The Phosphorylation and Absorption of Sugars in the Rat

2. SUGAR ABSORPTION *IN VIVO*, AND ITS RELATIONSHIP TO THE PHOSPHORYLATION OF SUGAR *IN VITRO*

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These experiments are intended to demonstrate that the rats of the stock used in the experiments on hexokinase activity (Hele, 1953) showed the same physiological behaviour as regards sugar absorption as animals described in the literature. Some information upon the rate of the hexokinase reaction catalysed by mucosa from the upper and lower halves of the intestine is also included.

EXPERIMENTAL

Sugar absorption *in vivo*. Sugar absorption was examined by the method of Cordier & Touzé (1948). The animals, of about 150 g. weight, were fasted for 48 hr. before the experiment. These animals, under ether anaesthesia, received an injection of 2 ml. of an isotonic sugar solution into the duodenum, and were killed by decapitation at intervals up to 1 hr. after the injection of the sugar. The small intestine was removed between ligatures, and its contents washed out as described by Cordier & Touzé. The washings were cleared of debris by boiling for 3 min. with 10 ml. of 0.1 M zinc sulphate and 10 ml. of 0.2 N sodium hydroxide, then filtering hot at the pump. The filtrate was made up to 500 ml., and the sugar content estimated by the method of Nelson (1944). It was observed during the course of these experiments that most of the sugar was absorbed from the upper quarter of the intestine.

The hexokinase reaction. The methods used were as described before (Hele, 1953). The intestine was cut into halves before weighing, and each half was weighed and treated separately.

RESULTS

Sugar absorption

Control experiments. Five animals received an injection of isotonic sodium chloride into the duodenum, and were sacrificed after 1 hr. Four of these animals showed no reducing substances in the intestinal washings, whilst one showed reducing substance equivalent to 1.5 mg. glucose.

The effect of time upon the rate of sugar absorption (Fig. 1). The animals used in these experiments were of similar, but not identical, stock with those used in

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Fig. 1. Sugar absorption from rat intestine. The animals received an injection of 2 ml. of an isotonic sugar solution into the duodenum, and were killed at intervals up to 1 hr. $\ominus\text{---}\ominus$, Galactose; $\circ\text{---}\circ$, glucose; $\bullet\text{---}\bullet$, fructose; $\odot\text{---}\odot$, mannose; $\times\text{---}\times$, xylose. The S.D. is indicated by vertical bars over each point.

Table 1. *Sugar absorption in the same stock of rats as those used for the studies on the hexokinase reaction*

(Values are expressed as mg. sugar absorbed in 30 min. after intraduodenal injection of 2 ml. isotonic sugar solution. The results are given as: mean $\pm$ S.D. (number of animals), extreme range of values.)

Galactose	Glucose	Fructose	Mannose	Xylose
64 $\pm$ 6 (5)	57 $\pm$ 4 (5)	28 $\pm$ 3 (5)	14 $\pm$ 3 (5)	15 $\pm$ 5 (5)
58-72	50-64	22-33	10-19	12-18

the *in vitro* experiments. The rate of absorption of glucose and galactose remained linear with time until all the sugar was absorbed. The absorption rate with the other sugars fell off after 15-30 min., when only part of the sugar had disappeared from the intestine.

The absorption rate ratio and the phosphorylation rate ratio. As only a limited number of rats of the same stock as those used in the *in vitro* experiments was available, absorption rate ratios could only be

Table 2. *Phosphorylation and absorption rate ratios*

(The phosphorylation- or absorption-rate ratio is defined as follows:

$$\frac{\text{mg. sugar absorbed or phosphorylated}}{\text{mg. glucose absorbed or phosphorylated}} \times 100.$$

The absorption-rate ratios were calculated from the data given in Table 1 and Fig. 1. The phosphorylation-rate ratios were calculated from the data of Hele (1953) relating to sugar phosphorylation at a sugar concentration of 0.0055 M, and an ATP concentration of 0.033 M.)

	Galactose Glucose	Glucose Glucose	Fructose Glucose	Mannose Glucose	Xylose Glucose
Phosphorylation-rate ratios	126	100	55	36	24
Absorption-rate ratios	112	100	52	25	26
Absorption-rate ratios: Cori (1925)	110	100	43	19	15
Wilbrandt & Laszt (1933)	115	100	45	33	30
Westenbrink (1936)	108	100	42	15	13

Table 3. *The hexokinase reaction in the upper and lower halves of the small intestine*

(The rate of the reaction was measured after 5 min. incubation, for all sugars, except galactose (2.5 min.). Concentrations were: glucose and galactose, 0.0055 M; fructose, mannose and xylose, 0.028 M; ATP, 0.033 M. The results are given as: mean $\pm$ S.D. (number of suspensions), extreme range of values.)

	Galactose	Glucose	Fructose	Mannose	Xylose
Upper half	0.86 $\pm$ 0.07 (5) 0.78–0.94	0.87 $\pm$ 0.10 (11) 0.72–1.02	0.97 $\pm$ 0.10 (12) 0.72–1.20	0.49 $\pm$ 0.07 (5) 0.24–0.60	0.26 $\pm$ 0.10 (5) 0.24–0.36
Lower half	0.11 $\pm$ 0.10 (5) 0.00–0.39	0.45 $\pm$ 0.12 (11) 0.21–0.75	0.48 $\pm$ 0.12 (12) 0.36–0.78	0.38 $\pm$ 0.09 (5) 0.21–0.48	0.39 $\pm$ 0.22 (5) 0.15–0.69

Table 4. *The total phosphorylating capacity of the mucosa*

(Hexokinase activity is expressed as 'mg. sugar disappearing/ml. suspension/5 min. (aqueous suspensions) or 2.5 min. (Ringer suspensions)'. The sugar concentration was 0.0055 M, the ATP concentration 0.033 M. The results are given as: mean $\pm$ S.D. (number of suspensions), extreme range of values. The values for total sugar phosphorylation in 24 hr. were corrected by applying a temperature coefficient for the hexokinase activity, $Q_{30-38} = 1.41$ (Long, 1952), and by adding to all values 30%, to compensate for the interference of the phosphatase reaction in the hexokinase assay (Hele, 1953).)

	Aqueous suspensions Glucose	Ringer suspensions		
		Glucose	Galactose	Fructose
Observed values:				
Body weight of rats (g.)	158 $\pm$ 8 (10) 140–170	147 $\pm$ 8 (10) 144–164	As for glucose —	— —
Suspension obtained (ml.)	13.5 $\pm$ 1.1 (10) 11.0–17.5	8.8 $\pm$ 1.1 (10) 8.0–12.0	As for glucose —	— —
Hexokinase activity	0.92 $\pm$ 0.12 (10) 0.75–1.14	0.64 $\pm$ 0.07 (10) 0.54–0.78	0.87 $\pm$ 0.07 (10) 0.75–1.02	0.32 $\pm$ 0.03 (10) 0.27–0.42
Calculated values:				
Sugar phosphorylated mg./total mucosa/1 hr.	146 120–185	132 115–166	178 153–216	68 58–89
Sugar phosphorylated g./total mucosa/24 hr.	3.5 2.9–4.4	3.2 2.8–4.0	4.3 3.8–5.2	1.6 1.4–2.1
Sugar phosphorylated g./total mucosa/24 hr., corrected	6.6 5.3–7.7	6.0 5.2–7.4	8.0 7.1–9.6	2.9 2.6–3.9

measured for one interval of time (30 min.) after the intraduodenal injection of the sugar solution. This represented a compromise between the point at which the rates with fructose, mannose, and xylose declined, and the point at which sufficient sugar had vanished from the intestine to give a significant decrease in sugar concentration (Table 1). Good correlation was obtained between the absorption-rate ratios of these animals, the absorption-rate ratios of the animals of related stock, the absorption-

rate ratios given in the literature, and the phosphorylation-rate ratios obtained from the experiments on the hexokinase reaction (Table 2).

The hexokinase reaction

The rate of the hexokinase reaction in the upper and lower halves of the small intestine (Table 3). Glucose and fructose were phosphorylated by tissue dispersions, prepared from the upper half of the intestine, at twice the rate given by dispersions pre-

pared from the lower half, and at rates similar to those shown by a dispersion prepared from whole intestine. Probably more hexokinase was present in the tissue suspensions obtained from the upper part of the intestine than the standard test system could measure. The phosphorylation of galactose was virtually confined to the upper half of the intestine. Mannose and xylose were phosphorylated at comparable rates in either half.

The total phosphorylating capacity of the mucosa. It was of interest to calculate the amount of sugar that could be phosphorylated by the whole mucosa in a given period of time, and to compare this with the amount of sugar absorbed during the same interval of time. Histological examination showed that all the epithelium of the mucosa passed into the suspension during preparation (Hele, 1953). Therefore, the sugar phosphorylated by the total volume of the suspension should be an indication of the phosphorylating capacity of the total absorbing surface of the small intestine. Table 4 shows that the total phosphorylating capacity of the suspension is more than sufficient to account for the phosphorylation of all the sugar absorbed during the experiments *in vivo*. It will also account for the phosphorylation of at least 50% of the carbohydrate consumed by an intact 150 g. rat in 24 hr., which amounts to about 7.5 g., chiefly as starch (Hele, 1953). After correction of the data on phosphorylation, the rate of the hexokinase reaction is sufficient to account for the phosphorylation of 70–100% of the carbohydrate consumed by the intact animal.

DISCUSSION

A relationship has been shown to exist between the relative rates of phosphorylation of certain sugars *in vitro*, at a sugar concentration of 0.0055M, and between the relative rates of absorption of these sugars *in vivo* from isotonic sugar solutions (0.3M). Raising the sugar concentration two- to three-fold results in an increase in the phosphorylation rates of the more slowly phosphorylated sugars, fructose, mannose and xylose, until these rates are close to those obtained for glucose and galactose at the same increased sugar concentration. A similar effect upon sugar absorption *in vivo* was described by Verzár and his co-workers (cf. Verzár & McDougall, 1936). These workers did not observe any sharp 'minimum' in their 'glucose concentration curve', such as is found with the phosphorylation reaction *in vitro*, but this could be explained by the existence *in vivo* of sufficient ATP to suppress this phenomenon.

A further parallelism between events *in vivo* and *in vitro* is shown by the fact that sugars in mixtures interfere with each other's phosphorylation rates (Hele, 1953). A similar effect of sugars in mixtures has been observed in studies on absorption rates (Cori, 1926; Cajori & Karr, 1935; Sobotka & Reiner,

1930). The observation also reported here, that certain sugars are more rapidly phosphorylated by dispersions from the upper half of the small intestine than by those from the lower half, is paralleled by the observations of Westenbrink (1936), Verzár & Wirz (1937) and Liem & Florey (1939), who found that these sugars are absorbed more rapidly from the upper part of the small intestine of the rat than from the lower.

The figures for the total phosphorylating capacity of the epithelium of the small intestine show that there is sufficient hexokinase present to account for the phosphorylation of all of the carbohydrate (7.5 g.) consumed by a 150 g. rat under completely physiological conditions in 24 hr. The rate of absorption as measured here by the method of Cordier & Touzé (1948) is only one-third (2.4 g. glucose in 24 hr.) of that of the intact animal. This can be attributed to the utilization of only a part of the total absorbing surface of the intestine during these experiments. These experiments reflected correctly the absorption-rate ratios, but gave no indication as to the total absorbing capacity of the intestine under normal conditions.

This striking parallelism between events *in vivo* and *in vitro* exists, although there is a marked difference between the sugar concentrations used *in vivo* (0.3M) and *in vitro* (0.0055M). It would be expected that *in vivo*, at a sugar concentration of 0.3M, the phosphorylation rates of all sugars would be the same, and that their absorption rates would follow suit. This is not so, and it must therefore be assumed that some factor, possibly membrane permeability, intervenes, causing the sugar concentration at the enzyme surface to behave as though it were some fifty times less than the sugar concentration in the intestinal lumen.

The evidence presented here suggests that the hexokinase of the intestinal mucosa is an integral part of the absorption mechanism for sugars. The other part of this mechanism involves the dephosphorylation of the esters formed. Since all the sugar monophosphates examined here were dephosphorylated at the same rate, it is unlikely that the dephosphorylation rate plays a part in deciding the relative absorption rates of the various sugars. These relative rates of absorption are determined by the affinity of the hexokinase for the different sugars. The distinction drawn between 'active' and 'passive' absorption is no longer a real one, for both are brought about by the same type of phosphorylation reaction. The effect of phlorrhizin or iodoacetate upon sugar absorption, whereby 'active' absorption is converted into 'passive' absorption may simply be due to the lowering of the concentration of available ATP in the mucosa, and to the effect of this upon the ability of the hexokinase to react with different sugars.

SUMMARY

1. The stock of rats used in the experiments upon the hexokinase reaction *in vitro* show the same behaviour regarding the absorption of sugar *in vivo* as do the rats used in experiments upon absorption described in the literature.

2. The phosphorylation-rate ratios for the sugars investigated are very similar to their absorption-rate ratios.

3. The rate of the hexokinase reaction catalysed by the intestinal mucosa is more than sufficient to account for the phosphorylation of all the sugar

absorbed during the experiments *in vivo*. It is sufficient to account for the phosphorylation of at least 50 % of the carbohydrate consumed by a normal 150 g. rat during 24 hr., and for the phosphorylation of 70–100 % of this carbohydrate after certain legitimate corrections have been applied to the figures for the hexokinase reaction.

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A Chromatographic Study of Uroporphyrins from Cases of Cutaneous, Acute and Congenital Porphyrria

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Chromatographic methods (Nicholas & Rimington, 1949, 1951; Nicholas, 1951; Chu, Green & Chu, 1951; Falk & Benson, 1953*a, b*) have made possible the isolation and characterization of isomeric porphyrins. A study of porphyria cases has revealed much variety in the urinary porphyrin pattern (Nicholas & Rimington, 1951; Rimington & Miles, 1951; Macgregor, Nicholas & Rimington, 1952), but final agreement has not yet been reached as to which particular isomer predominates in the different kinds of porphyria. Thus, while Waldenström (1934, 1935), Waldenström, Fink & Hoerb主ger (1935) and Mertens (1936, 1937) independently described the urinary porphyrin characteristic of acute porphyria as uroporphyrin III, Grinstein, Schwartz & Watson (1945) and Watson, Schwartz & Hawkinson (1945) considered that the 'Waldenström porphyrin' consisted only of uro-

porphyrin I together with smaller quantities of a heptacarboxylic porphyrin. Although there appears to be a good deal of clinical and chemical variability between cases of acute porphyria, nevertheless Nicholas & Rimington (1953), employing a number of available techniques (Kennard & Rimington, 1953), have confirmed Waldenström's original conclusion in several cases of undoubted acute porphyria. Porphyrria cutanea tarda (Waldenström, 1937), during exacerbations, may simulate acute porphyria closely, abdominal colic with or without nervous symptoms occurring and sometimes even porphobilinogen being found. However, the history of cutaneous lesions, presence of porphyrin in the plasma during the attack and high faecal porphyrin excretion during remission serve to distinguish it from acute porphyria. Some cases of porphyria, with cutaneous symptoms appearing late in life, seem to excrete uroporphyrin continuously in the urine (although perhaps with fluctuations of

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