Cows' milk protein-sensitive enteropathy

An important contributing cause of secondary sugar intolerance in young infants with acute infective enteritis

N. IYNGKARAN, K. DAVIS, M. J. ROBINSON, C. G. BOEY, E. SUMITHRAN, M. YADAV, S. K. LAM, AND S. D. PUTHUCHEARY

University Hospital, Kuala Lumpur

SUMMARY The effect of cows' milk protein (CMP) on the mucosal disaccharidases was investigated in 23 infants with acute infective enteritis. Jejunal biopsies performed before and after cows' milk provocation were subjected to histological examination and to mucosal disaccharidase enzyme (lactase, sucrase, and maltase) analyses. After milk challenge, changes in mucosal histology were observed in 18 infants, in 17 of them the levels of all 3 mucosal disaccharidases were much reduced. 10 of these infants developed diarrhoea and, in 6, the stools were positive for reducing sugar. It is concluded that CMP has a deleterious effect on the jejunal mucosa of young infants recovering from infective enteritis, so that in the management of young infants with sugar intolerance secondary to infective enteritis, CMP and lactose should be excluded from the diet.

Secondary sugar intolerance is a well recognised and common complication of acute infective enteritis (AIE) in young infants (Burke, *et al.*, 1965; Lifshitz *et al.*, 1971; Chandrasekaran *et al.*, 1975). The intolerance appears to be due to depletion of mucosal sugar-splitting enzymes secondary to damage to the small bowel mucosa caused by the primary infective agent (Weijers and Van de Kamer, 1962; Barnes and Townley, 1973). Exclusion of this sugar, however, does not always affect the diarrhoea. Recently cows' milk protein sensitive enteropathy (CMPSE) was shown to be an important factor prolonging diarrhoea in young infants with AIE (Iyngkaran *et al.*, 1978a). The present study was undertaken to determine the effect of cows' milk protein (CMP) on the intestinal oligosaccharidases in infants recovering from AIE.

Departments of Paediatrics, Pathology, Genetics, and Cellular Biology, and Medical Microbiology, University Hospital, Kuala Lumpur

N. IYNGKARAN, lecturer in paediatrics

K. DAVIS, visiting associate professor, department of pathology

M. J. ROBINSON, formerly professor and head of department of paediatrics

C. G. BOEY, biochemist

E. SUMITHRAN, lecturer in pathology

M. YADAV, associate professor, department of genetics and cellular biology

S. K. LAM, associate professor, department of medical microbiology

S. D. PUTHUCHEARY, associate professor, department of medical microbiology

Materials and methods

23 infants under age 12 months presenting with the clinical features of AIE, admitted to the Department of Paediatrics, University Hospital, Kuala Lumpur between March 1976 and March 1978 were studied. Investigations on admission included examination of the stools under the light and electron microscope for *Giardia lamblia* and virus, stool cultures for bacterial pathogens, and testing of stools for sugar by the method of Kerry and Anderson (1964).

After appropriate correction of fluid and electrolyte imbalances the infants were started on a formula free of CMP—such as Prosobee, Nutramigen, or Pregestimil (Mead Johnson). All infants remained in hospital until their stools were normal, they were feeding well, and were gaining weight. They were then discharged home, usually within 2 weeks of admission, the mothers being supplied with the appropriate CMP-free formula and instructed not to offer any other milk without first consulting us. About 6 or 8 weeks later the infants were readmitted for further study.

Further study.

Low-lactose cows' milk* provocation test

In most infants 'Al 110' (Nestlé) or 'Lactolac V' (Co-operatieve Condensfabriek Friesland, Holland)

* < 0.07% Lactose after reconstitution.

was used. If no reaction occurred after an initial feeding of 5 ml, the volume was doubled hourly for the first 4 hours and subsequently 3-hourly until daily fluid requirements were met.

Testing of stools

If diarrhoea occurred stools were tested for reducing sugar.

Pathogens

In all infants attempts were made to isolate parasitic, bacterial, and viral pathogens from the stools by culture and by examining stools under the light and electron microscope 12 to 24 hours before and 12 to 24 hours after milk challenge.

Jejunal biopsy

A proximal jejunal biopsy specimen taken with the Watson paediatric capsule just distal to the duodenojejunal junction was examined under dissecting microscope. The specimen was divided into three—one portion was fixed in gluteraldehyde for light and electron microscope studies, the 2nd was used for oligosaccharide enzyme determinations, and the 3rd for immunofluorescence studies. In addition a mucosal imprint was performed to detect *G. lamblia* (Kamath and Murugasu, 1974). Jejunal biopsies were performed (a) prechallenge, immediately before CMP challenge, and (b) postchallenge, 24 hours after the introduction of cows' milk, irrespective of the presence or absence of symptoms. Biopsies were graded by a method described previously (Iyngkaran *et al.*, 1978b).

Assay of intestinal disaccharidases. Portions of jejunal biopsy material were homogenised and incubated with the various disaccharidase substrates as described by Dahlquist (1968). Aliquots of the incubation were assayed for glucose at timed intervals for up to 2 hours using a Glucose Analyzer (Beckman Instruments Inc., Calif. 92634, USA). Disaccharidase activities are reported as μmol of disaccharide hydrolysed/min at 37°C per gram wet weight of tissue.

In 4 infants protein (micro-Kjeldahl method for total protein, Bauer *et al.*, 1974) and enzyme estimations were performed simultaneously. The enzyme levels in these 4 infants were expressed both as per gram wet weight of tissue and per gram protein. The percentage drop in the enzyme levels after milk provocation by either method was almost identical. In the other 19 cases the enzyme levels were expressed as per gram wet weight of tissue.

Results

Initial assessment. The 23 infants comprised 5 Malays, 11 Chinese, and 7 Indians (13 boys and 10

girls). The mean age at admission was 70 days (3–300). The mean duration of diarrhoea was 3.6 days (1–12). Dehydration was present in 13 infants, abdominal distension in 7, anal excoriation in 8, and oral thrush in 7. Interestingly, 8 infants had neonatal jaundice and 5 developed diarrhoea while on phototherapy in a ward with noninfectious patients.

At initial admission stools were positive for sugar in 13 infants; in the other 10, stools were not tested (Table 2). Food poisoning *Salmonella* was isolated from the stools of 4 infants, *Escherichia coli* from 2, and *Shigella* from one.

Assessment at milk challenge. At the time of readmission for milk challenge all the infants were clinically well and registered satisfactory weight gains: 3 were still excreting *Salmonella* and 4 were excreting *E. coli*. These organisms could not have been introduced during challenge studies as stools collected immediately before and after challenge were positive for the same organisms.

Biopsy findings (histological and enzymological). The prechallenge biopsy was normal in 17 infants and mildly abnormal in 6. The infants could be categorised into 4 groups according to the clinical and histological response to CMP after challenge.

Group A. Infants with histological and clinical relapse, symptoms appearing within 24 hours (8 infants) There was significant depression in the levels of all 3 disaccharidases. In 6 infants with diarrhoea, stools were tested and found to be positive for reducing sugar.

Group B. Infants with histological and clinical relapse but symptoms appearing at 3 and 14 days after milk provocation (2 infants) There was significant enzyme depression in both infants.

Exclusion of CMP produced prompt cessation of symptoms in infants of groups A and B.

Group C. Histological relapse but no clinical symptoms (8 infants) Disaccharidase levels were depressed in 7 of the 8 infants. Two have been lost to follow-up. The remaining 6 were thriving well on a CMP formula, when last seen 1 to 24 months after milk provocation but, in 2, initial weight gains were suboptimal.

Group D. No histological or clinical relapse (5 infants) Although some variability in the pre- and post-challenge enzyme values were observed, there was no consistent fall in enzyme levels as in the 17 patients in groups A, B, and C.

Although the reduction in the mean levels of the disaccharidases in group A was significantly greater than those in group C the levels in individual cases overlapped greatly.

The clinical features, Clinitest results, stool isolates, histological findings, and enzyme values in the 4 groups are summarised in Tables 1 to 5.

Table 1 Clinical features of 23 infants with acute enteritis

	Group A n=8	Group B n=2	Group C n=8	Group D n=5
Age (days) at initial admission				
Mean	24	3, 49	40	207
Range	(3-42)		(5-135)	(135-300)
Male/female	4/4	0/2	6/2	3/2
Ethnic group				
Malay	2	-	2	1
Chinese	3	1	4	3
Indian	3	1	2	1
Weight at initial admission (kg)				
Mean	3	3	3	6.5
Range	(2.4-6.5)	(2, 4)	(1.83-6.3)	(5-8.2)
Age (days) at onset of diarrhoea				
Mean	21	22.5	36	204
Range	(4-35)	(3, 42)	(4-130)	(131-288)
Duration of diarrhoea				
Mean	2.8	4	4.3	3.5
Range	(1-3)	(1, 7)	(1-12)	(2-6)

Table 2 Stool Clinitest results in 23 infants with acute enteritis at initial illness and after cows' milk challenge

Group	Clinitest	Initial illness	Postchallenge
A	Positive	6	6
	Not done	2	2
	Total	8	8
B	Positive	2	-
	Not done	-	2
	Total	2	2
C	Positive	5	No diarrhoea
	Not done	3	
	Total	8	
D	Positive	2	No diarrhoea
	Not done	3	
	Total	5	

Table 3 Stool isolates from 23 infants with acute enteritis during initial illness and milk provocation

Group	Pathogens	Initial illness	Pre- and postchallenge
A	<i>Salmonella</i>	2	2
B	-	-	-
C	<i>E. coli</i>	2	3
D	<i>E. coli</i>	1	1
	<i>Salmonella</i>	2	1
	<i>Shigella</i>	1	-

Table 4 Jejunal biopsy findings* in 23 infants with acute enteritis before and after cows' milk provocation

Group	Prechallenge	Postchallenge			
		Normal	Mild VA	Moderate VA	Severe VA
A	Normal 6 (1.2)	-	1 (7)	2 (8.8)	3 (12.5)
	Mild VA 2 (4)	-	-	1 (9)	1 (12)
	Total 8	-	-	-	-
B	Mild VA 2 (4)	-	-	2 (11)	-
C	Normal 7 (0.8)	-	6 (6.1)	1 (9.5)	-
	Mild VA 1 (4)	-	-	1 (11)	-
	Total 8	-	-	-	-
D	Normal 4 (1.8)	4 (1.7)	-	-	-
	Mild VA 1 (6)	-	1 (5.5)	-	-
	Total 5	-	-	-	-

VA=Villous atrophy.

*Figures in parenthesis denote mean score; normal histology=0-3, mild VA=4-7, moderate VA=8-11, severe VA >11.

Discussion

Secondary sugar intolerance complicating AIE in young infants appears to be due to depletion of the disaccharidases secondary to mucosal damage caused by the primary infective agent (Lifshitz *et al.*, 1971; Barnes and Townley, 1973). In the management of such infants the sugar is identified and excluded from the diet. Dietary manipulation becomes more difficult in those infants who do not improve with this simple approach. It often culminates in some being placed on an oral carbohydrate-free formula (Sirolac, CF1, Nestlé), with glucose given intravenously (Lifshitz *et al.*, 1970; Walker-Smith, *et al.*, 1973). However, a certain proportion of these infants continue to have diarrhoea despite the total exclusion of carbohydrate from the oral feeds. Harrison *et al.* (1976) suggested a possible association between CMPSE, lactose intolerance, and AIE. Recently, studies in young infants with AIE showed that the feeding of CMP during recovery damages the small bowel mucosa (Iyngkaran *et al.*, 1978a). Such damage could in itself perpetuate the diarrhoea or, by depleting the mucosal disaccharidases, contribute to the problem of secondary sugar intolerance.

18 of 23 infants with AIE in the present series had CMPSE by the criteria of Iyngkaran *et al.* (1978b). In 17 of these infants significant depression of the disaccharidases occurred after provocation with CMP. 10 of these infants had diarrhoea, 8 within 24 hours and 2, on days 3 and 14, after milk provocation. Stools were positive for reducing sugars in 6 infants. As the CMP formulae used in the provocation studies were glucose based and had negligible amounts of lactose, it is suggested that CMP not only impairs digestion and absorption of disaccharides but also of monosaccharides.

Table 5 Disaccharidase levels, mean (and range)* in 23 infants with acute enteritis before and after cows' milk provocation

Group	No. of cases	Disaccharidase	Prechallenge	Postchallenge	Percentage change†
A	8	Lactase	3.3 (0.7-5.8)	0.5 (0.1-1.4)	85 (76-97)
		Sucrase	4 (1.7-6.4)	1.6 (0.4-2.8)	69 (36-92)
		Maltase	18 (3.1-27)	5.7 (0.14-6)	68 (44-100)
B	2	Lactase	2.8 (2.7, 2.9)	1.1 (0, 2.3)	60 (100, 20)
		Sucrase	4 (4, 4)	2.7 (1.6, 3.9)	31 (60, 2)
		Maltase	23.6 (20, 27.2)	7.3 (1.9, 13.8)	70 (90, 49)
C†	8	Lactase	2.8 (0.9-6.1)	1.3 (0.7-2.4)	54 (5-90)
		Sucrase	4.3 (1.8-7.2)	2.3 (1.0-3.7)	46 (0-86)
		Maltase	14.9 (4.4-24.5)	12.1 (4.1-23.4)	19 (0-73)
D	5	Lactase	2.3 (1.4-3.4)	2.4 (1.4-4.1)	-4.0 (-20 to 0)
		Sucrase	3.0 (1.4-5.1)	2.9 (1.7-4.1)	2.0 (-20 to 20)
		Maltase	12.1 (7.7-17.3)	13.1 (7.9-17.1)	-2.5 (-20 to 12)

* $\mu\text{mol/min per g wet weight tissue}$.

†Included in this group is one infant who had histological changes without significant alterations in any of the disaccharidases.

‡Percentage change = $\frac{(\text{prechallenge} - \text{postchallenge})}{\text{Prechallenge}} \times 100$; positive values indicate decrease and negative values increase over the prechallenge.

Not all 17 infants with histological changes and enzyme depression developed symptoms. Similar observations have been made in coeliac disease (MacDonald *et al.*, 1965). The severity and extent of damage, the amount of lactose, the amount and type of CMP in the diet, the ability of the infants to digest the protein and render it harmless, their ability to mount an appropriate immunological reaction, and the presence of intercurrent enteric infections are some factors that may determine whether or not symptoms manifest in them.

The immunological mechanisms for the exclusion of foreign protein such as CMP by the intestinal mucosa are age dependent (Taylor *et al.*, 1973; Walker, 1975). In young infants this problem may become exaggerated in any situation such as AIE where damage to the intestinal mucosa further increases the permeability to foreign protein. The free passage of foreign antigen across the mucosa could then sensitise the intestinal mucosa to the protein. Subsequent re-exposure to the protein could then result in an immunological reaction at the mucosal level; this may lead to secondary damage of the small bowel mucosa and the attendant consequences of diarrhoea, secondary sugar intolerance, malabsorption, and failure to thrive (Harrison *et al.*, 1976; Iyngkaran *et al.*, 1978a). Therefore in the management of young infants with sugar intolerance secondary to AIE, a lactose- and CMP-free formula should be used and continued for some weeks after recovery.

The deleterious effects of CMP in infants with AIE and secondary sugar intolerance have gone unrecognised in the past because most of the lactose-free milk preparations such as Sobee, Prosobee, Nutramigen, and Pregestimil (Mead Johnson) are also free of CMP. Clearly more studies are needed

to confirm these findings and the magnitude of the problem. This would then pave the way for a review of some of the current concepts of secondary sugar intolerance complicating AIE and enable the formulation of a more rational approach to treatment.

We thank P. Thirukumar and S. Arichandran for technical assistance, and Sister Tang, Azizah, and the nursing staff for co-operation. This project was supported by funds from the Research Committee of the University of Malaya and in part by Bristol Myers (Research Division), New York, USA.

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Correspondence to Dr N. Iyngkaran, Department of Paediatrics, University Hospital, Kuala Lumpur, Malaysia.

Received 2 May 1978