13.1 Introduction

It has been well known since the reports of Dalla-Valle [6, 7] that the most characteristic feature of Hirschsprung’s disease (HD) is the absence of ganglion cells in the narrowed segment. The aganglionic segment starts at the anal ring and extends proximally for a variable length. A reliable diagnosis of HD on a hematoxylin-eosin (H&E) staining of a mucosal biopsy requires considerable experience. There is the risk of rendering a false-positive diagnosis of HD in cases of hypoganglionosis. A diagnosis of an ultrashort Hirschsprung segment of less than 3–4 cm in length above the anal ring cannot be established by an H&E staining or immunohistochemical reaction. In contrast to immunohistochemistry or H&E staining, the introduction of the enzyme histochemical acetylcholinesterase reaction (AChE) has made the morphological diagnosis of HD easier and more reliable [12, 19, 20, 31].

In addition, the parallel use of lactate dehydrogenase (LDH) enzyme histochemistry allows confirmation of hypoganglionosis of the submucous plexus because nerve cells can be fairly electively stained with a LDH reaction. Therefore, without an appropriate dehydrogenase reaction there is a risk of incorrect positive or negative results. The routine use of LDH and succinic dehydrogenase (SDH) reactions [3, 24, 30, 36] which allow the visualization of ganglia and nerve cells in the submucous and myenteric plexus has made it possible to detect other abnormalities of colonic innervation with symptoms of HD [15, 20, 26, 36, 37].

With an NADH diaphorase or LDH reaction a rapid assessment of the myenteric plexus is possible and can be performed intraoperatively during colon resection. It is important for the surgeon to determine if the oral resection border consists of an abnormally innervated or normally innervated colon. In the course of such a quick intraoperative investigation, the intensity of the staining can be continuously monitored and the incubation time kept to the minimum necessary for a reliable diagnosis (8–10 minutes). In contrast to immunohistochemistry, enzyme histochemistry offers remarkable flexibility and a much faster result. Today, enzyme histochemical kits are commercially available (Districhem, Oberwil, Switzerland, and Bio-Optica, Milan, Italy).

The aim of this chapter is to demonstrate the enzyme histochemical characteristics of HD and its differential diagnosis from other functional anomalies of intestinal innervation showing symptoms of HD [26].

13.2 Hirschsprung’s Disease

HD is characterized enzyme histochemically by the following easily identifiable features:
1. Absence of nerve cells in the submucous and myenteric plexus (ganglion cells are visualized by an LDH reaction (Fig. 13.1)); and
2. Typical increase in AChE activity in the parasympathetic nerve fibers of the lamina propria mucosae (Fig.
**Fig. 13.1**  
**a** Normal myenteric plexus.  
**b** Aganglionosis with empty plexus cleft (LDH enzyme histochemistry, ×150)

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**Fig. 13.2a,b**  
Acetylcholinesterase (AChE) reaction in a suction biopsy of rectal mucosa.  
**a** Normally innervated rectum mucosa without AChE activity in parasympathetic nerve fibers.  
**b** HD. Aganglionic rectum mucosa with characteristically increased AChE activity in parasympathetic nerve fibers of lamina propria mucosae (AChE reaction without counterstaining, ×90)