

Cows' milk protein-sensitive enteropathy

An important factor in prolonging diarrhoea of acute infective enteritis in early infancy

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SUMMARY The possible role of cows' milk protein in prolonging diarrhoea in very young infants with acute infective enteritis was studied in 14 infants, 9 under the age of 2 months and 5 older than 6 months. Bacterial pathogens were isolated from the stools of 4 infants from the younger age group. After appropriate initial treatment the infants were maintained on a cows' milk protein-free formula. 6 weeks later jejunal biopsies were performed before and 24 hours after challenge with a low lactose cows' milk protein formula. The immunoglobulin and complement levels in the serum and duodenal juice were also estimated at these times. Attempts to isolate bacterial and viral pathogens in stools were again made in all patients. The 5 older infants clinically tolerated cows' milk protein and their pre- and postchallenge jejunal biopsies were within normal limits. However, significant histological changes were observed in the postchallenge jejunal biopsies of all 9 infants under 2 months of age. In addition, 5 of these infants developed diarrhoea. This suggests that the jejunal mucosa of very young infants previously fed a cows' milk protein-based formula and who contract infective enteritis suffers damage when rechallenged with cows' milk protein.

There is general agreement that diarrhoea occurring in the first weeks of life is frequently prolonged and more hazardous than at any other period. The important factors responsible for this include the nature of the infection and the response of the young infant to infection, the frequency of sugar intolerance in young infants with infective enteritis (Burke *et al.*, 1965; Lifshitz *et al.*, 1971), dehydration and electrolyte imbalance which occur more rapidly in the smaller infant and which require great precision in the insertion, maintenance, and monitoring of intravenous infusions, and the prior nutritional status of the infant (Lifshitz *et al.*, 1971).

We believe that feeding with a cows' milk protein-based formula before the onset of acute infective enteritis is an additional factor in prolonging diarrhoea. Harrison *et al.* (1976) studied 25 children with cows' milk protein intolerance and have suggested that an acute attack of gastroenteritis, in damaging the small intestinal mucosa, may act as a triggering mechanism in cows' milk protein intolerance. Cows' milk protein-sensitive enteropathy has been suspected in the pathogenesis of intractable

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diarrhoea but objective proof has been lacking because satisfactory criteria for the diagnosis of cows' milk protein-sensitive enteropathy have not been available (Hyman *et al.*, 1971; Shwachman *et al.*, 1973). Recently Iyngkaran *et al.* (1978) proposed combined clinical and histological criteria for the diagnosis. We have applied these criteria in testing the following hypothesis put forward to explain the mechanisms involved—a small infant who contracts acute infective enteritis suffers damage and increased permeability of the gut mucosa by the aetiological agent. Gut permeability to cows' milk protein antigen may result in sensitisation so that a subsequent challenge with cows' milk protein may cause an immunological reaction on the gut wall resulting in additional mucosal damage, malabsorption, and continuing diarrhoea (Harrison *et al.*, 1976). To test this hypothesis the following study was designed.

Patients and methods

Infants under the age of 2 months presenting with the clinical features of acute infective enteritis, admitted to the Department of Paediatrics, University Hospi-

tal, between March and October 1976 were studied. All infants in this study had been fed a cows' milk-based formula before and up to the time of the episode of acute diarrhoea. Rehydration and correction of fluid and electrolyte imbalance were initially carried out intravenously. When the infant's condition improved and the diarrhoea was controlled, an oral 5% dextrose in $\frac{1}{4}$ isotonic saline mixture was offered. Those infants who tolerated this mixture were further studied for cows' milk protein-sensitive enteropathy. This required refeeding with a formula free of cows' milk protein, such as Pregestimil, Nutramigen, or Sobee. All infants were kept in hospital until the stools were normal, the infant was feeding well, and weight gains had started again. They were then discharged home, usually within 2 weeks of admission, the mothers being supplied with the appropriate formula free of cows' milk protein and instructed not to offer any other milk without consulting us. 6 weeks later the infants were admitted for further study.

Investigations at 6 weeks. (1) Low-lactose cows' milk provocation test. In the majority of the infants A1 110 (Nestle*) was used. In this test an initial feeding of 5 ml was offered. If no reaction occurred, the volume was doubled hourly for the first 4 hours and subsequently 3 hourly until daily fluid requirements were met. (2) Haemoglobin, total and differential white cell count before and after the reintroduction of cows' milk. (3) Determination of complement and immunoglobulins in duodenal juice before and 24 hours after milk challenge. (4) Determination of serum complement (C3, C4), serum immunoglobulins IgA, IgG, IgM, and IgE at 0, 1 $\frac{1}{2}$, 4, 12, and 24 hours after cows' milk challenge. (5) Testing of stools (if diarrhoea occurred) by the Clinitest method to exclude secondary sugar intolerance. (6) In all infants attempts were made to isolate parasitic, bacterial, and viral pathogens from the stools by culture, and by examination of stools under the light and electron microscope, 12 to 24 hours before and 12 to 24 hours after milk challenge. (7) Jejunal biopsy. The proximal jejunal biopsy specimen taken with the Watson paediatric capsule just distal to the duodenojejunal junction was examined under a dissecting microscope. Thereafter the specimen was divided into three portions, one fixed in glutaraldehyde for light and electron microscope studies, a second for enzyme determinations, and a third portion for immunofluorescence studies. In addition, a mucosal imprint was done to detect *Giardia lamblia* (Kamath and Murugasu, 1974). The following jejunal biopsies were performed: (a) prechallenge, and (b) post-challenge; this was performed 24 hours after the

*0.07% lactose after reconstitution.

introduction of cows' milk irrespective of the presence or absence of symptoms.

Results

Fourteen infants were studied, 9 under 2 months of age and 5 over 6 months. The latter 5 infants clinically tolerated cows' milk and their pre- and post-challenge jejunal biopsies were within normal limits. Significant mucosal changes were, however, observed in the postchallenge compared to the prechallenge jejunal biopsies from all the 9 infants under 2 months. In addition, 5 of these 9 infants developed diarrhoea. In 4 infants bacterial pathogens were isolated from stools during their initial admission and were still present at the time of the challenge studies 6 weeks later. The clinical and histological data of the 9 infants are summarised in Tables 1 and 2. The results of the immunological studies will be reported in full later.

Discussion

The 9 infants studied were all below the age of 6 weeks and had made a complete clinical recovery from an episode of acute infective enteritis. A small bowel biopsy taken 6 weeks after clinical recovery showed either normal histology or changes regarded as only marginally abnormal. A further biopsy taken 24 hours after the reintroduction of a cows' milk formula (A1 110) showed mucosal damage in all 9 infants. In addition, 5 infants developed diarrhoea, 3 within 48 hours, and 2 at 1 week and 2 weeks respectively after challenge and while receiving a cows' milk-based formula. All 5 responded satisfactorily when cows' milk protein was excluded from their diets. It is our belief that the mucosal changes shown in these infants are the result of re-exposure to cows' milk protein.

In 4 of the infants bacterial pathogens were isolated from stools and were still present 6 weeks later despite clinical recovery (Table 2). However, it is extremely unlikely that the change of milk formula would have provoked bacterial activity and mucosal damage within 24 hours—the time interval between the two biopsies. Furthermore, in 5 infants bacterial pathogens were not isolated at any stage.

Sugar intolerance was carefully excluded as an aetiological factor at the onset of the study and was excluded throughout the recovery period by testing of stools for reducing substances using the method of Kerry and Anderson (1964). Reducing substances were found in the stools of one infant when the cows' milk-based formula was introduced. As the quantity of lactose contained in A1 110 is less than 0.07%, it is unlikely that this small quantity of

Table 1 *Clinical profile of 9 infants with acute enteritis sensitive to cows' milk protein*

Case no.	Age (d)	Sex	Ethnic group	Birthweight (kg)	Type of feed since birth	Age at onset of diarrhoea (d)	Duration of diarrhoea (d)	Stool isolate* (virus and bacteria)	Sugar intolerance	Milk feed at discharge	Duration of illness (d)
1	4	M	Chinese	3.03	CMF	3	1	Neg	Neg	Sobee	14
2	8	M	Chinese	3.5	CMF	5	3	EPEC	„	Sobee	14
3	31	M	Indian	3.85	Breast for 1 week; CMF from 1 week	29	2	Neg	„	Nutramigen	10
4	9	F	Chinese	3.9	Breast for 6 days; CMF from 6 days	6	3	Neg	„	Sobee	2
5	28	F	Chinese	2.35	CMF	3	25	Salmonella	„	„	28
6	9	M	Malay	3.5	„	8	1	EPEC	„	„	6
7	28	F	Indian	2.88	„	25	3	Neg	„	„	3
8	9	M	Chinese	3.0	„	8	1	Neg	„	„	6
9	42	M	Chinese	3.4	„	28	14	Salmonella	„	Pregestimil	10

*EPEC=enteropathogenic *E. coli*.Table 2 *Clinical symptoms, jejunal mucosal changes, and stool isolates before and after challenge with cows' milk protein in 9 infants with acute enteritis sensitive to cows' milk protein*

Case no.	Stool isolates* at 1st admission		Stool isolates 12-24 h prechallenge		Stool isolates 12-24 h postchallenge		Prechallenge		Postchallenge	
	Virus	Bacteria	Virus	Bacteria	Virus	Bacteria	Symptoms	Histological† abnormality	Symptoms	Histological† abnormality
1	—	—	—	—	—	—	Nil	Normal (0)	Diarrhoea 7 h after challenge	Severe (13)
2	—	EPEC	—	EPEC	—	EPEC	„	Mild (4)	Nil	Moderate (11)
3	—	—	—	—	—	—	„	Normal (0)	Diarrhoea, weight loss, lethargy 2 w postchallenge	Moderate (9.5)
4	—	—	—	—	—	—	„	Normal (0)	Diarrhoea 48 h postchallenge	Moderate (9)
5	—	Salmonella	—	Salmonella	—	Salmonella	„	Mild (5.5)	Diarrhoea, weight loss 1 w postchallenge	Moderate (11)
6	—	EPEC	—	EPEC	—	EPEC	„	Normal (1)	Nil	Moderate (10)
7	—	—	—	—	—	—	„	Normal (3)	Vomiting, fever, lethargy, diarrhoea, 2 h postchallenge	Severe (13)
8	—	—	—	—	—	—	„	Normal (2)	Nil	Moderate (9)
9	—	Salmonella	—	Salmonella	—	Salmonella	„	Mild (4.5)	Nil	Moderate (8)

*— = negative.

†Figures in parentheses indicate scores: normal < 3, mild abnormality 3-7, moderate abnormality 8-11, severe abnormality > 11.

lactose would have produced the mucosal changes. In fact there is little or no evidence to suggest that lactose causes mucosal damage directly. It is much more likely that lactose intolerance had either persisted after the initial bowel infection or that it had occurred as the result of mucosal damage induced by the cows' milk protein present in the formula A1 110 (Liu *et al.*, 1967).

On the basis of this limited study we suggest that small infants who have been fed a cows' milk formula before the onset of acute infective enteritis should be fed a non-cows' milk protein formula and this should be continued for some weeks after recovery. It is clear that further work is necessary to confirm these observations, and if confirmed, to establish the actual incidence. We have duplicated this study in a

small group of 5 infants over the age of 6 months but could detect no evidence of either clinical disease or histological changes in the bowel mucosa. This further confirms our clinical experience that older infants tolerate cows' milk protein in the refeeding regimen after acute infective enteritis.

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