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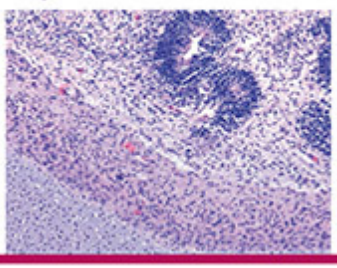

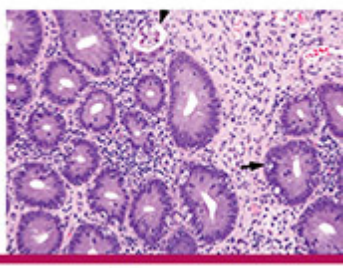

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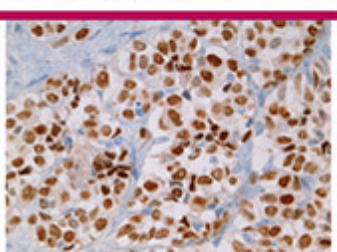

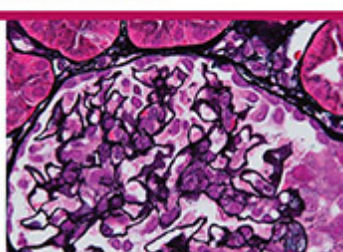

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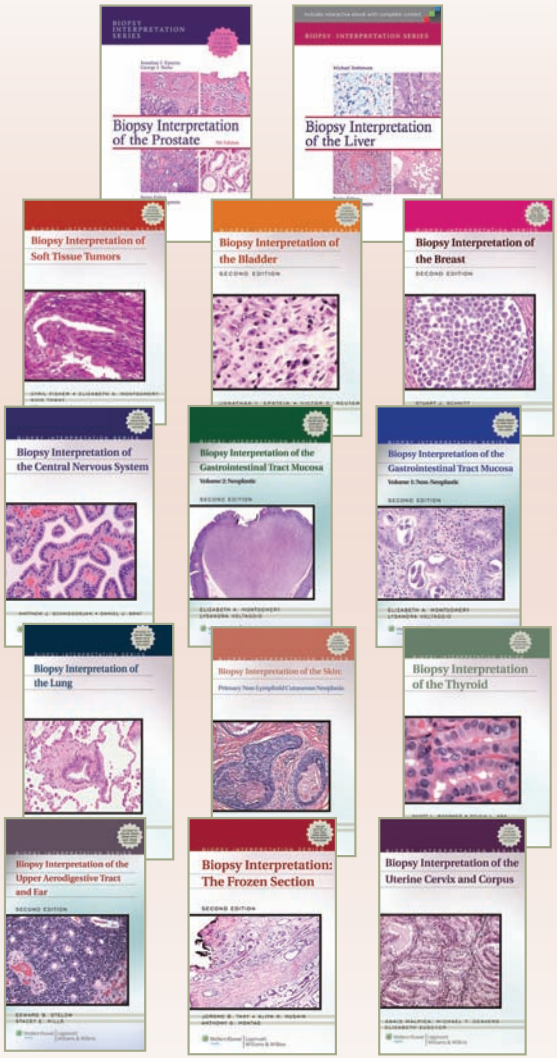

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For Ameena, my first one, whose early life experiences led me to do pediatric pathology.

For Ayesha, whose energy, enthusiasm, and organization are inspiring.

And for Omar, who keeps us all smiling.

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PREFACE

Many of the complex pediatric cases are treated in specialized children's hospitals; however, more and more children are first being diagnosed at general facilities. Thus, most practicing pathologists are dealing with pediatric specimens but not on a daily basis. Although there are some similarities between adult and pediatric biopsies, there are also significant differences. Many adult diseases do not or only very rarely occur in children, whereas the common pediatric lesions are uncommon in the adult. The spectrum of infectious organisms; types of tumors; and autoimmune, renal, pulmonary, cutaneous, gastrointestinal, and neurologic diseases have little overlap. The small size of the patient influences the type of biopsy that is possible; thus, triaging the tissue and use of judicious stains are critical.

This book addresses the common and not so common diseases that can be diagnosed by biopsy. The differential diagnosis and use of special techniques are emphasized. The latter include histochemical and immunohistochemical stains, electron microscopic examination, and molecular tests that one needs to do on limited samples. The appropriate tests need to be selected in view of the clinical setting, material available, and histologic findings on hematoxylin and eosin stain.

This book is organized by chapters devoted to each organ system. Experts in their fields have written each one and provided multiple illustrations. Advanced trainees and diagnostic pathologists will find it helpful to have a framework for handling pediatric biopsies in one text.

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Aliya N. Husain, MD

CONTENTS

1

GASTROINTESTINAL SYSTEM

Margaret H. Collins, MD, J. Todd Boyd, DO, and Rachel Sheridan, MD

The number of gastrointestinal (GI) tract endoscopies performed on children increased significantly over the past several decades. Among the reasons for the increase are the development of smaller and more flexible endoscopes, improved pediatric anesthesia, and increased awareness of diseases affecting the GI tract in children.

Not surprisingly, since pediatric GI endoscopies have become more common, the number of pediatric GI biopsies has also increased. This chapter presents the pathology of GI tract diseases found in endoscopic mucosal biopsies obtained from children emphasizing pathology more commonly encountered among children than adults. The first part of the chapter discusses diseases according to the affected anatomic site, and the second part of the chapter presents diseases organized by topic. For more global reviews of GI pathology—including, for example, epithelial carcinomas that are rarely encountered in children—and for pathology of resected GI specimens, the reader is referred to appropriate standard texts.

ESOPHAGUS

Gastroesophageal reflux is physiologic in both infants and adults. However, in some patients, reflux is associated with symptoms such as heartburn and chest pain and is then considered nonphysiologic, constituting gastroesophageal reflux disease (GERD).[1] Biopsies from patients who have GERD may be histologically normal or may show a variety of abnormalities such as erosions, acute and chronic inflammation, etc. Eosinophils are generally absent or present in small numbers. (See the following discussion of eosinophilic esophagitis versus GERD and proton pump inhibitor [PPI]–responsive esophageal eosinophilia.)

Esophageal infections occur mainly in immunocompromised patients, including those who receive chemotherapy or immunosuppressive therapies, most commonly in the setting of solid organ or bone marrow transplantation.[2] *Candida* esophagitis is the most common esophageal infection in immunocompetent hosts and may be the initial presentation of

disseminated disease in immunocompromised patients.[2] *Candida albicans* accounts for most cases, but other *Candida* species are also implicated. Focal or confluent white patches overlying an ulcerated mucosa are seen on endoscopy. Esophageal brushing or biopsy with culture confirms the diagnosis. In biopsies, budding yeasts and pseudohyphae are seen on the surface or in surface or deeper epithelium, associated with inflammation (Fig. 1.1). Fungal stains may demonstrate the organisms. Fungal esophagitis may coexist with viral esophagitis in immunocompromised patients.

Herpes simplex virus (HSV) esophagitis occurs primarily in immunocompromised patients, who may have life-threatening disease at the time of diagnosis.[2] In immunocompetent patients, the infection is self-limited and resolves spontaneously in 1 to 2 weeks.[3] Concomitant herpes labialis or oropharyngeal ulcers may be present. Symptoms include odynophagia, dysphagia, fever, and epigastric pain. Coalescent superficial ulcers with exudate are seen on endoscopy. Culture, polymerase chain reaction (PCR), or in situ hybridization confirms the diagnosis. In biopsies, ulcers and acute inflammatory exudate are present. Viral cytopathic effects are present in the squamous epithelium and include multinucleated cells, ground-glass nuclei, and dense eosinophilic inclusions with a thickened nuclear membrane and clear halo (Cowdry type A inclusions), which are best identified at the edge of the ulcer (Fig. 1.2).

Cytomegalovirus (CMV) esophagitis affects primarily immunocompromised patients who frequently have multisystem involvement. Symptoms and endoscopic findings are similar to HSV esophagitis. Viral cytopathic changes are best seen in endothelial and stromal cells deep within the ulcer base rather than at the edges and include basophilic intracytoplasmic inclusions

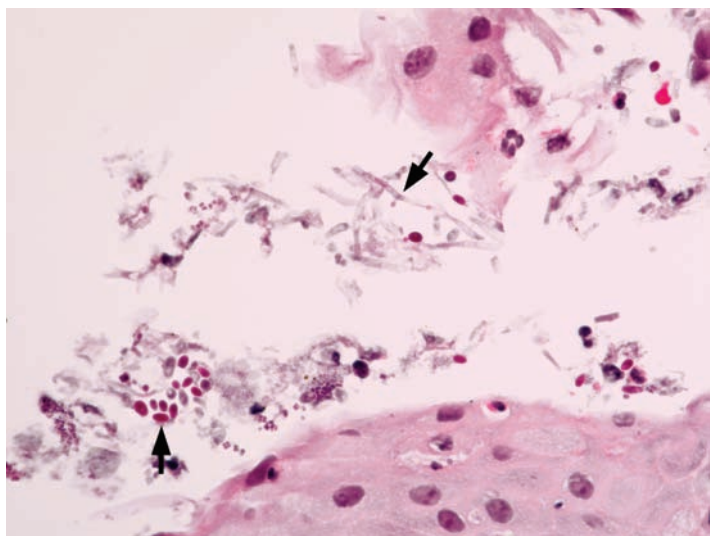


FIGURE 1.1 ***Candida* esophagitis.** *Arrows* point to pseudohyphae and yeast.

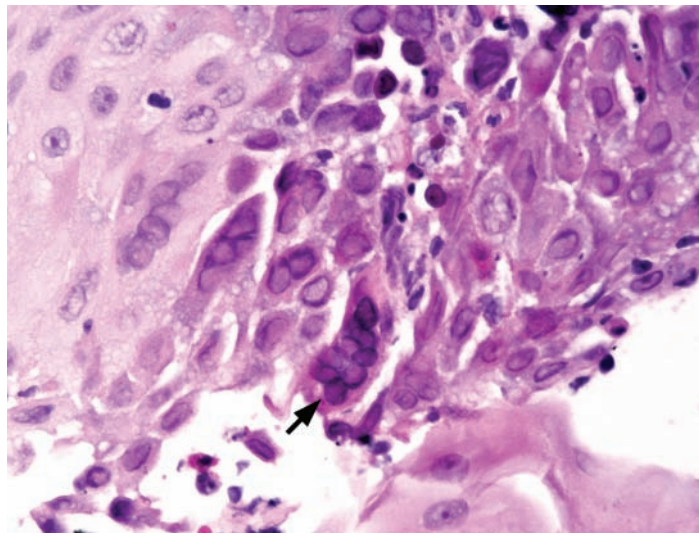


FIGURE 1.2 **Herpes esophagitis.** *Arrow* points to multinucleated cell showing viral cytopathic effect.

and the characteristic intranuclear "owl's eye" CMV inclusion. Viral inclusions may be rare or atypical. Diagnosis is confirmed by biopsy because cultures may reflect latent infection and not necessarily active disease.[2,4]

STOMACH

Children who have *Helicobacter pylori* infection may have high-grade gastritis[5] (Fig. 1.3). At least four biopsies, two from the antrum and two from the body, are recommended to increase the diagnostic yield because the infestation may be patchy.[6] Special stains and immunohistochemistry may be helpful to identify the organisms in biopsies, and in some cases, there are sufficient numbers of bacteria to be recognized on hematoxylin and eosin (H&E) stain (Fig. 1.4). The urease/*Campylobacter*-like organism (CLO) test is frequently positive in infected patients. Children may also have infections with *Helicobacter heilmannii* organisms.[7]

DUODENUM

Celiac disease (CD) is an immune-mediated enteropathy that is triggered by ingestion of gluten.[8] The population prevalence of CD is approximately 1% in the United States and 1.5% in Scandinavia and the United Kingdom.[9] CD occurs with higher frequency in certain populations, including patients who have immune disorders including diabetes mellitus, certain chromosomal abnormalities such as trisomy 21, selective immunoglobulin A (IgA) deficiency, etc.[10]

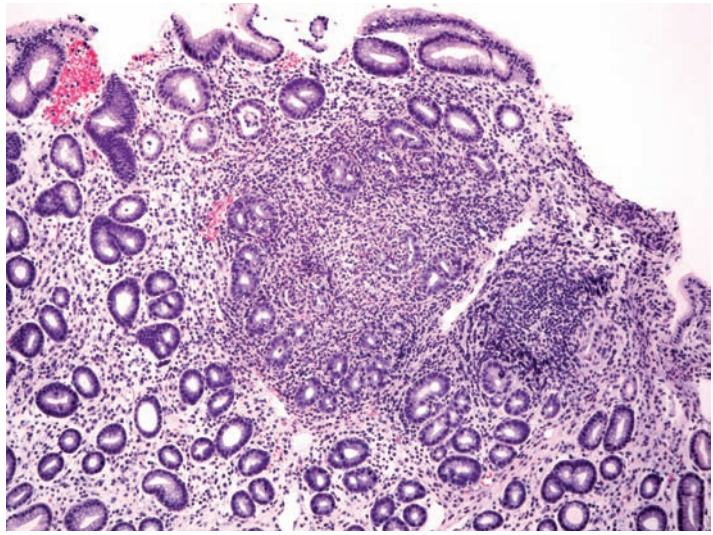
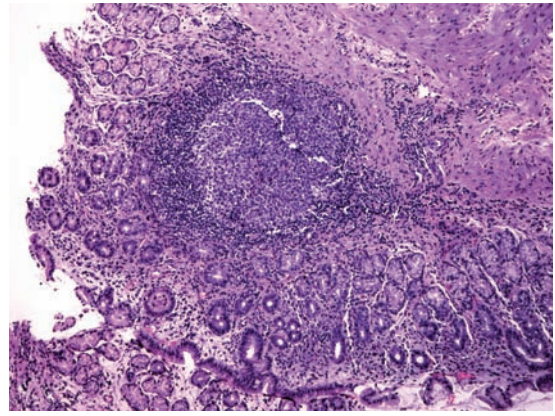


FIGURE 1.3 The differential diagnosis for the active gastritis illustrated in this photograph includes *H. pylori* gastritis; this patient, however, had UC. The presence of upper tract disease does not exclude a diagnosis of UC in children.



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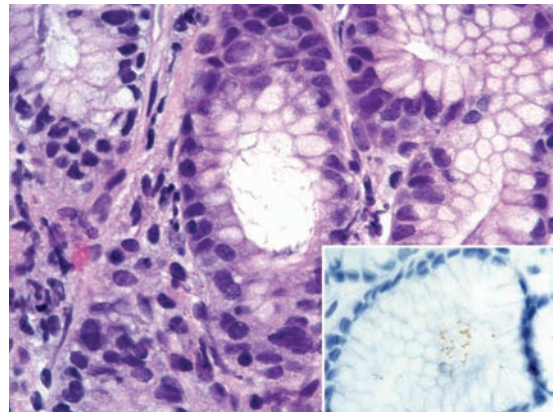

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FIGURE 1.4 **A:** Lymphoid follicles, as seen in this antral biopsy, correlate with the nodular gross appearance seen at endoscopy in patients with *H. pylori* gastritis. **B:** Numerous *H. pylori* organisms are seen in the lumen of the gland at the center of the photograph, and organisms stained with *H. pylori* antibody are seen in the *inset*.

Serologic tests may be helpful to evaluate patients suspected to have CD. Antigliadin (IgA) antibodies are the first autoantibodies to appear following intestinal exposure to gluten[11] but are diagnostically useful only in children younger than age 18 months. Tissue transglutaminase (tTGA) and endomysial antibodies (EMA) are the most sensitive antibody tests detecting IgA-class antibodies. Patients who have IgA deficiency should have immunoglobulin G (IgG) class CD-specific antibodies (IgG tTGA or IgG EMA) tests.[10] Most patients who have CD have HLADQ2/8 haplotype.

Duodenal biopsies from children and adults who have CD show similar changes, except that neutrophils are more prevalent and abundant in biopsies from children.[12] The pathologic changes may be patchy, of variable severity, and in some cases, restricted to the duodenal bulb.[10] The pathology report should include evaluation of biopsy orientation, degree of villous atrophy and crypt elongation, the villous–crypt ratio, number of intraepithelial lymphocytes (IEL), and grade based on Marsh-Oberhuber classification[10,13] (Fig. 1.5).

IEL are increased in CD duodenal biopsies. In normal duodenum, IEL are more prominent along the lateral edges of villi, decreasing from the base to the tip, the so-called decrescendo pattern. The original threshold value for CD diagnosis was 40 IEL per 100 enterocytes.[14]

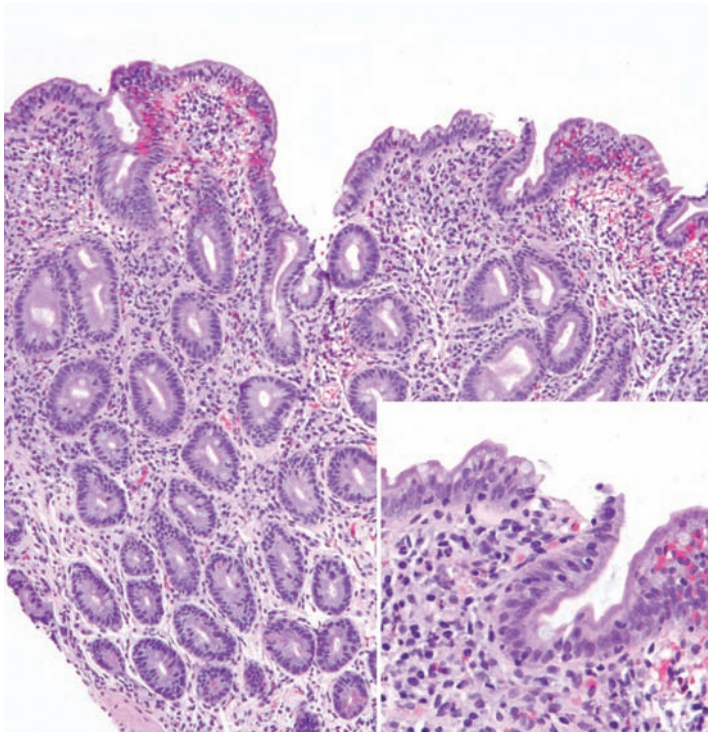


FIGURE 1.5 This duodenum biopsy from a patient with CD displays moderate villous blunting with increased IEL (Marsh-Oberhuber classification type 3b) better seen in the *inset*.

More recently, 25 or more IEL per 100 enterocytes are considered abnormal[11] (Table 1.1). Counting IEL per 20 epithelial cells at the tips of five villi or IEL per 50 enterocytes at the tips of two villi may substitute for counts per 100 enterocytes.[9] Immunohistochemistry to count IEL has been advocated, but it is not common practice.[15]

The histologic features of CD are not pathognomonic, and the diagnosis must be confirmed by response to gluten-free diet. The differential diagnosis includes cow's milk allergy, immunodeficiency, drug reaction, and infections.[10,16] eFigure 1.1 illustrates small bowel pathology in cystic fibrosis.

TABLE 1.1 Comparison of Marsh-Oberhuber and Corazza Classifications of Duodenal Biopsies in Celiac Disease

Marsh-Oberhuber	Morphology	Villi and Crypts	IEL/100 Enterocytes
Type 0	Normal mucosa	Both normal	>40
Type 1	Increased IELs	Both normal	>40
Type 2	Increased IELs	Villi normal and hyperplastic crypts	>40
Type 3a	Increased IELs	Mild villous blunting; crypts hypertrophic	>40
Type 3b	Increased IELs	Moderate villous blunting; crypts hypertrophic	>40
Type 3c	Increased IELs	Severe (flat) villous blunting; crypts hypertrophic	>40
Type 4	Increased IELs	Severe (flat) villous blunting; crypts atrophic	>40
Corazza	**Morphology**	**Marsh-Oberhuber Corresponding Classification**	**IEL/100 Enterocytes**
Grade A	Normal architecture	Type 1 and type 2 Type 0 deleted	>25
Grade B1	Atrophic, V:C ratio less than 3:1	Type 3a and type 3b	>25
Grade B2	Atrophic, villi absent	Type 3c Type 4 deleted	>25

IEL, intraepithelial lymphocytes; V:C, villous to crypt.

Modified from Walker MW, Murray JA. An update in the diagnosis of celiac disease. *Histopathology*. 2011;59:166–179; Robert M. Gluten sensitive enteropathy and other causes of small intestinal lymphocytosis. *Semin Diagn Pathol*. 2005;22:284–294.

ILEUM

Infections that are associated with granulomatous inflammation, including infections with organisms such as *Histoplasma*,[17] *Mycobacterium*,[18] and *Yersinia*,[19] may occur in the terminal ileum and mimic Crohn disease. Special stains and/or microbiologic culture may be helpful to correctly identify the causative agents.

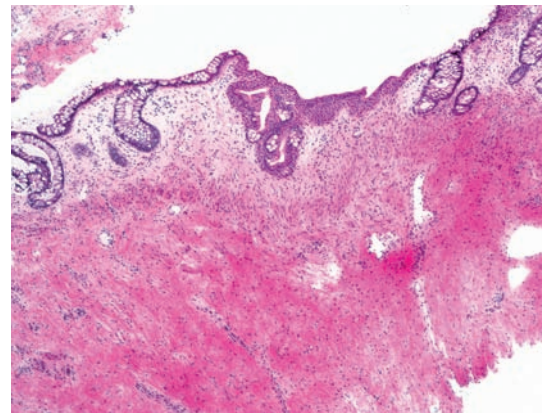
COLON

Hirschsprung disease (HD), a major genetic cause of functional intestinal obstruction with an incidence of 1 per 5,000 live births, is characterized by absence of intramural ganglion cells in the myenteric (Auerbach) and submucosal (Meissner) plexuses in the rectum and, in many cases, varying lengths of bowel proximal to the rectum.[20] HD may be familial; associated with syndromes such as trisomy 21, multiple endocrine neoplasia type 2, Waardenburg syndrome, etc.; or associated with other anomalies such as congenital heart disease, malrotation, genitourinary anomalies, etc.[21] Genes that have been implicated in isolated HD include *RET* and *EDNRB*.[21,22]

HD can be classified, according to the extent of aganglionic bowel, as short segment (SSHD, 80% of HD) and long segment (LSHD, 20% of HD).[21] Rarely, HD can involve the entire colon (total colonic aganglionosis) or entire bowel (total intestinal HD). The strong male preponderance in SSHD patients significantly decreases with more extensive aganglionosis.[23] Ultrashort segment HD involving the distal rectum[24] below the pelvic floor and aganglionic colon proximal to a normal segment of colon[25] have also been reported.

HD most commonly presents in newborns as failure to pass meconium, abdominal distension, bilious vomiting, or neonatal enterocolitis but may also be manifest as chronic constipation, abdominal distension, failure to thrive, and recurrent enterocolitis in older children (eFig. 1.2). In affected patients, contrast enema shows dilated proximal colon with narrowing toward the distal aganglionic bowel.[26]

The diagnosis of HD requires demonstration of the absence of ganglion cells from the rectum—a negative characteristic. The diagnosis is most commonly made in rectal biopsies[20,27] and must be confirmed by careful examination of the distal end of the resected bowel following surgical therapy. Rectal suction biopsies (RSB) procurement has low morbidity, does not require general anesthesia, and is preferred for neonates and young children.[28] However, interpretation of RSB is more challenging than full-thickness surgical biopsies because RSB contain only the superficial portion of the submucosa and not the more abundantly ganglionated deep submucosal and myenteric plexuses. Ideally, RSB measure at least 3 mm in diameter and contain an adequate amount of submucosal tissue that has at least the same thickness as the overlying mucosa.[20] In order to



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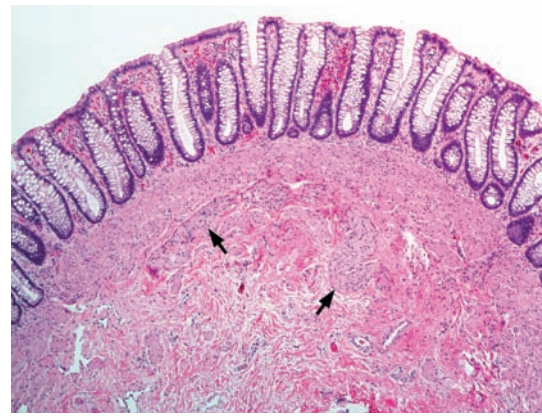

B

FIGURE 1.6 **A:** A specimen submitted for evaluation for HD may be inadequate for diagnosis even though there appears to be a lot of tissue: Transitional anal mucosa is seen on the surface of this biopsy indicating that the biopsy was obtained in the very distal rectum that is normally hypoganglionic and might lead to an incorrect diagnosis of HD. **B:** In contrast, this biopsy shows only colonic mucosa. In the submucosa, hypertrophic nerves are seen *(arrows)* but not ganglion cells.

avoid false-positive results, RSB should be obtained 2 to 3 cm proximal to the pectinate line because the distal 1 to 2 cm of rectum is normally hypoganglionic.[29] Therefore, a specimen containing squamocolumnar junction or transitional anal epithelium (Fig. 1.6) should be considered inadequate for evaluation if ganglion cells are not seen. RSB obtained after an initial inadequate specimen are often of better quality. Nevertheless, transmural rectal biopsies may be required in some cases.

There are various methods to histologically evaluate RSB for ganglion cells (Fig. 1.7). Most laboratories use H&E stains of serial sections, commonly over 50 but up to several hundred, or all sections obtained to exhaust the paraffin block.[30] If H&E stains only are used, numerous serial sections must be examined in order to establish the negative characteristic that a normal component of the tissue—ganglion cells—is absent from the tissue. Submucosal hypertrophic nerves (>40μ in diameter) are present in 90% of rectal biopsies from patients with HD and support the diagnosis[30,31] (see Fig. 1.6B). However, the original measurements were

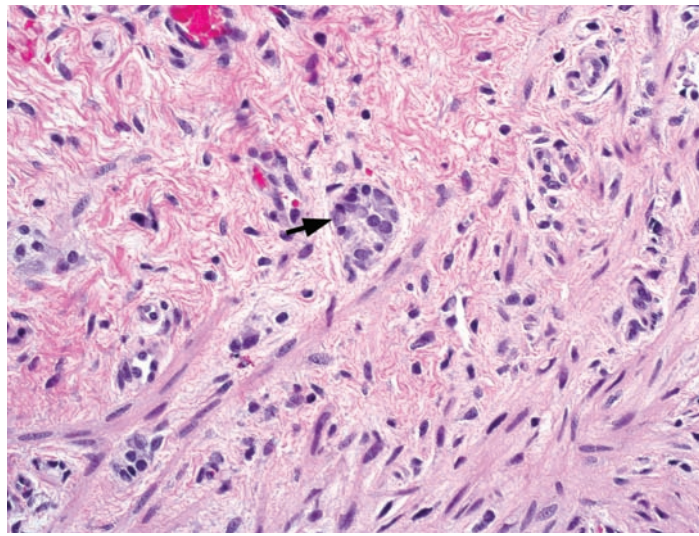

FIGURE 1.7 A cluster of ganglion cells *(arrow)* is seen in the submucosa of this formalin-fixed rectal biopsy from a 4-day-old infant.

obtained from infants and young children[32] and may be less suitable for older children.[33]

Some laboratories use alternative methods to establish the diagnosis of HD, and some use a combination of serial H&E sections and other stains. The most widely used stain other than H&E is acetylcholinesterase (AChE) histochemistry, which requires frozen tissue. Most laboratories that use AChE on RSB request several pieces to freeze one or more pieces for AChE stains and to fix one or more pieces in formalin; transmural biopsies may be divided with half frozen and half fixed in formalin. In our institution, a series of alternating H&E and AChE stains are prepared on pieces that are frozen and H&E stains are performed on pieces that are fixed in formalin. In a laboratory with experience performing AChE stains and with adequate biopsies, AChE stains combined with H&E stains have been shown to provide a diagnostic yield of 99%.[34] The advantage of AChE histochemistry is that it provides a positive finding (Figs. 1.8 to 1.11). In HD, AChE stains identify increased numbers of abnormally thick nerve fibers in the muscularis mucosa and lamina propria that are not seen on H&E stains. Conversely, normal RSB display relatively sparse, delicate AChE-positive fibers limited to the deep muscularis mucosa.

Nonetheless, interpretation of AChE stains is highly dependent on experience interpreting the stain and proper staining technique. Potential pitfalls are the lack of abnormal AChE pattern and absence of submucosal hypertrophic nerves in patients with total colonic aganglionosis,[35] equivocal AChE pattern in patients with trisomy 21,[36] and low sensitivity of

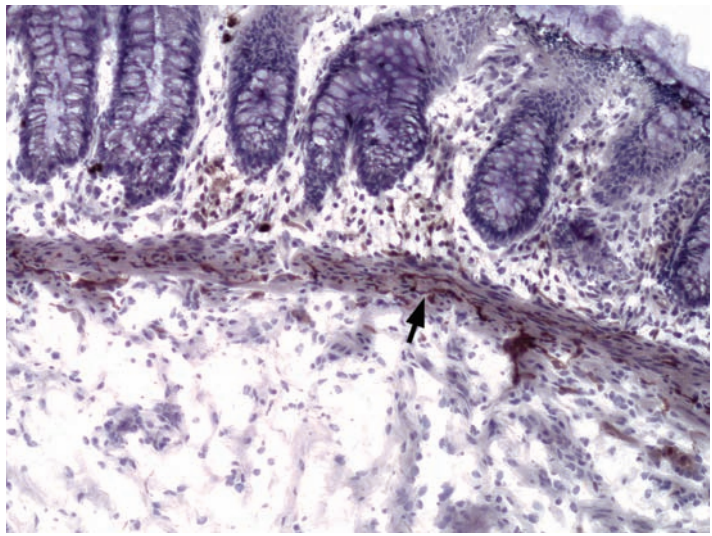

FIGURE 1.8 AChE stain on a normally ganglionated rectal biopsy shows delicate nerve fibers *(arrow)* in the muscularis mucosa.

AChE in biopsies from neonates.[34] Different diagnostic patterns encountered in RSB are summarized in Table 1.2.

Antibody to the calcium-binding protein calretinin stains delicate nerve fibers in the lamina propria of ganglionated rectal biopsies but not biopsies from HD—a negative finding[37–39] (Figs. 1.12 and 1.13).

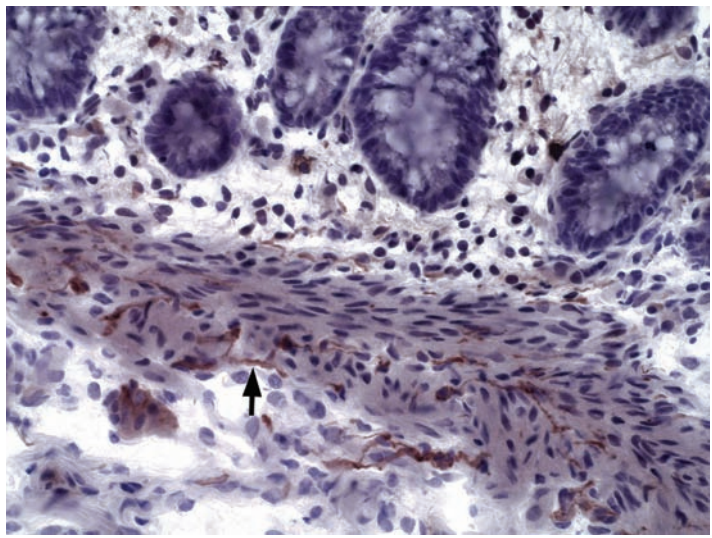

FIGURE 1.9 Higher power view of normal nerve fibers *(arrow)* in the muscularis mucosa of a normally ganglionated rectal biopsy.

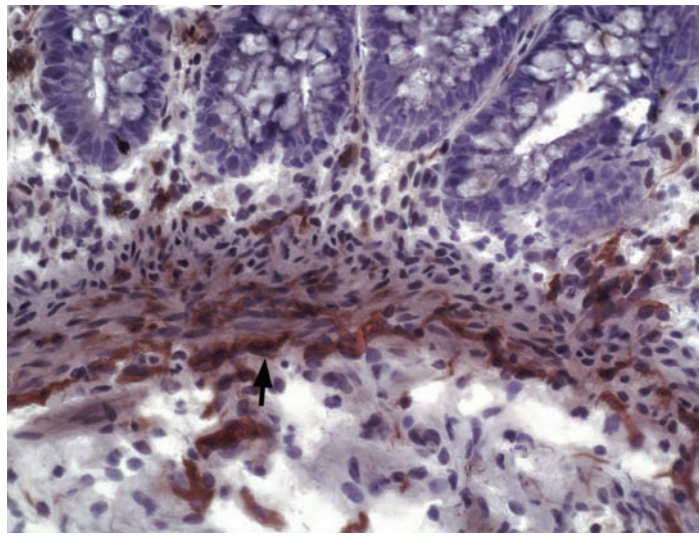

FIGURE 1.10 In contrast, AChE reaction demonstrates thick ropey nerves *(arrow)* in the muscularis mucosa from this rectal biopsy from a patient who has HD.

More studies evaluating the clinical use of this stain are required, but some advantages over AChE reaction are easier reproducibility and interpretation and its use in paraffin-embedded tissues. Currently, calretinin immunohistochemistry is an adjunct to and not a substitute for traditional methods of examining RSB.

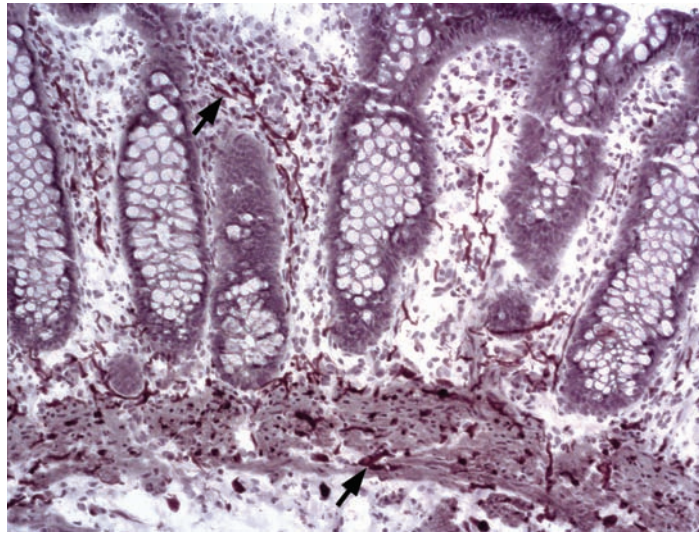

FIGURE 1.11 AChE stains numerous nerve fibers in the muscularis mucosa *(arrows)* and in the lamina propria including near the surface in this RSB from a patient with HD.

TABLE 1.2 Diagnostic Approach to Adequate[a] Rectal Suction Biopsies

Stain and Biopsy Feature				
Hematoxylin and Eosin		Acetylcholinesterase		
Ganglion Cells	Submucosal Nerves	Muscularis Mucosa/Lamina Propria Nerve Fibers	Interpretation	Comment
Present	Normal	Normal	Normal biopsy	Seek other causes for signs and symptoms.
Absent	Hypertrophic	Abnormal	Abnormal biopsy, consistent with HD	Proceed with definitive surgical therapy; confirm diagnosis at distal end of resected bowel.
Present	Hypertrophic	Normal or abnormal	Abnormal biopsy	Differential diagnosis includes HD TZ, mucosal ganglioneuromatosis
Absent	Normal	Equivocal or abnormal	Abnormal biopsy; abnormal calretinin may be helpful	Differential diagnosis includes HD TZ, LSHD, and TCA. Repeat biopsy may be necessary.
Absent	Normal	Normal	Abnormal biopsy; normal calretinin may be reassuring	Differential diagnosis includes normal biopsy if calretinin is normal, HD TZ

[a]Biopsy that includes submucosa at least as thick as the overlying mucosa does not show squamous or anal transitional epithelium, obtained from a site that permits recognition of short-segment disease.

HD, Hirschsprung disease; TZ, transition zone; LSHD, long-segment Hirschsprung disease; TCA, total colonic aganglionosis.

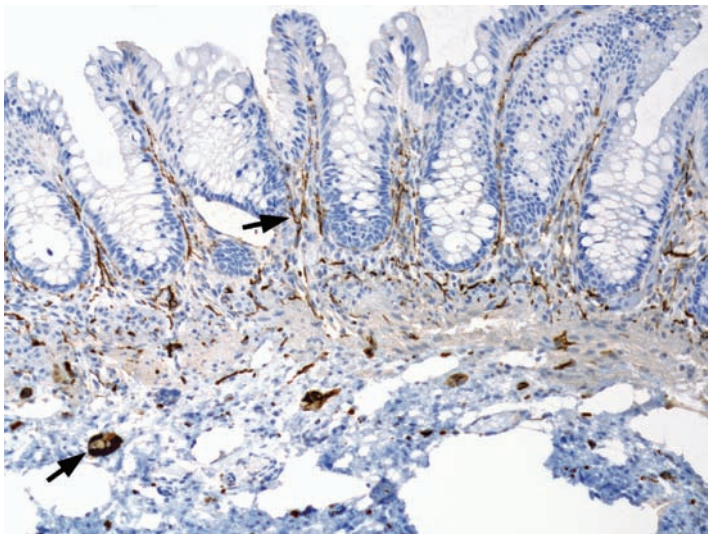

FIGURE 1.12 Calretinin antibody decorates delicate lamina propria nerve fibers *(arrow)* in the lamina propria from this rectal biopsy in a normal pattern.

HD therapy is surgical resection of the aganglionic bowel and creation of a neorectum that includes ganglionic bowel. Definitive surgical procedures (so-called pull-through operations) are performed if RSB or deeper rectal biopsies suggest the diagnosis and should be guided by intraoperative frozen section consultations to identify normally innervated bowel to create the neorectum. In HD, a transition zone occurs between aganglionic bowel and histologically normal

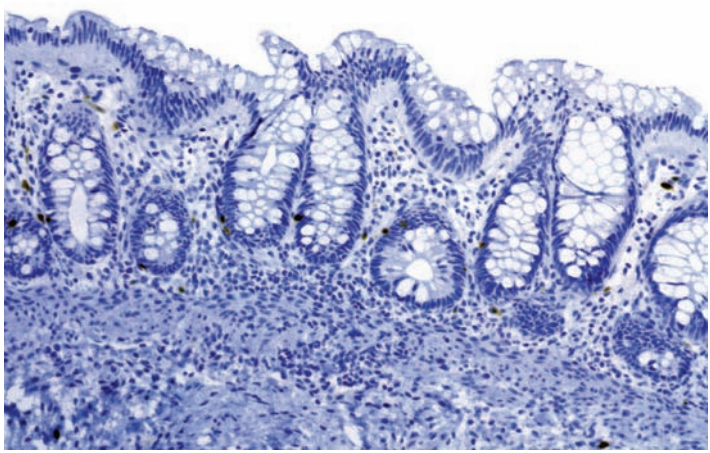

FIGURE 1.13 Nerve fibers in the lamina propria are not seen in this rectal biopsy from a patient who has HD that was stained with calretinin antibody. Stained cells are mast cells.