42. *The Oligosaccharides Synthesised from Maltose by* Escherichia coli.

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Washed cells of *E. coli* (Monod strain) have been incubated with maltose in the presence of iodoacetate, and the extra-cellular saccharides have been fractionated on a charcoal column. The fractions have been subjected to paper-chromatographic analysis, methylation and end-group assay, and oxidation with both hypoiodite and periodate. In addition, the molecular weights of their acetates have been determined. By these methods the saccharides have been shown to include glucose, maltose, and the lower members of the homologous series of glucose polymers containing the 1 : 4-α-glucosidic linkage.

In 1924, Grey (*Biochem. J.*, **18**, 712) reported that a strain of coliform bacteria, when incubated in a glucose medium, without aeration and in the presence of calcium carbonate to neutralise acids as produced, synthesised a starch-type polysaccharide, which could be extracted with aqueous oxalic acid. More recently, Monod and Torriani (*Compt. rend.*, 1948, **227**, 240) isolated from a strain of *Escherichia coli*, which was both maltose- and lactose-positive (Monod, Torriani, and Gribetz, *ibid.*, p. 315), an enzyme fraction which, under suitable conditions, catalysed the synthesis of starch-type products from maltose but not from glucose-1 phosphate or sucrose. It was clear that the enzyme system responsible for this synthesis did not include phosphorylase, the amylose-synthesising enzyme which is commonly found in plant and animal tissues and uses the Cori ester as its substrate. Neither was the active agent amylosucrase, an enzyme which has been shown by Hehre and his colleagues (*J. Bact.*, 1948, **55**, 197; *J. Biol. Chem.*, 1949, **177**, 267) to occur in *Neisseria perflava* and to catalyse the synthesis of a starch-type polysaccharide from sucrose. The French workers furnished evidence that the higher saccharides were in fact formed directly from maltose; the enzyme responsible was named by them "amylomaltase" and was shown to catalyse the reaction, n maltose $\rightleftharpoons$ n glucose + (glucose)$_n$. Thus the new enzyme, like phosphorylase, amylosucrase, and other enzymes responsible for the synthesis of polysaccharides, was a transglycosidase. The average degree of polymerisation of the mixture of glucose polymers resulting from amylomaltase action is normally quite small, but it has been demonstrated by Monod and Torriani (*Ann. Inst. Pasteur*, 1950, **78**, 65) that products of sufficiently high molecular weight to give a blue stain with iodine are produced when the glucose formed in the synthesis is continuously removed by means of glucose oxidase. Another contribution towards the elucidation of this intriguing topic was made in 1949 by Doudoroff, Hassid, Putman, Potter, and Lederberg (*J. Biol. Chem.*, 1949, **179**, 921), who reported a study of the metabolism of maltose by a suspension of a mutant of *E. coli* (strain W-327), which, in contrast to Monod's strain, was incapable of utilising either lactose or glucose. They fractionated the saccharides formed by resting cells of the organism, in the presence of iodoacetate, on the basis of their relative solubilities in alcohol, after having first removed glucose and maltose by fermentation and inorganic materials by ion-exchange resins. The fractions thus obtained consisted of a series of dextrins, possessing 4—6 glucose units per molecule.

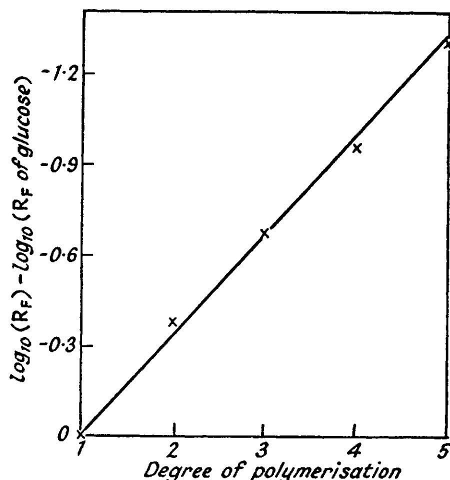
For some time we have been engaged, with our colleagues, in a study of Monod and Torriani's work. As part of that programme, we have examined the metabolism of maltose by *E. coli* (Monod strain) and have obtained results, reported herein, which are fully in accord with those obtained by Hassid and his co-workers, who used a different strain of the organism.

Washed cells of *E. coli* (Monod strain), which had been grown on a synthetic medium containing maltose as the sole source of carbon, were incubated with maltose in the presence of sodium hydrogen carbonate and sodium iodoacetate, under conditions similar to those employed by Hassid *et al.* (*loc. cit.*). After removal of the cells in the centrifuge, the supernatant liquid was fractionated by a purely chemical process, namely, by Whistler and Durso's method (*J. Amer. Chem. Soc.*, 1950, **72**, 677), later extended by Bailey, Whelan,

and Peat (*J.*, 1950, 3692), which involved passage of the solution through a charcoal column and subsequent elution of the column with aqueous solutions containing increasing concentrations of ethanol. In this way five fractions (A—E) were obtained (see Tables 1 and 4), the first as an aqueous solution and the rest as freeze-dried powders; they were not stained by iodine. In a second experiment, the fractions corresponding to C, D, and E were united and designated fraction F. As was expected, the amounts of inorganic material in the various eluates, as revealed by ash contents, decreased progressively with increasing concentration of ethanol (cf. Table 2).

Paper-chromatographic analysis of hydrolysates of the various fractions revealed, in each case, a single aldose component, having a R_F value identical with that of a reference glucose spot, and so it was very probable that each fraction was either glucose or an acid-labile derivative thereof. During paper-chromatographic analysis of the fractions themselves, five discrete spots, detectable by aniline hydrogen phthalate, were encountered; the distances moved by these spots (see Table 1), coupled with the corresponding figure for maltose (0·42), render it likely that the five spots represented glucose and the four lowest members of the homologous series of 1 : 4-α-linked glucose polymers, namely, maltose, maltotriose, maltotetraose, and maltopentaose. In conformity with this supposition, the plot of log (R_F) against degree of polymerisation was linear (see figure), as was the case also with a homologous series of dextrins obtained by Jeanes, Wise, and Dimler (*Analyt. Chem.*, 1951, **23**, 415) by α-amylolysis of amylose. Other slower-moving saccharides could not be isolated as discrete spots.

Paper chromatography of fractions A—E.



From the mode of its isolation, fraction A should have contained no sugars other than monosaccharides (cf. Whistler and Durso, *loc. cit.*); paper chromatography revealed glucose to be the only constituent, and optical-activity measurements indicated that this represented about 29% of the glucose units present initially in the maltose substrate. Fraction B should have consisted of disaccharide components and paper chromatography detected one such constituent, namely, maltose; this fraction had $[\alpha]_D$ (equil.) 100°, but, since its conversion into glucose by acid was only 76·5%, the true specific rotation of the carbohydrate moiety was 131°; the accepted value for maltose is 137°. Fraction C contained one major constituent, probably maltotriose, together with traces of glucose, maltose, and maltotetraose; this fraction gave a 95·1% conversion into glucose and had $[\alpha]_D$ (equil.) 151°, which corresponded to 159° for the carbohydrate portion; values of $[\alpha]_D$ reported for maltotriose are 163° (Blom and Rosted, *Acta Chem. Scand.*, 1947, **1**, 233), 158° (French, Levine, Pazur, and Norberg, *J. Amer. Chem. Soc.*, 1950, **72**, 1746), 160° (Sugihara and Wolfrom, *J. Amer. Chem. Soc.*, 1949, **71**, 3357), and 167° (Bailey, Whelan,

TABLE 1. *Paper chromatography of the oligosaccharide fractions.*

Fraction	Ethanol (%) in aqueous ethanol used for elution	R_F value of sugar component / R_F value of glucose: Main components	Trace components
A	0	1·00	—
B	5	0·42	—
C	15	0·21	0·11, 0·42, 1·00
D	25	0·05, 0·11	1·00
E	35	0·00—0·05	1·00
F	15, 25, 35	0·05, 0·11, 0·21	—

and Peat, *loc. cit.*). Fraction D appeared to be a mixture of maltotetraose and maltopentaose, whilst fraction E contained mainly maltopentaose and higher dextrins; in each of these two fractions there was a trace of glucose.

Confirmatory evidence regarding the nature of the oligosaccharide fractions under examination was obtained by methylation and end-group assay, by oxidation with hypoiodite and periodate, and by measurement of the molecular weights of their acetates.

TABLE 2. *Purities of the oligosaccharide fractions.*

Fraction	% of glucose units in substrate appearing in product *	Ash (%)	$[\alpha]_D^{20}$ in water (equil.)	Converion (%) into glucose by acid
B	24·4	6·4	100°	76·5
C	16·9 } 34·5	2·6	151	95·1
D	14·1 } 34·5	1·5	171	93·6
E	3·5 } 34·5	3·1	169	91·2
F	36·6	0·9	170	91·1

* Based on the conversion (%) into glucose by acid.

Methylation and end-group assay was conducted on fraction F (equivalent to C + D + E), which was already believed to be essentially a mixture of malto-triose, -tetraose, and -pentaose. This was treated with sodium and methyl iodide in liquid ammonia at —70° (cf. Freudenberg and Boppel, *Ber.*, 1938, **71**, 2505) until the methoxyl content of the product attained a constant value (49·8%). Thereafter, hydrolysates of the methyl ether were analysed, qualitatively and quantitatively, by filter-paper chromatography. An aniline hydrogen phthalate spray revealed two aldose components, which had R_F values identical with those of 2 : 3 : 6-trimethyl and 2 : 3 : 4 : 6-tetramethyl glucose, used as standards on the same chromatogram. There was no trace of a dimethyl glucose constituent, thus proving that the oligosaccharides contained in fraction F were virtually unbranched. The average chain length, determined by a method similar to that described by Flood, Hirst, and Jones (*J.*, 1948, 1679), was about 3·7.

The molecular weights of fractions C, D, and E were determined by oxidation with hypoiodite under the rigid conditions specified by Hirst, Hough, and Jones (*J.*, 1949, 928). On the assumption that the oxidation was quantitative, the degrees of polymerisation were calculated to be 2·8, 4·0, and 5·1, respectively. There are strong grounds for the belief that this assumption was valid, first, because, in each case, there was a linear relation between the weight of oligosaccharide taken and the quantity of hypoiodite consumed (Table 5), and, secondly, because it is known that the oxidation proceeds quantitatively with glucose, methyl ethers of glucose (Hirst, Hough, and Jones, *loc. cit.*; Barker, Bourne, and Wilkinson, *J.*, 1950, 3027), maltose (Bailey, Whelan, and Peat, *loc. cit.*; Barker and Bourne, unpublished results), and maltotriose (French *et al.*, *loc. cit.*). Moreover, Levine, Foster, and Hixon (*J. Amer. Chem. Soc.*, 1942, **64**, 2331) found that the chain lengths of starch dextrins determined by oxidation with hypoiodite agreed with those calculated from the potassium contents of salts of the dextrinic acids produced in the oxidation. Hassid *et al.* (*loc. cit.*) recorded agreement between the chain length of their "fraction 2," determined by hypoiodite oxidation, and the molecular weight of its acetate, measured by a modification of Rast's method; a discrepancy between the two corresponding values for their "fraction 3" was attributed by them to decomposition during the cryoscopic measurements. We have ourselves obtained excellent agreement between the results of the hypoiodite oxidations of fractions C and D and the molecular weights of the acetates of these fractions, determined by Barger's method (see Table 3); there was insufficient of fraction E to permit the isolation of an acetate. Attempts to assess the chain lengths of the oligosaccharide fractions by determining the formic acid produced

TABLE 3. *Molecular sizes of the oligosaccharide fractions.*

Method of measurement	Degree of polymerisation			
	Fraction C	Fraction D	Fraction E	Fraction F
Methylation and end-group assay	—	—	—	3·7
Hypoiodite oxidation	2·8	4·0	5·1	—
Barger's method on acetate	2·8	4·1	—	—

during treatment with periodate (cf. Brown, Dunstan, Halsall, Hirst, and Jones, *Nature*, 1945, **156**, 785) gave results some 25% higher than those of the methods already mentioned. This discrepancy is not regarded as significant because certain factors encountered during periodate oxidations of short-chain dextrins militate against accuracy; for example, it is difficult to choose the correct end-point for the final titration with alkali (cf. Meyer and Rathgeb, *Helv. Chim. Acta*, 1949, **32**, 1102), over-oxidation of reducing residues must be avoided (cf. Hassid and Potter, *J. Amer. Chem. Soc.*, 1948, **70**, 3488), and destruction of formic acid occurs if the pH falls too low (cf. Meyer and Rathgeb, *loc. cit.*).

The behaviour of fractions C, D, and E towards soya-bean β-amylase was in accordance with the foregoing conclusions (see Table 7), and with the observation of other workers that maltotriose is attacked only very slowly by this enzyme (cf. Bailey, Whelan, and Peat, *loc. cit.*; French *et al.*, *loc. cit.*). Fraction C, known to be essentially maltotriose, showed an increase in reducing power of only 3% in 17·5 hours and 14% in 41·5 hours. The corresponding figures for fraction D were 97 and 105%, and for fraction E 199 and 228%. Thus, in the last two cases there was an initial rapid reaction, followed by a relatively slow one; paper-chromatographic analysis revealed that, at the end of the first stage, the products were maltose and maltotriose, the latter being responsible for the subsequent slow reaction.

In conclusion, it has thus been shown that incubation of a washed culture of *E. coli* with maltose in the presence of iodoacetate afforded, in the extracellular solution, a mixture of glucose, unchanged maltose, and higher homologues of maltose. Of the glucose units present initially in the substrate approximately 29% were converted into free glucose, 24% remained as maltose, and 35% were converted into higher saccharides. An examination is now being made of a polysaccharide which is stored intracellularly by cultures of *E. coli* (Monod strain) when grown on a synthetic medium containing maltose; details of this work will be published later.

Experimental

Isolation of Escherichia coli.—*E. coli* (Monod strain) was grown at 35° for 22 hours in an aerated synthetic medium (6·5 l.), containing maltose as the sole carbon source, as described by Monod and Torriani (*loc. cit.*). The cells were collected in a Sharples continuous centrifuge and washed by the passage of distilled water (5 l.). The bacterial sludge was freeze-dried to a yellow powder (4·94 g.), which was stored at 1° until required.

Isolation of the Oligosaccharides synthesised from Maltose by E. coli.—A digest (40 c.c.), containing maltose hydrate (2·888 g.), freeze-dried *E. coli* (1·20 g.), sodium hydrogen carbonate (3·36 g.), and sodium iodoacetate (0·0166 g.), was incubated at 32° for 3 hours, during which time a stream of carbon dioxide was bubbled through the suspension. Excess of trichloroacetic acid was added, and the precipitate, collected by the centrifuge, was washed with 6% trichloroacetic acid solution (20 c.c.). The supernatant liquid and washings were combined and fractionated on a charcoal column (17 × 3·4 cm.) by Whistler and Durso's method (*loc. cit.*). The column was washed as shown in Table 4. Each of the eluates, with the exception of the first, was concentrated to a small volume (*ca.* 25 c.c.), filtered, and freeze-dried to a white solid, which was subsequently dried to constant weight at 60° in a vacuum over phosphoric oxide.

Table 4. *Yields of the oligosaccharide fractions.*

Eluant	Oligosaccharide product Fraction	g.	Eluant	Oligosaccharide product Fraction	g.
Water, 800 c.c.	A	—	25% Ethanol, 700 c.c. ...	D	0·402
5% Ethanol, 1400 c.c. ...	B	0·874	35% Ethanol, 400 c.c. ...	E	0·103
15% Ethanol, 900 c.c. ...	C	0·479			

In a duplicate experiment, in which maltose hydrate (2·908 g.) was employed, the fractions corresponding to fractions C, D, and E were united. The product (1·081 g.) was designated fraction F.

Examination of the Oligosaccharides.—(a) *Ash content.* The oligosaccharide (20 mg.) was heated in a micro-muffle until there was no further change in weight.

(b) *Optical activity.* The specific rotations were determined in aqueous solution (*c*, 0·5—1·0).

(c) *Paper-chromatographic analysis.* Spots of the de-ionised aqueous eluate, and of solutions (2%) of fractions B—F, together with reference spots of solutions (2%) of maltose and glucose,

were applied to a sheet of filter paper (Whatman No. 1), which was then irrigated for 66 hours with the upper phase of a mixture of *n*-butanol (40%), ethanol (10%), water (49%), and ammonia (1%). The sheet was dried and stained with aniline hydrogen phthalate, as described by Partridge (*Nature*, 1949, **164**, 443).

(d) *Reducing sugars liberated during acidic hydrolysis.* (i) Identification. The oligosaccharide (10 mg.) was heated at 100° for 5 hours with 2N-sulphuric acid (0·50 c.c.) in a sealed tube. The solution was diluted with water (2·00 c.c.) and freed from ions by means of an apparatus similar to that described by Consden, Gordon, and Martin (*Biochem. J.*, 1947, **41**, 590). The hydrolysate, together with a reference solution of glucose (2%), was submitted to paper-chromatographic analysis, as described above. The aniline hydrogen phthalate spray revealed only one component in each of the hydrolysates derived from fractions B—F; this component had an R_F value identical with that of the glucose reference spot. A second chromatogram, which had been irrigated for only 24 hours, failed to show any faster-moving component.

(ii) Determination of glucose. A sample (13—20 mg.) of the oligosaccharide was hydrolysed for 3 hours with 7% sulphuric acid at 100°, and the glucose produced was determined by cuprimetric titration, as described by Bourne, Donnison, Haworth, and Peat (*J.*, 1948, 1687). In the calculations of the percentage conversions into glucose, fractions B, C, D, and E were assumed to be di-, tri-, tetra-, and penta-saccharides, respectively. A small correction (1·5%) was made for the loss in reducing power which occurs when glucose itself is treated with acid under these conditions (Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224).

Methylation and End-group Assay of Fraction F.—(a) *Methylation.* Fraction F (0·942 g.) was treated with sodium and methyl iodide in liquid ammonia at −70°, according to the general method of Freudenberg and Boppel (*loc. cit.*). After four additions of the methylating reagents had been made, the ammonia was allowed to evaporate, with exclusion of moisture; the last traces of ammonia were removed at 55°/15 mm. Extraction of the residue with dry ether (Soxhlet) and evaporation of the extract afforded a glass (0·794 g.), having OMe 47·1%, and $[\alpha]_D^{17}$ +166° (*c*, 0·71 in chloroform). Part (0·721 g.) of this material was treated four more times with the methylating agents; the product, isolated as before, was again a glass (0·557 g.), having OMe 49·8%, and $[\alpha]_D^{17}$ +169° (*c*, 0·30 in chloroform). Further treatments with sodium and methyl iodide did not increase the methoxyl content.

(b) *Hydrolysis and end-group assay.* The hydrolysate was prepared and analysed by a method which was essentially that used by Flood, Hirst, and Jones (*loc. cit.*) and later by Hirst, Hough, and Jones (*loc. cit.*). Minor modifications introduced by Barker, Bourne, and Wilkinson (*loc. cit.*) were also incorporated in the method. Chromatography of the hydrolysate, as described above, showed two spots only; these had R_F values identical with those of 2 : 3 : 6-trimethyl and 2 : 3 : 4 : 6-tetramethyl glucose, used as standards on the same chromatogram. As a result of several independent quantitative determinations, based on oxidation with hypoiodite, it was calculated that the molecular composition of the mixture was trimethyl glucose 72·7 and tetramethyl glucose 27·3%; these values correspond to an average chain length of 3·7 glucose units for fraction F.

In control experiments, glucose and maltose hydrate were methylated with sodium and methyl iodide, and gave syrupy products, having OMe 60·9 and 53·7%, respectively. These products were subjected to methanolysis, hydrolysis, and paper-chromatographic analysis, as described above. The chromatogram obtained in the case of glucose showed an intense spot with the same R_F value as 2 : 3 : 4 : 6-tetramethyl glucose, and a very faint spot, which was probably due to a trimethyl glucose. The chromatogram derived from maltose showed two intense spots, corresponding in position to 2 : 3 : 6-trimethyl and 2 : 3 : 4 : 6-tetramethyl glucose, respectively.

Oxidation of Fractions C, D, *and* E *with Hypoiodite.*—A series of solutions of these fractions was oxidised with iodine in alkaline solution, and the excess of iodine was determined by sodium thiosulphate, as described by Hirst, Hough, and Jones (*loc. cit.*). The results quoted in Table 5 show that there was a linear relation in each case between the amount of oligosaccharide employed and the amount of iodine consumed. The chain lengths recorded in Table 5 were calculated on the assumption that the oxidation was stoicheiometric, and on the basis of the conversions into glucose shown in Table 2.

Acetylation of Fractions C *and* D.—In the standard method the saccharide (*ca.* 100 mg.) was suspended in a mixture of acetic anhydride (2·0 c.c.) and pyridine (2·0 c.c.) and left at room-temperature for 48 hours. Ice-water (50 c.c.) was added and, after 24 hours, the precipitate was collected by filtration. It was washed with water (100 c.c.) and dried at 60° in

TABLE 5. *Oxidation of fractions* C, D, *and* E *with hypoiodite.*

Weight (mg.)	Equiv. vol. (c.c.) of 0·01-N-$Na_2S_2O_3$	Chain length	Weight (mg.)	Equiv. vol. (c.c.) of 0·01N-$Na_2S_2O_3$	Chain length	Weight (mg.)	Equiv. vol. (c.c.) of 0·01N-$Na_2S_2O_3$	Chain length
Fraction C			*Fraction* D			*Fraction* E		
1·589	0·63	2·85	2·423	0·70	3·89	2·123	0·49	4·76
3·178	1·30	2·76	4·846	1·34	4·07	3·539	0·78	4·99
3·989	1·64	2·74	6·058	1·68	4·05	5·662	1·17	5·34
4·767	1·98	2·71	7·269	1·99	4·11	8·493	1·80	5·20
	Mean	2·77		Mean	4·03		Mean	5·07

a vacuum over phosphoric oxide. In order to avoid fractionation of the product, no attempt was made to crystallise it.

In a control experiment, maltose hydrate (110·1 mg.) gave a product (172 mg., 83%), $[\alpha]_D^{17}$ +57·3° (*c*, 0·70 in chloroform) (Found : Ac, 50·4. Calc. for $C_{28}H_{38}O_{19}$: Ac, 50·7%). β-Maltose octa-acetate has $[\alpha]_D^{20}$ +62·6° (in chloroform).

Acetylation of fraction C (98·5 mg.) gave a product (135 mg.), $[\alpha]_D^{17}$ +106·8° (*c*, 0·46 in chloroform) (Found : Ac, 49·4. Calc. for $C_{40}H_{54}O_{27}$: Ac, 49·0%).

In the same way, fraction D (99·4 mg.) afforded an acetate (157 mg.), $[\alpha]_D^{17}$ +127·7° (*c*, 0·71 in chloroform) (Found : Ac, 48·6. Calc. for $C_{52}H_{70}O_{35}$: Ac, 48·0%).

Molecular weights. The molecular weights of the acetylated oligosaccharides were determined by Barger's method (*J.*, 1904, 286), β-glucose penta-acetate being employed as the standard substance and ethyl acetate as the solvent. Alternate drops (5—7 mm. in length) of the standard solution and of a solution of the acetylated oligosaccharide were drawn into a capillary tube (length, 13 cm.; diameter, 1—2 mm.), and the extremities of the tube were sealed in a small flame. The lengths of the five centre drops were measured, with the aid of a travelling microscope, after 1 hour and again after 17 hours. For each acetate, the experiment was repeated several times, with glucose penta-acetate solutions of different concentrations. The isopiestic condition was determined by plotting the concentrations of the standard solutions against the algebraic difference of Δl (unknown) and Δl (known), where Δl was the summation of any increase or decrease in length occurring during the interval between the measure-

TABLE 6. *Molecular weights of the acetates of fractions* C *and* D.

Concn. of acetate (% by wt.)	Concn. of glucose penta-acetate (% by wt.)	Δl (unknown)	Δl (known)	Difference
Fraction C				
8·021	5·409	−0·836	+0·563	−1·399
8·021	4·222	−0·353	+0·086	−0·439
8·021	3·158	+0·030	−0·230	+0·260
8·021	2·646	+0·545	−0·329	+0·874
Fraction D				
10·56	5·409	−0·955	+0·581	−1·536
10·56	4·222	−0·237	+0·128	−0·365
10·56	3·776	−0·159	+0·101	−0·260
10·56	3·158	+0·143	−0·071	+0·214
10·56	2·646	+0·266	−0·227	+0·493

ments. The results are recorded in Table 6. They correspond to molecular weight, C 920 and D 1270, and degree of polymerisation, C 2·8 and D 4·1.

β-*Amylolysis of the Oligosaccharides*.—(a) *Determination of reducing power.* The digests employed contained the oligosaccharide (*ca.* 8 mg. in 2 c.c. of water), M-acetate buffer (pH 4·7; 1 c.c.), and a 0·3% solution (1 c.c.) of soya-bean β-amylase (prepared by the method of Bourne, Macey, and Peat, *J.*, 1945, 882). The reducing powers of the digests were determined at intervals by means of the Shaffer–Hartmann copper reagent (*J. Biol. Chem.*, 1921, **45**, 365), a correction being applied in each case for the reducing power of the enzyme sample. The results are shown in Table 7.

(b) *Chromatographic analysis of* β-*amylolysis products.* After the above digests had been incubated for 17·5 hours, aliquot portions were subjected to paper-chromatographic analysis,

TABLE 7. *Changes in reducing power* (*as* % *of that of maltose*) *during* β-*amylolysis.*

Time (hours) :	0	17·5	41·5
Fraction C	61·9	63·7	70·3
Fraction D	40·9	80·7	83·7
Fraction E	25·8	77·2	84·4

as described above. In each case, aniline hydrogen phthalate spray revealed two components; these had R_F values identical with those of maltose and maltotriose, used as standards on the same chromatogram. The proportion of maltotriose, judged from the intensities of the stains, appeared to be greatest in the products from fraction C and least in those from fraction E.

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