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THE DIGESTION AND ABSORPTION OF SUCROSE BY THE INTACT RAT

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Since sucrose is an important dietary sugar both in man and animals, knowledge about its digestion and absorption is of considerable interest. During recent studies of the digestion and absorption of disaccharides in man, performed in our laboratory with an intubation technique, we found sucrose to be absorbed in the distal part of the small intestine, whereas glucose and lactose were absorbed in its proximal part (Dahlqvist & Borgström, 1961; Borgström, Dahlqvist, Lundh & Sjövall, 1957). Our experimental conditions did not permit the calculation of the total amount of the different sugars absorbed per unit time. The disaccharidase activity in the intestine could not be measured, since these enzymes are localized inside the mucosal cells, and are practically absent from the intestinal content.

We have therefore performed an investigation of the digestion and absorption of sucrose in the intact rat, compared with the absorption of an equivalent amount of a mixture of glucose and fructose. The sugars have been given together with an unabsorbable reference substance, so that the rate of absorption, the absorption index for the sugars in the different parts of the digestive tract, and the total amount of sugar absorbed could be measured.

The amounts of sucrose, glucose and fructose recovered have been measured individually. The rate of hydrolysis of sucrose *in vivo* has also been compared with the rate of hydrolysis of this disaccharide by homogenates of the intestine *in vitro*.

METHODS

Animals. Albino rats of both sexes of the Sprague-Dawley strain were used. The weight of the rats varied between 151 and 223 g, with a mean of 185 g. Before the experiment the rats fasted for 16–20 hr, with free access to water.

Sugars. Sucrose and glucose were obtained from Baker Chem. Co. (Phillipsburg, N.J., U.S.A.) and fructose from Pfanstiehl Lab. Inc. (Waukegan, Ill., U.S.A.). All sugars were of analytical grade purity.

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Expression of the weight of sugars. On complete hydrolysis 1·00 g of sucrose will yield 1·05 g of invert sugar (glucose plus fructose). In order to simplify the calculations in the present paper, the amount of sucrose found in the analyses has always been expressed as the weight of the invert sugar obtained on hydrolysis.

Reference substance. Polyethylene glycol (mol. wt. 4000) was obtained commercially. This substance is not absorbed in the intestine, and can thus be used as a reference substance for calculating the absorption index of water-soluble materials (Sperber, Hydén & Ekman, 1953; Borgström *et al.* 1957; Hydén, 1960; Dahlqvist & Borgström, 1961). It also makes possible the calculation of the intestinal transit of the water phase of a meal through the gastro-intestinal tract.

Absorption tests. The fasting rats were given either 760 mg sucrose (on hydrolysis this amount will yield 800 mg invert sugar) or 800 mg of an equimolar mixture of glucose and fructose, and 50 mg of polyethylene glycol, dissolved in 4 ml. water. The solution was given by stomach tube while the animals were lightly anaesthetized with ether. After the time allowed for absorption, the rats were again narcotized with ether and the abdomen was opened by a mid-line incision. Clamps were attached to the cardia, pylorus, ileum just proximal to caecum and to two loops of the small intestine, in order to avoid movement of the content of the gastro-intestinal tract during the subsequent preparation and to divide the small intestine into three approximately equal parts.

The entire gastro-intestinal tract was then removed. The small intestine was divided into three equal parts by adjusting the two clamps mentioned above. The segments of the gastro-intestinal tract thus obtained were: (1) stomach; (2) upper, (3) middle, (4) lower third of the small intestine; (5) large intestine (caecum and colon).

The time used for the preparation of these segments was less than 5 min. Each segment was immediately put into a test-tube containing 5–10 ml. distilled water, heated in a boiling water-bath for 2 min to inactivate the intestinal invertase, and then homogenized with distilled water in an Ultra-Turrax homogenizer. The homogenate of each segment was diluted to 100 ml. with distilled water.

Analytical methods

Determination of polyethylene glycol. Procedures for the turbidimetric determination of polyethylene glycol in different biological materials have been described by Hydén (1955). In our experiments polyethylene glycol was determined as follows: reagents; (1) Trichloroacetic acid solution; 150·0 g trichloroacetic acid and 25·0 g $BaCl_2$ dissolved in water to 500 ml. and filtered. (2) Barium hydroxide solution; 47·3 g $Ba(OH)_2$ dissolved in CO_2-free water to 1000 ml. and filtered; stored under protection from CO_2. (3) Barium chloride solution; 10·0 g $BaCl_2.2H_2O$ dissolved in water to 100 ml. (4) Zinc sulphate solution; 10·0 g $ZnSO_4.7H_2O$ dissolved in water to 200 ml. (5) Standard polyethylene glycol solution; 100·0 mg polyethylene glycol dissolved in water to 100 ml.

Procedure. In a centrifuge tube 5·0 ml. of the homogenate was mixed with 2·0 ml. of the barium hydroxide solution, 1·0 ml. of the barium chloride solution and 2·0 ml. of the zinc sulphate solution. The contents were mixed after each addition. The tubes were centrifuged at 1000 *g* for 5 min. 5·0 ml. of the clear supernatant was transferred to another test-tube and mixed with 5·0 ml. of the trichloroacetic acid solution. At the same time a stopwatch was started. The tube was left standing at room temperature (20° C). Exactly 15 min after the sample had been mixed with the trichloroacetic acid solution, the turbidity was measured in a spectrophotometer at a wave-length of 450 mμ, in 1 cm cuvettes.

Each day a standard series was prepared from a set of test-tubes containing 0·0, 0·1, 0·3, 0·5, 0·7, 0·9, and 1·1 ml. of the standard polyethylene glycol solution, diluted with water to 5·0 ml. and treated in the same way as the homogenates.

Precipitation of protein was performed with the zinc sulphate and barium hydroxide solutions (adjusted to give an ion-free supernatant) described by Somogyi (1945). For

4·0 ml. of the homogenate 2·0 ml. of each of the precipitation reagents was added. After centrifugation at 1000 *g* for 10 min the protein-free supernatant was decanted into a clean test-tube. This supernatant was used for the subsequent analysis for sugars.

Determination of glucose. A suitable amount of the protein-free supernatant (0·1–0·5 ml.) was diluted with water to 0·5 ml. After the addition of 3·0 ml. of the tris-glucose oxidase reagent (Dahlqvist, 1961*b*) the tube was immersed in a water-bath at 37° C for 1 hr for the colour to be developed. The intensity of the colour produced was measured spectrophotometrically against a blank without sugar at a wave-length of 420 mμ, in 1 cm cuvettes. The amount of glucose was calculated from a standard curve with glucose (0·01–0·05 mg). If the sample contained more than 0·05 mg of glucose the determination was repeated with a smaller sample.

Determination of fructose. A portion of the protein-free supernatant (0·5–2·0 ml.) was diluted with water to 2·0 ml. After the addition of 2·0 ml. of the 3,5-dinitrosalicylate reagent of Sumner (1924) (most readily prepared as described by Hostettler, Borel & Deuel, 1951) the tubes were heated in a boiling water-bath for 10 min, and then cooled with tap water. After the addition of 20·0 ml. of distilled water to each tube the content was mixed, and the intensity of the red colour measured in a spectrophotometer against a blank without sugar at a wave-length of 530 mμ, in 1 cm cuvettes.

The amount of reducing sugar (glucose plus fructose) present was calculated from a standard series, containing 0·5–4·0 mg glucose. Glucose and fructose yield the same extinction coefficient with the 3,5-dinitrosalicylate reagent. The amount of fructose present in the sample was calculated as the amount of reducing sugar minus the amount of glucose as determined with the tris-glucose oxidase reagent (see above).

Determination of sucrose. A portion of the protein-free supernatant (0·5–1·0 ml.) was diluted with water to 1·0 ml. Then 1·0 ml. of a solution of yeast invertase ('analytical'; Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.), 0·2 g/100 ml. 0·1 M sodium acetate–acetic acid buffer pH 4·5, was added. The tube was placed in a water-bath at 37° C for 1 hr, during which time the sucrose was completely hydrolysed by the enzyme. Then 2·0 ml. of the 3,5-dinitrosalicylate reagent was added, and the amount of reducing sugar was determined as described above. The amount of sucrose was calculated as the amount of reducing sugar present after yeast invertase digestion, minus the amount present before.

Specificity and accuracy of the analytical methods. In homogenates of the gastro-intestinal tract of fasting rats to which only 4 ml. of water had been given, less than 1 mg of polyethylene glycol and less than 2 mg of sugar were found by the methods described above. When known amounts of polyethylene glycol, glucose, fructose and sucrose were added to these homogenates, 95–98 % of the different substances were recovered.

Determination of the rate of hydrolysis of sucrose in vitro *by tissue homogenates.* The gastro-intestinal tract of fasting rats was removed and divided into segments as described above. The stomach and the large intestine were cut open and the contents removed. Each segment was weighed, and then homogenized for 2 min in an Ultra-Turrax homogenizer with 4 parts of 0·9 % NaCl. The tube was chilled with crushed ice during the homogenization. The homogenate was centrifuged at 1000 *g* for 10 min. The opalescent supernatant, which contains the disaccharidases (Borgström & Dahlqvist, 1958), was analysed for invertase activity as described by Dahlqvist (1960). One unit of invertase liberates 1 mg invert sugar from sucrose in 60 min under the specified conditions.

Determination of sugar in urine. To determine the amount of sugar in the urine after the administration of sucrose, the urine was collected in a beaker under a glass ball which separated the urine from the faeces. The amount of urine obtained during 24 hr was diluted to 50 ml. Then 0·5 ml. samples were analysed for carbohydrate with the anthrone method, performed as described by Scott & Melvin (1953) but with heating for 7·5 min at 100° C instead of 16 min at 90° C.

Calculation of the results

The terms used, as defined below, are similar to those used by Reynell & Spray (1956*a*). However, it was unnecessary to use a control animal for the calculations, since in our experiments the marker and sugar were recovered quantitatively at zero time.

Gastric emptying is the percentage of the marker which has left the stomach during the period of time used for absorption. It is calculated in the following way,

$$\text{Gastric emptying} = \frac{P_m - P_s}{P_m} \times 100,$$

where

P_m = amount of polyethylene glycol in the solution fed; and
P_s = amount of polyethylene glycol recovered from the stomach.

Intestinal transit. The transit through a segment of the small intestine is that percentage of the amount of marker entering the segment during the time used for absorption which has moved on to the next segment during the same period of time. The intestinal transit is calculated from the formula

$$\text{Intestinal transit} = \frac{P_d}{P_d + P_i} \times 100,$$

where

P_d = amount of polyethylene glycol recovered from all the segments of the intestinal tract located distally to the segment under consideration; and

P_i = amount of polyethylene glycol recovered from the segment of intestine under consideration.

Total amount of sugar absorbed. This is the amount (mg) of sugar that has disappeared from the gastro-intestinal tract. It is calculated as the amount of sugar fed minus the total amount of sugars (sucrose, glucose and fructose together) recovered from the entire gastro-intestinal tract after the time allowed for absorption.

Rate of sugar absorption. This is the total amount (mg) of sugar absorbed per hour, calculated from the values obtained during the first 2 hr after intubation.

Total absorption of glucose. This is the amount of glucose (fed either as free glucose or as the glucosyl component of sucrose) that has been removed from the gastro-intestinal tract. It is calculated as the amount of glucose fed minus the amount of glucose recovered (both in free form and as the glucosyl component of sucrose) from the entire gastro-intestinal tract.

Total absorption of fructose. This is defined and calculated in the corresponding way to that described for glucose.

Rate of absorption of glucose and fructose. This is the total number of milligrams of glucose or fructose absorbed per hour. It is calculated from the values for the total absorption of glucose or fructose obtained for the ½, 1, 1½, and 2 hr absorption periods.

Absorption index. This is the percentage of glucose or fructose which has been absorbed from that fraction of the solution fed which is present in the stomach or in an intestinal segment at the time the animal is killed. The amount of polyethylene glycol recovered in the segment under consideration is taken to represent the fraction of the solution fed which is in the segment. If the administered meal or solution contains both water and fat, the movement of polyethylene glycol indicates the movement of the *water* phase (Borgström, Dahlqvist & Lundh, 1962). The absorption index is obtained by comparing the ratio of glucose or fructose to polyethylene glycol found in that segment of the gastro-intestinal tract to the ratio of the sugar to polyethylene glycol in the solution fed. It is calculated from the formula

$$\text{Absorption index} = 100\left(1 - \frac{S_i \cdot P_m}{P_i \cdot S_m}\right),$$

where

S_l = mg of the sugar recovered from the relevant segment;
P_m = mg of polyethylene glycol in the solution fed;
P_l = mg of polyethylene glycol recovered from the relevant segment; and
S_m = mg of the sugar in the solution fed.

The absorption indices for glucose are calculated by using S_l = total amount (mg) of glucose recovered from the segment under consideration, i.e. free glucose + glucosyl component of sucrose. The absorption indices for fructose are calculated in the corresponding way.

Information about the site and completeness of absorption can be obtained from the absorption indices. For example, if the indices for the distal third of the small intestine and colon are 100 % during the whole time that the sugars are being absorbed, it can be concluded that the sugars are absorbed before they reach the distal third of the small gut. The values for the distal third of the small intestine are an indication of the completeness of the small-intestinal absorption of the sugar concerned. Similarly, if the indices for the stomach were zero, no gastric absorption would have taken place.

Statistical calculations

The statistical calculations used in this paper have been performed according to the method of Bailey (1959).

RESULTS

Recovery of the reference substance

The recovery of the polyethylene glycol administered varied with the technique used in preparation of the material for analysis. In preliminary experiments the intestine was cut open and rinsed with water. With this technique only about 80 % of the amount of polyethylene glycol given was recovered. This is in accordance with the findings of Aberdeen, Shepherd & Simmonds (1960), who used a similar technique for absorption studies in rats. When the segments of the gastro-intestinal tracts were homogenized as described in the present paper, the recovery of polyethylene glycol was between 95 and 100 %.

Gastric emptying

The percentage gastric emptying was the same whether sucrose or the corresponding monosaccharides were given (Fig. 1). After $\frac{1}{2}$ hr about 50 %, after 1 hr about 75 % and after 2 hr more than 90 % of the meal had entered the small intestine.

Transit through the small intestine

There was no obvious difference in the transit of the sugar-polyethylene glycol solution through the small intestine whether the sugar fed was sucrose or an equimolar mixture of glucose and fructose (Table 1). In both cases transit was most rapid through the upper third and decreased progressively in the more distal segments. From Table 1 and Fig. 1 it can be seen that the solutions fed had passed through the stomach and proximal

third of the small intestine after 2 hr, and that they were in the distal third of the small intestine or colon after 3 hr.

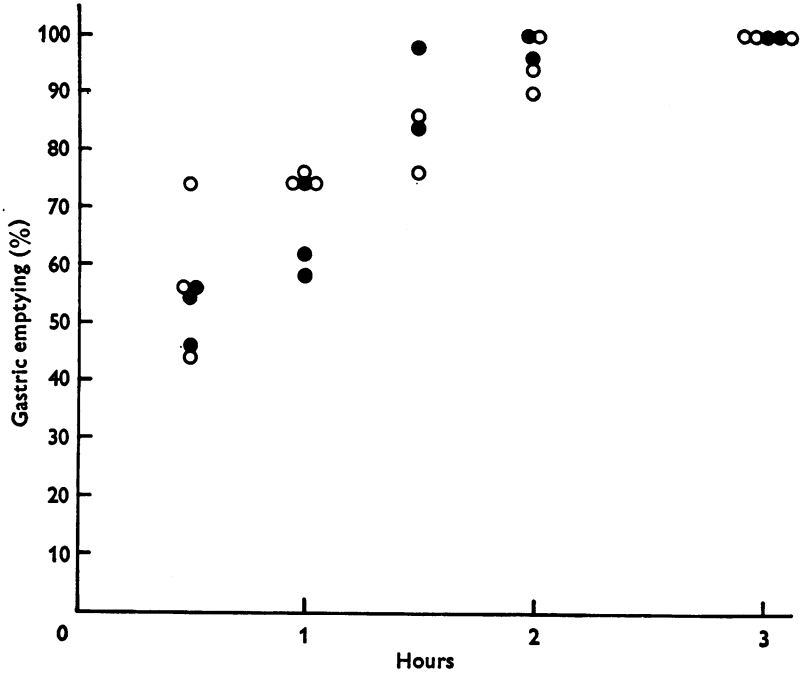

Fig. 1. Gastric emptying calculated from the amount of polyethylene glycol recovered from the stomach at different times after the ingestion of 800 mg sugar and 50 mg polyethylene glycol in 4 ml. of water. In Figs. 1–4, ● = glucose and fructose fed; ○ = sucrose fed.

TABLE 1. Mean transit of the sugar + polyethylene glycol solution through the small intestine at various intervals of time after feeding

Time after intubation (hr)	Number of animals	Sugar fed	Small intestine		
			upper third	middle third	lower third
0·5	3	Glucose + fructose	64	28	0
	3	Sucrose	55	33	0
1	2	Glucose + fructose	86	69	14
	3	Sucrose	73	52	0
1·5	2	Glucose + fructose	95	67	40
	2	Sucrose	82	62	4
2	3	Glucose + fructose	93	75	45
	2	Sucrose	98	77	29
3	2	Glucose + fructose	98	98	59
	3	Sucrose	100	90	53
4	1	Glucose + fructose	100	100	62
	1	Sucrose	98	96	27

Rate of absorption of sugars

The total amount of sugar absorbed at different times after the feeding of 800 mg, either as sucrose or as a mixture of glucose and fructose, is seen in Fig. 2. For the first 2 hr the absorption proceeded practically linearly. During this time 600–700 mg of the sugar was absorbed in both cases. The absorption of the remainder of the sugar took place more slowly, and was completed by 4 hr. The regression lines for the linear period during the first 2 hr were calculated. For the glucose–fructose line the regression coefficient was 320, and the formula for the regression line $y = 30{\cdot}7 + 320x$.

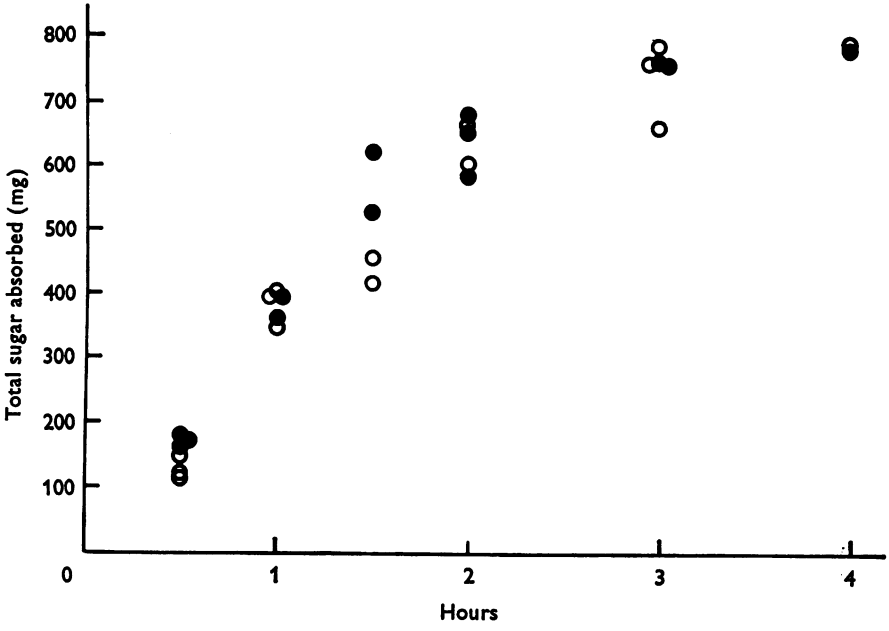

Fig. 2. Total amount of sugar absorbed at different times after the ingestion of 800 mg, either as sucrose or as an equimolar mixture of glucose and fructose.

For the sucrose line the regression coefficient was 332, and the formula for the regression line $y = 13{\cdot}7 + 332x$. The residual variances were assumed to be equal after examination of the variance ratio, and the two regression coefficients were not significantly different as tested by Student's t test. For the first 2 hr the rate of sugar absorption when glucose and fructose were fed was 348 ± 37 mg/hr (mean $\pm$ S.D.). The corresponding figure when sucrose was fed was 313 ± 58 mg/hr. Examination of the variance ratio showed that the unknown variances σ_1^2, and σ_2^2 could be assumed to be equal and the means were not significantly different when examined by Student's t test. Therefore there was no significant difference in the rate

of total sugar absorption for the first 2 hr, whether the glucose-fructose mixture or sucrose was administered.

The total amount of glucose absorbed at varying intervals of time after the administration of the two sugar solutions is shown in Fig. 3. Again absorption appears to proceed linearly during the first 2 hr. The equation for the regression line for glucose absorption when glucose and fructose were fed was $y = 64 + 164x$. When sucrose was fed, the equation for the regression line was $y = 32 + 171x$. The regression coefficients were not

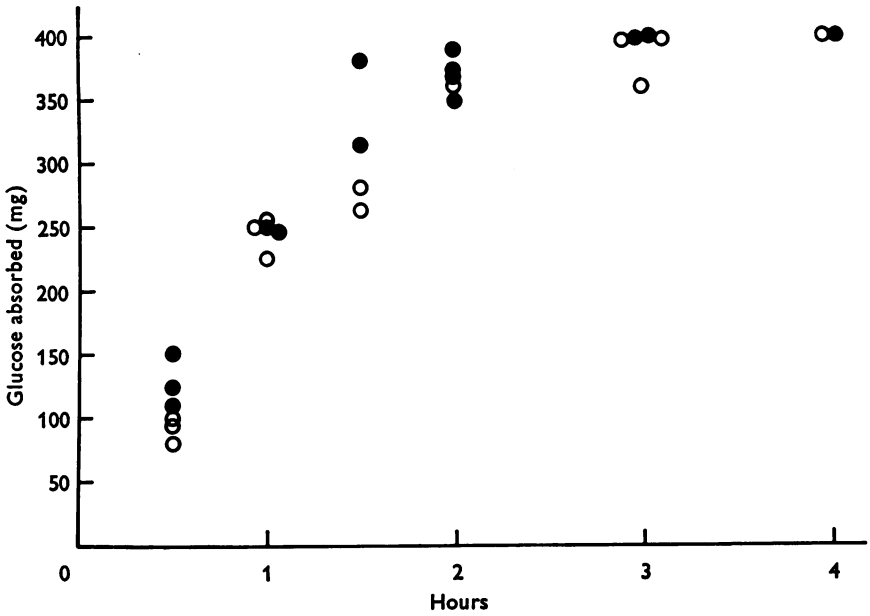

Fig. 3. Total absorption of glucose at different times after the ingestion of 800 mg sugar, either as sucrose (○) or as an equimolar mixture of glucose and fructose (●).

significantly different when compared as described above. For the linear period, the rate of glucose absorption when the monosaccharide mixture was fed was 228 ± 39 mg/hr (mean $\pm$ S.D.). When sucrose was fed, the rate of glucose absorption was 200 ± 32 mg/hr. The two means were not significantly different when tested as described above, and therefore the rates of glucose absorption were similar whether the monosaccharides or the disaccharide was fed.

The total amount of fructose absorbed at varying intervals of time after the two sugar solutions were administered is shown in Fig. 4. Comparison with Fig. 3 shows that the fructose is absorbed somewhat more slowly than the glucose. The equation for the regression line when

glucose and fructose were fed was $y = -33 + 156x$. The corresponding equation when sucrose was fed was $y = -26 + 143x$.

The rate of fructose absorption when glucose and fructose were fed was 119 ± 40 mg/hr and when sucrose was fed it was 112 ± 34 mg/hr. Again there was no significant difference between the regression coefficients and the mean rates of absorption for the first 2 hr.

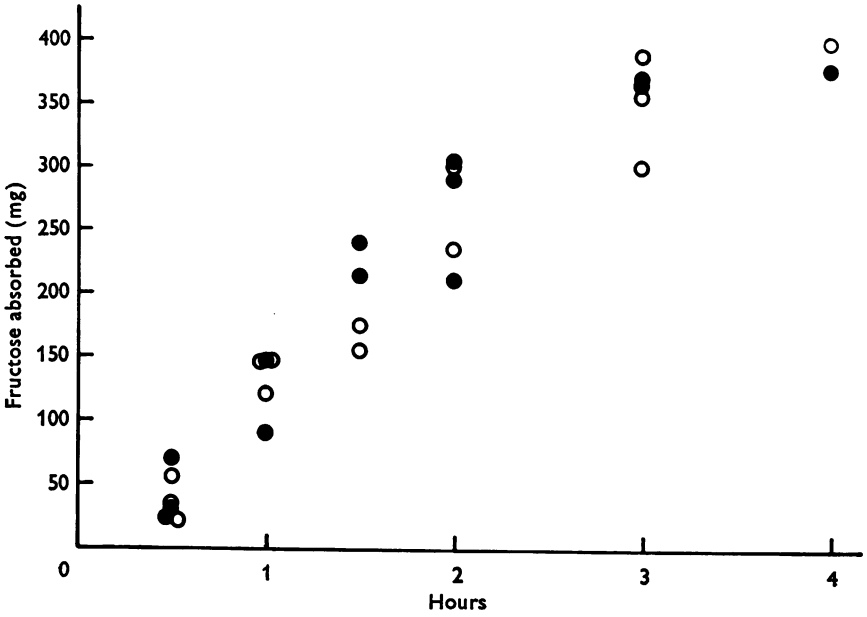

Fig. 4. Total absorption of fructose at different times after the ingestion of 800 mg sugar, either as sucrose (○) or as an equimolar mixture of glucose and fructose (●).

Type of sugar recovered from the various segments of the intestinal tract

The amount and type of sugar recovered from each segment of the gastro-intestinal tract at varying times after the feeding of 800 mg sucrose, or the same amount of an equimolar mixture of glucose and fructose, are seen in Figs. 5 and 6. In both cases only minute amounts of glucose were recovered from the distal third of the small intestine and the large intestine. This indicates that almost all the glucose is absorbed before the lower third of the small intestine is reached. In each sample in which sugar was recovered from the large intestine fructose was present, showing that some of the fructose is absorbed in the distal third of the small intestine and the colon. It is mainly fructose which reaches the lower parts of the gastro-intestinal tract.

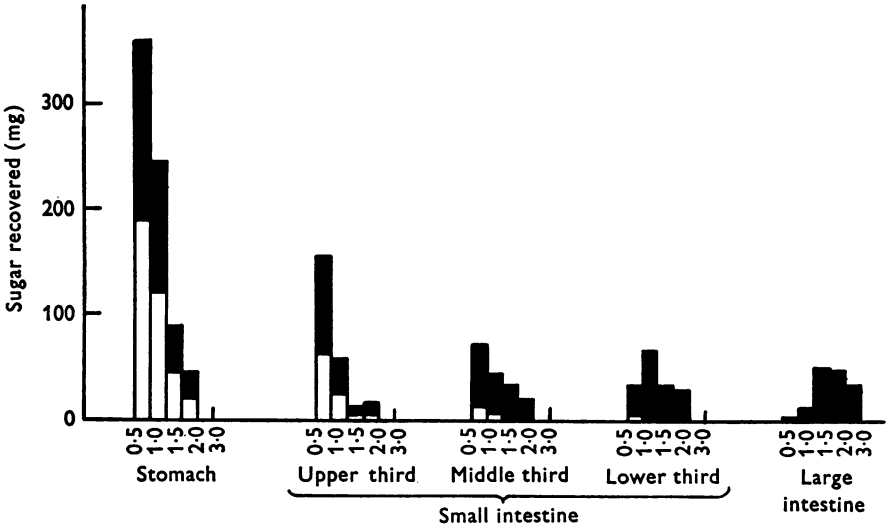

Fig. 5. Amounts of glucose (□) and fructose (■) recovered from the different parts of the gastro-intestinal tract at different times after the ingestion of 800 mg of an equimolar mixture of glucose and fructose. Number below each column indicates hours after ingestion.

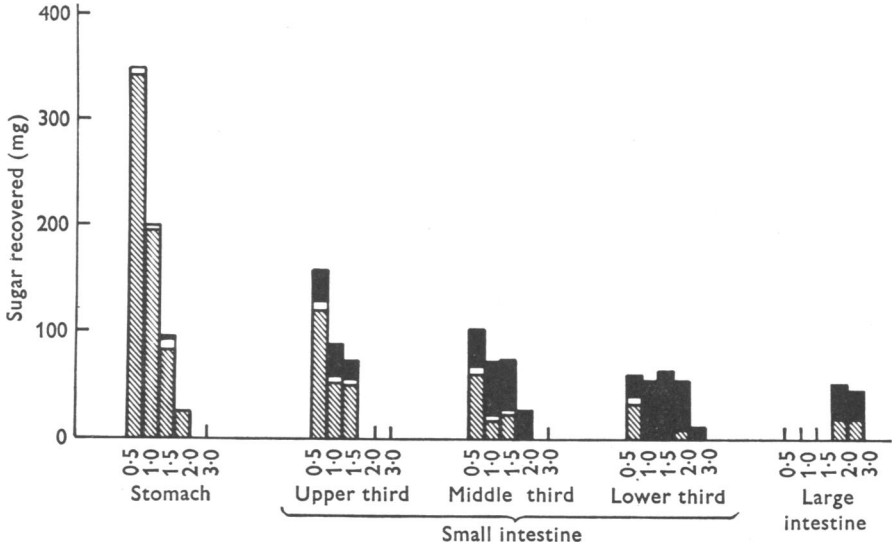

Fig. 6. Amounts of sucrose (▨), glucose (□) and fructose (■) recovered from the different parts of the gastro-intestinal tract at different times after the ingestion of 800 mg sucrose. Number below each column indicates hours after ingestion.

Gastric and intestinal absorption indices

The mean absorption indices for glucose and fructose at different levels in the gastro-intestinal tract, calculated from the amount of sugar and polyethylene glycol recovered from the individual segments, are shown in Tables 2 and 3. Very little, if any, glucose was absorbed by the stomach. Except for the half-hour indices, more than 94% of the glucose was absorbed from that fraction of the solution fed which had reached the lower third of the small intestine. (Examination of Figs. 5 and 6 shows that only a few milligrams of glucose reached the lower third of the small

TABLE 2. Mean absorption indices for glucose for the stomach and the intestinal segments after varying times following intubation

Time after intubation (hr)	Number of animals	Sugar fed	Stomach	Small intestine			Large intestine
				upper third	middle third	lower third	
0·5	3	Glucose + fructose	4	12	89	82	—
	3	Sucrose	0	25	56	52	—
1	2	Glucose + fructose	8	12	89	97	97
	3	Sucrose	3	58	84	94	—
1·5	2	Glucose + fructose	0	66	99	99	99
	2	Sucrose	3	40	79	98	—
2	3	Glucose + fructose	0	83	99	97	98
	2	Sucrose	0	94	89	94	—
3	2	Glucose + fructose	—	100	100	100	99
	3	Sucrose	—	—	100	100	96
4	1	Glucose + fructose	—	—	—	100	100
	1	Sucrose	—	—	—	100	100

The absorption index can only be calculated for those segments which contain a measurable amount of polyethylene glycol.

TABLE 3. Mean absorption indices for fructose for the stomach and the intestinal segments after varying times following intubation

Time after intubation (hr)	Number of animals	Sugar fed	Stomach	Small intestine			Large intestine
				upper third	middle third	lower third	
0·5	3	Glucose + fructose	9	0	22	11	—
	3	Sucrose	1	5	20	21	—
1	2	Glucose + fructose	1	0	22	36	48
	3	Sucrose	4	32	44	52	—
1·5	2	Glucose + fructose	0	25	62	66	55
	2	Sucrose	4	16	37	57	—
2	3	Glucose + fructose	0	49	75	76	34
	2	Sucrose	0	—	68	75	51
3	2	Glucose + fructose	—	100	100	100	85
	3	Sucrose	—	—	95	92	89
4	1	Glucose + fructose	—	—	—	100	91
	1	Sucrose	—	—	—	99	100

intestine and that none reached the colon in the first half hour.) The results were similar whether the sugar was fed as glucose plus fructose or as sucrose.

The absorption indices for fructose vary more than do those for glucose. It is obvious, however, that in the first 2 hr a considerable amount of fructose has not been absorbed from the fractions of the meal reaching the lower third of the small intestine and the colon. It is concluded that, with the dosage employed, fructose is absorbed by all parts of the small intestine and also by the colon. The conclusion that a considerable fraction of the fructose is absorbed by the large intestine is also supported by the fact that considerable amounts of fructose (up to 70–80 mg or 17–20 % of the amount fed) have been recovered from the large intestine at ½–2 hr after ingestion.

The location of invertase (saccharase) in the digestive tract measured in vitro

The intestinal invertase is known to exert its action inside the mucosal cells (Dahlqvist & Borgström, 1961; Miller & Crane, 1961). In accord with this concept, no invertase could be detected in rat pancreatic juice collected with a catheter. The pancreatic juice had very powerful amylase and weak maltase activity.

The invertase activity of the different segments of the gastro-intestinal tract was measured in tissue homogenates (Table 4). The stomach con-

TABLE 4. Mean invertase (sucrase) activity of homogenates of different parts of the gastro-intestinal tract of three fasting rats. One unit of invertase hydrolyses sucrose 1 mg/hr at 37° C, 0·028M substrate and pH 6·5

Segment	Number of animals	Mean weight of tissue (g)	Total units of invertase (mean ± S.D.)
Stomach	3	1·5	4 ± 1
Small intestine			
Upper third	3	2·6	143 ± 3
Middle third	3	2·1	139 ± 29
Lower third	3	2·6	87 ± 28
Caecum	3	1·4	5 ± 1
Colon	3	1·8	3 ± 1
Caecal content	2	1·1	1 ± 1

TABLE 5. Invertase activity of homogenates of the whole small intestine in the fasting state and at intervals after the feeding of 800 mg sucrose

Time after feeding 800 mg sucrose (hr)	Number of animals	Mean weight of small intestine with contents (g)	Total units of invertase (mean ± S.D.)
0 (fasting)	8	7·0	419 ± 86
0·5	6	11·4	485 ± 150
1·0	4	11·1	472 ± 114
2·0	3	10·1	313 ± 74

tained a significant but very weak invertase activity. This gastric invertase has been localized histochemically to the basal cells of the glands of the gastric mucosa (Dahlqvist & Brun, 1962). The small intestine had powerful invertase activity, distributed through the whole small intestine, but highest in the proximal two thirds. This localization of the invertase activity is different from that in the pig small intestine, in which the activity is highest in the distal two thirds (Dahlqvist, 1961*a*). The caecum, colon and caecal contents contain only small amounts of invertase. As is seen in Table 4, more than 96 % of the total invertase activity in the rat intestine is present in the small intestine.

To elucidate whether the invertase activity of the small intestine increased during the absorption of sucrose, the invertase activity in homogenates of the whole small intestine of fasting rats was compared with that of similar homogenates from rats which had previously been given 800 mg of sucrose (Table 5). No significant increase was found.

Location of the hydrolysis of sucrose in vivo

Very little absorption of sugar occurred in the stomach (Tables 2 and 3) and only insignificant amounts of free monosaccharides were recovered from the stomach after feeding sucrose (Fig. 6). It can thus be concluded that neither the weak invertase activity in the gastric mucosa nor the hydrochloric acid are of any importance for the hydrolysis of ingested sucrose.

In the small intestine sucrose disappeared, and fructose formed by its hydrolysis accumulated. Considerable amounts of unhydrolysed sucrose were recovered from the proximal segment of the small intestine, while the amounts of unhydrolysed sucrose recovered from the distal segment of the small intestine and from the large intestine were small (Fig. 6). The main hydrolysis of sucrose therefore occurred in the proximal two thirds of the small intestine.

Comparison of the rate of hydrolysis of sucrose in vivo *and* in vitro

To be able to calculate the rate of hydrolysis of sucrose *in vivo* it is necessary to know if part of the disaccharide is being absorbed into the circulation without hydrolysis in the intestinal wall. Parenterally administered sucrose is known to be more or less quantitatively excreted into the urine (Verzár & McDougall, 1936). In a few experiments the urine was collected from rats after 800 mg sucrose and 50 mg polyethylene glycol, dissolved in 4 ml. water, had been given by the stomach tube. Less than 10 mg carbohydrate (less than 1·5 % of the dose given) was recovered in the urine collected during 48 hr.

When, in another experiment, 50 mg sucrose in 0·5 ml. water was

injected intraperitoneally, 97–98% of the sugar was excreted into the urine within 24 hr. These results indicate that after oral feeding no significant amount of sucrose is absorbed into the blood unhydrolysed.

The course of the hydrolysis of sucrose in the intact rat, with the assumption made that all sucrose that had disappeared from the intestine had also been hydrolysed, is seen in Fig. 7. The total rate of hydrolysis in the experiments where digestion had proceeded for 0·5–1 hr was calculated at 525 ± 73 mg/rat/hr (mean $\pm$ S.D.). This is somewhat faster than the average initial rate of hydrolysis of 0·028 M sucrose by homogenates of the small intestine at optimal pH and 37° C (Table 5).

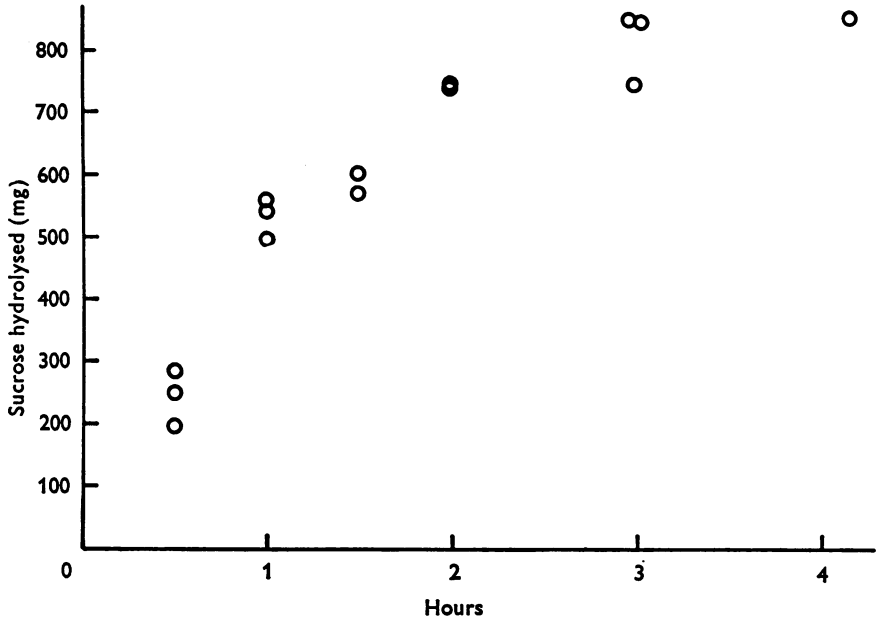

Fig. 7. The course of the hydrolysis of sucrose in the intact rat after the peroral administration of 800 mg of the disaccharide.

DISCUSSION

As was shown by Reynell & Spray (1956*a*), the use of a water-soluble unabsorbable marker in absorption studies permits the calculation of the rates of gastric emptying and intestinal transit as well as the gastric and intestinal absorption indices. Information about the site of absorption can be obtained. The technique used thus yields more detailed information than that of Cori (1925) who fed sugar solutions by stomach tube and measured the amount of sugar recovered from the entire intestinal tract at the end of the experimental period.

With the technique used, polyethylene glycol is a very suitable reference

substance for absorption studies in the rat. The recovery of nearly 100% of the amount of polyethylene glycol fed makes it clearly superior to the phenol red used by Reynell & Spray (1956*a*, *b*). The reason why lower amounts of polyethylene glycol are recovered when the intestine is only washed out with water or saline, as noted by Aberdeen *et al.* (1960) and verified by us, may be that the rinsing of the space between the villi is inefficient. When longer absorption times are used, the solution reaches the lower parts of the intestine where the villi are not so numerous, and consequently more of the polyethylene glycol is recovered (Aberdeen *et al.* 1960). By homogenization of the intestine this difficulty is overcome.

Whether the sugar was given as disaccharide (sucrose) or as free monosaccharides (glucose plus fructose), there was little difference in the rate of passage of the solution through the gastro-intestinal tract, the rate of total sugar absorption, the rate of absorption of each of the two monosaccharide components, or in the locus in the intestine where these were absorbed. In both cases the glucose component was mainly absorbed in the upper third of the small intestine, in good accordance with the results of Reynell & Spray (1956*b*). The slower absorption of the fructose component, in contrast, occurred in the whole small intestine. To some extent fructose passed into the large intestine.

The locus of sucrose absorption thus differs from that in man, where the degree of absorption of sucrose was low in the upper two thirds of the small intestine (Dahlqvist & Borgström, 1961). In the human experiments the glucose and fructose were not measured individually, but the major fraction of the sugar recovered from the lower part of the jejunum was unhydrolysed sucrose.

Chain, Mansford & Pocchiari (1960) have reported that the absorption of sucrose by rat intestine *in vitro* leads to a higher concentration of fructose on the serosal side than the absorption of free fructose at an equimolar concentration. We were not able to find any more effective absorption of fructose from sucrose than from mixtures of glucose and fructose *in vivo*. Although the hydrolysis of sucrose occurs intracellularly (Dahlqvist & Borgström, 1961; Miller & Crane, 1961) the fructose seems to diffuse readily back into the intestinal lumen. The intracellular hydrolysis of sucrose thus occurs externally to the rate-limiting step of fructose absorption.

The intestinal invertase activity does not seem to be rate-limiting for the absorption of sucrose. The disaccharide seems to undergo virtually 100% hydrolysis during absorption, since no sugar appears in the urine. The invertase is chiefly localized in the small intestine, and is highest in its proximal two thirds, in contrast to the pig (Dahlqvist, 1961*a*). A comparison of the rate of sucrose hydrolysis *in vivo* and *in vitro* indicates that

the enzyme is very efficient *in vivo*. The hydrolysis *in vivo* proceeded faster than the hydrolysis of sucrose by homogenates of rat small intestine at 0·028 M substrate concentration, optimal pH and 37° C *in vitro*. This is in contrast to the results obtained with dipeptidases by Newey & Smyth (1960). They showed that the dipeptidase activity of homogenates was considerably greater than that of the intact absorbing intestine. The intestinal invertase activity did not increase when sucrose solution was fed before the animal was killed.

SUMMARY

1. The absorption of sucrose has been studied in the intact rat with the aid of a water-soluble unabsorbable reference substance polyethylene glycol. Sucrose absorption was also compared with the absorption of an equivalent amount of its component monosaccharides, glucose and fructose.

2. Polyethylene glycol was recovered virtually quantitatively with our technique, which permitted calculation of the rates of gastric emptying and intestinal transit and of the absorption indices at different sites.

3. The rates of absorption of the glucose and fructose components did not differ significantly whether the sugar was fed as the disaccharide or as the monosaccharide mixture.

4. The hydrolysis of sucrose was not rate-limiting for its absorption.

5. After the intracellular hydrolysis of sucrose, the fructose which was liberated diffused back into the intestinal lumen to a large extent, and was then absorbed at a lower level than the glucose component. Part of the fructose was absorbed in the large intestine.

6. The efficiency of the small-intestinal invertase was high *in vivo* when compared with its efficiency *in vitro*.

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