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THE ABSORPTION OF SUCROSE, MALTOSE AND HIGHER OLIGOSACCHARIDES FROM THE ISOLATED RAT SMALL INTESTINE

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Several workers have studied the absorption of sugars from preparations *in vitro* of the small intestine of various species (e.g. Fisher & Parsons, 1950; Darlington & Quastel, 1953; Wilson & Vincent, 1955). In general, quantitative results have been obtained only with monosaccharides because of the limitations of the analytical methods. The availability of an automatic scanning method for paper chromatography of radioactive substances (Chain, Frank, Pocchiari, Rossi, Ugolini & Ugolini, 1956) has made it possible to examine quantitatively the absorption of glucose, maltose and the higher oligosaccharides contained in a starch hydrolysate (similar to liquid glucose, B.P. Codex 1959), and of sucrose, from the isolated surviving rat small intestine.

METHODS

Animals. Male Wistar rats weighing 200–250 g and maintained on a standard diet were used.

Radioactive materials. Uniformly labelled ^{14}C-glucose, ^{14}C-sucrose and ^{14}C-fructose were obtained from the Radiochemical Centre, Amersham, Bucks. ^{14}C-labelled tobacco leaf starch was prepared by a modification (P. F. Langley & K. D. Rapson, personal communication) of the method of Porter & Martin (1952). Hydrolysis was carried out with 10 parts (w/v) of 0·1 N-HCl at 145° C for 45 min. This procedure was comparable with that used to produce liquid glucose from maize starch. This product was analysed chromatographically and shown to correspond closely with a typical liquid glucose (Table 1), the data for which were obtained partly from analyses published by the Technical Advisory Bureau, Corn Industries Research Foundation (1956). All sugars were diluted with the corresponding inert material to give a specific activity of about 1·0 μc/mg.

Intestinal preparation. The preparation of the rat small intestine was similar to that described by Fisher & Parsons (1949). An oxygenated (95 % O_2, 5 % CO_2) Krebs–Ringer bicarbonate buffer solution was circulated by gas (95 % O_2, 5 % CO_2) lift through the whole length of the small intestine (from the duodenojejunal flexure to the caecum) through cannulae inserted into both ends of the lumen in an ether-anaesthetized rat. The intestine was then removed from the animal and suspended in a bath containing 50 ml. of Krebs–Ringer solution which was also circulated by gas lift. By this technique the tissue was prevented from being subjected to anoxia at any time, and thus the associated disturbances demonstrated by Fisher & Parsons (1949) were avoided.

Perfusion conditions. All perfusions were carried out for 1 hr at 37° C with an initial volume of 50 ml. of Krebs–Ringer bicarbonate buffer at pH 7·2 containing an initial concentration of 0·5 % (w/v) of each sugar, except where otherwise stated. 1 ml. of 0·3 % phenol red/50 ml. of buffer was added to the inner circuit to reveal any minute pinholes in the neighbourhood of the cannulae. As soon as the circulation was set up, a 1 ml. sample was removed and analysed for total radioactivity and the initial circulating volume thus calculated to correct for saline entrapped in the lumen during the operation procedure. The course of the absorption of the different sugars was followed by taking 1 ml. samples from the inner and outer circuits at intervals during the next 60 min. These were immediately replaced by 1 ml. of Krebs–Ringer buffer to prevent volume changes, appropriate corrections for this sampling being made in the calculations.

TABLE 1

	Anhydrous sugars (%)	
	Hydrolysed tobacco starch	Liquid glucose from maize starch
Glucose	16·2	18·9
Maltose	11·4	14·0
Maltotriose	10·0	11·0
Maltotetraose	9·4	9·7
Maltopentaose	8·6	8·4
Higher oligosaccharides	44·4	38·0
	100·0	100·0

At the end of the perfusion period the contents of the inner and outer circuits were both drained and both circuits washed with 50 ml. of buffer circulated for 1 min. From the total radioactivity in the washings obtained from the inner and outer circuits a correction was made to allow for the loss in volume due to incomplete drainage of the circuits. A correction for evaporation losses was also applied.

Preparation of samples for chromatography. Paper chromatograms were made of tissue extracts and the samples of inner and outer circulating fluids. The tissue extracts were prepared as follows. The intestine, after measurement of its length, was transferred into 10 ml. of ice-cold 60 % alcohol and homogenized in a Waring Blendor. After homogenization the solution was centrifuged and the supernatant collected. The centrifuged tissue was again homogenized in 10 ml. alcohol (this time in a Potter–Elvehjem homogenizer) and centrifuged; and this procedure was repeated three times. The combined extracts were then evaporated to dryness in a Craig rotary evaporator. The extract was then dissolved in 1 ml. of water and samples removed for paper chromatography. The alcohol-insoluble material was kept for determination of residual radioactivity.

The samples of inner and outer fluids removed during and at the end of the 60 min perfusion were freeze-dried and reconstituted in one-fifth of the original volume, in order to provide sufficient radioactivity for precise counting on paper chromatograms.

Paper chromatography. The concentrated samples were analysed by both uni- and bi-dimensional paper chromatography. Each sample was chromatographed in at least two solvent systems at 20° C. The following solvents were used:

A. *n*-butanol:ethanol:water–2:1:1.
B. *n*-butanol:acetic acid:water–40:11:25.
C. *sec.*-butanol:formic acid (88 %):water–5:1:1.
D. phenol:ammonia (0·880):water–80:1:20.
E. phenol:acetic acid:water–80:1:20.

For the separation of the components of starch hydrolysate, unidimensional chromatograms were run for 60 hr in solvent *A*. Bidimensional chromatograms were run first in

solvent *A* for 36 hr and then in solvent *C* for 36 hr. Lactate was determined on unidimensional chromatograms run for 19 hr in solvent *B*. Both lactate and amino acids were determined on bidimensional chromatograms run first in solvent *C* for 16 hr and then in solvent *D* for 19 hr. Glucose, fructose and sucrose were determined on bidimensional chromatograms run either for 36 hr in solvent *A* and then for 36–48 hr in solvent *C*, or for 19 hr in solvent *B* followed by 24 hr in solvent *E*.

In order to obtain clearly defined spots on the chromatograms of samples containing large amounts of sucrose with small amounts of glucose, desalting was carried out with Amberlite MB3 resin in the bicarbonate form (Woolf, 1953).

To prevent losses of lactate by volatilization, all chromatograms used for the separation of lactate were placed in an atmosphere of ammonia vapour immediately after removal from the solvent tank.

Quantitative determination. The paper chromatograms of the radioactive sugars and their metabolites were examined by the automatic scanning device of Chain *et al.* (1956) in its modified form (Frank, Chain, Pocchiari & Rossi, 1959).

The radioactivity of the insoluble residue was determined by counting thin films of the solid material on aluminium planchets. Correction for self-absorption and counter geometry were applied to make the results comparable with measurements of the radioactivity counted on paper.

RESULTS

Rates of absorption

The mean absorptions of the different sugars calculated from the quantitative evaluation of the scanned chromatograms are given in Table 2. At comparable concentration (0·5 % (w/v)) the highest rates of disappearance of total carbohydrate from the lumen and the highest rates of appearance of glucose on the serosal side were obtained when starch hydrolysate or glucose were perfused. The figures given for the experiments with glucose are in close agreement with the published rates of Fisher & Parsons (1953). The rate of disappearance of sucrose was greater than that of fructose and more fructose appeared on the serosal side after perfusion with sucrose

TABLE 2. Absorption of carbohydrates during perfusion of rat small intestine with various sugars

Results expressed as mg carbohydrate/cm (mean ± S.E.) after 1 hr perfusion carried out at 37° C in 50 ml. of oxygenated (95 % O_2, 5 % CO_2) Krebs–Ringer bicarbonate buffer at pH 7·2. All sugars were used at a concentration of 0·5 % (w/v) except where indicated.

Sugar perfused	No. of expts.	Disappearance of carbohydrate from inner fluid			Appearance of carbohydrate in outer fluid		
		Glucose	Fructose	Total	Glucose	Fructose	Total
Starch hydrolysate	8	—	—	1·98 ± 0·05	0·96 ± 0·04	nil	0·96 ± 0·04
Glucose	4	1·81 ± 0·03	—	1·81 ± 0·03	1·18 ± 0·03	nil	1·18 ± 0·03
Glucose-fructose mixture	3	1·22 ± 0·04	0·34 ± 0·03	1·56 ± 0·03	0·57 ± 0·08	0·06 ± 0·02	0·62 ± 0·09
Glucose-fructose mixture*	2	1·77 ± 0·01	0·68 ± 0·02	2·45 ± 0·01	0·98 ± 0·01	0·11 ± 0·01	1·09 ± 0·01
Sucrose	4	—	—	1·15 ± 0·05	0·30 ± 0·02	0·17 ± 0·02	0·47 ± 0·02
Fructose	3	—	0·79 ± 0·05	0·79 ± 0·05	nil	0·11	0·11

* 1·0 % (w/v) concentration used in these experiments.

(a)
1
2
3
4
5
6

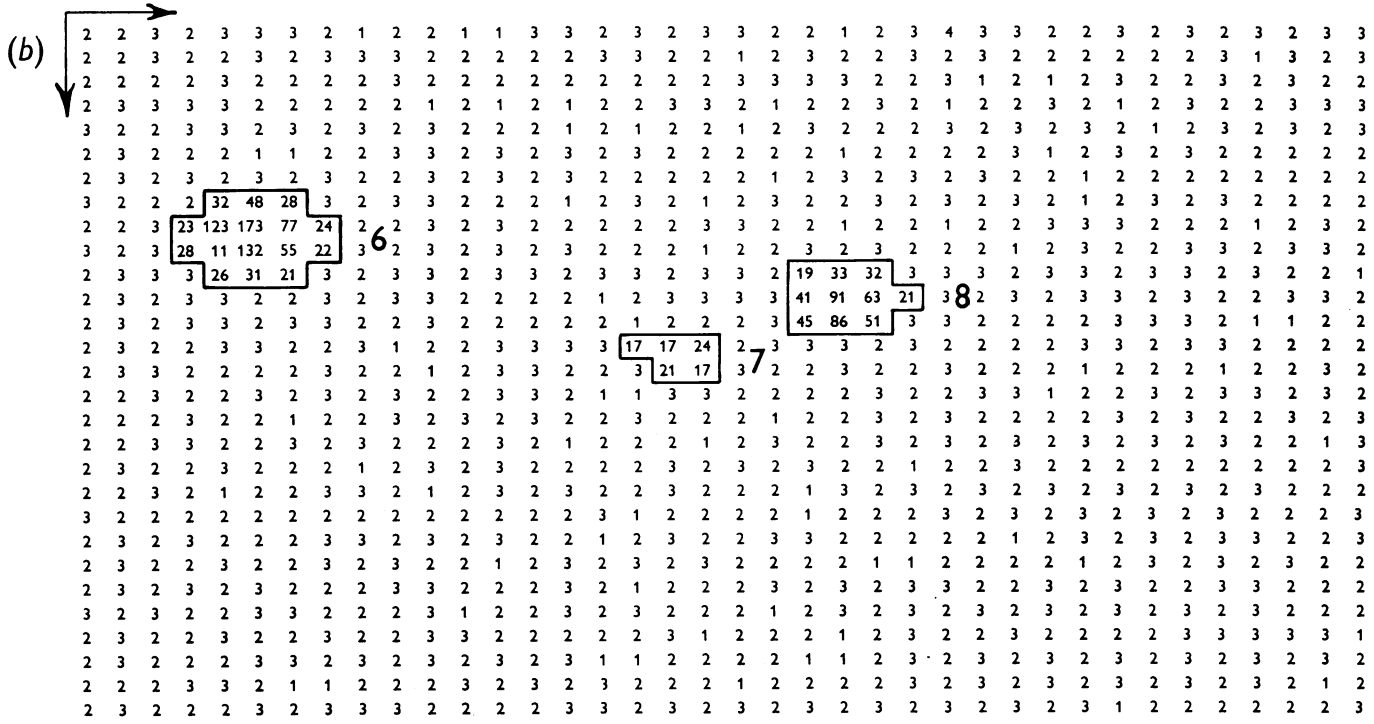

Fig. 1. Number maps of radiochromatograms of: (*a*) starch hydrolysate remaining in the inner fluid after perfusion for 1 hr; (*b*) serosal fluid after perfusion with starch hydrolysate. Chromatogram (*a*) developed in → solvent *A*, ↓ solvent *C*; chromatogram (*b*) in → solvent *C*, ↓ solvent *D*. (1) polysaccharides, (2) maltopentaose, (3) maltotetraose, (4) maltotriose, (5) maltose, (6) glucose, (7) alanine, (8) lactate.

than after perfusion with fructose. The rate of disappearance of fructose was about 45 % of the rate observed with glucose, in good agreement with the results of Cori (1925) and Verzár & McDougall (1936) with *in vivo* techniques. With a mixture of glucose and fructose, each in a concentration of 0·5 % (w/v), the rates of absorption were not significantly different from the rates observed with each of the sugars perfused alone.

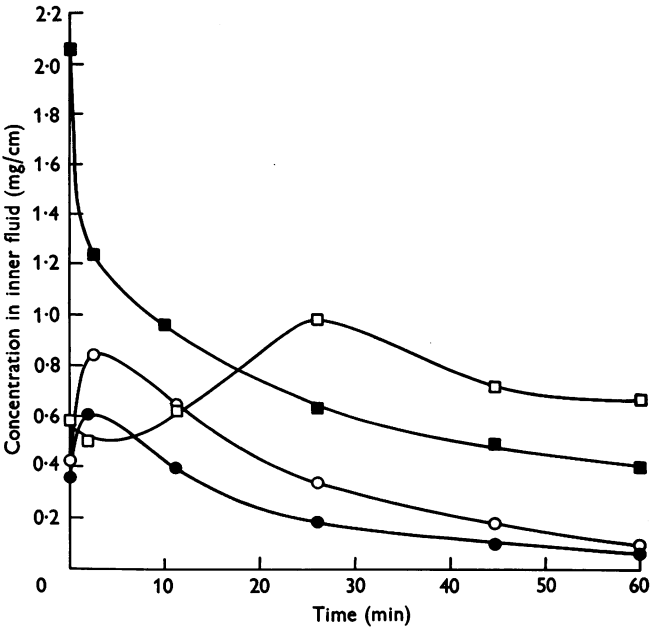

Fig. 2. Composition of the inner fluid at various periods during perfusion of the rat small intestine with starch hydrolysate; each point represents the mean of two experiments. □ Glucose, ● maltose, ○ maltotriose, ■ maltotetraose + polysaccharides.

Starch hydrolysate. The composition of the inner fluid during perfusion with starch hydrolysate is shown in Fig. 3, and an autoscanner number map of a chromatogram of the inner fluid at the end of the perfusion is shown in Fig. 1(*a*). The concentration of the higher oligosaccharides decreased rapidly while the maltose concentration rose steeply, indicating strong amylase activity in the lumen (Fig. 2). Following the steep rise, the maltose concentration diminished and during the first 30 min the glucose concentration increased, indicating maltase activity. Of the sugars in starch hydrolysate, only glucose appeared on the serosal side (see Fig. 1(*b*)).

The time course of the absorption of starch hydrolysate from the lumen and the appearance of glucose on the serosal side is given in Table 3. The rate of disappearance of starch hydrolysate from the inner fluid during the

first half hour was 2·7 mg/cm/hr, whereas in the second half hour it dropped to 1·54 mg/cm/hr. The corresponding rates of appearance on the serosal side were 0·68 mg/cm/hr in the first half hour and 1·32 mg/cm/hr in the second half hour. Although the concentration of free glucose in the lumen was never higher than 0·16 % (w/v), the rate of appearance of glucose on the serosal side during perfusion with starch hydrolysate was as high as the mean rate obtained with 0·5 % (w/v) glucose in the lumen.

TABLE 3. Rate of absorption of carbohydrates during perfusion of rat small intestine with starch hydrolysate

Results expressed as mg carbohydrate/cm intestine/hr (mean of 4 expts.) during perfusion carried out with 0·5 % (w/v) starch hydrolysate.

Time (min)	Rate of disappearance of carbohydrate from lumen	Rate of appearance of glucose in serosal fluid
0–10	3·00	0·60
10–20	2·76	0·60
20–30	2·34	0·84
30–40	1·92	1·32
40–50	1·56	1·32
50–60	1·14	1·32

Sucrose. The composition of the inner circuit during perfusion with sucrose is shown in Fig. 3. Sucrose was not completely hydrolysed during the perfusion. The concentration of glucose in the lumen was small and tended to fall during the second 30 min of perfusion, as its rate of absorption was greater than its rate of formation from sucrose. The concentration of fructose in the lumen rose during the perfusion because of its lower rate of absorption.

The appearance of glucose and fructose in the serosal circuit during perfusion with sucrose is compared in Fig. 4 with the glucose appearing after perfusion with starch hydrolysate. With starch hydrolysate the concentration of glucose on the serosal side was approximately twice the sum of the glucose and fructose concentrations from sucrose. With a 1 : 1 glucose-fructose mixture (total concentration 0·5 % (w/v)) the amount of sugar appearing on the serosal side was still only half that obtained with 0·5 % (w/v) starch hydrolysate.

Fructose was found to be absorbed in the rat small intestine without epimerization to glucose. In the guinea-pig, however, 36 % of the carbohydrate appearing on the serosal side after the perfusion was found to be glucose (Table 4).

Because fructose was removed from the lumen at a slower rate than glucose, the proportion of fructose to glucose in the lumen rose steeply during the perfusions with glucose-fructose mixture (1 : 1). At the end of the 1 hr perfusion the carbohydrate in the lumen consisted of 84–97 %

fructose whereas the serosal fluid carbohydrate consisted of 89–93% glucose.

Metabolism of sugars and transport of water. With all the sugars studied some conversion to lactate was observed. Lactate was found in both the inner and outer circuits and in the extract of the intestinal tissue. The

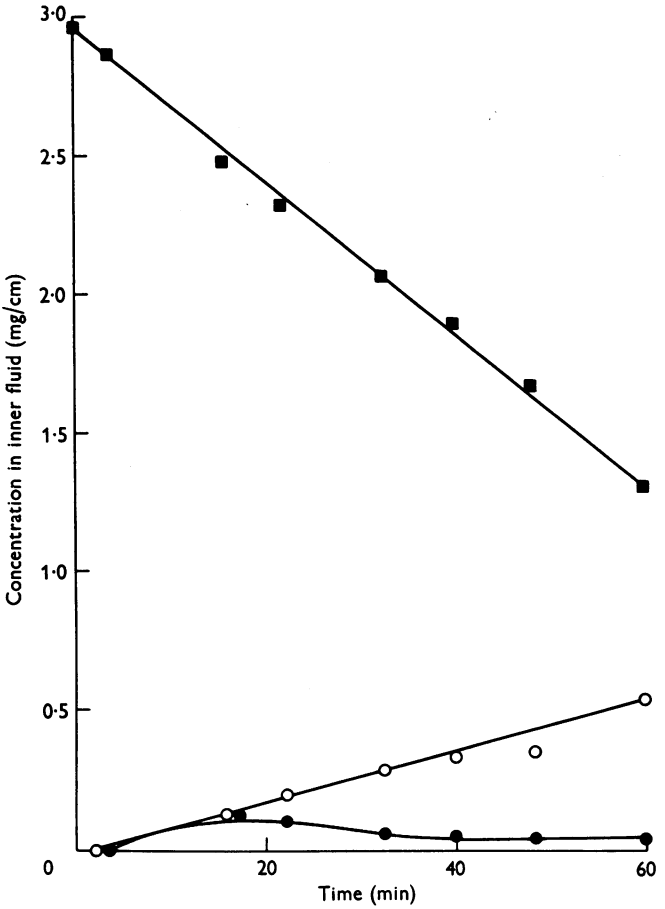

Fig. 3. Composition of the inner fluid at various periods during perfusion with sucrose; each point represents the mean of four experiments. ■ Sucrose, ● glucose, ○ fructose.

distribution of this lactate and the total determined is shown in Table 5. With all the sugars examined alanine was detected in trace amounts in the intestinal extracts and low residual radioactivity was associated with the insoluble intestinal residue.

The observation of Fisher & Parsons (1950), that during absorption of glucose there was considerable water transport against a large osmotic

pressure gradient was confirmed. In the present experiments a loss of approximately 150 μl./cm of intestine was observed when starch hydrolysate or glucose was perfused. This was reduced to about 100 μl./cm with sucrose and to 50–60 μl./cm with fructose perfusions.

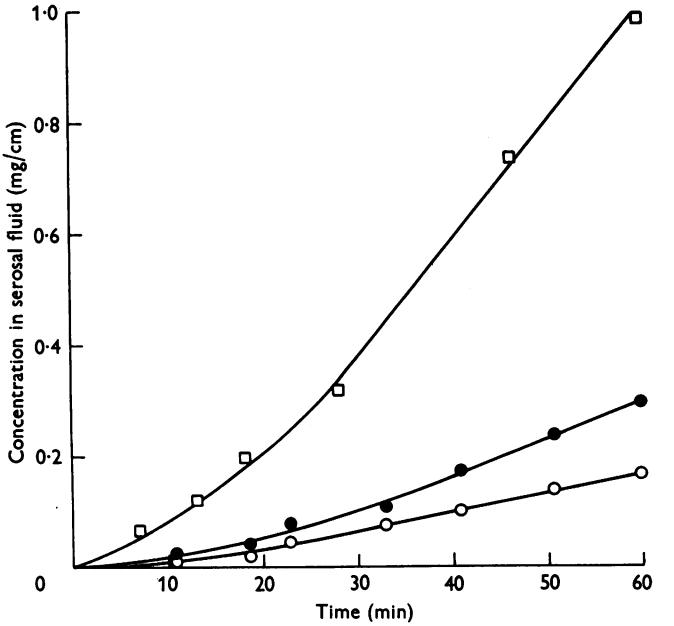

Fig. 4. Appearance of sugars at various times in the serosal fluid after perfusion of the rat small intestine with starch hydrolysate or sucrose; each point represents the mean of four experiments. □ Glucose after perfusion with starch hydrolysate, ■ glucose after perfusion with sucrose, ○ fructose after perfusion with sucrose.

TABLE 4. Absorption of fructose during perfusion of guinea-pig small intestine

Results expressed as mg sugar/cm intestine after perfusion for 1 hr with 0·5 % (w/v) fructose.

Disappearance of fructose from lumen	Appearance of sugar on serosal side		
	Fructose	Glucose	Total
0·91	0·14	0·08	0·22

TABLE 5. Formation and distribution of lactate after perfusion with various sugars

Results expressed as sugar converted to lactate, mg/cm/hr. Experimental conditions as given in Table 2.

	Inner circuit	Outer circuit	Intestinal extract	Total ± S.E.
Starch hydrolysate	0·091	0·331	0·088	0·510 ± 0·052
Sucrose	0·064	0·205	0·063	0·332 ± 0·041
Fructose	0·055	0·051	0·057	0·163 ± 0·022
Glucose	0·086	0·178	0·085	0·349 ± 0·033
Glucose-fructose 1:1	0·094	0·224	0·098	0·416 ± 0·052

DISCUSSION

The rate of disappearance of carbohydrate from the lumen of the isolated rat small intestine was certainly not less when starch hydrolysate was used than when 0·5 % (w/v) glucose was used, although with starch hydrolysate the concentration of glucose in the lumen never rose above 0·16 % (w/v). It is of interest to compare the observed rates of disappearance from the lumen and appearance on the serosal side with the results of Fisher & Parsons (1953) using various glucose concentrations in the inner circuit. With a 0·16 % (w/v) glucose concentration in the lumen, the rate of disappearance from the lumen of glucose would be expected to be around 1 mg/cm/hr. As this 1 mg/cm/hr of glucose corresponds to the glucose utilization rate for the intestine (Fisher & Parsons, 1953) practically no glucose would be expected on the serosal side under these conditions. The appearance of 1·32 mg/cm/hr (Table 2) of glucose on the serosal side in the second half hour of the starch hydrolysate perfusions, when the glucose concentration was at most 0·16 % (w/v), cannot therefore be accounted for solely by glucose absorption. Something other than glucose must be absorbed from the lumen and this substance must be supplying the needs of the intestinal wall for glucose.

The time course of the absorption of starch hydrolysate from the lumen was markedly different from the published results of Fisher & Parsons (1953) with glucose. The rate of glucose absorption from a solution containing initially 0·5 % (w/v) was 2·54 mg/cm/hr in the first 30 min but only 1·18 mg/cm/hr in the second 30 min (Fisher & Parsons, 1953). With starch hydrolysate the corresponding rates were 2·70 mg/cm/hr and 1·54 mg/cm/hr (Table 3). The rates of appearance of glucose in the serosal fluid with perfused glucose (Fisher & Parsons, 1953) were 1·16 mg/cm/hr for the first 30 min and only 0·10 mg/cm/hr for the last 30 min. With starch hydrolysate the corresponding rates were 0·68 mg/cm/hr and 1·32 mg/cm/hr (Table 3). With starch hydrolysate, therefore, an apparent time lag was observed before the maximum rate of appearance of glucose in the serosal fluid was attained.

From these differences in the results of glucose and starch hydrolysate perfusions there is definite evidence for the direct absorption of maltose, maltotriose or the higher oligosaccharides of starch hydrolysate from the lumen of the rat small intestine. When the comparisons between glucose and starch hydrolysate are made in terms of the glucose equivalent, the initial concentration of starch hydrolysate becomes 0·53 % (w/v). On average the rates of disappearance of starch hydrolysate from the lumen are 8–9 % greater when thus expressed than when expressed as weights of total carbohydrate. The mean rate of disappearance of 0·53 % (w/v) starch

hydrolysate then becomes $2{\cdot}15 \pm 0{\cdot}05$ mg/cm/hr, which is 19 % greater in terms of glucose equivalent than the rate of disappearance from 0·5 % (w/v) glucose (Table 1). From the kind of relation known to exist between glucose concentration and rate of absorption (Fisher & Parsons, 1953) the 19 % difference in rate could not result solely from the 6 % difference in concentrations. The differences described above between the rates of absorption of starch hydrolysate and glucose in the first and second half hours are similarly accentuated by conversion to glucose equivalent.

Additional evidence for the absorption of higher saccharides was provided by the results of the perfusions with sucrose. Sucrose, although only partially inverted during the perfusion, was removed from the lumen faster than fructose, and the rate of appearance of fructose on the serosal side was higher with sucrose in the lumen than with fructose alone. Sucrose must therefore be absorbed as such by the mucosa with subsequent breakdown to glucose and fructose in the intestinal wall. Somewhat similar evidence for the absorption of sucrose in the guinea-pig was presented by Fridhandler & Quastel (1955). These authors, using much higher sucrose concentrations than in the present experiments, were able to demonstrate the presence of unchanged sucrose on the serosal side. In the present experiments radioactive sucrose was not found on the serosal side and only traces were detected in the alcoholic extract of the intestine.

Fructose absorption follows different pathways in the rat and guinea-pig. In the former species fructose was absorbed at a higher rate than the published rate for sorbose (Cori, 1925; Darlington & Quastel, 1953), which was considered to be absorbed by passive diffusion. In the guinea-pig partial epimerization to glucose was observed, confirming the results of Darlington & Quastel (1953). Ginsberg & Hers (1959) have put forward a hypothesis for this species difference, based on the presence or absence of glucose-6-phosphatase activity in the mucosa. It is of interest that some water transport was observed across the intestinal wall during fructose absorption. Although the rate of water transport was much lower here than during glucose perfusion, it is evident that this water transport is not specifically linked with glucose absorption in the rat small intestine.

Only trace amounts of alanine and insoluble material were formed from the sugars, and the lactate produced by utilization of the sugars during absorption formed only 15–25 % of the total sugar disappearing from the lumen. The distribution of this lactate was found to vary according to the amount of water transport associated with the absorptive process.

SUMMARY

1. The absorption from the isolated rat small intestine of sucrose, a starch hydrolysate similar to liquid glucose B.P. Codex 1959, glucose and

fructose was studied by means of a quantitative radioactive paper-chromatographic technique.

2. From the observed rates of absorption of starch hydrolysate and the published results for glucose absorption it was concluded that something other than glucose was absorbed during perfusion with starch hydrolysate, and that this substance (maltose, maltotriose or a higher saccharide) was able to supply the needs of the intestinal wall for glucose.

3. Only glucose appeared on the serosal side after starch hydrolysate perfusion. Sucrose was partially inverted during absorption and both glucose and fructose appeared on the serosal side.

4. The perfusions with sucrose led to a higher concentration of fructose on the serosal side than after perfusions with fructose. It was concluded that sucrose was being absorbed as such by the mucosa, with subsequent break-down to glucose and fructose in the intestinal wall.

5. The rates of disappearance from the lumen of starch hydrolysate and glucose were about twice those of sucrose and fructose. The rate of appearance of sugar on the serosal side after perfusion with starch hydrolysate or glucose was twice that of sucrose and nearly ten times that of fructose.

6. No conversion of fructose to glucose was observed in perfusion of the rat small intestine with fructose, whereas in the guinea-pig considerable conversion occurred.

7. All sugars examined were converted to lactate during the absorption process to the extent of 15–25 % of the total sugar disappearing from the lumen. Traces of alanine were found in the intestinal wall together with small amounts of radioactive insoluble material.

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