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PHLORIZIN HYDROLASE: A β-GLUCOSIDASE OF HAMSTER INTESTINAL BRUSH BORDER MEMBRANE

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SUMMARY

A β-glucosidase activity catalyzing phlorizin hydrolysis to phloretin and glucose is present in the microvillus membrane of hamster intestinal brush border.

The pH optimum, K_m and the effects of Tris, galactono- and gluconolactones, sulfhydryl reagents and heavy metals were studied. This β-glucosidase is distinct from the other brush border glycosidases documented in the literature.

The presence of this enzyme serves to rationalize the divergent observations that cellular entry of low concentrations of mucosally added phlorizin has not been detected although high concentrations have been reported to inhibit intracellular metabolism.

INTRODUCTION

Phlorizin, a naturally occurring plant glucoside, has found frequent use as a potent, competitive inhibitor of the glucose transport system in mammalian intestine[1,2]. Although a variety of other aliphatic and aromatic glucosides have been found to be actively transported by mammalian intestine and to share a common pathway with glucose[3], phlorizin appears to be different. Phlorizin strongly interacts with the transport system, which provides for cellular accumulation of other glucosides, yet it is, itself, not accumulated by epithelial cells, at least to an extent measurable chemically[3] or autoradiographically[4]. Interaction of phlorizin with the intestinal wall has been said to involve two binding sites; one for the glucose moiety which is the same as the initial binding site of glucose transport and another for the aglycone, phloretin[5]. Phloretin is not a potent inhibitor of sugar transport in the mammalian intestine. A degree of inhibition by phloretin comparable to that by phlorizin requires concentrations at least 1000 times greater. However, the phloretin moiety binds and the presence of two membrane binding sites, one of them not identical with the binding site of transport, may explain the failure of phlorizin to be transported. However, if phlorizin does not enter the cell, inhibition of cellular metabolism by high concentrations of phlorizin[6] would appear to be anomalous.

Recently, an additional new finding which must be taken into account in attempts to understand the interaction of phlorizin with mammalian intestine has been made. We have observed a rapid hydrolysis of phlorizin by hamster intestine brush borders with the release of free glucose. Extensive studies of this phenomenon

have now documented the presence at the brush border membrane of a hitherto unrecorded enzymic activity; namely, a β-glucosidase active against phlorizin and not identical with any previously known brush border enzyme. Although earlier workers recorded β-glucoside-splitting activity by everted sacs and rings of hamster small intestine[1,7] and by homogenates of rat and pig intestinal mucosa[8,9], positive identification of the site of the activity was not made.

MATERIALS AND METHODS

Most compounds used were obtained from the same commercial sources previously noted[1,3]. Gentiobiose and cellobiose were obtained from Sigma Chemical Co., St. Louis, Mo. In a preliminary communication we reported that cellobiose had considerable inhibitory action on β-glucosidase[10]. The particular preparation of cellobiose used, however, was obtained from a different commercial source and was found to be grossly contaminated as revealed by uncharacteristic absorption in the 5.6–5.8 μ region of the infrared spectrum. The cellobiose obtained from Sigma Chem. Co., which was used in all experiments reported below, did not exhibit any unusual absorption bands and showed a spectral absorption comparable to that described by KUHN[11]. Phlorizin, phloretin, salicin and arbutin were recrystallized from ethanol–water before use.

Most of this study was carried out with hamster intestinal brush borders isolated as previously and extensively described[12]. In brief, animals were starved overnight and killed by stunning. The small intestine was excised and its contents washed out with cold isotonic saline. The mucosa was removed by scraping, homogenized in cold 5 mM EDTA (pH 7.4) and the brush borders separated by centrifugation. In experiments where further localization was desired, the brush borders were disrupted with 1 M Tris (pH 7.4) and the resultant fractions, which include one composed only of microvillus membranes, were separated on a glycerol density gradient in the manner detailed by EICHHOLZ AND CRANE[13]. A few experiments with everted sacs were carried out by the method of WILSON AND WISEMAN[14].

Assay methods

Unless otherwise stated, β-glucosidase was measured at pH 6.0 in McIlvaines citrate–phosphate buffer[15] using as substrate phlorizin or *p*-nitrophenol-β-D-glucoside at concentrations of 2.5 mM and at 37°. β-Galactosidase activity was measured under similar conditions using *p*-nitrophenol-β-D-galactoside (2.5 mM) as substrate. Ordinarily the reaction was arrested by the addition of 0.19 M $ZnSO_4$ and protein-free filtrates prepared by further addition of 0.3 N $Ba(OH)_2$ according to SOMOGYI[16]. However, when very low concentrations of substrates were used, as in the determination of Michaelis' constants, the reaction was stopped by immersing the tubes in a boiling water bath for 3 min so as to avoid excessive dilution of the hydrolysis products to be assayed.

Disaccharidases were assayed according to the method of DAHLQVIST[17].

When phlorizin was used as substrate, liberated glucose was measured by the reducing sugar method of SOMOGYI[18]. Inasmuch as phloretin, the aglycone released upon hydrolysis of phlorizin, also causes some reduction of Cu^{2+}, standards for comparison were prepared from equimolar mixtures of glucose and phloretin. Unhy-

drolyzed phlorizin contributes no color in this reaction. In experiments using *p*-nitrophenyl glycosides, liberated *p*-nitrophenol was measured at 420 mμ in a Beckman DU spectrophotometer after addition of 0.05 M Na_2CO_3.

Protein was determined by the method of LOWRY *et al.*[19]. Specific activity is measured as nmoles of substrate hydrolyzed per min per mg protein.

RESULTS AND DISCUSSION

The presence and locus of β-glucosidase activity in hamster intestine

In our earliest studies of the effects of incubation of phlorizin with isolated brush borders, the fact that glucose was liberated was indicated by a small but positive color reaction with the Tris–glucose oxidase reagent. Careful investigation of the phenomenon revealed that phloretin and, to a lesser extent, phlorizin interfere in the assay reaction and reduce the amount of color produced. When we turned to SOMOGYI's reducing sugar method[18], brush border catalysis of phlorizin hydrolysis was very evident. Accordingly, the ability of brush borders to catalyze hydrolysis of a variety of glycosides was tested.

TABLE I

HYDROLYSIS OF β-GLYCOSIDES BY HAMSTER BRUSH BORDERS

The assay mixture contained buffer, 10 μmoles of substrate and 1.0 mg of brush border protein in a vol. of 1 ml. Gentiobiose, cellobiose and lactose splitting was assayed by the glucose oxidase method, the others by reducing sugar. Incubations were for 60 min at 37°.

Substrate	*Specific acticity (nmoles/min per mg)*
Phlorizin	48.0
p-Nitrophenyl-β-glucoside	42.0
Cellobiose	10.8
Salicin	8.0
Arbutin	4.0
Phenyl-β-glucoside	3.4
Gentiobiose	Nil
Methyl-β-glucoside	2.8
Lactose	58.0
p-Nitrophenyl-β-galactoside	35.8
p-Nitrophenyl-β-xyloside	1.15
Methyl-β-xyloside	0.02
p-Nitrophenyl-β-glucuronide	0.67
Methyl-β-arabinoside	0.02

The results are assembled in Table I. Under the conditions of the experiment, glucosides and galactosides were hydrolyzed to the greatest extent during the arbitrary time interval of incubation. Also aryl glycosides were hydrolyzed to a greater extent than the corresponding alkyl analogues. Only one compound tested; namely, gentiobiose-(6-*O*-(β-glucopyranosyl)-D-glucopyranose) was not hydrolyzed by brush borders nor, indeed, by any kind of preparation from hamster intestine. Although it at first seemed possible that one enzyme might account for the hydrolysis of both glucosides and galactosides, further experiments indicated the presence of two separate activities.

From reports in the literature[20–25], it may be concluded that there are at least two intestinal β-galactosidases; one, a particle-bound or brush border enzyme optimally active near neutral pH and preferentially hydrolyzing lactose, and the other, a cytoplasmic enzyme with a more acid pH optimum and no pronounced specificity for the aglycone. There was no information on the possible number of β-glucosidases. Studies of the effect of pH on hydrolysis of a representative β-glucoside and β-galactoside by brush borders and brush border-free homogenates gave information on this point. The results are shown in Fig. 1. Hamster intestine shows two β-galactosidase activities; namely, a cytoplasmic, presumable lysosomal, β-galactosidase, optimally active at pH 3.5, and a brush border β-galactosidase with an optimum at pH 5.5. There is, however, only one apparent β-glucosidase activity, namely that in the brush border. Little or no activity could be detected in the brush border-free homogenates

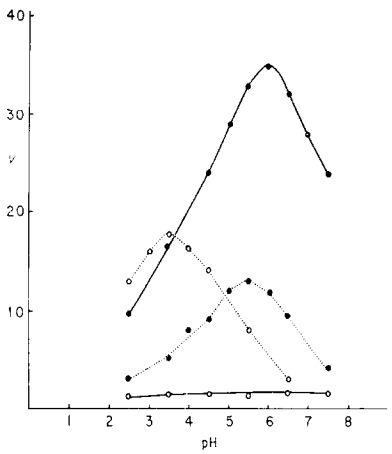

Fig. 1. β-Galactosidase and β-glucosidase activities in the intestinal mucosa. The assay methods are described in the text. Citrate–phosphate buffers were used at all pH values. ●, experiments with brush borders; ○, experiments with brush border-free homogenates. ———, β-glucosidase; ·····, β-galactosidase. The substrates used were, in both instances, the *p*-nitrophenylglycoside at a concentration of 2.5 mM. Incubation time was 10 min excepting incubation of brush border-free homogenate with β-glucoside which was for 60 min. V = specific activity.

TABLE II

LOCALIZATION OF β-GLUCOSIDASE IN HAMSTER INTESTINAL MUCOSA

The mucosal homogenate is a preparation of mucosal scrapings suspended and homogenized in 5 mM EDTA. The supernatant fraction is that remaining after a low-speed centrifugation to remove brush borders.

Fraction	*Specific activity (nmoles/min per mg)*	
	Hydrolysis of phlorizin	*Hydrolysis of p-nitrophenyl-β-glucoside*
Mucosal homogenate	3.95	3.26
Brush borders	48.5	38.4
Supernatant fraction	0.69	0.86

over a wide range of pH and with up to 60 min of incubation. As expected then and shown in Table II, all the β-glucosidase activity of a mucosal homogenate is recovered in isolated hamster brush borders at a greater than 10-fold increase in specific activity over that in the homogenate. A similar localization of β-glucosidase activity in the brush borders obtained from rat and mouse intestine was also observed.

Although this evidence for localization of β-glucosidase activity would ordinarily satisfy us, we felt the implications of this finding required some additional effort. It has lately become increasingly evident that a number of glycosidases are present in the lysosomes of various mammalian tissues including the intestine, and in the latter the lysosomes have been reported to be particularly concentrated in the apical area of the mucosal cells[26–33]. Moreover, β-glucosidase activity of liver lysosomes has been reported to be membrane bound[30]. Inasmuch as some subjacent cytoplasm adheres to some brush borders in even the most successful preparations, the possibility of lysosomal enzyme contamination of our brush border preparations seemed very real. To eliminate this possibility, we carried out the following experiment. Intact everted sacs of hamster intestine were incubated with phlorizin on the mucosal side only. The free glucose liberated with time was assayed in the mucosal medium, in the carefully blotted tissue sac and in the serosal fluid. We found no measurable glucose in either the tissue or the serosal fluid, whereas an average of 0.95 mM glucose (4 experiments) was contributed linearly over 15 min to the mucosal medium. These results can be explained only if the β-glucosidase responsible for phlorizin hydrolysis is peripherally situated in the brush border. Glucose would be liberated preferentially into the medium because it would be prevented entry and accumulation in the cell by the presence of the phlorizin serving as substrate.

As expected on the basis of the foregoing, the β-glucosidase activity of hamster intestine was found in the membrane fraction when isolated brush borders were treated in the manner described by EICHHOLZ AND CRANE[13]. As shown in Table III, Fraction C, which consists of microvillus membranes[34], has a specific activity for β-glucosidase that is several-fold increased over that of the brush borders. This is in accord with earlier observations[35] on other brush border hydrolases.

The enzymic activities present in the microvillus membrane can be sequentially removed by the action of papain; the digestive disaccharidases are removed at the earliest period of papain action, the peptidases after longer periods of digestion, and some alkaline phosphatase and most trehalase remain bound to the residual

TABLE III

LOCALIZATION OF β-GLUCOSIDASE ACTIVITY IN FRACTIONS OF TRIS-DISRUPTED BRUSH BORDERS

The substrate used was phlorizin at a concentration of 2.5 mM. Fraction C contains the membranes of the microvilli.

Fraction	*Specific activity (nmoles/min per mg)*
A	nil
B	52.0
C	208.0
C^1	50.0
D	30.0

membrane[36]. Like the digestive disaccharidases, β-glucosidase is also cleaved from the membrane by papain and is found in the same fraction. About 75 % of the activity is recovered (Table IV).

TABLE IV

RELEASE OF β-GLUCOSIDASE FROM THE MICROVILLUS MEMBRANE BY PAPAIN

The isolated membrane fraction (Fraction C, Table III) was digested for the time period indicated under the conditions described by EICHHOLZ[36].

Digestion with papain (min)	*Activity recovered (%)*		
	Sucrase	*Lactase*	*β-Glucosidase*
5	80	70	76
45	98	86	75

Properties of brush border β-glucosidase

pH dependence and activation by sodium phosphate

It has been pointed out that the kinetic characteristics of sodium activation of intestinal transport of sugars bear a strong resemblance to sodium activation of sucrase, and it has been suggested that these two functions share a common site[37]. Although there is evidence from which to conclude that these two functions do not occur at the same site, the possibility of a close spatial relationship between two

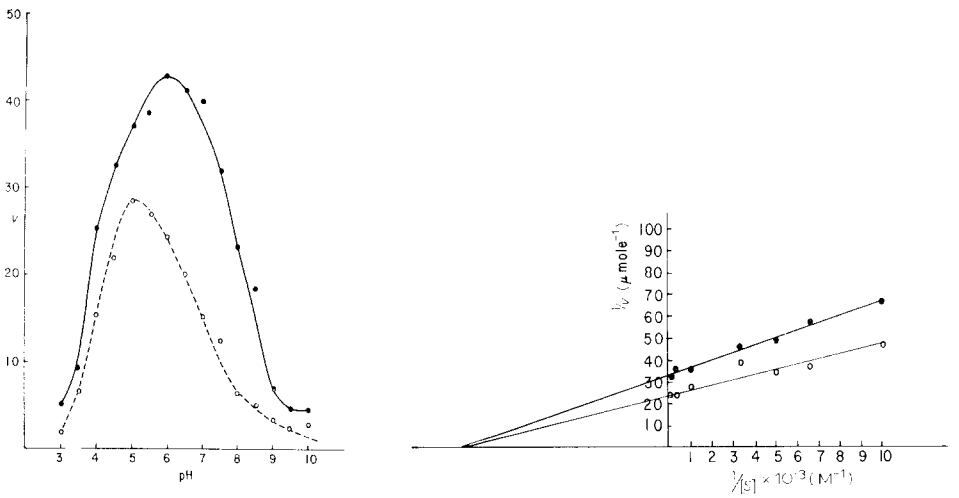

Fig. 2. The pH dependence of β-glucosidase in the presence and absence of sodium phosphate. The buffers used were: citric acid–KOH, pH 3.0–6.0; Tris–maleic acid, pH 5.0–8.5; and ammediol–HCl, pH 8.0–10.0. When sodium phosphate was added (0.05 M), care was taken to correct pH shifts. ○ - - ○, sodium phosphate absent; ● —●, sodium phosphate present. V = specific activity.

Fig. 3. Lineweaver–Burke plots for β-glucosidase with and without added sodium phosphate. The substrate was phlorizin. The reaction (in 1 ml) was stopped by immersion in boiling water. Reducing value was measured on the entire assay mixture at substrate concentrations below 1 mM or in aliquots of the assay mixture at higher concentrations. The reliability of the assay at low concentrations of reducing sugar was increased by replicate determinations. ●- -●, in the presence of sodium phosphate; ○— ○, in the absence of sodium phosphate.

such dissimilar but sequentially operative functions cannot lightly be dismissed. There is merit in the suggestion that the same entity may catalyze both. Consequently, the presence of a β-glucosidase which has no obvious important digestive function in the same region of the cell as the sugar carrier was of great potential interest. Studies designed to elucidate the relationship, if any, were carried out.

Fig. 2 shows that β-glucosidase has a pH optimum at 5.0 when tested in buffers containing no Na^+. Addition of Na^+ as NaCl changed neither the pH optimum nor the velocity of reaction. However, when sodium phosphate was added to the buffer systems, there was a substantial increase in reaction rate accompanied by a shift of pH optimum from 5.0 to 6.0. Further studies showed that this effect was produced only when Na^+ and PO_4^{3-} were present together (Table V). As to the question of a possible relationship between transport and membrane β-glucosidase, it may be noted that sodium phosphate activation of β-glucosidase differs markedly from Na^+ activation of hamster intestinal sugar transport and hamster sucrase. Sodium phosphate does not appear to influence the K_m of β-glucosidase. All of the effect is an enhancement of the maximal velocity (Fig. 3). Contrary to this, the effects of Na^+ on transport and sucrase, specifically in the hamster, are clearly on the carrier–sugar and enzyme–sugar affinities[37,38]. Moreover, the K_i for phlorizin binding at the carrier site for sugars is on the order of 10^{-6} M (ref. 3), whereas the K_m for phlorizin hydrolysis by the β-glucosidase is 10^{-4} M (Fig. 3). These disparities seem to rule out any possibility that β-glucosidase and sugar transport share a common site.

TABLE V

ACTIVATION OF BRUSH BORDER β-GLUCOSIDASE BY SODIUM PHOSPHATE

The substrate was 2.5 mM phlorizin. All buffers and additions of salts were at a concentration of 0.05 M, pH 6.0. Incubation was for 10 min at 37°.

Buffer	*Specific activity (nmoles/min per mg)*
Tris chloride	32.0
Tris chloride *plus* NaCl	32.0
Tris chloride *plus* sodium phosphate	42.0
Tris phosphate	33.0
Tris phosphate *plus* NaCl	42.0
Potassium phosphate	32.0
Potassium phosphate *plus* NaCl	42.0
Sodium phosphate	42.0

A search of the literature shows that the properties we have found are more or less characteristic of β-glucosidases. Some preparations of β-glucosidase from other sources are activated by citrate–phosphate buffer and neutral salts and are reported also to exhibit shifts in pH optima depending on the nature of the buffer used[39]. The reasons for such effects remain obscure.

Specificity

The presence of cellobiase activity in intestinal tissue inseparable from and closely associated with lactase activity has led some to conclude that β-glucosidase and lactase activities are exerted by the same emzyme[9,23], despite evidence that β-glucosidase, hetero-β-galactosidase and lactase are separable activities in the rat[8].

In recent years β-glucosidase has been separated from β-galactosidase in other tissues like brain[40] and liver[30]. Consequently it should not be entirely unexpected that hamster intestinal β-glucosidase emerges as distinct from brush border β-galactosidase, that is, from lactase. The experiments which prove this follow.

Effect of Tris

Tris is a competitive inhibitor of several carbohydrases including intestinal lactase[41,42]. Our first positive indication that brush border β-glucosidase activity was not due to lactase was the finding that brush border hydrolysis of phlorizin remained unchanged in the presence of the Tris used for Na^+ replacement in experiments on Na^+ activation (Table V). Carrying this observation further, it is shown in Table VI that hydrolysis of phlorizin remains quite unaffected by concentrations of Tris strongly inhibitory to lactase, sucrase and cellobiase. Tris inhibition is attributed to the unionized free base because it increases with alkaline pH[41]. However, even at higher pH ranges, Tris did not affect phlorizin hydrolysis. For contrast, note that the sensitivity of the hydrolysis of cellobiose to Tris is similar to that of sucrose and lactose in spite of the fact that it is a β-glucoside.

TABLE VI

EFFECT OF TRIS ON THE HYDROLYSIS OF SEVERAL GLYCOSIDES BY BRUSH BORDER ENZYMES

Concn. of Tris (mM)	*Inhibition of hydrolysis (%)*			
	Sucrose	*Lactose*	*Cellobiose*	*Phlorizin*
1	35	5	0	0
5	68	24	18	0
10	86	50	30	0
50	95	95	65	0
100	99	99	64	5

The fact that cellobiose hydrolysis was not completely inhibited by Tris suggests that lactase is not the only enzyme capable of splitting this β-glucoside. Although we at first believed that cellobiose inhibited β-glucosidase activity and thus could account for the discrepancy in Tris inhibition, further investigation revealed that the sample of cellobiose used was grossly impure. Sigma cellobiose does not inhibit β-glucosidase activity (see below). Consequently, there is a modest basis for an inference that a second β-glucosidase, *i.e.*, an enzyme distinct from both lactase and "phlorizin hydrolase", is present in these tissue and membrane preparations.

Effect of heavy metals and sulfhydryl reagents

Since Tris is without effect on brush border β-glucosidase, there is no basis for assuming that imidazole groups implicated in the active centers of Tris-sensitive carbohydrases[41,42] are involved in the catalytic site of this glucosidase. Tests for sensitive sulfhydryl groups were consequently made. At 1 mM, Ca^{2+}, Ba^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+} and Hg^+ were without effect as were the sulfhydryl reagents *p*-chloromercuribenzoate (1 mM), *N*-ethyl maleimide (1 mM) and iodoacetate (10 mM). However, the enzyme was inhibited by 0.1 mM Ag^+ and Hg^{2+} to 48 % and 26 %, respectively.

The inhibition caused by Ag^+ and Hg^{2+} to an activity not inhibited by Tris or

sulfhydryl reagents is rather curious. However, this heavy metal inhibition is not reversible by dialysis and may reflect a partial denaturation similar to that described for calf lactase by WALLENFELS AND FISCHER[42].

Inhibition by lactones

Various glycosidases are competitively inhibited by aldonolactones corresponding to the substrates in configuration[43]. Accordingly, the effects of glucono- and galactonolactone were tested. The results, displayed in Table VII, show clear differences between brush border β-glucosidase, brush border lactase and cytoplasmic β-galactosidase. β-Glucoside hydrolysis was powerfully inhibited by gluconolactone but not at all by galactonolactone, even at very high concentrations ($5 \cdot 10^{-2}$ M). On the other hand, lactose and cellobiose hydrolysis was inhibited by gluconolactone as well as galactonolactone. Cytoplasmic β-galactosidase was not inhibited by gluconolactone but was sensitive to galactonolactone.

TABLE VII

EFFECTS OF LACTONES ON INTESTINAL β-GLYCOSIDASE ACTITIVIES

K_i values were determined by DIXON's graphical method[45] in which $1/v$ is plotted against the concentration of the inhibitor (lactone) using two or more different concentrations of substrate. Lactose and cellobiose were tested at 1, 5 and 10 mM, *p*-nitrophenyl-β-galactoside at 1, 2.5 and 5 mM, and phlorizin, at 0.5, 1 and 2 mM. Gluconolactone was used at 0.025, 0.05, 0.1, 0.25 and 0.5 mM. Galactonolactone was used at 0.25, 0.5, 1.0, 2.5, and 5.0 mM. All activities were assayed at optimal pH.

	Substrate	K_i (*M*)	
		Gluconolactone	*Galactonolactone*
Brush borders	Phlorizin	$1.8 \cdot 10^{-4}$	Not inhibited
	Lactose	$2.75 \cdot 10^{-4}$	$2 \cdot 10^{-3}$
	Cellobiose	$3.00 \cdot 10^{-4}$	$1.9 \cdot 10^{-3}$
	p-Nitrophenyl-β-galactoside	$2.1 \cdot 10^{-4}$	$1.3 \cdot 10^{-3}$
Brush border-free homogenate	*p*-Nitrophenyl-β-galactoside	Not inhibited	$1.35 \cdot 10^{-3}$

In comparing the K_i values given in Table VII, it should be noted that the K_i values with galactonolactone, which were 10 times greater than those with gluconolactone, may be an artificial result of using the γ-lactone (galactono 1–4 lactone). The inhibitory species is thought to be the δ (1–5) lactone, which is formed from the γ-lactone in solution[44].

Specificity for C-4 configuration in the glycone

Additional evidence that β-glucosides (other than cellobiose) and β-galactosides do not interact with the same catalytic site on the brush border was obtained from kinetic studies. Using equal relative concentrations ($\Theta = [S]/K_m$) (ref. 45) of *p*-nitrophenyl-β-glucoside ($K_m = 5 \cdot 10^{-4}$ M) and *p*-nitrophenyl-β-galactoside ($K_m = 6 \cdot 10^{-3}$ M) the mixed substrate velocity was always the sum of the individual velocities obtained with the two substrates separately (Table VIII). Moreover, in other experiments, it was found that lactose and cellobiose, even at relatively high concentrations (100 mM), did not affect the hydrolysis of *p*-nitrophenyl-β-glucoside whereas the

TABLE VIII

RATES OF HYDROLYSIS OF *p*-NITROPHENYL-β-GLUCOSIDE AND *p*-NITROPHENYL-β-GALACTOSIDE ALONE AND IN MIXTURE

$[S]/K_m$	*p*-Nitrophenol liberated/min per mg protein (nmoles)		
	p-Nitrophenyl-β-glucoside	*p*-Nitrophenyl-β-galactoside	*p*-Nitrophenyl-β-glucoside plus *p*-nitrophenyl-β-galactoside
2.0	29.0	36.4	66.0
1.0	23.0	28.8	53.0
0.8	20.0	24.8	44.0
0.5	14.8	14.8	29.0
0.4	12.0	13.8	25.4
0.2	8.9	8.75	17.3

hydrolysis of the galactoside analogue was considerably (80 %) depressed by both. It may be concluded that the β-galactosides are hydrolyzed at a different site.

Comments on the specificity of lactase

The seemingly anomalous behavior of cellobiase activity in being a property of lactase rather than of phlorizin hydrolase requires comment. It is known that glycosidases from various sources exhibit a rather rigid specificity for the glycone residue of the substrate molecule and are less discriminatory with regard to the aglycone, although the latter may strongly influence enzyme–substrate affinity[46]. Intestinal lactase is a β-galactosidase which hydrolyses lactose much more readily and hetero-β-galactosides much less readily than do β-galactosidase from other sources[42]. Thus, it seems possible that lactase is unusual in having considerable specificity for both the glycone and aglycone moieties of the substrate molecule. It appears to prefer a β-galactosyl glycone and a glucosyl "aglycone". On this basis, lactose (4-(β-D-galactoside)-D-glucose), cellobiose (4-(β-D-glucoside)-D-glucose) and even a hetero-β-galactoside (*p*-nitrophenyl-β-galactoside) could be imagined to undergo hydrolysis at the same site since these substrates meet the specificity required of at least one of the two portions of the molecule, *i.e.*, either the glycone, the aglycone, or both. It would not be expected that hetero-β-glucosides, like *p*-nitrophenyl-β-glucoside and phlorizin, would undergo hydrolysis, since they fail to meet the specificity required of either the glycone or aglycone. Some support for this analysis seems to be provided by the inhibition of brush border lactase by both galactono- and gluconolactones (Table VII) and the absence of inhibition by a hetero-β-glucoside (Table VIII).

The permeability of mucosal cells to phlorizin

The anomaly of the apparent inhibition of cellular metabolism by high concentrations of mucosally added phlorizin, a compound which in low concentrations appears not to enter the mucosal cells, was mentioned in the INTRODUCTION. One possible answer to this anomaly comes out of the present work. It seems possible that inhibition of cellular metabolism is accomplished by phloretin rather than phlorizin. Formation of phloretin as a product of phlorizin hydrolase activity would occur most markedly at concentrations of phlorizin above 10^{-4} M, the K_m of the enzyme. Phloretin,

in contrast to phlorizin, is a lipid soluble molecule which can penetrate the cell in the absence of a specific transport system. The result of this combination of factors could be an inhibition of cellular metabolism by phloretin which would not readily be distinguishable from a direct effect of phlorizin especially as phlorizin is known to inhibit a number of enzymes[2].

On the twin bases of this analysis of the significance of phlorizin hydrolase and the failure of intracellular phlorizin to be detected when the compound is present luminally at low concentrations, there is adequate reason for taking the position that phlorizin cannot penetrate the brush border membrane of hamster epithelium. Whether this position will remain tenable depends upon the results of a complete reinvestigation of hitherto well-accepted older work.

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