Small intestinal biopsy in cow's milk protein allergy in infancy

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Fontaine, J. L., and Navarro, J. (1975). *Archives of Disease in Childhood*, 50, 357. **Small intestinal biopsy in cow's milk protein allergy in infancy.** Intestinal biopsies were performed in 31 infants with cow's milk protein intolerance. Mucosal damage was constant, partial villous atrophy being the most usual finding. Though these changes are nonspecific, small intestinal biopsy can be a useful investigation in these patients. With a milk-free diet, recovery of normal mucosal appearance is reached between 3 and 13 months.

Only a few authors have published the results of intestinal biopsy in cow's milk protein intolerance in infancy, and no clear conclusions have been established concerning the degree of alteration of small intestinal mucosa, which in some cases has been considered to be normal (Freier *et al.*, 1969; Lubos, Gerrard, and Buchan, 1967).

Our aim in the present study has been to evaluate small intestinal mucosal damage in this disease, and to observe whether it is constant and specific and how it is related to the clinical symptoms.

Material and methods

Mucosal specimens were studied in 31 patients. Ages ranged from 3 to 7 months, except for one who was 20 months. Details of these cases are summarized in Table I.

Diagnosis of cow's milk protein intolerance was based upon the following points: onset of symptoms soon after the first cow's milk exposure; clinical recovery with a milk-free diet; relapse with cow's milk challenge. Malabsorption and immunological features were studied, but were not considered as providing essential criteria for diagnosis.

Intestinal biopsy was performed with a paediatric Crosby-Kugler capsule in the distal duodenum or proximal jejunum. Specimens were viewed with a dissecting microscope, and histological slides were made

(haematoxylin-pholxin-safran and PAS stains). Jejunal biopsies were carried out in all the cases while still taking cow's milk in their diet, with the exception of 5 children (Cases 1, 8, 10, 11, and 21) who had been on a milk-free diet for between 5 days and 2 months. In 19 cases recovery of the mucosa was studied while the patient was maintained on a cow's milk-free but gluten-containing diet.

Results

Initial features. In one infant (Case 1), the intestinal mucosa was normal; this infant had been on a milk-free diet for 2 months when biopsied. In the remaining 30 cases, including 4 infants biopsied while on a milk-free diet, small intestinal mucosal damage was observed (Table I). The dissecting microscope and histological appearances could be graded as follows.

Slight histological lesions: 4 cases (Fig. 1). The villi are either broadened or branched, but are not shortened. The cellular density of the lamina propria is slightly increased.

Partial villous atrophy: 25 cases. Under the dissecting microscope the villi are flattened and separated by tortuous ridges (like brain convolutions). Under the light microscope the length of villi is obviously reduced and the cellular infiltration of the lamina propria is increased (plasma cells,

Case no.	Sex	Age at which milk introduced (d)	Age of onset of symptoms (d)	Vomiting	Diarrhoea	Shock	Milk challenge		
							Vomiting	Diarrhoea	Shock
1	M	15	20	—	+	—	—	+	—
2	M	2 m	2 m	—	+	—	+	+	—
3	F	1st	First days	+	+	—	+	+	—
4	M	1st	7	+	+	—	+	+	—
5	F	1st	15	+	—	—	—	—	—
6	F	1 m	1 m	+	+	—	+	+	+
7	M	4	4	+	+	+	+	+	—
8	M	14	15	+	—	—	+	+	—
9	M	1st	6	—	+	—	+	+	+
10	F	1st	21	+	+	—	+	+	—
11	F	1st	15	+	+	—	+	+	—
12	M	1st	8	+	+	—	+	+	+
13	F	5	5	+	+	—	—	+	—
14	F	1st	20	—	+	—	—	+	—
15	M	1st	15	+	+	—	+	+	+
16	M	1 m	1 m	—	+	—	—	+	—
17	F	1st	17	+	+	—	—	+	—
18	F	1st	2 m	+	+	—	+	+	+
19	M	1st	3	—	+	—	—	+	—
20	F	1st ?	1 m	+	+	—	—	+	—
21	F	1st	First days	+	+	—	—	+	—
22	F	1st	12	+	+	—	+	+	—
23	M	1st	20	+	+	—	—	+	—
24	F	1st	7	+	+	—	—	+	—
25	F	1st	2	+	+	+	—	+	—
26	M	1st	First days	—	+	—	—	+	—
27	F	1st	First days	—	+	—	—	+	—
28	F	1st	First days	+	+	—	+	+	+
29	F	1st	First day	—	+	+	+	+	+
30	F	1st	First days	—	+	+	+	+	+
31	F	6 w	2 m	+	—	—	+	+	+

*+, >3.5 g/d; —, <3.5 g/d.

†9 h urinary excretion; ‡lymphoblastic transformation test.

§PVA I, II, or III, partial villous atrophy, grade I, II, or III; SHL, slight histological lesions; SVA, subtotal villous atrophy.

ND, not done.

eosinophilic granulocytes, and round cells). The villous epithelium contains an increased number of goblet cells.

Three grades of partial villous atrophy can be recognized according to height of the villi. Grade I (5 cases): height of villi between $\frac{1}{2}$ and $\frac{3}{4}$ of the mucosal thickness (Fig. 2). Grade II (12 cases): height of villi between $\frac{1}{4}$ and $\frac{1}{2}$ of the mucosal height (Fig. 3). Grade III (8 cases): height of villi less than $\frac{1}{4}$ of the mucosal thickness (Fig. 4).

Subtotal villous atrophy: 1 case (Fig. 5). Under the dissecting microscope, the mucosa is quite flat with a mosaic pattern. Histological lesions are much more severe than in previous cases. No correlation was observed between the degree of

mucosal damage and the clinical features such as malabsorption.

Recovery on a cow's milk-free diet. The results obtained in the 19 patients studied during recovery are summarized in Table II. Complete normalization of the intestinal mucosal appearances occurred in 4 cases (Cases 9, 19, 24, and 31) between 3 and 13 months on the diet. However, the biopsies were not performed at the same intervals and after the same dietary period in each patient.

Discussion

While some authors have found normal small intestinal mucosa in this condition (Freier *et al.*,

cow's milk protein allergy

Allergic symptoms	Steatorrhea*	Xylose test (%)†	Blood eosinophilia (%)	Serum p-lactoglobulin antibodies	α-Lactalbumin antibodies	LTT† (%)	First intestinal biopsy	
							Result§	Age (m)
Eczema	+	29	0	1/32 000	1/1024	—	Normal	4
—	ND	32	0	1/2048	1/2048	β-lacto 6·2 α-lacto 7·2	PVA III	3
—	—	12·4	2	1/1024	1/128	<4	PVA II	4
—	ND	ND	3	1/572	1/1024	β-lacto 10·2	SHL	3 1/2
—	ND	11	0	1/128	1/64	β-lacto 10	PVA II	6
—	+	36	4	1/62 000	1/128	<4	PVA III	3
—	—	12·7	2	1/130 000	1/100 000	β-lacto 6·4	PVA II	3
—	+	19	10	1/130 000	1/2048	α-lacto 4·2	PVA III	3 1/2
—	—	8	0	1/256	1/64	<4	PVA I	3 1/2
—	+	15	7	1/16	1/128	<4	PVA III	3
Eczema	—	ND	9	1/64	1/128	<4	PVA III	3
—	ND	ND	0	1/1024	1/1024	β-lacto 10 α-lacto 6·6	PVA I	3
Eczema	ND	ND	9	1/8192	1/1024	<4	PVA I	6
—	—	19	0	1/256	1/32	β-lacto 9·8	SHL	3 1/2
—	ND	17	0	1/256	1/256	β-lacto 4	PVA I	6
—	—	46	6	1/16	1/64	ND	SHL	7
—	—	25	0	1/1024	1/64	—	PVA III	3
—	ND	6·8	0	1/256	1/1024	ND	PVA II	4
—	ND	18	0	1/4	1/4	ND	PVA II	5
—	ND	15·2	0	1/128	1/2048	<4	PVA III	4 1/2
—	—	ND	0	1/64	1/64	β-lacto 5·2	PVA III	3
—	—	12·5	2	ND	ND	β-lacto 5·8	PVA II	4
—	ND	ND	8	1/512	1/32	β-lacto 6·7	PVA II	4
Eczema	—	ND	ND	1/256	1/64	α-lacto 7·2	PVA II	4
—	ND	ND	ND	1/64	1/64	ND	PVA II	4
—	—	ND	3	1/64	1/128	ND	PVA II	4
—	ND	11	1	1/128	1/64	β-lacto 7·2	PVA II	5
Eczema	+	ND	0	1/32 000	1/1024	<4	SVA	20
—	—	14	2	1/128	1/32	ND	SHL	5
Eczema	—	ND	0	1/64	1/32	α-lacto 5·8	PVA II	7
—	—	ND	3	1/134 000	1/268 000	β-lacto 5·2	PVA II	6
—	—	ND	—	—	—	ND	PVA I	6

1969; Lubos *et al.*, 1967) their cases were biopsied from 1 to 6 months after the beginning of a cow's milk-free diet. Other authors have observed small intestinal mucosal damage (Lamy *et al.*, 1963) including partial or subtotal villous atrophy (Freier *et al.*, 1969; Kuitunen, Visakorpi, and Hallman, 1965; Kuitunen *et al.*, 1973; Shmerling, 1970; Loeb *et al.*, 1971). Visakorpi and Immonen (1967) in 10 infants with intolerance to cow's milk noted intestinal mucosal damage in all, but association with gluten intolerance did not allow clear conclusions.

The mucosal changes, though constant, lack distinctive histological features. Eosinophilic infiltration of the lamina propria was not as conspicuous as might have been expected in an allergic

disorder, or as has been observed in rectal mucosa by Silver and Douglas (1968). We have noted the same histological lesions not only in patients with coeliac disease who have been on a gluten-free diet for a short time, but also in miscellaneous disorders such as prolonged nonspecific enterocolitis, transient exudative enteropathy, congenital disaccharidase deficiencies, intestinal parasitic infestation, and acute gastroenteritis (Barnes and Townley, 1973).

Nevertheless, small intestinal biopsy remains an informative investigation in cow's milk protein intolerance. Case 5 illustrates this. After a typical clinical onset, reintroduction of milk at 5 months was perfectly tolerated without any digestive symptoms and with normal weight gain. But two biopsies, after one and 6 months of normal diet,

FIG. 1.—Case 4. *Slight histological alterations: broadened villi without atrophy, moderately increased cellular infiltration of the lamina propria. (Haematoxylin-Phloxin-Safran $\times 84$.)*

FIG. 2.—Case 9. *Partial villous atrophy, grade I. (Haematoxylin-Phloxin-Safran. $\times 84$.)*

FIG. 3.—Case 5. *Partial villous atrophy, grade II.* (Haematoxylin-Phloxin-Safran. $\times 84$.)

FIG. 4.—Case 20. *Partial villous atrophy, grade III.* Haematoxylin-Phloxin-Safran. $\times 84$.)

FIG. 5.—Case 27. Subtotal villous atrophy. (Haematoxylin-Phloxin-Safran. $\times 84$.)

TABLE II
Effects of cow's milk-free diet on intestinal mucosa in 19 cases

Case no	Biopsy (and age)			
	1st	2nd	3rd	4th
1	Normal (4 m)	PVA II (6 m)	SHL (10 m)	PVA I (22 m)
2	PVA III (3 m)	PVA III (6 m)	PVA I (10 m)	
5	PVA II (6 m)	PVA II (7 m)	PVA II (12 m)	
7	PVA II (3 m)	SHL (10 m)		
8	PVA III (3 1/2 m)	PVA II (6 m)	PVA II (7 m)	
9	PVA I (3 1/2 m)	Normal (9 m)		
12	PVA I (3 m)	PVA I (6 m)		
13	PVA I (6 m)	PVA I (8 m)	PVA I (13 m)	
17	PVA III (3 m)	PVA II (8 m)		
18	PVA II (4 m)	PVA II (12 m)	SHL (18 m)	
19	PVA II (5 m)	Normal (18 m)		
20	PVA III (4 1/2 m)	PVA III (8 m)	PVA I (14 m)	
21	PVA III (3 m)	PVA II (6 m)		
22	PVA II (4 m)	PVA II (9 m)	PVA I (13 m)	
23	PVA II (4 m)	PVA II (6 m)	PVA I (10 m)	
24	PVA II (4 m)	PVA II (8 m)	Normal (16 m)	
26	PVA II (5 m)	PVA I (14 m)		
29	PVA II (7 m)	SHL (16 m)		
31	PVA I (6 m)	Normal (9 m)		

PVA, partial villous atrophy (type I, II, or III); SHL, slight histological lesions.

showed an unchanged villous atrophy, and only after 6 months of cow's milk-free diet was mucosal improvement observed.

Mucosal recovery under the milk-free diet and ultrastructural changes which occur soon after the milk challenge, as Kuitunen *et al.* (1973) observed in their study, confirm that intestinal damage in these cases is cow's milk induced, though the mechanism remains unknown.

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