Cows' milk-sensitive enteropathy

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SUMMARY Serial small intestinal biopsies related to withdrawal and challenge with cows' milk are described in 5 infants with cows' milk protein intolerance. On the basis of these serial observations the existence of a cows' milk-sensitive enteropathy in infancy is clearly established. The cows' milk-sensitive mucosal lesion varied in its severity ranging from a partly flat mucosa to a mild degree of partial villous atrophy. Intraepithelial lymphocyte counts rose after a positive milk challenge, but on only one occasion to a level outside the normal range.

The technique of serial biopsies related to dietary milk enables a firm diagnosis of cows' milk intolerance to be made upon the basis of a single milk challenge. Clinically this appears to be a temporary condition which disappears by the age of 2 years.

Small intestinal mucosal abnormalities in children with a malabsorption syndrome induced by cows' milk were first reported by Lamy *et al.* in 1963, and have been widely confirmed subsequently (Kuitunen *et al.*, 1965; Shiner *et al.*, 1975a; Fontaine and Navarro, 1975; Walker-Smith, 1975). Kuitunen *et al.* (1973) described a milk provocation test in children who had previously responded to a cows' milk-free diet. They observed morphological changes in the small intestinal mucosa when there was a clinical relapse on milk challenge. It has been suggested that routine histological examination of small intestinal biopsies before and after a cows' milk challenge is the most helpful way to diagnose cows' milk allergy (Walker-Smith, 1975, 1976; Shiner *et al.*, 1975b). Yet sceptics continue to deny the very existence of cows' milk protein intolerance, and in Britain until recently, this entity has been an uncommon clinical diagnosis.

The purpose of this paper is firstly to provide firm evidence for the existence of cow's milk-sensitive enteropathy in infancy based upon serial small intestinal biopsies related to dietary milk intake, and secondly to recommend the use of such serial biopsies in order to diagnose cows' milk protein intolerance after a single milk challenge.

Patients and methods

Five infants admitted to the Queen Elizabeth Hospital for Children were selected for this report. They were chosen because all had serial biopsies related to change in dietary intake of cows' milk and because biopsy was used to establish the diagnosis in each case.

All 5 infants had developed acute diarrhoea, with vomiting in 3 between the ages of 1 and 8 weeks (Table 1). One infant was being fully breast-fed at that time and had never had cows' milk (Case 5), but the remainder were bottle fed. In each case an initial

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Table 1 Clinical data and acute gastroenterological symptoms in the 5 infants

Case no.	Sex	Initial symptoms	Age at first symptom (w)	Breast feeding and duration	Ethnic origin	Evidence for gastroenteritis at time of initial symptoms
1	F	Acute diarrhoea	3	10 d	Indian	<i>E. coli</i> O127—stool culture
2	M	Acute diarrhoea and vomiting	2	—	English	Other family members acute diarrhoea and vomiting
3	M	Acute diarrhoea and vomiting	3	—	English	—
4	F	Acute diarrhoea and vomiting	1	—	Indian	—
5	M	Acute diarrhoea	1	1 w	West Indian	(a) Rising antibody titre to Nebraska calf antigen (b) <i>E. coli</i> O55—stool culture

clinical diagnosis of acute gastroenteritis was made, but in only 3 was there conclusive evidence for this, namely identification of a known aetiological agent or contact with gastroenteritis in other family members (Table 1). Their ethnic origins were diverse. Cases 1-3 had initially been admitted to other hospitals.

Each infant had milk removed from his diet at some point in initial management. Cases 1 and 3 were given a trial of expressed breast milk but this was discontinued as they developed watery stools with an excess of reducing substances, i.e. evidence of lactose intolerance. Cases 1-4 had an immediate return of diarrhoea and vomiting on reintroduction of cows' milk to their diet. Case 1 subsequently developed intractable diarrhoea and had gross malnutrition at the time of her referral aged 10 weeks. She was managed with intravenous feeding and after recovery was given a lactose-free, milk-free diet based upon chicken meat. Case 5, although hitherto apparently completely breast fed, developed total monosaccharide intolerance and became dehydrated when given an oral electrolyte mixture containing in turn glucose and fructose, finally requiring intravenous fluids. On recovery he was also given a diet based on chicken meat. He apparently was never given milk until the time of milk challenge, although it is possible that he had some milk in a neonatal unit, unknown to the medical staff. The remaining 3 children were given a commercial milk-free formula.

Each infant had a first biopsy as soon as possible after admission, the time interval depending on their clinical condition (Table 2). In all except Case 2, this meant they were already on a milk-free diet. The duration of the milk-free diet ranged from 10 days to 4½ months at the time of this initial biopsy. All children thereafter continued on a strict milk-free diet. A second (prechallenge) biopsy was performed while still on this milk-free diet as a preliminary to a cows' milk challenge (Table 2). After each infant had had a second biopsy which showed a normal or near-normal small bowel mucosa, they were given a lactose challenge

(Harrison and Walker-Smith, 1977) followed by a cows' milk challenge as follows.

Day 1: lactose 2 g/kg was given as an oral load after an overnight fast. No diarrhoea having developed, a feed containing lactose 7 g/100 ml was then given for 24 hours. As no diarrhoea followed the lactose load in this group, the challenge proceeded. But had diarrhoea with ½% or more reducing substances occurred, a diagnosis of lactose intolerance would have been made, the milk challenge postponed, and the infant returned to a milk-free diet (Harrison and Walker-Smith, 1977).

Day 2: fresh cows' milk 5 ml was given at 9 am and 10 ml at 10 am. If no clinical symptoms appeared by midday, and subsequently if no clinical relapse occurred, each infant was then graded onto a cows' milk formula, or whole cows' milk was used if the infant was over 6 months of age at the time of challenge.

The infants were observed carefully for the appearance of vomiting, fever, loss of weight, and diarrhoea. Loose stools were tested for reducing substances and occult blood. When significant symptoms appeared milk was stopped and a third intestinal biopsy was performed as soon as was considered clinically appropriate, usually within 24 hours. Having then demonstrated mucosal deterioration, each child was placed back onto a milk-free diet.

A fourth biopsy was performed in 3 children after a further period on a milk-free diet (see Table 2), in these and the remaining children cows' milk was subsequently reintroduced to their diet without a clinical relapse, except Case 1 who relapsed again on a second challenge but who did not do so after a third challenge. All biopsies were performed as a part of routine management in order to make a therapeutic decision.

Small intestinal biopsy specimens were examined under the dissecting microscope, photographed, and their appearances classified (Walker-Smith, 1967). Cryostat sections were stained with haematoxylin and eosin (H & E) and with Scharlach R in all cases, and the tissue embedded in ester wax, sectioned, and

Table 2 *Biopsy data of the 5 infants*

Case no.	Initial biopsy grading	Time already on milk-free diet	Prechallenge biopsy	Time on milk-free diet (m)	Postchallenge biopsy	Final biopsy
1	±	19 d	Challenge 1 not done; challenge 2, ±	9	++/+++	Normal
2	++	On cows' milk	N	3½	++	Not done
3	±	10 d	N	3½	+/N	Not done
4	++	37 d	±/+	7	++	+
5	+/N	4½ m	N	7½	+	±

Biopsy gradings: see text.

stained with H & E and with Mann's stain for eosinophils. When a double-port capsule (Kilby, 1976) was used, the second specimen was fixed in formol saline and embedded in ester wax or paraffin. Although a number of other stains, including those for various enzymes, were used they only confirmed the grading of mucosal changes based upon H & E stained sections. Final assessment of the mucosal appearance was based upon a combination of both gross and histological findings.

Biopsy specimens were graded as follows. (a) *Normal*: tall thin ridge-like villi; tongues, leaves, and fingers in any combination. On section the villi appeared at least twice as long as the crypts. (b) $\pm$: normal dissecting microscopic appearance with sections showing borderline or doubtful changes. (c) $+$: dissecting microscopy showing normal villi or thickened and shorter villi. Histologically there was a minor degree of partial villous atrophy although the picture varied from case to case. This grade included mucosa with not more than two of the following features: epithelial abnormality, shortening or thickening of villi, increased plasma cells or eosinophils in the lamina propria, elongation of crypts. (d) $++$: short thick ridges on dissecting microscopy. All specimens in this grade showed increased intraepithelial lymphocytes and/or pseudostratified epithelium covering short, frequently wide ridges, moderate increase of cells in the lamina propria and hyperplasia of crypts. (e) $+++$: classical flat mucosa. (f) Patchy lesions (e.g. $+/++$), i.e. where the lesion was nonuniform.

The number of intraepithelial lymphocytes was quantified in each case by one of us (A.P.). A normal range of 10.5–31.5 intraepithelial lymphocytes per 100 epithelial cells counted was used. This range was obtained by calculating the mean intraepithelial lymphocyte count in 16 histologically normal biopsies, and taking 2 standard deviations on each side of it. Disaccharidase activity was assayed in each biopsy (Burgess *et al.*, 1964).

Results

First biopsy. There was a wide range of gradings for these biopsies (see Table 2). The children were continued on a milk-free diet for varying periods of time depending on their clinical progress, i.e. rate of weight gain and general well being.

Second biopsy. The duration of a milk-free diet in these children ranged from 3½ to 9 months at time of second biopsy. Three of these biopsies were unequivocally normal, one had one area of borderline abnormality and one had a patchy lesion with one area of borderline abnormality and another of mild partial villous atrophy.

Third biopsy. All challenges were clinically positive but in all cases significant symptoms took more than 48 hours to appear, and in one up to 7 days (Fig. 1). Once significant symptoms appeared, milk was withdrawn from the diet and the child had a third biopsy as soon as possible. All biopsies showed a significant deterioration. The time interval to biopsy after stopping milk on return of significant symptoms (Fig. 1) ranged from 15 to 20 hours in all except the first challenge in Case 1, where the interval was 7 days, the infant developing severe diarrhoea and requiring intravenous fluids because of dehydration. Despite this interval in this case, the mucosa was in part completely flat. Serial biopsies from Case 1 who had two positive challenges are illustrated in Fig. 2.

Fourth biopsy. This was normal in 2 cases and showed a mild degree of partial villous atrophy in the third child. This represented an improvement from the third biopsy. The children tolerated cows' milk at the ages of 21, 19, 14, 19, and 16 months respectively.

Morphology of biopsies (Table 2, Fig. 2). Patchy lesions were observed in 5 of the 19 specimens examined. Although most were illustrated in a single specimen, the importance of using a double-port capsule is stressed as the two specimens may differ, and in Case 3 this was of critical importance. The variability of severity of mucosal changes in response to milk challenge is clearly shown in Fig. 2, with different mucosal response in the two positive challenges in the same child.

Fig. 1 Duration of milk challenge and milk-free diet before postchallenge biopsy. The numbers represent Cases 1–5, Case 1 having been challenged twice.

Fig. 2 Small intestinal mucosa in Case 1. (H & E $\times 50$.) (1) Initial biopsy: short villi with numerous goblet cells and increased cells in lamina propria ($\pm$). (2) Postchallenge biopsy: flat surface with columnar epithelium, moderately increased cells in lamina propria, and elongated crypts (+++). (3) Prechallenge biopsy: short villi with increased cells in lamina propria ($\pm$). (4) Postchallenge biopsy: short villi with pseudo-stratified epithelium, moderately increased cells in lamina propria, and elongated crypts (++). (5) Final biopsy: normal mucosa.

Intraepithelial lymphocytes. There was a rise in the numbers of epithelial lymphocytes in each post-challenge biopsy as compared to the prechallenge biopsy, although in one challenge only did the level rise above the range found in histological normal biopsies (Fig. 3).

Disaccharidase activity. Disaccharidase activity rose in the second biopsy after a milk-free diet only to fall again after mucosal relapse with a positive milk challenge (see Fig. 4). Although these serial observations of disaccharidase activity were a helpful guide to mucosal remission and relapse, individual levels did not correlate with the severity of the mucosal morphology (Harrison and Walker-Smith, 1977).

Discussion

This study shows both the existence of a cows' milk-sensitive enteropathy in infancy, and the use of serial small intestinal biopsies before and after a cows' milk challenge as a diagnostic technique. The mucosal lesion at the time of first biopsy was of variable severity. This was in part probably related to the length of time already on a milk-free diet. In all children there was improvement in morphology after a milk-free diet and in most a return to normal. In all there was a significant deterioration after a further milk challenge. However, the mucosal lesion was markedly variable in its severity, ranging from a partly flat mucosa to a mild degree of partial villous

Fig. 3 Intraepithelial lymphocyte counts in pre- and postchallenge biopsies.

Fig. 4 Mean disaccharidase activity related to cows' milk intake in 5 infants with milk-sensitive enteropathy.

atrophy. *Pari passu* with these serial morphological changes disaccharidase activity rose on a milk-free diet and fell after a positive milk challenge. Intraepithelial lymphocyte levels rose after a positive milk challenge, but on only one occasion to a level outside the normal range. A pre- and postchallenge biopsy is essential for accurate interpretation of postchallenge appearances, because of this variable severity. Monitoring of disaccharidase activity and the intraepithelial lymphocyte count provides additional useful evidence for mucosal relapse after challenge especially when less severe morphological changes

occur. In practice a fourth biopsy may not routinely be necessary.

In the past various immunological phenomena have been studied before and after milk challenge but in clinical practice they have not been very helpful in making a firm diagnosis. Unlike the recommendations of Goldman *et al.* (1963), where three positive clinical challenges are demanded before the diagnosis can be made, this technique of serial small intestinal biopsies enables a firm diagnosis of cows' milk intolerance to be made upon the basis of a single milk challenge. In this way the diagnosis of cows' milk protein intolerance is removed from a purely clinical response to cows' milk withdrawal and challenge, as still recommended by standard texts.

The use of a preliminary lactose challenge enables continuing lactose intolerance to be excluded as a cause of milk intolerance before proceeding to a milk challenge. In the past the distinction between lactose intolerance and cows' milk protein intolerance has often been confused, especially as it is clear that a positive cows' milk challenge may lead to a fall in disaccharidase activity usually accompanied by evidence of clinical lactose intolerance although not invariably (Harrison *et al.*, 1976).

The mucosal lesion described here is clearly similar to that found in children with untreated coeliac disease, although usually less severe. Both clinical and mucosal relapse appear to occur more quickly after an elimination diet and challenge than is the case in children with coeliac disease tested in a similar way in relation to gluten. As previously reported (Harrison *et al.*, 1976), it appears to be triggered by an attack of acute gastroenteritis at least in some cases. Case 5 was remarkable as he apparently developed symptoms on first contact with cows' milk at time of milk challenge after recovery from acute gastroenteritis.

Clinically, in these and other studies, intolerance to cows' milk appears to disappear by the age of 2 years, although it is still uncertain whether a mucosal lesion persists beyond this age. In practice, a second milk challenge may not require serial biopsies, but it is recommended that this be performed in hospital because of occasional severe reactions.

Whether the syndrome of cows' milk-protein intolerance is identical with the syndrome of cows' milk-sensitive enteropathy described here is not clear. Only close collaboration between immunological and gastroenterological observations will clarify this point.

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References

- Burgess, E. A., Levin, B., Mahlanabis, D., and Tonge, R. E. (1964). Hereditary sucrose intolerance: levels of sucrase activity in jejunal mucosa. *Archives of Disease in Childhood*, **39**, 431-443.
- Fontaine, J. L., and Navarro, J. (1975). Small intestinal biopsy in cow's milk protein allergy in infancy. *Archives of Disease in Childhood*, **50**, 357-362.
- Goldman, A. S., Anderson, D. W., Jr., Sellers, W. A., Saperstein, S., Kniker, W. T., and Halpern, S. R. (1963). Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics*, **32**, 425-443.
- Harrison, B. M., and Walker-Smith, J. A. (1977). Reinvestigation of lactose intolerant children: lack of correlation between continuing lactose intolerance and small intestinal morphology, disaccharidase activity and lactose tolerance tests. *Gut*, **18**, 48-52.
- Harrison, B. M., Kilby, A., Walker-Smith, J. A., France, N. E., and Wood, C. B. S. (1976). Cow's milk protein intolerance: a possible association with gastroenteritis, lactose intolerance and IgA deficiency. *British Medical Journal*, **1**, 1501-1504.
- Kilby, A. (1976). Paediatric small intestinal biopsy capsule with two ports. *Gut*, **17**, 158-159.
- Kuitunen, P., Visakorpi, J. J., and Hallman, N. (1965). Histopathology of duodenal mucosa in malabsorption syndrome induced by cow's milk. *Annales Paediatrici*, **205**, 54-63.
- Kuitunen, P., Rapola, J., Savilahti, E., and Visakorpi, J. K. (1973). Response of the jejunal mucosa to cow's milk in the malabsorption syndrome with cow's milk intolerance. *Acta Paediatrica Scandinavica*, **62**, 585-595.
- Lamy, M., Nezelof, C., Jos, J., Frèzal, J., and Rey, J. (1963). La biopsie de la muqueuse intestinale chez l'enfant; premiers résultats d'une étude des syndromes de malabsorption. *Presse Médicale*, **71**, 1267-1270.
- Shiner, M., Ballard, J., and Smith, M. E. (1975a). The small intestinal mucosa in cow's milk allergy. *Lancet*, **1**, 136-140.
- Shiner, M., Ballard, J., Brook, C. G. D., and Herman, S. M. D. (1975b). Intestinal biopsy in the diagnosis of cow's milk protein intolerance without acute symptoms. *Lancet*, **2**, 1060-1063.
- Walker-Smith, J. A. (1967). Dissecting microscope appearance of small bowel mucosa in children. *Archives of Disease in Childhood*, **42**, 626-630.
- Walker-Smith, J. A. (1975). Cow's milk protein intolerance: transient food intolerance of infancy. *Archives of Disease in Childhood*, **50**, 347-350.
- Walker-Smith, J. A. (1976). Milk intolerance in infancy. *Journal of Maternal and Child Health*, **1**, 39-45.

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