

Lactose absorption kinetics in Zambian African subjects

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1. Using a double-lumen tube perfusion system, solutions of lactose (50, 125 and 250 mmol/l) were introduced into the upper jejunum of six Zambian African subjects. By reference to a non-absorbable marker, polyethylene glycol, mol. wt 4000, the rates of absorption of lactose from each solution were calculated for a 300 mm jejunal segment.
2. In three subjects total lactase activity of the jejunal mucosa and brush-border lactase and other disaccharidase activities were estimated. The jejunal total and brush-border lactase activities were low. Jejunal morphology was normal for African subjects.
3. All subjects suffered abdominal colic and diarrhoea during and after the lactose perfusions. The kinetic curves for lactose were very shallow, and with all perfused solutions there was a net movement of water into the jejunal lumen. The limited number of subjects, and the low and narrow range of enzyme activity, did not permit correlation between lactose absorption rate and lactase activity.
4. In Zambian African subjects with adult hypolactasia, the jejunal mucosa absorbs a very small proportion of the perfused lactose.

Specific hypolactasia is present in adult members of most ethnic groups (Cook & Kajubi, 1966; Cook, 1969, 1973*b*). Only a minority of the world's human population has a high concentration of jejunal lactase (β -D-galactosidase galactohydrase; EC 3.2.1.23) in adult life. Adult hypolactasia has been demonstrated in a very high proportion (approximately 100%) of Zambian Africans (Cook, Asp & Dahlqvist, 1973). This condition is associated with a high concentration of the enzyme at birth, and the concentration falls at a variable time during the first few years of life (Cook, 1967*a*). A similar situation exists in most other mammals.

Lactase in the brush border of the enterocyte is necessary for the hydrolysis of dietary lactose to its monosaccharides before absorption into the portal circulation. Although the enterocyte contains other β -galactosidases their role, if any, in lactose absorption is not clear.

The absorption kinetics of lactose in adults with hypolactasia have been investigated in a small group of subjects in London (McMichael, Webb & Dawson, 1967). Our communication reports results for a group of Zambian African adults in whom brush-border lactase activity is known to be very low (Cook *et al.* 1973). It was originally intended to correlate the absorption rates of lactose and the activities of brush-border lactase; this was made impossible by the small number of subjects and the low and narrow range of lactase activity.

Table 1. Details of the six Zambian African male subjects given jejunal perfusions of three lactose solutions containing 50, 125 and 250 mmol/l

Subject no.	Age (years)	Body-wt (kg)	Tribe*	Clinical diagnosis	Haemo-globin (g/l)	Serum protein (g/l)			Stool parasites	Tube position (distance of proximal opening of Treitz) (mm)
						Albumin	Total globulin	γ -Globulin		
1	23	54	Lala	Acute malaria (recovered)	40	37	37	18	Hook-worm	110
2	19	57	Tumbuka	Right lower lobar pneumonia (recovered); <i>Schistosoma haematobium</i> infection	118	36	46	24	None	180
3	31	68	Kaonde	Acute gastroenteritis (recovered); <i>Schistosoma haematobium</i> infection	141	36	27	9	None	220
4	52	50	Sala	Cirrhosis of unknown aetiology	122	40	66	41		100
5	56	64	Tonga	Hypochromic anaemia of unknown aetiology	44	30	24	7	None	140
6	44	45	Nsenga	Bronchial asthma	157	42	44	18		190

* Brelsford (1965).

EXPERIMENTAL

Subjects studied

Table 1 gives details of the six Zambian African male subjects. They were all in-patients at The University Teaching Hospital, Lusaka, and agreed to take part after the procedure and purpose of the study had been explained. They were taken at random from one male medical ward. None of the subjects was clinically malnourished or suffered from intestinal disease. Table 1 also summarizes results of haemoglobin and serum protein determinations, and stool parasites.

Jejunal enzyme activities and histology

Samples of jejunal mucosa were taken at 160, 120 and 170 mm past the ligament of Treitz in subjects nos 2, 3 and 6 respectively (Cook & Kajubi, 1966). The specimens were immediately divided and part was frozen (-20°) and transported by air to Sweden in solid CO_2 (Cook *et al.* 1973). Enzyme activity was estimated by the methods of Dahlqvist (1968), Dahlqvist & Asp (1971) and Asp & Dahlqvist (1972). Enzyme activity was estimated as total lactase activity and also as the separate activities of brush-border lactase, acid β -galactosidase and hetero β -galactosidase. Enzyme activity is expressed as units per g jejunal mucosal protein. One unit of enzyme activity is that which hydrolyses $1 \mu\text{mol}$ substrate/min at 37° (Asp, Berg, Dahlqvist, Jussila & Salmi, 1971). Part of each biopsy specimen was examined with a dissecting microscope and was then placed in 10% formol saline for histological examination.

Perfusion technique

A double-lumen tube (Portex MLT/B, external diameter 4.2 mm) was used for the perfusion studies (Cook, 1971 *a, b*). There was a proximal opening into one lumen 300 mm from the end and three others into the other lumen at the end of the tube. The tube was swallowed the previous evening (16–18 h before the test), and sips of water were permitted during that period. The position of the proximal opening in relation to the ligament of Treitz was determined before (Table 1) and after the investigations, and on no occasion had the tube moved more than 50 mm in a distal direction.

Three solutions (A, B and C) containing lactose (Hopkin & Williams Ltd, Chadwell Heath, Romford, England) (50, 125 and 250 mmol/l respectively) were perfused in order of ascending concentration through the proximal opening at a constant rate of 12.0 ml/min. All solutions were made iso-osmotic with sodium chloride and contained polyethylene glycol (PEG; mol. wt 4000) (5.0 g/l) as a non-absorbable marker. After an equilibration period of 35 min, three successive 10 min samples which were siphoned from the distal opening of the tube were collected. They were immediately frozen and stored for analysis. Duplicate estimates for lactose were made by the method of Asatoor & King (1954), and for glucose by a glucose oxidase (β -D-glucose: oxygen oxidoreductase; EC 1.1.3.4) method (Marks, 1959). PEG was estimated in duplicate by the turbidimetric method of Hydén (1955). Samples of all perfusion solutions were similarly treated and estimates were made for lactose,

Table 2. Results of the jejunal biopsy examinations for appearance under the dissecting microscope and histological examination in the six Zambian African male subjects given perfusions of three lactose solutions containing 50, 125 and 250 mmol/l

Subject no.	Appearance under dissecting microscope	Surface epithelium	Plasma cell infiltration	Neutrophil leucocyte infiltration	Iron staining in:		Mucosal index*
					Epithelium	Stroma	
2	Thin leaves	Normal	Distinct increase	Normal	-	-	3.3
3	Broad leaves with occasional 'fingers'	Normal	Slight increase	Normal	+	+	3.7
6	Thin leaves	Normal	Distinct increase	Normal	-	-	3.6

* Total mucosal height divided by subvillous height.

Table 3. Results of the jejunal biopsy examinations for specific activities (U/g protein) of disaccharidases, brush-border lactase and β -galactosidases in the six Zambian African male subjects given perfusions of lactose solutions containing 50, 125 and 250 mmol/l

Subject no.	Disaccharidases			Brush-border lactase	Acid β -galactosidase	Hetero β -galactosidase
	Total lactase	Sucrase*	Trehalase†			
2	1.2	69	13	0.3	4.3	2.4
3	4.0	98	24	2.9	4.9	1.8
6	2.2	95	23	1.2	4.2	2.4

* β -D-fructofuranoside fructohydrolase; EC 3.2.1.26.

† α , α -Glucoside-1-glucohydrazase; EC 3.2.1.28.

glucose and PEG at the same time as those on the intestinal fluid. The rates of absorption of lactose and water were calculated from standard formulas (Sladen & Dawson, 1968).

RESULTS

Jejunal morphology and enzyme activities

Table 2 summarizes the results of appearances of the jejunal biopsy specimens under the dissecting microscope and details of the histological examinations in three of the six Zambian African male subjects investigated. Table 3 summarizes the results for specific activities of disaccharidases, brush-border lactase and β -galactosidases in samples of the same biopsy specimens. Concentrations of sucrase and trehalase are similar to those in a larger group of Zambian African adults (Cook *et al.* 1973) and also in adult African subjects without hypolactasia (Cook & Dahlqvist, 1968).

Lactose absorption

All subjects suffered abdominal colic during the lactose perfusions and diarrhoea (either two or three fluid stools) soon after the investigation.

Fig. 1 summarizes the results for lactose absorption for the six subjects; results for the absorption of glucose ($n = 10$) and galactose ($n = 4$) in similar Zambian African subjects (Cook, 1971*b*), which were obtained by the same methods, are included for comparison. The SD for the three 10 min collection periods for each perfusion was 0.09 mmol/min per 300 mm jejunum ($n = 18$) (Snedecor & Cochran, 1967; Cook, 1971*b*). The reproducibility of the results was not as good as that reported for glycine, glucose and galactose (Cook, 1971*a, b*). The mean slope of the lactose curve was very shallow compared with those for glucose and galactose. The mean rate of lactose absorption was always less than 0.2 mmol/min per 300 mm jejunum. In two of the six kinetic curves for lactose there was a suggestion of saturation kinetics as the curve seemed to plateau at the higher lactose concentrations. Whether or not the plot for mean values of lactose absorption conforms to saturation kinetics cannot be determined, however, as the values were very low. Fig. 2 gives comparative results for lactose absorption rates in the six subjects; the area under

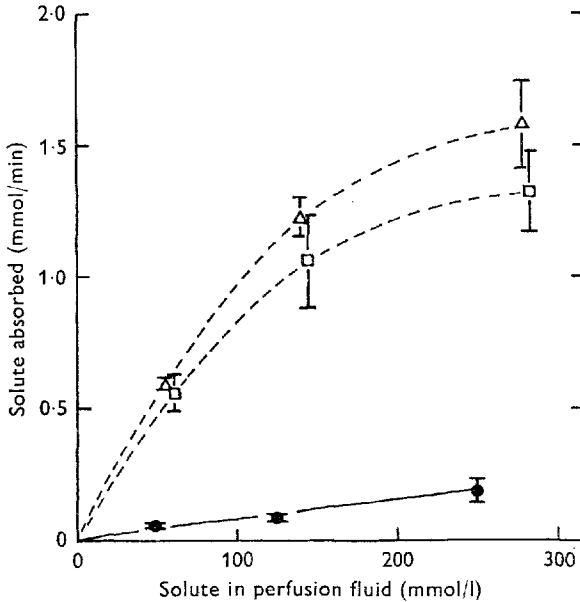


Fig. 1. Mean rates of absorption of lactose (●), glucose (△) and galactose (□) (mmol/min per 300 mm jejunum) in the six Zambian African male subjects given jejunal perfusions of solutions containing lactose (50, 125, 250 mmol/l), and in other subjects given glucose (56, 139, 278 mmol/l) and galactose (56, 139, 278 mmol/l). The vertical bars represent the standard errors of the mean. The results for glucose and galactose perfusions were reported by Cook (1971*b*).

each kinetic curve is shown. Brush-border lactase concentrations were estimated for three subjects; subject no. 2 had the lowest concentration and the flattest absorption curve. Glucose was not detectable in any of the specimens of perfusion fluid or in any of the siphoned samples of intestinal fluid.

Net water movement

Fig. 3 summarizes the net water movement during the lactose perfusions. The SD for the three 10 min collection periods for each perfusion was 1.13 ml/min per 300 mm jejunum ($n = 18$). In all subjects, at each concentration of lactose, the results show a net transfer of water into the jejunal lumen; this observation differs from results of glucose and galactose perfusions (Cook, 1971*b*) when there was a net outflow of water. The mean net movement into the jejunum seemed to increase linearly with increasing concentrations of lactose in the perfusion fluid. Fig. 2 gives comparative values for mean net water movement into the jejunal lumen in the six subjects; the area above the curve is shown for each subject. The highest net water movement was found in subject no. 5. There was no significant correlation between lactose absorption and water movement in the different subjects and no relationship between the net water movement and serum protein concentration.

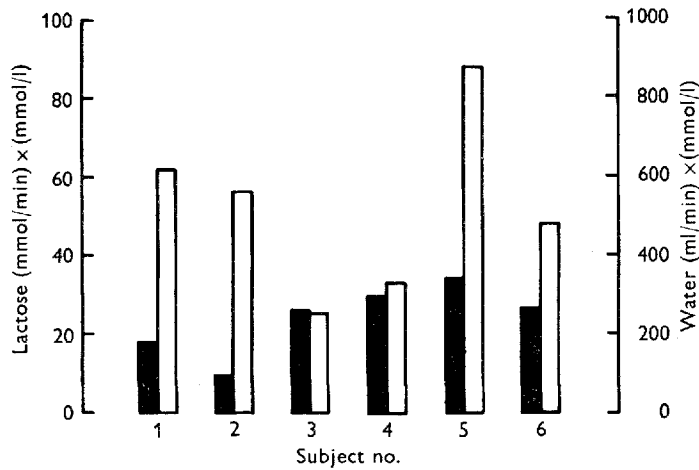


Fig. 2. Comparison of amounts of lactose absorbed from the jejunal lumen (■) and net water movement to the lumen (□) in the six Zambian African male subjects given jejunal perfusions of lactose (50, 125, 250 mmol/l). The area under each lactose kinetic curve was calculated and expressed as (mmol/min per 300 mm jejunum) × mmol/l, and the area above each curve for water movement was calculated and expressed as (ml/min per 300 mm jejunum) × mmol/l.

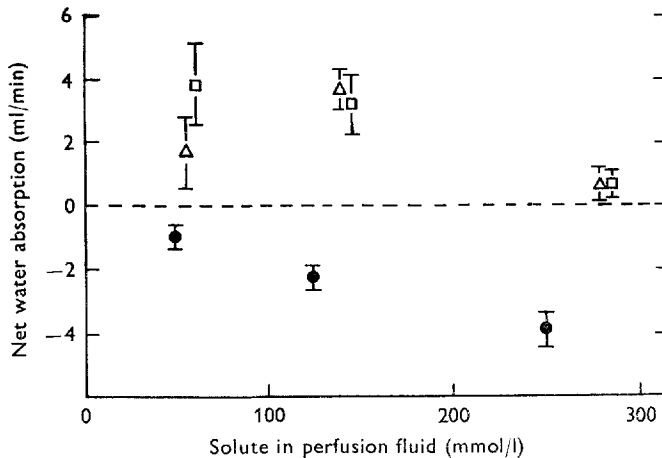


Fig. 3. Net water movement (ml/min per 300 mm jejunum) across the jejunal mucosa in the six Zambian African male subjects given jejunal perfusions of solutions containing lactose (50, 125, 250 mmol/l) (●), and in other subjects given glucose (56, 139, 278 mmol/l) (△) and galactose (56, 139, 278 mmol/l) (□). The vertical bars represent the standard errors of the mean. The results for glucose and galactose perfusions were reported by Cook (1971*b*).

DISCUSSION

This study clearly showed that there was very little transfer of lactose across the jejunal mucosa during jejunal perfusions of lactose in adult Zambian African subjects, the majority of whom have hypolactasia (Cook *et al.* 1973). The subject with the lowest brush-border lactase activity had the flattest lactose kinetic curve. The mean kinetic curve was considerably flatter than that reported for four 'lactase-

deficient' subjects studied in London by McMichael *et al.* (1967). Those authors reported an absorption rate of 0.29 mmol/min per 300 mm jejunum from a solution containing 146 mmol/l, approximately three times the rate expected from our results. The different perfusion rate (20 ml/min) seems unlikely to have produced the marked difference between the two investigations. Asp & Dahlqvist (1972) have shown that mean brush-border lactase activity is higher in Finnish subjects with adult hypolactasia (4.1 U/g protein) than in Zambian African subjects (1.8 U/g protein), and it is therefore probable that the difference in absorption rate resulted from the different levels of residual lactase activity in the two groups. It was impossible to determine from the present study whether the kinetic curve for lactose was linear or whether it conformed to saturation kinetics.

The amount of lactose absorbed was higher than expected from the calculated amount of brush-border lactase in a 300 mm section of jejunum. If it is assumed that a 10 mm section of jejunum contains 1 g mucosa, the total brush-border lactase activity in subjects 2, 3 and 6 would be approximately 1.5, 15 and 6 U respectively (V_{\max} values). One unit of enzyme activity is the amount of enzyme required to hydrolyse 1 μ mol lactose/min; therefore, the maximum hydrolytic activity of a 300 mm jejunal section would be 1.5, 15 and 6 μ mol/min. The amount of lactose actually absorbed in the experiments was fifteen to fifty times higher. It seems probable therefore that *in vivo*, adults with hypolactasia absorb some intact lactose, possibly by simple diffusion; this is consistent with a previous observation of lactosuria after lactose ingestion in Ugandan African adults with hypolactasia (Cook & Howells, 1968).

The total amount of lactose given to each subject was approximately 115 g in a period of 195 min, although a considerable proportion was siphoned from the distal opening of the perfusion tube. Symptoms of 'intolerance' were evident in all subjects. Although milk and lactose are unlikely to produce significant ill-health in Zambian African adults, it seems clear that a substantial energy loss must occur. A previous investigation has shown that when 50 g lactose (equivalent to approximately 1 l milk) is given orally approximately 80% of Zambian African adults experience 'intolerance' (i.e. colic or diarrhoea, or both) (Cook *et al.* 1973). The present study in which lactose was infused into the jejunum does not add to that result; the rate of gastric emptying and dilution of the lactose solution in the upper gastrointestinal tract are important factors which influence disaccharide 'intolerance'.

There was a net water movement towards the jejunal lumen with all concentrations of lactose perfused which contrasted with the outward movement associated with glucose and galactose. This observation was the same as that of McMichael *et al.* (1967), although the different perfusion rates in the two studies did not permit comparison of rates. The inverse relationship between the rates of lactose absorption and net water absorption was not significant, although the number of subjects studied was small. It seems clear that much of the colic and diarrhoea caused by dietary lactose in Zambian African subjects is the result of an osmotic effect (Christopher & Bayless, 1971). Such an effect is not specific to lactose, however, and is produced by other compounds if present at high concentration in the intestinal lumen (Cook, 1973*a*).

The present investigation re-emphasized the probable practical importance of hypolactasia. Low brush-border lactase activity before weaning is likely to be of considerable practical significance (Cook, 1967*a, b*).

In most Zambian Africans, the curve for blood glucose after oral administration of 50 g lactose is not flat and rises to a mean of about 100 mg/l (Cook *et al.* 1973). The present study indicates that this rise occurs despite very low brush-border lactase activity and lactose absorption rates.

The concentrations of acid and hetero β -galactosidases in our subjects were similar to those demonstrated in Scandinavian adults, most of whom do not have hypolactasia (Asp *et al.* 1971; Cook *et al.* 1973). Acid β -galactosidase, which hydrolyses both lactose and synthetic β -galactoside substrates, is located in the lysosomes of the enterocyte; it seems unlikely that it has a role in the digestion of dietary lactose. Hetero β -galactosidase is present in the cytoplasm and hydrolyses synthetic substrates but not lactose.

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