Column Chromatography of Human Small-Intestinal Maltase, Isomaltase and Invertase Activities

By A. DAHLQVIST AND U. TELENUS

*Research Department of the Hospital and Department of Physiological Chemistry,
University of Lund, Lund, Sweden*

(Received 3 July 1968)

1. The maltase, isomaltase and invertase (sucrase) activities of solubilized mucosal preparations from human jejunum and ileum were studied with column chromatography on anion-exchange (diethylaminoethyl- and triethylaminoethyl-)cellulose and Sephadex G-200 gel. 2. On ion-exchange cellulose columns both kinds of enzyme preparations yielded two major disaccharidase peaks. The first peak contained maltase Ia (= isomaltase) and maltase Ib (= invertase). The second peak contained maltase II and maltase III. 3. On Sephadex G-200 gel columns jejunal preparations yielded the corresponding peaks as on ion-exchange columns, but the peaks appeared in the reverse order in the effluent. The ileal preparation studied yielded a single peak on gel columns, containing all the activities studied and eluted with the 'void volume'. 4. Precipitation with ethanol did not affect the behaviour of the enzymes during ion-exchange chromatography. When gel filtration was performed after ethanol precipitation of the enzymes, however, two peaks were obtained also with the ileal preparation, and subfractionation of the invertase was obtained with both kinds of preparations. 5. The second peak from ion-exchange chromatograms, containing maltase II and maltase III, on concentration was found to have very weak isomaltase activity, probably exerted by these enzymes as such. This activity accounts for only about 1% of the total isomaltase activity of the mucosa. 6. The results support the concept of the specificity of the human small-intestinal disaccharidases previously described after heat-inactivation experiments. The subfractionation of the invertase that under certain conditions is seen on Sephadex G-200 columns appears most likely to be an artifact. Consequently the nomenclature for the human maltose-, isomaltose- and sucrose-splitting enzymes proposed by another research group after gel-filtration chromatography studies should be abandoned. It seems more logical to keep the nomenclature based on heat inactivation [maltase Ia (= isomaltase), maltase Ib (= invertase or sucrase), maltase II and maltase III] until increased knowledge about the specificity and structure of these enzymes makes possible a more rational nomenclature.

Knowledge about the specificity of the human small-intestinal disaccharidases is of importance both for theoretical reasons and for the understanding of congenital and acquired enzyme defects of the small intestine. A study of the human small-intestinal disaccharidases, with heat inactivation being used for the separation of different enzymes, has previously been published (Dahlqvist, 1962). The relation between the intestinal invertase and isomaltase activities has also been studied kinetically, with sucrose and isomaltose in mixture as substrates (Dahlqvist, Auricchio, Semenza & Prader, 1963). The present paper deals with a column-chromatography study of the human small-intestinal maltase, isomaltase

and invertase (sucrase) activities, which was performed to obtain further information about the specificity of the enzymes responsible for these activities.

MATERIALS AND METHODS

Intestinal preparations

Intestine from adult humans. (a) Ileal preparation. A 58 cm.-long piece of macroscopically normal ileum was obtained from a 69-year-old male, operated for a cancer coli. This preparation for practical reasons had to be stored at -20° for 6 months before it was used for experiments (see below).

(b) Jejunal preparations. Several pieces of macroscopically normal jejunum from adult humans were

obtained after operations for pancreatic tumour or resections of the stomach. These preparations were in most cases used for chromatography within 2 weeks after the operation. In some occasions samples were used that had been stored at -20° for several months. The results then obtained did not differ from those obtained with fresh preparations.

Intestine from human foetus. The jejunum from an about 20-week-old human foetus was obtained on legal abortion, performed by Caesarean section.

Homogenization of the mucosa

The mucosa was removed from the intestinal wall with a glass microscope slide and homogenized with 4 vol. of ice-cold water in an Ultra-Turrax homogenizer. The flask was chilled with crushed ice before and during the homogenization. Homogenates prepared in this way contain about 20–30 mg. of protein/ml.

Solubilization of the disaccharidases

To 10 ml. of mucosal homogenate, prepared as described above, were added: 1.2 ml. of 0.5 M-potassium phosphate buffer, pH 7.0, 0.65 ml. of a freshly prepared cysteine solution (10 mg. of cysteine hydrochloride/ml. in water) and 0.15 ml. of a papain preparation (crystalline papain suspension, 20 mg. of papain/ml.; Mann Research Laboratories, New York, N.Y., U.S.A.). After incubation at 37° for 1 hr. the solution was chilled with ice and then centrifuged in a Spinco preparative ultracentrifuge at 100 000g for 1 hr. to remove insoluble material. The solubilized disaccharidases were present in the clear supernatant.

In a few experiments the insoluble disaccharidases present in the mucosal homogenate were first spun down in the ultracentrifuge at 100 000g for 1 hr., then suspended in potassium phosphate buffer and solubilized with papain as described above.

Precipitation with ethanol

The supernatant containing the solubilized disaccharidases was well chilled with crushed ice, and 2 vol. of ethanol (previously chilled to -20°) was added slowly under stirring. The mixture was left for 30 min. in an ice bath and was then centrifuged in the Spinco ultracentrifuge at 6000g (rotor 50; 10 000 rev./min.) for 15 min. The sediment was dissolved in 0.01 M-potassium phosphate buffer, pH 7.0, to the original volume. Before use for chromatography the solution was dialysed against an appropriate buffer solution for 24 hr. at 4° . If turbidity appeared on dialysis, the solution was centrifuged clear. This did not remove any of the enzyme activities studied.

Ion-exchange chromatography

DEAE-cellulose (medium grade) was obtained from Sigma Chemical Co. (St Louis, Mo., U.S.A.). TEAE-cellulose* was prepared from DEAE-cellulose by ethylation as described by Porath (1957).

The chromatography columns were prepared in the following way: 1 g. of DEAE- or TEAE-cellulose was

stirred mechanically with 100 ml. of 0.01 M-potassium phosphate buffer, pH 7.0, at room temperature for 1 hr.; it was then packed into a 20 cm.-long glass column (1 cm. diam.) and rinsed with 100–200 ml. of the same buffer.

To this column was applied 2–4 ml. of a solubilized enzyme preparation containing 5–10 mg. of protein/ml. Elution was performed with potassium phosphate buffer, pH 7.0, by continuously increasing the molarity up to 0.10 M. The chromatography was performed at 4° with a flow rate of 5–10 ml./hr. and a fraction volume of 2–3 ml. The buffer concentration in the fractions collected was determined conductimetrically.

Gel-filtration chromatography

A 1 g. portion of Sephadex G-200 (Pharmacia AB, Uppsala, Sweden) was mixed with 300 ml. of 0.05 M-potassium phosphate buffer, pH 7.0, and allowed to swell at room temperature for at least 24 hr. with occasional mixing. Mechanical stirring was not used, since this was found to decrease markedly the flow rate of the columns. In some experiments other kinds of buffer were used.

After swelling, the gel was packed into a 50 cm.-long glass column (1 cm. diam.). The gel column obtained had a length of about 40 cm. It was rinsed with at least 100 ml. of 0.05 M-potassium phosphate buffer, pH 7.0, before use.

To this column was applied 2 ml. of solubilized disaccharidase solution, containing 5–10 mg. of protein/ml., and dialysed against the same buffer as had been used for the preparation of the column. Chromatography was performed at 4° with 0.05 M-potassium phosphate buffer, pH 7.0, a flow rate of 1 ml./hr. and a fraction volume of 0.5–1.0 ml.

For characterization of the dimensions of the Sephadex columns separate chromatograms were run in which a mixture of a high-molecular-weight dextran (blue dextran, mol.wt. 2000 000; Pharmacia AB) and NaCl was applied. The blue dextran was assayed in the effluent by measuring E_{630} and the NaCl by conductimetry. The dextran was completely excluded from the gel and thus indicated the 'void volume' of the column; the NaCl penetrated freely into the gel and indicated the 'total volume'.

Heat inactivation

Heat-inactivation experiments were performed in 0.01 M-sodium phosphate buffer, pH 7.0, as described previously (Dahlqvist, 1959, 1962).

Assay of disaccharidase activity

Disaccharidase activities were assayed either with the two-step method of Dahlqvist (1964) or with the one-step method of Messer & Dahlqvist (1966). These two methods gave identical results when the isomaltase, invertase or trehalase activities were measured. The maltase activity, however, was 40% lower when measured with the one-step method than when measured with the two-step method. This difference is known to be caused by interference of maltose with the coupled glucose oxidase reaction, i.e. with the assay system rather than with the disaccharidase activity as such (Messer & Dahlqvist, 1966). In accordance with this the difference between the one-step and the two-step assay of maltase activity was the same in each of the

* Abbreviation: TEAE-cellulose, triethylaminoethyl-cellulose.

maltase peaks separated on chromatography (see below) as for the crude preparation. The values given in the Figures for maltase activity refer to assay with the two-step method.

Assay of protein

Protein was assayed with the method of Lowry, Rosebrough, Farr & Randall (1951), with the modified reagent B described by Eggstein & Kreutz (1955). A freshly prepared solution of human serum albumin (Kabi AB, Stockholm, Sweden) was used for the preparation of a standard curve.

RESULTS

Solubilization of the disaccharidases

The disaccharidases were mainly insoluble in the homogenates, but incubation with papain solubilized the major part of the maltase, isomaltase and invertase activities. The trehalase, in contrast, was not solubilized (Table 1). This accords with the results of a previous study (Auricchio, Dahlqvist & Semenza, 1963).

The amount of papain used in the present investigation for the solubilization of the disaccharidases was considerably smaller than the amount used in the previous study (0.015 mg. of papain/mg. of mucosal protein as compared with 0.3 mg. of papain/mg. of mucosal protein).

Precipitation of the solubilized disaccharidases with ethanol

In earlier studies of the disaccharidases present in pig intestine, precipitation of the solubilized enzymes with ethanol, redissolving in buffer and removal of those components that had become insoluble on this treatment by ultracentrifugation were found to result in a 50-fold purification of

the maltase, isomaltase and invertase activities (Borgström & Dahlqvist, 1958). With human enzymes this procedure caused a much smaller degree of purification. However, the behaviour of the human disaccharidases on subsequent gel-filtration chromatography was found to be influenced by whether ethanol precipitation had been performed or not (see below).

The presence of potassium phosphate buffer protected the disaccharidase activity during precipitation with ethanol. The recovery of the disaccharidases after precipitation in the presence of potassium phosphate buffer was 85–100%, but if the enzyme solution had been dialysed against water before the precipitation the recovery was only 10–20%. Addition of potassium phosphate buffer to the dialysed enzyme solution before precipitation protected it against this inactivation.

Ion-exchange chromatography

Intestine from adult humans. (a) Ileal preparation. Chromatography of the papain-solubilized enzyme, without previous precipitation with ethanol, on DEAE- or TEAE-cellulose columns yielded two large peaks of maltase activity (Fig. 1). The isomaltase and invertase activities were located in the first of the two maltase peaks. Heat inactivation of the enzyme activities in the two peaks showed that the enzyme activities of the first peak were rather heat-sensitive, and that the maltase activity in this peak was inactivated partly in parallel with the isomaltase and partly in parallel with the invertase. This peak thus seems to contain the two fractions of maltase that have previously been named maltase Ia and maltase Ib (Dahlqvist, 1962). The maltase activity of the second large peak from the ion-exchange chromatography was much more stable against heat, and seemed to correspond to the maltase II and maltase III according to the nomenclature suggested after heat-inactivation studies on crude homogenate (Dahlqvist, 1962).

When the chromatography fractions were analysed for disaccharidase activities, no isomaltase or invertase activity could be detected in the second large maltase peak. When a number of fractions from this peak were combined, dialysed against water, freeze-dried and dissolved in a small volume, however, weak isomaltase and invertase activity could be detected that each amounted to about 2% of the maltase activity. This isomaltase and invertase activity might either be exerted by the enzymes that were mainly present in the second peak or by 'tailing' of the enzymes from the first peak. Heat-inactivation studies showed that the invertase activity of the concentrated second maltase peak was much more heat-sensitive than the maltase activity, whereas most of the isomaltase

Table 1. *Solubilization of the disaccharidases*

The disaccharidases are essentially completely insoluble in homogenates of the mucosa, but are solubilized by papain in the presence of K⁺ ions without inactivation. The Table shows the fraction of the disaccharidase activity that remains after papain digestion and the fraction that is soluble after papain digestion, all expressed as percentages of the original activity of the ileal homogenate. Incubation had been performed with 0.015 mg. of papain/mg. of mucosal protein at 37° for 1 hr.

	Total activity remaining after papain digestion (% of original activity)	Activity soluble after papain digestion (% of original activity)
Maltase	91	59
Isomaltase	93	81
Invertase	84	73
Trehalase	90	10

Fig. 1. TEAE-cellulose chromatogram of the solubilized ileal disaccharidases. For clarity, the chromatogram is presented in two parts: (a) ●, protein; Δ, concn. of buffer; (b) ●, maltase; ○, isomaltase; □, invertase. Two enzyme peaks were obtained, the first one of which contained maltase Ia (=isomaltase) and maltase Ib (=invertase), and the second one maltase II and maltase III.

was equally as heat-stable as the maltase. This indicated that the invertase activity of the second peak is caused by tailing, and the isomaltase activity found in this peak to a large extent may be exerted by the heat-stable maltases (maltase II and maltase III). In this respect the human enzymes have a specificity similar to that previously found in the pig (Dahlqvist, 1960a).

In some chromatograms an additional very small peak was seen, a little ahead of the first large maltase peak; this peak contained maltase and isomaltase activities (around fraction no. 24 in Fig. 1). The enzymes in this minor peak were not further studied in the present investigation since it was not found in all chromatograms. Later experiments have, however, shown that it is a reproducible and constant finding if sufficiently large amounts of enzymes are applied to the column and the enzyme assay is performed in a sensitive enough way (H. McMichael & A. Dahlqvist, unpublished work). This peak accounts for less than 1% of the total disaccharidase activity of the mucosa and therefore probably lacks physiological importance.

Fig. 2. DEAE-cellulose chromatogram of the solubilized disaccharidases from the jejunum of an about 20-week-old human foetus. For clarity, the chromatogram is presented in two parts: (a) ●, protein; Δ, concn. of buffer; (b) ●, maltase; ○, isomaltase; □, invertase. Only one peak was obtained, corresponding to the first large peak in Fig. 1. This fits with the results of heat-inactivation experiments, which have shown that at this stage of foetal development maltase II and maltase III are missing, although maltase Ia (=isomaltase) and maltase Ib (=invertase) are well developed (Dahlqvist & Lindberg, 1966).

The results of the ion-exchange chromatography were not influenced by precipitation of the solubilized enzymes with ethanol before application to the column.

(b) *Jejunal preparations.* Ion-exchange chromatography of the jejunal preparations gave the same results as described for the ileal preparation above. Ethanol precipitation of the solubilized enzyme had no influence on the results.

Intestine from human foetus. One ion-exchange chromatogram was run with a solubilized preparation of jejunal mucosa from a human foetus, obtained at about the twentieth week of intra-uterine life. At this time heat inactivation has shown that maltase II and maltase III were still missing, although the maltase Ia (=isomaltase) and maltase Ib (=invertase) activities were well developed (Dahlqvist & Lindberg, 1966). In

accordance with this only one peak was obtained in the ion-exchange chromatogram, corresponding to the first large peak of the chromatogram of adult preparations (Fig. 2).

Gel-filtration chromatography

Gel-filtration chromatography was performed only with the adult human preparations.

(a) Ileal preparation. Sephadex G-200 chromatography of the ileal preparation solubilized with papain but not precipitated with ethanol yielded only a single peak containing all the three disaccharidase activities studied (Fig. 3). This peak was eluted close to the 'void volume' of the column, indicating a large molecular size.

Attempts were made to alter the behaviour of the enzymes on the Sephadex G-200 columns by varying the concentration of the potassium phosphate buffer between 0.01 and 0.10M, performing the chromatography in 1M-glycine buffer, pH 7.0, which because of its strong polarity has been found to split intermolecular bonds between protein molecules (Porath & Ui, 1964), or further digestion of the solubilized disaccharidases with a large excess of papain (0.5 mg. of papain/mg. of mucosal protein at 37° for 1 hr.). In some experiments the particle-bound disaccharidases also were isolated from the soluble proteins of the homogenate by ultracentrifugation before incubation with papain. None of these treatments had any effect on the results of Sephadex chromatography except that a broad glucose peak, which appeared after the isomaltase peak, was larger at low buffer concentration (0.001M). This release of glucose apparently resulted from the action of isomaltase (which also has dextranase activity) (Dahlqvist, 1963; H. McMichael & A. Dahlqvist, unpublished work) on the dextran in the gel particles. That the effect was stronger in dilute buffers may depend on a more extensive swelling of the gel.

When the solubilized disaccharidases were precipitated with ethanol and redissolved before application on the column, however, two peaks were obtained. Maltase and invertase were present in both of these peaks, but isomaltase was only present in the more slowly eluted one (Fig. 4).

(b) Jejunal preparations. The jejunal preparations differed from the ileal one in that, when the solubilized preparations were applied to a Sephadex column without previous precipitation with ethanol, two peaks of disaccharidase activity were obtained (Fig. 5). Both these peaks contained maltase, but only the second one contained measurable amounts of isomaltase and invertase activity. The two peaks apparently correspond to those seen in the ion-exchange chromatograms, but appear in the reverse order in the effluent from the columns.

Fig. 3. Sephadex G-200 chromatogram of the solubilized ileal disaccharidases without previous ethanol precipitation of the enzymes. For clarity, the chromatogram is presented in two parts: (b) ●, protein; (c) ●, maltase; ○, isomaltase; □, invertase. (a) Control chromatogram performed on the same column with blue dextran (●), indicating the 'void volume', and NaCl (○), indicating the total volume of the column. Most of the protein present is strongly retarded and appears around the total volume. This is probably chiefly oligopeptides, and shows that the papain digestion has been effective. In spite of this the disaccharidases are eluted as a single peak close to the 'void volume', indicating a large molecular size.

When ethanol precipitation was performed before the chromatography a fraction of the invertase activity (and presumably a corresponding fraction of the maltase Ia activity) was recovered in the first maltase peak, whereas the major fraction of these activities was still recovered in the second maltase peak (Fig. 6).

As with the ileal preparation a broad glucose peak appeared if the chromatogram was run at a low buffer concentration.

Fig. 4. Similar chromatogram to that shown in Fig. 3, but after that the enzyme preparation had been precipitated with ethanol in the cold and redissolved in buffer. For clarity, the chromatogram is presented in two parts: (b) ●, protein; (c) ●, maltase; ○, isomaltase; □, invertase. (a) Control chromatogram performed on the same column with blue dextran (●), indicating the 'void volume', and NaCl (○), indicating the total volume of the column. The oligopeptide peak has disappeared (because it was not precipitated by the ethanol). The disaccharidases are now retarded on the column, and separated into two major peaks.

DISCUSSION

The results of the ion-exchange chromatography support the previously reported results of heat-inactivation studies (Dahlqvist, 1962; Dahlqvist *et al.* 1963). The enzymes previously named maltase Ia (=isomaltase) and maltase Ib (=invertase) are obtained in one common peak, and the enzymes named maltase II and maltase III in

Fig. 5. Sephadex G-200 chromatogram of solubilized jejunal disaccharidases without previous ethanol precipitation of the enzymes. The column used was similar to that used for the chromatograms shown in Figs. 3 and 4. □, Maltase; ○, isomaltase; ●, invertase. Two disaccharidase peaks were obtained, not completely separated from each other. The first peak contained maltase II and maltase III, whereas the second peak contained maltase Ia (=isomaltase) and maltase Ib (=invertase). There was no separation between the isomaltase and invertase activities. (The weak isomaltase activity of maltase II and maltase III could not be demonstrated without previous concentration of the enzyme peak.)

Fig. 6. Sephadex G-200 chromatogram of solubilized jejunal disaccharidases after ethanol precipitation of the enzymes. □, Maltase; ○, isomaltase; ●, invertase. As in Fig. 5 two major disaccharidase activities were obtained, but some of the invertase was now present in the first peak, separated from the isomaltase. This first small invertase peak is the one that was designated invertase 1 by Semenza and co-workers (Semenza & Auricchio, 1962; Semenza, Auricchio & Rubino, 1965; Auricchio, Semenza & Rubino, 1965). It consists probably of a fraction of the invertase that has been dissociated from the isomaltase, and consequently is not retained on the column (Kolínková & Semenza, 1967).

another. In addition, a further small maltase-isomaltase peak was seen, which was, however, not further characterized in the present investigation,

and which accounted for only a very small fraction of the total disaccharidase activity.

The finding that the isolated and concentrated two heat-stable maltases (maltase II and maltase III) have weak isomaltase activity accords with the specificity of the corresponding enzymes in the pig (Dahlqvist, 1960a,b). In the previously published heat-inactivation study of the human disaccharidases isomaltase activity could not be measured in this heat-stable maltase fraction, but it was also stated that an isomaltase activity exerted by these enzymes, and equally weak in relation to the maltase activity as that in the pig, would not have been detected under the conditions used in the experiments with the human preparation (Dahlqvist, 1962). Later E. Eggermont (personal communication) in ultracentrifugation studies and Semenza and co-workers (Semenza *et al.* 1965; Auricchio *et al.* 1965) in gel-filtration studies have stated that the two more heat-stable human maltases also have weak isomaltase activity. Our present results thus perfectly agree with this statement. The isomaltase activity of the heat-stable enzymes is only 2% of their maltase activity and accounts for only about 1% of the total isomaltase activity of crude homogenates of human small-intestinal mucosa.

The results of the gel-filtration chromatography are more difficult to interpret. The solubilized enzymes of the ileal preparation were eluted close to the 'void volume', with no separation between the different activities measured. After the enzyme had been precipitated with ethanol and redissolved before chromatography, however, a separation between the different activities was obtained. Nevertheless the same preparation could be separated into several peaks on ion-exchange chromatography without previous ethanol precipitation. The jejunal preparations in contrast were separated into two peaks both on gel-filtration chromatography and on ion-exchange chromatography without previous ethanol precipitation. In this case also, however, the precipitation with ethanol resulted in an alteration in the results of the gel-filtration chromatography so that the invertase activity was separated into two components after such a precipitation.

According to Semenza and co-workers (Semenza & Auricchio, 1962; Semenza *et al.* 1965; Auricchio *et al.* 1965), who reported a similar fractionation of the invertase on Sephadex chromatography, the corresponding maltase activity (maltase Ib) is also subfractionated in a similar way. The two invertase fractions have the same heat-sensitivity, have identical pH-activity curves and are activated to the same degree by Na^+ ions (Auricchio *et al.* 1965). They also have approximately the same K_m value for sucrose as substrate. This similarity between the

enzymic properties of the two invertase fractions, plus the fact that they can only be separated after previous precipitation with ethanol, suggests that the fractionation may be an artifact. Kolínská & Semenza (1967) have made observations that seem to explain the occurrence of this artifact. The major parts of the invertase and isomaltase in the mucosa are present bound together as 'hyphozymes'. They therefore appear in a common peak in chromatograms. Since isomaltase interacts with the Sephadex gel as with a substrate, this peak is retained on the column. A small fraction of the invertase has, however, become dissociated from the isomaltase. This minor invertase fraction is not retained, but eluted close to the 'void volume', thus forming the peak designed as invertase 1 by Semenza and co-workers (Semenza & Auricchio, 1962; Semenza *et al.* 1965; Auricchio *et al.* 1965). This explanation fits well with our finding that under certain conditions glucose was liberated from the Sephadex by enzymic hydrolysis.

With this background the new nomenclature proposed by Semenza and co-workers for the intestinal maltase, invertase and isomaltase activities (Semenza & Auricchio, 1962; Semenza *et al.* 1965; Auricchio *et al.* 1965) should be abandoned. This nomenclature distinguishes between two invertases (invertase 1 and invertase 2) that are probably only two forms of the same enzyme, and five maltases (maltases 1-5), of which maltase 3 and maltase 4 probably also are identical. The suggestion that this nomenclature should be based on the molecular size of the different enzymes, and that this should be a better foundation for nomenclature than the heat-stability (Auricchio *et al.* 1965), also seems unjustified, since the separation of these enzymes on Sephadex does not seem to be based on their molecular size. The nomenclature based on heat-stability [maltase Ia (= isomaltase), maltase Ib (= invertase or sucrase), maltase II and maltase III] (Dahlqvist, 1962) seems to give a better description of the number of enzymes actually present, and also has the advantage that the fractionation on which the nomenclature is based can be performed with homogenates that have not been subjected to any solubilization procedure. Since the solubilization of the intestinal disaccharidases involves digestion with proteolytic enzymes, this procedure offers possibilities for partial degradation of the enzymes, which in turn may give rise to artificial separations on chromatography.

However, the heat-inactivation nomenclature of the disaccharidases is also to be regarded as an arbitrary one, which may be replaced with a more rational nomenclature when we have obtained more detailed knowledge about the specificity and structure of the enzymes present.

The subfractionation of the invertase on gel

filtration after ethanol precipitation implies a partial separation of invertase from isomaltase. Heat-inactivation experiments have shown that isomaltase is more heat-sensitive than invertase (Dahlqvist, 1962), which suggested that the two activities were exerted by two different enzymes. However, patients with sucrose intolerance as a result of the congenital absence of small-intestinal invertase always seem to lack intestinal isomaltase activity as well, i.e. the inherited congenital defect seems to affect the two enzyme activities simultaneously (Auricchio, Dahlqvist, Mürset & Prader, 1963). Because of this finding it has been questioned whether different heat-inactivation properties are sufficient to characterize the two activities as exerted by two separate enzymes (Launiala, Perheentupa, Visakorpi & Hallman, 1964). Further experiments have, however, shown that the two activities can be separated, not only by heat inactivation, but also by the fact that kinetic experiments with the two substrates in mixture show that sucrose and isomaltose are hydrolysed independently, i.e. one does not inhibit the hydrolysis of the other, which would be the case if they were hydrolysed by the same enzyme (Dahlqvist *et al.* 1963). These kinetic experiments have been verified by Auricchio *et al.* (1965).

As was mentioned above, the separation of the intestinal disaccharidases into two peaks on Sephadex G-200 columns seems not to be related to different molecular size, as was first believed (Auricchio *et al.* 1965), but to a substrate-enzyme interaction between the gel and isomaltase, which retains the isomaltase (maltase Ia), and the invertase (maltase Ib) when the latter protein molecule is associated with the isomaltase molecule (Kolinská & Semenza, 1967). Then the effect of ethanol precipitation on the behaviour of the jejunal enzymes during Sephadex G-200 chromatography may be the result of an action of ethanol either on the activity of isomaltase, causing a partial inactivation, or on the link between isomaltase and invertase, causing a partial dissociation of these two enzymes. The ileal isomaltase behaved differently in the present study since this enzyme was not retained on the Sephadex column until after it had been precipitated with ethanol. This may reflect a change in the protein molecule, making the active centre of the ileal isomaltase accessible for interaction with the

polysaccharide gel after ethanol precipitation. It is remarkable, however, that, although the ileal isomaltase was not retained on the Sephadex until after ethanol precipitation, it could liberate glucose from the Sephadex before such precipitation had been performed. The effect of ethanol precipitation on the human small-intestinal disaccharidases should be subjected to further research.

This investigation has been supported by grants from the Swedish Nutrition Foundation and the Swedish Medical Research Council (Project no. 13X-157). The authors are indebted to the staff of the Surgical Clinic of the University Hospital for making human intestinal preparations removed during operations available for study. Mrs Karin Thörnqvist, Miss Maj Britt Dahl, Mrs Anita Olsson and Mrs Birgitta Norén are acknowledged for skilful technical assistance.

REFERENCES

- Auricchio, S., Dahlqvist, A., Mürset, G. & Prader, A. (1963). *J. Pediat.* **62**, 165.
- Auricchio, S., Dahlqvist, A. & Semenza, G. (1963). *Biochim. biophys. Acta*, **73**, 582.
- Auricchio, S., Semenza, G. & Rubino, A. (1965). *Biochim. biophys. Acta*, **96**, 498.
- Borgström, B. & Dahlqvist, A. (1958). *Acta chem. scand.* **12**, 1997.
- Dahlqvist, A. (1959). *Acta chem. scand.* **13**, 945.
- Dahlqvist, A. (1960a). *Acta chem. scand.* **14**, 72.
- Dahlqvist, A. (1960b). Dissertation: University of Lund.
- Dahlqvist, A. (1962). *J. clin. Invest.* **41**, 463.
- Dahlqvist, A. (1963). *Biochem. J.* **86**, 72.
- Dahlqvist, A. (1964). *Analyt. Biochem.* **7**, 18.
- Dahlqvist, A., Auricchio, S., Semenza, G. & Prader, A. (1963). *J. clin. Invest.* **42**, 556.
- Dahlqvist, A. & Lindberg, T. (1966). *Clin. Sci.* **30**, 517.
- Eggstein, M. & Kreutz, F. H. (1955). *Klin. Wschr.* **33**, 879.
- Kolinská, J. & Semenza, G. (1967). *Biochim. biophys. Acta*, **146**, 181.
- Launiala, K., Perheentupa, J., Visakorpi, J. & Hallman, N. (1964). *Pediatrics, Springfield*, **34**, 615.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Messer, M. & Dahlqvist, A. (1966). *Analyt. Biochem.* **14**, 376.
- Porath, J. (1957). *Ark. Kemi*, **11**, 97.
- Porath, J. & Ui, N. (1964). *Biochim. biophys. Acta*, **90**, 324.
- Semenza, G. & Auricchio, S. (1962). *Biochim. biophys. Acta*, **65**, 173.
- Semenza, G., Auricchio, S. & Rubino, A. (1965). *Biochim. biophys. Acta*, **96**, 487.