Serial Rectal Biopsy in Ulcerative Colitis During the Course of a Controlled Therapeutic Trial of Various Diets

RALPH WRIGHT, M.D., D.PHIL., M.R.C.P., and
SIDNEY R. TRUELOVE, M.D., F.R.C.P.

A NUMBER OF STUDIES suggest that in some patients ulcerative colitis may be an allergic disease related to particular foods, of which cow's milk appears to be the most important.[1–6] In order to obtain objective evidence of this possibility, we have carried out a controlled therapeutic trial of various diets. During the course of the trial, the patients were seen at monthly intervals for a period of 1 year for clinical assessment and for investigations which included rectal biopsy, circulating eosinophil count, and immunological studies. The results of the therapeutic trial, of the immunological studies, and of the circulating eosinophil counts have been reported in detail elsewhere.[7–9] The purpose of the present article is to report the results obtained from serial rectal biopsy.

MATERIAL AND METHOD

PATIENTS STUDIED

Seventy-seven patients with ulcerative colitis in relapse were allotted at random to 3 different diets, a milk-free diet, a gluten-free plus milk-free diet, and a dummy diet which was essentially normal. The patients were seen at monthly intervals for assessment and investigations which included rectal biopsy, total eosinophil count, and immunological studies. The initial relapse and subsequent relapses during the course of the trial were treated with a standard course of oral prednisolone, 20 mg. daily, and daily rectal infusions of 100 mg. hydrocortisone hemisuccinate. The oral prednisolone was used for 6 weeks and the local hydrocortisone for 8 weeks. If the response was poor, the dose was doubled. At the end of the year the patients were asked to go back to a normal diet.

The relative effectiveness of the 3 diets on the clinical course of the disease was judged by the number of relapses of ulcerative colitis developing during the trial period. The milk-free diet was superior to the gluten-free plus milk-free and the dummy diets. More patients on the milk-free diet remained well for the year than on the other diets and fewer had repeated relapses. The best estimate was that a milk-free diet is likely to be beneficial to about 1 in 5

From the Nuffield Department of Clinical Medicine, University of Oxford, Oxford, England.

TABLE 1. EXAMPLE OF GRADING OF SERIAL RECTAL BIOPSY SPECIMENS OF PATIENTS ON MILK-FREE DIETS

Serial No.	*Classification*	*Dilated vessels*	*Glandular structures*	*Cellular infiltration*	*Polymorphs*	*Plasma cells or lymphocytes*	*Eosinophils*	*Crypt abscesses*	*Ulceration*	*Abnormal epithelial cells*	*Clinical state*
08-01-1	Severe	+	Fair	++	+	+	++	+	+	++	R*
08-01-2											S*
08-01-3	Mild	+	Fair	+	−	+	+	−	−	+	S
08-01-4	NN*	+	Good	±	−	±	±	−	−	−	L*
08-01-5	NN	±	Good	±	−	±	+	−	−	−	
08-01-6	NN	−	Good	±	−	+	+	−	−	±	
08-01-7	Moderate	+	Fair	++	+	+	++	+	+	++	
08-01-8											
08-01-9	Mild	+	Fair	+	−	++	++	−	−	+	
08-01-0	Mild	±	Good	+	−	+	±	−	−	+	
08-02-1	Mild	+	Good	++	±	++	++	±	−	++	R
08-02-2	Mild	++	Good	+	−	+	±	−	−	+	S
08-02-3	NN	±	Good	±	−	−	−	−	−	±	L
08-02-4	Mild	±	Fair	+	−	+	±	−	−	+	

*R indicates relapse; S, treatment with systemic corticosteroid drugs; NN, near normal; and L, treatment with local corticosteroid drugs.

patients with ulcerative colitis, with a suggestion that the proportion might be higher in patients in their first attack of the disease.

The results obtained with the milk-free plus gluten-free diet were similar to those obtained with an ordinary diet, but it was thought that the relative failure of this group was due to the fact that patients found it difficult to adhere to the diet strictly and that milk protein had inadvertently been included in the diet.

Rectal Biopsy

Rectal biopsy specimens were obtained at sigmoidoscopy 4–6 in. from the anal verge, with a Truelove-Salt suction biopsy instrument. The biopsy specimens were fixed in 10% formol-saline and embedded in paraffin wax. Several sections were cut from each specimen and stained with hematoxylin and eosin.

Each specimen was given a code number and the histologic sections were examined by one of us without any knowledge of the clinical activity of the ulcerative colitis or the nature of the diet. Only the code number was known at the time of examination; the clinical data were recorded subsequently for analysis.

The following features were examined and graded, using a simple code (Table 1): (1) dilated vessels; (2) infiltration with polymorphonuclear leukocytes, plasma cells or lymphocytes, and eosinophils; (3) crypt abscesses; (4) superficial ulceration; (5) abnormal epithelial cells; and (6) glandular structure.

The degree of inflammation in each specimen was classified as follows, based on the classification of Truelove and Richards[10]: no significant inflammation, mild inflammation, moderate inflammation, or severe inflammation.

No Significant Inflammation (Fig. 1A)

It was considered that there was no significant inflammation if the mucosa was not ulcerated, if the surface and glandular epithelial cells were intact, and if lymphocytes, plasma cells, and eosinophils in the lamina propria were scanty. The degree of cellularity was graded by comparison with that found in biopsies from healthy subjects, or from patients with the irritable colon syndrome. Giant epithelial cells, sparse and stunted glands, and dilated vessels were frequently seen in the patients with ulcerative colitis during remission, but were not regarded as indicating inflammation in the absence of other abnormalities.

Mild Inflammation (Fig. 1B)

Inflammation was graded as mild if there was a definite increase in lymphocyte, plasma cell, or eosinophil infiltration of the lamina propria, usually with dilated vessels, interstitial hemorrhage, and edema.

Moderate Inflammation (Fig. 1C)

A heavier infiltration of cells with occasional crypt abscesses and small areas of ulceration, indicating a more marked degree of inflammation, was graded as moderate.

Severe Inflammation (Fig. 1D)

Indications of severe inflammation were a marked loss of glandular structure, crypt abscesses, and extensive ulceration, with a heavy infiltration of cells, many of them polymorphonuclear leukocytes.

The distinction between the different grades is not clear cut. However, repeated examination usually produced closely similar results and there was close agreement when some of the sections were graded by a second observer using the same criteria.

RESULTS

During the course of the trial, 658 biopsy specimens were taken from the 77 patients in the therapeutic trial, an average of more than 8 specimens per patient. This is a smaller number than the number of times the patients at-

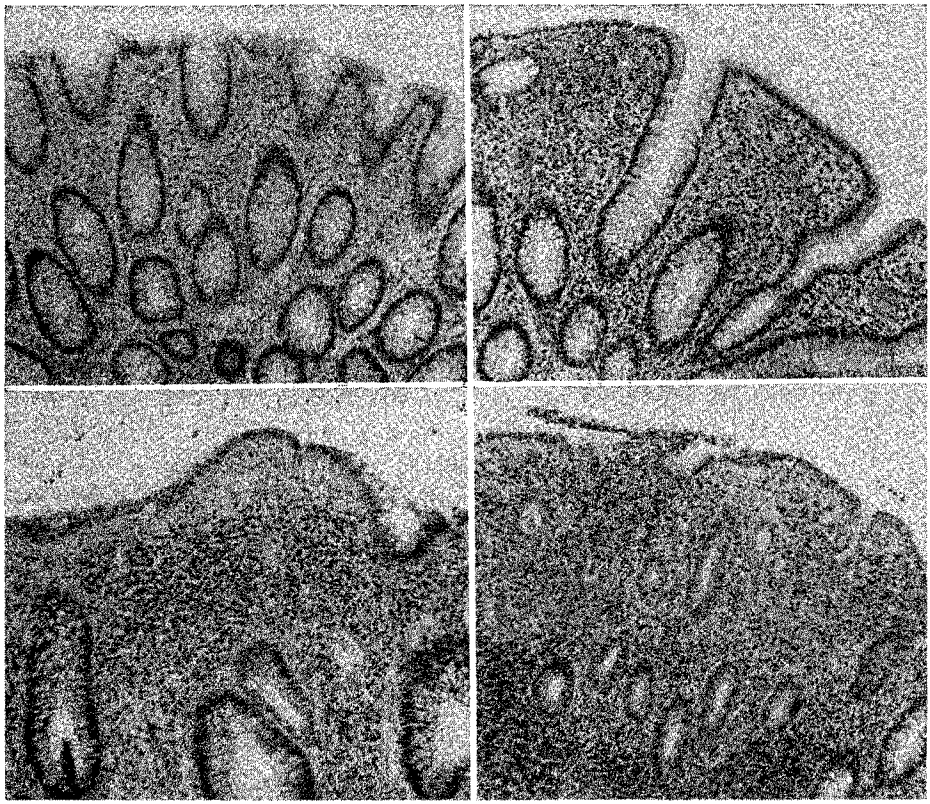

Fig. 1. Selected rectal biopsy specimens from 1 patient (see Table 1), to illustrate the 4 grades of inflammation used in analyzing the data. **A.** Specimen 08-01-6, showing no significant inflammation. **B.** Specimen 08-01-3, showing mild inflammation. **C.** Specimen 08-01-7, showing moderate inflammation. **D.** Specimen 08-01-1, showing severe inflammation.

tended for clinical assessment, but for various practical reasons it was not always feasible to take a specimen on every attendance. However, the number of specimens taken from each dietary group was closely similar and there is no reason to suppose that the results are biased in favor of any one dietary group.

Severity of Inflammation

Figure 2A shows that the 3 dietary groups were closely similar as regards the severity of inflammation shown by the biopsy specimens taken at the time when the patients were admitted to the trial. Figure 2B shows that the patients

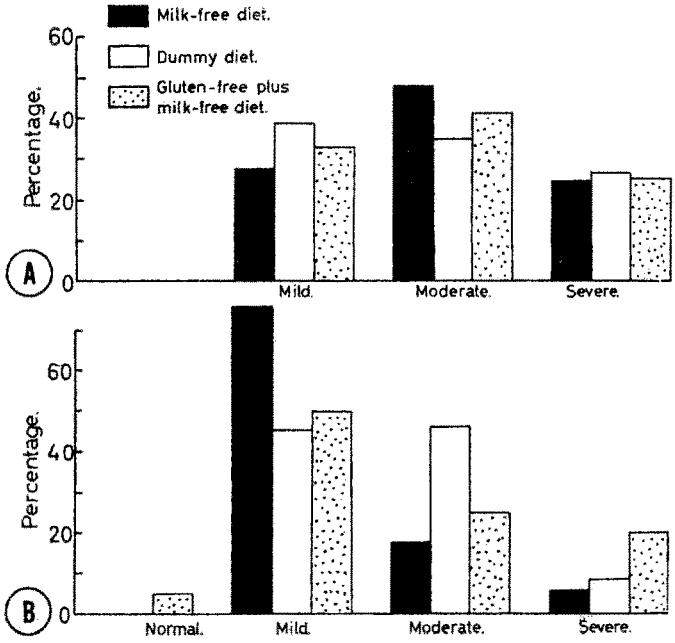

Fig. 2. Histologic findings on rectal biopsy of patients in the 3 dietary groups at 2 different stages in trial. **A.** On admission to trial, when histologic picture was closely similar for the 3 groups. **B.** When in relapse during course of trial, and not under corticosteroid treatment, showing that milk-free group had, on average, milder histologic relapses than the other groups.

on a milk-free diet were more likely to show only mild inflammation in the biopsy specimen when they relapsed during the course of the trial than were the patients in the other 2 dietary groups. In other words, these results suggest that a milk-free diet often lessens the severity of a relapse of ulcerative colitis as judged histologically.

Biopsy Results Related to Clinical Results

Quite apart from the severity of inflammation at the time of a clinical relapse, the patients on a milk-free diet differed from the patients on the other 2 diets in having a smaller number of relapses. When the clinical relapses are plotted as cumulative totals as in Fig. 3A, the curve for the patients on a milk-free diet consistently runs below that for the patients on a dummy (normal) diet. The curve for the patients on a diet excluding both milk and gluten is

interesting, because it starts to run close to that for the milk-free diet but swings up to approximate that for the dummy diet by the end of the trial; this would fit in with our impression that patients found this severely restricted diet difficult to adhere to.

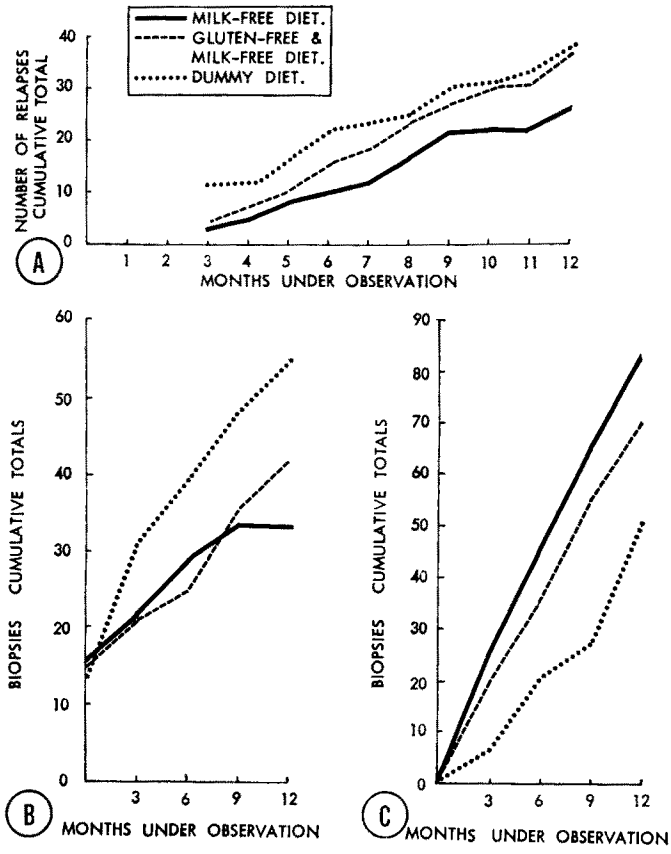

Fig. 3. Relationship between clinical course as judged by cumulative total of relapses and histologic findings, also based on cumulative totals. **A.** Clinical relapses in the 3 dietary groups. **B.** Rectal biopsy specimens showing moderate or severe inflammation. **C.** Rectal biopsy specimens showing no inflammation.

When the results of examining the biopsy specimens are plotted in a similar manner, there are interesting comparisons to be made with the curves obtained from the clinical relapses. In Fig. 3B, the number of biopsy specimens showing moderate or severe inflammation are shown as cumulative totals for the 3 dietary groups, and these curves show that as the trial proceeded, the milk-free dietary group had the smallest number of specimens with pronounced inflammation, although the 3 dietary groups were similar in this respect at the beginning of the trial. As with the clinical relapses, the gluten-free plus milk-free dietary group ran initially in parallel with the milk-free diet group, but diverged from it towards the dummy group as the trial progressed.

When the converse of this situation is examined, namely, the cumulative

totals of biopsy specimens showing no significant inflammation, as in Fig. 3C, the curves are reversed in their relative positions. The milk-free dietary group shows the largest number of such essentially normal biopsy specimens while the patients on a dummy diet show the least, with patients on the diet excluding both gluten and milk occupying an intermediate position.

It is striking that such close similarities exist between the clinical results of the trial and the results as judged by serial biopsy, especially in view of the biopsy specimens having been assessed in a "blind" manner, as described earlier under Methods.

Relationship of Rectal Biopsy Findings to Sigmoidoscopic Findings

The sigmoidoscopic findings were graded according to the following criteria:

Normal A smooth mucosa, not hyperemic and with a visible vascular pattern; not friable

Mild A mucosa showing hyperemia and granularity and with some friability as evidenced by punctate intramucosal hemorrhages when it is rubbed with gauze

Moderate A hyperemic, edematous, granular mucosa which is frankly hemorrhagic

Severe A severely hemorrhagic mucosa, often with gross ulceration

In Table 2, the relationship between the sigmoidoscopic gradings and the biopsy gradings in terms of severity of inflammation are shown in a contingency table. There was a good correlation between the sigmoidoscopic and the histological findings although in a few instances there was marked discrepancy. It can also be seen that the biopsy specimen was more likely to show inflammation when the sigmoidoscopic findings were normal, than the reverse. Thus, of 256 specimens taken when the sigmoidoscopic picture was judged to be normal, there were 105 (38.6%) which showed inflammation. By contrast, of the 209 biopsy specimens judged to show no significant inflammation, all but 43 (20.6%) came from patients with a normal sigmoidoscopic picture.

Predictive Value of Sigmoidoscopic and Biopsy Findings

It is of interest that both the sigmoidoscopic findings and the rectal biopsy findings after a single standard course of corticosteroid treatment have some

TABLE 2. RECTAL BIOPSY FINDINGS AND SIGMOIDOSCOPIC FINDINGS

	Degree of inflammation on biopsy specimen				
Sigmoidoscopic grading	*None*	*Mild*	*Moderate*	*Severe*	*Total*
Normal	166	103	2	1	272
Mild	30	83	22	1	136
Moderate	11	87	44	23	165
Severe	2	31	38	14	85
Total	209	304	106	39	658

TABLE 3. SIGMOIDOSCOPIC FINDINGS 2 MONTHS AFTER START OF DIET AND NO. OF CLINICAL RELAPSES DURING REMAINDER OF TRIAL YEAR

Dietary group	*Inflamed (No. of relapses)*					*Not inflamed (No. of relapses)*					*Not known*	*Grand total*
	0	*1*	*2*	*3*	*Total*	*0*	*1*	*2*	*3*	*Total*		
Milk-free	2	2	1	2	7	8	6	3	1	18	1	26
Gluten-free plus milk-free	1	2	1	4	8	7	5	3	3	18	1	27
Dummy (normal)	2	4	2	5	13	2	1	1	3	7	4	24
All groups	5	8	4	11	28	17	12	7	7	43	6	77
Remaining symptom-free (%)					17.9					39.5		

TABLE 4. RECTAL BIOPSY FINDINGS 2 MONTHS AFTER START OF DIET AND NO. OF CLINICAL RELAPSES DURING REMAINDER OF TRIAL YEAR

Dietary group	*Inflamed (No. of relapses)*					*Not inflamed (No. of relapses)*					*Not known*	*Grand total*
	0	*1*	*2*	*3*	*Total*	*0*	*1*	*2*	*3*	*Total*		
Milk-free	1	3	2	2	8	6	3			9	9	26
Gluten-free plus milk-free	3	5	1	2	11	4		2	1	7	9	27
Dummy (normal)	2	4	2	3	11	1	1		1	3	10	24
All groups	6	12	5	7	30	11	4	2	2	19	28	77
Remaining symptom-free (%)					16.7					57.9		

TABLE 5. PROPORTION OF PATIENTS SHOWING NO INFLAMMATION ON SIGMOIDOSCOPY AND ON RECTAL BIOPSY 2 MONTHS AFTER START OF TRIAL

Dietary group	*Sigmoidoscopic findings*			*Rectal biopsy findings*		
	No. of observations	*Patients with no inflammation*		*No. of observations*	*Patients with no inflammation*	
		No.	*%*		*No.*	*%*
Milk-free	25	18	72.0	17	9	52.9
Gluten-free plus milk-free	26	18	69.2	18	7	38.9
Dummy (normal)	20	7	35.0	14	3	21.4

value in predicting the course of the disease during the remainder of the trial year. Table 3 shows the results of sigmoidoscopy at 2 months after entry into the trial in relation to the number of relapses subsequently suffered. It will be seen that among the patients with normal sigmoidoscopic findings at that time, 39.5% remained symptom-free during the remainder of the trial period, compared with 17.9% of those with abnormal sigmoidoscopic findings. The results are consistent for the 3 dietary groups.

Table 4 shows comparable data for the rectal biopsy findings at 2 months after entry into the trial. It will be seen that 57.9% of the patients with no significant inflammation in the biopsy specimen remained symptom-free during the rest of the year, compared with only 16.7% of those with evidence of inflammation in the biopsy specimen. In other words, the rectal biopsy has greater predictive value than the sigmoidoscopic findings.

Another point is that sigmoidoscopy and biopsy are both much more likely to show no inflammation in patients on a milk-free diet and on a gluten-free plus milk-free diet than in patients on a dummy (normal) diet (Table 5) at 2 months from admission to the trial—i.e., at conclusion of standard course of corticosteroid treatment. By these criteria, results for the gluten-free plus milk-free diet are little different from those for the milk-free diet, which supports the finding that the patients on the gluten-free plus milk-free diet initially do well clinically in the therapeutic trial—even though later in the trial year their results approximated those with a dummy (normal) diet, a shift which we judge may have been due to difficulty in adhering to this particular diet.

DISCUSSION

Rectal biopsy is a relatively safe procedure when a suitable instrument is employed, but it carries a definite, though small, risk of causing brisk hemorrhage from the biopsy site. In the present study, 5 episodes of bleeding sufficient to demand blood transfusion took place (0.76%). Such brisk bleeding is most likely to occur when the mucosa is normal or near-normal; the probable explanation for this is that the normal mucosa is pliable and is readily sucked into the hole of a suction instrument so that the knife may cut across blood vessels of appreciable size in the submucosa, whereas the inflamed, edematous mucosa of active ulcerative colitis is less readily sucked in so that the biopsy specimen is usually not so deep.

There was a particular reason for performing serial biopsy on the patients undergoing this formal trial of different diets. Practical considerations made it unfeasible for us to conduct the dietary trial in a "double-blind" manner, but it was arranged for the rectal biopsy specimens to be given code numbers and assessed "blindly" so that a completely objective estimate of the degree of inflammation would be available. Thus a set of data was obtained which could be related to the clinical findings. In the event, the results of the rectal biopsy study confirm and reinforce the clinical findings in respect to the

therapeutic value of a milk-free diet in a certain proportion of patients with ulcerative colitis.

We have discussed in detail elsewhere the possible explanations for the beneficial effect of a milk-free diet in a certain proportion of patients with ulcerative colitis, a proportion which we estimate to be about 1 in 5.[7] In summary, the main possibilities are:

1. Ulcerative colitis can be a primary allergic disease and cow's milk an important allergen.
2. Secondary food allergy may occur in ulcerative colitis and be responsible for perpetuating the disease.
3. Milk may contain substances other than proteins which may be harmful to patients with ulcerative colitis. For example, recent work raises the possibility that lactose intolerance through a deficiency of intestinal lactase may account for the ill effects of cow's milk in a minority of patients with ulcerative colitis. We judge this particular possibility to be unlikely because we have provoked relapses of ulcerative colitis by reintroducing milk into the diet of patients keeping well on a milk-free diet and it takes days or weeks before symptoms arise, whereas symptoms arise within a few hours when lactose is administered to patients with lactase deficiency.[11]
4. Milk may modify the intestinal flora in some way which is often harmful to patients with ulcerative colitis.

SUMMARY

In the course of a controlled therapeutic trial of 3 different diets in ulcerative colitis, serial rectal biopsy was performed on 77 patients, each of whom was on a specific diet for 1 year. In all, 658 rectal biopsy specimens were taken, an average of more than 8 per patient.

The rectal biopsy specimens were examined "blind," that is, without knowledge of the clinical state of the patient when the biopsy was performed. The degree of inflammation was graded as not significant, mild, moderate, or severe. On entry into the dietary trial, the patients in the different groups were closely similar as regards the histologic picture in the rectal biopsy specimen.

During the course of the trial, patients on a milk-free diet had fewer clinical relapses than patients on a normal "dummy" diet. Patients on a diet excluding both milk and gluten were intermediate, and we judge that some of these patients failed to adhere to their severely restricted diet.

The histologic findings in the rectal biopsy specimen showed a close parallelism with these clinical results. There was a close but not perfect correlation between sigmoidoscopic findings and rectal biopsy findings. The rectal biopsy was more likely to show inflammation when the sigmoidoscopic appearance was normal than the reverse.

If a patient was sigmoidoscopically or histologically normal at the end of a

standard course of corticosteroid treatment after admission to the trial, this was a favorable sign in relation to the clinical course for the remainder of the year. A normal biopsy specimen gave a better prediction than a normal sigmoidoscopic appearance.

The possible ways in which a milk-free diet may be beneficial are briefly discussed.

S. C. T.
Nuffield Department of Clinical Medicine
University of Oxford
The Radcliffe Infirmary, Oxford, England

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