

# The Surgical Pathology of Malabsorption

## (How not to sprue-up a small bowel biopsy)

Joel K. Greenson, M.D.\*  
Professor of Pathology  
University of Michigan Medical School  
Ann Arbor, Michigan 48109-0054  
facjkgmd@umich.edu

### Malabsorption-Definition.

- A. The term malabsorption is used to encompass broadly any type or degree of dysfunction in uptake of any substance that is normally absorbed (or retained) by the small intestine. "Malabsorption" in this sense can be caused by a wide variety of disorders affecting the small intestine.
- B. The malabsorption syndrome more narrowly describes the constellation of clinical findings that includes diarrhea and steatorrhea and variable secondary changes, such as weight loss and evidence of vitamin deficiencies resulting from reduced absorption of nutrients.

### Introduction:

All too often endoscopic biopsies of the small bowel to rule out malabsorption are taken from the two worst places to evaluate villous architecture, either the duodenal bulb or the terminal ileum. Although both sites are the easiest for endoscopists to biopsy, they are the least reliable in terms of evaluating small bowel architecture. In a perfect world, only distal duodenal or proximal jejunal biopsies would be sent for such evaluation, and perfectly oriented sections would magically emerge from every histology lab. The harsh reality is that the deck is stacked against the pathologist from the beginning and diagnostic errors are easy to make if one is not familiar with anatomic variations incumbent upon this region of the gut. The combinations of peptic duodenitis or prominent Peyer's patches and tangential sections can easily lead to misdiagnoses. It is important to realize that the vast majority (>90% in my practice) of small bowel biopsies are normal.

While there are several different approaches to classifying small bowel biopsy changes, the most problematic cases for surgical pathologists are those that involve non-specific inflammatory changes. The poster child for this is, of course, celiac disease.

CELIAC DISEASE (Synonyms): gluten-induced enteropathy, celiac sprue, "non tropical sprue"

1. A well-characterized entity in which epithelial injury caused by dietary wheat gluten (and more particularly, its alcohol-soluble fraction, gliadin)

accounts for the malabsorption.

2. The cardinal features in patients with symptomatic celiac disease during different stages of therapy with a gluten-free diet (GFD) are:

a. Before gluten-free diet:

- Clinical and lab evidence of the malabsorption syndrome
- Flat jejunal biopsy (Absent or severely blunted villi) with:  
Surface epithelium that is thinned, injured  
Intraepithelial lymphocytes increased at surface
  - Chronic inflammation increased in lamina propria
  - Crypt mitoses increased; crypts elongated ("hyperplastic").
  - Some patients may have normal villi with increased IELs**

b. Gluten-free diet – short term (1wk-3 mos)

- Marked clinical improvement
- Diminished surface epithelial injury
- Reduced number of intraepithelial lymphocytes
- Villi return - partially

c. Gluten-free diet - Long Term (greater than 3 mos.)

- Villi gradually become normal
- Mitotic hyperactivity gradually subsides
- Chronic inflammation much diminished

d. Gluten restored to diet

- Rapid return of all lesions and malabsorption.
- Early increase in intraepithelial lymphocytes and injury to epithelium over villi

3. Diagnostic approaches to celiac disease.

a. Endoscopic biopsy of the distal duodenum (preferably beyond the bulb to avoid confounding peptic disease) is usually satisfactory, but an even more distal specimen may be needed at times.

b. Serum anti-reticulin, anti-endomysial, and anti-gliadin antibodies; these are used to screen and to check on effectiveness of a GFD. Anti-endomysial antibodies have historically been the most sensitive and specific for celiac disease. Antibodies to tissue transglutaminase (a closely related factor to anti-endomysial antibodies) may now be the single most sensitive and specific test for celiac disease.

c. Unequivocal evidence of improvement on a GFD is needed for a definitive diagnosis of symptomatic celiac disease.

4. Pathogenesis. The mechanism(s) by which gluten injures the epithelium have not been fully established. However, immunologic, genetic, and environmental factors all seem to be important
  - a. Immune mechanisms: Multiple observations favor the conclusion that celiac disease is an autoimmune disorder occurring in the context of a combination of genetic and environmental factors, including the following:
    - i. Serum antibodies to gluten and its gliadin fraction are virtually always present in active celiac disease. A recent study showed subepithelial deposition of activated complement, IgG and IgM in proportion to circulating anti-gliadin levels, suggesting humoral epithelial injury. Anti-reticulin antibodies also correlate with disease activity, and have diagnostic specificity.
    - ii. Presence of numerous intraepithelial lymphocytes, chiefly in the injured surface epithelium (these are mostly T cells, but with a high proportion gamma/delta types). This is highly consistent with cell-mediated injury that is somehow facilitated by gluten.
    - iii. Steroid Rx can cause patient improvement comparable to that with a GFD.
    - iv. Patients with celiac diseases show a highly significant excess of other autoimmune diseases (e.g., insulin dependent diabetes mellitus)
  - b. Genetic factors. These include:
    - i. A strong tendency for celiac disease to run in families (11-22% occurrence in first degree relatives). Also a high (70%) concordance for celiac diseases in identical twin pairs.
    - ii. A high correlation between celiac disease and presence of HLA-B8, DR3(DR17), DR7, and DQ2 histocompatibility loci. The strongest association (95%) is with a specific HLA DQ2 molecule that is seen in only 20-30% of the general population. The genetics seem to be "complex" and a multigenic HLA associated susceptibility is favored.
    - iii. Overlap with the blistering skin condition dermatitis herpetiformis(DH), including similar mucosal alterations, and overlap in HLA markers, and improvement in the skin lesions on a GFD.
  - c. Environmental factor(s). It seems likely that an environmental factor is a necessary precondition, along with appropriate genetic make-up and gluten ingestion, for an individual to develop celiac disease. Support for this conclusion comes from:
    - i. The fact that only one member of an identical twin pair is sometimes affected by celiac disease.
    - ii. Evidence of a possible role for viral infection: In one study serologic findings of prior exposure to an adenovirus(Type 12) were unusually common. In addition, a Type 12 adenovirus antigen shares an amino acid sequence with gliadin. Although more recent studies have not confirmed a direct correlation between celiac diseases and adenovirus12, the concept remains an attractive paradigm.

d. General hypothesis of celiac diseases pathogenesis (simplified):  
Gluten + Specific HLA Receptor + environmental factor (?viral infection) --> Sensitization --> Autoimmune susceptibility to gluten --> Lymphocyte/lymphokine induced epithelial injury = Celiac disease.

5. Variant and related forms of celiac disease. These include:

- a. Latent celiac diseases. This term is applied to persons who are asymptomatic for malabsorption but who nevertheless show evidence of gluten sensitivity. This can be accompanied by small bowel mucosal changes, and it is important for pathologists to be aware that these changes can fall anywhere along a long spectrum of severity. In some persons with latent celiac disease, the mucosal lesion approaches that seen in the full-blown symptomatic disorder while individuals at the other extreme show normal mucosa. A common denominator among these patients can be elevated anti-gliadin antibody production.
- b. Dermatitis herpetiformis. (See 4.-b-iii, above)
- c. "Collagenous sprue" A probable variant manifestation of celiac disease in which a flat small intestinal biopsy also shows a thick subepithelial collagen band.

6. Important complications of celiac disease include:

a. Refractory celiac disease and refractory ("unclassified") sprue.

The common denominator of "refractory celiac disease" is late failure to respond to a gluten-free diet. I limit use of "refractory sprue" to those patients with flat biopsies who have never shown gluten sensitivity and for whom the relevance of gluten is therefore unproven. Others also use "refractory sprue" to cover those with celiac diseases. Refractory celiac disease is sometimes seen with peptic duodenitis and distinctive radiological changes ("bubbly bulb"). Refractory sprue cases often have a poor prognosis and many feel these cases are lymphoproliferative disorders. Loss of CD8 staining and/or T-cell gene rearrangements can be helpful in proving clonality.

b. Small intestinal ulceration (with or without refractory celiac disease).

c. Malignancy: These include primary intestinal lymphoma - "enteropathy-associated T cell lymphoma" or EATCL. It may present clinically as refractory celiac disease or sprue. Other tumor types are: small intestinal adenocarcinoma, and squamous carcinoma of the esophagus.

REFERENCES (Celiac disease):

1. Baer, AX, Bayless, T.M., and Yardley, J.H. Intestinal ulceration and malabsorption syndromes. *Gastroenterology* 79:754-765,1980.
2. Bayless, T.M., Kap elowitz, R.F. and Shelley, W.M., et. al.: Intestinal ulceration - a complication of celiac disease. *New Eng.J. Med.*276:996-1002,1967.
3. Falchuk,Z.M.,Gebhard,R.L.,Sessoms,C.and Strober, W.: An in vitro model of gluten-sensitive enteropathy. Effect of gliadin on intestinal epithelial cells of patients with gluten-sensitive enteropathy in organ culture. *J. Clin.Invest.* 53:487-500,1974.
4. Gebhard,R.L.,Falchuk,Z.H.,Katz,S.L,Sessoms,C.,Rogentine,G.N.,Strober, W.: Dermatitis herpetiformis. Immunological concomitants of small intestinal disease and the relationship to histocompatibility antigens. *J. Clin.Invest.*54:98,1974.
5. Swinson,C.M.,Slavin, G.,Coles,E.C.,Booth,C.C.: Coeliac disease and malignancy. *Lancet*1:111-115,1983.
6. Holdstock, G.,Eade, D.E., Isaacson,P.,and Smith, C.L. Endoscopic duodenal biopsy in coeliac disease and duodenitis. *Scand.J.GastroenteroL*14:717,1979.
7. Jones, B.,Bayless,T.M.,Hamilton,S.R.,and Yardley, J.H.: The bubbly bulb and celiac disease. *Am. J.Roentgen.*142:119,1984.
8. Kagnoff, M.F.: Immunology and disease of the gastrointestinal tract. In: Sleisinger, M.H. and Fordtran, J.S.: *Gastrointestinal disease*. Philadelphia, Saunders,1993,5th Ed., pgs.45-86.
9. Katz, A.J., Falch a k, Z.M.: Definitive diagnosis of gluten-sensiti ve enteropathy: Use of an in vitro organ culture model. *Gastroenterology* 75:695-700, 1978.
10. Rubin, C.E.,et.aL: Studies of celiac disease. I. Apparent identical and specific nature of duodenal and proximal jejunal lesion in celiac disease and idiopathic sprue. *Gastroenterology* 38:28-49, 1960.
11. Samloff,I.M.,David,J.S.,and Schenk, E.A.: A clinical and histochemicalstudy of gluten-free diet. *G astroenterology* 48:1 55, 1965.
12. Trier, J.S., Falchak, Z.M., Carey, M.C., et. al.: Celiac sprue and refractory sprue (clinical conference). *Gastroenterology* 75:307-31 6, 1978.
13. Unsworth, D.J., Leonard, N.L., Fry, L.: Antireticulin and antigliadin antibodies in dermatitis herpetiformis and celiac disease. In: Beutner EH, Chorzelski TC, Kumar V(eds): *Immunopathology of the Skin*, ed 3. New York, John Wiley & Sons Inc.,1987,Chap 25.
14. Weinstein, W.M.,Saunders, D.R.,Tytgat, G.N., et.aL: Collagenous sprue an unrecognized type of malabsorption. *New Eng.J. Med.*283:1297-1301,1970.
15. Whitehead, R.: Primary lymphadenopathy complicating idiopathic steatorrhea. *Gut* 9:569-575,1948.
16. Yardley, J.H., Bayless, T.M., Norton, J.H., et. al.: Celiac disease: A study of the jejunal epithelium before and after a gluten-free diet. *New Eng. J. Med.* 267:1173-1179,1962.
17. Yardley, J.H.: Malabsorption disorders. In: Min g SC, Goldman H (ed s.) *Pathology of the gastrointestinal tract*. Philadelphia, Saunders, 1992, Chap 29.
18. Halstensen,T.S.,Hvatum, M., Scott, H.,Fausa, O.,Brandtzaeg,P.: Association of subepithelial deposition of activated complement and immunoglobulin G and M response to gluten in celiac disease. *Gastroenterology* 102:751-759,1992.
19. Arranz,E.,Ferguson,A.: Intestinal antibody pattern in celiac diseases: Occurrence in patients with normal jejunal biopsy histology. *Gastroenterology* 104:1263-1272,1993.

20. Marsh, M.N.: Gluten sensitivity and latency: Can patterns of intestinal antibody secretion define the "silent majority?" *Gastroenterology* 104:1150-1153, 1993.
21. Lawler, M., Humphries, P., O'Farrelly, C., Hoey, H., Sheils, O., Jeffers, M., O'Briain, D.S., Kelleher, D.: Adenovirus E1A gene detection by polymerase chain reaction in both the normal and celiac duodenum. *Gut* 35:1226-1232, 1994.
22. Collin, P., Reunala, T., Pukkala, E., Kyrilainan, O., Pasternack, A.: Coeliac disease-associated disorders and survival. *Gut* 35:1215-1218, 1994.
23. Catassi, C., Ratsch, I-M, Fabiani, E., Rossini, M., Bordicchia, F., Candela, F., Coppa, G.V., Giorgi, P.L.: Coeliac disease in the year 2001 exploring the iceberg. *Lancet* 343:200-203, 1994.
24. Cronin, C., Shanahan, F.: A significant step in the celiac puzzle. *Gastroenterology* 1998;114:1338-40.
25. Colin P. New diagnostic findings in Coeliac disease. *Ann Int Med* 31:399-405, 1999.
26. Farstad IN, Johansen F-E, Vlatkovic L, Johansen J et al. Heterogeneity of intraepithelial lymphocytes in refractory sprue: potential implications of CD30 expression. *Gut* 51:372-378, 2002.

### TROPICAL SPRUE (POST-INFECTIVE TROPICAL MALABSORPTION)

1. Definition (Baker and Mathan): "Intestinal malabsorption of unknown etiology, occurring among residents in, or visitors to, the tropics". Despite the fact that a single etiologic agent has not been identified, there is much evidence that an infection initiates and sustains tropical sprue: a) It occurs in certain specific geographic areas (eg, West Indies, Indian subcontinent) and enteric infections are common in these locations. b) In some areas it is epidemic. c) Aerobic enterobacteria colonize the patient's small intestine and these may be toxin producing. (Note that this differs from the stasis syndrome in which anaerobic bacterial overgrowth is central (see below)). d) Recovery after treating tropical sprue with broad-spectrum antibiotics is usually rapid and dramatic. e) Some have postulated that a Protozoan infection such as cyclospora may play a role.

The important role played by infection in tropical sprue has led to the alternative designation "Post-infective tropical malabsorption".

2. Other factors such as epithelial damage may also contribute to the condition. Along with diarrhea and malabsorption, folate, and sometimes B-12 deficiencies are commonly present. In severe tropical sprue there can be resulting diminution in epithelial mitosis accompanied by nuclear enlargement - changes that are the epithelial counterpart to maturational derangements in the marrow and macrocytic anemia (megaloblastic changes). Genetic or ethnic predisposition has also been suggested.
3. The mucosal lesion in tropical sprue is of "nonspecific" type with epithelial blunting, chronic inflammation, etc. A completely flat biopsy like that often seen in celiac disease is rare in tropical sprue, but epithelial dysfunction, as in celiac disease, is central to pathogenesis. Unlike celiac disease, in which mucosal changes are

greatest in the proximal small bowel, lesions in the ileum are as prominent as those in the jejunum in tropical sprue. This fits well with the resulting secondary B-12 and folate deficiency states (which are not common in celiac disease). Intraepithelial lymphocytes are increased in tropical sprue, although they may be more numerous in the crypts than in the villi.

#### REFERENCES (Tropical Sprue):

1. Baker, Z. T. and Mathan, V. I.: Syndrome of tropical sprue in India. *Am. J. Clin. Nutr.* 21:984, 1968.
2. Schenk, E. A., Samloff, I. M. and Klipstein, F. A.: Morphologic characteristics of jejunal biopsy in celiac disease and tropical sprue. *Am. J. Path.* 47:765, 1965.
3. Swanson, V. L. and Thomassen, R. W.: Pathology of the jejunal mucosa in tropical sprue. *Am. J. Path.* 46:511, 1965.
4. Swanson, V. L., Wheby, M. S. and Bayless, T. M.: Morphologic effects of folic acid and vitamin B-12 on the jejunal lesion of tropical sprue. *Am. J. Path.* 49: 167, 1966.
5. Trier JS, Donnelly SM. Case records of the Massachusetts General Hospital. *New Engl J Med* 322:1067-1075, 1990.
6. Wheby, M. S., Swanson, V. L. and Bayless, T. M.: Comparison of ileal and jejunal biopsies in tropical sprue. *Am. J. Clin. Nutr.* 24:117-123, 1971.
7. Cook GC. Aetiology and pathogenesis of postinfective tropical malabsorption (tropical sprue). *Lancet* 1:721-723, 1984.
8. Cook GC. Postinfective malabsorption (includes tropical sprue). In: Bouchier AD, Alan RN, Hodgson JF, Deighley M RB, eds. *Gastroenterology. Clinical practice and science.* Philadelphia, Saunders. 1993. Pgs. 522-537
9. Cook GC. "Tropical sprue": Some early investigators favored an infective cause, but was a coccidian protozoan involved? *Gut*; 40:428-429.

#### STASIS (BLIND-LOOP) SYNDROME / BACTERIAL OVERGROWTH

1. The causes of stasis in the small bowel include motor/neural disorders such as diabetic neuropathy and scleroderma as well as structural lesions such as diverticula and surgical anastomoses.
2. The pathophysiology of stasis is largely due to anaerobic bacteria that deconjugate bile salts, deplete vitamin B12 and damage surface epithelium.
3. Stasis in the small bowel (regardless of the underlying etiology) may result in abnormal inflammatory changes in the mucosa (although some patients with malabsorption may have normal biopsy findings). As with most small bowel disorders, the histologic features are non-specific. There is generally mild to moderate villus blunting which may be accompanied by an increase in lamina propria mononuclear cells and focal neutrophilic infiltrates in the epithelium. At low-power these changes may mimic partially developed or partially treated celiac disease. Stasis/bacterial overgrowth typically lacks

the intense intraepithelial lymphocytosis of celiac disease. These findings may be focal/patchy. Since the appropriate clinical history is often lacking in these cases, one should think of this diagnosis whenever a small bowel looks "a little funny".

#### REFERENCES (Scleroderma and Stasis (Blind-Loop) Syndrome):

1. Ament, M.E., Shimoda, S.S., Saunders, D.P. and Rubin, C.E. 1972. Pathogenesis of steatorrhea in three cases of small intestinal stasis syndrome. *Gastroenterology* 63:728.
2. Cave DR, Compton CC, et al. A 21-year-old man with progressive gastrointestinal stasis, hepatomegaly, and a neurologic disorder – familial visceral myopathy. *Case records of the Massachusetts General Hospital. New Engl J Med* 322:829-841, 1990.
3. Glanella, R.A., Rout, W.R., and Toskes, P.P. Jejunal brush border injury and impaired sugar and amino acid uptake in the blind-loop syndrome. *Gastroenterology* 67:965, 1974.
4. Goldstein, F. Mechanisms of malabsorption and malnutrition in the blind-loop syndrome. *Gastroenterology* 61:780, 1971.
5. Hendel L, Kobayashi T, Petri M.: Ultrastructure of the small intestinal mucosa in progressive systemic sclerosis (PSS). *Gastroenterology* 95:41-46, 1987.
6. Krishnamurthy S, Schuffler, M.D.: Pathology of neuromuscular disorders of the small intestine and colon. *Gastroenterology* 93:610-639, 1987.
7. Schuffler M.D., Beegle R.G.: Progressive systemic sclerosis of the gastrointestinal tract and hereditary hollow visceral myopathy: Two distinguishable disorders of intestinal smooth muscle. *Gastroenterology* 77:664-671, 1979.

#### WHIPPLES DISEASE

Definition: A rare chronic infectious disease characterized by massive accumulation of PAS-positive macrophages in the lamina propria and in other organs. The causative agent is now felt to be a novel rod-shaped bacterium, *Tropheryma whippelii*, an actinomycete that was recently identified by molecular techniques. The Whipple bacillus occurs both extracellularly and within PAS-positive granules in the macrophages. The macrophagic granules are believed to be phagosomes that result from bacterial phagocytosis and digestion.

Malabsorption in Whipple's disease is due predominantly to blockage of nutrient movement in the lamina propria and in the mesenteric lymphatic drainage system by PAS positive macrophages. Lipid accumulation in the mucosa and mesenteric lymph nodes is a prominent feature in the small intestine, and led to Whipple's name for the disorder- "intestinal lipodystrophy".

Although Whipple's disease almost always includes involvement of the small intestine and presence of malabsorption, it is in fact a systemic illness that also affects other organs, especially the heart, brain, eyes, joints and R-E system. At the same time, a few well-studied patients have been reported in whom PAS positive macrophage infiltration of the small intestine was not seen. The lesion can also go undetected when present in the small intestine. Thus a small bowel biopsy that gives no histopathologic evidence of Whipple's disease does not necessarily rule out the diagnosis there or at other sites. With expanded use of more sensitive molecular diagnosis, recognition of such cases should become more common (see below under Diagnosis). Pathologists and gastroenterologists need to be aware of these possibilities when asked to examine the GI tract in patients with suspected non-GI lesions of Whipple's disease (e.g., in the brain).

Evidence of host-related, and possibly genetically determined increased susceptibility to Whipple's disease in patients who develop the disorder has been sought by several investigators. Such studies are hampered by the rarity of Whipple's disease and the need to conduct studies on both the active and treated infection. There is now, however, increasing evidence that patients with Whipple's disease may have innate defects in their T cells and mononuclear cells.

Diagnosis: Jejunal biopsy is usually still the optimal method for diagnosis and verification of Whipple's disease. Sampling errors because of patchy distribution of the lesion or restriction of PAS positive macrophages to the submucosa are potential diagnostic problems. Diagnosis can be suggested by endoscopic recognition of enlarged, lipid-choked villi, which are also optimal for biopsy detection, or by demonstration of PAS positive macrophages in the liver, lymph nodes, spleen, tonsil, brain, heart, etc. However, demonstration of organisms and/or granules by EM is usually needed for definitive diagnosis of Whipple's disease from tissues outside the small intestine because PAS positive inclusions in those locations are most often due to causes other than Whipple's disease.

Availability of the new molecular techniques for recognizing Whipple's disease should make a major contribution to its diagnosis and study in the future. Use of PCR methods to demonstrate the *T. whippelii* organism has already led to diagnosis of the disorder in peripheral blood, in intestinal biopsy specimens that did not show lesions histopathologically, and in vitreous fluid from the eye. In addition, the former observation led to the realization that the RBC's in peripheral blood smears from patients with Whipple's disease can show numerous adherent rod-shaped bacteria with Whipple's bacillus.

6. Follow-up: PCR methods may be a better way to monitor when the infection has been adequately treated. PAS positive macrophages remain in the small bowel for many months to years after eradication of the Whipples Bacillus, hence H & E morphology is not useful for monitoring therapy. Demonstration of intact bacteria by EM is a useful but expensive and time-consuming technique.

## REFERENCES (Whipple's disease)

1. Whipple's disease: A 62 year odyssey from death to diagnosis. *Neurology* 45:617-625, 1995.
2. Marth T, Rous Matthias, von Herbay A, Meuer SC, Feurle GE. Persistent reduction of complement receptor 3 alpha-chain expressing mononuclear blood cells and transient inhibitory serum factors in Whipple's disease. *Clin Immunol and Immunopathol* 72:217-226, 1994.
3. Kuhajda FP, Belitsos NJ, Keren DF, Hutchins GM. A submucosal variant of Whipple's disease. *Gastroenterology* 82:46-50, 1982.
4. Petrides PE, Muller-Hoehnan J, Fredricks DN, Relvian DA. PCR Analysis of *T. whippelii* DNA in a case of Whipple's disease: effect of antibiotics and correlation with histology. *Am J of Gastroenterol* 93:1579-82; 1998.
5. Volpicelli NA, Salyer WR, Milligan FD, Bayless TM, Yardley JH: The endoscopic appearance of the duodenum in Whipple's disease. *Johns Hopkins Med.J.* 138:19-23, 1976.
6. Lowsky R, Archer GL, Fyles G, Minden M, Curtis J, Messner H, Atkins H, Patterson B, Willey BM, McGeer A. Brief report: Diagnosis of Whipple's disease by molecular analysis of peripheral blood. *New Engl J Med* 331:1343-1346, 1994.
7. Rickman LS, Freeman WR, Green WRG, Feldman ST, Sullivan J, Russack V, Relman DA. Brief report: Uveitis caused by *Tropheryma whippelii* (Whipple's bacillus). *New Engl J Med* 332:363-366, 1995.

## Giardia lamblia:

*Giardia lamblia* was the first protozoa discovered in the human intestine, as Leeuwenhoek describe the organism in his own stools in 1681. Giardiasis is reportedly the most prevalent gut parasite in the United States and Britain. While the prevalence of giardiasis has been quoted as 7.4% in the U.S., it may be as high as 50% in some underdeveloped nations. A study of American travelers to Leningrad over a four year period found that 23% were infected with the organism.

While many patients with giardia are asymptomatic, a wide variety of gastrointestinal symptoms may occur. Some patients report acute onset diarrhea with greasy foul-smelling non-bloody stools while others report abdominal distension/bloating, pain, and flatulence. Weight loss and malabsorption may occur and the disease may persist for months if unrecognized/untreated.

The organism is often transmitted by contaminated drinking water (fecal/oral), however, intimate contact may also spread the disease as evidenced by the high prevalence of infection in "gay bowel disease." Both endemic and epidemic infections can be seen. The organism is ingested as a cyst present in contaminated food or drinking water. In the stomach, duodenum, and upper small intestine, the parasite transforms into a trophozoite. In the lower small intestine and colon, the organisms form back into cysts which are passed in the stool

A number studies have found that children and patients with hypogammaglobulinemia, agammaglobulinemia, IgA deficiency, and/or achlorhydria have a higher incidence of

infection with giardia. However, a humoral immunodeficiency state is not necessary for infection. Immunosuppressed patients are more likely to have long-term infections with chronic diarrhea and malabsorption, while immunocompetent patients are more likely to have an acute self-limited diarrheal illness or become asymptomatic carriers.

### **Histopathology:**

While the diagnosis of giardiasis is best made on ova and parasite exams or via a duodenal fluid aspirate, identification of the trophozoite on routine H & E stained sections of the proximal small bowel is often possible. The trophs are pear-shaped and exhibit two nuclei from a ventral view. From a lateral view the trophs have a sickled shape. In some cases large numbers of the organisms can be found clumped together in the intravillous spaces, while in others only one or two parasites will be evident in serial sections. Some trophs may be seen attached to the epithelial surface and there are reports of tissue invasion by the organism. On H&E stained sections the trophs often have a smudgy blue-gray appearance which can easily be overlooked as extravasated mucus or intraluminal debris. By focusing up and down one may appreciate the two nuclei of the troph peering back through the microscope like eyes on a face. A trichrome, giemsa, PTAH or PAS stain may help bring out the detail of the organisms. In general, it is a good idea not to diagnose giardiasis unless you can see the organisms' nuclei.

The histologic changes seen in the small intestinal mucosa are quite varied. Many patients have completely normal appearing small bowel biopsies, while some have minor non-specific villous blunting and a modest increase in lamina propria mononuclear cells. Other patients have had more substantial pathologic changes including cases of near total villus atrophy reminiscent of untreated sprue. Patients with hypogammaglobulinemia often have the most profound histologic changes, having been referred to as "hypogammaglobulinemic sprue". Fortunately, these patients' biopsies often show significant improvement following the eradication of Giardia.

### **Biopsy techniques and ancillary studies:**

While giardia can be found anywhere in the small bowel, the distal duodenum/proximal jejunum are the best sites to biopsy for reasons discussed previously. Touch preps of the biopsy may be made to aid in the diagnosis of giardia, as the organisms are easily shed onto a glass slide during this procedure. While special stains such as PAS, PTAH, trichrome, or giemsa may accentuate the details of the organisms, H & E stained sections are usually all that are needed to make the diagnosis.

The organism can also be identified in duodenal fluid aspirates and more recently by using the "string test". Examination of the stool will often demonstrate the cyst form of the organism. In cases of severe diarrhea the trophozoite may also be found in the stool.

## REFERENCES (Giardiasis):

1. Rodrigues Da Silva J, Coutinho SG, Dias LB, Figueiredo ND. Histopathologic Findings in Giardiasis: A Biopsy Study. *American Journal of Digestive Diseases* 9:355-365, 1964.
2. Hartong WA, Gourley WK, Arvanitakis C. Giardiasis: Clinical Spectrum and Functional-Structural Abnormalities of the Small Intestinal Mucosa. *Gastroenterology* 77:61-69, 1979.
3. Levinson JD, Nastro LJ. Giardiasis With Total Villous Atrophy. *Gastroenterology* 74:271-275, 1978.
4. Ament ME, Rubin CE. Relation of Giardiasis To Abnormal Intestinal Structure and Function in Gastrointestinal Immunodeficiency Syndromes. *Gastroenterology* 62:216-226, 1972.
5. Babb RR, Peck OC, Vescia FG. Giardiasis: A Cause of Traveler's Diarrhea. *JAMA* 217:1359-1362, 1971.
6. Saha TK, Ghosh TK. Invasion of Small Intestinal Mucosa By *Giardia Lamblia* In Man. *Gastroenterology* 72:402-405, 1977.
7. Knight R. Progress Report Intestinal Protozoa. *Gut* 19:940-953, 1978.
8. Sun T. The Diagnosis of Giardiasis. *The American Journal of Surgical Pathology* 4:265-271, 1980.
9. Brasitus TA. Parasites and Malabsorption. *The American Journal of Medicine* 67:1058-1064, 1979.
10. Moore GT, Cross M, McGuire D, Mollohan CS, Gleason NN, Healy G R, Newton LH. Epidemic Giardiasis At A Ski Resort. *The New England Journal of Medicine* 281:402-407, 1969.
11. Yardley J H.: Pathology of Chronic Gastritis and Duodenitis. In Goldman H, Appelman HD, Kaufman N, (eds.): *Gastrointestinal Pathology*. Willams and Wilkins, Baltimore, 1990, pp 69-143.
12. Abrams G D.: Infectious Disorders of the Intestines. In Ming SC, Goldman H, (eds.): *Pathology of the Gastrointestinal Tract*, W.B. Saunders Co. Philadelphia 1992, pp621-642.

## G. CRYPTOSPORIDIOSIS

1. These are coccidial organisms related to Isospora, Toxoplasma and (remotely) Plasmodia. More common in animals than man, but now known that it is a zoonosis, causing short-term diarrheal illness in immunocompetent humans, persistent and even lethal intestinal illness in immunodeficient and AIDS patients (See Sections and J.) Can be epidemic. Roberts found 11% of cryptosporidia in duodenal aspirates from asymptomatic carriers.
2. Organisms occur as numerous round, small (2-4 um) basophilic bodies lying within the brush border, especially upper villi. However, EM study demonstrates that they are actually intracytoplasmic, as with coccidia and related protozoans.
3. Cryptosporidia can infect colon as well as small intestine. (See Riddell lecture, this course). Also reported in bronchi, pancreatic ducts and gallbladder.
4. Diagnosis is usually made from tissue or smear. Diagnosis from stool requires recognition of oocyst form. Acid-fast stain is best for demonstrating oocysts.

### REFERENCES (Cryptosporidiosis):

1. Current, W.L., Reese, N.C., Ernst, J.V., Bailey, W.S., Heyman, M.B., and Weinstein, W. M. Human cryptosporidiosis in immunocompetent and immunodeficient persons. Studies of an outbreak and experimental transmission. *New England J. Med.* 308:1252-1257, 1983.
2. Garcia, L.S., Brucker, D.A., Brewer, T.C., and Shimizu, R.Y.: Techniques for the recovery and identification of cryptosporidium oocysts from stool specimens. *J. Clin. Microbiol.* 18:1 58, 1983.
3. Meisel, J.L., Perera, D.R., Meligro, C. and Rubin, C.E.: Overwhelming watery diarrhea associated with cryptosporidium in an immunosuppressed patient. *Gastroenterology* 70:1156-1160, 1978.
4. MacKenzie WR, Hoxie NJ, Proctor ME, et al. A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. *N Engl J Med* 1994; 331:1 61-7. (A first-rate epidemiologic study.)
5. Nime, F.A., Burek, J.D., Page, D.L., Holscher, M.A., Yardley, J.H.: Acute enterocolitis in a human being infected with the protozoan cryptosporidium. *Gastroenterology* 70:592-598, 1978.
6. Roberts WG, Green PH R, Ma J, Carr M, Ginsberg AM: Prevalence of cryptosporidiosis in patients undergoing endoscopy: Evidence for an asymptomatic carrier state. *Am J Med* 87:537, 1989.

## MICROSPORIDIOSIS

### **BACKGROUND.**

The phylum Microspora consists of primitive protozoa that are characterized by the production of unique spores. These spores contain a coiled hollow tube (polar tubule) that can be extruded and through which infective material (sporoplasm) can be injected into host cells. Since the main hosts for these organisms are arthropods and fish, these protozoa received scant medical attention until brought to light in the study of diarrhea in AIDS patients. Although there are over a hundred genera and about a thousand species in this phylum, only a handful of species have thus far been detected in man, and these are collectively designated by the non-taxonomic term microsporidiosis.

The 2 major microsporidia seen in the gut are *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* (formerly *Septata intestinalis*). In conventional H&E stained biopsies, these organisms are quite difficult to detect due to their minute size and poor staining qualities. Formerly, diagnosis rested on electron microscopy of tissue samples, but diagnosis is now being rendered more frequently by light microscopy (special stains) on biopsies, nasal smears, stool, sputum, and urine samples.

**ENTEROCYTOZOON BIENEUSI.** *E. bieneusi* was first described in 1985, and is still the major microsporidial infection in man, particularly in AIDS patients with diarrhea. Prospective studies of HIV-associated chronic diarrhea indicate a prevalence of 9 to 16% in patients evaluated by stool studies to detect microsporidia. Recently, this organism was reported to have caused a self-limited traveler's diarrhea in a non-immunocompromised individual, and it is likely that this occurs more frequently but is not recognized. While originally described as limited to small bowel, this organism has subsequently been detected in the biliary system.

**Clinical Symptoms.** The most commonly reported symptoms with *Enterocytozoon bieneusi* infections are chronic diarrhea, anorexia, and weight loss, and less frequently - abdominal pain, nausea, vomiting and fever. These latter symptoms are more common in patients with concomitant biliary disease which may produce a clinical picture of cholangitis or cholecystitis. Not all patients develop diarrhea and the exact relationship between infection and clinical illness has been disputed. Those individuals with chronic debilitating disease usually have advanced AIDS with very low CD4 counts. Treatment is primarily supportive as there is no effective drug to treat this particular infection.

**Diagnosis by Electron Microscopy.** Two phases of the lifecycle of *E. bieneusi* can be identified by EM, a proliferative phase (merogony) and a spore-forming phase (sporogony). In the proliferative phase, the organisms are small rounded objects with 1 to 6 nuclei, and are more electron-lucent than the surrounding cytoplasm of the surrounding host cell. Very few structures are present in the cytoplasm of the organisms with empty clefts (electron lucent inclusions) being the major feature. The herald of the spore-forming phase is the presence of stacks of electron dense discs. At later stages, the discs aggregate end-to-end to form the longer, curved profiles of polar tubes. During sporogony, the nuclei continue to divide, resulting in large organisms

with up to twelve nuclei with each nucleus surrounded by several coils of polar tube. Finally, these large multinucleated forms break up into immature spores (sporoblasts) which then develop into mature spores. Mature spores are extremely electron dense, have a single nucleus, and an extrusion apparatus consisting of several coils of polar tube, and anchoring disc, and a polaroplast. The coils of polar tube are in 2 rows. Both phases of the life cycle are localized to the apical portion of enterocytes (supranuclear), and multiple or all stages of the life cycle can be identified in a single cell. All phases of the life cycle lie free within the host cytoplasm, with no limiting vacuole or membrane. Frequently, *E. bienewisi* (which lack their own mitochondria) are closely apposed to host mitochondria, presumably siphoning off energy.

**Diagnosis by Light Microscopy.** Although *E. bienewisi* has been reported in normal small bowel mucosa, it is more frequently detected in biopsies showing some histologic abnormalities. The highest concentrations of organisms tend to be in villous tips which have a degree of blunting, focally increased intraepithelial lymphocytes, and some shedding of enterocytes; and it is in these abnormal areas that the hunt for organisms should be concentrated. On conventional H&E stains, the organisms appear as small pale blue structures in the supranuclear cytoplasm which may be surrounded by a halo or indent the nucleus. H&E diagnosis alone is unreliable and needs to be supported by special stains for the spores. The spores are gram positive and are usually clustered together in the apical cytoplasm of enterocytes at villus tips. The spores are also highlighted by modified Trichrome stains, Warthin-Starry, and in 1 micron plastic sections by toluidine blue or azure II methylene blue. Unfortunately, the proportion of mature spores to multinucleated forms is unpredictable and maybe low. Biopsies of jejunum have a higher diagnostic yield than duodenum or ileum, although the latter sites may be diagnostic.

**Diagnosis by Stool Examination.** Spores are shed in stool but are difficult to detect due to their small size and resemblance to yeast. Current recommendation for detecting spores of *E. bienewisi* are using fixed smears and screening with an optical brightener such as calcofluor white (that binds the chitin in the spore wall) and then confirming with a modified trichrome stain. By the modified trichrome stain, the spores are seen as small pink ovals, approximately 1.5 x 0.5 microns, which have a polar cleared zone, giving the appearance of a band around the middle of the spore. Although they also stain pink, small yeast such as *Candida* are rounder and more homogeneous with no internal structures. Bacteria and most fecal debris stain only with the counterstain.

**ENCEPHALITIZOON INTESTINALIS.** A second microsporidian that involves the GI tract, *E. intestinalis* (formerly *Septata intestinalis*), was first described in 1992. But in addition to infecting the small bowel and biliary system, *E. intestinalis* can disseminate to involve the respiratory system, urinary system, and conjunctiva. Although *E. intestinalis* is less frequent than *E. bienewisi*, it is important to recognize since effective therapy is available.

**Clinical Symptoms.** The primary clinical complaints are severe watery diarrhea and weight loss followed by a sclerosing cholangitis-like picture with RUQ pain and fever, with ultrasound showing a diseased biliary system. Other symptoms include sinusitis, bronchitis, and conjunctivitis. While spores are frequently seen in urine, urogenital symptoms are surprisingly minimal and creatinine is usually normal. Clinical symptoms respond rapidly and dramatically to albendazole therapy, but relapses can occur following cessation of therapy.

**Diagnosis by EM.** The various proliferative and spore-forming stages of *E. intestinalis* occur together within a cluster, with the late stages centrally located and the earlier stages more peripheral in the clusters. The early proliferative phase consists of relatively simple structures with 1 to 4 nuclei, and are embedded in a fine fibrillar network secreted by the organism. The sporogonic phase is heralded by the accumulation of electron-dense material along the cell surface giving it a scalloped appearance that then gives way to a thick membrane, which eventually becomes the exospore coat. Cells with the thick membrane continue to undergo nuclear and cytoplasmic division and when the last cell division is completed, spore-specific organelles appear including the polar tube. As the spores mature, an electron-lucent space develops around each spore within the fibrillar network, giving the appearance that the spores are suspended in a honeycomb. The mature spores measure 2 x 1.5 microns, have a single nucleus, and 5 turns of the polar tube in a single row.

**Diagnosis by Light Microscopy.** Small bowel biopsies findings are similar to those *E. bienewisi*, but differences do exist. Again, organisms appear as indistinct, small bluish structures in the apical cytoplasm of enterocytes. The organisms are somewhat more refractile (perhaps due to heavier spore production). Gram stain will highlight the spores in the enterocytes, but in *E. intestinalis* infection will also show spores in the macrophages, fibroblasts and endothelial cells of the lamina propria. Thus gram staining can help speciate the 2 major microsporidia seen in the gut. In addition to being found in the small bowel, the organisms can be seen in the biliary system, as well as in urine, nasal smears, and sputum (free and in cells). The use of exfoliative and aspirated cytologic methods to detect human microsporidiosis is assuming increasing importance with a gram or Weber chromotome stain done to identify spores.

**Diagnosis by Stool Exam.** Screening with calcofluor white and confirmation with a modified trichrome stain is the current suggested method for detecting the spores of *E. intestinalis*. As in *E. bienewisi*, the spores are oval and by trichrome stain pink with a stripe, but the spores of *E. intestinalis* are larger, measuring approximately 2.5 x 1.5 microns.

Comparison of Microsporidia in the Gut:

	<i>E. bienersi</i>	<i>E. intestinalis</i>
Disseminated disease	No	Yes
Major sites of infection	Small bowel Biliary system	Small bowel Biliary system Respiratory (sinuses, bronchi) Genitourinary (kidney, bladder)
Small bowel bx	Organisms in apical enterocytes Few Gram + spores	Organisms in apical enterocytes and lamina propria cells Many Gram + spores
Spores	1-1.5x.5 microns Double row polar tubules	2-2.5x1.5 microns Single row polar tubules
E M	Electron dense discs	"Septate" parasitophorous vacuoles
Effective therapy	No	Yes

## REFERENCES (Microsporidiosis):

1. Cali A, Owen R L. Microsporidiosis In: Balows A, ed., Laboratory diagnosis of infectious disease: Principles and practices. New York. Springer-Verlag.1988. Vol 1,Chap 98,Pgs 929-950.
2. Cali A, Owen R L.Intracellular development of Enterocytozoon, a unique microsporidian found in the intestine of AIDS patients. J Protozool 37:1 45-155, 1990.
3. Desportes I, LeCharpentier Y, Galian A, Bernard F, Cochand-Priollet B, Lavergne A, Ravisse P, Modigliani R: Occurrence of a new microsporidian Enterocytozoon bienewisi occurring in the enterocytes of a human patient with AIDS. J Protozool 132:250-254, 1985.
4. Orenstein J M, Chiang J, Steinberg W, Smith PD, Rotterdam H, Kotler DP. Intestinal microsporidiosis as a cause of diarrhea in human immunodeficiency virus-infected patients. Hum Pathol 21:475-481, 1990.
5. Greenson JK, Belitsos PC, Yardley JH, Bartlett JG. AIDS enteropathy-occult enteric infections and duodenal mucosal alterations in chronic diarrhea. Ann Intern Med 114:366-372, 1991.
6. Asmuth DM, DeGirolami PC, Federman M, Ezratty CR, Pleskow DK, Desai G, Wanke CA. Clinical features of microsporidiosis in patients with AIDS. Clin Infect Dis 1994; 18:819-25.
7. van Gool T, Hollister WS, Schattenkerk JE, van den Bergh Weerman MA, Terpstra WJ, van Ketel R J, Canning EU. Diagnosis of Enterocytozoon bienewisi Microsporidiosis in AIDS patients by recovery of spores from faeces. Lancet 336:267-268, 1990.
8. Orenstein J M, Zierdt W, Zierdt C, Kotler DP. Identification of spores of enterocytozoon-bienewisi in stool and duodenal fluid from AIDS patients. Lancet 336:1127-1128, 1990.
9. van Gool T, Snijders F, Reiss P, Schattenkerk J K M E, Weerman M A v d B, Bartelsman J F W M, Bruins J J M, Canning E U, Dankerk J. Diagnosis of intestinal and disseminated microsporidial infections in patients with HIV by a rapid new fluorescence technique. J Clin Pathol 1993;46:694-699.
10. Wanke CA, Mattia AR. Case records of the Massachusetts General Hospital. Case 51-1993. New Engl J Med 1993;329:1946-1954.
11. Rabeneck L, Gyorkey F, Genta RM, Gyorkey P, Foote LW, Risser J M. The role of Microsporidia in the pathogenesis of HIV-related chronic diarrhea. Ann Intern Med 1993;119:895-9.
12. van Gool T, Canning EU, Gilis H, van den Bergh Weerman MA, Eeftink Schattenkerk J K, Dankert J. Septata intestinalis frequently isolated from stool of AIDS patients with a new cultivation method. Parasitology 1994;109 (Part 3):281-9.
13. Giang TT, Kotler DP, Garro ML, Orenstein J M. Tissue diagnosis of intestinal microsporidiosis using the chromotrope-2 R modified trichome stain. Arch Pathol Lab Med 1993;117:1249-51.
14. Weber R, Bryan RT, Owen RL, Wilcox CM, Gorelkin L, Visvesvara GS. Enteric Opportunistic Infections Working Group. Improved light-microscopic detection of

microsporidia spores in stool and duodenal aspirates. New Engl J Med 1992;326:161-6.

15. Orenstein J M, Tenner M, Kotler DP. Localization of infection by the microsporidian *Enterocytozoon bienersi* in the gastrointestinal tract of AIDS patients with diarrhea. AIDS 1992;6:195-7.

## I. IMMUNODEFICIENCY ASSOCIATED MALABSORPTION/DIARRHEA

1. General: Principal forms affecting the GI tract are summarized in Table 5. These can be usefully divided into PRIMARY and SECONDARY forms. No attempt at a complete review is made here. A few major points will be highlighted while AIDS-related bowel disease is covered in a separate section (see below). For a brief general discussion see Kagnoff (Ref. 4, pgs. 127-131).
2. Tissue Examination: It is good practice to keep immunodeficiency states in mind during examination of all small bowel biopsies. Histopathologically, important features to consider include:
  - a. Inflammatory and other general alterations to mucosa.
  - b. Presence of secondary infectious agents. These may be viral, bacterial, fungal, or parasitic. Special stains may be needed (Giemsa, AFB, agent-specific immunoperoxidase, etc.).
  - c. Status of the lymphoid tissue. Special stains to characterize lymphoid tissue (B vs. T cells; IGA, M, G secretion status; CD4, CD8, T cells, etc).
3. Primary immunodeficiency syndromes: The majority is rare. The most important are selective IgA deficiency and Common variable Immunodeficiency. All may be associated with GI infections. Selective Iga deficiency is a congenital disorder in which Iga secretion is absent, but IgM and IgG secretion are present. Accordingly, IgA-containing plasma cells, but not others, are absent in the ucosa. Patients may develop giardiasis, but in general it has milder G.I. effects than common variable immunodeficiency. May be associated with other immunologically based disorders, e.g, celiac disease.

Common variable immunodeficiency (CVI syndrome): This is not a single entity but a group of conditions. With various underlying abnormalities, many of them immune regulatory, including hyperactive T cell suppression or reduced T helper function. CVI appears after infancy and often is first discovered in adulthood. There is variable deficiency of IgA, IgM, and IgG, but IgA and IgM production are typically reduced or absent. Expect mucosa to show reduced or absent plasma cells (H&E); stain for Ig isotypes. Lymphoid nodularity in mucosa can be marked, visible grossly and by x-ray study. Clinical findings are variable. In a detailed study of 109 patients with

CVI, recurrent bacterial illnesses were common to all, 22% had autoimmune disease, 22% had chronic lung disease, 15% cancer, 13% hepatitis, and 9% malabsorption. Diarrhea or a sprue-like illness is a typical finding, and this is commonly caused by giardiasis, but other pathogens (parasites, viruses, etc.) can also be present. Histologically, there can be marked alterations to mucosa with celiac-like villous blunting and absence, features that can improve with eradication of pathogen.

5. Secondary Immunodeficiency. Relative deficiency of humoral and/or cellular immunity can occur in a variety of conditions and situations. Immunodeficiency can result from immunosuppressive therapy, destruction of the immune system, and infections (including AIDS- for which see below). In protein losing enteropathy serum antibody is depleted by escaping into the lumen from the mucosa. Lymphocytes may also be depleted.

## IMMUNODEFICIENCY SYNDROMES AND CONDITIONS GI-Related Disorders (Major Forms)

### PRIMARY IMMUNODEFICIENCY

- Selective IgA deficiency
- Common Variable Immunodeficiency
- X-Linked Agammaglobulinemia (Bruton)
- Thymic hypoplasia(DiGeorge)
- Severe Combined Immunodeficiency Diseases (SCID)

### SECONDARY IMMUNODEFICIENCY

- Drug-Induced (Steroids, Cytotoxic)
- Acquired Immune Deficiency Syndrome (AIDS)
- Other (non-AIDS) infections
- Graft-vs-Host (Post-BM Transplant)
- Malnutrition and Debilitation
- Protein-losing enteropathy (PLE)-associated

### REFERENCES (Immunodeficiency):

1. Ament, M.E., Ochs, H.D.,Davies,S.O. Structure and function of the gastrointestinal tract in primary immunodeficiency syndromes: A study of 39 patients. *Medicine* 52:227-248, 1973.
2. Ament, M.E. and Rubin, C.E.: Relation of giardiasis to abnormal intestinal structure and function in gastrointestinal immunodeficiency syndromes. *Gastroenterology* 62:216-226, 1972.

3. Hermans, P.E., Huizenga, K.A., Hoffman, H.N, et al: Dysgammaglobulinemia associated with nodular lymphoid hyperplasia of the small intestine. *Am. J. Med.* 40:78-89, 1966.
4. Kag n off M.F. Immunology and inflammation of the gastrointestinal tract. In: Sleisinger, M.H. and Fordtran, J.S.: *Gastrointestinal disease*. Philadelphia, Saunders, 1993, 5th Ed., pgs.45-86.
5. Nagura, H., Kohler, P.F. and Brown, W. R.: Immunocytochemical characterization of the lymphocytes in nodular lymphoid hyperplasia of the bowel. *Lab. Invest.* 40:66, 1979.
6. Tytgat, G.N., Huibregtse, K., Schellekens, P.T.A. and Feltkamp Vroom, T.H.: Clinical and immunologic observations in a patient with late onset immunodeficiency. *Gastroenterology* 76:1458, 1979.
7. Yardley, J.H. and Hendrix, T.R. Immunologic status in patients with giardiasis. *Gastroenterology* 78:421, 1980.
8. Cunningham-Rundles C. Clinical and immunologic analysis of 103 patients with common variable immunodeficiency. *J Clin Immunol* 1989;9:22-33.

\*Much if not most of the information in this handout was originally compiled by John H. Yardley, M.D.