THE HYDROLYSIS OF SUCROSE, LACTOSE, AND CELLOBIOSE BY SMALL INTESTINAL MUCOSA *IN VITRO*.

RELATIONSHIP TO LAXATION IN THE RAT PRODUCED BY THESE DISACCHARIDES *IN VIVO*[1]

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The ingestion of lactose in fairly large amounts is followed by laxation in many species of animals. The available evidence suggests that this laxation occurs because lactose is rather slowly hydrolyzed in the digestive tract; hence, unsplit molecules remain for a rather long time in the lumen and exert a prolonged osmotic (hydragogue) effect. This mechanism is suggested by direct experiments in the dog (Albertoni, 1891; Röhmann and Nagano, 1903), and by indirect evidence in several other species (reviewed by Fischer and Sutton, 1949). To further test the idea that slow hydrolysis rate and laxative action are related in the rat, it seemed desirable to compare the hydrolysis and laxative action of several disaccharides. In addition to lactose, the disaccharides sucrose and cellobiose were chosen for study. Sucrose was chosen because, even at high levels in the diet, it does *not* produce much diarrhea in the rat (Mitchell, 1927). Cellobiose was studied, on the other hand, because the very low "absorption coefficient" of this disaccharide in the rat suggested that the rate of intestinal hydrolysis of cellobiose was slow (Vaniman and Deuel, 1944). Thus, cellobiose in the gut would be expected to produce diarrhea readily.

The present paper gives (a) the relative laxative actions of sucrose, lactose, and cellobiose administered by stomach tube to fasted rats; and (b) the relative rates of the hydrolysis of these three disaccharides catalyzed by rat small intestinal mucosa *in vitro*, and the effects of calcium gluconate and calcium lactate thereupon. Limited data on the activity of certain disaccharidases in calf and pig mucosa also are given.

METHODS

Male rats of a modified Wistar strain were obtained from the colony of the Department of Agricultural Biochemistry of The Ohio State University.

The rats were fed a stock diet containing 60 percent yellow corn meal, 18 percent casein (commercial), 14 percent linseed oil meal, 6 percent alfalfa leaf meal, 1 percent sodium chloride, and 1 percent calcium carbonate, *ad libitum*, plus whole milk *ad libitum*.

When the laxative action of disaccharides was studied, nine mature rats were housed in individual cages with wire screen floors. The rats' feces dropped through the screens onto papers. The papers were changed every day. The rats were fasted for 24 hours, at the end of which time they averaged 282 gm. in weight. Then, 5.0 ml. of a solution containing a known amount of a disaccharide in distilled water was administered by stomach tube. The rats were then allowed the stock diet *ad libitum*, but no milk. On each succeeding day, the feces were examined and the diarrhea classified according to the method of Riggs and Beaty (1947). When no more diarrhea occurred, feed was withdrawn again, and the procedure was repeated with a different disaccharide or a

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different amount of disaccharide. A number of such trials were conducted with each rat.

Small intestinal mucosa was prepared for use in the *in vitro* studies of disaccharidase action as follows. Calf and pig intestines were obtained as soon as the viscera were removed in the usual slaughter procedure. Sections of intestine were washed out with tap and distilled water. The mucosa was stripped out by hand without slitting the gut open, blended in a Waring blendor, and preserved with toluene in the cold for one or more days. Later, aliquots were ground with sand in a mortar, diluted with approximately ½ volume of distilled water, and stored in the cold until used. For the rat preparations, mucosa was stripped from the entire cleaned small intestine immediately after chloroforming the rats. The mucosa was ground with sand and toluene, diluted (usually), and stored in the cold until used. Exceptions to this procedure were that for one rat preparation (R1) the ground mucosa was used directly without dilution, and for another rat preparation (R2) a turbid, toluene water extract of mucosa was prepared (table 2).

The enzymatic hydrolysis of the disaccharides *in vitro* was studied by a procedure based on that developed by Cajori (1935) for dog mucosal lactase. The digestion mixtures contained 1.0 or 2.0 ml. of mucosa preparation, 2.0 ml. of 0.2 M sodium acetate - acetic acid buffer, and 100 mg. of a disaccharide added either in 2 ml. of a 5 percent or 1.0 ml. of a 10 percent (w/v) solution in distilled water. The final total volume of the reaction mixture was 5.0 ml. The proportion of mucosa preparation, either ⅕ or ⅖, in the total digestion mixture was the same for all experiments with any one preparation. However, there were differences both in the amount of dilution of the original mucosa preparations and in the proportion of mucosa in the digestion mixture with different preparations, so that the results with different preparations are not directly comparable one with another. The buffer was at pH 5.6 in the rat and pig experiments, and at pH 5.4 in the calf experiments, except where otherwise noted in table 2. The enzymatic reaction was started by mixing the substrate solution with the other components at 39.5° C. The mixtures were incubated at 39.5° C for the lengths of time given in table 2. Aliquots of the digestion mixture after incubation were clarified according to Cajori (1935). The total monosaccharides liberated in the enzymatic hydrolysis were estimated in the clarified filtrate by a slight modification of the Tauber-Kleiner (1932) method, and the percentage hydrolysis of the disaccharide substrate was calculated. Control digestion mixtures were prepared and incubated using boiled mucosa, and treated as given above. These boiled controls never gave any appreciable reduction of the acid-copper reagent of Tauber and Kleiner (1932), indicating that the hydrolysis observed with the fresh mucosa preparations was entirely enzymatic.

RESULTS

The effects of disaccharides upon laxation are given in table 1. Up to 2.5 gm. of sucrose administered by stomach tube under the conditions described had no laxative effect in the rat in any of the trials. Lactose first initiated laxation at the level of 1.2 gm. per dose. Cellobiose first initiated laxation sometimes at the level of 0.5 gm., and always at the level of 0.8 gm.

Table 2 gives the results of the enzyme studies. It shows that rat, calf, and pig mucosa catalyzed the hydrolysis of both lactose and cellobiose. In all three species, the amount of lactose split was double or more the amount of cellobiose split in the same length of time. The differences between lactase and cellobiase in the rat and in the calf were not due to hydrogen ion effects since all of the determinations were conducted at the optimum pH for the enzyme activities. The pH optima for activity were determined in preliminary experiments to be as

TABLE 1

Laxative action of disaccharides administered by stomach tube to fasted rats

Sugar	Amount given (gm. sugar/5.0 ml. of solution)	No. of trials	Laxation
Sucrose	0.5	6	None
	2.5	5	None
Lactose*	0.5	6	None
	0.8	5	None
	1.2	5	Slight to moderate
	1.5	5	Slight to moderate
	2.0	2	Moderate to severe
	2.5	5	Moderate to severe
Cellobiose†	0.5	5	None to moderate
	0.8	5	Slight to severe

*Eastman, 98% β, form initially dissolved at room temperature just before administration.
†Eastman; the cellobiose solutions were heated to aid solution, then cooled and made to volume.

TABLE 2

Relative rates of hydrolysis of three disaccharides by small intestinal mucosa in vitro, *and effects of calcium gluconate and calcium lactate thereupon*

Mucosa source*	Mucosa preparation		Incubation time (hours)	Substrate	% hydrolysis		
	No.	Nature			Alone	With calcium salts† Gluconate	Lactate
Rat	R1	Ground	3	Lactose	23.7	8.3	25.8
Rat	R2	Ground, diluted,	22½	Cellobiose	12.6	7.3	
		centrifuged; turbid	3	Lactose	27.4	9.5	
		supernate used	3¾	Sucrose	47.0	48.5	
Rat	R3	Ground, diluted	21½	Cellobiose	28.3	26.6	
			5	Cellobiose	11.8	10.0	
			2	Cellobiose	9.1		
			2	Lactose	16.6		
			2	Sucrose	43.5		
Rat	R5	Ground, diluted	2	Cellobiose	7.7	6.7	
			2	Lactose	19.8	10.0	19.5
Rat	R6	Ground, diluted	2	Lactose	19.0	10.7	
			2	Sucrose	65.8	64.7	
Pig	P1	Ground, diluted	2½	Cellobiose	6.8		
			2½	Lactose	17.6	9.9	
			4	Cellobiose	9.8	7.4	
			4	Lactose	24.7		
Calf	C1	Ground, diluted	2½	Lactose	45.1		
			2⅔	Cellobiose	12.0		
			16⅓	Cellobiose	31.0		
			16⅓	Sucrose‡	0.0		
			28¼	Sucrose§	0.0		

*Each of the rat preparations contained pooled mucosa from three or more rats. The pig preparation was obtained from one pig of unknown sex, age, or weight. The calf mucosa was obtained from an Ayrshire bull calf which weighed 395 lbs.
†1% or 2% (w/v) in the reaction mixture; when both levels of salts were tested, the results were close and were averaged.
‡This test was made at both pH 6.0 and 6.8.
§This test was made at pH 6.0.

follows: rat lactase, 5.6; rat cellobiase, 5.0 to 5.6; calf lactase, 5.4 to 5.6; calf cellobiase 5.0 to 6.0; pig lactase, 5.6 (The optimum pH for pig cellobiase activity was not determined).

Table 2 shows also that sucrose hydrolysis by rat mucosa is about 2 to 3 times as great as lactose hydrolysis in the same length of time under our conditions at pH 5.6. The optimum pH for rat sucrase activity was not determined; however, if the optimum for the sucrase is somewhat different from pH 5.6 this would merely increase the difference between sucrase and lactase activity *in vitro*, and would not alter the fact that sucrase is considerably more active than lactase in the rat. Detailed studies of the kinetics of the hydrolysis of the different disaccharides were not carried out with the crude mucosa preparations. Therefore, for the present, the relative hydrolysis rates must be regarded only as approximate estimates of the relative orders of magnitude of the three enzyme activities *in vitro*.

Sucrose was not hydrolyzed at all by calf mucosa under our conditions (table 2). This confirms the early work of Fischer and Niebel (1896).

Table 2 shows also that the presence of calcium gluconate in the reaction mixtures markedly inhibited, but did not completely abolish, lactose hydrolysis by rat (and also, pig) mucosa. Calcium lactate had no significant effect upon rat lactase. This indicates that the inhibition by calcium gluconate was due to gluconate ions rather than to calcium ions. Calcium gluconate inhibited rat and pig cellobiase also, but the degree of inhibition was rather slight in most of the trials. Calcium gluconate did not inhibit rat sucrase.

DISCUSSION

Several observations reported herein are consistent with previous evidence that slow intestinal hydrolysis is a primary factor in the laxation which results from the ingestion of certain disaccharides. First, we have shown herein that the relative activities of three disaccharidases in rat small intestinal mucosa *in vitro* are cellobiase $<$ lactase $<$ sucrase, while the relative laxative potencies of the corresponding disaccharides tested in the rat are cellobiose $>$ lactose, with no laxation due to sucrose under our conditions. The relative cellobiase and lactase activities agree well with the relative rates of disappearance from the rat gut of cellobiose (Vaniman and Deuel, 1944) and lactose (Cori and Cori, 1928; Coryell and Christman, 1943; Fischer and Sutton, 1953). Second, we have shown that gluconate inhibits mucosal lactase *in vitro*. This is consistent with the finding of Mitchell *et al.* (1939) that gluconate in the diet markedly intensified the diarrhea produced by feeding lactose to rats. These workers showed that the effect of gluconate was not exerted with glucose, galactose, sucrose, starch, or dextrin in the diet. Thus, our finding that gluconate had no effect upon rat sucrase *in vitro* also is completely consistent with the *in vivo* experiments of Mitchell *et al.* (1939). The present experiments, considered together with the previous evidence (Fischer and Sutton, 1949; Bailey *et al.*, 1956), leave no doubt that hydrolysis rate is an important factor in the laxation produced by disaccharides.

On the other hand, the rate of intestinal absorption of the monosaccharide end products of disaccharide hydrolysis does *not* appear to be an important factor contributing to the laxation produced by cellobiose or lactose in the rat. This is because glucose, galactose, or a mixture of the two monosaccharides is absorbed from the gut generally faster than cellobiose or lactose is hydrolyzed (Cori, 1925; Cori and Cori, 1928; Coryell and Christman, 1943). Therefore, large numbers of monosaccharide molecules do not accumulate in the gut lumen during the absorption of lactose (Coryell and Christman, 1943) or cellobiose (Vaniman and Deuel, 1944), so a deleteriously high osmotic (hydragogie) action cannot be due to the monosaccharide products.

The finding in rat, pig, and calf small intestinal mucosa of an enzyme which catalyzes cellobiose hydrolysis confirms the original report of the existence of such an enzyme in animals (Porcher, 1910). (In Porcher's work it was not entirely clear what animals were tested for cellobiase; in other experiments reported in the same paper donkeys and dogs were used.) Oppenheimer (1925) stated that the cellobiase action observed by Porcher was due to microorganisms. In our work the intestines were washed out thoroughly and toluene was always present during storage and incubation of the mucosa preparations so that the hydrolysis of cellobiose almost certainly was due to an animal enzyme rather than to microbial action. The presence in intestinal mucosa of an enzyme which catalyzes the hydrolysis of various aryl-β-D-glucosides has been reported (Thomas and Frouin, 1909; Steensholt and Veibel, 1943); it is not known whether the hydrolysis of these compounds is due to the same enzyme as is the hydrolysis of cellobiose reported herein. The presence of cellobiase in rat mucosa explains the physiological evidence (intestinal absorption, glycogen formation, and ketolytic action) which indicates that the normal rat can utilize cellobiose to some extent (Vaniman and Deuel, 1944). The fact that cellobiose had a ketolytic action *in vivo* equivalent to that of glucose (Vaniman and Deuel, 1944) indicates that the cellobiose disappearance from the gut *in vivo* probably was not due to microorganisms since the microbial production of non-ketolytic fermentation products would have made cellobiose a less active ketolytic agent than glucose. Thus, enzymatic and physiological evidence establish the presence of cellobiase in the rat, and enzymatic evidence establishes the presence of this enzyme in the calf and pig.

Several observations indicate that marked differences exist among mammals in the ability to hydrolyze different disaccharides. First, the occurrence and the relative activity of the various disaccharidases vary in different species (this paper; Fischer and Niebel, 1896). Second, there may be differences between species in the time of first appearance of the different disaccharidases; these enzymes may appear first in embryonic life (Needham, 1931; Heilskov, 1951) or in early postnatal life (Bailey *et al.*, 1956). Third, the persistence of each disaccharidase and the changes in its activity at different ages of life may vary in different species (Plimmer, 1906; Heilskov, 1951; Bailey *et al.*, 1956). Fourth, the total activities in the small intestinal mucosa (of the rat, at least) may be affected by prolonged feeding of a disaccharide (lactose) (Fischer, 1955b, 1956). Fifth, other constituents of the diet may affect the hydrolyses directly (as has been shown herein with calcium gluconate) or indirectly (Fischer and Sutton, 1949). All of these observations suggest that suitable levels of intake of a particular disaccharide must be determined separately for each species, for each stage of development, and for each dietary regimen.

SUMMARY

The relative laxative actions of three disaccharides administered by stomach tube to fasted rats was cellobiose > lactose, and no laxation with sucrose under our conditions. The relative disaccharidase activity of rat small intestinal mucosa *in vitro* was sucrase > lactase > cellobiase. Calcium gluconate, but not calcium lactate, inhibited lactase strongly (but not completely under our conditions). Gluconate inhibited cellobiase slightly, but did not inhibit sucrase. These results, in conjunction with information from other experiments, establish that slow hydrolysis of disaccharides is responsible for the induction of the laxation which follows the ingestion of certain disaccharides by the rat.

Cellobiase activity was detected in the small intestinal mucosa of a calf and a pig. In the mucosa of both species, as in the rat, the hydrolysis of cellobiose *in vitro* was slower than that of lactose. In pig mucosa, as in the rat, gluconate inhibited lactase strongly and cellobiase slightly.

The absence from bovine small intestinal mucosa of sucrase was confirmed.

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