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STUDIES ON INTESTINAL SUCRASE AND SUGAR TRANSPORT

VII. A METHOD FOR MEASURING INTESTINAL UPTAKE. THE ABSORPTION OF THE ANOMERIC FORMS OF SOME MONOSACCHARIDES*

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SUMMARY

1. A procedure for measuring intestinal uptake is presented. It allows randomization of small-intestinal pieces, yields relatively small 'extracellular space' and allows the determination of unidirectional flux.

2. The intestinal uptake of the anomeric forms of some monosaccharides has been measured. The β-form of 6-deoxy-D-glucose is better absorbed than its α-form. In the case of D-glucose and of 3-*O*-methyl-D-glucose, no statistically significant difference could be ascertained between the anomeric forms.

3. Some speculations on the possible role of sucrase and of mutarotase during the intestinal absorption of sugars are presented.

INTRODUCTION

Although many studies have been performed, mainly by CRANE, WILSON, and their associates (reviewed by CRANE[2,3]), on the effect on absorption of the configuration of the various carbon atoms of the sugar, little is known of the requirements at C-1 (ref. 4). The anomeric forms of monosaccharides do not seem to have ever been investigated in this respect, probably because of the technical difficulties involved. One reason for investigating this problem is to provide further information on the relationship between sucrase and the sugar transport system in the small intestine. In fact, sucrase liberates the glucose moiety of the substrate in the α-form[1], and the glucose moiety of sucrose is known to be better absorbed than free glucose[5].

For the purpose of the present investigation it was necessary to develop a procedure to measure intestinal uptake. Since it might have wide application, a detailed description is given.

MATERIALS AND METHODS

Chemicals

α-D-Glucose: a Fluka (Buchs, S.G., Switzerland) product. Gas chromatographic analysis[1] showed that it contained less than 1% of the β-form. β-D-Glucose was a Sigma (St. Louis, Mo.) product.

* VI, ref. 1.

3-*O*-Methyl-D-glucose: a product of Ayerst, McKenna and Harrison (Montreal, Canada). It had an initial $[\alpha]_D$ of 103.5° ($[\alpha]_D$ at equilibrium: 55.5°; 10 % in water; room temp.). The $[\alpha]_D$ of the α-form is 104.5° and of the β-form 31.9° (refs. 6-8). The batch used thus contained 1–2 % of the β-form.

6-Deoxy-D-glucose was a generous gift from Professor E. HARDEGGER[9] (ETH, Zurich). It had an initial $[\alpha]_D$ of 64° ($[\alpha]_D$ at equilibrium: 29°; 8.2 % in water; room temp.; literature[10]: 63.1° and 29.1°, respectively). It thus contained only traces, if any, of the β-form.

Preparation of tissue

The hamsters were killed by a blow on the neck. The intestines were dissected out, everted, washed with 0.9 % NaCl, then with pre-gassed Krebs–Henseleit buffer. They were then cut into pieces 1.2–1.5 cm long, randomized as recommended by CRANE AND MANDELSTAM[11], and placed in a pre-gassed Krebs–Henseleit buffer. These pieces were then mounted in the apparatus demonstrated in Fig. 1. The tissue was carefully placed exactly in the center hole so that the edges of the tissue were excluded from the medium by pressure from the rubber ring. In some experiments a broad rubber band was tightened around the 'sandwich' to reduce any leaking of substances from cuts in the tissue into the medium. This rubber sealing was, however, soon found not to affect the results, and was thus omitted. With some experience the mounting of tissue required only a few minutes.

Incubation conditions

The incubation medium had the following composition: 0.05 M sodium maleate buffer* (pH 6.5), 0.06 M NaCl, 0.006 M KCl, 0.010 M Tris–HCl. This solution was gassed with O_2 shortly before the preincubation. The final concentration of sugar (0.005 or 0.010 M) was obtained by adding a calculated amount of the solid crystalline sugar immediately before incubation, or by adding a small volume of a known amount of sugar equilibrium mixture. Experiments on the absorption of glucose were performed in the presence of L-glyceraldehyde (0.005 M) (see under RESULTS AND DISCUSSION).

4–5 Pieces (each held in the tissue-holding apparatus of Fig. 1) were placed in a beaker containing 20 ml of the incubation medium (± glyceraldehyde) at 37 or at 25° in a metabolic incubator of the Dubnoff type (Gallenkamp), and preincubated for 3–4 min. The sugar was added and the beakers were gently shaken at the same temperature during the incubation time (generally 3 min). At the end of the incubation the apparatuses were removed and rinsed with cold Krebs–Henseleit buffer. The tissue at the center of each apparatus was cut out with the punch (see legend of Fig. 1), gently blotted dry and prepared for analysis as described below. The remaining tissue was discarded.

Analytical procedures

The 4 or 5 pieces of tissue which had been incubated in the same beaker were processed together. After being weighed on a torsion balance, they were deproteinized

* Phosphate buffers were avoided because they are known to increase the mutarotation rate[12–14]. A slightly acidic pH was preferred, in order to approach the pH of minimum mutarotation rate[15].

according to NELSON[16], *i.e.*, they were homogenized in 1–1.5 ml of 5 % $ZnSO_4 \cdot 7\ H_2O$. An equal volume of exactly matched CO_2-free 4.73 % $Ba(OH)_2 \cdot 8\ H_2O$ was then added and the samples were spun down. Aliquots of the media were subjected to the same procedure. Alternatively, deproteinization was carried out with $HClO_4$ *plus* KOH.

3-*O*-Methyl-D-glucose was determined by a reducing method[16]; 6-deoxy-D-glucose, by the method of DISCHE AND SHETTLES[17]; D-glucose, by the glucose–oxidase–peroxidase method[18]. In all cases, tissue blanks, *i.e.*, from intestine incubated in the absence of sugar were subtracted. The results are expressed as μmoles of sugar taken up per min per ml of tissue water (assumed to be 80 % of the tissue wet wt.[11]) and were corrected for the 'extracellular' space.

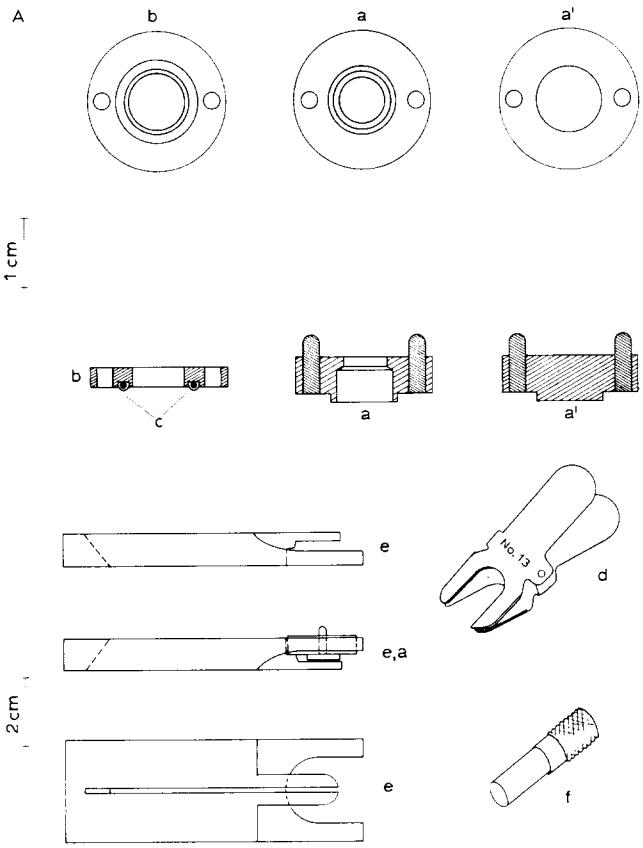
The extracellular space was determined with [^{3}H]mannitol (Amersham, 500 μC/μmole) (mannitol concentration in the medium: 0.38 nmole/ml). After deproteinization as above, ^{3}H was determined in a scintillation counter. As internal standard *n*-[^{3}H]hexadecane (Amersham) was used. Fig. 2 reports the values of the extracellular space found at various incubation times. Since the extracellular space was found to be rather consistently, 8 % after 3 min of incubation, it was not determined in each experiment; the uptake data were simply corrected for this value.

RESULTS AND DISCUSSION

The method

For the present investigation, it was advantageous to establish a procedure which would permit an adequate randomization of intestinal pieces, yield a small extracellular space, and allow only the mucosal side to be exposed to the medium. As BIHLER AND CRANE[19] originally pointed out, the extracellular space increases when the small intestine is cut. This extracellular space may actually be, in part, 'intracellular space', because interepithelial cell membranes do not offer great resistance to diffusion[20]. Thus, the methods based on randomization of cut intestinal pieces[11,21] expose both mucosal and serosal sides to the medium and are subject to a large correction for the extracellular space. The methods using isolated intestinal cells[22–24] are likely to suffer similar limitations. On the other hand, the classical everted-sac technique[25], or other methods using intact intestinal sacs (recently

Fig. 1.A. Tissue-holding apparatus. This consists of three pieces, a lower component (a), an upper (b), and a rubber ring (c), which fits into the slot of the face of piece b. The ring c has a cross-sectional diameter of 1.4 mm and the slot has a depth of 1.2 mm, so that the lower and upper halves, (a) and (b), do not quite touch even when held together with the clamp (d). Component a has two pins and component b has corresponding slots, which assure the exact alignment of the hole which is through the center of each piece. The holder (e), which facilitates the assembling of the apparatus, is shown from the top view and from the side with piece a in place. In order to mount the pieces of tissue, piece a is held by holder e, the cylinder of everted intestine is placed onto it, piece b with the rubber ring is put onto the tissue, and the whole 'sandwich' is held together with clamp d. The outer diameter of the punch (f) (0.78 cm) is slightly smaller than the diameter of the hole in component b, but slightly larger than the diameter of the hole in component a. At the end of the incubation, the punch is used to cut the tissue onto the protruding border of the hole of component a. A modified lower component (a') without a center hole was used to incubate broad pieces of intestine (*e.g.* from rabbit); the serosa surface was placed on piece a'. In this arrangement, therefore, the intestine cut by the punch has an area of approx. 0.48 cm^2 (serosal area). Pieces a, a', b, and e are made of plexiglass, pieces d and f of stainless steel. B. Tissue-holding apparatus. a, e: holder e and lower piece a during the mounting of the intestine. b, c: upper part b with rubber ring c. g: everted hamster intestine ready for incubation. h: punching after incubation. →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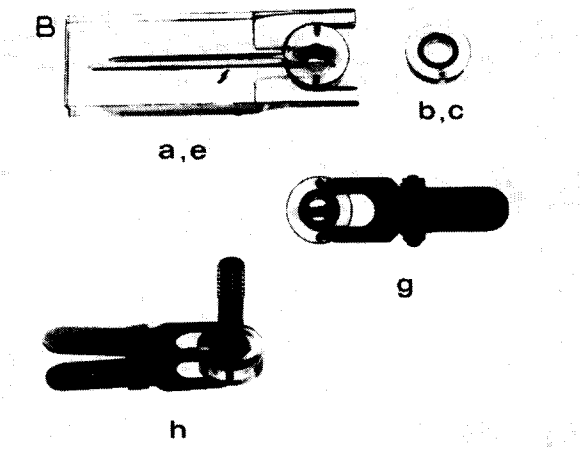
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reviewed by PARSONS[26]), or even the newly developed procedure for measuring unidirectional flow[27] do yield small extracellular space values, but require large pieces of intestine or rather complicated equipment for each sample; they make it impractical to randomize an adequate number of specimens.

The procedure described in detail in this paper combines the advantages of methods which use small-intestinal pieces (easy randomization) with advantages of the methods which use relatively intact, large intestinal pieces (small extracellular space; possibility of exposing to the medium either the mucosa or the serosa, or both). The extracellular space obtained with the present method, although larger than the one obtained with intact tissues[19], is smaller than that obtained with cut intestinal pieces of comparable size (Fig. 2). Furthermore, it permits very short incubation times (Fig. 3) and so inables an easy measurement of unidirectional flow. This method has been mainly used with hamster intestine but it was also applied successfully in our laboratory to rat and rabbit intestine. In the latter case, the intestine was cut open and layered onto part a' (Fig. 1) with the mucosal side facing up.

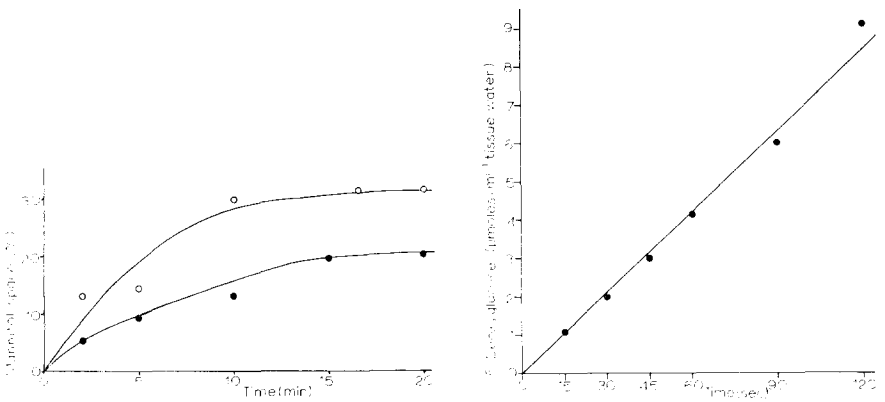

Fig. 2. Mannitol space in pieces of hamster small intestine: ○, free intestinal pieces; ●, pieces mounted in the tissue-holding apparatus of Fig. 1. The free pieces and the mounted ones (punched part) had comparable size (four pieces of 45–50 mg fresh wt. in each incubation mixture). Incubation temperature, 37°.

Fig. 3. Time-course of the uptake of 6-deoxy-D-glucose (equilibrium mixture) in hamster small intestine at 37°. The pieces of intestine were mounted in the apparatuses of Fig. 1, with the mucosal side only exposed to the medium. 6-Deoxy-D-glucose in the medium: 10 mM. The data are not corrected for the negligible extracellular space. The unidirectional flow of 6-deoxyglucose from the mucosal medium into the tissue (J_{mc}) is, therefore, 4.2 μmoles·min^{-1}·ml^{-1} tissue water.

One of the disadvantages of our method, however, is that it requires a relatively large volume of medium (20 ml), and consequently, greater amounts of expensive chemicals. Another disadvantage is that the intestine of very young animals may be too fragile for the rather strong clamps used in the standard procedure.

The specificity of the small-intestinal sugar transport system for the conformation of C-1 of some free monosaccharides

The configurational requirements of the intestinal sugar transport system for most carbon atoms of the substrates have been very thoroughly investigated, mainly by CRANE, WILSON and their associates (reviewed by CRANE[2,3]). Apart from the work of LANDAU, BERNSTEIN AND WILSON[4], however, little is known about the effect of

changes in the configuration of C-1. Using a series of α- and β-glycosides, and measuring the accumulation ratios in intestinal sacs, these authors did not detect a significant preference for either configuration. The uptake of α/β-anomers of free monosaccharides apparently has never been investigated in this system, although similar studies were carried out on the sugar transport system in erythrocytes[28,29].

TABLE I

UPTAKE OF SUGARS IN HAMSTER SMALL INTESTINE FROM THE MUCOSAL SIDE AT 37°

Uptake is given in μmoles·min^{-1}·ml^{-1} tissue water ± S.D. Unless stated otherwise, the substrates were 10 mM.

Sugars	*n*	*Starting from*			*P**
		α-form	*Equilibrium mixture*	*β-form*	
6-Deoxy-D-glucose	25	2.74 ± 0.77	3.04 ± 0.62		<0.02
3-*O*-Methyl-D-glucose	15	1.81 ± 0.54	1.71 ± 0.69		n.s.
D-Glucose (5 mM)	9	2.56 ± 0.86	2.57 ± 0.72		n.s.
D-Glucose (at 25°)	9	1.53 ± 0.93	1.73 ± 1.04		n.s.
D-Glucose (5 mM)	15	5.27 ± 1.35		5.76 ± 1.38	n.s.

* Calculated from the differences in uptake in paired experiments; n.s. = non significant.

Our data on a few free aldohexoses (Table I) are consonant with the conclusions of LANDAU, BERNSTEIN AND WILSON[4] on glycosides. In fact, the differences in uptake between the anomeric forms are small, and only in the case of 6-deoxy-D-glucose was a statistically significant difference ascertained. At the present time it is not clear why such a difference could not be detected for 3-methylglucose, for example. Other inconsistencies of this kind have been pointed out recently by CRANE[3]. Apparently, however, whenever a change in configuration affects intestinal sugar uptake, an equatorial hydroxyl group makes the sugar more absorbable. This conclusion, which was reached for other carbon positions by CRANE[2] and by ALVARADO[30], seems to hold for C-1 also, although this position is a rather 'tolerant' one. The few monosaccharides we investigated all show a 'normal' mutarotation. The anomeric forms involved were therefore the α-pyranosic and the β-pyranosic only. The short incubation times used, together with the strong functional asymmetry of the small-intestinal sugar transport system, make the uptake a measure of the unidirectional flux (see also Fig. 3). Thus, the ambiguity related to the dual nature of carrier kinetics[31] is avoided* and a larger uptake indicates a smaller K_m and/or a larger maximum velocity. In the case of glucose, a different metabolic utilization of the two anomeric forms, combined with the short incubation times used, may have been a source of error. Although this error is likely to be minimal because of the high mutarotase activity present in the tissue (see below), we still tried to reduce it by using the hamster as an experimental animal (hamsters utilize less glucose than other species[25]) and by adding L-glyceraldehyde to the incubation medium. Although never tested on the small intestine, this substance reportedly inhibits glucose utilization in a variety of tissues[32,33,51]. A few

* The two groups of scientists who investigated the same problem in erythrocytes, *i.e.* in a symmetric transport system, arrived at contrasting conclusions in spite of having obtained coinciding results[28,29].

experiments were also carried out in the absence of L-glyceraldehyde; they also failed to show any difference in the uptake of the two anomeric forms of glucose.

Some speculations on the role of sucrase and mutarotase in connection with intestinal sugar transport

In 1961 MILLER AND CRANE[5] showed that the glucose moiety of sucrose is better absorbed than free glucose by hamster intestine, *i.e.*, the tissue-glucose to medium-glucose ratio is larger if the intestine is incubated in sucrose than in free glucose (see also ref. 35). This observation was interpreted as showing a close spatial relationship of sucrase and sugar carrier. In 1967 we showed that intestinal sucrase liberates the glucose moiety of the substrate in the α-form only[1]. Thus, an alternative explanation to the observation of MILLER AND CRANE became possible: if the sugar transport system happened to prefer the α-form of glucose over its β-form, the kinetic advantage of the glucose liberated by sucrase would be explained by the identity of the anomeric form arising from sucrose with that possibly preferred by the carrier. The data presented in this paper (Table I) show, however, that this hypothetical alternative is not tenable.

Thus, the kinetic advantage of the glucose moiety of sucrose over free glucose unequivocally shows a close spatial relationship between sucrase and the sugar carrier, as MILLER AND CRANE[5] originally suggested. To explain this kinetic advantage, at least three possibilities can be envisaged: (a) Sucrase liberates α-glucose and fructose very close to their carriers; these monosaccharides, therefore, are transported through the membrane before they diffuse into the medium. For this, no special anatomical arrangement needs to be postulated; a simple Nernst diffusion film 0.01–0.001 cm thick[36] suffices. The local hyperconcentration in the immediate proximity of the carrier is responsible for the kinetic advantage of glucose. (Fructose also can yield tissue to medium ratios larger than the one under these conditions[5].) (b) Sucrase may act as a permease (defined by KEPES[37]), transferring free glucose onto the carrier. However, the development of intestinal sucrase is not accompanied by any detectable decrease of the apparent K_m for the intestinal uptake of free glucose (G. SEMENZA AND V. COLOMBO, unpublished observations). (c) Sucrase may act as a transglucosidase, transferring the glucosyl moiety onto the carrier which would be the 'natural acceptor' for such transglucosidase activity. This sucrase-induced asymmetry of the carrier, rather than a local hyperconcentration of free substrate, would be the reason for the kinetic advantage of the glucosyl moiety of sucrose. This hypothesis, which at present cannot be proved or disproved, would explain why most intestinal membrane-bound hydrolases have strong transferase activity[38], whereas most soluble hydrolases, *e.g.*, pancreatic hydrolases, do not.

It should be noted that these possibilities are not mutually exclusive. Possibility (c) fails to account for the kinetic advantage of the fructose moiety quoted above. Intestinal sucrase, in fact, has no transfructosidase activity[39] (G. SEMENZA AND V. COLOMBO, unpublished observations) (see, however, ref. 40).

As pointed out earlier by other workers, a remarkable parallelism exists between mutarotase activity and sugar transport capacity. Mutarotase activity is high in tissues which can transport sugar actively[41], is inhibited by phlorizin[41], and has a substrate specificity similar to the sugar transport system[41,42]. It has thus been suggested that mutarotase is the carrier for sugar transport across the membrane[41,43].

This hypothesis was criticized because 1,5-anhydroglucitol and the glucosides (which obviously do not mutarotate) are absorbed by the sugar transport system[44], the mutarotase activity is not associated with the brush borders of columnar cell[45] which certainly contain the sugar transport system[46,47], and because the similarity in substrate specificity between mutarotase and sugar transport system is less marked than previously believed[3,48]. Finally, the rate of enzyme-catalyzed mutarotation of glucose in the intestine is at least one order of magnitude greater than that of glucose absorption (ref. 48)*. Still, the parallelism between mutarotase and sugar transport is too puzzling to be dismissed as merely coincidental. The broad tolerance of the sugar transport system for the configuration of C-1 (Table I), together with the narrow specificity of some of the first enzymes in some metabolic pathways[49,50] indicates a possible metabolic role for mutarotase in one of the first steps of intermediary metabolism, rather than in the transport proper.

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* We have identified the anomeric forms of the absorbed 3-*O*-methylglucose, starting from either α-3-*O*-methylglucose or from its equilibrium mixture in the medium. In every case we found that the tissue 3-methylglucose had a composition (36–42% α- and 64–58% β-form) similar to that of the equilibrium mixture (32.5% α-form; 67.5% β-form[7,8]). (The incubations were carried out exactly as described here under MATERIALS AND METHODS. After incubation, the tissue was punched, frozen in liquid air, lyophilized, directly persilylated and chromatographed as described elsewhere for sucrase[1]. Unpublished experiments with H.-CH. CURTIUS AND M. MÜLLER.)

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