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Cows' milk protein-sensitive enteropathy

Combined clinical and histological criteria for diagnosis

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SUMMARY Cows' milk protein enteropathy is recognised as a significant cause of persistent diarrhoea and malabsorption in young infants, but there are as yet no generally accepted diagnostic criteria. A combined clinical and histological approach to the diagnosis of cows' milk protein-sensitive enteropathy has been used in 15 patients, and the following set of criteria are proposed. (1) Clinical disease (diarrhoea with or without vomiting) while receiving cows' milk protein. (2) Clinical improvement on a diet free of cows' milk protein. (3) Normal or mildly abnormal histology of jejunal mucosa when taken 6–8 weeks after symptoms subside. (4) Histological relapse, with or without clinical relapse, after re-exposure to cows' milk protein.

Intolerance to cows' milk protein has been reported in recent years as a cause of persistent diarrhoea and failure to thrive in Caucasian infants (Goldman *et al.*, 1963; Gryboski, 1967; Freier *et al.*, 1968; Kuitunen *et al.*, 1975). Diagnosis has hitherto been based on certain clinical criteria described by Goldman *et al.* (1963). (1) The development of symptoms which subside after dietary elimination of milk. (2) Symptoms which recur within 48 hours of milk challenge. (3) Reactions to three such challenges should be positive and have a similar onset, duration, and clinical features.

These criteria, while basically sound, are frequently impractical and we believe lead to underdiagnosis. Recently other criteria have been suggested, the emphasis being placed on the demonstration of intestinal mucosal damage by the toxic effect of cows' milk protein rather than on clinical observations (Walker-Smith, 1975; Shiner *et al.*, 1975a, b). We have used both a clinical and histological approach to the diagnosis of cows' milk protein intolerance in a prospective study.

Material and methods

Fifteen infants with diarrhoea clinically suspected to be due to intolerance to cows' milk protein were included in the study. Treatment initially involved elimination of cows' milk and substitution of a formula free of cows' milk (soy protein based, Pregestimil, or breast milk). When a satisfactory response was obtained, assessed clinically by lack of symptoms and satisfactory weight gain, the infant was discharged and the parents instructed not to introduce any new food item without our knowledge. 6 to 8 weeks later the infant was readmitted for further study. The following studies were performed.

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 total daily fluid requirements were met.

Jejunal biopsy. The proximal jejunal biopsy specimen taken with the Watson paediatric capsule at or 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* by the method of Kamath and Murugasu (1974). The following jejunal biopsies were performed: (a) prechallenge; (b) postprovocation; this was performed 24 hours after the introduction of cows' milk irrespective of the presence or absence of symptoms.

Jejunal biopsy appearances were classified using four parameters: (a) villous morphology on dissecting microscope; (b) villous/crypt height ratio; (c) epithelial cells; (d) cellular infiltration.

The severity of mucosal changes was scored

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*Protein fraction consists of casein, lactose content 0·07% at normal reconstitution.

(method modified from Townley *et al.*, 1965). With regard to dissecting microscope appearances, a pattern of leaves was accepted as normal and scored 0; broad leaves 1, ridges 2, convolutions 3, and flat mucosa 4. A villous/crypt height ratio of 3:1 or more was accepted as normal (score 0). A ratio of 2:1 scored 2 points, 1:1, 4 points, and a reversed ratio, 6 points. The height of villous epithelial cells (over the upper third of the villi) was compared with crypt cells (in the bases of the crypt). A normal mucosa is characterised by villous cells that are taller than crypt cells (score 0). Mucosal damage resulting in villous cells being of the same height as crypt cells was scored as 1 and, a reversal of this ratio obtained a score of 2. Cellular infiltration of the lamina propria was subjectively graded as normal, mild, moderate, and severe, and was scored respectively as 0, 1, 2, and 3. The scores were weighted according to the generally recognised importance of the parameters and the ease with which changes can be recognised. On the basis of this classification biopsies have been assessed as *normal* (score <3), *mildly abnormal* (4–7), *moderately abnormal* (8–11), and *severely abnormal* (>11).

Other investigations. Hb, total and differential white cell count, before and after the reintroduction of cows' milk. Determination of serum complement (C3 and C4), serum immunoglobulins IgA, IgG, IgM, and IgE at 0, 1½, 4, 12, and 24 hours after cows' milk challenge. Determination of complement and immunoglobulins in duodenal juice before and 24 hours after milk challenge. In those infants who developed diarrhoea after cows' milk challenge 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. Testing of stools (if diarrhoea occurred) by the Clinitest method to detect secondary sugar intolerance.

Results

Fifteen infants clinically suspected of having cows' milk protein intolerance were studied between November 1975 and October 1976. In 4 infants no significant alteration was noted in the jejunal mucosa 24 hours after challenge with cows' milk. These 4 infants continue to thrive well on cows' milk formula.

The clinical details of the 11 patients in whom significant changes were noted in the jejunal mucosa after challenge with cows' milk are summarised in Table 1. The 11 infants, 10 Chinese and one Indian, were all under 3 months of age at admission. 8 were under 2 weeks of age when they first contracted diarrhoea. The average duration of diarrhoea before admission to our hospital was 3·5 weeks (range 1–6 weeks). Total intravenous hyperalimentation for prolonged periods was needed in 4 infants before diarrhoea settled and oral feeds were tolerated. The clinical, histological, haematological, and serum complement data before and after provocation with cows' milk are summarised in Tables 2–4. Figs. 1 and 2 show the normal and abnormal histological appearances of the jejunal mucosa taken before and 24 hours after challenge with cows' milk in Case 3.

Discussion

The criteria of Goldman *et al.* (1963) are sound but are frequently not practical, as many mothers refuse to accept the frequent milk challenges required. Moreover, reactions so produced may be extremely serious and even life threatening. For these reasons many clinicians have modified Goldman's criteria and accept one positive challenge as diagnostic of cows' milk protein intolerance. This has tended to result in undue parental concern, misdiagnosis, and frequently a financial burden to parents who are unable to afford the special formulae. We disagree with this latter approach as it is difficult to interpret the results of a single positive milk challenge. In addition, the possibility of an intercurrent bowel infection producing the symptoms cannot be excluded. To compound the difficulty, some workers (Kuitunen *et al.*, 1975) have shown that it may take up to a month in some infants exposed to cows' milk protein to develop the typical gastrointestinal symptoms of malabsorption. There are also difficulties in differentiating cows' milk protein enteropathy from secondary sugar intolerance by response to elimination diets alone (Liu *et al.*, 1967; McNeish 1974).

Walker-Smith (1975) has suggested that sensitivity of the infant's small intestinal mucosa to cows' milk may be shown by using serial biopsies in addition to a single milk challenge. The studies of Shiner *et al.* (1975a,b) have shown that routine light microscopy of the pre- and postchallenge jejunal biopsies may be helpful in diagnosis. A combined clinical and histological approach seems to provide the best means to diagnosis, and the following set of criteria for the diagnosis of cows' milk protein intolerance are proposed. (1) Clinical disease (diarrhoea with or without vomiting) while receiving cows' milk protein. (2) Clinical improvement on a diet free of cows' milk protein. (3) Normal or mildly abnormal histology of jejunal mucosa when taken 6–8 weeks after symptoms subside. (4) Histological relapse with or without clinical relapse after re-exposure to cows' milk protein. These criteria closely follow those laid down

Table 1 *Clinical features of 11 infants with cows' milk protein intolerance at first admission*

Case no.	Age at first admission (w)	Sex	Ethnic group	Birthweight (kg)	Type of feed since birth*	Symptoms			Signs		
						Age of onset	Duration (w)	Nature	Weight (kg)	Abdominal distension	Other features
1	5	M	Chinese	3·25	Breast fed 2 w, CMF from 2 w	2 w	3	Diarrhoea, erythematous rash, rhinorrhoea, cough	3·95	+	Erythematous rash over neck, back, groin
2	4	F	Indian	3·2	CMF	1 w	3	Diarrhoea	3·45	—	—
3	4	F	Chinese	2·7	,,	5 d	3	Diarrhoea, bilious vomiting, abdominal distension	2·35	+	Marasmic with rash over scalp, neck, groin; oral thrush
4	4	F	,,	3·2	,,	2 d	4	Diarrhoea, vomiting, abdominal distension, erythematous rash, rhinorrhoea	2·45	+	Marasmic with rash over scalp, neck, groin; oral thrush
5	6	F	,,	3·0	,,	4 d	5	Diarrhoea	3	+	Marasmic
6	6	F	,,	4·0	,,	5 d	5	,,	4·29	+	Mildly wasted with oral thrush
7	7	M	,	3·6		5 d	6		2·56	+	Marasmic, staphylococcal septicaemia with septic arthritis
8	3 m	F	,,	—	,	11 w	1	,,	6·15	—	—
9	5	F	,,	3·0	,,	1 w	4	,,	3·0	—	Mildly wasted; perianal excoriation
10	6	F	,,	3·4		4 w	2		3·8	+	Perianal excoriation
11	9	M	Indian	2·5	Breast fed 4 w CMF from 4 w	6 w	3	,,	3·9	—	Perianal excoriation; mild wasting

*CMF = cows' milk formula.

Table 2 *Onset and nature of clinical relapse and mucosal changes after provocation with cows' milk protein*

Case no.	*Interval between provocation and clinical relapse*	*Nature of clinical relapse*	*Postchallenge (24 hours) jejunal biopsy**
1	14 d	Diarrhoea, erythematous rash, rhinorrhoea, cough	Moderately abnormal (7·5)
2	4 d	Diarrhoea	Moderately abnormal (10)
3	½ h	Vomiting, fever, lethargy, diarrhoea	Severely abnormal (13)
4	2 w	Erythematous rash, persistent rhinorrhoea	Moderately abnormal (9)
5	No symptoms up to 5 w after challenge	—	Moderately abnormal (11)
6	3 d	Diarrhoea	Moderately abnormal (9)
7	2 d	Diarrhoea	Severely abnormal (12)
8	No symptoms up to 1 w after challenge	—	Moderately abnormal (10)
9	No symptoms up to 2 w after challenge	—	Moderately abnormal (9·5)
10	No symptoms up to 4 w after challenge	—	Moderately abnormal (8)
11	2 h	Vomiting, fever, lethargy, diarrhoea	Severely abnormal (13)

*Numbers in parentheses denote score: normal >3; mild abnormality 4–7; moderate abnormality 8–11; severe abnormality >11.

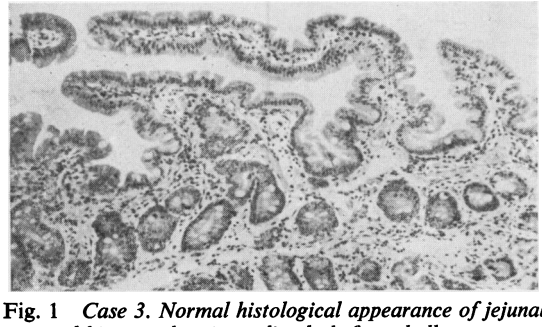

Fig. 1 *Case 3. Normal histological appearance of jejunal mucosal biopsy taken immediately before challenge. (H & E ×50.)*

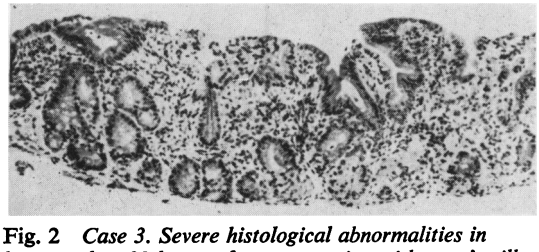

Fig. 2 *Case 3. Severe histological abnormalities in biopsy taken 20 hours after provocation with cows' milk protein. (H & E ×50.)*

by the European Society for Paediatric Gastroenterology (Meeuwisse, 1970) for the diagnosis of gluten enteropathy.

The 11 cases studied fulfil all of the criteria. The postprovocation biopsies in all showed obvious changes in mucosal pattern when compared to the prechallenge biopsies. For technical reasons biopsies on 2 infants were not performed until 5 days and 18 days after the reintroduction of cows' milk when these infants developed symptoms. In 9 infants the postprovocation biopsies were performed 24 hours after introduction of cows' milk. Of the latter 9 infants, 5 developed symptoms at ½, 2, 48, 72 hours, and 2 weeks respectively after introduction of cows' milk. The 4 remaining infants have not yet developed symptoms.

On the basis of Goldman's criteria (Goldman *et al.*, 1963) which specifies that symptoms should appear within 48 hours of challenge, only 3 of our cases would be regarded as suffering from cows' milk protein intolerance. We believe, however, that cows' milk protein intolerance is a spectrum of varying degree. The appearance and the severity of the symptoms are determined by the severity and extent of mucosal damage and the lactose content of the milk used in the provocation test. Similar observations have been made in patients with gluten enteropathy (MacDonald *et al.*, 1965). There is no doubt that in Cases 1 and 2 because of the long time relationships between reintroduction of cows' milk protein, the development of symptoms, and repeat biopsy, it could be argued that the mucosal changes are the result of some other factors, e.g. intercurrent infection. While such a possibility cannot be discounted, it is most unlikely as parasites, bacteria, and viruses were looked for and not found.

The jejunal mucosa of 'normal' inhabitants from tropical areas when compared to those of Caucasians from temperate zones may appear mildly abnormal (Creamer, 1974), and this has been attributed to a 'non-specific tropical enteropathy'. Clearly this possibility could not be excluded entirely. We find, however, the jejunal mucosal appearances of normal Malaysian infants under 6 months of age very similar to those of their Caucasian counterparts.

The protein content of A1 110 is only purified casein. Of the 5 major protein fractions in cows' milk, β-lactoglobulin has been shown to be more often responsible for the manifestations of cows'

Table 3 *Histological findings* of jejunal mucosa before and 24 hours after challenge with cows' milk protein in 11 patients†*

Case no.	Prechallenge						Postchallenge					
	Villous morphology (stereomicroscope)	*Villous/ crypt ratio*	*Epithelial cell/crypt cell ratio*	*Cellular infiltrate*	*Histological abnormality*	*Total score*	*Villous morphology*	*Villous/ crypt ratio*	*Epithelial cell/crypt cell ratio*	*Cellular infiltrate*	*Histological abnormality*	*Total score*
1	Leaves (0)	3:1–2:1 (1)	>1:1–1:1 (0·5)	Normal (0)	Normal	1·5	Broad leaves and ridges (1·5)	2:1 (2)	<1:1 (2)	Moderate (2)	Moderate	7·5
2	Broad leaves (1)	2:1–1:1 (3)	1:1 (1)	Mild (1)	Mild	6	Broad leaves (1)	<1:1 (6)	1:1 (1)	,,	Moderate	10
3	Leaves (0)	3:1–2:1 (1)	>1:1 (0)	Normal (0)	Normal	1	Convolutions (3)	<1:1 (6)	<1:1 (2)	,,	Severe	13
4	Broad leaves (1)	3:1–1:1 (2)	1:1 (1)	Normal (0)	Mild	4	Ridges (2)	2:1–1:1 (3)	<1:1 (2)	,,	Moderate	9
5	Ridges (2)	2:1–1:1 (3)	1:1 (1)	Mild (1)	Mild	7	Ridges and convolutions (3)	1:1–<1:1 (5)	1:1 (1)	,,	,,	11·0
6	Leaves (0)	3:1 (0)	>1:1 (0)	Normal (0)	Normal	0	Broad leaves (1)	1:1 (4)	1:1 (1)	,,	,	9·0
7	Broad leaves (1)	3:1–2:1 (1)	1:1 (1)	Mild (1)	Mild	4	Convolutions (3)	1:1–<1:1 (5)	<1:1 (2)	,,	,,	12·0
8	Leaves (0)	3:1–1:1 (2)	>1:1 (0)	Mild (1)	Normal	3	Ridges (2)	2:1–1:1 (3)	<1:1 (2)	,,	,,	9·0
9	Broad leaves (1)	3:1–1:1 (2)	>1:1 (0)	Mild (1)	Mild	4	Broad leaves and ridges (1·5)	2:1–<1:1 (4)	<1:1 (2)	,,	,,	9·5
10	Leaves (0)	3:1–1:1 (2)	1:1–<1:1 (1·5)	Mild (1)	Mild	4·5	Broad leaves and) ridges (1·5	1:1 (4)	1:1–<1:1 (1·5)	Mild (1)	,,	8
11	Broad leaves (1)	3:1–1:1 (2)	>1:1 (0)	Mild (1)	Mild	4	Ridges and convolutions (3)	<1:1 (6)	<1:1 (2)	Moderate (2)	Severe	13

*Numbers in parentheses denote score.
†In Cases 1 and 2 biopsies were performed 18 and 5 days after challenge.

Table 4 *Eosinophilia (absolute count $\times 10^9/l$) and serum complement levels (mg/100ml) before and 24 hours after challenge with cows' milk in 11 patients*

	Prechallenge			*Postchallenge*		
Case no.	*Eosinophils*	*Complement (C3)*	*Complement (C4)*	*Eosinophils*	*Complement (C3)*	*Complement (C4)*
1	Nil	100	58	0·186	68	25
2	Nil	142	24·6	0·628	134	12·4
3	0·034	63	31·4	Nil	63	31·4
4	1.0	84	87·4	0·316	76	79·6
5	0·072	76	53·5	Nil	76	59
6	0·43	54	19	0·157	56	21
7	0·138	96	87·4	0·588	70	78
8	—	66	44	—	66	42·5
9	0·19	58	26·6	0·377	58	20·4
10	0·096	82	42	0·3	84	42
11	0·505	72	67·6	Nil	67	67·6

milk protein intolerance (Bleumink and Young, 1968; Freier *et al.*, 1969). Goldman *et al.* (1963) studied 45 infants with proved cows' milk protein sensitivity and showed that in infants suffering from anaphylaxis, diarrhoea, or vomiting, β-lactoglobulin was not more frequently toxic than the other milk proteins tested. Visakorpi and Immonen (1967) showed that 3 of 4 patients each reacted to casein and β-lactoglobulin. The response of our patients to casein appears similar to those studied by Goldman *et al.*

Eosinophilia and/or changes in complement levels, although occurring in some cases, was not seen in all. In addition, the demonstration of increased type-specific immunocytes (IgA, IgG, IgM, IgE) in the postchallenge jejunal mucosa by immunofluorescence studies was very variable (the immunological studies will be reported in full). These results could be consistent with different immunological responses in different patients, or in the same patient at different times.

It is at present unclear just when the postchallenge biopsy should be performed. Our view is that when it is done 24 hours after challenge, changes are very likely to occur, as all cases biopsied at 24 hours in this series were abnormal. However, it would be necessary to study a larger number of infants in order to predict that changes will invariably occur at 24 hours in all cases of cows' milk protein enteropathy.

Despite biopsy changes, 3 infants did not develop symptoms until several days later and 4 infants have not yet developed symptoms. The latter are making satisfactory clinical progress on a cows' milk formula. This has important implications for management and we would suggest the following approach. (a) Infants with postchallenge biopsy changes and symptoms indicate exclusion of cows' milk protein from the diet. (b) Infants with biopsy changes without symptoms may be managed with a cows' milk formula, but will need careful follow-up and the exclusion of cows' milk protein should gastrointestinal or any other allergic symptoms appear within a reasonable time after provocation, e.g. <3 months.

It could be argued that continuing cows' milk feeds in infants with mucosal abnormality but without symptoms may be unwise, as continued antigen challenge in the presence of an abnormal intestinal mucosa could place the child at risk to further allergic disease, but at present there is no firm evidence to support this view.

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References

Bleumink, E., and Young, E. (1968). Identification of the atopic allergen in cows' milk. *International Archives of Allergy and Applied Immunology*, **34**, 521–543.

Creamer, B. (1974). (Editor.) The small intestinal mucosa—geographical considerations. *The Small Intestine*, p. 16. Heinemann, London.

Freier, S., Kletter, B. Gery, I., Lebenthal, E., and Geifman, M. (1969). Intolerance to milk protein. *Journal of Pediatrics*, **75**, 623–631.

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.

Gryboski, J. D. (1967). Gastrointestinal milk allergy in infants. *Pediatrics*, **40**, 354–362.

Kamath, K. R., and Murugasu, R. (1974). A comparative study of four methods for detecting *Giardia lamblia* in children with diarrheal disease and malabsorption. *Gastroenterology*, **66**, 16–21.

Kuitunen, P., Visakorpi, J. K., Savilahti, E., and Pelkonen, P. (1975). Malabsorption syndrome with cow's milk intolerance. *Archives of Disease in Childhood*, **50**, 351–356.

Liu, H.-Y., Tsao, M. U., Moore, B., and Giday, Z. (1967). Bovine milk protein-induced intestinal malabsorption of lactose and fat in infants. *Gastroenterology*, **54**, 27–34.

MacDonald, W., Dobbins, W., and Rubin, C. E. (1965). Studies of the familial nature of celiac sprue using biopsy of the small intestine. *New England Journal of Medicine*, **272**, 448–456.

McNeish, A. S. (1974). The role of lactose in cow's milk intolerance (Abst.). *Acta Paediatrica Scandinavica*, **63,** 652.

Meeuwisse, G. (1970). Diagnostic criteria in coeliac disease. *Acta Paediatrica Scandinavica*, **59,** 461–463.

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. (1975b). Intestinal biopsy in the diagnosis of cow's milk protein intolerance without acute symptoms. *Lancet*, **2,** 1060–1063.

Townley, R. R. W., Khaw, K. T., and Shwachman, H. (1965). Quantitative assay of disaccharidase activities of small intestinal mucosal biopsy specimens in infancy and childhood. *Pediatrics*, **36,** 911–921.

Visakorpi, J. K., and Immonen, P. (1967). Intolerance to cow's milk and wheat gluten in the primary malabsorption syndrome in infancy. *Acta Paediatrica Scandinavica*, **56,** 49–56.

Walker-Smith, J. (1975). Cow's milk protein intolerance. *Archives of Disease in Childhood*, **50,** 347–350.

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