Cellobiose:mannitol differential permeability in small bowel disease

S HODGES, S P ASHMORE, H R PATEL, AND M S TANNER

Department of Child Health, University of Leicester

SUMMARY Cellobiose and mannitol absorption were studied in patients with suspected abnormal function of the small bowel mucosa. The urinary cellobiose:mannitol ratio was increased in subtotal villous atrophy, iron deficiency anaemia, and small intestinal Crohn's disease. The test seems a sensitive indicator of the integrity of small bowel mucosa.

A number of non-invasive dual sugar permeability tests have been proposed, based on the theory that villous atrophy reduces absorption of monosaccharides, and mucosal damage increases permeability of disaccharides, particularly if the test solution is hyperosmolar.¹ The disaccharide cellobiose and the sugar alcohol mannitol, given orally in hyperosmolar solution, provide a sensitive test of mucosal function of the small bowel in adults.² The ratio between percentage recoveries of the two probe molecules in a timed urine collection—the cellobiose:mannitol ratio—is raised if the mucosa is damaged. Factors common to the urinary excretion of both molecules such as failure of ingestion of the fixed dose, adequacy of timed urine collections, rapidity of intestinal transit and renal clearance, are eliminated. We have therefore evaluated the test in children with suspected disease of the small intestine.

Patients and methods

A jejunal biopsy and the cellobiose mannitol test were carried out at the same time on 49 occasions in 48 children (mean age 4.8 years, range 6 month–16 years) with symptoms suggestive of malabsorption. After fasting overnight and emptying the bladder a 1500 milliosmolar solution (cellobiose 33 g/l, mannitol 13 g/l, lactose 133 g/l, and sucrose 133 g/l) was given in a dose of 150 ml/1.73 m². An hour later a jejunal biopsy was taken under fluoroscopic control. After the biopsy the patients were encouraged to drink water to promote a diuresis. Urine was collected for five hours. Cellobiose was measured enzymatically and mannitol colorimetrically as previously described by Strobel *et al*² and the ratio of their percentage recoveries calculated. The biopsy specimen was processed for conventional

histological examination and reported without knowledge of the cellobiose:mannitol ratio.

The cellobiose mannitol test was also carried out in 13 healthy children (mean age 6.8 years, range 1.6–11.66) who were either of normal stature (n=9) or of short stature with normal growth velocity (>25th centile height velocity) and no intestinal symptoms (n=4), seven healthy adults, and three children with moderate Crohn's disease of the small bowel (score 50–60 Lloyd-Still classification³).

Non-parametric statistical analysis was by the Mann-Whitney U test.

Results

The test solution was well tolerated; no children had diarrhoea despite the lactose load and no difficulties accompanied the performance of the two tests together.

There was no significant difference between the cellobiose:mannitol ratio in normal adults and children (figure, table). Eight biopsy specimens showed subtotal villous atrophy; the cellobiose:mannitol ratios in these cases were significantly greater than in both normal controls (p<0.01) and children with normal biopsy specimens (p<0.01). A cellobiose:mannitol ratio of 0.035 corresponded to two standard deviations above the mean for our

Figure 1 Cellobiose:mannitol ratios for normal subjects and patients.

Table Median (interquartile range) cellobiose:mannitol ratios and percentage recovery of cellobiose and mannitol in normal subjects and patients

	Normal adults (n=7)	Normal children (n=13)	Subtotal villous atrophy (n=8)	Normal jejunal biopsy specimen, not iron deficient (n=34)	Normal jejunal biopsy specimen, iron deficient (n=5)
Cellobiose:mannitol ratio	0.009 (0.0092)	0.016 (0.015)	0.24 (0.147)	0.027 (0.039)	0.105 (0.054)
Cellobiose recovery (%)	0.29 (0.22)	0.34 (0.24)	1.56 (1.04)	0.14 (0.41)	1.53 (0.93)
Mannitol recovery (%)	33.7 (10.0)	23.6 (13.7)	4.66 (4.69)	9.4 (13.4)	13.13 (8.35)

normal children. Fifteen of 39 children with normal biopsy specimens had cellobiose:mannitol ratios of greater than 0.035. Five had iron deficiency anaemia (haemoglobin concentration <100 g/l, mean corpuscular volume <70, and serum ferritin concentration <12 µg/l), two had food allergies, two had diarrhoea, one had recently had gastroenteritis, one had a family history of coeliac disease and positive antibodies to reticulin, and four were considered normal. Two children with coeliac disease on gluten free diets whose cellobiose:mannitol ratios were 0.089 and 0.095, respectively, had increased lymphocytes in the lamina propria but normal villi and crypts.

Individual sugar recoveries showed significantly decreased mannitol ($p<0.01$) and increased cellobiose ($p<0.01$) in those with subtotal villous atrophy when compared with normal controls. Similar cellobiose recovery was seen in iron deficiency anaemia and subtotal villous atrophy (table).

Discussion

The cellobiose:mannitol ratio in normal children and adult controls corresponds well with previously published adult values.²

Hyperosmolar solutions have a potential risk of inducing osmotic diarrhoea, but this did not occur in the subjects studied.

If the test was used as a screen for subtotal villous atrophy and a value of 0.035 (two standard deviations above the mean for normals) was taken, above which a jejunal biopsy was undertaken, the sensitivity would be 100%, the specificity 62%, and the predictive value 35%. Taking a value of 0.1 would not reduce the sensitivity but would increase the specificity to 87% and the predictive value to 62%.

A study in adults using this hyperosmolar test solution has shown functional abnormality despite normal crypt and villous architecture,² particularly if the intraepithelial lymphocyte count is high. By contrast, using lactulose and mannitol in an isotonic solution in children, abnormalities of permeability in the presence of increased intraepithelial lympho-

cytes were not found.⁴ Thus increasing tonicity of the test solution seems to increase sensitivity and reduce specificity. The two children in our study with increased intraepithelial lymphocytes both had high cellobiose:mannitol ratios, as did some of our patients with normal jejunal morphology, all of whom had biopsies taken because of suspected malabsorption. This might represent a functional abnormality of small intestinal mucosa, particularly in the five patients with iron deficiency anaemia who had appreciably increased cellobiose absorption. Reversible mucosal damage with associated steatorrhoea has been previously described in children with severe iron deficiency⁵; mucosal damage not apparent on light microscopy may have been present in these patients.

We have shown by giving a hyperosmolar solution that there is increased absorption of disaccharides in children with subtotal villous atrophy. This has previously been shown in adults² but not in children.⁶

The cellobiose:mannitol permeability test is a sensitive and well tolerated non-invasive screening test of small bowel function. Values of more than 0.1 suggest that a jejunal biopsy specimen should be taken. The test may be used to monitor a gluten challenge, and possibly also the response to treatment in small bowel Crohn's disease. Difficulties in interpretation may occur in patients with iron deficiency anaemia.

We thank Dr IC Talbot for histological reports on the jejunal biopsy specimens.

References

- 1 Laker MF, Menzies IS. Increase in human intestinal permeability following ingestion of hypertonic solutions. *J Physiol* 1977;265: 881-94.
- 2 Strobel S, Byrdon WG, Ferguson A. Cellobiose/mannitol sugar permeability test complements biopsy histopathology in clinical investigation of the jejunum. *Gut* 1984;25:1241-6.
- 3 Lloyd-Still JD, Green OC. A clinical scoring system for chronic inflammatory bowel disease in children. *Dig Dis Sci* 1979;24: 620-5.
- 4 Nathavitharan KA, Lloyd DR, Raafat F, Brown GA, McNeish AS. Urinary mannitol:lactulose excretion ratios and jejunal mucosal structure. *Arch Dis Child* 1988;63:1054-7.

⁵ Naiman JL, Oski FA, Diamond LK, Vawter GF, Shwackman H. The gastrointestinal effects of iron deficiency anaemia. *Pediatrics* 1964;33:88-99.

⁶ Hamilton I, Hill A, Bose B, Bouchier IAD, Forsyth JS. Small intestinal permeability in paediatric practice. *J Pediatr Gastroenterol Nutr* 1987;6:697-701.

Correspondence to Dr S Hodges, Department of Child Health, Clinical Sciences Building, Leicester Royal Infirmary, PO Box 65, Leicester LE2 7LX.

Accepted 8 December 1988

Focal glomerulosclerosis treated with heparin

D R LINES, M COLEMAN, AND A GALLUS

Department of Paediatrics, Flinders University of South Australia

SUMMARY A boy with focal glomerulosclerosis as a result of nephrotic syndrome became unresponsive to corticosteroids and cyclophosphamide. He was given prolonged subcutaneous heparin with reduction in proteinuria, return of corticosteroid sensitivity, and no further deterioration (possibly improvement) in histological appearance. He remained completely well after five years.

Focal glomerulosclerosis usually progresses to death of the kidneys. It commonly presents with nephrotic syndrome, and initial histological examination may miss the focal lesions, and lead to a mistaken diagnosis of nephropathy with minimal lesions.¹ We report a 9 year old boy who presented in this way, and became unresponsive to corticosteroids and cyclophosphamide. His subsequent cure seemed to be the result of treatment with heparin.

Case report

A boy aged 9.5 years presented with severe oedema, hypoproteinaemia (total serum protein concentration 44 g/l), and a 24 hour urinary protein excretion of 1.36 g. His course is illustrated in the figure. Treatment with corticosteroids resulted in clinical remission and the disappearance of the proteinuria. Three months later he relapsed, but again responded to treatment with steroids.

After a further three months he relapsed and was referred to one of us (DRL). Prednisolone 40 mg/day was given and examination of a renal biopsy specimen under electron microscopy showed 15 glomeruli with no evidence of focal glomerulosclerosis, but evidence of fusion of the podocytes consistent with minimal change nephropathy. His urine became free of protein after six days, when cyclophosphamide 2.5 mg/kg/day was added to his treatment regimen for eight weeks. After four

weeks he relapsed; he responded to steroids but six days before completing the course of cyclophosphamide he relapsed again. A course of prednisolone did not clear his proteinuria completely, and after eight weeks reduction in dosage was attempted, but increased oedema and proteinuria required higher doses. After his sixth relapse he was given a further course of cyclophosphamide (4.8 mg/kg/day) for eight weeks.

During the next four months he became unresponsive to prednisolone 2 mg/kg/day, his urinary protein excretion remained between 4 and 11 g/day requiring intravenous courses of protein and diuretics for anasarca. Examination of a second biopsy specimen showed 31 glomeruli with widespread mild mesangial expansion, sclerosis, and hypercellularity. Discrete, sclerosing, segmental lesions were present and there was almost complete podocyte fusion. The appearance was considered typical of focal glomerulosclerosis.

After a further intravenous course of protein for anasarca it was decided to give heparin, initially intravenously for three days, but there was no obvious response. Indomethacin 25 mg three times a day for a month did not reduce the urinary protein excretion, and further intravenous protein was given.

At the age of 10.8 years a further intravenous course of heparin was given for 3.5 days, but this did not help and nephrectomy for the control of proteinuria and anasarca was considered. The parents requested a longer trial of heparin, which was then given subcutaneously for three months with a target blood concentration of 0.2 U/ml. The range achieved was 0.11 to 0.64 on an average daily dose of 550 U/kg/day. Frequent 24 hour specimens of urine were examined to determine protein excretion and these are summarised in the figure. Proteinuria rapidly decreased, and remained low but after three months the effect seemed to lessen. From day 5 fluid retention was less than it had been