3. Phosphoproteins of brain contain similar phosphoseryl sequences. This has been shown both by comparison of rates of migration of phosphopeptides separated from partial hydrolysates and by a technique involving isotopic dilution.

4. The results are discussed in relation to the possible metabolic role of brain phosphoproteins in the intact tissue.

I am indebted to Dr J. Williams for helpful correspondence during the initial stages of this work, and Dr M. J. H. Smith, Kings College Hospital Medical School, for permission to use an automatic recording apparatus (constructed on the premises) for the detection of radioactivity in chromatographic strips.

REFERENCES

Burnett, G. & Kennedy, E. P. (1954). *J. biol. Chem.* **211**, 969.
Flavin, M. (1954). *J. biol. Chem.* **210**, 771.
Heald, P. J. (1957). *Biochem. J.* **66**, 659.
Heald, P. J. (1958). *Biochem. J.* **68**, 580.
Heald, P. J. (1959). *Biochem. J.* **73**, 132.
Heald, P. J. (1960). *Phosphorus Metabolism of Brain.* Oxford: Pergamon Press Ltd.
Heald, P. J. (1961*a*). *Biochem. J.* **78**, 340.
Heald, P. J. (1961*b*). *Proc. 4th int. Symp. Neurochem.* Oxford: Pergamon Press Ltd.
Judah, J. D. (1960). *Nature, Lond.*, **187**, 506.
Kennedy, E. P. & Smith, S. W. (1954). *J. biol. Chem.* **207**, 153.
Rabinowitz, M. & Lipmann, F. (1960). *J. biol. Chem.* **235**, 1043.
Rose, S. P. R. & Heald, P. J. (1961). *Biochem. J.* (in the Press).
Troll, W. & Cannan, R. K. (1953). *J. biol. Chem.* **200**, 806.
Wade, H. E. & Morgan, D. M. (1954). *Biochem. J.* **49**, 286.
Williams, J. & Sanger, F. (1959). *Biochim. biophys. Acta*, **33**, 294.

Biochem. J. (1961) **80**, 514

The Action of a *Lactobacillus bifidus* Dextranase on a Branched Dextran

By R. W. BAILEY, D. H. HUTSON and H. WEIGEL
Chemistry Department, Royal Holloway College, University of London, Englefield Green, Surrey

(*Received* 23 *February* 1961)

It has been shown that an extracellular dextranase of a rumen strain of *Lactobacillus bifidus*, when incubated with the essentially unbranched dextran of *Streptococcus bovis*, produces isomaltotriose, isomaltotetraose, isomaltopentaose, isomaltohexaose and traces of isomaltoheptaose, but no glucose or isomaltose (Bailey & Clarke, 1959). A study of the action of the *L. bifidus* dextranase on various dextrans revealed that the mixture of oligosaccharides produced varied with the type, and degree of branching, of the dextran (Bailey, Hutson & Weigel, 1960). We have now examined in detail the action of this dextranase on the branched dextran elaborated by *Leuconostoc mesenteroides* (*Betacoccus arabinosaceous*, Birmingham strain).

EXPERIMENTAL

Dextranase. The dextranase was isolated from cell-free culture fluid of a rumen strain of *Lb. bifidus* by one of us (R.W.B.) in the Laboratories of the Plant Chemistry Division, D.S.I.R., Palmerston North, New Zealand, according to the method described by Bailey & Clarke (1959).

Dextrans. Leuconostoc mesenteroides (Birmingham strain) dextran was synthesized from sucrose. The dextran was from the same batch as was that used for structural studies of the dextran, which was shown to contain, as well as α-1:6-linkages, 12–15% of α-1:3-branch linkages (Barker, Bourne, Bruce, Neely & Stacey, 1954). A virtually unbranched dextran (Bailey, 1959) was isolated from a sucrose-containing culture of *S. bovis* (strain I) (Bailey & Oxford, 1958).

Paper chromatography and ionophoresis. The solvents used for paper chromatography were: (*a*) the upper layer of ethyl acetate–water–pyridine (2:2:1, by vol.) (Jermyn & Isherwood, 1949); (*b*) ethyl acetate–water–pyridine–acetone (Malpress & Hytten, 1958); (*c*) upper layer of butanol–ethanol–water (4:1:5, by vol.); (*d*) ethyl acetate–acetic acid–water (9:2:2, by vol.); (*e*) butanol–benzene–pyridine–water (5:1:3:2, by vol.). Ionophoresis was carried out at about 50 v/cm. in borate solution, pH 10 (Foster, 1953), and in molybdate solution, pH 5·5 (Bourne, Hutson & Weigel, 1959). It will be noticed that some of the M_S (mobility with respect to sorbitol) values reported here differ slightly from those reported earlier. This is due to the application of smaller quantities, thus allowing a more accurate determination of the rates of migration. In all cases comparison was made with known compounds.

The reagents used for the detection of compounds were: (*a*) silver nitrate in acetone–ethanolic sodium hydroxide (Trevelyan, Procter & Harrison, 1950); (*b*) aniline hydrogen phthalate (Partridge, 1949); (*c*) *p*-anisidine–HCl (Hough, Jones & Wadman, 1950); (*d*) aniline–diphenylamine–

phosphoric acid (Schwimmer & Bevenue, 1956); (*e*) triphenyltetrazolium chloride (Feingold, Avigad & Hestrin, 1956).

Dextranase digests. Standard digests were prepared from dextran solution (15 mg. in 2 ml. of water), dextranase solution (5 mg. in 1 ml. of water) and 0·2M-citrate buffer, pH 5·5 (2 ml.). The digests were incubated under a layer of toluene at 37° for 60 hr.

Reducing sugars. Reducing sugars produced were determined at time intervals by the cuprimetric method of Shaffer & Hartmann (1921). The results were calculated in terms of isomaltotriose (Fig. 1). The solutions were desalted with Bio-Deminrolit, pretreated with carbon dioxide and evaporated to dryness *in vacuo*. The residues were dissolved in water (0·2 ml.) for chromatographic analysis.

RESULTS

Oligosaccharides produced by the action of the dextranase on Leuconostoc mesenteroides *dextran*

The rate of liberation of reducing sugars from *L. mesenteroides* dextran by *Lb. bifidus* dextranase is compared with that from *S. bovis* dextran in Fig. 1. The amounts of reducing sugars released indicates the effect of branching on the enzymic hydrolysis. Paper chromatography in solvent (*b*) of the digest containing *L. mesenteroides* dextran revealed, after 30 hr. incubation, the formation of three oligosaccharides, A, B and C, with $R_{\text{isomaltotriose}}$ (R_{IMT}) values of 0·75, 0·50 and 0·35 respectively, in addition to isomaltotriose, isomaltotetraose, isomaltopentaose, isomaltohexaose and isomaltoheptaose, also obtained from *S. bovis* dextran. Glucose, isomaltose and oligosaccharides with a degree of polymerization greater than seven glucose units were absent from the digest containing *S. bovis* dextran. Glucose and isomaltose were also absent from the digest containing *L. mesenteroides* dextran, but a large amount of unresolvable material with $R_{\text{IMT}} < 0{\cdot}20$ was produced.

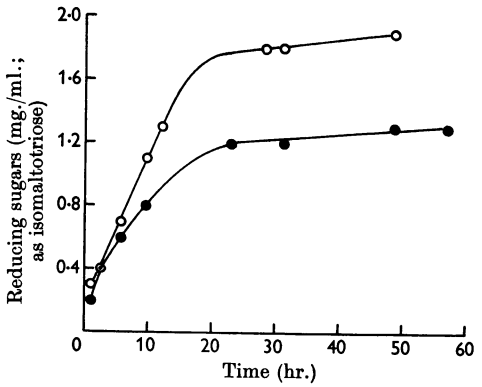

Fig. 1. Liberation of reducing sugars in dextran–dextranase digests. Solutions contained dextran (7·5 mg./ml.; 10 ml.), dextranase (5 mg./ml.; 5 ml.) and citrate buffer (0·2M, pH 5·5; 10 ml.). ○, *S. bovis* dextran; ●, *L. mesenteroides* dextran.

Table 1. *Yields of oligosaccharides produced by the action of* Lb. bifidus *dextranase on* Leuconostoc mesenteroides (*Birmingham strain*) *dextran*

Solutions containing dextran (15 mg./2 ml. of water), dextranase (5 mg./1 ml. of water) and citrate buffer (0·2M, pH 5·5; 2 ml.) were incubated at 37° for 60 hr. Oligosaccharides were resolved by chromatography in solvent (*b*) and determined by the anthrone method. Grading scheme in visual estimation: + (trace) to + + + + (intense).

	L. mesenteroides dextran (% of recovered material)	*S. bovis* dextran (by visual estimation)
Isomaltotriose	8·8	+ + + +
Isomaltotetraose	9·4	+ + + +
Tetrasaccharide A	3·3	Absent
Isomaltopentaose	11·4	+ + + +
Pentasaccharide B	3·5	Absent
Hexasaccharide fraction	8·6	+ + +
Heptasaccharide fraction	13·1	+
Unresolvable fraction	41·9	Absent

A part of the digest containing *L. mesenteroides* dextran (3·76 mg. of the original dextran; corrected for moisture content) was fractionated by paper chromatography in solvent (*b*). The glucose equivalent of each eluted fraction was determined by the anthrone method (Yemm & Willis, 1954). The results are shown in Table 1. Qualitative results obtained from *S. bovis* dextran are included for comparison.

A digest (500 ml.) containing *L. mesenteroides* dextran (1·90 g.) was incubated under standard conditions. After desalting, the mixture of oligosaccharides was fractionated by paper chromatography in solvent (*b*) on Whatman no. 3 filter paper. Purification of the fractions containing oligosaccharides A and B by the same paper-chromatographic method, followed by desalting and freeze-drying, yielded chromatographically pure oligosaccharide A (30 mg.) and B (30 mg.).

Structural examination of oligosaccharide A. Oligosaccharide A could be detected on paper chromatograms with spray reagents (*a*), (*b*), (*c*) (green spot) and (*d*) (pink spot). During ionophoresis in borate solution (pH 10) it migrated with the same M_G (mobility with respect to glucose) as isomaltotetraose (0·65). When estimated by the Shaffer & Hartmann (1921) method oligosaccharide A (5 mg.) had a reducing power equivalent to 1·15 mg. of glucose. This corresponded to 85·2 % of the calculated value for a tetrasaccharide. It was not possible to determine the ash and moisture contents.

Oligosaccharide A (250 μg.) was heated for 4 hr. at 100° in 1·5N-HCl (1 ml.) and the solution evaporated to dryness *in vacuo* over solid NaOH to

remove the acid. The hydrolysate contained a single component which was chromatographically identical with glucose. A partial hydrolysate, obtained by heating for 1 hr. at 100° in N-HCl and deionizing with Amberlite IRA-400 (carbonate), was shown by paper chromatography to contain isomaltotriose, isomaltose and glucose.

Oligosaccharide A (12 mg.) was reduced with potassium borohydride (Bragg & Hough, 1957). The solution was deionized by treatment with Amberlite IR-120 (H^+) followed by evaporation and repeated distillation with dry methanol. Oligosaccharide A alcohol was purified and isolated by paper chromatography in solvent (*a*) (R_G 0·32). Ionophoresis in molybdate solution (pH 5·5) (Bourne *et al.* 1959) showed that it migrated with the same M_S as 6-*O*-α-isomaltotriosylsorbitol (0·50).

Oligosaccharide A alcohol was partially hydrolysed by heating for 4 hr. at 100° in 1% aqueous oxalic acid (5 ml.). The hydrolysate was deionized with Amberlite IRA-400 (carbonate) and fractionated by paper chromatography in solvent (*a*) (see Table 2) into 5 components in addition to unchanged material (about 40%, visual estimation on chromatograms). Each fraction was subjected to ionophoresis in molybdate solution (pH 5·5), when fractions 3, 4 and 6 were resolved into trisaccharide D and trisaccharide E alcohol, isomaltose and 6-*O*-α-D-glucopyranosylsorbitol, D-glucose and sorbitol respectively. The identity of the products of hydrolysis with the corresponding named compounds was confirmed by ionophoresis in borate solution.

Trisaccharide D, which was immobile during ionophoresis in molybdate solution (pH 5·5), was eluted and, after removal of molybdate with Amberlite IR-120 (H^+) and Amberlite IRA-400 (carbonate), reduced with potassium borohydride (Bragg & Hough, 1957). The solution was deionized as described above. Ionophoresis in molybdate solution (pH 5·5) revealed the presence of trisaccharide F alcohol (M_S 0) and trisaccharide E alcohol (M_S 0·62).

To separate solutions of oligosaccharide A (500 μg.) and isomaltotetraose (500 μg.) in water (1 ml.) was added 0·03 M-sodium periodate (0·5 ml.). After standing for 16 hr. at room temperature the excess of periodate was destroyed with 0·03 M-ethylene glycol (0·5 ml.). Hydrochloric acid was added to give 2 N-HCl solutions. These were heated for 6 hr. at 100° and then evaporated *in vacuo* over solid NaOH. Although paper chromatography in solvent (*a*) of the hydrolysate of oxidized oligosaccharide A revealed an intense spot of glucose, the hydrolysate of oxidized isomaltotetraose was shown to contain only traces of glucose. Measured portions of the hydrolysates (containing 250 μg. of original oligosaccharides) were fractionated by paper chromatography in solvent (*a*). The components corresponding to glucose were eluted with water and determined by the benzidine method (Jones & Pridham, 1954), with glucose as a standard. The yields of glucose from oligosaccharide A and isomaltotetraose corresponded to 27% and 5% respectively of the glucose present in a tetrasaccharide containing glucose only.

Structural examination of oligosaccharide B. The methods were as for oligosaccharide A. Oligosaccharide B could be detected on paper chromatograms with spray reagents (*a*), (*b*), (*c*) (green spot) and (*d*) (pink spot). During ionophoresis in borate solution (pH 10) it migrated with the same M_G as isomaltopentaose. Complete hydrolysis gave a single reducing compound which was chromatographically identical with glucose, whereas a partial hydrolysate was shown to contain, by paper chromatography in solvents (*a*) and (*b*), isomaltotetraose, isomaltotriose, isomaltose and glucose. Oligosaccharide B alcohol migrated during ionophoresis in molybdate solution (pH 5·5) with the same M_S as 6-*O*-α-isomaltotetraosylsorbitol (0·44). A partial acid hydrolysate of oligosaccharide B alcohol showed, on chromato-

Table 2. *Fractionation of products from partial hydrolysis of oligosaccharide A alcohol*

Paper chromatography in solvent (*a*)		Ionophoresis in molybdate solution	
Fraction no.	R_G	M_S	Identity
1	0·32	0·50	Oligosaccharide A alcohol
2	0·45	0·60	6-*O*-α-Isomaltosylsorbitol
3	0·55	0	Trisaccharide D
		0·60	Trisaccharide E alcohol
4	0·75	0	Isomaltose
		0·75	6-*O*-α-D-Glucopyranosylsorbitol
5	0·85	0	Nigerose
6	1·0	0	Glucose
		1·0	Sorbitol

graphy in solvents (*a*) and (*d*), in addition to unchanged material (about 50%, visual estimation), intense spots corresponding to glucose and 6-*O*-α-isomaltotriosylsorbitol. Only very small traces of isomaltose, 6-*O*-α-D-glucopyranosylsorbitol and sorbitol were detectable.

When oligosaccharide B (500 μg.) was oxidized with sodium periodate and hydrolysed in the same way as oligosaccharide A and isomaltotetraose, the yield of glucose corresponded to 24% of the glucose present in a pentasaccharide containing only glucose.

Action of the dextranase on 6-O-α-*isomaltohexaosyl- and* 6-O-α-*isomaltoheptaosyl-sorbitol*

6-*O*-α-Isomaltohexaosylsorbitol (8 mg.) and 6-*O*-α-isomaltoheptaosylsorbitol (8 mg.), prepared by reduction of isomaltoheptaose and isomaltooctaose respectively with potassium borohydride, were separately dissolved in 0·25 M-citrate buffer (pH 5·5) (1 ml.) and incubated with dextranase (4 and 6 mg. respectively) for 26 hr. at 37°. Paper ionophoresis in molybdate solution of the deionized digest containing 6-*O*-α-isomaltohexaosylsorbitol revealed, in addition to non-migrating material, the presence of two components which migrated at the same rate as 6-*O*-α-isomaltosyl- and 6-*O*-α-isomaltotriosyl-sorbitol respectively. The non-migrating material was eluted from the paper and deionized. Paper chromatography in solvent (*a*) revealed components corresponding to isomaltotriose and isomaltotetraose. 6-*O*-α-Isomaltosylsorbitol, 6-*O*-α-isomaltotriosylsorbitol, 6-*O*-α-isomaltotetraosylsorbitol, isomaltotriose, isomaltotetraose and isomaltopentaose were identified in a similar manner in the digest containing 6-*O*-α-isomaltoheptaosylsorbitol. Visual examination of the paper ionograms and chromatograms suggested that all the products were present in approximately equal quantities.

DISCUSSION

The evidence presented shows that oligosaccharide A is a reducing tetrasaccharide of glucose. The results of the partial acid hydrolysis of tetrasaccharide A and the reduction product showed the presence of an isomaltotriose unit, together with another glucose unit joined by a linkage of a more labile type. One glucose unit was shown to be resistant to oxidation by periodate. This demonstrates the presence of one 1:3-glucosidic linkage in tetrasaccharide A. As the 1:3- as well as the 1:6-glucosidic linkages in dextran are of the α-type it is reasonable to assume that the 1:3-glucosidic linkage in tetrasaccharide A also is an α-1:3-linkage.

There are four possible structures (I–IV) (Fig. 2) for a tetrasaccharide containing an isomaltotriose unit to which a fourth glucose unit is joined by an α-1:3-glucosidic linkage. The results of periodate oxidation eliminate structures (III) and (IV). The migration of tetrasaccharide A alcohol during ionophoresis in molybdate solution conclusively eliminates structures (III) and (IV) because a 3-*O*-substituted sorbitol does not form a complex with molybdate (Bourne *et al.* 1959). This migration and the reaction of tetrasaccharide A with triphenyltetrazolium chloride also eliminate the possibility of substitution at $C_{(4)}$- and $C_{(2)}$-hydroxyl groups respectively of the reducing glucose unit.

The structure of tetrasaccharide A was elucidated further by a partial hydrolysis of the reduction product (V or VI) (Fig. 3). Products which would be expected from both structures, namely 6-*O*-isomaltosylsorbitol (VII), isomaltose (XII), 6-*O*-α-D-glucopyranosylsorbitol (XIII), nigerose (XIV), glucose (XV) and sorbitol (XVI), were identified by paper chromatography and ionophoresis. As would be expected if (VI) were present, two trisaccharide alcohols were obtained (Table 2). One of these (fraction 2) was identical with 6-*O*-isomaltosylsorbitol (VII), the only trisaccharide alcohol expected from (V). The other, trisaccharide E alcohol, must have structure (IX), which is consistent with its M_S.

The possible structures of the reducing trisaccharide D of fraction 3 are (VIII) and (X). Ionophoresis in molybdate solution (pH 5·5) of the reduced trisaccharide D revealed trisaccharide E alcohol (IX) and a non-migrating trisaccharide F alcohol. As 3-*O*-substituted sorbitols do not migrate, trisaccharide F alcohol must have structure (XI), and hence trisaccharide D must have contained both (VIII) and (X).

These results show that tetrasaccharide A is a mixture of 3^3-glucosylisomaltotriose (I) and 3^2-glucosylisomaltotriose (II).

The results obtained with oligosaccharide B provide evidence that it is a reducing pentasaccharide B containing an isomaltotetraose unit to which a fifth glucose unit is joined by a 1:3-

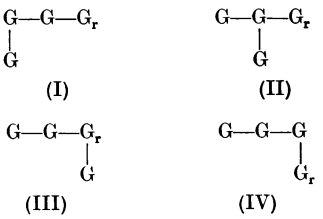

Fig. 2. Possible structures of a tetrasaccharide containing an isomaltotriose unit and a fourth glucosyl unit joined by an α-1:3-link. —, α-1:6-link; |, α-1:3-link; G , reducing glucose unit; G, glucosyl unit.

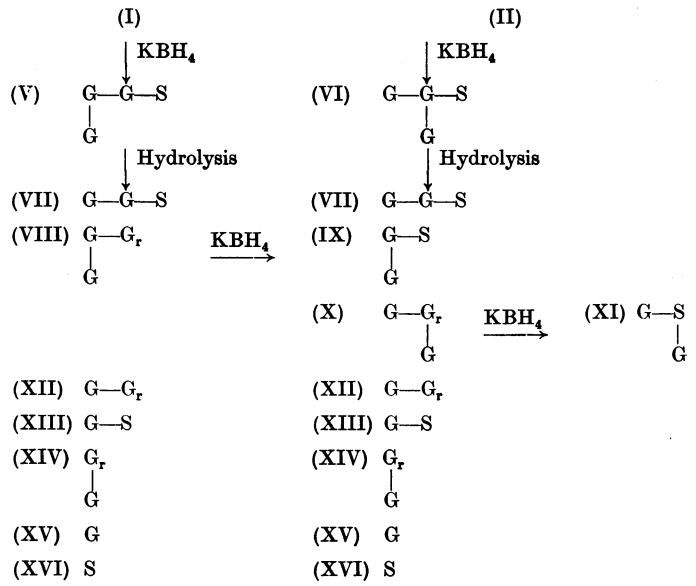

Fig. 3. Products of reduction-hydrolysis of tetrasaccharides (I) and (II). —, α-1:6-link; |, α-1:3-link, G_r, reducing glucose unit; G, glucosyl unit; S, sorbitol.

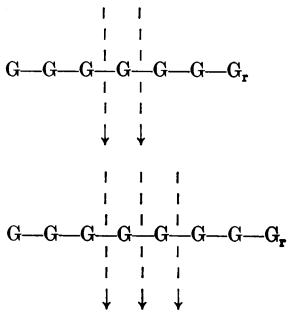

Fig. 4. Action of *Lb. bifidus* dextranase on isomaltodextrins, —, α-1:6-Link; G_r, reducing glucose unit; G, glucosyl unit; - - →, alternative points of hydrolysis.

glucosidic linkage similar to that in (I) or (II). The fact that tetrasaccharide A is a mixture of 3^3-glucosylisomaltotriose and 3^2-glucosylisomaltotriose suggests that pentasaccharide B is also a mixture of pentasaccharides with these types of structures.

Tetrasaccharides (I) and (II) and pentasaccharide B could have arisen by enzymic hydrolysis of *L. mesenteroides* dextran or by chemical or enzymic transglucosylation action on isomaltodextrins. The latter possibility is unlikely as, in this case, they would be expected to have been produced in the control digest containing *S. bovis* dextran. The presence of a 1:3-glucosidic linkage in tetrasaccharides (I) and (II) and pentasaccharide B indicates that the enzyme cannot hydrolyse the 1:3-linkage. We believe that the present work is the first occasion in which oligosaccharides containing the branch linkages of the original dextran have been found.

Bailey & Clarke (1959) established that the action of *Lb. bifidus* dextranase involves random hydrolysis of the dextran chain and that the smallest isomaltodextrin readily hydrolysed is isomaltoheptaose. The action of the enzyme on 6-*O*-α-isomaltohexaosyl- and 6-*O*-α-isomaltoheptaosylsorbitol shows that it has no preference for a particular type of chain end, a result which is in accordance with the properties of an endopolysaccharase. It is probable, therefore, that the dextranase hydrolyses dextran or an isomaltodextrin containing not less than seven glucose units at a point not less than three glucosidic linkages from a chain end, as shown in Fig. 4.

The production of 3^3-glucosylisomaltotriose (I) and 3^2-glucosylisomaltotriose (II) and pentasaccharide B suggests that this type of hydrolysis of dextran by the dextranase is not inhibited by the presence of 1:3-branch linkages. The structure of these products and the absence from the digest of oligosaccharides in which the reducing glucose unit is substituted at $C_{(3)}$ suggests that the α-1:6-link on the reducing side of the branch point of the main dextran chain is resistant to hydrolysis by the dextranase. The branching of *L. mesenteroides* dextran has been reported to occur once in each 6 or 7 anhydroglucose units (Barker *et al.* 1954). It can be calculated that dextrans of regular structures

with branch links on every sixth, seventh and eighth anhydroglucose unit respectively will yield, on hydrolysis with *Lb. bifidus* dextranase, oligosaccharides in unequal proportions. The approximately equal quantities of isomalto-triose, -tetraose and -pentaose produced indicate that the branching of this dextran is random. The presence in the digest of much material containing more than six glucosyl units is further support for a random branching of the dextran molecule.

It is significant that the 'branching' in tetrasaccharide A and pentasaccharide B consists of only one glucosyl unit. 3^3-Glucosylisomaltotriose (I) and 3^2-glucosylisomaltotriose (II) could arise from a part of the dextran molecule where the branching was a single glucosyl unit, or from parts of a randomly branched dextran molecule. Bovey (1959), on the grounds of physical measurements, has suggested that 80% of the branches in *L. mesenteroides* (NRRL-B512) dextran consist of only one glucosyl unit.

SUMMARY

1. *Lactobacillus bifidus* dextranase has been shown to hydrolyse *Leuconostoc mesenteroides* (Birmingham strain) dextran to a complex mixture of oligosaccharides.

2. In addition to isomalto-triose, -tetraose, -pentaose, -hexaose and unresolvable material of degree of polymerization greater than 6, a tetrasaccharide (A) and a pentasaccharide (B) were isolated.

3. Tetrasaccharide (A) was shown to be a mixture of 3^3-glucosylisomaltotriose and 3^2-glucosylisomaltotriose. Pentasaccharide (B) contained one glucose unit joined through a 1:3-linkage to a glucose unit, other than the reducing one, of isomaltotetraose and is probably a mixture of isomers.

4. The dextranase has been shown to hydrolyse 6-*O*-α-isomaltohexaosyl- and 6-*O*-α-isomaltoheptaosyl-sorbitol at two and three alternative glucosidic linkages respectively.

5. The implications of the results, as far as dextranase action and dextran structure are concerned, have been discussed.

The authors are indebted to Professor E. J. Bourne for his interest and encouragement, and to the Department of Scientific and Industrial Research for financial assistance.

REFERENCES

Bailey, R. W. (1959). *Biochem. J.* **71**, 23.
Bailey, R. W. & Clarke, R. T. J. (1959). *Biochem. J.* **72**, 49.
Bailey, R. W., Hutson, D. H. & Weigel, H. (1960). *Nature, Lond.*, **186**, 553.
Bailey, R. W. & Oxford, A. E. (1958). *J. gen. Microbiol.* **19**, 130.
Barker, S. A., Bourne, E. J., Bruce, G. T., Neely, W. B. & Stacey, M. (1954). *J. chem. Soc.* p. 2395.
Bourne, E. J., Hutson, D. H. & Weigel, H. (1959). *Chem. & Ind.* p. 1047.
Bovey, F. A. (1959). *J. Polymer Sci.* **35**, 167.
Bragg, P. D. & Hough, L. (1957). *J. chem. Soc.* p. 4347.
Feingold, D. S., Avigad, G. & Hestrin, S. (1956). *Biochem. J.* **64**, 351.
Foster, A. B. (1953). *J. chem. Soc.* p. 982.
Hough, L., Jones, J. K. N. & Wadman, W. H. (1950). *J. chem. Soc.* p. 1702.
Jermyn, M. A. & Isherwood, F. A. (1949). *Biochem. J.* **44**, 402.
Jones, J. K. N. & Pridham, J. B. (1954). *Biochem. J.* **58**, 288.
Malpress, F. H. & Hytten, F. E. (1958). *Biochem. J.* **68**, 708.
Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
Schwimmer, S. & Bevenue, A. (1956). *Science*, **123**, 543.
Shaffer, P. A. & Hartmann, A. F. (1921). *J. biol. Chem.* **124**, 425.
Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature, Lond.*, **166**, 444.
Yemm, E. W. & Willis, A. J. (1954). *Biochem. J.* **57**, 508.

Biochem. J. (1961) **80**, 519

The Purification and Properties of a Factor containing Vitamin B_{12} concerned in the Synthesis of Methionine by *Escherichia coli*

BY M. A. FOSTER, K. M. JONES* AND D. D. WOODS
Microbiology Unit, Department of Biochemistry, University of Oxford

(*Received* 27 *January* 1961)

Evidence has been obtained that cobalamin and folic acid are concerned in the synthesis of methionine from homocysteine by *Escherichia coli* strain PA15, a serine- or glycine-requiring auxotroph (Gibson & Woods, 1960; Cross & Woods, 1954; Szulmajster & Woods, 1960; Guest, Helleiner, Cross & Woods, 1960). (In this paper, the terms folic acid and vitamin B_{12} will be used in general reference to the two families of compounds; names in common use will be used in reference to specific

* Guinness Research Fellow in Microbiological Biochemistry.