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Chapter 2

Delayed passage of meconium;
clinical implications and pathophysiology

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submitted

General introduction

In healthy term infants, the passage of meconium occurs in 95% within 24 hours and 99% within 48 hours of birth^{1,2}. This percentage however dramatically drops to 66% in preterm (≤ 32 weeks gestation) and low birth weight (< 2500 grams) infants^{1,2}. Most likely, this delay simply reflects impaired maturation, but from a clinical point of view, it should alert the clinician as it may be the first sign of a defecation disorder.

Especially in premature infants, delayed maturation of the motor mechanisms of the gut might play a role. In this fragile group of infants delayed passage of meconium may cause significant morbidity (intestinal perforation) and even mortality. At first instance, a 'wait and see policy' is often used in these infants. However, if these infants develop feeding problems and abdominal distension, enemas and/or laxatives are given to relieve colonic distension and will be continued until a normal defecation pattern has been developed. However, in those infants who do not develop a normal defecation pattern in the following weeks, Hirschprung's disease (HD) should be excluded.

To date, the development of colorectal motility is poorly understood in preterm infants. It is suggested that preterm infants may have a delayed transit of luminal contents through the colon, however colonic motility has never been directly assessed in these infants³⁻⁵. Recently, a study demonstrated that delayed passage of meconium may result from a delayed maturation of interstitial cells of Cajal⁶. Interstitial cells of Cajal function as pacemaker cells coordinating the electro-mechanical activity of the gut^{7,8}.

Factors other than motility may also contribute to the delay in meconium passage. The meconium of premature infants differs in composition (glycoprotein, saccharides, calcium, copper, iron and phosphorus), making it more viscous and thus more difficult to be expelled⁹.

Importantly however, delayed passage of meconium may result from the absence of the enteric nervous system, as typically described in HD. This is a developmental disorder of the enteric nervous system characterized by the absence of ganglion cells along a variable portion of the distal intestine starting from the internal anal sphincter. More than 90% of the infants with HD fail to pass meconium within 24 hours after birth. If unrecognised, HD will lead to severe constipation, abdominal distension, enterocolitis, toxic megacolon and even death. Therefore it is of clinical

importance especially in these young infants to be alert and to identify this disorder in an early stage.

To diagnose HD, anorectal manometry evaluating the rectoanal inhibitory reflex (RAIR) can be performed. Two other tests are also employed in the diagnostic work-up of patients suspected for HD. At contrast enema of the colon, a calibre change with a dilated normal colon to a narrowed aganglionic bowel is typically present. Lastly, a rectal suction biopsy is taken to evaluate cholinesterase activity and the presence or absence of enteric neurones^{2,10}.

Finally, delayed passage of meconium might also be the first sign of functional constipation. This is a common disorder and symptoms vary from a relative mild short-lived bowel problem to severe chronic constipation with faecal impaction and encopresis. The pathophysiology underlying functional constipation is undoubtedly multifactorial, and not well understood. Clearly, functional constipation can result from abnormal function of the different players involved, including the colon, the rectum and the sphincter complex. No specific organic cause can be found in approximately 90% of the infants¹¹. Compared to infants with Hirschsprung's disease, infants with functional constipation often demonstrate a milder clinical pattern in most cases responsive to laxative treatment. We will not further discuss functional constipation in this chapter but rather focus on the latest findings regarding the pathophysiology, symptomatology, diagnostic workup and treatment of Hirschsprung's disease in infancy and childhood.

Hirschsprung's disease

HD is a developmental disorder of the enteric nervous system (ENS) characterized by the absence of ganglion cells along a variable portion of the distal intestine. This results in a functional obstruction caused by dysmotility of the diseased segment, lack of propagation of peristaltic waves into the aganglionic colon, and an abnormal or absent relaxation of this segment and of the internal anal sphincter^{12,13}.

Clinical presentation

Approximately 80 to 90% of all cases of HD have typical clinical symptoms and are diagnosed during the neonatal period. Delayed passage of meconium is the cardinal symptom in neonates with HD. Indeed over 90% of patients fail to pass meconium within 24 hours after birth¹³. This is in strong contrast with healthy neonates who pass meconium within 24 hours in 95% of the cases.

The usual presentation of HD in the neonatal period is constipation, abdominal distension and vomiting during the first days of life. However, about a third of the babies with HD present with diarrhoea without obvious distension and vomiting. In these cases, one should be alert as diarrhoea in HD is in the majority of cases a symptom of enterocolitis. The latter remains the commonest cause of morbidity and mortality in this disease. Adequate therapy is necessary to prevent toxic megacolon, a life threatening condition characterized by the sudden onset of marked abdominal distension, bile-stained vomiting, fever and signs of dehydration, and shock.

In many cases of HD a rectal examination causes passage of meconium and relief of the acute intestinal obstruction. These babies may have normal bowel movements for a few days or weeks and then present again with signs and symptoms of intestinal obstruction. In contrast to children with functional constipation,

Table 1. Presenting symptoms: chronic constipation versus classic Hirschsprung's disease.

Symptoms	Chronic constipation	Hirschsprung's disease
Encopresis	Common	Rare
Abdominal pain	Occasionally	Common
Stool size	Large	Normal
Failure to thrive	Rare	Common
Stool-withholding behaviour	Common	Rare
Abdominal faecal mass	Common	Rare
Ampullary faeces	Common	Rare
Anorectal examination	Dilated ampulla	Narrow
Enterocolitis	Never	Frequent (20 – 58%) ⁶⁵

encopresis is often absent in children with HD (table 1). On the other hand, in a minority, HD may be manifested as chronic constipation with or without abdominal distension, manageable with changes in feeds, laxatives, suppositories or enemas.

From the above, it is clear that early differentiation between HD and functional constipation is necessary to prevent severe complications of HD.

Epidemiology and genetics

HD is a rare disorder and its incidence is estimated to be approximately 1 in 5000 live births with a male to female ratio of 4:1¹³. The majority of children with HD (93%) are born a term with a normal birth weight¹³.

The length of the affected segment varies and appears to determine the recurrence risks and mode of inheritance. Short-segment or classical HD, involving the rectum and sigmoid accounts for 75-80% of cases¹⁴⁻¹⁶.

In the remainder of cases, the aganglionic segment extends more cranial and may even affect the entire colon. In case of total colonic aganglionosis, a familial incidence of 15% to 21% has been reported increasing up to 50% in total intestinal aganglionosis¹⁵. As the length of aganglionic segment increases, the recurrence risk to siblings increases¹⁴.

Although genetic factors are definitely involved, there is no clear pattern of inheritance and most investigators have found a sex-modified multifactorial mode of inheritance¹³. So far, eight genes are known to be involved in patients with HD, namely the proto-oncogene RET (RET), glial cell line derived neurotrophic factor (GDNF), neuroturin (NTN), endothelin B receptor (EDNRB), endothelin 3 (EDN3), endothelin converting enzyme 1 (ECE1), SOX10, and SIP1 genes (table 2).

Many studies indicate the receptor tyrosine kinase (RET) as the major susceptibility gene for HD¹⁷⁻¹⁹. Currently, approximately 50 RET mutations have been reported in HD patients accounting for 50% of familial and 15-20% of sporadic cases of HD¹⁷⁻¹⁹. RET mutations are also associated with multiple endocrine neoplasia type 2 (MEN2), and medullary thyroid carcinoma^{20,21}.

In the majority (70%) however, HD occurs as an isolated trait (non-syndromatic HD). Association with chromosomal abnormalities, other birth defects, and syndromes with Mendelian patterns of inheritance occurs in 30% (syndromatic HD). A chromosomal abnormality is associated with HD in 12% of cases with trisomy 21 (Down syndrome) being by far the most frequent (>90%), involving

Table 2. Genes involved in HD.

Gene	Product	Map location	Trans-mission	Frequency in HD patients	ref
RET	Co-receptor for GDNF	10q11.2	AD	50% familial cases 15% sporadic cases	59
GDNF	Ligand for RET	5p13	AD	Rare	60
NTN	Ligand for RET	19p13	AD	Single family reported	61
EDNRB	Receptor for EDN3	13q22	AR/AD	5-10%	22
EDN3	Ligand for ETB	20q13	AR/AD	5-10%	62
ECE1	ET3 converting enzyme	1p36	AD	Rare	63
SOX10	Transcription factor	22q13	AD	<5%	61
SIP1	Encoding Smad interacting protein-1	2q22	Spo	Rare	64

AD: autosomal dominant, AR: autosomal recessive. Spo: sporadic

2-10% of ascertained HD cases²². Associated congenital anomalies are found in 18% of HD patients and includes central nervous system anomalies, genitourinary abnormalities and other gastrointestinal malformations. Furthermore, HD has been reported in several known syndromes including Smith-Lemli-Opitz syndrome, Multiple Endocrine Neoplasia (MEN) type 2, X-linked hydrocephalus, congenital central hypoventilation syndrome and Waardenburg syndrome type 4²².

Aetiology and Pathogenesis

Hirschprung's disease is characterized by the absence of ganglion cells in the myenteric (Auerbach) and submucosal (Meissner) plexus of the distal bowel extending proximally from the internal anal sphincter for varying distances. The absence of ganglion cells has been attributed to failure of migration of neural crest cells between the fifth and twelfth week of gestation²³. The neurons and glia of the enteric nervous system (ENS) are all derived from precursor cells from the central nervous system (CNS) primordium. These cells, termed neural crest cells, are produced from the entire length of the neural axis but only certain tightly defined regions of the neural crest give rise to the ENS. These precursor cells first migrate from the CNS primordium into the oral and anal ends of the intestinal tract early in embryonic life. Subsequently, these cells migrate along the gut to colonize the entire intestine. The ENS precursor cells differentiate into a range of neuron types and glial cells, and form the complex circuitry necessary for ENS function²⁴.

HD is regarded as a neurocristopathy. The latter is defined as a syndrome or tumour that arises secondary to an abnormality in neural crest cell development.

In the case of HD, the enteric nervous system (ENS) is inadequately formed secondary to the arrest of vagal neurocrest cell emigration to the hindgut. The earlier the arrest of migration, the longer the aganglionic segment. Aganglionosis is generally limited to the rectum (75%), although in rare cases the aganglionosis may affect the entire colon, the small intestine or the entire gut¹⁴⁻¹⁶.

The absence of enteric ganglia containing cholinergic neurons, interneurons and non-adrenergic non-cholinergic (NANC) neurons is supposed to be the most important pathological finding in HD. The absence of ganglion cells is accompanied by increased cholinergic and adrenergic nerve fibres because regulation on the development of these nerve fibres is lacking²⁵. These abnormalities in innervation explain the inability of the aganglionic segment to relax. The wave of relaxation normally preceding each propulsive contraction does not occur and furthermore, the rectoanal inhibitory reflex (RAIR) to rectal distension is lacking²⁵.

Rectoanal inhibitory reflex

The presence of the rectoanal inhibitory reflex (RAIR) is a key factor in the normal defecation process. The RAIR is elicited by distension of the rectal wall which stimulates mechanoreceptors located in the rectum (figure 1). This leads to activation of intramural inhibiting neurons, located in the myenteric plexus, relaxing the internal anal sphincter (IAS)²⁶.

Previous animal studies have shown that NO is the main inhibitory neurotransmitter released by these neurons²⁷. Blockade of the NO biosynthesis reduced the relaxation of muscle strips of the IAS and impaired the RAIR^{27,28}.

Recently, evidence was provided that the nitrergic innervation is dependent on the presence of an intact network of interstitial cells of Cajal (ICC)^{29,30}. ICC are

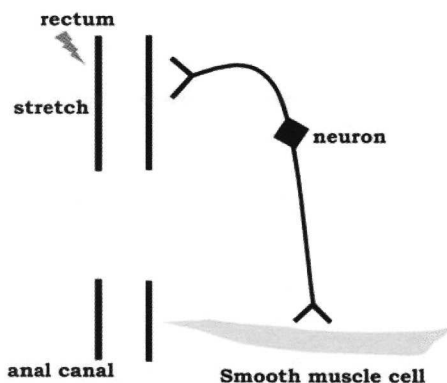


Figure 1. Triggering of mechanoreceptors in the gut initiates the RAIR.

cells of mesenchymal origin and are distributed within the tunica muscularis of the gastrointestinal tract. They function as pacemaker cells coordinating the electromechanical activity of the gut^{7,8}. A second subset of ICC located in the muscular layer have been shown to mediate the nitrergic neurotransmission in the stomach and lower oesophageal sphincter (LES)²⁹. Furthermore, ICC have been shown to play a role in the afferent limb of the RAIR³¹.

Diagnosis

Due to the risk of significant complications, it is of clinical importance to differentiate infants with HD from infants with functional constipation. Contrast enema (CE), anorectal manometry (ARM) and rectal suction biopsy (RSB) are the most common tests used in the diagnostic work-up of HD. There is a large variation in sensitivity and specificity of these tests. Therefore, the appropriate diagnostic approach for HD is still a matter of debate³²⁻³⁵. In most centres however, the reference standard for diagnosing HD is a full-thickness rectal biopsy (FTB). This test provides the most definitive answer, but is invasive and requires general anesthesia.

Contrast enema

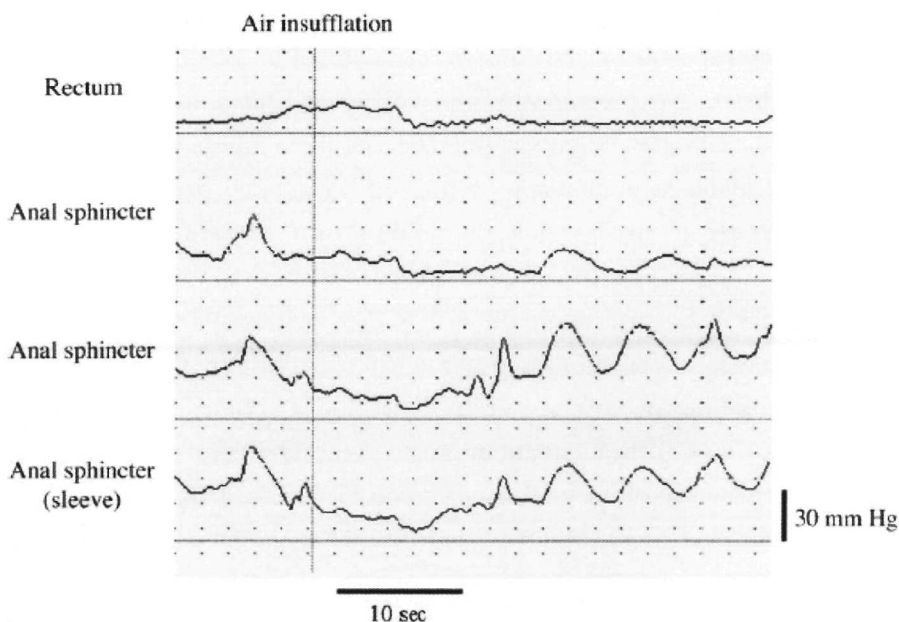
One of the first investigations to evaluate low intestinal obstruction in infancy to assist in differentiating between meconium ileus, distal atresias, meconium plug syndrome, and Hirschsprung's disease is a contrast enema³⁶. To imitate the natural situation it is important to perform a contrast enema in the unprepared colon³⁷. However, even in experienced hands a contrast enema is difficult to evaluate. The classical finding in patients with HD is a calibre change between the small or normal-sized distal aganglionic segment and the dilated proximal ganglionic bowel³⁸. In some cases, an abnormal mucosal pattern can be present indicating the presence of enterocolitis. In young children, or in case of total aganglionosis, the calibre change is however more difficult to demonstrate^{34,39-41}. On the other hand, rectal enemas and even digital rectal examinations may decompress the distended proximal bowel with distortion of the calibre change leading to false-negative test results. Contrast enema might be presumed helpful for the surgeon to assess the localization of the calibre change and thus the length of the aganglionic segment. However, the assessment of length of aganglionosis has often been questioned in the literature⁴².

Anorectal manometry

Anorectal manometry is a relatively safe and non-invasive technique. It measures pressures in the anorectal region and provides a way of quantifying the function of the internal and external sphincters. Although this technique is widely used, lack of standardization in methodology (open-tipped perfusion, closed triple-balloon, pressure transducers) has resulted in inconsistent data^{43,44}.

The main indication to perform this procedure is to demonstrate the presence of the rectoanal inhibitory reflex (RAIR) (figure 2). The RAIR can be elicited by rectal balloon distension with small volumes of air (1-60 mL). Term and premature infants older than 26 weeks' postmenstrual age (PMA) have a normal developed RAIR to rectal distension^{45,46}. False-positive test results may result from insufficient inflation of the balloon in children with a megarectum as larger volumes are required in these children to elicit a RAIR⁴⁷. Furthermore, technical factors such as an air leak in the circuit or an incorrect position of the catheter in the sphincter complex can also cause false-positive or negative test results. On the other hand, one must emphasise that artefacts are common in agitated or crying infants and results should be interpreted with caution.

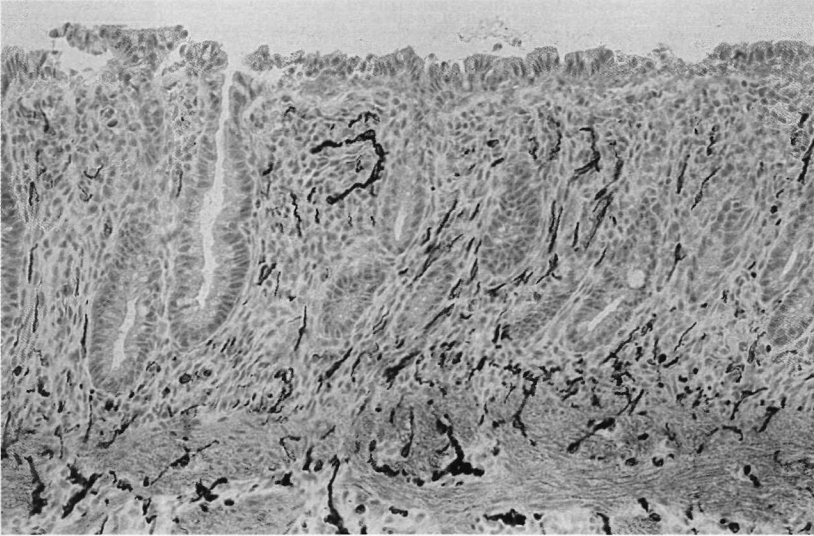
Figure 2. Anorectal pressure recordings in an 29 week old premature infant showing an RAIR elicited by air insufflation.



Histology

Classically, the diagnosis of HD is made on histochemical evaluation of the rectum. This typically shows absence of ganglion cells in the submucous and myenteric plexus and a typical increase in acetylcholinesterase (AChE) activity in the parasympathetic nerve fibres of the lamina propria mucosae, muscularis mucosae and circular muscles (figure 3)³⁸. There are 2 methods to obtain tissue for histochemical evaluation;

Figure 3. Increased acetylcholinesterase activity in the aganglionic bowel.



1. Rectal suction biopsy

A suction biopsy can be performed with no anaesthetic and little risk; the biopsies are taken above the anal margin (2 and 4 cm anterior and posterior). This should reduce the risk to sample from the normal aganglionic anal canal or to miss an ultrashort segment of HD. The instrument used for biopsies must be sharp to get good-sized specimens. However, as the specimens are small, the examination remains difficult even for experienced pathologists³⁹. The introduction of the AChE activity on a suction biopsy has made the morphological diagnosis of HD easier and more reliable^{38,48}. Possible causes for false-negative test results are: sampling error, superficial biopsy material that lacks muscularis mucosa, technical variations in staining technique and the experience of individual pathologists. Furthermore, in neonates a false-negative result may occur due to immaturity of

the submucous plexus³⁸. Possible complications of this procedure are perforation, bleeding and infection.

2. Full thickness biopsy

The classical method of rectal biopsy involves a full thickness biopsy of rectal mucosa and underlying muscle. However this requires general anaesthesia and suturing of the biopsy site⁴⁹. As with the suction biopsy possible complications of this procedure are perforation, bleeding and infection. Absence of ganglion cells in a full thickness biopsy confirms the diagnosis of HD.

Differential diagnosis

Other causes of intestinal obstruction should be considered when abdominal distension and failure to pass meconium occur in a newborn infant. The following organic disorders are important to exclude; meconium ileus (resulting from cystic fibrosis), intestinal malformations such as lower ileal and colonic atresia or congenital strictures, malrotation (which needs immediate surgical action) and functional intestinal obstruction resulting from maternal infection, maternal intoxication, or congenital hypothyroidism. Furthermore, ENS anomalies such as hypoganglionosis, hyperganglionosis, intestinal neuronal dysplasia type B (IND B) and immaturity of the submucous and myentric plexus should be considered. These anomalies lead to chronic intestinal pseudo-obstruction and can be differentiated from HD by histological stainings. A full thickness biopsy is needed to diagnose hypoganglionsos and IND B (giant ganglia) and lactic dehydrogenase (LDH) reaction is needed to verify the maturity of the ganglia³⁸.

Treatment

Once the diagnosis of HD has been confirmed by rectal biopsy examination, the infant should be prepared for laparotomy. In inconclusive rectal biopsies the clinical picture will drive the persistence of repeat biopsy attempts and if necessary full thickness biopsy sampling to confirm the clinical apparent diagnosis of HD. If the newborn has enterocolitis complicating HD, the child will require correction of dehydration and electrolyte imbalance by infusion of appropriate fluids. It is essential to decompress the bowel immediate and permanently. Deflation of the intestine may be carried out initially by rectal irrigations through a canule. When

the baby is clinically stable a colostomy will be performed.

For many years, the vast majority of cases of HD are diagnosed in the neonatal period⁵⁰. Laparotomy will be performed between 6-12 months of age. The advantages of operating in the first year are that the colonic dilatation can be quickly controlled by washouts and at operation the calibre of the pull-through bowel is near normal allowing for an accurate anastomosis that minimizes leakage and cuff infection^{51,52}.

A number of different operations have been described for the treatment of HD. Currently, many centres are performing one-stage pull-through operation in the newborn with minimal morbidity and encouraging results^{22,51,52}.

The basic principle is to bring the ganglionic bowel down to the anus. During surgery, biopsies for frozen sections are taken to determine the level of transition and a colostomy is placed proximal to the transition zone. The three most commonly used operation techniques are the rectosigmoidectomy developed by Swenson, the retrorectal transanal approach developed by Duhamel and the endorectal procedure developed by Soave. Recent innovations are the transanal endorectal pull-through and the laparoscopic approach to all three techniques. For more detailed information about surgery procedures, we refer to an excellent review by Geerdes et al.⁵³.

Prognosis

Surgical reconstruction for HD provides near-normal gastrointestinal function for the majority of children⁵⁴. In general the most commonly encountered post-operative problems include constipation (more often with Duhamel technique), incontinence, enterocolitis (more often with Swenson and Soave technique) and the overall impact of the disease on lifestyle⁵³. Other complications such as fistulae and obstructions are quite rare⁵⁵.

Although gastrointestinal function improved after surgery, long-term follow-up reveals significant residual problems. Unfortunately, most reviews of HD are hampered by the fact that follow-up periods are rather short and may not adequately reflect long-term outcome and lifestyle. One of the more complete works on this subject is performed by Moore et al.^{56,57}. These authors described that the overall quality of life was quite good, with 94% of children becoming well adjusted members of society. Although patients had low weight for age, this generally corrected with time. Additionally, developmental milestones and school performance were satisfactory in most patients (95% and 82%, respectively). Clearly, patients with

poor functional outcome will have a greater tendency to have more psychosocial problems. In general, Moore's review of quality of life issues were quite positive in children after an endorectal procedure. Factors which were predictive of poorer quality of life issues were faecal incontinence (2.5 – 13.6%) and poor family report.

Another recently published study showed that patients with HD encountered "overall" physical health problems compared to healthy subjects, but HD patients did not show additional pain or limitations in functioning due to physical problems⁵⁸. Psychosocial functioning had the most important effect on the quality of life of patients with HD, while faecal incontinence and constipation had almost no effect on their quality of life.

Summary and conclusion

Delayed passage of meconium in (very) premature infants may result from several causes, such as impaired maturation, functional constipation and the most serious always to be considered, Hirschsprung's disease. In premature infants, delayed maturation of the motor mechanisms of the gut is suggested to be the key factor. In the majority of these infants, defecation problems will resolve in the following weeks. However, if infants do not develop a normal defecation pattern, Hirschsprung's disease (HD) should be excluded. Delayed passage of meconium in HD (>90%) can lead to severe constipation, abdominal distension, enterocolitis, toxic megacolon and even death. To prevent these severe complications, it is of clinical importance especially in these young infants to be alert and to identify this disorder in an early stage.

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