The Invertase Activity of Rabbit Small Intestine

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Most recent publications describing the properties of invertase refer to enzyme preparations obtained from plants, bacteria, fungi, protozoa or the honey bee (Edelman, 1956; Allen & Bacon, 1956; Pavlinova & Kursanov, 1956; Christie & Porteous, 1957; Carnie & Porteous, 1959*a*; Howard, 1959). Borgström & Dahlqvist (1958), Dahlqvist & Borgström (1959) and Dahlqvist (1958, 1959*a-c*, 1960*a-f*, 1961) have provided the only recent systematic accounts of the properties of an invertase obtained from an animal source, namely hog intestine.

The characterization of the invertase activity of the mucosal cells of rabbit small intestine is now described. A brief account of some of the work has been published (Carnie & Porteous, 1959*b*).

EXPERIMENTAL

Animals. Dutch rabbits of either sex and weighing 1.5–3.0 kg. were kept on a diet of cabbage, rabbit cake and water for several days before an experiment. For the 24 hr. before an experiment all solid food was withheld, the cages were cleaned of faeces twice during the starvation period, but water was allowed *ad libitum*. The primary purpose of starvation was to free the small intestine of partially digested food.

Isolation of mucosal cells of small intestine. A single rabbit was used for each experiment and killed under light chloroform anaesthesia by a sharp blow across the neck. The complete small intestine was rapidly excised and transferred to chipped ice. The chilled intestine was cut into approximately 15 cm. lengths, fat and omentum were trimmed away and the lumen was washed out with 0.9% NaCl or with 0.25M-sucrose at 2°. Each length of trimmed and washed intestine was cut open longitudinally, laid mucosal side uppermost on a cold glass plate and the mucosal layer removed with the aid of a glass slide into a tared beaker immersed in chipped ice. All subsequent manipulations were carried out in pre-cooled vessels immersed in chipped ice or contained in centrifuges operated at 2–5°.

Cell homogenization. The weighed mucosal cell preparation (usually 30 g.) was suspended in 4 vol. of ice-cold freshly prepared 0.25M-sucrose. This suspension was treated in a Waring Blendor at approximately 7500 rev./min. for 1 min., then for four 1 min. periods at approximately 15 000 rev./min. with 15 sec. intervals between each maceration.

Centrifuging. The disrupted cell preparation was centrifuged at 10 000*g* for 15 min. (MSE 13 centrifuge, rotor no. 69142), the sediment discarded and the supernatant suspension centrifuged at 100 000*g* for 45 min. (Spinco model L centrifuge, rotor no. 40). The supernatant solution (which was free of invertase activity) was removed. The sediment (hereafter called the 10⁶*g* fraction) was used as the source of enzyme for further experiments.

Reagents. Sucrose, lactose and glucose were AnalaR reagents (British Drug Houses Ltd.); fructose was obtained from Hopkin and Williams Ltd., turanose from British Drug Houses Ltd. and maltose from Thomas Kerfoot, Ashton-under-Lyne, Lancs. Uniformly (U) labelled [U-¹⁴C]glucose and [U-¹⁴C]fructose, specific activity 20 μ C/mg., were obtained from The Radiochemical Centre, Amersham, Bucks. All sugars except maltose (which contained a trace of maltotriose) were pure when tested by the chromatographic procedure detailed below. Sodium dihydrogen phosphate dihydrate, KH₂PO₄, (NH₄)₂SO₄, trichloroacetic acid, sodium acetate, trihydrate and acetic acid were AnalaR reagents. Sodium deoxycholate was purchased from Hopkin and Williams Ltd. Butan-1-ol was fractionally redistilled before use; ethanol was refluxed over KOH and distilled from CaO.

Chromatography. Sugars were separated by descending chromatography at 22–24° during 24–36 hr. on Whatman no. 54 paper with the butan-1-ol-acetic acid-water (4:1:5, by vol.) solvent of Partridge (1948). Where samples from an enzyme-catalysed reaction were to be chromatographed the starting points of the chromatogram were pretreated with 5 μ l. of 0.01M-HgCl₂ to stop enzyme activity. Each starting point normally received 5–10 μ l. of a solution that contained initially 0.1–0.5M-sucrose. Free and combined monosaccharides were detected on air-dried chromatograms with the benzidine-trichloroacetic acid reagent of Bacon & Edelman (1951). Free and combined ketoses were detected with the urea-phosphoric acid reagent of Wise, Dimler, Rist & Davis (1955).

Radioactivity measurements. ¹⁴C-Labelled sugars separated by paper chromatography were located with the benzidine-trichloroacetic acid spray reagent. A vertical line, representing the track of the chromatographically separated sugars, was drawn from the starting point to the lower end of the paper. Two parallel lines were drawn 1.5 cm. on either side of this line and the resulting strip of paper was cut along horizontal lines spaced at 1.5 cm. intervals to give serial sections of paper, 1.5 cm. $\times$ 3.0 cm. Each section was bisected and the radioactivity of each square of paper determined with a halogen-quenched end-window Geiger-Müller counter and a type N 530D autoscaler (Ekco Electronics Ltd., Essex). Since the activities of different areas of the chromatograms were expected to differ widely the time required to record 300 counts was determined for each square of paper; these values were converted into counts/min. and corrected for the back-

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ground count. Scanning and spraying of the residual chromatogram showed that all radioactive and benzidine-positive material had been accounted for.

Determinations of pH. All measurements were made at 18–20° with a Model 30 pH meter (Electronic Instruments Ltd., Richmond, Surrey) standardized at the same temperature.

Determinations of total nitrogen. Samples were analysed by Kjeldahl digestion for 18–24 hr. with the catalyst of Chibnall, Rees & Williams (1943), followed by distillation (Markham, 1942) of the NH_3 under alkaline conditions into boric acid–bromocresol green–methyl red solution (Conway & O'Malley, 1942) and titration of the trapped NH_3 with approximately 0.01 N- H_2SO_4 .

Determinations of free reducing sugar. Eluates from chromatograms and samples from enzyme-assay systems were analysed for free reducing sugars by the colorimetric method of Nelson (1944). Glucose and fructose gave identical calibration curves.

Determinations of total reducing sugar. Eluates from chromatograms were subjected to hydrolysis in 0.25 N-HCl or in 0.5% oxalic acid at 100° for 30–60 min. followed by neutralization of the cold hydrolysate with 0.25 or 0.22 N-NaOH, respectively. The neutralized solution, after being made to a known volume with water, was analysed for reducing sugar as detailed above.

Determinations of total fructose. Eluates from chromatograms were analysed by the colorimetric method of Roe, Epstein & Goldstein (1949).

Assay of invertase activity. In assaying the invertase activity of the 10^5g fraction and subfractions, in determining the kinetic characteristics of partly purified invertase and in experiments concerned with the formation of oligosaccharides by a partially purified invertase, deproteinization of samples removed from the incubation system was found to be unnecessary. Buffered substrate was preincubated at 37° for 10 min. Addition of the enzyme preparation (at 2°) brought the volume to 5 ml. Two controls were always incubated at the same time, one without enzyme and one without substrate. After incubation at 37° for a known time samples were measured directly into 2 ml. of the alkaline CuSO_4 reagent (Nelson, 1944); enzyme activity was thus terminated immediately. The contents of the Nelson reaction tubes were made to 4 ml. with water before the determination of free reducing sugars.

In using this system to determine optimum conditions for the assay of the hydrolytic activity of the enzyme the composition and pH of the buffer, the substrate concentration, the enzyme concentration and the time of incubation were varied in turn. In following oligosaccharide formation in this system the pH was held at 6.7 in 0.04 M- Na_2HPO_4 – NaH_2PO_4 buffer; the initial substrate concentration and the time of incubation at 37° were varied as stated in the text.

Enzyme unit. An invertase unit is defined as the amount of enzyme required to produce 1 μmole of free hexose from 0.1 M-sucrose in 0.04 M- Na_2HPO_4 – NaH_2PO_4 buffer, pH 6.7, when incubated at 37° for 60 min.

Specific enzyme activities. These are expressed as the number of enzyme units/mg. of total nitrogen.

RESULTS

Stabilization of the invertase activity. The particulate invertase preparation (10^5g fraction) lost

approximately 50% of its activity on storage for 1 week at –20°; attempts to stabilize the activity were combined with attempts to solubilize the enzyme. The 10^5g fraction from 20 g. of mucosal cells was suspended in 100 ml. of water and dialysed for 48 hr. at 2° against distilled water; the non-diffusible material was termed fraction 1. A similar portion of the 10^5g fraction was suspended in 100 ml. of sodium deoxycholate (0.5%) at pH 7.5 in 0.1 M- KH_2PO_4 – Na_2HPO_4 buffer at 2° for 5 min. Centrifuging (100 000g for 60 min. at 2°) yielded a clear supernatant extract, which after dialysis as before gave fraction 2 with a 1.7-fold increase in activity over fraction 1. Approximately 80% of the activity of fraction 2 was retained in the supernatant (fraction 3) when fraction 2 was stored at –20° for 24 hr., thawed and centrifuged (100 000g for 60 min. at 2°); the remainder of the activity was recovered from the sediment. A further portion of the deoxycholate extract (before dialysis) was incubated for 10 min. at 37° with $\frac{1}{2}$ vol. of butan-1-ol, centrifuged (1000g for 20 min. at 2°) and the aqueous layer dialysed as before. The non-diffusible material contained the same number of enzyme units as did fraction 1. Storage of this material at –20°, followed by thawing and centrifuging (100 000g for 60 min. at 2°), yielded a clear supernatant (fraction 4) containing 60% of the activity of fraction 1; the remainder of the activity was recovered from the sediment. Fraction 3 (specific activity 70) and fraction 4 (specific activity 180) appeared to contain the invertase in solution. Fraction 1, when treated with butan-1-ol, gave an aqueous layer which, after dialysis, was devoid of enzyme activity.

Fractions 3 and 4 retained all their invertase activity for at least 10 days when stored at 2°, and for several weeks when stored at –20°, although both fractions became slightly turbid on thawing. Fraction 3 when freshly isolated became slightly turbid under enzyme-assay conditions whereas fraction 4 remained water-clear under the same conditions.

Kinetic characteristics of the invertase. Fraction 4 was used to determine the kinetic characteristics of the invertase and the optimum conditions for its assay. The hydrolytic activity of the invertase was optimum at pH 6.7 (Fig. 1). The hydrolytic activity–substrate concentration plot for the enzyme (Fig. 2) showed that at pH 6.7 the enzyme became saturated with substrate at a concentration of 0.1 M-sucrose. A double-reciprocal plot (Lineweaver & Burk, 1934) of the data shown in Fig. 2 was consistent with the formation of a substrate-activated enzyme–substrate complex (Dixon & Webb, 1958). A linear double-reciprocal plot, obtained over a restricted range of substrate concentration, is shown in Fig. 3, from which apparent

K_m 6.2 mM-sucrose and V_{max} 3.2 μ moles of reducing sugar/hr. were determined.

At pH 6.7 (0.04 M-sodium phosphate buffer) and with an initial sucrose concentration of 0.1 M it was further shown that hydrolytic activity was proportional to enzyme concentration over a tenfold range of enzyme concentration and that hydrolysis was proportional to time for at least 120 min.

Fig. 1. Effect of pH on the hydrolytic activity of an invertase preparation (fraction 4) obtained from the mucosal cells of rabbit small intestine. Incubation was for 60 min. ●, Phosphate buffers (described in the Experimental section); ○, 0.04 M-acetate buffer.

Fig. 2. Effect of sucrose concentration on the hydrolysis of sucrose by an invertase preparation (fraction 4) obtained from the mucosal cells of rabbit small intestine. Incubation was for 60 min., with phosphate buffer, pH 6.7 (see Fig. 1).

From these data the optimum conditions used for enzyme assay were established. The enzyme concentration was always adjusted, unless otherwise stated, so that no more than 6 % of the initial substrate was hydrolysed.

In separate experiments it was shown that replacement of the Na_2HPO_4 - NaH_2PO_4 buffer, pH 6.7, by Na_2HPO_4 - KH_2PO_4 buffer of the same molarity and pH value decreased the activity of the enzyme by approximately 30 %. This effect has not been further investigated.

Oligosaccharide formation by an intestinal invertase preparation. The 10⁵g fraction, fraction 3 and fraction 4 contained maltase, invertase and trehalase activity (Carnie & Porteous, 1962).

Incubation of fraction 3 enzyme with sucrose in 0.04 M-phosphate buffer, pH 6.7, followed by chromatographic separation of the substrate and reaction products, showed that sucrose, glucose, fructose and a single oligosaccharide with a mobility 0.6 of that of sucrose (R_{sucrose} 0.6) could be detected with the benzidine spray reagent. This oligosaccharide gave a positive (brown) reaction to the benzidine spray reagent and a blue reaction with the urea-phosphoric acid reagent, which indicated that it contained both aldose and ketose sugars. The results of several experiments are shown in Table 1. Oligosaccharide formation increased with increasing initial substrate concentration; for a given initial substrate concentration oligosaccharide formation at first increased with time of incubation, then decreased again. This latter semi-quantitative observation is consistent with the quantitative results of Bealing & Bacon (1953), who showed that oligosaccharides formed by mould invertase are themselves hydrolysed by the enzyme.

Fig. 3. Lineweaver-Burk plot of data shown in Fig. 2. ●, Data obtained over the range 0.005-0.5 M-sucrose (upper abscissa); ○, data obtained over the range 0.02-0.1 M-sucrose (lower abscissa).

A trace of a second oligosaccharide ($R_{\text{sucrose}} 0.25$) was occasionally detected with the benzidine spray reagent when the enzyme was incubated with 0.5 M-sucrose until approximately 17 % apparent hydrolysis of initial substrate had occurred.

In the above-mentioned experiments, samples taken from the two control tubes of the assay system failed to show the presence of any oligosaccharide and none was found when the enzyme was incubated with a mixture of glucose and fructose in the absence of sucrose.

In an incubation system containing 10 % (v/v) of ethanol and 0.1 M-sucrose neither ethyl glucoside nor ethyl fructoside was produced by the enzyme and no inhibition of apparent hydrolytic activity occurred (cf. Bacon, 1952; Bealing, 1953; Christie & Porteous, 1957).

Incubation of the same enzyme preparation with 0.1 M-maltose in place of sucrose gave free glucose and two oligosaccharides, neither of which was identical (as judged by chromatographic mobility and reaction to spray reagents) with those formed from sucrose. That with greater mobility was possibly maltotriose since it appeared in the same position as the impurity in maltose. The other oligosaccharide was possibly a tetrasaccharide. Neither was analysed. When 0.1 M-lactose replaced sucrose in the incubation system neither hydrolysis nor oligosaccharide formation could be detected.

The formation of the oligosaccharide ($R_{\text{sucrose}} 0.6$) from sucrose and of two oligosaccharides from maltose was confirmed when fraction 4 enzyme replaced fraction 3 enzyme in similar experiments.

Analysis of the oligosaccharide ($R_{\text{sucrose}} 0.6$) formed from 0.5 M-sucrose by fraction 3 enzyme in four experiments and eluted from chromatograms by the method of Dent, Stepka & Steward (1947) gave the results shown in Table 2. In Expts. 1 and 2 the apparent free reducing sugar values accounted for approximately 5 % of the total reducing sugar; in Expts. 3 and 4 the corresponding value was 2 %. The free reducing sugar values in Expts. 2,

3 and 4 (Table 2) were within the limits of error of the analytical procedure. It appeared therefore that the oligosaccharide had poor reducing power or was a non-reducing sugar that accounted for 3-4 % of all sugars present in the incubation system (Table 2). Separate experiments showed that under the conditions of hydrolysis used in Expts. 1 and 2 (Table 2) the oligosaccharide was completely hydrolysed to glucose and fructose only. Under the conditions of hydrolysis used in Expts. 3 and 4 (Table 2) a trace of the oligosaccharide remained, the other products being glucose and fructose only. On the assumption that the small amount of apparent free reducing sugar (Table 2) could be ignored, the glucose content of the oligosaccharide was obtained as the difference between the total reducing sugar and the total fructose determinations. The ratio of glucose to fructose was thus determined in the four experiments as 1.32, 1.16, 0.82, 0.90. The first of these values did not take account of any sugars present in the chromatographic paper (Table 2); the mean of the three other determinations is 0.96.

Incorporation of [$U^{14}C$]glucose and of [$U^{14}C$]fructose into the products of action of invertase on sucrose. Four reaction tubes were set up containing (1) 0.5 M-sucrose, 0.04 M-phosphate buffer, pH 6.7; (2) as (1) but with 30 μ C of [$U^{14}C$]fructose (about 1.5 mg.) added initially; (3) as (1) but with 30 μ C of [$U^{14}C$]glucose (about 1.5 mg.) added initially; (4) as (1) but with sucrose omitted and replaced by 0.5 M-glucose and 0.5 M-fructose. A drop of toluene and of chloroform was added to each tube and the contents were incubated at 37° for 5 min. before bringing the volume to 1 ml. by adding to each a dialysed deoxycholate extract of the 10⁶g fraction of mucosal cells (fraction 2). After incubation for 24 hr., 10 % apparent hydrolysis of the sucrose had occurred; a sample (5 μ l.) was removed from each incubation tube and applied to chromatograms, which were prepared, developed, sprayed and cut into sections as described above.

Table 1. *Oligosaccharide formation at various sucrose concentrations*

Fraction 3 invertase was incubated with different initial concentrations of sucrose for various times at 37° and pH 6.7 and the amount of oligosaccharide ($R_{\text{sucrose}} 0.6$) estimated. O, Relative amounts of oligosaccharide formed as judged visually from spot size and intensity. H, Apparent hydrolysis (%) as determined by direct analysis of reaction mixture for free reducing sugars.

Initial sucrose concn. (M) ... Incubation time (hr.)	Amount of oligosaccharide formed							
	0.05		0.1		0.25		0.5	
	O	H	O	H	O	H	O	H
2	0	17	—	—	—	—	—	—
4	—	—	1	—	—	—	—	—
5	0	42	—	23	—	—	—	3.7
10	—	—	2	39	—	—	—	—
12	0	80	3	45	3	23	2	10
24	—	—	1	85	4	45	16	20

Table 2. *Analysis of the oligosaccharide ($R_{\text{Sucrose}} 0.6$)*

Sucrose (0.5M) was incubated at 37° and pH 6.7 for 24 hr. with fraction 3 enzyme until 11–17% apparent hydrolysis had occurred (Table 1). In Expt. 1, 5 μ l. of the incubation system was applied to each of 20 separate points along the starting line of a chromatogram; in the other three experiments 5 μ l. was applied to each of eight points. Values quoted refer to the total oligosaccharide subsequently eluted from the individual chromatograms. Total reducing sugar was determined after hydrolysis with HCl (Expts. 1 and 2) or with oxalic acid (Expts. 3 and 4).

Expt.		Free reducing sugar (as glucose) (μ g.)	Total reducing sugar (as glucose) (μ g.)	Total ketose (as fructose) (μ g.)
1	Oligosaccharide	40	744	320
	Paper blank	—	—	—
	Net	40	744	320
2	Oligosaccharide	24	280	128
	Paper blank	12	12	4
	Net	12	268	124
3	Oligosaccharide	12	220	120
	Paper blank	8	16	8
	Net	4	204	112
4	Oligosaccharide	4	236	128
	Paper blank	0	8	8
	Net	4	228	120

Fig. 4. Radioactivity of chromatographically separated sugars formed during incubation of rabbit intestinal invertase (fraction 2) with sucrose and [^{14}C]fructose.

Fig. 5. Radioactivity of chromatographically separated sugars formed during incubation of rabbit intestinal invertase (fraction 2) with sucrose and [^{14}C]glucose.

Samples from tube (4) showed the presence of glucose and fructose only. Samples from tubes (1), (2) and (3) showed the presence (benzidine spray reagent) of the oligosaccharide ($R_{\text{Sucrose}} 0.6$) mentioned above, sucrose, glucose and fructose. Figs. 4 and 5 show the results obtained on determining the radioactivity in successive sections of the paper to which a sample from tubes (2) and (3) respectively had been applied. Separate experiments showed that the [^{14}C]glucose and [^{14}C]fructose when chromatographed in the presence of carrier glucose and fructose respectively were free of any other radioactive or benzidine-positive material.

Fig. 4 indicates that the intestinal invertase formed sucrose and oligosaccharide ($R_{\text{Sucrose}} 0.6$) by transglucosylation from sucrose to free fructose. The radioactive areas of the chromatogram corresponded to benzidine-positive areas. The results shown in Fig. 5 complement those shown in Fig. 4; the invertase preparation did not form either sucrose or oligosaccharide ($R_{\text{Sucrose}} 0.6$) by transfructosylation from sucrose to free glucose. Two areas of the chromatogram (Fig. 5) that failed to react with the benzidine spray reagent became labelled in the presence of [^{14}C]glucose. These areas of the chromatogram lay immediately above and

below the oligosaccharide of $R_{\text{sucrose}} 0.6$. It was subsequently shown that the material in these areas ($R_{\text{sucrose}} 0.8$ and 0.25) reacted slowly with an alkaline AgNO_3 reagent. Sucrose and the oligosaccharide ($R_{\text{sucrose}} 0.6$) also reacted slowly with this reagent.

DISCUSSION

Partially purified preparations (fractions 3 and 4) of the invertase and maltase activities of the mucosal cells of rabbit upper intestine have been obtained. The activity of either of these preparations was considerably more stable than that of the crude starting material. In experiments designed to determine the optimum substrate concentration of the enzyme it was found that hydrolytic activity increased smoothly with increasing initial substrate concentration to approach maximum activity at 0.1M -sucrose. There was no discontinuity in the plot of enzyme activity against initial substrate concentration and no decrease in activity with increasing substrate concentration up to 0.5M -sucrose. This contrasts with the situation reported for an invertase preparation obtained from three mixed species of protozoa (Christie & Porteous, 1957; Carnie & Porteous, 1959*a*) and for the invertase and maltase activity of hog intestine (Dahlqvist, 1960*e*). Evidence consistent with the formation of a substrate-activated enzyme-substrate complex (Fig. 3) must be treated with reserve until a more rigorously purified invertase preparation has been obtained from rabbit intestine.

It has been shown that incubation of sucrose with the partially purified invertase from rabbit intestine gave rise to three oligosaccharides ($R_{\text{sucrose}} 0.8$, 0.6 and 0.25). All three sugars reacted slowly with an alkaline silver nitrate reagent but only the sugar of $R_{\text{sucrose}} 0.6$ reacted with a benzidine spray reagent and with the ketose-sensitive urea-phosphoric acid spray reagent.

Whereas plant, bacterial, fungal and protozoal invertases appear to be β -fructosidases (Edelman, 1956; Allen & Bacon, 1956; Christie & Porteous, 1957; Howard, 1959), the honey-bee enzyme (White & Maher, 1953) and hog intestinal invertase (Dahlqvist, 1958, 1960*c*; Dahlqvist & Borgström, 1959) are α -glucosidases. In the present work it has been shown that the rabbit intestinal invertase also exhibited α -glucosidase activity, since it apparently transferred the α -glucosyl residue, but not the β -fructosyl residue, of sucrose to a suitable acceptor such as fructose. In this transfer reaction sucrose was resynthesized (Fig. 4); the only other invertase known to transfer a glycosyl residue to a hydroxyl group other than that of a primary alcohol or of water is a mould invertase (Edelman & Bealing, 1953).

The oligosaccharide ($R_{\text{sucrose}} 0.6$) was also presumably formed by transglucosylation from sucrose (Figs. 4 and 5); if the acceptor in this instance was sucrose, some of which had already become labelled with $[^{14}\text{C}]$ fructose, the product of the transfer reaction would have been a trisaccharide with a glucose:fructose ratio of 2. Hydrolysis of the sugar ($R_{\text{sucrose}} 0.6$) showed that it contained glucose and fructose only and analysis showed that it had a glucose:fructose ratio approximating closely to unity (Table 2); its chromatographic mobility was consistent with that of a disaccharide. It would appear therefore that this sugar was formed by transglucosylation from sucrose to free fructose to give a disaccharide. Since the sugar also appeared to be essentially non-reducing it seemed improbable that it was iso-maltulose, leucrose or maltulose [6-O- , 5-O- or $4\text{-O-}(\alpha\text{-D-glucopyranosyl})\text{-D-fructose}$ respectively]. Turanose [$3\text{-O-}(\alpha\text{-D-glucopyranosyl})\text{-D-fructose}$] was shown to have marked reducing power, giving about one-third of the extinction of an equal weight of glucose in the Nelson reaction; the new sugar was also distinguished from turanose since the latter had a chromatographic mobility essentially identical with that of sucrose in the solvent system used to separate sucrose and the new disaccharide. On the assumption that inversion about the anomeric carbon of glucose did not take place during transglucosylation, and that the glucosyl radical retained the pyran ring structure, the remaining possibilities are that the new disaccharide is $1\text{-O-}(\alpha\text{-D-glucopyranosyl})\text{-D-fructose}$ (Avigad, 1959), $2\text{-O-}(\alpha\text{-D-glucopyranosyl})\text{-}\alpha\text{-D-fructofuranoside}$, $2\text{-O-}(\alpha\text{-D-glucopyranosyl})\text{-}\alpha\text{-D-fructopyranoside}$ or $2\text{-O-}(\alpha\text{-D-glucopyranosyl})\text{-}\beta\text{-D-fructopyranoside}$. All of these sugars would be expected to react very slowly with the usual chromatographic alkaline silver nitrate reagents and to show little if any reducing power when tested with the alkaline copper sulphate reagent of Nelson (1944).

Transglucosylation from sucrose to the fructose moiety of another molecule of sucrose that had already become labelled with $[^{14}\text{C}]$ fructose (Fig. 4) would, as mentioned above, give a trisaccharide with a glucose:fructose ratio of 2. Subsequent hydrolysis of this trisaccharide by the enzyme so as to release the α -glucosyl residue of the original sucrose molecule would leave a glucosylfructose labelled in the fructose moiety (cf. Fig. 4). If the glucosylfructose ($R_{\text{sucrose}} 0.6$) was in fact formed by this mechanism, rather than by transglucosylation to free fructose, it could only have the first of the four possible structures mentioned above, assuming again that inversion about the anomeric carbon atom of glucose did not take place during transglucosylation and that the glucosyl residue retained its pyran ring structure.

SUMMARY

1. Invertase and maltase have been detected in a centrifugally isolated particulate fraction of disrupted mucosal cells of rabbit small intestine. The preparation was free of lactase activity.

2. The crude enzyme preparation has been partially purified and a relatively stable and apparently soluble form of the two activities obtained.

3. The kinetic characteristics of the invertase activity have been determined.

4. Oligosaccharide formation by the enzyme preparation incubated with sucrose or with maltose has been demonstrated. In the presence of the latter substrate two oligosaccharides were formed, one of which was possibly maltotriose and the other possibly a tetrasaccharide.

5. The invertase activity has been characterized as an α -glucosidase. On incubation with sucrose the enzyme incorporated [^{14}C]fructose into sucrose and into another apparently non-reducing, benzidine-positive, glucosylfructose. Two benzidine-negative sugars were detected as products of the action of the enzyme on sucrose in the presence of [^{14}C]glucose.

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The Metabolism of Potassium L-Serine O^[35S]-Sulphate in the Rat

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Dodgson, Lloyd & Tudball (1961a) and Dodgson & Tudball (1961) reported the presence in mammalian tissues of an enzyme system capable of bringing about the desulphation of L-serine O-sulphate *in vitro*. The enzyme is mainly located in the cell sap of rat liver and therefore coexists with the

enzyme system which is responsible for the sulphation of alcohols, phenols and steroids (see Spencer, 1960; Roy, 1956). Dodgson & Tudball (1961) have pointed out that the coexistence of desulphating and sulphating mechanisms could explain why it has not been possible to achieve the