Investigation of the Starch Components of a Synthetic Cereal Species

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A comparative study of structural features of the starch components of an alien genome combinant (synthetic cereal species) and the parental cereal species was conducted. Investigations involving chemical and enzymatic degradation and ultracentrifugation showed no digressions in the structural nature of the starch components. This indicated that, at the genetic level, no significant biogenetic interactions were present. Based on preliminary agronomic tests, the alien genome combinant may serve as an efficient source of cereal carbohydrate and protein.

Earlier investigations on the rye-wheat species hybrid indicated that, with some notable exceptions, a general hybrid effect was present when various characteristics of the storage proteins (Yong and Unrau, 1964, 1966) and some enzymes (Lee and Unrau, 1969) were considered. The synthetic rye-wheat species has been assigned the genome complement "AABBRR" since its parents, tetraploid durum wheat, *Triticum durum* var. Stewart, and diploid rye, *Secale cereale* var. Prolific, have the genome connotations "AABB" and "RR," respectively. Investigation of the storage protein of the synthetic species (AABBRR) by gel electrophoresis showed that new protein bands were present while some bands characteristic of one or both parents were absent (Yong and Unrau, 1964). In some instances, marked deviations in the amino acid composition of protein fractions existed in the hybrid species (Yong and Unrau, 1966). Although the α-amylase in the alien genome combinant (hybrid species) possessed physical characteristics that were generally intermediate (hybrid) in nature, the enzyme did possess a distinct electrophoretic mobility, indicating an altered electrical charge density and/or molecular size as well as a distinct amino acid composition (Lee and Unrau, 1969). It is significant that both parental species have distinctly diverged genetically, and it is therefore noteworthy that the alien genome combination resulted in a surprisingly harmonious biosynthetic unit.

Although amylose and amylopectin are structurally much less complex than proteins, it was considered desirable to initiate investigations to ascertain whether any detectable structural alterations existed in these glucans as they occur in the synthetic species.

EXPERIMENTAL METHODS AND RESULTS

Extraction of Starch. ALKALINE EXTRACTION. Starch present in flour was solubilized using 20% sodium hydroxide solution containing 0.1% sodium borohydride to minimize alkaline oxidation of the reducing end-residues. The mixture was heated (80° to 90° C) for 4 hr under nitrogen, after which the turbid solution was cooled, followed by neutralization with glacial acetic acid. The solution was centrifuged (3000 rpm) to remove insoluble material, and the starch precipitated by addition of four volumes of ethanol. In a similar manner, starch was extracted from common white potato.

HOT WATER EXTRACTION OF STARCH. To approximately 1 l. of water containing sodium borohydride (400 mg) was added 20 g of flour (prewetted with ethanol to afford proper suspension) and the mixture boiled for 6 hr with a constant stream of oxygen-free nitrogen bubbled through the solution. After cooling and centrifugation to remove insoluble material, trichloroacetic acid (5 g) was added. The precipitate was removed by centrifugation, and the solution concentrated under vacuum using a silicone base antifoam to prevent frothing. The starch was precipitated with ethanol as described above. Starch was extracted from common white potato in an identical manner.

NITROGEN DETERMINATION. Small aliquots of dry starch (30 to 40 mg) were digested with conc. sulfuric acid (1.5 ml) in small borosilicate tubes placed in suitably heated copper blocks. To the brown solutions was added, after cooling, 30% hydrogen peroxide (0.5 ml), and the tube and contents again heated. After a second treatment with hydrogen peroxide, perfectly clear solutions were obtained which were consequently made to volume with distilled water (Lanni and Dillon, 1950). Nitrogen as ammonium ion was determined using stabilized Nessler's reagent (Ballantine, 1957) (Table I).

Fractionation of Amylose and Amylopectin. Amylose was separated from amylopectin using a described procedure (Montgomery and Senti, 1958) with only minor modifications. Cyclohexanol was used as the complexing agent. Amylose was purified by repeated complex formation until a constant blue value with iodine was attained (Gilbert and Spragg, 1964) (Table I). The supernatant solution contained mainly amylopectin. Amylose still present in the amylopectin solution was partially removed by adsorption on cellulose paper column (1 in. diameter, 18 in. long) (Winkler, 1962). The product obtained after two successive filtrations is referred to as amylopectin I. More highly purified amylopectin was obtained after a series (four) of treatments with cyclohexanol, followed by further successive (three) filtrations through cellulose paper columns to give finally a product referred to as amylopectin II.

Determination of Amylose Content in Isolated Starches. The amylose content of the isolated starches was determined colorimetrically (Gilbert and Spragg, 1964). A highly purified sample of potato amylose (six precipitations with cyclohexanol) was used as a standard. This sample gave a slightly higher blue value than commercially available potato amylose (Table I).

Ultracentrifugation. Solutions of amylose (1%) in phosphate buffer (pH 7.6), and 0.5% and 0.1% sodium hydroxide were subjected to ultracentrifugation (60,000 rpm). Molecular weight estimations were made using sedimentation values from three different concentrations (0.25, 0.5, and 1%).

The sedimentation coefficients reduced to standard condi-

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Table I. Properties of Starch and Amylose

Source	Nitrogen Content[a] %				Amylose in Starch %		Blue[b] Value			
	Alkali extraction		Hot water extraction		Alkali extraction	Hot water extraction	Alkali extraction		Hot water extraction	
	Starch	Amylose	Starch	Amylose			Starch	Amylose	Starch	Amylose
AABB	0.17	nil	0.25	nil	25–26	24–25	0.29	1.03	0.27	1.09
RR	0.25	nil	0.31	nil	25–26	24–25	0.29	1.01	0.28	1.11
AABBRR	0.21	nil	0.29	nil	26–27	25–26	0.29	1.07	0.27	1.13
Potato	0.11	nil	0.16	nil	24–25	24–25	0.30	1.16	0.32	1.18
MSD (P 0.5)	0.05		0.06		dns[c]	dns	dns	0.06	dns	0.05

[a] Minimum significant difference (msd) at P 0.5 level between extraction procedure, 0.4. [b] Msd at P 0.5 between extraction procedures for amylose blue value, 0.04. [c] Differences not statistically significant (dns) at P 0.5 level.

tions were estimated from the slope of the line from a plot of log (distance of boundary to axis of rotation) *vs.* time (Schachman, 1957). A plot of S *vs.* concentration of polysaccharide and extrapolation to zero concentration gives $S^0_{20,w}$. Sedimentation values ($S_{20,w}$) thus obtained for the different amyloses were used in molecular weight calculations in which 6.5×10^{-7} was taken as an average diffusion coefficient (D), 0.75 as apparent specific volume ($\bar{v}$), and 1.00 as the density of the medium (p). Traces of sedimentation patterns for the amyloses (in 0.1*N* sodium hydroxide solution) obtained from hot water extracted starch are shown in Figure 1.

Amylopectin solutions (2% in phosphate buffer, pH 7.6, and 2% in 0.5*N* and 0.1*N* sodium hydroxide) were similarly subjected to ultracentrifugation (60,000 rpm) and photographs of resulting patterns (0.5*N* sodium hydroxide solutions) taken at regular intervals (Figure 2). Ultracentrifugation of 2% solution (phosphate buffer, pH 7.6) of amylopectin I showed a broad peak fronted by a sharp peak, due to the presence of amylose. Suspension in 0.5% and 0.1% sodium hydroxide showed a similar pattern. Similar runs were made of amylopectin II in which the amylose peak was absent. An approximate molecular weight range was estimated using the method referred to above using concentrations of 0.5, 1.0 and 2.0% (Table V).

Methylation. Samples (approx. 400 mg) of purified amylose and amylopectin were subjected to methylation first by the Haworth procedure (dimethylsulfate and alkali), followed by that of Kuhn *et al.* (1957), and finally exhaustive methylation using the Purdie reagents (methyl iodide and silver oxide). Complete methylation was adjusted by the complete disappearance of the hydroxyl band in the infrared spectrum (liquid film, sodium chloride plates). The fully methylated polysaccharide was depolymerized by dissolving the syrupy material in 75% sulfuric acid (Croon *et al.*, 1960) and, after 1 hr, the solution was diluted with ice water to about 1*N* acid concentration. The solution was boiled for 8 hr, followed by neutralization (barium carbonate), evaporation (*in vacuo*) of the filtrate and paper (Whatman No. 1) chromatographic examination of the product using methylethyl ketone–water azeotrope as the developing solvent, and *p*-anisidine-trichloroacetate as the spray reagent (Hough *et al.*, 1950). The following partially methylated sugars were identified by comparison to authentic compounds.

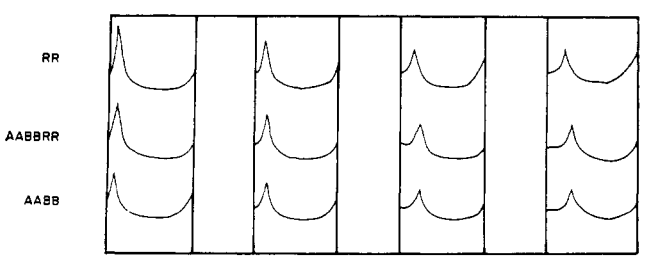

Figure 1. Ultracentrifuge patterns of amyloses

Table II. Molar Ratios of Partially Methylated Sugars from Methylated Amyloses and Amylopectins

Source	Starch extraction method	Tetra-*O*-methyl-D-glucose	Tri-*O*-methyl-D-glucose[a]	Di-*O*-methyl-D-glucose
Amylose				
AABB	alkali	1	200 ± 10	Trace
	hot water	1	310 ± 15	Trace
RR	alkali	1	210 ± 10	Trace
	hot water	1	310 ± 15	Trace
AABBRR	alkali	1	230 ± 10	Trace
	hot water	1	340 ± 15	Trace
Potato	alkali	1	250 ± 10	Trace
	hot water	1	360 ± 15	Trace
Amylopectin				
AABB	alkali	10	325 ± 15	9
	hot water	10	330 ± 15	9
RR	alkali	10	320 ± 15	9
	hot water	10	325 ± 15	9
AABBRR	alkali	10	330 ± 15	9
	hot water	10	330 ± 15	9
Potato	alkali	10	330 ± 15	9
	hot water	10	335 ± 15	9

[a] Msd (P 0.5 level) (trimethylglucose from amylose) between species, alkali extraction, 32; hot water extraction, 35; between extraction procedure, 25. Differences in trimethylglucose from amylopectin not statistically significant.

AMYLOSE. 2,3,4,6-Tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose. A trace of what was believed to be 2,3-di-*O*-methyl-D-glucose was also evident (same R_f value of authentic compound). The ratio of the component sugars was determined by use of the phenol-sulfuric acid method (Dubois *et al.*, 1956) using 2,3,6-tri-*O*-methyl-D-glucose as a standard.

AMYLOPECTIN. 2,3,4,6-tetra-*O*-methyl-D-glucose; 2,3,6-tri-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-glucose were identified. The molar ratios are summarized in Table II.

CONFIRMATION OF IDENTITY OF PARTIALLY METHYLATED SUGARS. The identity of the *O*-methyl sugars were further confirmed as follows.

Silylation of *O*-methyl Glucital Mixture. A small portion (about 10 to 20 mg) of mixture in water (10 ml) was reduced with $NaBH_4$ (10 mg). The residue left after evaporation was silylated (TMS, HMDS, pyridine) (Sweeley *et al.*, 1963) and

Table III. Determination of *O*-Methyl-D-Glucitols as the TMS-Derivatives by Gas-Liquid Chromatography

Source	Starch Extraction Method	2,3,4,6-tetra-*O*-Methyl Glucitol %	2,3,6-tri-*O*-Methyl Glucitol %	2,4,6-tri-*O*-Methyl Glucitol %	2,3-di-*O*-Methyl Glucitol %
Amylose					
AABB	alkali	0.5	99	...	trace
	hot water	0.3	99	...	trace
RR	alkali	0.5	99	...	trace
	hot water	0.3	99	...	trace
AABBRR	alkali	0.5	99	...	trace
	hot water	0.3	99	...	trace
Potato	alkali	0.6	99	...	trace
	hot water	0.3	99	...	trace
Amylopectin					
AABB	alkali	3.6	92	0.3	3.4
	hot water	3.6	92	0.3	3.4
RR	alkali	3.6	92	0.4	3.4
	hot water	3.8	91	0.4	3.5
AABBRR	alkali	3.6	92	0.2	3.4
	hot water	3.6	92	0.2	3.4
Potato	alkali	3.6	92	0.1	3.5
	hot water	3.6	92	0.1	3.4

analyzed by vapor phase chromatography (vpc) using a column ($^1/_4$'' × 5') of 20% SE30 on Chromosorb W. Amylose: A major peak corresponding to tri-*O*-methyl, minor peak corresponding to tetra-*O*-methyl, and a trace of di-*O*-methyl glucitol was evident. The compounds were identified by comparison to the retention time of authentic compounds. Amylopectin: Major peak for tri-*O*-methyl glucitol and well defined peaks for tetra- and di-*O*-methyl glucitol were evident and identified by comparison to retention times of authentic compounds. A second tri-*O*-methyl glucitol (minor amount) was also evident and believed to be 2,4,6-trimethyl glucitol (Table III).

Degradation of Methyl Glucitols. The tetra-*O*-methyl glucitol obtained via borohydride reduction of 2,3,4,6-tetra-*O*-methyl glucose, $[\alpha]_D^{20}$ + 80°, H_2O (c, 1.2), was not degraded.

Amylose. Tri-*O*-methyl glucitol (from 2,3,6-tri-*O*-methyl glucose) was oxidized (IO_4^-) followed by reduction (BH_4) and demethylation (reflux 30 min with 40% HBr) gave ethylene glycol, L-threitol, and a very small trace of hexitol which may have its origin in periodate resistant 2,4,6-tri-*O*-methyl glucose. The compounds were identified by vpc analysis of the TMS derivatives. Similar treatment of the trace of dimethyl glucitol gave L-threitol identified as above. Amylopectin: Tri-*O*-methyl glucitol fraction gave ethylene glycol, L-threitol (from 2,3,6-tri-*O*-methyl), and a trace of hexitol believed to have arisen from 2,4,6-tri-*O*-methyl hexitol. Dimethyl (2,3-di-*O*-methyl) glucitol fraction gave L-threitol as expected. Identification was achieved through vpc analysis of TMS derivatives and comparison of retention time to authentic compounds.

Periodate Oxidation of Amyloses and Amylopectins. Small amounts (100 to 200 mg aliquots) of the prepared amyloses and amylopectins were subjected to periodate oxidation according to the method of Abdel-Akher and Smith (1951) (Tables IV and V). The expected 1:1 molar ratio in moles of periodate uptake per mole of glucose was observed for both amyloses and amylopectins. This value is expected for normal amylose and amylopectin. Formic acid produced during the periodate oxidation was determined as described by Shasha and Whistler (1964). The amount of formic acid liberated was used to estimate the number of nonreducing end units in the amylopectins and was also used to approximate the molecular weight of the amyloses (Table V).

The formaldehyde liberated during the periodate oxidation

Table IV. Periodate Degradation Results for Amyloses and Amylopectins from Hot Water and Alkali Extracted Starch

Species	Molar ratio: Glucose	Glycerol	Erythritol[a]: Hot Water extraction	Erythritol[a]: Alkali extraction
Amylose				
AABB	0.18	1	252	190
RR	0.16	1	278	185
AABBRR	0.15	1	270	185
Potato	0.11	1	350	290
Amylopectin				
AABB	0.54	1	13.6	13.7
RR	0.54	1	13.1	13.1
AABBRR	0.62	1	13.2	13.3
Potato	0.43	1	14.1	14.0

[a] Msd (P 0.5 level) for erythritol formed from amylose: between species, hot water extraction, 20; alkali extraction, 15: between extraction procedure, 35. Differences for erythritol from amylopectin not statistically significant at P 0.5 level.

of reduced (sodium borohydride) amylose and amylopectin was determined using chromotropic acid (Lambert and Neish, 1950). The procedure involved dialysis of the oxidation mixture to avoid interference from the polyaldehydes (Unrau and Smith, 1957). Molecular weights estimated from formaldehyde produced are given in Table V.

Smith Periodate Degradation. Smith periodate degradations were carried out as described elsewhere (Hay *et al.*, 1965). Chromatographic separation of degradation products were carried out on Whatman No. 1 and 3MM paper for analytical and preparative purposes, respectively, using the descending technique and solvent systems ethyl acetate : acetic acid : water (8:2:2) and ethyl acetate : pyridine : water (8:3:2). Compounds were detected with *p*-anisidine trichloroacetic acid (Hough *et al.*, 1950), ammoniacal silver nitrate (Partridge, 1946), and periodate-permanganate reagents (Lemieux and Bauer, 1954).

DETERMINATION OF ERYTHRITOL, GLYCEROL AND GLUCOSE IN RESIDUE

Aliquots of the residue were separated by paper (Whatman No. 1) chromatography using ethyl acetate : pyridine : water (8:3:2). The sections corresponding to erythritol and

Table V. Molecular Weights of Amyloses and Amylopectins

	HCOOH Titration[a]		H_2CO Formation[b]		Methylation[c]		Ultracentrifuge[d]	
	Alk.	Hw	Alk.	Hw	Alk.	Hw	Alk.	H
Amylose	($\times 10^4$)	($\times 10^4$)	($\times 10^4$)	($\times 10^4$)	($\times 10^4$)	($\times 10^4$)	($\times 10^4$)	($\times 10^4$)
AABB	2.54	3.12	3.41	3.85	3.24	5.20	3.95	5.65
RR	2.42	2.95	3.23	3.75	3.41	5.20	3.90	5.70
AABBRR	2.51	3.15	3.90	4.31	3.72	5.52	3.95	5.95
Potato	2.75	3.60	3.82	4.35	3.90	5.60	4.35	6.05
Msd (P 0.5)	0.12	0.22	0.14	0.23	0.15	0.18	0.13	0.18
Amylopectin			($\times 10^5$)	($\times 10^5$)			($\times 10^5$)	($\times 10^5$)
AABB	...	...	1.3	1.9	...	...	1.8	2.4
RR	...	...	1.1	1.4	...	...	1.5	1.9
AABBRR	...	...	1.3	1.9	...	...	1.9	2.5
Potato	...	...	1.8	2.6	...	...	...	...
Msd (P 0.5)			0.2	0.3			0.2	0.4

[a] Msd (P 0.5) between extraction procedure, 0.25. [b] Msd (P 0.5) between extraction procedure, amylose, 0.21; amylopectin, 0.3. [c] Msd (P 0.5) between extraction procedure, 0.28. [d] Msd (P 0.5) between extraction procedure, amylose, 0.40; amylopectin, 0.3.

Table VI. Paper Chromatographic Analysis of α-Amylolysis Products (Starch Extracted with Alkali-$NaBH_4$)

Sample	Oligosaccharides	G_6 R_G = 0.125	G_3 R_G = 0.266	G_2 R_G = 0.51	G_1 R_G = 1.00
Amyloses					
AABB	6.2	9.1	42.1	33.3	9.1
RR	6.8	12.2	46.1	26.8	8.1
AABBRR	6.0	11.6	44.6	28.8	9.1
Potato	4.3	8.2	41.3	38.5	7.8
MSD	1.2	1.6	dns	3.1	dns
Amylopectins					
AABB	27.7	5.8	29.5	27.3	9.9
RR	27.6	5.9	30.9	25.7	9.9
AABBRR	28.8	5.9	30.0	24.7	10.6
Potato	27.5	5.3	26.5	31.5	8.9
MSD	dns	dns	3.2	3.5	dns
Starches					
AABB	29.6[a]		33.8	27.5	9.1
RR	26.2		28.8	32.6	12.4
AABBRR	27.8		31.5	29.4	11.5
Potato	26.1		27.5	35.6	10.3
MSD	dns		dns	3.7	dns

[a] This section includes oligosaccharide and G_6, R_G = 0.0–0.173.

glycerol were excised and eluted with distilled water. The relative amount of each was determined by the periodate-chromotropic acid method (Lambert and Neish, 1950). Since glucose occurred only in trace amounts, glucose standard was applied as the marker, and the corresponding location was eluted with water. The amount of glucose present was determined by the phenol-sulfuric acid method. Molar ratios of glucose : glycerol : erythritol after mild and complete acid hydrolysis were essentially the same (Table IV).

IDENTIFICATION OF COMPONENTS

Whatman No. 3MM paper was used to resolve larger quantities of the residue in ethyl acetate : acetic acid : water (8:2:2). Glycerol: To the dry syrup was added a 10% molar excess of *p*-nitrobenzoyl chloride and pyridine (3 ml), and the mixture was heated for 1 hr. Excess aroyl halide was decomposed by the addition of a few drops of water, followed by addition of an excess of saturated sodium bicarbonate. After storing overnight, the solid was collected by filtration, washed with water, and recrystallized from acetone. The tri-*p*-nitrobenzoate of glycerol had melting point and mixed melting point 187–190° C.

Erythritol was converted to the tetra-*p*-nitrobenzoate as described for glycerol, melting point, and mixed melting point 248–250° C.

ENZYMIC DEGRADATION: α-AMYLASE DEGRADATION

α-Amylase from *Bacillus subtilus* (Nutritional Biochemicals) was used. The method was that of Fisher and Stein (1961) as modified by Whelan (1964). It basically consists of incubation of a starch and enzyme mixture, followed by measurement of reducing sugars with alkaline sodium 3,5-dinitrosalicylate reagent. The activity of the enzyme was 8.76 units per mg of protein.

The original polysaccharide concentration in the incubation mixtures was determined using the phenol-sulfuric acid method (Dubois *et al.*, 1956) using glucose as the standard, and the results were interpreted as total glucose content. A constant reducing value was obtained after three or four reincubations, after which time the enzyme was still active, since a sample of the final solution when added to the control solution (substrate with deactivated enzyme) hydrolyzed the starch within 2 hr, as observed by a change in the iodine absorption color. The hydrolyzate, after heat of inactivation of the enzyme, was deionized (Amberlite IR 120 and Duolite A4) and the residue left after evaporation analyzed by descending paper chromatography using Whatman No. 1 paper, ethyl acetate : acetic acid : water (8:2:2) as solvent and 36-hr development time. The reducing sugars were located using the *p*-anisidine trichloroacetic acid spray reagent (Hough *et al.*, 1950). Glucose, maltose, maltotriose, maltohexaose, and oligosaccharides were found to be present, and the relative amounts were determined by the phenol-sulfuric acid method (Dubois *et al.*, 1956) after elution of these from the paper with water (Table VI).

HYDROLYSIS OF STARCHES, AMYLOSES, AND AMYLOPECTINS WITH β-AMYLASE

β-amylolysis of starch, amylose, and amylopectin was carried out using a standard procedure (Whelan, 1964). The amount of β-limit dextrin present was determined using the phenol-sulfuric acid method, after paper chromatographic (Whatman No. 1) separation of the hydrolysis mixture (limit dextrin remaining at the origin (Table VII)).

DISCUSSION

Isolation and Purity of Amylose and Amylopectin. Although Bourne *et al.* (1948) reported that the cyclohexanol-

Table VII. Formation of Limit Dextrins from β-Amylolysis of Amylose, Amylopectin and Starch

	Extraction Procedure	Percent Conversion to Maltose[a] AABB	RR	AABBRR	Potato
Amylose	alkali	99+	99+	99+	99+
	hot water	99+	99+	99+	99+
Amylo-pectin	alkali	62	61	61	60
	hot water	57	57	56	54
Starch	alkali	64	66	68	64
	hot water	60	61	63	58

[a] Differences between species and extraction procedures not statistically significant at P 0.5 level.

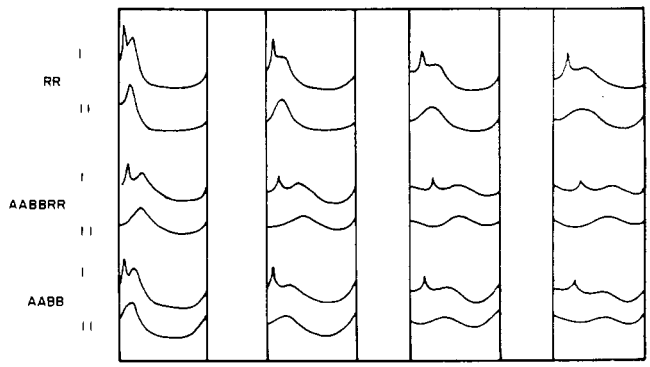

Figure 2. Ultracentrifugation patterns of amylopectin fractions

amylose complex contained a higher proportion of amylopectin than the thymol-amylose complex, this is believed to be unlikely in the investigation reported here because of the repeated fractionations that were employed. This is supported by Sarko *et al.* (1964) who reported that, by using the cyclohexanol fractionation method, 99% purity was easily obtained by two fractionations, while a third fractionation gave essentially pure amylose (Figure 1). Amylopectin free from amylose was relatively difficult to prepare. Only after repeated cellulose adsorption chromatography in conjunction with three to four additional treatments with cyclohexanol could pure amylopectin be obtained (Figure 2).

Different plant species and even different varieties within a species exhibit specific amylose—amylopectin ratios. The present investigation showed that durum (AABB) starch contained 25 to 26% amylose, rye (RR) starch 25 to 26% amylose, and Triticale (AABBRR) starch 26 to 27% amylose. These values are consistent with the reported values for wheat starch (Greenwood, 1964); hence no difference in the overall gene action is apparent.

Analyses of variance between species and between extraction procedure (Table I) indicated statistically significant differences in nitrogen content. Such differences would be expected between extraction procedures in which the alkali-$NaBH_4$ extraction has long been known to hydrolyze protein, and the method has been routinely used in glycogen extraction from liver tissue. The lower nitrogen content of potato starch is not unexpected. The higher blue value for potato starch and amylose is probably a reflection of higher molecular weight and greater regularity in helical structure of the amylose.

Molecular Weight and Structural Features of Isolated Amyloses and Amylopectins. The values from formic acid titrations and formaldehyde production upon periodate oxidation of borohydride reduced amyloses were in relatively good agreement. The amyloses from the three cereal species possessed very similar molecular weights (Table V) and in a similar range, as observed by Wolfrom and Khadem (1965), and Foster (1965). Values for degree of polymerization (d.p.) (wheat amylose) and 1000 and 1500 (potato amylose) have been reported by Greenwood (1956) and Banks and Greenwood (1968), using physical and enzymatic methods. As is evident in a general comparison of the molecular weights of amyloses isolated from starch which were extracted in two different procedures, a very low incidence of bond breakage could result in a drastic reduction in d.p. (and molecular weight). It is unlikely that such a process can be random, since a wide range of molecular sizes should then result and this would be detected as a broad peak upon ultracentrifugation. It is notable that the amyloses isolated from hot water extracted starch (Figure 1) sediment as a sharp peak, as did amylose samples from alkali extracted starch (not shown). In the latter case, a significant reduction in molecular weight was observed.

In the case of the amylopectins, the degree of branching (Table IV) and molecular weight (Table V) of the three species are again essentially the same. The degree of branching, 19 to 21 units, was consistent with the value reported by Greenwood (1964) for wheat amylopectin and the molecular weights were within the range reported for amylopectins prepared under ordinary conditions. As was the case for amyloses, amylopectin obtained from starch which had been extracted with alkali possessed a lower molecular weight.

Periodate Degradation and Methylation Studies. The molar ratio of erythritol to glycerol may be used as a measure of the d.p. of amylose, since glycerol could be derived only from the nonreducing end unit of the chain while erythritol arises from the internal chain units. The sum of the molar ratio of these components represents an estimate of the d.p. of the molecule from which the molecular weight can easily be determined. The values obtained (270 to 280) from amylose obtained from hot water extracted starch agree reasonably well with those obtained using other methods. The use of alkali significantly reduced the molecular size (Table V). In the case of amylopectin, this ratio constitutes the basis for the determination of the ratio of nonterminal to terminal nonreducing units within the molecule, and a value of 14 to 15 was obtained as compared to the value of 19 to 21 obtained from formic acid titration. These values furnish an estimate of the length of the repeating unit in branched polysaccharides.

The ratios of 2,3,4,6-tetra-*O*-methyl to 2,3,6-tri-*O*-methyl-D-glucose in the amyloses from the cereal species were quite comparable, and the d.p. reflected in these values were in reasonable agreement with those obtained by other methods. Degradation due to alkali extraction of starch was again rather evident (Table II). Ultracentrifuge sedimentation gave consistently higher molecular weight values. Methylation analysis also indicated in all three samples a low incidence of branched amylose-like molecules, as shown by the small amount of 2,3-di-*O*-methyl-D-glucose. This is consistent with the results from α-amylolysis. The ratios of *O*-methyl glucoses obtained from fully methylated amylopectins were similar and in good agreement with values for repeating unit length obtained by other methods (*e.g.*, formic acid titrations in periodate oxidation). As would be expected, a slight depolymerization due to alkali extraction of starch is not reflected in the value for repeating unit length.

α-Amylolysis of Starch and Its Components. Although 100% conversions to maltose have been claimed for α-amylol-

ysis of amyloses, paper chromatographic studies indicated the presence of oligosaccharides of greater size than maltose. A similar result has been reported by Whelan (1964) using barley-malt amylase. Structural irregularities which are now known to occur in amyloses could prevent complete hydrolysis.

Some features observed in the α-amylolytic hydrolyses indicated the presence, as mentioned above, of linkage irregularities. The percentages of conversion to maltose of amyloses, amylopectins, and starches were essentially identical for the three cereal species. The kinetics of hydrolysis as well as the distribution of α-limit dextrins were also essentially the same. Potato amylose and amylopectin used as reference material differed considerably from these species. The results indicated that the polymeric materials obtained from the cereal species were structurally very similar.

β-Amylolysis of Starch and Its Components. The general properties and action pattern of β-amylase have been discussed in detail by French (1960) and Manners (1962). In the present investigation, amylose was degraded completely by β-amylase, amylopectin 60 to 61%, and starch 64 to 68%. A small difference in the values for whole starch may have been due to the presence of linear or essentially linear low molecular weight "amylose" type molecules which were not present in the amylose fraction because of the isolation procedures employed.

β-Amylolysis of amylose present a somewhat complicated picture. Various values ranging from 70% upwards have been reported (Peat *et al.*, 1952). It is now known that abnormal linkages that are resistant to β-amylolytic attack exist (Cowie *et al.*, 1958). Individual amyloses appear to differ in both d.p. and the β-amylolysis limit indicated that variation exists in the relative proportion and distribution of barrier to β-amylolysis (Cowie *et al.*, 1957; Geddes *et al.*, 1957).

The β-Amylolysis limit of amylopectin was consistent with the values reported by various workers (Manners and Wright, 1962; Peat *et al.*, 1952). The amounts of β-limit dextrin isolated from amylopectin and starch of the three cereal species are in reasonable agreement. The β-amylolysis of starch was only slightly greater than that of amylopectin. If starch is composed only of "linear" amylose and amylopectin, the β-amylolysis would be expected to be much higher. This observation has been attributed to the existence of a third intermediate type polysaccharide (5 to 10) in some starch, notably in potato, rubber seed, and wheat (Greenwood, 1964; Perlin, 1958).

Within the limits of experimental procedures employed, the starch components from the parental and the synthetic (alien genome combinant) species do not appear to possess obviously significant structural differences. This does not preclude the possibility of subtle modifications in the fine structure of the amylose and amylopectin of the synthetic species which may be difficult to detect by experimental methods employed. Although in some instances statistical significance in differences has been shown (see Tables for minimum significant differences at P 0.5 level), it is difficult to attach undue biological significance to these observations at the present time. Significant differences between extraction procedures are not surprising, and these are only incidental to the original objective of this investigation. In the same sense, statistically significant differences between measured characteristics of potato amylose and amylopectin *vs.* these same components of starch from the cereal species, including the alien genome combinant, are also not surprising.

The synthetic species has, under field conditions, been shown to be capable of producing higher yields of grain, particularly under adverse conditions, than either of the parent species. The rheological properties of dough mixes from flour from the rye-durum wheat combinant definitely appear to be intermediate when compared to those from the parent species (Unrau and Jenkins, 1964). A considerable amount of the AABBRR flour can be blended with very strong flours from certain spring wheat varieties. It might be noted here that the spring wheat–rye genome combinant (AABBDDRR) quite strongly exhibits the effect of the spring wheat genome (AABBDD) (Unrau and Jenkins, 1964), and morphologically more closely resembles common spring wheat than is the case for the genome combinant (AABBRR) concerned in this investigation. How broad the spectrum of utility of AABBRR may become will of course depend on general acceptability of this source of cereal carbohydrate and protein and the ultimate specific uses that it may advantageously serve.

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