with water.

Tubes 350-940 were found to contain a sugar corresponding to 2:3:6-tri-β-methyl-D-glucose, and tubes 990-1150 a mixture of the 2:3-, 2:6- and 3:6-di-β-methyl-D-glucoses.

The tubes were suitably combined, the solvent removed at 40°/20 mm., and the residue dissolved in water and filtered through charcoal to remove waxy impurities. After further concentration the residue was dissolved in acetone and the last traces of impurity removed by filtration, before the solvent was evaporated completely.

The column was then washed with n-butanol, 50% saturated with water, (1 litre), and with water (1 litre). These extracts were evaporated to dryness under reduced pressure purified as above, and examination by paper chromatography indicated the presence of a monomethyl glucose and glucose in trace quantity.

Four fractions were thus obtained.

Fraction/-
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Tube No.</th>
<th>R&lt;sub&gt;g&lt;/sub&gt; found</th>
<th>Sugar suspected</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>140-200</td>
<td>1</td>
<td>2:3:4;6-tetra-O-methyl-D-glucose</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>201-350</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>351-940</td>
<td>0.82</td>
<td>2:3:6-tri-O-methyl-D-glucose</td>
<td>3.530</td>
</tr>
<tr>
<td></td>
<td>941-990</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>991-1150</td>
<td>0.56</td>
<td>2:3:6-di-O-methyl-D-glucose</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>1151-1300</td>
<td></td>
<td>3:6-D-glucose</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Butanol and water extracts</td>
<td>0.26 monomethyl glucose</td>
<td>0.10 glucose</td>
<td>trace</td>
</tr>
</tbody>
</table>

Weight of sugar recovered 3.658 (88%).

**Examination of the fractions**

**Fraction 1.**

Chromatographic examination indicated the presence of a single substance (R<sub>g</sub> 1.0) identical to that of 2:3:4;6-tetra-O-methyl-D-glucose.

The syrup (27 mg.) was dissolved in water (25 ml.) and two portions (1 ml.) were quantitatively examined by the method of Hough, Hirst and Jones (97). The portions were oxidised with buffered hypiodite as follows: the samples were placed in "Quickfit" boiling tubes and treated with iodine (1 ml., 0.1N) and phosphate buffer solution (ref.164) (pH 11.4, 2 ml.), the tubes being sealed with stoppers moistened with potassium iodide solution (10%). The tubes were kept in the dark for four hours before/-. 
before the stoppers were washed with water and the solution acidified with sulphuric acid (2 ml., 2N) and the excess iodine titrated with standard thiosulphate (0.01N). A blank was run concurrently.

<table>
<thead>
<tr>
<th>Volume of thiosulphate used to titrate solution (ml.) (0.0102N)</th>
<th>Volume of iodine required to oxidise sugar (ml.) (0.0102N)</th>
<th>Wt. of sugar sample oxidised (mg.)</th>
<th>Wt. of sample (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>9.069</td>
<td>-</td>
<td>1.06 (46.3%)</td>
</tr>
<tr>
<td>1</td>
<td>8.567</td>
<td>0.402</td>
<td>0.50</td>
</tr>
<tr>
<td>2</td>
<td>8.589</td>
<td>0.420</td>
<td>0.52</td>
</tr>
</tbody>
</table>

A portion (1 mg.) of the syrup was hydrolysed by heating at 100° with sulphuric acid (1 ml., 2N) for five hours. Re-examination by paper chromatography showed the presence of an additional sugar (Rf 0.82) corresponding to 2:3:6-tri-O-methyl-D-glucose.

**Hydrolysis of fraction 1.**

Fraction 1 (22 mg.) was hydrolysed by heating at 100° with hydrochloric acid (10 ml., 1%) for five hours. The acid was neutralised with silver carbonate and the silver chloride removed by filtration, and washed. After further removal of silver as before, the filtrate was evaporated to dryness under reduced pressure. Yield 21 mg.

The hydrolysate was separated on Whatman 3 MM papers using benzene–ethanol–water (149:45:15:v/v) as solvent and running for six hours, the requisite portions being cut off and extracted in a Soxhlet apparatus/—
apparatus with acetone and water to give fractions (1a) 10.5 mg. and (1b) 9 mg.

Investigations of fractions (1a) and (1b).

Fraction 1a - tetra-O-methyl-D-glucose.

Chromatographic examination indicated the presence of a single substance (R$_g$ 1.0), identical to 2:3:4:6-tetra-O-methyl-D-glucose.

The fraction crystallised on seeding with a crystal of authentic 2:3:4:6-tetra-O-methyl-D-glucose and was obtained in the form of thin needles which were tiled. M. P. 78-80° which was not depressed on admixture with an authentic sample of 2:3:4:6-tetra-O-methyl-D-glucose.

From the above results the amount of 2:3:4:6-tetra-O-methyl-D-glucose was calculated to be 12.8±0.4 mg., corresponding to the presence of one non-reducing terminal-group per 510±20 glucose residues.

Fraction 1b (9 mg.)

The presence of a single substance (R$_g$ 0.82), corresponding to 2:3:6-tri-O-methyl-D-glucose was indicated by paper chromatography. The fraction failed to crystallise on standing several weeks in the cold and was not further examined.

Fraction 2. (3.580 g.)

On chromatographic examination, this fraction showed one spot (R$_g$ 0.83) corresponding to 2:3:6-tri-O-/
Q-methyl-D-glucose. It crystallised on standing and was recrystallised twice from dry ether. Hypocodite oxidation showed that it was 95% pure.

Yield of crystalline material 2.123g.
M.P. 115-117° alone or mixed with authentic 2:3:6-tri-Q-methyl-D-glucose,
\[ [\alpha]_{D}^{18} + 94° \rightarrow + 70° \] (C, 1.0 in water)
OCH₃, 41.4% (Theoretical 41.9%)

Rotation of 2:3:6-tri-Q-methyl-D-glucose in cold methanolic hydrogen chloride solution.

2:3:6-tri-Q-methyl-D-glucose (0.095g) was dissolved in methanolic hydrogen chloride solution (10 ml., 2%) at room temperature and the rotation of the solution observed at intervals using a 2 dm. tube.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Observed Rotation</th>
<th>[\alpha]_{D}^{18}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+1.20</td>
<td>+63.0</td>
</tr>
<tr>
<td>1.5</td>
<td>+0.30</td>
<td>+15.8</td>
</tr>
<tr>
<td>2</td>
<td>+0.09</td>
<td>+ 4.2</td>
</tr>
<tr>
<td>5</td>
<td>-0.07</td>
<td>- 3.7</td>
</tr>
<tr>
<td>4</td>
<td>-0.16</td>
<td>- 8.4</td>
</tr>
<tr>
<td>5</td>
<td>-0.31</td>
<td>-17.4</td>
</tr>
<tr>
<td>7</td>
<td>-0.63</td>
<td>-33.2</td>
</tr>
<tr>
<td>24</td>
<td>-0.64</td>
<td>-33.7</td>
</tr>
</tbody>
</table>

Fraction 3. Di-Q-methyl glucose fraction (51 mg.)

Chromatographic examination revealed two spots (Rg 0.59) corresponding to 2:3-di-Q-methyl glucose, and/—
and \( R_g \ 0.53 \) corresponding to 2:3- and 2:6-di-O-methyl glucoses.

Quantitative estimation of the mixture of di-O-methyl glucoses by paper chromatography indicated that there was 7.5% of 2:3-di-O-methyl-D-glucose, corresponding to 4 mg. in the fraction.

The fraction had \( OCH_3 \ 29.0\% \) (Theoretical 29.7\%)
\[
[\alpha]_D^18 + 58^0 \rightarrow -11^0 \quad (C, 0.4, \text{ in methanolic hydrogen chloride, } 2\%: 10 \text{ ml.}) \quad (8 \text{ hours constant}).
\]

**Estimation of 2:6-di-O-methyl-D-glucose present in the di-O-methyl-fraction by the method of Bell (101) using sodium metaperiodate.**

The solution of fraction 3 (40 mg.) in methanolic hydrogen chloride solution was heated at 100\(^0\) for three hours. A slight excess of sodium bicarbonate was added before evaporating off the alcohol. Water (2.6 ml.) was then added to the residue. The solution was found to be non-reducing to Fehling's solution. The pH was adjusted to 7 (bromothymol blue) by the addition of dilute acetic acid and sodium metaperiodate (2 ml., 0.5M) was added.

After leaving for five hours at room temperature the consumption of periodate was determined with sodium arsenite (0.05M) after the addition of phosphate buffer (2 ml., 0.5M: pH 7.5) and excess potassium iodide.

It/-
It was found that the uptake of sodium metaperiodate was 0.79 moles per methyl-di-2-methyl-glucoside unit. This indicated the presence of 79% of 2;6-di-5-methyl glucose in the fraction.

After the above estimation, ethylene glycol (1 ml.) was added to the solution to destroy excess periodate. The solution was evaporated to dryness before extracting the residue with chloroform. These extracts were evaporated off and the residue hydrolysed with sulphuric acid (2 ml., 2%) by heating at 100° for 5 hours. After neutralisation the solution was examined by paper chromatography when two substances were indicated, one (Rf 0.59) corresponding to 2;5-di-5-methyl glucose and the other (Rf 0.53) corresponding to 3;6-di-5-methyl glucose.

**Fraction 4.**

Chromatographic examination of this fraction indicated that two sugars were present in trace quantity. These spots (Rf 0.26) and (Rf 0.11) corresponded to mono methyl glucose and glucose respectively.

The fraction was not further examined.
Periodate oxidation of the malt amylose—estimation of the formic acid liberated from the end-groups (64)

Freeze-dried amylose (0.262g.) was dissolved in water (50 ml.) containing potassium chloride (2.5g) and sodium metaperiodate (20 ml., 0.5M) was added. The mixture was continuously shaken in a stoppered bottle, completely surrounded with black cloth. At intervals the shaking was stopped and the solid matter allowed to settle. Samples (10 ml.) of the clear supernatant liquid were withdrawn, and ethylene glycol (0.2 ml.) was added with shaking. After standing for one hour the solutions were titrated from a microburette with sodium hydroxide (0.01M) using methyl red as indicator. After the samples had been withdrawn the shaking was again started. A blank was run concurrently with the experiment.

As the oxidised amylose was not removed from the flask when a sample of liquid was withdrawn for titration, corrections were applied to the experimental titres apart from the first. The results were as follows:

Weight of amylose (A) 0.262g.
Total volume of solution 60 ml.
10 ml. samples withdrawn.

Time/—
Time of shaking (hours) | Corrected volumes of sodium hydroxide (0.01N) used per 10 ml. sample (ml.)
---|---
144 | 0.232
168 | 0.253
201 | 0.271
214 | 0.286
262 | 0.305

Estimated titre after 150 hours shaking 0.240 ml.

Yield of formic acid (moles) from 162g. amylose 0.00885

No. of glucose residues per mole formic acid 113

No. of glucose residues per non-reducing terminal group 339

(It is assumed that 2 molecules of formic acid are released from the reducing end-group and one from the non-reducing end-group).

The experiment was repeated and the results were as below:

Weight of amylose (b) 0.255 g.
Total volume of solution 60 ml.
10 ml. samples withdrawn.
<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Corrected volumes of sodium hydroxide (0.01N) per 10 ml. sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>169</td>
<td>0.230</td>
</tr>
<tr>
<td>186</td>
<td>0.255</td>
</tr>
<tr>
<td>211</td>
<td>0.295</td>
</tr>
<tr>
<td>234</td>
<td>0.319</td>
</tr>
<tr>
<td>258</td>
<td>0.350</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking = 0.215 ml.

Yield of formic acid (moles) from 162g. amylose = 0.00820

No. of glucose residues per mole formic acid = 122

No. of glucose residues per non-reducing terminal group = 366

The average of the two results indicates the presence of one non-reducing terminal group per 350±30 glucose residues.

Hydrolysis of the amylose after oxidation with periodate

After the last sample had been removed in (b) above ethylene glycol (1.0 ml.) was added and the solution shaken overnight to destroy excess periodate. The amylose solution was then dialysed until free from oxidant and the solution reduced in volume (to 100 ml.)

Sodium metaperiodate (15 ml., 0.3 M.) was then added and the periodate uptake determined in the following manner. Portions (1 ml.) were withdrawn and to each sodium arsenite (5 ml., 0.1N.) was added. The reagents were mixed and allowed to stand for 15 minutes when the excess arsenite was titrated against iodine (0.1N.), using starch as indicator. The oxidation was/
was carried out at room temperature.

The results showed that the periodate uptake was negligible indicating that the amyllose was completely oxidised.

Excess periodate was destroyed by ethylene glycol as before and the oxidised amyllose dialysed until free from oxidant. The solution was then evaporated to dryness under reduced pressure, and the residue hydrolysed with sulphuric acid (30 ml., 4 N.) by heating at 100° for six hours. The acid was neutralised with barium carbonate, the barium sulphate removed by filtration and washed. When the filtrate was evaporated to dryness a very small white residue remained. This was dissolved in water (0.1 ml.) and the solution examined by paper chromatography. No sugar was found to be present.

The Amylopectin Fraction

Isolation of the Malt Amylopectin from the Crude Starch

The method adopted was D, using 20% aqueous pyridine and butanol which gave the fraction richest in amylopectin and in highest yield in the preliminary fractionation experiments.

Crude dry starch (60g.) suspended in water (200 ml.) was added to water (1800 ml.) at 90° containing pyridine (400 ml.) and vigorously stirred for three hours at this temperature. It was then subjected to five minutes high speed stirring in an Ato/-
Ato-Mix Disperser, before being cooled to $70^\circ$ when the insoluble fibrous material was removed at the centrifuge. The solution was then heated to $90^\circ$ and butanol (400 ml.) slowly added with mechanical stirring and the resulting paste stirred at this temperature for five minutes. It was then transferred to Dewar flasks and allowed to cool slowly for 60 hours during which the amylose complex settled. This was removed at the centrifuge (2,500 r.p.m. for 5 minutes) and the mother liquor concentrated to 1500 ml. The amylopeptin was precipitated by pouring this liquor into alcohol ($4\frac{1}{2}$ l.), but settled as a light brown precipitate which was washed several times with butanol-saturated water. It was then dispersed in water (1 litre) and concentrated to ca 250ml. This solution was freeze dried and gave a fine white powder as the product. The process was repeated on a further 60g. crude starch. Yield of amylopeptin from two fractionations 41g.

This represents ca 60% of the total amylopeptin in the original crude starch.

**Examination/-**
Examination of the Malt Amylopectin

Optical rotations

(A) In sodium hydroxide solution (N)

\[ \left[ \alpha \right]_{D}^{16} + 149^\circ \ (c, 1.1) \]

(B) In perchloric acid solution (30%)

\[ \left[ \alpha \right]_{D}^{16} + 170^\circ \ (c, 0.72) \]

Moisture Content 16%

Protein Content 0.52% (%age nitrogen x6.25)

Hydrolysis with sulphuric acid A.

A small sample (50 mg.) of the amylopectin was hydrolysed with sulphuric acid (2%) in a sealed tube at 100° for seven hours. The procedure was the same as in the case of the whole starch.

Glucose was the only sugar detected on the chromatogram.

Hydrolysis with sulphuric acid B. and estimation of the glucose with alkaline hypoiodite.

The procedure was the same as in the case of the whole starch.

Weight of amylopectin sample 0.1085g.

Weight of glucose found 0.1158g.

This constitutes 96.0% of the theoretical yield of glucose from the amylopectin sample.

Amylose/-
POTENTIOMETRIC IODINE TITRATION OF MALT AMYLOPECTIN

TECHNIQUE A.
Amylose Determinations

Blue Value 0.085. Since the Blue Value for pure amylose is assumed to be 1.40, this is equivalent to 6.0% amylose.

The white powdered amylopectin dispersed readily in hot water on stirring. This solution gave a blue coloration on the addition of one drop of iodine solution (0.1N) but with the addition of more iodine the coloration changed to a reddish-purple.

Potentiometric iodine titration

Technique A (59, 63)

The procedure adopted was the same as in the case of the whole starch.

The iodine was added in 0.5 ml. portions, the additions being made with stirring and seven minutes allowed for equilibrium to be established before readings were taken.

Weight of amylopectin sample 40.58 mg.

Iodine solution 0.00096N.

Volume/
Plotting the concentration of free iodine against the amount bound by 100 g. amylopectin, the amylopectin is found to take up 0.37% of its own weight of iodine.

Now since pure amylose takes up 21.5% of its own weight of iodine, the %age amylose in the amylopectin can be calculated as follows:

\[ \text{Technique/} = \frac{0.37}{21.5} \times 100 = 1.73\% \]
Technique B (68)

Two determinations were carried out using this procedure. The % amylose in the amylopectin fraction were 0.4 and 0.7 assuming that amylose takes up 19% of its own weight of iodine by the method.

Methylation of the amylopectin (c.f.16)

Amylopectin (24g.) was dispersed in cold water (180 ml.) and sodium hydroxide (120 ml.; 30%) added. It was further dispersed by the addition of more water (60 ml.). The containing flask was immersed in cold water and the methylation carried out with vigorous mechanical stirring in an atmosphere of nitrogen and at room temperature. Sodium hydroxide (400 ml.; 30%) and dimethyl sulphate (200 ml.) were added dropwise over a period of six hours and the stirring continued for a further 18 hours. This procedure was repeated three more times.

Acetone (500 ml.) was then added and the stirring continued for two hours when the acetone was removed under reduced pressure, and the insoluble partially methylated amylopectin came to the surface. The liquid was decanted from the containing vessel and the partially methylated amylopectin dispersed in acetone (500 ml.) and water (100 ml.). The paste was then methylated by the simultaneous addition of sodium hydroxide (400 ml.; 30%) and dimethyl sulphate (200 ml.). A further seven similar treatments were given.

The/-
The product was isolated and purified by washing with boiling water until free from sulphate and by precipitation from chloroform solution by pouring into light petroleum (b.p. 40-60°).

Yield of methylated amylopectin 24.4g.

\( \text{OCH}_3 \) 43.0%

This yield is 80% of the theoretical.

**Fractionation of the methylated amylopectin**

The methylated amylopectin (24.4g.) was fractionated by dissolution in light petroleum (b.p. 60-80°) - chloroform solutions, using increasing percentages of chloroform and heating under reflux on a water bath at 65°.

<table>
<thead>
<tr>
<th>Percentage chloroform</th>
<th>Fraction</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>A</td>
<td>1.4g</td>
</tr>
<tr>
<td>14</td>
<td>B</td>
<td>20.7g</td>
</tr>
<tr>
<td>15</td>
<td>C</td>
<td>2.1g</td>
</tr>
<tr>
<td>16</td>
<td>D</td>
<td>0.2g</td>
</tr>
</tbody>
</table>

**Fraction A.** \( \text{OCH}_3 \) 42.3%: \( [\alpha]_D^{15} + 193^\circ \) (G, 0.5 in chloroform)

**Fraction B.** \( \text{OCH}_3 \) 43.2%: \( [\alpha]_D^{15} + 200^\circ \) (G, 0.5 in chloroform)

**Fraction C.** \( \text{OCH}_3 \) 40.1%: \( [\alpha]_D^{15} + 194^\circ \) (G, 0.5 in chloroform)

**Fraction D.** \( \text{OCH}_3 \) 20.2%

**Viscosities/-**
Viscosities (c.f. 21)

The viscosities of the methylated fractions were determined in m-cresol in an Ostwald viscometer at 20°.

Results were as follows:

<table>
<thead>
<tr>
<th>Concentration of fraction in solution (%)</th>
<th>Fraction A</th>
<th>Fraction B</th>
<th>Fraction C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Average time of flow in seconds</td>
<td>Solution $T_2$</td>
<td>379</td>
<td>493</td>
</tr>
<tr>
<td></td>
<td>Solution $T_1$</td>
<td>261</td>
<td>261</td>
</tr>
<tr>
<td>Specific viscosity</td>
<td>$\gamma_{sp}$ i.e. $\frac{T_2 - T_1}{T_1}$</td>
<td>0.450</td>
<td>0.884</td>
</tr>
<tr>
<td></td>
<td>$\gamma_{sp/c}$ (gm./100 ml.)</td>
<td>1.13</td>
<td>2.21</td>
</tr>
</tbody>
</table>

When these figures were referred to the graph obtained by Hirst and Young (21), showing the relationship between $\gamma_{sp/c}$ and the molecular weights for starches it was found that the apparent molecular weights of the fractions were:

- Fraction A: 135,000
- Fraction B: 320,000
- Fraction C: 70,000

Further methylation of the methylated amylopectin by the method of Purdie (187)

Methylated amylopectin fraction B (6.0 g.) was treated with boiling neutral methyl iodide (120 ml.) and dry silver oxide (40 g.) was added in small portions every half hour over an eight hour period.
Chloroform was then added to a volume of 250 ml. and the solution was heated under reflux for an hour before being filtered. The silver oxide was extracted with hot chloroform (150 ml.) a further 3 times. The combined solution and extracts were concentrated to a small volume (100 ml.) and the amylopectin precipitated by pouring into well stirred light petroleum (b.p. 40–60°).

Yield of product 5.2 g. - 85% theoretical -

\[
\text{fraction B'}
\]

\[
\text{OCH}_3\ 45.5\%
\]

\[
\left[\alpha\right]_{D}^{15} + 200^\circ \ (c, 0.5 \ in \ chloroform).
\]

\[
\gamma sp/c 2.21 \ molecular \ weight \ of \ 320,000
\]

**Attempted fractionation of methylated fraction B'**

Methylated fraction B' (5.2 g.) was treated as before with light petroleum (b.p. 60–80°) - chloroform solutions and refluxed at 65°. The whole fraction dissolved at a chloroform percentage of 14. The fraction was therefore essentially homogeneous.

**Hydrolysis of the methylated amylopectin on a small scale and examination of the products by paper chromatography.**

Fraction B' (50 mg.) was heated with methanolic hydrogen chloride (1 ml.; 4%) in sealed tubes at 100° for 6 hours. After this hydrolysis the tubes were cooled and cautiously opened. After removal of the solvent/
solvent the residual syrups were hydrolysed for 7 hours with hydrochloric acid (5 ml. : 4%), neutralised with silver carbonate and filtered. Silver was removed from the filtrate by hydrogen sulphide and basic and acidic ions by treatment with "Amberlite" resins IR-100 and IR-4B. The clear solution was then concentrated to a thin syrup at 40°C under reduced pressure. The syrup was examined by paper chromatography.

Quantitative estimation of the mixture of methylated sugars by alkaline hypoiodite was carried out by the method of Hirst, Hough and Jones (97). A sodium hydroxide-phosphate buffer (pH 11.4) (164) was used in place of the carbonate-bicarbonate buffer pH 10.6.

The results were as follows:

<table>
<thead>
<tr>
<th>Sugar indicated</th>
<th>Rf found</th>
<th>Sugar present in mixture %</th>
<th>(average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:3:4:6-Tetra-O-methyl-D-glucose</td>
<td>1.0</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>2:3:6-tri-O-methyl-D-glucose</td>
<td>0.84</td>
<td>66.1</td>
<td></td>
</tr>
<tr>
<td>2:3-di-O-methyl-D-glucose</td>
<td>0.65</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>2:6-di-O-methyl-D-glucose</td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monomethyl glucose</td>
<td></td>
<td>faint trace</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td>faint trace</td>
<td></td>
</tr>
</tbody>
</table>

The dimethyl sugars were not separated in this case but were estimated together.
Hydrolysis of the methylated amylopectin fraction B'

Methylated amylopectin fraction B' (3.32 g.) was hydrolysed by heating with methanolic hydrogen chloride (200 ml.; 1%) until the rotation was constant (5 hours). The solution was then cooled and neutralised with silver carbonate. The silver chloride was filtered off and washed well with hot dry methanol. Excess silver was removed by the passage of hydrogen sulphide through the solution, and the insoluble silver sulphide was removed by filtration. The filtrate was evaporated to a fine syrup. Yield 3.66 g. (96% of theoretical).

The syrupy glycosides were hydrolysed by boiling with hydrochloric acid (160 ml.; 2%) until the rotation was constant (5 hours). The solution was cooled and neutralised with silver carbonate as above. Excess silver was removed as above and the solution was shaken for 2 hours with resins "Amberlite" IR-100 and IR-4B to complete the removal of ions before being evaporated to dryness under reduced pressure to give a clear syrup.

Yield 3.35 g. (95% of theoretical).

Separation of the methylated glucose on a cellulose column.

A column of powdered cellulose (64 x 3 cm.) was prepared and washed and tested as described by Hough, Jones and Wadman (100). The procedure followed was the same as for the case of the methylated amyllose (see p.116). Weight of the syrup transferred 3.05 g. 2100/-
2100 tubes were obtained each containing 4-5ml. solvent. Tubes 130-309 were found to contain a sugar corresponding to 2:3:4:6- tetra-¿-methyl-D-glucose. The presence of no other sugar was indicated.

Tubes 775-1148 were found to contain 2:5:6-tri-¿-methyl-D-glucose, and 1728-1910 a mixture of 2:3:6-tri-¿-methyl-D-glucose and 2:5-di-¿-methyl-D-glucose. After all the tetra-¿-methyl-D-glucose had been collected from the column the eluting solvent was changed to light petroleum (b.p. 100-120°) 50%, -n-butanol 50%, saturated with water.

The tubes were suitably combined, the solvent was removed at 40°/20 mm., and the residue dissolved in water and filtered through charcoal to remove waxy impurities. After further concentration the residue was dissolved in acetone and the last traces of impurity were removed by filtration.

The column was then washed with n-butanol, 50% saturated with water (1 litre) and this extract evaporated to dryness under reduced pressure, the residue being purified as above. It was examined and found to contain sugars corresponding to two or more dimethyl glucoses.

The column was washed with water (1 litre). The aqueous extract was treated as above and examination by paper chromatography showed the presence of monomethyl glucose and glucose.

Fraction/-
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Tube No.</th>
<th>R&lt;sub&gt;g&lt;/sub&gt; found</th>
<th>Sugar suspected</th>
<th>Weight(gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120-209</td>
<td>1.0</td>
<td>2:3:4:6-tetra-O-methyl-D-glucose</td>
<td>0.329 g.</td>
</tr>
<tr>
<td>2</td>
<td>210-774</td>
<td>-</td>
<td>2:3:6-tri-O-methyl-D-glucose</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>775-1148</td>
<td>0.84</td>
<td>2:3:6-tri-O-methyl-D-glucose</td>
<td>2.508 g.</td>
</tr>
<tr>
<td>2</td>
<td>1149-1727</td>
<td>-</td>
<td>2:3-di-O-methyl-D-glucose</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1728-1910</td>
<td>0.65, 0.84</td>
<td>2:3-di-O-methyl-D-glucose</td>
<td>0.160 g.</td>
</tr>
<tr>
<td>2</td>
<td>1911-2000</td>
<td></td>
<td>3:6-di-O-methyl-D-glucose</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Butanol extract</td>
<td>0.58</td>
<td>2:6-di-O-methyl-D-glucose, 3:6-di-O-methyl-D-glucose</td>
<td>0.070 g.</td>
</tr>
<tr>
<td>5</td>
<td>Aqueous extract</td>
<td>0.28</td>
<td>monomethyl glucose, 0.09 glucose</td>
<td>trace, trace</td>
</tr>
</tbody>
</table>

Weight of sugar recovered 2.867g (93%)

Examination of the fractions

1) Fraction 1. (0.329 g.)

Chromatographic examination indicated the presence of a single substance (R<sub>g</sub> 1.0) identical to that of 2:3:4:6-tetra-O-methyl-D-glucopyranose. Quantitative determination by the method of Hough, Hirst and Jones (97) revealed the presence of 49% of tetramethyl glucose.

Two portions were weighed and oxidised with buffered hypoiodite as follows: The portions were dissolved in water (5 ml.) and treated with iodine (1 ml: 0.1N) and buffer solution (pH 11.4; 2 ml.) in "Quickfit" boiling tubes which were sealed with stoppers moistened with potassium iodide solution (10%). After keeping the tubes in the dark for 4 hours/
hours, the stoppers were washed with water, and the solutions acidified with sulphuric acid (2 ml.; 2N) and titrated with sodium thiosulphate (0.01N).

A blank was run concurrently.

<table>
<thead>
<tr>
<th>Volume of thiosulphate used to titrate soln. (ml.)</th>
<th>Vol. of iodine required to oxidise sugar (ml.)</th>
<th>Weight of sugar oxidised (mg.)</th>
<th>Weight of sample (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>9.466</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>8.054</td>
<td>1.412</td>
<td>1.71</td>
</tr>
<tr>
<td>2</td>
<td>8.255</td>
<td>1.231</td>
<td>1.48</td>
</tr>
</tbody>
</table>

A portion (10 mg.) of the syrup was hydrolysed by heating at 100° with sulphuric acid (1 ml.; 2%) for 5 hours. Re-examination by paper chromatography showed the presence of an additional substance (Rq 0.84) corresponding to 2:3:6-tri-O-methyl-D-glucose.

Hydrolysis of fraction 1

Fraction 1 (310 mg.) was hydrolysed by heating at 100° with hydrochloric acid (20 ml.; 1%) for 5 hours. The acid was neutralised with silver carbonate and the silver chloride filtered off and washed, excess silver being removed as before. The filtrate was evaporated to dryness under reduced pressure.

Yield 290 mg.

The hydrolysate was separated on Whatman 3MM papers, using benzene-ethanol-water (149:45:15 v/v) as solvent, and running for 4 hours, the requisite portions being cut off and extracted in a Soxhlet apparatus/
apparatus with acetone and water to give fractions (la) 105 mg., and (lb) 118 mg.

Investigation of fractions la and lb.

Fraction la - tetra-Q-methyl-D-glucose.

Chromatographic examination indicated the presence of a single substance, \( R_G \ 1.0 \) which corresponded to 2:3:4:6-tetra-Q-methyl-D-glucopyranose. Hydrolysis with sulphuric acid (2%), and re-examination by paper chromatography indicated that no other sugar was present. Hypoiodite oxidation by the method already described showed that the sugar was 99% pure.

The fraction crystallised on standing and was recrystallised twice from light petroleum (b.p. 40-60°). It was obtained in the form of long needles which were tilled and washed with petroleum, the filtrate and washings being retained and evaporated.

Yield of crystalline material 70 mg.

M.P. 86-88° C which was not depressed on admixture with an authentic sample of tetra-Q-methyl-D-glucopyranose.

\[ [\alpha]_D^{18} + 98^0 \rightarrow + 83^0 \ (c, 0.4 \ \text{in water}) \]

\( \text{OCH}_3 52.0\% \) (theoretical 52.5%)

C 51.2%, H 8.5% (calculated C 51.2%, H 8.5%)

From the above results the amount of tetra-Q-methyl-D-glucose was calculated to be 160 ± 3 mg., corresponding to one non-reducing terminal group per 17-18 glucose residues.

Preparation/-
Preparation of 2:5:4:6-tetra-O-methyl-D-glucose anilide

Tetramethyl glucose (70 mg.) was dissolved in absolute alcohol (3 ml.) and freshly distilled aniline (0.1 ml.) added, the mixture being heated under reflux for 2 hours. When it was cooled in ice, crystals separated and were recrystallised from dry ether/light petroleum, (b.p. 40-60°), (1:1).

Yield 45 mg.

M, P. 136-138° alone or mixed with authentic tetra-O-methyl-D-glucopyranose anilide.

Found

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>4.3%</td>
</tr>
<tr>
<td>OCH₃</td>
<td>39.4%</td>
</tr>
</tbody>
</table>

Calculated for C₁₆H₂₅O₅N₂

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>4.5%</td>
</tr>
<tr>
<td>OCH₃</td>
<td>39.9%</td>
</tr>
</tbody>
</table>

Fraction I - tri-O-methyl-D-glucose (112 mg.)

The presence of a single substance (R₄ 0.84) corresponding to 2:5:6-tri-O-methyl-D-glucose was indicated by paper chromatography and hydrolysis by sulphuric acid and re-examination by paper chromatography confirmed this result. Hypoiodite oxidation showed that it was 98% pure.

It crystallised on standing and was recrystallised from dry ether.

Yield of crystalline material 60 mg.

M, P. 115-117° alone or mixed with authentic 2:5:6-tri-O-methyl-D-glucose.

\[ [\alpha]^{18}_D + 68 \rightarrow + 68° \ \text{(c, 0.4 in water)} \]

\[ [\alpha]^{18}_D + 67 \rightarrow -35° \ \text{(10 hours constant, c, 0.4 in cold dry methanolic hydrogen chloride 2%)} \]

OCH₃ 41.2% calculated 41.9%

Fraction/
Fraction 2 - tri-0-methyl-D-glucose (2.308 g.)

Fraction 2 was found to be chromatographically pure, giving one spot, \((R_g 0.84)\) corresponding to 2:3:6-tri-0-methyl-D-glucose. It crystallised on standing and was recrystallised twice from dry ether. Hypoiodite oxidation showed that it was 93% pure.

Yield of crystalline material 1.24 g.

M.P. 115-117° alone or mixed with authentic 2:3:6-tri-0-methyl-D-glucose.

\([\alpha]_{D}^{18} + 90 \rightarrow + 66° \) (c, 1.0 in water).

\([\alpha]_{D}^{18} + 67 \rightarrow + 54° \) (10 hours constant, c, 1.0 in cold methanolic hydrogen chloride, 2%).

C, 49.5% (calculated 49.9%)

H, 8.2%: (calculated C, 49.1%; H, 8.1%).

Fraction 3 - tri-0-methyl and di-0-methyl-D-glucose
(160 mg.)

Fraction 3 when run on the chromatogram gave 2 spots, \((R_g 0.84)\) corresponding to 2:3:6-tri-0-methyl-D-glucose, and \((R_g 0.65)\) corresponding to 2:3-di-0-methyl-D-glucose. The fraction was separated on Whatman 3MM papers with butanol-ethanol-water \((4:1:5 \, v/v)\) as solvent and extracted as above into fractions \((3a)\) 28 mg.; and \((3b)\) 95 mg.

Fraction 3a

Fraction 3a crystallised on standing and was recrystallised from dry ether.

M.P. 114-116° alone or mixed with authentic 2:3:6-tri-0-methyl-D-glucose.

Fraction/—
Fraction 3b

Chromatography indicated the presence of a single sugar, \( (R_g 0.65) \) — corresponding to 2:3-di-O-methyl-D-glucose. Hydrolysis of a portion and re-examination by paper chromatography confirmed this result. Hypoclorite oxidation indicated that the sugar was 98% pure.

The fraction was obtained as a pale yellow syrup which failed to crystallise on standing for 2 weeks in the cold.

\[
\text{OCH}_3: 29.3\% \quad \text{(calculated } 29.7\%) \\
[\alpha]^{18}_D + 106 \rightarrow +68^\circ \quad (c, 0.4 \text{ in water}).
\]


Preparation of 2:3-di-O-methyl-D-gluconolactone

2:3-Di-O-methyl-D-glucose (80 mg.) was dissolved in water (5 ml.) and treated with liquid bromine (1 ml.) and kept at room temperature for 5 days until non-reducing, being occasionally shaken during this period. After removal of the bromine by aeration the solution was neutralised with silver carbonate and the silver bromide removed by filtration, excess silver being precipitated by hydrogen sulphide. The solution was again aerated to remove soluble gases and was concentrated to a syrup at 40°/20 mm. The syrup was transferred to a micro-distillation apparatus and distilled at high vacuum (Bath/-
(bath temperature 160–165 °/0.005 mm.), a thick syrup, which partially crystallised on standing overnight, being obtained.

Yield 60 mg. (75% theoretical)

OCH₃ 28.5% (calculated 30.1%)

Preparation of 2:5-di-O-methyl-D-glucosphenylhydrazide

The 2:5-di-O-methyl-D-gluconolactone was dissolved in anhydrous ether by heating under reflux for 3 hours. On addition of freshly distilled phenylhydrazine (0.8 gm.) a white precipitate formed, and after 4 hours refluxing it was removed by filtration. Additional material was recovered from the filtrate by further treatment with phenylhydrazine and concentration of the reaction mixture.

Yield of crude material 75 mg. (90% of theoretical).

After 2 recrystallisations from ethanol it was obtained in the form of short white needles.

Yield 35 mg.
M. P. 160–162°C.

Found: OCH₃ 19.2%. Calculated 19.7%

Fraction 4 - di-O-methyl-D-glucose (70 mg.)

Paper chromatography indicated that this fraction, (Rₖ 0.58) was 2:6- or 3:6-di-O-methyl-D-glucose or a mixture of the two. Hydrolysis and re-examination of the fraction on the chromatogram did not reveal the presence of any other sugar. Hypoiodite oxidation showed the purity of the sugar to be 95%.

The/-
The fraction was obtained as a pale syrup which failed to crystallise on standing for 2 weeks in the cold.

\[
\text{CDE}_{6} \quad 29.0\% \text{ (calculated 29.7\%).}
\]

\[
\left[\alpha\right]_{D}^{18} + 74 \rightarrow + 58^\circ \text{ (c, 0.5 in water).}
\]

\[
\left[\alpha\right]_{D}^{18} + 60 \rightarrow - 10^\circ \text{ (8 hours constant; c, 0.5 in methanolic hydrogen chloride 2\%).}
\]

**Estimation of the 2:6-di-O-methyl-D-glucose present in fraction 4.** (101)

The solution of fraction 4 (35 mg.) in methanolic hydrogen chloride was heated at 100° for 3 hours, the solution then neutralised and sodium metaperiodate (2 ml.; 0.3M) was added. After standing for 5 hours at room temperature the amount of periodate consumed was determined by titration with sodium arsenite (0.05N), after the addition of phosphate buffer (2 ml.; 0.5M, pH 7.5) and excess potassium iodide.

It was found that the uptake of periodate was 0.53 moles per di-O-methyl-D-glucoside unit, i.e. 53% of the dimethyl fraction is 2:6-di-O-methyl-D-glucose.

**Estimation of the 3:6-di-O-methyl-D-glucose present in fraction 4.**

Ethylene glycol (1 ml.) was added to the above solution to destroy excess periodate, and it was then evaporated to dryness and extracted several times with chloroform. The extracts were evaporated to dryness and the residue hydrolysed with sulphuric acid (2 ml.; 8%) by/-
by boiling for 6 hours. The solution was neutralised and examined by paper chromatography which showed the presence of a single substance, (Rg 0.59), corresponding to 3:6-di-β-methyl-D-glucose. Quantitative estimation of the substance by alkaline hypoiodite showed that 14.5 mg. was present.

**Fraction 5**

Chromatographic examination indicated that 2 substances were present. One (Rg 0.23) corresponded to monomethyl glucose, whilst the other (Rg 0.09) corresponded to glucose.

The fraction was not examined further.
The action of methanolic oxalic acid on the methylated amylopectin (OCH$_3$ 43.5%) (21)

The methylated amylopectin (3.6 g.) was allowed to remain overnight in a mixture of methanol (120 ml.) and water (40 ml.). The containing flask was placed in a thermostat at 44.3° when a warm solution of oxalic acid dihydrate (2g.) in methanol (30 ml.) and water (10 ml.) was added. Nitrogen was constantly passed through the solution. The apparent pH of the reaction mixture was 2.74. At varying intervals of time samples were removed and the amylopectin fractions isolated as follows: The solution (ca. 15 ml.) was neutralised with sodium hydroxide (2N) before the methanol was removed at 40° under reduced pressure. After water (10-15 ml.) had been added to the flask the sample was heated under reflux, resulting in the precipitation of the partially dis-aggregated amylopectin. This material was removed by filtration through a sintered 363 funnel, the filtrate again being heated under reflux and any further precipitate removed by filtration. The white dried sample was then dissolved in acetone, filtered and the solution evaporated to dryness. The product, light brown in colour and brittle in texture was dried over phosphorus pentoxide at 40°.

The filtrates were tested for reducing properties but no reduction was shown to Fehling's solution in any sample. The method of Jeanloz (172) was adopted as a more sensitive test. After the filtrate had been made/-
made neutral to aqueous methyl orange, sodium hydroxide—sodium hydrogen phosphate buffer (15 ml.; pH 11.4) was added followed by iodine (20 ml.; 0.5N). The filtrate was allowed to stand for 1½-2 hours before being acidified with sulphuric acid (4N) when the excess iodine was titrated with sodium thiosulphate (0.05N). Blank estimations were carried out using distilled water, and a methanol—oxalic acid—water mixture. No increase in the reducing power of the filtrates was determined during the complete experiment, although a slight difference, which remained sensibly constant throughout, was found in each case.

Three filtrates were retained and investigated to ensure that no glucoside formation was occurring between any liberated oligosaccharide and methanol. The filtrates were combined the volume adjusted to ca 20 ml. and hydrolysed by heating at 100° with hydrochloric acid (10 ml.; 2N) for eight hours. After neutralising with silver carbonate, deionising by resin treatment, the solution was evaporated to dryness and the resulting residue examined by paper chromatography. A trace of 2:5:6-tri-O-methyl-D-glucose was found, probably because of incomplete precipitation of the partially disaggregated amylpectin from solution.

When the reaction had continued for 2011 hours and a sample removed, the remaining solution was heated under reflux on the water-bath, before isolation of/-
of the final product as in the previous occasions.

The specific rotations were measured in dry chloroform at a concentration of 1%. The specific viscosities were measured in an Ostwald viscometer at 20°.

The experiment was repeated at 70.5°, pH 2.74 and continued for 150 hours.

Reaction/-
DISAGGREGATION OF METHYLATED MALTED AMYLOPECTIN

TEMPERATURE 44.3°C

TEMPERATURE 70.5°C

\[ \frac{\eta_{sp}}{c} \]

\[ \eta_{sp} / c \]

TIME (HOURS)
<table>
<thead>
<tr>
<th>Sample disaggregation (hrs.)</th>
<th>Time of Reaction at 44.3°</th>
<th>Wt of sample 3.6 g.</th>
<th>pH 2.74</th>
<th>Specific Rotation $\left[\alpha\right]_D$</th>
<th>Viscosity $\nu_2$</th>
<th>T1 (secs.)</th>
<th>$\nu_2^sp$</th>
<th>c(g./100ml.)</th>
<th>$\nu_2^sp/c$</th>
<th>Fehling's Reduction</th>
<th>Hypoiodite Solution T</th>
<th>Blank filtrate T</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0</td>
<td>+202°</td>
<td>265</td>
<td>482</td>
<td>0.819</td>
<td>0.413</td>
<td>1.98</td>
<td>-ve</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S2</td>
<td>48</td>
<td>-</td>
<td>265</td>
<td>461</td>
<td>0.739</td>
<td>0.437</td>
<td>1.69</td>
<td>-ve</td>
<td>24.85</td>
<td>24.10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S3</td>
<td>336</td>
<td>-</td>
<td>262</td>
<td>361</td>
<td>0.377</td>
<td>0.442</td>
<td>0.85</td>
<td>-ve</td>
<td>24.27</td>
<td>23.87</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S4</td>
<td>504</td>
<td>-</td>
<td>262</td>
<td>329</td>
<td>0.258</td>
<td>0.396</td>
<td>0.65</td>
<td>-ve</td>
<td>20.26</td>
<td>19.74</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S5</td>
<td>714</td>
<td>+209°</td>
<td>263</td>
<td>311</td>
<td>0.184</td>
<td>0.374</td>
<td>0.49</td>
<td>-ve</td>
<td>19.33</td>
<td>19.30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S6</td>
<td>1200</td>
<td>-</td>
<td>263</td>
<td>297</td>
<td>0.129</td>
<td>0.398</td>
<td>0.33</td>
<td>-ve</td>
<td>20.06</td>
<td>19.62</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S7</td>
<td>1512</td>
<td>-</td>
<td>259</td>
<td>288</td>
<td>0.112</td>
<td>0.401</td>
<td>0.28</td>
<td>-ve</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S8</td>
<td>2011</td>
<td>-</td>
<td>259</td>
<td>285</td>
<td>0.100</td>
<td>0.395</td>
<td>0.25</td>
<td>-ve</td>
<td>19.33</td>
<td>19.30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S9</td>
<td>-</td>
<td>+209°</td>
<td>259</td>
<td>282</td>
<td>0.089</td>
<td>0.388</td>
<td>0.23</td>
<td>-ve</td>
<td>20.06</td>
<td>19.62</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### Disaggregation at 70.5°. pH 2.74.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time of Disaggregation (hrs.)</th>
<th>Specific Rotat. $[\alpha]_D$</th>
<th>Viscosity $\eta$</th>
<th>Viscosity $\eta$</th>
<th>$c$ (g./100ml)</th>
<th>$\eta$ sp/c</th>
<th>Fehling's Reduction solution</th>
<th>Hypoiodite Blank filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0</td>
<td>202°</td>
<td>264</td>
<td>488</td>
<td>0.848</td>
<td>0.434</td>
<td>1.95</td>
<td>-ve</td>
</tr>
<tr>
<td>S2</td>
<td>3</td>
<td>-</td>
<td>264</td>
<td>457</td>
<td>0.731</td>
<td>0.454</td>
<td>1.61</td>
<td>-ve</td>
</tr>
<tr>
<td>S3</td>
<td>5</td>
<td>-</td>
<td>264</td>
<td>420</td>
<td>0.591</td>
<td>0.439</td>
<td>1.34</td>
<td>-ve</td>
</tr>
<tr>
<td>S4</td>
<td>12</td>
<td>208°</td>
<td>264</td>
<td>390</td>
<td>0.477</td>
<td>0.427</td>
<td>1.10</td>
<td>-ve</td>
</tr>
<tr>
<td>S5</td>
<td>30</td>
<td>-</td>
<td>262</td>
<td>328</td>
<td>0.252</td>
<td>0.385</td>
<td>0.65</td>
<td>-ve</td>
</tr>
<tr>
<td>S6</td>
<td>42</td>
<td>-</td>
<td>262</td>
<td>319</td>
<td>0.218</td>
<td>0.392</td>
<td>0.56</td>
<td>-ve</td>
</tr>
<tr>
<td>S7</td>
<td>66</td>
<td>209°</td>
<td>267</td>
<td>306</td>
<td>0.146</td>
<td>0.371</td>
<td>0.39</td>
<td>-ve</td>
</tr>
<tr>
<td>S8</td>
<td>90</td>
<td>-</td>
<td>267</td>
<td>301</td>
<td>0.127</td>
<td>0.382</td>
<td>0.33</td>
<td>-ve</td>
</tr>
<tr>
<td>S9</td>
<td>150</td>
<td>-</td>
<td>259</td>
<td>288</td>
<td>0.112</td>
<td>0.402</td>
<td>0.28</td>
<td>-ve</td>
</tr>
<tr>
<td>S10</td>
<td></td>
<td>208°</td>
<td>259</td>
<td>285</td>
<td>0.100</td>
<td>0.417</td>
<td>0.24</td>
<td>-ve</td>
</tr>
</tbody>
</table>
Kinetics of Disaggregation Reactions

The velocity constants have been calculated for each reaction, the specific viscosity/concentration ratios used in the calculations being obtained by graphical interpolation to eliminate errors of experiment in the viscosity determinations.

<table>
<thead>
<tr>
<th>Temperature 44.3°C</th>
<th>Time (hours)</th>
<th>( \frac{\eta_{sp}}{c} )</th>
<th>( K \times 10^{-7} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1.45</td>
<td>3.89</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>1.11</td>
<td>4.14</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>0.91</td>
<td>3.81</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>0.76</td>
<td>3.60</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>0.65</td>
<td>3.44</td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>0.56</td>
<td>3.36</td>
<td></td>
</tr>
<tr>
<td>700</td>
<td>0.45</td>
<td>3.57</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>0.41</td>
<td>3.43</td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td>0.35</td>
<td>3.79</td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td>0.34</td>
<td>3.43</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Temperature 70.5° Time (hours) $\gamma_{sp/g}$ $x 10^{-6}$

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.95</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>1.04</td>
<td>6.10</td>
</tr>
<tr>
<td>25</td>
<td>0.73</td>
<td>6.02</td>
</tr>
<tr>
<td>40</td>
<td>0.44</td>
<td>6.47</td>
</tr>
<tr>
<td>50</td>
<td>0.42</td>
<td>5.43</td>
</tr>
<tr>
<td>75</td>
<td>0.35</td>
<td>5.70</td>
</tr>
<tr>
<td>100</td>
<td>0.34</td>
<td>6.47</td>
</tr>
<tr>
<td>125</td>
<td>0.33</td>
<td>6.18</td>
</tr>
<tr>
<td>250</td>
<td>0.29</td>
<td>5.92</td>
</tr>
</tbody>
</table>

Hence for $T_1$, 44.5° $k_1 = 5.67 \times 10^{-7}$

For $T_2$, 70.5° $k_2 = 6.04 \times 10^{-6}$

From these figures (1) the energy of activation

$E = 20,010$ cal.

(2) the collision number

$A = 10^{7.31}$

Examination of the final sample of the disaggregated malt amylopectin.

In the second disaggregation experiment (at 70.5°) nine samples were withdrawn before the final product was isolated. Nine samples of 15 ml. each correspond to 2.43 g. and the final product was 0.81 g. in weight, giving a yield of over 90% for the experiment.

Hydroxyl groupings freed on disaggregation were methylated with Purdie's regents (167, see p. 135) the product isolated, dissolved in chloroform, and precipitated/-
precipitated by pouring the chloroform solution into petroleum ether (b.p. 40-60°). The yield was 0.48g. (from 0.55 g.) 0CH₂ 45.2%.

An attempted fractionation of the product was carried out by dissolution in petroleum ether (bp, 60-80°) - chloroform mixtures. The majority of the amylopectin dissolved at 11% chloroform, and the remainder at 13%, leaving an insoluble residue.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>%age chloroform</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>0.34g</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>0.06g</td>
</tr>
</tbody>
</table>

Fraction 1 had [α]_D^16 + 209° (c, 0.5 in chloroform)

$$[β]_{SP/C} = 0.22$$ (c, 0.44 in m-cresol)

$$\eta = 0.19$$ (Intrinsic viscosity c = 0)

A sample of fraction 1 (0.15 g.) was hydrolysed with methanolic hydrogen chloride solution (10 ml.; 2%) to glycosides which were converted to the corresponding sugars by aqueous hydrochloric acid (10 ml.; 0.4%) (see p.115). The methylated sugars were chromatographed and estimated by the method of Hough, Hirst and Jones (97, see p.119)

The results were as follows:

Sugar/-
Sugar indicated | Rq found | Molar percent
--- | --- | ---
2:3:4:6-tetra-O-methyl-D-glucose | 1.0 | 5.4
2:3:6-tri-O-methyl-D-glucose | 0.81 | 88.2%
2:3-di-O-methyl-D-glucose (at least 50% of "di" fraction) | 0.60 | 6.3%
2:6) 3:6)-di-O-methyl-D-glucose | 0.54 |

These results are equivalent to one non-reducing terminal group per 19 glucose residues in the final "disaggregation" sample.

\[\beta\]-Amylalysis of the malt amylopectin fraction.

Preparation of standard curves (with the Shaffer-Somogyi reagent 60 (173) as modified by Hames and Cattle (174) for glucose and maltose.

The concentration of the reducing sugars, glucose and maltose, were determined polarimetrically in 4Dm tubes by taking a solution (ca. 2%) of the sugars and finding the final equilibrium value of the rotation.

Glucose | Concentration from polarimeter | 2.64%
Maltose | " " | 1.73%

Each solution was diluted 20 times so that

1 ml. glucose solution = 1.320 mg.
1 ml. maltose solution = 0.865 mg.

The Shaffer-Somogyi reagent, calibrated against the reducing sugars would determine 0.2 - 2.5 mg. glucose and 0.5 - 4 mg. maltose in 5 ml. of aqueous samples provided the time of heating (15 minutes) was strictly adhered to.

The/-
STANDARD CURVES FOR THE SHIFFER-3010071 REAGENT

GLUCOSE

MALTOSE
The following results were obtained for the standard curves:

<table>
<thead>
<tr>
<th>Glucose Blank (9.90 ml.)-average Weight of titre of sodium thiosulphate (0.01N) (ml.)</th>
<th>Maltose Blank (9.90 ml.)-average Weight of titre of sodium thiosulphate (0.01N) (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of Sugar (mg.)</td>
<td>Average titre of Sugar (mg.)</td>
</tr>
<tr>
<td>2.64</td>
<td>3.08</td>
</tr>
<tr>
<td>1.96</td>
<td>6.05</td>
</tr>
<tr>
<td>1.32</td>
<td>4.20</td>
</tr>
<tr>
<td>0.66</td>
<td>2.20</td>
</tr>
<tr>
<td>0.26</td>
<td>0.70</td>
</tr>
</tbody>
</table>

- Amylolytic of potato starch and waxy amyllopectin

Solutions (ca. 0.2%) of the starch and the amyllopectin were prepared and the concentration determined by Pirt and Whelan's method (175). The starches were hydrolysed in 0.1% solution in 1.5N sulphuric acid at 100°C for 2 hours and the liberated glucose estimated by the Somogyi reagent after cooling and neutralising with sodium hydroxide (5N) to faint alkalinity to phenolphthalein and then just back to neutrality with sulphuric acid (2N, 2 drops).

The weight of glucose found from the curve was multiplied by a factor which allowed for loss during the hydrolysis.

1 ml. volumes of the starch and amyllopectin solutions were treated as described and the results were as follows:

Potato/-
<table>
<thead>
<tr>
<th>Time/ml.</th>
<th>Potato Starch</th>
<th>Malt amylopectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Blank - titre of solution</td>
<td>Blank - titre of solution</td>
</tr>
<tr>
<td></td>
<td>Sodium thiosulphate</td>
<td>Sodium thiosulphate</td>
</tr>
<tr>
<td></td>
<td>(0.01N) ml.</td>
<td>(0.01N) ml.</td>
</tr>
<tr>
<td>8.20</td>
<td>6.48</td>
<td>6.48</td>
</tr>
<tr>
<td>8.10</td>
<td>6.52</td>
<td>6.47</td>
</tr>
<tr>
<td>8.08</td>
<td>6.42</td>
<td>6.42</td>
</tr>
</tbody>
</table>

The enzyme used was a sample of crystalline β-amylase from the sweet potato, which was free from maltase and α-amylase and had no Z enzyme activity (146), kindly presented by Dr Manners.

Initially the action of the β-amylase on the potato starch was studied as follows — the digest contained starch solution (20 ml.) (2.43 mg. /ml.), 0.2M acetate buffer of pH 4.6 (10 ml.), distilled water (19 ml.) and β-amylase solution (1 ml.). The digest was incubated at 37° and aliquot portions (5 ml.) were analysed for each determination. A blank containing all but the enzyme solution was also run. The experiment was repeated using the amylopectin solution (20 ml.; 1.92 mg. /ml.)
<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Blank Sodium thiosulphate (0.01N)</th>
<th>Amylopectin Sodium thiosulphate (0.01N)</th>
<th>Average Blank - Average starch titre sodium thiosulphate (0.01N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>9.73 (ml.) 4.98</td>
<td>6.86 (ml.) 2.88</td>
<td>2.92 = 1.6 mg. maltose</td>
</tr>
<tr>
<td>2</td>
<td>9.71 5.05</td>
<td>6.79 2.95</td>
<td>1.57 mg. amylopectin</td>
</tr>
<tr>
<td>4</td>
<td>9.72 4.97</td>
<td>6.54 3.21</td>
<td>1.77 mg. maltose</td>
</tr>
<tr>
<td>4</td>
<td>9.78 4.92</td>
<td>6.60 3.15</td>
<td>1.63 mg. amylopectin</td>
</tr>
<tr>
<td>24</td>
<td>9.76 4.95</td>
<td>6.69 3.05</td>
<td>1.7 mg. maltose</td>
</tr>
<tr>
<td>24</td>
<td>9.71 4.89</td>
<td>6.62 3.12</td>
<td>1.65 mg. amylopectin</td>
</tr>
<tr>
<td>90</td>
<td>9.73 4.98</td>
<td>6.60 3.14</td>
<td>1.7 mg. maltose</td>
</tr>
<tr>
<td>90</td>
<td>9.75 4.88</td>
<td>6.64 3.10</td>
<td>1.67 mg. amylopectin</td>
</tr>
</tbody>
</table>

Time of incubation (hours) 2 4 24 90

Percentage conversion to maltose A) potato starch 52 53 53 53
B) Malt amylopectin 41 44 44 44

Isolation/-
Isolation of \( \alpha \)-amylase from barley flour

The enzyme was prepared according to D.H. Northcote (169) by extracting barley flour (100 g.) with water (100 ml.) for 1 hour. After the resulting solution had been dialysed it contained \( \alpha \)- and \( \beta \)-amylases and maltase. It was stored at pH 3.6 and 4° for 1 week to destroy \( \alpha \)-amylase activity.

\( \beta \)-Amylase was extracted by ethanolic fractionation of the solution according to the method of Halsall, Hirst, Hough and Jones (56). The fraction which was precipitated when the alcohol concentration was 60-80% was removed on the centrifuge, washed with alcohol, ether and dried over phosphorous pentoxide in a vacuum desiccator.

The percentage hydrolysis of potato starch to maltose on prolonged incubation with the \( \beta \)-amylase thus prepared was 55, indicating the absence of \( \alpha \)-amylase. Maltose was the only sugar present in the digest as shown by paper chromatography, indicating that no maltase was present.

Isolation of the limit dextrin from malt amylopectin

The following digest was set up; amylopectin solution (180 ml.: 3.8 mg./ml.) (Concentration by Pirt and Whelan's method, 175), 0.2M acetate buffer of pH 4.6, (100 ml.), distilled water (125 ml.) \( \beta \)-amylase solution (25 ml.). The digest was incubated at 37° and aliquot portions (2 ml.) were analysed for each determination. A blank on a smaller scale containing all but the enzyme solution was also run.
The course of the hydrolysis was as follows:

<table>
<thead>
<tr>
<th>Time of incubation</th>
<th>24 hrs.</th>
<th>48 hrs.</th>
<th>90 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage conversion to maltose</td>
<td>40</td>
<td>47</td>
<td>47</td>
</tr>
</tbody>
</table>

After boiling for 10 minutes to inactivate the enzyme and filtering to remove co-agulated protein, the solution was dialysed for 2 days. The dextrin was precipitated with alcohol, and dissolved in water (50 ml.) before freeze-drying.

Yield 0.274 g.

Protein Content 5.2% (% age nitrogen x 6.25)

Attempted removal of protein from the limit dextrin

The dextrin (0.244 g.) was dispersed in water (100 ml.) and shaken for one hour with butanol (100 ml.) after which the liquor was allowed to settle. A brown layer formed between the butanol phase and the dextrin dispersion which was then removed in a separating funnel. The dextrin was precipitated with alcohol and dispersed in water before freeze-drying.

Yield of dextrin 0.208 g.

Protein Content 0.32% (% age nitrogen x 6.25)

Potentiometric iodine titration - Technique R (68)

It was found that there was no appreciable amount of iodine bound, but that there was approximately 0.6% "impurity" present. A reddish coloration was produced in a solution of the dextrin on the addition of a few drops of iodine (0.1N).
Periodate oxidation of the limit dextrin from malted barley amylopectin — estimation of the formic acid liberated from the end-groups (64).

The dextrin (0.118 g.) was dissolved in water (50 ml.) containing potassium chloride (2.5 g.) and sodium metaperiodate added. The procedure was then as on p. 125 for the oxidation of the amylose.

10 ml. samples withdrawn. Total volume 60 ml.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Corrected volume of sodium hydroxide (0.01N) per 10 ml sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>161</td>
<td>1.48</td>
</tr>
<tr>
<td>185</td>
<td>1.51</td>
</tr>
<tr>
<td>209</td>
<td>1.56</td>
</tr>
<tr>
<td>233</td>
<td>1.59</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 1.44 ml.

Yield of formic acid from 162 g. polysaccharide 0.118 moles

Number of non-terminal glucose residues per non-reducing terminal group 8.5

Isolation of the limit dextrin from the amylopectin from the barley starch — (Plumage Archer and Spratt Archer).

The procedure was the same as in the case of the dextrin from the malt amylopectin. The preparation of the barley amylopectin is outlined on p. 167.
The course of the hydrolysis with β-amylase was as follows:

<table>
<thead>
<tr>
<th>Time of incubation (hours)</th>
<th>3</th>
<th>22</th>
<th>46</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage conversion to maltose</td>
<td>50.7</td>
<td>58.8</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>Weight of amylopectin used</td>
<td>0.721g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield of dextrin (after protein removal using butanol)</td>
<td>0.266 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein Content</td>
<td>0.23% (%age nitrogen x6.25)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Optical rotation**

In sodium hydroxide solution (N)

\[ [\alpha]_D^{153°} + 153° \] (c, 0.5)

Potentiometric iodine titration. Technique B (68)

The binding power of this dextrin was negligible. The presence of ca 0.2% "impurity" was indicated by the curve. It also gave a reddish coloration in solution on addition of a few drops of iodine (0.1N).

Periodate oxidation of the -limit dextrin from barley amylopectin - estimation of the formic acid liberated from the end-groups (64)

Carried out as described for amylase p. 125.

Weight of sample | 0.144 g |
Total volume of solution | 60 ml |
10 ml samples withdrawn.

Time/
### Time of shaking

<table>
<thead>
<tr>
<th>(hours)</th>
<th>Corrected volume of sodium hydroxide (0.01N) used per 10 ml. sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>161</td>
<td>1.56</td>
</tr>
<tr>
<td>185</td>
<td>1.67</td>
</tr>
<tr>
<td>209</td>
<td>1.76</td>
</tr>
<tr>
<td>233</td>
<td>1.87</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking: 1.53ml.

Yield of formic acid from 162 g. polysaccharide: 0.103 moles

Number of non-terminal glucose residues per non-reducing terminal group: 9.7
Starches from barley and malt

Preparation of the starch from barley

The variety of the barley was a mixture of Spratt Archer and Plumage Archer from which the malted barley whose starch, amylose and amyllopectin have been investigated, was produced.

A crude starch was obtained by the same technique as used in the case of the malted barley starch but it was given two additional extractions with 95% methanol.

Original weight of barley 100g.
Yield of crude starch 53g.

A sample of this crude starch (50g.) was stirred for one hour with sodium bisulphite solution (1½ litre; 0.1%) to remove contaminating protein material. When the solid matter was removed on the centrifuge (1000 r.p.m.; 10 minutes) the heavier starch granules sedimanted before the rich protein and husk fraction, forming a double layer. The latter was scraped off, a process which entailed a heavy loss of the starch. The starch was stirred with the aqueous sulphite and the procedure repeated twice.

The starch was finally dispersed in cold water (50 ml.) and freeze dried. Yield 5g.

Examination/-
Examination of the barley starch

Amylose content (By potentiometric titration -
technique B)  22.6%

Optical rotation. In sodium hydroxide solution (N)

\[ [\alpha]_D^{18} + 154^\circ \ (c, 1.0) \]

Protein content  0.13% (% nitrogen X6.25).

On microscopic examination the starch was seen
to be in granular form, the granules being ovoid to
spherical in shape.

Preparation of the amylopectin from the crude barley
starch by using 20% aqueous pyridine and butanol.

The procedure was the same as for the malt
amylopectin (p. 128)

Weight of crude starch 10g.

Yield of amylopectin  4.6g.

Examination of the amylopectin

Amylose content (by potentiometric titration -
technique B)  0.70%

Optical rotation. In sodium hydroxide solution (N)

\[ [\alpha]_D^{18} + 148^\circ \ (c, 1.2) \]

Protein content  0.29% (%age nitrogen X6.25).

Starches and Amylopectins from Ymer Malt and Ymer Barley

Crude starches were prepared as in the case of
Spratt Archer and Plumage Archer malt, and heated
under reflux for five hours with two additional
volumes of methanol (95%).

The amylopectins were isolated using the method
with/-
with 20% aqueous pyridine and butanol, while the whole starches were obtained by chloral hydrate extraction (32, 137).

<table>
<thead>
<tr>
<th></th>
<th>Ymer Barley</th>
<th>Ymer Malt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original weight of grain</td>
<td>100 g.</td>
<td>100 g.</td>
</tr>
<tr>
<td>Yield of crude starch</td>
<td>59 g.</td>
<td>49 g.</td>
</tr>
<tr>
<td>Chloral hydrate extraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight of crude starch</td>
<td>10 g.</td>
<td>10 g.</td>
</tr>
<tr>
<td>Yield of whole starch</td>
<td>7.2 g.</td>
<td>5.9 g.</td>
</tr>
</tbody>
</table>

Examination of the starches from Ymer malt and Ymer barley.

Amylose content (By potentiometric titration-technique B).

- Ymer barley starch: 22.5%
- Ymer malt starch: 26.8%

Optical rotation. In sodium hydroxide solution (N)

- Ymer barley starch: \([\alpha]_D^{18} + 156^0\ (c, 1.1)\)
- Ymer malt starch: \([\alpha]_D^{18} + 149^0\ (c, 0.9)\)

Protein content (% nitrogen \(X6.25\))

- Ymer barley starch: 0.16%
- Ymer malt starch: 0.29%

Amylopectin

<table>
<thead>
<tr>
<th></th>
<th>Ymer barley</th>
<th>Ymer Malt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of crude starch</td>
<td>10 g.</td>
<td>10 g.</td>
</tr>
<tr>
<td>Yield of amylopectin</td>
<td>5.1 g.</td>
<td>4.2 g.</td>
</tr>
</tbody>
</table>
Examination of the amylpectins from Ymer malt and Ymer barley.

Amylose content (By potentiometric titration - technique B)

Ymer barley amylopectin 0.40%
Ymer malt amylopectin 4.19%

Optical rotations. In sodium hydroxide solution (N)

Ymer barley amylopectin $[\alpha]_D^{18} + 149^\circ$ (c, 0.86)
Ymer malt amylopectin $[\alpha]_D^{18} + 144^\circ$ (c, 0.92)

Protein content (% nitrogen x6.25)

Ymer barley amylopectin 0.15%
Ymer malt amylopectin 0.42%
Periodate Oxidation Studies on the Whole Starches and Amylopectins from Malt and Barley.

Estimation of the formic acid liberated from the end-groups (64).

The procedure was the same as in the case of the amylose (p.12). The amylopectins remained in solution during the oxidation process and were withdrawn along with the samples for titration. No corrections were therefore made to the titration figures for the amylopectins.

The results were as follows.

Flumace Archer and Spratt Archer Malt-Whole Starch.

A. Weight of sample 0.5000g.

Total volume of solution 120 ml.

20 ml. samples withdrawn.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Corrected volumes of sodium hydroxide (0.01N) used per 20 ml. sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>2.161</td>
</tr>
<tr>
<td>144</td>
<td>2.274</td>
</tr>
<tr>
<td>192</td>
<td>2.600</td>
</tr>
<tr>
<td>212</td>
<td>2.793</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 2.280 ml.

Yield of formic acid (moles) from 162g. starch 0.045 moles

Number of non-terminal glucose residues per non-reducing terminal group 22

Calculated number of non-terminal glucose residues per non-reducing terminal group in the amylopectin fraction 17
The oxidation was repeated and the results were as follows:

B. Weight of sample 0.5005g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Corrected volumes of sodium hydroxide (0.01N) per 20 ml sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>162</td>
<td>2.490</td>
</tr>
<tr>
<td>186</td>
<td>2.550</td>
</tr>
<tr>
<td>210</td>
<td>2.613</td>
</tr>
<tr>
<td>240</td>
<td>2.665</td>
</tr>
<tr>
<td>260</td>
<td>2.713</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 2.470 ml.

Yield of formic acid (moles) from 162g starch 0.043 moles

Number of non-terminal glucose residues per non-reducing terminal group 21

Calculated number of non-terminal glucose residues per non-reducing terminal group in the amylopectin fraction 16

---

Plumage Archer and Spratt Archer Malt - Amylopectin

A. Weight of sample 0.5004g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Volume of sodium hydroxide (0.01N) used per 20ml sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>3.278</td>
</tr>
<tr>
<td>150</td>
<td>3.688</td>
</tr>
<tr>
<td>171</td>
<td>3.814</td>
</tr>
<tr>
<td>190</td>
<td>3.925</td>
</tr>
<tr>
<td>261</td>
<td>4.350</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 3.690 ml.

Yield of formic acid (moles) from 162g polysaccharide 0.0717 moles
Number of glucose residues per non-reducing terminal group: 14

B. Weight of sample: 0.4595g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Volume of sodium hydroxide (0.01N) used per 20 ml. sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>162</td>
<td>3.440</td>
</tr>
<tr>
<td>186</td>
<td>3.555</td>
</tr>
<tr>
<td>210</td>
<td>3.650</td>
</tr>
<tr>
<td>240</td>
<td>3.795</td>
</tr>
<tr>
<td>260</td>
<td>3.935</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking: 3.375 ml.

Yield of formic acid (moles) from 162g. polysaccharide: 0.0714 moles

Number of glucose residues per non-reducing terminal group: 14

Hydrolysis of amylopectin after oxidation by periodate.

Amylopectin (0.9847 g.) was dissolved in water (110 ml.) containing potassium chloride (5 g.) and sodium metaperiodate (30 ml.; 0.3M) in a glass stoppered bottle at room temperature, and shaken for 10 days. The excess periodate was then destroyed by shaking with ethylene glycol (2.5 ml.) for 24 hours. The oxidized amylopectin solution was dialysed until free from oxidant (test with potassium iodide and sulphuric acid, and diphenylamine and sulphuric acid). It was then evaporated to dryness.

The periodate oxidised amylopectin was dissolved/-
dissolved in sodium hydroxide (0.2N; 30 ml.) and sodium borohydride (0.250 g.) added. After standing overnight it was neutralised with glacial acetic acid and evaporated to dryness, dissolved in water and dialysed for 4 days, before being hydrolysed in sulphuric acid (1.5N) by heating at 100°C for 5 hours. Ribose (20 mg.) was added as reference sugar before the solution was neutralised with barium carbonate and the barium sulphate removed by filtration and washed. The filtrate was concentrated and examined by paper chromatography.

Glucose was found to be present. Quantitative examination by the method of Flood, Hirst and Jones (68) revealed the presence of 0.74 g. glucose per 100 g. amylpectin.

**Estimation of formic acid liberated from end-groups.**

**Plumage Archer and Spratt Archer Barley-Whole Starch.**

A. Weight of sample 0.435g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Corrected volume of sodium hydroxide (0.01N) used per 20 ml. sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>161</td>
<td>1.45</td>
</tr>
<tr>
<td>185</td>
<td>1.56</td>
</tr>
<tr>
<td>209</td>
<td>1.65</td>
</tr>
<tr>
<td>233</td>
<td>1.79</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 1.40 ml.

Yield of formic acid (moles) from 162g. starch 0.031 moles.

Number of glucose residues per non-reducing terminal group in the starch 32

Calculated/-
Calculated number of glucose residues per non-reducing terminal group in the amylopectin fraction

B. Weight of sample 0.456 g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Corrected volume of sodium hydroxide (0.01N) used per 20 ml. sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td>1.55</td>
</tr>
<tr>
<td>191</td>
<td>1.68</td>
</tr>
<tr>
<td>211</td>
<td>2.04</td>
</tr>
<tr>
<td>234</td>
<td>2.21</td>
</tr>
<tr>
<td>264</td>
<td>2.33</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 1.55 ml.

Yield of formic acid from 162 g. starch 0.033 moles

Number of glucose residues per non-reducing terminal group in the starch

Calculated number of glucose residues per non-reducing terminal group in the amylopectin fraction

Plumage Archer and Spratt Archer Barley - Amylopectin

A. Weight of sample 0.454 g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Volume of sodium hydroxide (0.01N) used per 20 ml. sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>161</td>
<td>1.82</td>
</tr>
<tr>
<td>185</td>
<td>1.91</td>
</tr>
<tr>
<td>209</td>
<td>2.02</td>
</tr>
<tr>
<td>233</td>
<td>2.08</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 1.77 ml.

Yield of formic acid (moles) from 162 g. polysaccharide 0.038 moles

Number/-
Number of glucose residues per non-reducing terminal group 26

B. Weight of sample 0.498 g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Volume of sodium hydroxide (0.01N) used per 20 ml sample (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>165</td>
<td>2.08</td>
</tr>
<tr>
<td>189</td>
<td>2.19</td>
</tr>
<tr>
<td>261</td>
<td>2.62</td>
</tr>
<tr>
<td>284</td>
<td>2.69</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 1.98 ml.
Yield of formic acid from 162 g polysaccharide 0.0336 moles.

Number of glucose residues per non-reducing terminal group 26

Ymer Malt - Whole Starch

A. Weight of sample 0.452 g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Corrected volume of sodium hydroxide (0.01N) used per 20 ml sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td>1.67</td>
</tr>
<tr>
<td>191</td>
<td>1.71</td>
</tr>
<tr>
<td>211</td>
<td>1.77</td>
</tr>
<tr>
<td>234</td>
<td>1.86</td>
</tr>
<tr>
<td>264</td>
<td>1.91</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 1.65 ml.
Yield of formic acid (moles) from 162 g starch 0.0355 moles.
Number of glucose residues per non-reducing terminal group in the starch 28
Calculated number of glucose residues per non-reducing terminal group in the amylopectin 20
B. Weight of sample 0.463g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Corrected volume of sodium hydroxide (0.01N) per 20ml sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>1.71</td>
</tr>
<tr>
<td>162</td>
<td>1.77</td>
</tr>
<tr>
<td>186</td>
<td>1.88</td>
</tr>
<tr>
<td>258</td>
<td>2.40</td>
</tr>
<tr>
<td>281</td>
<td>2.60</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 1.75 ml.

Yield of formic acid (moles) from 162g starch 0.0366 moles.

Number of glucose residues per non-reducing terminal group in the starch 27

Calculated number of glucose residues per non-reducing terminal group in the amyllopectin fraction 20

Ymer Malt - Amylopectin

A. Weight of sample 0.541 g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Volume of sodium hydroxide (0.01N) per 20 ml. sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>161</td>
<td>2.72</td>
</tr>
<tr>
<td>185</td>
<td>2.80</td>
</tr>
<tr>
<td>209</td>
<td>2.89</td>
</tr>
<tr>
<td>233</td>
<td>2.93</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 2.69 ml.

Yield of formic acid (moles) from 162g polysaccharide 0.0485 moles.

Number of glucose residues per non-reducing terminal group 20
**B. Weight of sample** 0.502g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Volume of sodium hydroxide (0.01N) per 20 ml. sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td>2.92</td>
</tr>
<tr>
<td>193</td>
<td>2.93</td>
</tr>
<tr>
<td>219</td>
<td>3.26</td>
</tr>
<tr>
<td>240</td>
<td>3.33</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 2.85 ml.

Yield of formic acid (moles) from 162g.

polysaccharide 0.0554 moles

Number of glucose residues per non-reducing terminal group 18

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**Ymer Barley - Whole Starch**

**A. Weight of sample** 0.488g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Corrected volume of sodium hydroxide (0.01N) per 20 ml. sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>161</td>
<td>1.85</td>
</tr>
<tr>
<td>185</td>
<td>2.03</td>
</tr>
<tr>
<td>209</td>
<td>2.15</td>
</tr>
<tr>
<td>235</td>
<td>2.39</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 1.76 ml.

Yield of formic acid from 162g. starch 0.035 moles

Number of glucose residues per non-reducing terminal group in the starch 29

Calculated number of glucose residues per non-reducing terminal group in the amylopectin 22

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B. Weight of sample 0.420g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Corrected volume of sodium hydroxide (0.01N) per 20 ml. sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td>1.36</td>
</tr>
<tr>
<td>191</td>
<td>1.45</td>
</tr>
<tr>
<td>211</td>
<td>1.48</td>
</tr>
<tr>
<td>234</td>
<td>1.60</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 1.34 ml.

Yield of formic acid (moles) from 162g starch 0.031 moles.

Number of glucose residues per non-reducing terminal group in the starch 32

Calculated number of glucose residues per non-reducing terminal group in the amylopectin 25

Ymer Barley - Amylopectin

A. Weight of sample 0.308g.

<table>
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<th>Time of shaking (hours)</th>
<th>Volume of sodium hydroxide (0.01N) per 20 ml. sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>161</td>
<td>1.27</td>
</tr>
<tr>
<td>165</td>
<td>1.32</td>
</tr>
<tr>
<td>209</td>
<td>1.40</td>
</tr>
<tr>
<td>233</td>
<td>1.45</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 1.23 ml.

Yield of formic acid (moles) from 162g polysaccharide 0.040 moles

Number of glucose residues per non-reducing terminal group 25

B. /-
B. Weight of sample 0.498g.

<table>
<thead>
<tr>
<th>Time of shaking (hours)</th>
<th>Volume of sodium hydroxide (0.01N) per 20 ml sample (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>163</td>
<td>2.19</td>
</tr>
<tr>
<td>187</td>
<td>2.23</td>
</tr>
<tr>
<td>201</td>
<td>2.31</td>
</tr>
<tr>
<td>225</td>
<td>2.34</td>
</tr>
<tr>
<td>251</td>
<td>2.61</td>
</tr>
</tbody>
</table>

Estimated titre after 150 hours shaking 2.15 ml.

Yield of formic acid (moles) from 162g polysaccharide 0.042 moles

Number of glucose residues per non-reducing terminal group 24
SUMMARY

The Starch and Related Components from Plumeage
Archer and Spratt Archer Malt.

(1) The malt starch had an amylose content of 23 to 28%.

(2) On fractionation, amylose and amyllopectin were obtained which were considered to be pure components from their respective iodine-binding powers.

(3) When the amylose was methylated a fraction was isolated and hydrolysed, yielding 2:3:4:6-tetra-<i>Q</i>-methyl-D-glucose (9.32%) (molar percent), 2:3:6-tri-<i>Q</i>-methyl-D-glucose (98.1%), and di-<i>Q</i>-methyl-D-glucoses (1.6%). It was therefore concluded that the amylose fraction could be represented for the most part by unbranched chains of ca 300 glucose residues, linked together by <i>α</i>-1:4-bonds.

(4) From the periodate oxidation of the amylose, the formic acid liberated from the end-groups was equivalent to a chain of ca 350 glucose residues, confirming the result of the methylation study.

(5) Methylation investigations were carried out on the amyllopectin and a fraction was isolated which, on hydrolysis, yielded 2:3:4:6-tetra-<i>Q</i>-methyl-D-glucose (5.7%), 2:3:6-tri-<i>Q</i>-methyl-D-glucose (87.4%), 2:3-di-<i>Q</i>-methyl-D-glucose (4.3%), 2:6-di-<i>Q</i>-methyl/-
methyl-D-glucose (1.5%), and 3:6-di-2-methyl-D-glucose (1.1%). The amount of non-reducing end-group represented a "repeating unit" of 17-18 glucose residues in the amylopectin while the di-2-methyl fraction showed that the majority of the inter-chain bonds were of the 1:6-variety.

(6) The methylated amylopectin had a molecular weight of 320,000, and was "disaggregated" in methanolic oxalic acid solution in two experiments at 44.3° and 70.5°. The final sample had a molecular weight of ca 17,000 and the apparent chain length was the same as the original methylated sample. This indicated that the diminution in molecular size resulted from the rupture of inter-chain bonds of which at least two different types were present in the molecule. The energy of activation of the reaction was calculated to be 80,010 calories and the collision number \(10^{+7.31}\).

(7) Periodate oxidation of the malt starch showed the presence of one non-reducing terminal group per 21-22 glucose residues, which was equivalent to an apparent chain length of 16-17 in the amylopectin fraction. Similar treatment of the malt amylopectin indicated that the chain length was shorter, namely 14 glucose residues.

(8) \(\beta\)-Amylolysis of the malt amylopectin gave a maltose conversion of 44%, while the conversion from the action of \(\beta\)-amylase on the amylopectin isolated from/-
from the starch of Plumage Archer and Spratt Archer barley was 59%. A shortening of the external chain of the amylopectin had therefore occurred during the malting procedure. The β-limit dextrins isolated after both experiments had similar apparent chain lengths from periodate oxidation studies and represented an internal chain of 6-7 glucose residues.

(9) From the periodate oxidation of the starch from Plumage Archer and Spratt Archer barley, the presence of one non-reducing terminal-group per 30-32 glucose residues was indicated, equivalent to an apparent chain length of 23-24 in the amylopectin fraction. Periodate oxidation of the amylopectin itself gave a similar result. The amylose content of the barley starch was 22.6%.

(10) Periodate oxidation and potentiometric iodine titration investigations were carried out on the starches and amylopectins from Ymer malt and Ymer barley. The results were in close agreement with those obtained in the study of the malt and barley of the Plumage Archer and Spratt Archer variety.
ACKNOWLEDGEMENT

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