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# PEG-400 Excretion in Patients with Crohn's Disease, Their First-Degree Relatives, and Healthy Volunteers

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*An altered small bowel permeability may be implicated in the pathogenesis of Crohn's disease. Intestinal permeability, using polyethylene glycol 400 (PEG 400) as the orally ingested probe, was assessed in 45 patients with Crohn's disease (ileal* N = *14, ileocolonic* N = *9, colonic* N = *10, postresection* N = *12), 20 first-degree relatives, and 31 controls. PEG 400 excretion was measured using a direct injection HPLC method, and results are expressed as percent of dose recovered in urine (median and range). No quantitative differences in the recovery of PEG-400 were found [Crohn's patients 21.9% (6.1–39.9), relatives 23.7% (4.9–39.9), controls 25.0% (4.5–39.7)]. In all groups, the composition of ingested and recovered PEG-400 was similar and no selective permeability to any molecular weight species was found. Disease site did not influence probe recovery [ileal 23.8% (7.7–30.6), ileocolonic 22.6% (14.4–33.8), colonic 27.8% (9.5–33.5)]. Resected patients had significantly lower PEG-400 recovery [18.8% (8.1–39.9)] than nonresected patients [23.5% (6.1–33.8%)* P < *0.02]. The data suggest either that altered intestinal permeability is not a factor in Crohn's disease or that PEG-400 is not a suitable probe.*

**KEY WORDS:** polyethylene glycol 400; intestinal permeability; small bowel permeability; Crohn's disease.

Small bowel permeability has been reported to be altered in a wide variety of disorders (1–4). Although several different probes have been used to document this phenomenon (5–7), the mechanisms of altered small bowel permeability remain unknown. In patients with Crohn's disease an increased bowel permeability may serve as a route for antigenic absorption with a resultant autoimmune response and the subsequent inflammatory changes that are characteristic of this condition. There is a correlation between some of the extraintestinal manifestations of Crohn's disease and circulating immune complexes that have been isolated from patient sera, and the latter may be the mechanism of tissue injury (8–10). Furthermore, a defect in macrophage degradation ability, especially to an increased antigenic load associated with an increased bowel permeability, has also been suggested (11).

In Crohn's disease, both an increase and a decrease in small bowel permeability to polyethylene glycol 400 (PEG-400) have been documented (5, 14, 15). An increased small bowel permeability in first-degree relatives of patients with Crohn's disease has also been noted (5).

In view of the possible importance of the concept of antigen absorption vis à vis Crohn's disease, the

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TABLE 1. SEX AND AGE DISTRIBUTION

| *Group* | N | *Males* | *Females* | *Age* |
|---|---|---|---|---|
| Crohn's | 45 | 19 | 26 | 39.8 (19–67) |
| Relatives | 20 | 8 | 12 | 33.3 (11–55) |
| Controls | 31 | 6 | 25 | 42.4 (24–72) |

relatively small groups studied thus far, and the conflicting data reported with PEG-400, we decided to use this marker to investigate small bowel permeability in a larger series of patients and their first-degree relatives. This would, in addition, allow evaluation of the possible influence of disease extent on permeability.

## MATERIALS AND METHODS

The subjects included 45 patients with Crohn's disease attending on an outpatient basis at the gastrointestinal clinic, 20 first-degree relatives of 13 patients, and 31 healthy controls (Table 1). On the basis of small bowel enema and either double-contrast barium enema or colonoscopic examination, the Crohn's group was further subdivided into patients with ileal disease ($N$ = 14), ileocolonic disease ($N$ = 9), and colonic involvement ($N$ = 10). The remaining 12 patients had undergone previous resections of varying length (Table 2). The relatives and the controls (an age- and sex-matched group of clinic staff) were in excellent health and, in particular, had no evidence of any bowel disease, either on history or physical examination. There was no history of ingestion of alcohol or nonsteroidal antiinflammatory drugs in the 48-hr period preceding the test, by any of the subjects studied. Informed consent was obtained from all subjects, and the study was approved by the University of Cape Town Medical School Ethics Committee.

After an overnight fast each person drank 200 ml of water containing 5.6 g PEG-400 (Sigma Chemical Company, St. Louis, Missouri). During the following 6 hr while urine was collected there was unrestricted intake of water. No food was allowed. The total volume of urine collected from each patient was measured and aliquots stored at −20° C until further analysis. Analysis of PEG-400 by high-performance liquid chromatography (HPLC) was performed using a method of direct injection (13). While nine peaks were separated and detected, only the six main components were individually analyzed. A separate standard curve for concentrations of PEG-400 from 0.2 to 1.6 g/liter was plotted for each batch of urine samples. The concentration of PEG-400 in each sample was determined and the results expressed as the percentage of the ingested dose excreted in 6 hr. Recovery of added PEG-400 varied from 94.1 to 101.8%. Reproducibility of the method was assessed by analysis of aliquots of a single urine sample in 10 separate runs and a coefficient of variation (CV) of 3.3% obtained. The standard error of estimation between duplicate determinations was 0.87% ($N$ = 100). Seven controls participated more than once with an interval of five months between the tests. These data were used to establish the intraindividual variation. Results from the second test only were used in intergroup comparisons. The mean value on the first occasion was 28.14 ± 1.56. The mean value on the second occasion was 24.94 ± 1.48. The standard error due to the intraindividual variation was thus 2.69%. The test–retest reliability coefficient was $r$ = 0.74.

**Statistical Methods.** Because results are expressed as percentages, normal distribution cannot be assumed. Results are expressed as medians and ranges. Statistical differences between more than two groups were analyzed using Kruskal-Wallis one-way analysis of variance. The Mann-Whitney test was used to calculate statistical significance when there were only two comparisons.

TABLE 2. EXTENT OF RESECTIONS IN CROHN'S PATIENTS

| *Patient* | *Small Bowel* | *Large Bowel* |
|---|---|---|
| 1 | 20 cm | proximal right colon, cecum |
| 2 | 25 cm | cecum, proximal ascending colon |
| 3 | less than 5 cm | |
| 4 | 50 cm | cecum |
| 5 | 20 cm | 10 cm right colon |
| 6 | 20 cm | right hemicolectomy |
| 7 | more than 20 cm | descending, transverse colon |
| 8 | | right hemicolectomy |
| 9 | 20 cm | cecum |
| 10 | less than 5 cm | right hemicolectomy |
| 11 | ileum | cecum, right hemicolectomy |
| 12 | less than 10 cm | right hemicolectomy |

## RESULTS

The composition of PEG in the urine from controls, patients, or their relatives, was similar to that in the ingested material (Figure 1).

Patients with Crohn's disease excreted 21.9% (6.1–39.9) of 5.6 g PEG-400 ingested during the 6 hr, their relatives 23.7% (4.9–39.9), and the controls

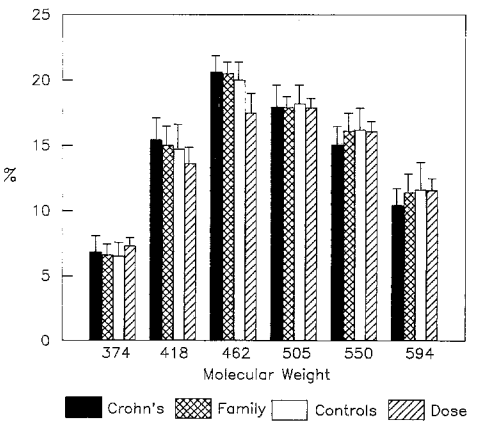


**Fig 1.** The composition of PEG-400 in urine from patients with Crohn's disease, their relatives, controls, and in the ingested dose. The means and standard deviations are shown.

25.0% (4.5–39.7). Crohn's patients with ileal disease excreted 23.8% (7.7–30.6), those with ileocolitis 22.6% (14.4–33.8), and those with colonic involvement 27.8% (9.5–33.5). Nonresected Crohn's patients excreted more [23.5% (6.1–33.8)] than patients who had undergone previous resections [18.8% (8.1–39.9)] ($P < 0.02$).

## DISCUSSION

The present investigation showed no difference in the total amounts of PEG-400 excreted in the Crohn's group when compared with controls. This is in contrast with other reports where either an increased (5) or a decreased excretion (14, 15) has been shown. The reason for these conflicting data is not clear. However, since values reported for Crohn's patients are similar (14, 15), differences may well relate to the variation in the results for controls. In the study that demonstrated a raised PEG-400 excretion by the Crohn's patients and their relatives (5), the controls excreted only 4% as opposed to the 16–30% found in the present and in other studies (4, 15, 16).

The complexity of the methodology used by others may also account for variation in results. The methodology used in the study by Hollander et al (5) involves lyophilization of samples and inaccuracy may occur during the extraction and freeze-drying processes. Indeed, these processes are usually performed in batches. Loss of sample in a single batch (possibly the whole control group), may thus have been responsible for the intergroup differences reported. The direct injection method used in the present study decreased technical error by eliminating several preparative steps (CV = 3.3% vs 7.4% of the extraction method) (17).

An inverse relationship between the molecular size and the amount of PEG recovered in the urine, after an oral load of PEG-1000, PEG-600, and PEG-400, has been reported in patients and controls (6, 14, 18–21). This relationship is not found consistently, however (7, 15, 16), and in the present study, no evidence of preferential uptake of any of the individual polymers of PEG-400 was seen in any group. The similarity of results for controls and patients with Crohn's disease suggests that any selective filtering is independent of inflammatory change.

PEG-400 excretion was apparently uninfluenced by the localization and extent of Crohn's disease. While no other studies using PEG-400 have compared regional involvement, similar findings with oral carbohydrate probes have been reported recently (22). This is in contrast to the findings of Olaison et al (18) using PEG-1000, where patients with colonic Crohn's absorbed less than those with ileal Crohn's disease. Oral probes may have differing physiochemical characteristics and pathways of permeation across the gut mucosa, and these factors may complicate any comparison of results.

The present investigation showed significantly lower PEG 400 excretion in patients with previous resections. This finding is compatible with a loss of surface area available for absorption and is in agreement with other data (5, 12). Surprisingly, the opposite has also been found, and an increased excretion associated with longer resection has been reported (14). Factors other than a previous bowel resection would appear to have influenced these results. Olaison et al, using PEG-1000 (6), and Hollander, using carbohydrate probes (22), were unable to document differences between resected and nonresected patients.

The mechanism of the purported altered small bowel permeability reported in the literature remains obscure. Whether this is a primary or secondary phenomenon is also not clear. The increased small bowel permeability in relatives of patients with Crohn's disease documented by Hollander et al (5) suggested that the defect may be at a genetic level. As no quantitative or qualitative differences between the controls and the relatives of patients could be documented, our study cannot confirm this observation.

The results of the present study should not be construed as evidence against the concept of altered permeability in patients with Crohn's disease since such differences have been documented with other probes. They do, however, cast doubt on the use of PEG-400 as a suitable probe.

## REFERENCES

1. Serrander R, Magnusson KE, Sundqvist T: Acute infections with *Giardia lamblia* and rotavirus decrease intestinal permeability to low-molecular weight polyethylene glycols (PEG-400). Scand J Infect Dis 16:339, 1984
2. Bjarnson I, Peters TJ: *In vitro* determination of small intestinal permeability—demonstration of a persistent defect in patients with coeliac disease. Gut 25:145–150, 1984
3. Serrander R, Magnusson KE, Kihlstroom E: Acute *Yersinia* infections in man increase intestinal permeability for low-molecular weight polyethylene glycols (PEG-400). Scand J Infect Dis 18:409–413, 1986

4. Robinson GM, Orrego H, Israel Y, Devenyi P, Kapur BM: Low-molecular-weight polyethylene glycol as a probe of gastrointestinal permeability after alcohol ingestion. Dig Dis Sci 26:971–977, 1981
5. Hollander D, Vadheim CM, Brettholz E, Peterson GM, Delahuny T, Rotter I: Increased intestinal permeability in patients with Crohn's disease and their relatives. Ann Intern Med 105:883–885, 1986
6. Olaison G, Sjodahl R, Tagesson C: Decreased gastrointestinal absorption of peroral polyethyleneglycols (PEG 1000) in Crohn's disease. Acta Chir Scand 153:373–377, 1987
7. Olaison G, Leandersson P, Sjodahl R, Tagesson C: Intestinal permeability to polyethylene glycol 600 in Crohn's disease. Preoperative determination in a defined segment of the small intestine. Gut 29:196–199, 1988
8. Jewell DP, MacLennan KP, Truelove SC: Circulating immune complexes in ulcerative colitis and Crohn's disease. Gut 13:139, 1972
9. Nielsen H, Hyltoft-Peterson P, Svehag SE: Circulating immune complexes in ulcerative colitis. I. Correlation to disease activity. Clin Exp Immunol 31:72, 1978
10. Nielsen H, Hyltoft-Peterson P, Svehag SE: Circulating immune complexes in ulcerative colitis. II. Correlation with serum protein concentrations and complement conversion products. Clin Exp Immunol 31:81, 1978
11. Ward M: The pathogenesis of Crohn's disease. Lancet 2:903, 1977
12. Heuman R, Sjodahl R, Tagesson C: Passage of molecules through the wall of the gastrointestinal tract. Acta Chir Scand 148:281–284, 1982
13. Young GO, Ruttenberg D, Wright JP: Measurement of Polyethylene glycol-400 in urine using direct injection high performance liquid chromatography. Clin Chem 36:1800–1802, 1990
14. Magnusson KE, Sundqvist T, Sjodahl R, Tagesson T: Altered intestinal permeability to low-molecular-weight polyethyleneglycols (PEG-400) in patients with Crohn's disease. Acta Chir Scand 149:323–327, 1983
15. Jenkins RT, Goodacre RL, Rooney PJ, Bienenstock J, Sivakumaran T, Walker WH: Studies of intestinal permeability in inflammatory diseases using polyethylene glycol-400. Clin Biochem 19:298–302, 1986
16. Blatzinger JG, Rommel K, Eknauer R: Elimination of low molecular weight polyethylene glycol-400 in the urine following an oral load, as a measure of intestinal permeability. J Clin Chem Clin Biochem 19:265–266, 1981
17. Delahunty T, Hollander D: New liquid-chromatographic method for measuring polyethylene glycol in urine. Clin Chem 32(2):351–353, 1986
18. Olaison G, Sjodahl R, Leandersson P, Tagesson C: Abnormal intestinal permeability pattern in colonic Crohn's disease. Scand J Gastroenterol 24:571–576, 1989
19. Tagesson C, Andersson PA, Bolin T, Kallberg M, Sjodahl R: Passage of molecules through the wall of the gastrointestinal tract. Scand J Gastroenterol 18:481–486, 1983
20. Philipsen EK, Batsberg W, Christensen AB: Gastrointestinal permeability to polyethylene glycol: An evaluation of urinary recovery of an oral load of polyethylene glycol as a parameter of intestinal permeability in man. Eur J Clin Invest 18:139–145, 1988
21. Chadwick VS, Phillips SF, Hofman AF: Measurements of intestinal permeability using low molecular weight polyethylene glycols (PEG-400). I. Chemical analysis and biological properties of PEG 400. Gastroenterology 73:241–246, 1977
22. Katz KD, Hollander D, Vadheim CM, McElree C, Delahunty T, Dadufalza VD, Krugliak P, Rotter JI: Intestinal permeability in patients with Crohn's disease and their healthy relatives. Gastroenterology 97:927–931, 1989