

JAMES H. GRENDALL, M.D.
Division of Digestive Diseases
New York Hospital—Cornell Medical Center
New York, New York 10021

1. Lüthen R, Niederau C, Niederau M, Ferrell LD, Grendell JH. Influence of ductal pressure and infusates on activity and subcellular distribution of lysosomal enzymes in the rat pancreas. *Gastroenterology* 1995;109:573–581.
2. Steer ML, Meldolesi J. The cell biology of experimental pancreatitis. *N Engl J Med* 1987;316:144–150.
3. Saluja A, Saluja M, Villa A, Leli U, Rutledge P, Melodolesi J, Steer M. Pancreatic duct obstruction in rabbits causes digestive zymogen and lysosomal enzyme colocalization. *J Clin Invest* 1988;84:1260–1266.
4. Ohshio G, Saluja A, Steer ML. Effects of short-term pancreatic duct obstruction in rats. *Gastroenterology* 1991;100:196–202.
5. Lüthen R, Grendell JH, Niederau C. Glutathione and ATP levels, subcellular distribution of enzymes, and the permeability of the duct system in rabbit pancreas following intravenous administration of alcohol and caerulein. *Dig Dis Sci* 1994;115:139–150.
6. Resau JH, Marzella M, Trump BF, Jones RT. Degradation of zymogen granules by lysosomes in cultured pancreatic explants. *Am J Pathol* 1984;115:139–150.
7. Gorelick FS, Modlin I, Leach SD, Carangelo R, Katz M. Intracellular proteolysis of pancreatic zymogens. *Yale J Biol Med* 1993;65:407–420.

Viewpoints in Intestinal Permeability

Dear Sir:

We read with great interest the review article on intestinal permeability by Bjarnason et al.¹ They provided a general overview of the area and discussed various issues concerning intestinal permeability. As alluded to by the authors, many of the areas are controversial and need further clarification. We would like to offer a different perspective on some of the controversial issues discussed by the authors.

Bjarnason et al. indicated that "significant lipid solubility of PEG 400 has been claimed" by some investigators,² and therefore the permeation route of polyethylene glycol (PEG) 400 may depend on its lipid solubility. Contrary to the earlier claim that PEG 400 is a lipophilic molecule, we have recently shown PEG 400 to be strongly hydrophilic.³ We determined the lipid-water partitioning of PEG 400 using two different methods and have found PEG 400 to have a petroleum ether–water partition coefficient (k_d) of 8.4×10^{-6} and a hexane–water k_d of 1.5×10^{-5} . Similarly, Phillipsen et al.⁴ also found the PEG 400 hexane–water k_d to be $<8 \times 10^{-5}$. These k_d values indicate that for every 1.5 parts of PEG 400 present in hexane (a lipid solvent), 100,000 parts of PEG 400 were present in water. Therefore PEG 400 is not lipophilic (lipid loving) but a lipophobic (lipid fearing) or hydrophilic (water loving) molecule. The earlier study,² which reported a k_d of 10^{-2} in petroleum ether–water mixture, appeared to have used inadequate separation techniques and thus resulted in an erroneously high k_d value.

Previously, we examined the mechanism of intestinal permeation of PEG 400 and 900 by in vivo recycling perfusion of rat intestinal segments.^{5,6} The intestinal absorption rate of PEG was linearly related to concentration (first-order kinetics), indicating passive diffusion. As shown by brush border membrane vesicle studies, there was negligible transmembrane flux of PEG.⁵ Under all experimental conditions tested, PEG net absorption followed changes in water flux.^{5,6} When water absorption was increased pharmacologically or through osmotic gradient manipulations, PEG absorption increased. Similarly, when water secretion was induced, PEG absorption decreased. In fact, the

solvent drag coefficient of PEG 400 was 0.1, confirming a strong dependence on water fluxes. Additionally, the mechanism of intestinal permeation of PEG 400 was similar to other well-known extracellular markers such as mannitol and inulin.^{7,8} These findings clearly indicated that PEG 400 was absorbed passively through paracellular aqueous pathways and not through enterocyte apical membranes.

As pointed out by Bjarnason et al.,¹ intestinal permeation rate of PEG 400 is higher than the other permeability probes, mannitol, lactulose, and ⁵¹Cr–ethylenediaminetetraacetic acid (EDTA). Based on its smaller cross-sectional diameter, the intestinal permeation rate of PEG 400 would be expected to be higher.⁸ It is important to note that although PEG 400 (molecular weight, 400 g/mol) has a higher molecular weight than other probes (mannitol, 182 g/mol), lactulose (342 g/mol), ⁵¹Cr-EDTA (341 g/mol), the cross-sectional diameter of PEG 400 (5.3 Å) is smaller than the other permeability probes (mannitol, 6.7 Å; lactulose, 9.5 Å; and ⁵¹Cr-EDTA, 11.5 Å).⁹ Previous studies have shown an excellent correlation between molecular size (cross-sectional diameter) of the probes and their intestinal epithelial permeation rate in both clinical and in vitro systems.^{9,10} The cross-sectional diameter of a permeability probe is a more reliable predictor of transepithelial permeation rate than the molecular weight of a probe because the molecular size of molecules can vary greatly among molecules with the same molecular weight.¹⁰ Therefore, based on its cross-sectional diameter, the intestinal permeation rate of PEG 400 is expected to be higher than other probes having larger cross-sectional diameters.

Based on its molecular size, we believe that PEG 400 "behaves" more like the smaller probe mannitol than the larger probes lactulose and ⁵¹Cr-EDTA. In patients with celiac disease, this may explain the decreased intestinal permeation of PEG 400 and monosaccharides, whereas lactulose and ⁵¹Cr-EDTA are increased. In Crohn's disease, although the permeation rates of lactulose and ⁵¹Cr-EDTA have been consistently found to be increased, the permeation rates of monosaccharides appear to be unchanged or decreased, and conflicting results have been reported with PEG 400. It is important to note that earlier studies (before 1990) seemed to be divided on whether the permeation rate of PEG 400 is increased, whereas the three most recent studies (Teahon et al., 1992; Ruttenberg et al., 1992; and Munkholm et al., 1994) suggest either a slight decrease or no significant change in permeation rate of PEG 400. The more recent findings may reflect advances in detection methods and have greater accuracy. Other possibilities were discussed in the review by Bjarnason et al.¹

Finally, Bjarnason et al.¹ took the view that the increase in intestinal permeability in clinically healthy relatives of patients with Crohn's disease is "within the variability of the method or due to transient disorder," which causes an increase in intestinal permeability. It is our view that the increase in intestinal permeability in these healthy relatives reflects the intrinsic permeability defect present in this subpopulation. There is some dispute as to whether relatives of patients with Crohn's disease as a group have an increased intestinal permeability in each of these studies,^{11–13} but approximately 10% of relatives had increased intestinal permeability, whereas a smaller proportion of controls had increased intestinal permeability. If the observed increase in intestinal permeability was strictly caused by experimental variability or unrelated conditions that increase intestinal permeability, then the proportion of patients with increased intestinal permeability should be similar in both healthy relatives and in controls. Also, there should be random occurrence as to whether healthy relatives or controls as a group have greater increase in intestinal permeability. However, in each of the studies, a larger number of healthy relatives have consistently had increased intestinal permeability than

the controls. Because only up to 10% of relatives of patients with Crohn's disease actually develop Crohn's disease, it was suggested by May et al.¹³ that only a small proportion of relatives may have increased intestinal permeability. Thus, it would require large numbers of patients to detect the small percentage of the relatives who have increased intestinal permeability.

Other evidence supporting a preexisting permeability disorder in healthy relatives includes observations by Pironi et al.¹⁴ and Hilsden et al.¹⁵ that healthy relatives of patients with Crohn's disease have an exaggerated response to a "permeability stressing" agent (aspirin) compared with controls. Additionally, an immunologic alternation in circulating B cells was found in 100% of relatives with an increase in intestinal permeability and in none of the controls or relatives with normal intestinal permeability, indicating a correlation between increase in intestinal permeability and immunologic alteration.¹⁶ A long-term follow-up of healthy relatives is required to determine whether a preexisting intestinal permeability disorder in these patients actually leads to the development of Crohn's disease. Until such time, the controversy concerning the role of intestinal permeability in patients with Crohn's disease will unlikely be resolved.

In conclusion, we agree with Bjarnason et al.¹ that there have been many scientific advances in both the clinical and basic scientific understanding of intestinal permeability, but many controversial issues still exist and there is much room for further advances in this "exciting and rewarding area of future research."

THOMAS Y. MA, M.D., Ph.D.
Gastroenterology Division
Department of Medicine
Long Beach VA Medical Center and
University of California, Irvine-College of Medicine
Long Beach, California 90822

PAVEL KRUGLIAK, M.D.
Gastroenterology Department
Soroka Medical Center and
Faculty of Health Science
Ben-Gurion University of the Negev
Beer-Sheva, Israel

1. Bjarnason I, MacPherson A, Hollander D. Intestinal permeability: an overview. *Gastroenterology* 1995;108:1566–1581.
2. Ukabam SO, Cooper BT. Small intestinal permeability to mannitol, lactulose, and polyethylene glycol 400 in coeliac disease. *Dig Dis Sci* 1984;29:809–816.
3. Ma TY, Hollander D, Krugliak P, Katz K. PEG 400, a hydrophilic molecular probe for measuring intestinal permeability. *Gastroenterology* 1990;98:39–46.
4. Phillipsen EF, Batsberg W, Christensen AB. Gastrointestinal permeability to polyethylene glycol as a parameter of intestinal permeability in man. *Eur J Clin Invest* 1988;18:139–145.
5. Krugliak P, Hollander D, Ma TY, Tran D, Dadufalza VD, Katz KD, Le K. Mechanism of polyethylene glycol 400 permeability of perfused rat intestine. *Gastroenterology* 1989;97:1164–1170.
6. Hollander D, Koyama S, Dadufalza V, Tran DQ, Krugliak P, Ma TY, King KY. Polyethylene glycol 900 permeability of rat intestinal and colonic segment in vivo and brush border membrane vesicles in vitro. *J Lab Clin Med* 1989;113:505–515.
7. Ma TY, Hollander D, Krugliak P, Truong H. Is the small intestinal tight junctions truly "tight" to inulin permeation. *Am J Physiol* 1991;260:G669–676.
8. Krugliak P, Hollander D, Schlaepfer CC, Nguyen H, Ma TY. Mechanism and sites of mannitol permeability of small and larger intestine in the rat. *Dig Dis Sci* 1994;38:796–801.
9. Hollander D, Rickets D, Boyd CAR. Importance of "probe" molecular geometry in determining intestinal permeability. *Can J Gastroenterol* 1988;2(Suppl A):35A–38A.
10. Ma TY, Hollander D, Bhalla D, Nguyen H, Krugliak P. IEC-18, a nontransformed small intestinal cell line for studying epithelial permeability. *J Lab Clin Med* 1992;120:329–341.
11. Teahon K, Smethurst P, Levi AJ, Menzies IS, Bjarnason I. Intestinal permeability in patients with Crohn's disease and their first degree relatives. *Gut* 1992;33:320–323.
12. 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* 1989;97:927–931.
13. May GR, Sutherland LR, Meddings JB. Is small intestinal permeability really increased in relatives of patients with Crohn's disease? *Gastroenterology* 1993;104:1627–1632.
14. Pironi L, Miglioli M, Ruggeri E, Dallasta MA, Ornigotti L, Valpiani D, Barbara L. Effect of non-steroidal anti-inflammatory drugs (NSAID) on intestinal permeability in first degree relatives of patients with Crohn's disease (abstr). *Gastroenterology* 1992;102:A679.
15. Hilsden RJ, Meeding J, Sutherland LR. Aspirin provokes an exaggerated increase in the lactulose/mannitol permeability index in first degree relatives of Crohn's patients (abstr). *Gastroenterology* 1989;95:A834.
16. Yacyshyn BR, Meddings JB. CD45RO expression on circulating CD19⁺ B cells in Crohn's disease correlates with intestinal permeability. *Gastroenterology* 1995;108:132–137.

Reply. We appreciate the highlighting of the two issues raised by Drs. Ma and Krugliak.

Indeed we are sympathetic to their views, which are supported by valid scientific data. However, it is our view that the jury is still out on the issues raised. Furthermore, it is important to remember that controversy is unavoidable and in abundance in such a rapidly developing field. The ultimate "truth" often turns out to be a compromise between the two extremes.

INGVAR BJARNASON, M.D.
Department of Clinical Biochemistry
King's College School of Medicine and Dentistry
London, England

DANIEL HOLLANDER, M.D.
School of Medicine
University of Kansas Medical Center
Kansas City, Kansas

Chronic Pancreatitis and Pancreatic Cancer

Dear Sir:

In their recent article on pancreatitis and pancreatic cancer, Bansal and Sonnenberg¹ conclude that chronic pancreatitis is a risk factor for pancreatic cancer. I would like to point out that among the various potential risk factors of pancreatic cancer, smoking is well established.² Because most patients with chronic pancreatitis are heavy smokers, I wonder whether the patients studied by Bansal and Sonnenberg were smokers and, if so, why the authors did not mention this fact and why this was not taken into account in calculating the risk. Isn't it possible that the increased risk they noted may have been caused by smoking rather than chronic pancreatitis?

In the Materials and Methods section of their paper, the authors state that pancreatic pseudocyst was considered a manifestation of chronic pancreatitis. However, it is well known that pancreatic pseudocyst is also a complication of acute pancreatitis; could this have