7. *Ionophoresis of Carbohydrates. Part III.* Behaviour of Some Amylosaccharides and their Reaction with Borate Ions.*

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Paper ionophoresis of a wide range of amylosaccharides has been studied using alkaline borate and other buffers. Amylose and amylopectin were found to have a characteristic behaviour and could be separated from each other. Evidence is presented which indicates that, in addition to known reactions (Part II*), borate ions appear to react in a unique manner with certain amylosaccharides. Application of paper ionophoresis to the *Neisseria perflava* polysaccharide (Barker, Bourne, and Stacey, *J.*, 1950, 2884) and to the synthetic amylosaccharides obtained by the action of mixtures of potato P- and Q-enzyme on D-glucose 1-phosphate (Barker, Bourne, Peat, and Wilkinson, *J.*, 1950, 3022) has provided additional information on the structure of these polysaccharides. The reaction of polyglucosans with borate ions is commented on.

The migration of acidic polysaccharides in paper ionophoresis has been studied in some detail (Rienits, *Biochem. J.*, 1953, **53**, 79; Pasternak and Kent, *Research*, 1952, **5**, 486; Greenway, Kent, and Whitehouse, *Research*, 1953, S **6**; Ricketts, Walton, and Saddintgon, *Biochem. J.*, 1954, **58**, 532; cf. Gardell, Gordon, and Åqvist, *Acta Chem. Scand.*, 1950, **4**, 907) but only brief reports of the examination of neutral polysaccharides by this technique have been made (Greenway *et al.*, *loc. cit.*; Tiselius and Flodin, *Adv. Protein Chem.*, 1953, **8**, 461; Michl, *Monatsh.*, 1952, **83**, 737; Preece and Hobkirk, *Chem. and Ind.*, 1955, 257). The electrophoretic behaviour of amylosaccharides in agar jelly in the presence of iodine–iodide has been studied in detail by Mould and Synge (*Biochem. J.*, 1954, **58**, 585).

Migration of certain carbohydrate derivatives, in paper ionophoresis, is facilitated by the formation of negatively charged complexes with the borate ions present in the aqueous alkaline media employed as the conducting solution (see Part II and the references cited therein). The aims of this investigation were to examine the reaction of neutral polysaccharides with borate ions in the light of knowledge of the reactions which occur with mono- and oligo-saccharides (Part II) and to evaluate the paper electrophoresis of neutral polysaccharides.

In preliminary experiments potato amylose (B.V. 1·35) and amylopectin (B.V. 0·18) were examined ionophoretically in a borate buffer (pH 10; cf. Foster, *J.*, 1953, 982) under standard conditions (see Experimental section). The amylosaccharides could be detected on the neutralised paper by spraying it with ethanolic iodine. The amylopectin appeared as a reddish-brown zone and the amylose as a violet zone, as might be expected from the well-known nature of the complexes of these polysaccharides with iodine in solution. This method of detection was found to be specific for the amylosaccharides. Alternatively the iodine-vapour method of Brante (*Nature*, 1949, **163**, 651) as applied to polysaccharides by Greenway *et al.* (*loc. cit.*) may be employed; this method has been claimed as able to detect a range of polysaccharides (Greenway *et al.*, *loc. cit.*). Some of the results obtained are represented diagrammatically (A, B_1, B_2, B_3, B_4) in Table 1, from which it may be seen that whilst the amylopectin underwent appreciable migration toward the anode, with

* Part II, *J.*, 1955, 1778.

little absorption of the polysaccharide on the paper in the path of migration, the pattern of the amylose movement depended on the amount of the polysaccharide introduced on to the paper (cf. Greenway *et al.*, *loc. cit.*). Thus, when a small amount of amylose was introduced on to the paper and subjected to ionophoresis, it was largely absorbed at the origin, although there was slight migration toward the anode (B_1). The strong absorption on the paper of the amylose in ionophoresis is not surprising since it has long been known

TABLE 1. *Diagrammatic representation of the ionophoretic migration of some amylosaccharides.*

	Amylosaccharide	Migration pattern *	Buffer
		Origin zone ⊕ ←	
A	Amylopectin (1·0%)		Borate
B_1	Amylose (0·25%)		,,
B_2	Amylose (1·0%)		,,
B_3	Amylose (2·0%)		,,
B_4	Amylose (0·25%) + amylopectin (1·0%)		,,
C	Amylopectin (1·0%)		Glycine
D	Amylose (0·25%)		,,
E	Glycogen (rabbit liver) (1·0%)		Borate
F	*Neisseria perflava* polysaccharide		,,
G	Synthetic polysaccharide I*b*		,,
	,, ,, II*b*		,,
	,, ,, III*b* & IV		,,

Each of the above diagrams represents the distribution of the polysaccharide, after ionophoresis under standard conditions (see Experimental), in a cross-section of the paper perpendicular to its plane and parallel to the direction of the electric field.

* Representation of zones : ——— main zone of polysaccharide, – – – – – intermediate amounts of polysaccharide, ····· traces of polysaccharide. The figures in parentheses refer to the concentrations of the aqueous solutions of the polysaccharide introduced on to the paper (see Experimental).

that amylose is absorbed on cotton cellulose (see Gilbert, Greenwood, and Hybart, *J.*, 1954, 4454, and references cited therein). It might be expected that there would be a limit to the number of amylose molecules that can be absorbed per unit area of paper. Below this limit, as was observed in practice, no migration of the amylose would be expected to occur, and, above the limit, the extent of migration would be expected to depend on the amount of amylose introduced on to the paper. This is borne out by the patterns of movement B_1, B_2, and B_3. There was appreciable absorption of the amylose on the paper in the path of movement. The retention of a considerable amount of amylose at the origin zone in B_1, B_2, B_3, and B_4 may have been due to some irreversible absorption of the polysaccharide on the paper when the aqueous solutions of amylose were introduced on to the paper. The pattern of migration B_4 reveals that the mobility of amylopectin is not influenced by the presence of amylose.

In order to assess the influence of borate ions on the movement of amylose and amylopectin in ionophoresis, the behaviour of these polysaccharides in phosphate (pH 10·0) and glycine (pH 11·0) buffers was examined. From Table 1 (C and D) it may be seen that in glycine buffer the amylose remained largely absorbed at the origin whilst the amylopectin underwent slight migration toward the anode but much less than in borate buffer (the amount of amylose introduced on to the paper in B_1 and D was identical, as was the amount of amylopectin in A and C). The behaviour of amylose and amylopectin in phosphate

buffer was similar to that in glycine buffer. That there was true migration of the amylopectin in the non-borate buffers was supported by the fact that the slight migration occurred toward the anode, *against* the electroendosmotic flow. This slight movement may have been due to the presence of phosphate groups in the amylopectin and/or the formation of complexes between phosphate ions or glycine molecules with amylopectin (evidence which appears to support this will be described later). The migration of amylose in ionophoresis in borate buffer is undoubtedly due to complex formation with borate ions.

Ionophoresis of amylose samples with D.P. >4000, 415, and 135 showed that, in each case, there was considerable absorption at the origin. The molecular size of amylosaccharides at which absorption on paper becomes appreciable is not precisely known although it would appear to be between the rather wide limits D.P. 6—135. In the glycine buffer, ionophoresis of the oligosaccharides in the series maltose—maltohexaose revealed that there was no absorption, since all the members of the series moved identically with methyl α-D-xylopyranoside under the influence of the electroendosmotic flow. Under the same conditions α- and β-Schardinger dextrin (*cyclo*maltohexoside and *cyclo*maltoheptoside respectively) were not absorbed on the paper.

The possibility that polysaccharides may be absorbed on, or may otherwise interact with, the paper in ionophoresis, makes it difficult to compare the results obtained by this technique with those obtained by electrophoresis in free solution. Northcote (*Biochem. J.*, 1954, **58**, 353) has studied in detail the behaviour of a range of neutral polysaccharides on electrophoresis in free solution in borate buffer (pH 9·2). Potato amylopectin ($\mu = 3{\cdot}6 \times 10^{-5}$ cm.^{2} v^{-1} sec.$^{-1}$) was observed to migrate more rapidly than amylose ($\mu = 3{\cdot}1 \times 10^{-5}$ cm.^{2} v^{-1} sec.$^{-1}$). In filter-paper ionophoresis we have observed a similar relation: $\mu = 0{\cdot}51$—$0{\cdot}69 \times 10^{-6}$ cm.^{2} v^{-1} sec.$^{-1}$ for amylopectin, and for amylose (Table 1, B_3) the migrating zone had $\mu = 0{\cdot}35$—$0{\cdot}57 \times 10^{-6}$ cm.^{2} v^{-1} sec.$^{-1}$ (see Table 3). It would appears from these mobilities that, in ionophoresis, the paper support for the electrolyte has a general retarding effect on the migrating polysaccharides. Northcote (*loc. cit.*) reported that electrophoresis, in free solution in borate buffer (pH 9·2), of a mixture of potato amylose and amylopectin resulted in a reduced mobility of both components compared with the migration of each separately. We have not observed this effect in paper ionophoresis; the mobilities of potato amylose and amylopectin were identical when the polysaccharides were run separately or in admixture (Table 1, B_4).

It is conceivable that the absorption of polysaccharides on the paper in ionophoresis may be used to advantage in certain cases, as, for example, in the separation of amylose and amylopectin, by controlling the amount of the amylose introduced on to the paper. On the other hand, a possible disadvantage that might arise is the difficulty in assessing accurately the intensity of reaction of a polysaccharide with borate ions (which is essential for information on polysaccharide structure) if appreciable absorption occurs.

Several attempts to reduce the absorption of amylose in paper ionophoresis were made. Tiselius and Flodin (*loc. cit.*) report that the inclusion of anionic and neutral detergents in the buffer solution reduced the absorption of certain proteins in paper electrophoresis. Introduction of Teepol (mixed sodium alkanesulphonates) into the alkaline borate buffer almost completely prevented the migration of amylose and amylopectin. The reason for this action is at present unknown. Dissolution of amylose or amylopectin in molten urea (Clark, *Nature*, 1951, **168**, 876), followed by paper ionophoresis in alkaline borate or in alkaline borate containing 10% of urea, of an aqueous solution of the melt resulted in substantially slower migration of the polysaccharides. Ionophoresis of untreated amylose in borate buffer containing 40% of urea resulted in considerably reduced absorption of the polysaccharide. An amount of amylose (0·25% aqueous solution) which gave the migration pattern B_1 (Table 1) in alkaline borate gave a migration pattern similar to B_3 in the borate–urea buffer. The migration pattern of the amylopectin was not appreciably changed under these conditions. Conditions were not discovered whereby absorption of the polysaccharides could be eliminated completely. The high percentage of urea in the buffer system did not interfere seriously with the detection of the polysaccharides.

The blue values of the amylose and amylopectin (1·35 and 0·18 respectively) described above, indicated that each should contain a trace of the other. It was observed that in the

borate–urea buffer, where the coloration of the polysaccharides with iodine appeared to be intensified, ionophoresis of amylopectin revealed the presence of a small amount of intensely blue-staining material (most probably amylose) remaining at the origin. In the case of amylose a reddish zone (amylopectin) could be distinguished clearly, migrating just ahead of the amylose (the properties of amylose and amylopectin after ionophoresis will be described in a subsequent paper). Ethanolic iodine was used in these detections. In the normal borate buffer, ethanolic iodine did not reveal the traces but the iodine vapour method was effective in this respect. Thus, ionophoresis would appear to offer a means of checking the purity of amylose and amylopectin preparations. It should be emphasised that, since the method for the detection of amylopectin is considerably less sensitive than for amylose, precautions should be taken concerning the amounts of polysaccharide introduced on to the paper.

In Part II, evidence was presented which indicated that, in the alkaline media used in ionophoresis, borate ions reacted exclusively with the 4 : 6-hydroxyl groups in methyl α- and β-D-glucopyranoside. Thus in the amylosaccharides, which are 1—4α-linked, reactions of this type should occur solely at the non-reducing chain ends of the polysaccharide. On analogy with 4-*O*-methyl-D-glucose (M_G value 0·24; see Foster, *loc. cit.*) the reducing ends of the chains would be expected to react with borate ions. Because of the greater number of non-reducing end groups, amylopectin, on reaction with borate ions, would be expected to acquire a greater net negative charge, and hence to have a higher electrophoretic mobility than amylose. The contribution to the net negative charge, from borate-ion reaction with the reducing chain ends, would be expected to be the same for amylose and amylopectin since there is only one reducing end group per molecule in both polysaccharides. Glycogen, because of its shorter average chain length, and consequent greater percentage of non-reducing end groups per molecule, would be expected to migrate more rapidly than amylopectin in ionophoresis. These predictions are borne out by the results in Table 1 and by those of Northcote (*loc. cit.*).

The rate of migration of amylose is, however, greater than might be expected in view of its very long chain and the assumption that only the D-glucose units at the ends of the chains react with borate ions. Thus, other, hitherto unrecognised, reactions of the polysaccharide with borate ions probably occur. The well-known tendency for the helical chains of amylosaccharides to entrap other molecules, *e.g.*, iodine, butanol, etc., within the helices suggests that borate ions might be entrapped in a similar manner, thus conferring a negative charge on the molecule (cf. Mould and Synge, *loc. cit.*). Although direct proof of this could not be obtained some support was provided by the fact that α- and β-Schardinger dextrin were found to undergo appreciable migration on ionophoresis in borate buffer (Table 3). Reaction of individual D-glucose units in the cyclic dextrins is precluded (cf. Part II).

Ionophoresis of amylosaccharides may, in certain cases, provide information on their structure in addition to that obtained by other methods, for example, in the case of the amylosaccharides obtained by the action of variously composed mixtures of P- and Q-enzymes (from potatoes), on D-glucose 1-phosphate (Barker, Bourne, Peat, and Wilkinson, *J.*, 1950, 3022; Barker, Bourne, and Wilkinson, *J.*, 1950, 3027).

A series of polysaccharides were prepared which in certain cases (low Q : P ratio) could be separated into fractions which were soluble in water. The water-soluble fractions I*b*, II*b*, and III*b* (Barker *et al.*, *loc. cit.*), which had blue values 0·82, 0·44, and 0·22 respectively, were examined ionophoretically. With higher Q : P ratios, fractions were obtained which were completely soluble in water, *e.g.*, IV (B.V. 0·15). It was inferred from detailed studies by Barker *et al.* (*loc. cit.*) that there was a gradation of properties from amylose-type to amylopectin type polysaccharides as the Q : P ratio increased. Further, certain fractions, *e.g.*, I*b*, were dissimilar to any fractions isolatable from natural starch. Ionophoresis was found to reveal other differences between these polysaccharides and the components of natural starch. Polysaccharides I*b* and II*b* were found to contain two components with ionophoretic behaviour and staining properties with iodine similar to those of potato amylose and amylopectin (Table 1, A and B_1) but they migrated more slowly. Polysaccharide II*b* contained less of the amylose type component than I*b*. The

polysaccharides III*b* and IV exhibited unusual diffuse migration (Table 1, G), not previously encountered with amylosaccharides, and gave a reddish stain with iodine. Ionophoretically the polysaccharides III*b* and IV would seem to be somewhat different from natural amylopectin. It is of interest that the operation of an artificial mixture of P- and Q-enzymes on D-glucose 1-phosphate produces a range of polysaccharides whereas the naturally operating enzymes tend to produce the two distinct types, amylose and amylopectin.

Ionophoresis of the polysaccharide elaborated by *Neisseria perflava* revealed an unusual behaviour. The structure of this polysaccharide has been studied in detail (Barker, Bourne, and Stacey, *J.*, 1950, 2884; Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 994) and it has been recognised as of the glycogen type with an average chain length of 11—12 units. In ionophoresis it was found to remain stationary at the origin and gave a migration pattern (Table 1, F) very different from that of rabbit-liver glycogen (Table 1, E). Ionophoresis of a range of glycogens, *viz.*, ox liver, hog round worm, bee drone larvæ, bass liver, human liver, sheep tapeworm, dog liver, frog liver, chicken liver, guinea-pig liver, northern-pike liver [samples very kindly supplied by Professor F. Smith and described by Abdel-Akher and Smith (*loc. cit.*)] revealed a general behaviour essentially similar to that of rabbit-liver glycogen (Table 1, E). A detailed study of the electrophoretic behaviour of these glycogens will be published elsewhere (Professor F. Smith, personal communication). The ionophoretic behaviour of the *N. perflava* polysaccharide, as a glycogen-type polysaccharide, would therefore appear to be anomalous. The *N. perflava* polysaccharide has been observed to have a surprisingly low activating power in the conversion of amylose into amylopectin by the Q-enzyme isolated from *Polytomella coeca* (Barker, Bebbington, and Bourne, *J.*, 1953, 4051). It was suggested (Barker *et al.*, *loc. cit.*) that this might be due to the entanglement of the chains of the polysaccharide since the activating power depends on non-reducing chain ends. The behaviour of the *N. perflava* polysaccharide in ionophoresis would indicate either that the polysaccharide does not react with borate ions because of its structure or because of steric hindrance, or that it is strongly absorbed on the paper. Since the molecule appears to be of the glycogen type (Barker *et al.*, *loc. cit.*; Abdel-Akher and Smith, *loc. cit.*), and since in ionophoresis in non-borate buffers it did not migrate under the influence of the electroendosmotic flow, it appears that absorption is taking place. Since no measurement of the molecular weight of the polysaccharide has been made, the origin of any absorption effects is obscure at present.

Whilst the ability of borate ions to form complexes with carbohydrates is well known, there is little evidence to show that phosphate groups may behave similarly. When using alkaline non-borate buffers for comparison purposes, it was observed that members of the series maltose–maltohexaose and α- and β-Schardinger dextrin moved only under the influence of the electroendosmotic flow and identically with methyl α-D-xylopyranoside (cf. Part II) in glycine buffer (pH 11·0). In phosphate buffer (pH 10·0), however, all these compounds migrated toward the anode, with respect to methyl α-D-xylopyranoside. Although the precise origin of the migrations in phosphate buffer is unknown the results indicate that care should be taken in assessing the migration of carbohydrates in phosphate buffers in ionophoresis (cf. Goldwasser and Mathews, *J. Amer. Chem. Soc.*, 1955, **77**, 3135).

In considering the behaviour of polyglucosan in paper ionophoresis it appears that at least three factors must be taken into account: (1) solubility in the buffer solution, (2) absorption on the paper, and (3) mode of reaction with borate ions.

Clearly, paper ionophoresis can be applied only to polysaccharides which are soluble in the buffer system. Some aspects of absorption effects have been discussed above. The reaction of polyglucosans with borate ions is dependent upon the structure of the polysaccharide and two types of complex formation have been observed which involve (1) individual D-glucose residues and (2) D-glucose residues in different parts of the same molecule. No evidence has been obtained which suggests that more than one carbohydrate molecule can simultaneously react with a borate ion. Reaction of borate ions with D-glucose units within the polysaccharide chains would be expected to occur only if both the 4- and the 6-hydroxyl group are unsubstituted (cf. Part II). The reaction will be influenced

by the configuration of the glycosidic links along the polysaccharide chain. For polysaccharide chain ends, reaction with borate ions will be controlled, at the non-reducing end, by the configuration of the glycosidic linkage attaching it to the polysaccharide chain (Part II) and, at the reducing end, by the point of attachment of the remainder of the polysaccharide (cf. Part I, *J.*, 1953, 982). Reaction of the borate ions with D-glucose units in different parts of the same molecule will depend on the shape of the polysaccharide molecule and this appears to be especially favoured in the amylosaccharides.

A consideration of the ionophoretic behaviour of other neutral polysaccharides is reserved for a future communication.

Experimental

General Procedure for the Ionophoresis of Polysaccharides.—Whatman No. 3 paper was used throughout, although no significant differences were observed when No. 3MM paper was used. The technique for the preparation of the paper has been described in detail elsewhere (Foster, *Chem. and Ind.*, 1952, 1050). The amylosaccharides were introduced on to the paper in a rectangular zone of width 0·7 cm. (parallel to the direction of the applied electric field) and of optional length, by a single application of the appropriate amylosaccharide solution, from a capillary tube. The width of the rectangle corresponds to the origin zone in Table 1. Aqueous solutions of the polysaccharides were used and it was important to ensure that the spots of polysaccharide introduced on to the paper were kept moist. This precaution materially reduced the absorption of certain polysaccharides, especially amylose. If necessary the preparation of the paper may be reversed in that it is first soaked in the buffer solution, and blotted so that the origin line is surrounded by a dry zone on to which the solutions of polysaccharides may be introduced.

For the apparatus previously described (Foster, *loc. cit.*) the standard conditions adopted for the ionophoreses were 500 v for 4 hr. (final current 24 milliamp. for borate buffer).

Buffer Solutions.—The compositions of the buffer solutions employed in ionophoreses were as follows.

(a) *Borate* (pH 10·0) : boric acid (7·44 g.) and sodium hydroxide (4·0 g.) per l. of solution.

(b) *Glycine* (pH 11·0) : glycine (4·503 g.), sodium chloride (3·505 g.), and sodium hydroxide (1·8 g.) per l. of solution.

(c) *Phosphate* (pH 10·0) : disodium hydrogen phosphate (8·905 g.) and sodium hydroxide (0·33 g.) per l. of solution.

Detection of the Amylosaccharides on Paper.—After ionophoresis of the amylosaccharides the paper was dried (100—110°), neutralised by immersion in water–ethanol–concentrated hydrochloric acid (10 : 1 : 1 by volume) and dried. The location of the amylosaccharides was revealed (1) by spraying the paper lightly with ethanolic iodine (0·4%) and allowing the excess of iodine to volatilise, or (2) by allowing the paper to remain in an atmosphere of iodine vapour for 1 hr. (cf. Greenway *et al.*, *loc. cit.*). The colours so produced tended to fade slowly although in some cases they were still clearly discernible after several months. The colours could be regenerated by the application of either of the above methods. The colours observed are listed in Table 2.

Table 2. *Colours produced by reaction of iodine with some amylosaccharides on paper.*

Amylopectin	Reddish-purple	*N. perflava* polysaccharide	Dark brown
Amylose	Blue	α-Schardinger dextrin	Brown
Glycogens	Yellow-brown	β-Schardinger dextrin	Yellow

Sensitivity of the Method for the Detection of Amylose and Amylopectin.—The ethanolic iodine spray described above was more sensitive for the detection of amylose (limit 0·1 μg.) than for amylopectin (1·0 μg.) on neutral paper without ionophoresis. As the amount of amylose or amylopectin decreased towards the limit of detection the colours produced faded with increasing rapidity. After ionophoresis under standard conditions 0·1 μg. of amylose could still be detected since it was absorbed at the origin and remained in a compact zone. The amylopectin, because of weak absorption during migration, gave a rather diffuse zone at the limits of detection (1·0 μg.). With mixtures of amylose amd amylopectin and ionophoresis under standard conditions 0·3 μg. of amylose could be detected in the presence of 10 μg. of amylopectin, and 2 μg. of amylopectin in the presence of 2·5 μg. of amylose.

Mobilities and M_G Values.—Mobilities and M_G values were determined after ionophoresis under standard conditions. The limits quoted for the mobilities were obtained from the leading and trailing edges of the migrating zones; 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and methyl

TABLE 3. *Mobilities and M_G values of some amylosaccharides in ionophoresis in borate buffer* (pH 10).

Amylosaccharide	M_G	Mobility (cm.^{2} v^{-1} sec.$^{-1}$)	Amylosaccharide	M_G	Mobility (cm.^{2} v^{-1} sec.$^{-1}$)
D-Glucose	1·00	2·26—2·74 × 10^{-5}	Glycogen (rabbit liver)...	0·31	0·61—0·90 × 10^{-5}
Maltose	0·36	0·81—1·08 × 10^{-5}	α-Schardinger dextrin ...	0·14	—
Amylose	0·18	0·35—0·57 × 10^{-5}	β-Schardinger dextrin ...	0·12	—
Amylopectin	0·25	0·51—0·69 × 10^{-5}	Amylose *	—	3·1 × 10^{-5}
			Amylopectin *	—	3·6 × 10^{-5}

* Values quoted by Northcote (*loc. cit.*) for electrophoresis in free solution in 0·05M-borate buffer, pH 9·2, at 0°.

α-D-xylopyranoside were used to determine the extent of the electroendosmotic flow. M_G values were determined as previously described (Foster, *Chem. and Ind.*, 1952, 828); distances were measured from the centres of the migrating zones. Mobilities and M_G values are listed in Table 3.

The authors thank Dr. S. A. Barker, Dr. E. J. Bourne, and Dr. G. A. Gilbert for generous provision of samples, and Professor F. Smith for specimens of various glycogens.

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