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Case report

Enteric nervous system and endocrine cells demonstrated in the gut in teratomas

Ryuichiro Hirose[1], Tomoaki Taguchi[1], Sachiyo Suita[1], Osami Nada[2]

[1] Department of Pediatric Surgery, Faculty of Medicine 60, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812, Japan
[2] Department of Medical Technology, Kyushu University School of Health Sciences 90, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812, Japan

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Abstract. A case of retroperitoneal teratoma, showing considerable morphological development presented as an encapsulated and pedunculated tumour with a seemingly mature intestinal loop. Markedly complex intramural nerve plexuses and numerous epithelial endocrine cells were revealed immunohistochemically in the gut tissue. Ten other mature teratomas containing gastrointestinal tissues were examined for comparison, but neither intramural ganglia nor nervous networks were found in the gut components, despite the presence of amine- and/or peptide-containing endocrine cells in all intestinal mucosa linings. Enteric endocrine cells were found to occur irrespective of the differentiation of intestinal layers or the occurrence of neural elements. These findings suggest that the epithelial endocrine cells of intestinal mucosa do not have the same origin as enteric neurons, but are rather of endodermal origin. This invertebrate well-formed teratoma, containing a highly organized enteric nervous system, suggests that teratoma and fetus in fetu are related entities distinguished by the presence of a vertebral axis.

Key words: Teratoma – Fetus in fetu – Enteric nervous system – Epithelial endocrine cell – Immunohistochemistry

Introduction

Teratomas are composed of various components, derived from three primitive germ layers and co-ordinated tissue formation, influenced by contiguous tissue is frequently seen in these tumours. Gastrointestinal mucosa is often found within teratomas (Willis 1935; Berry et al. 1969), while enteric ganglia and neurons in the gut tissue are rarely seen. There have been few descriptions of the occurrence of enteric ganglia in gut components within teratomas (Willis 1935; Heifetz et al. 1988). This paper presents a rare case of a teratoma, with marked morphological development which included a highly-organized enteric nervous system. Immunohistochemical investigation of neurons and endocrine cells in the gut tissue within the tumour demonstrated several interesting findings, in contrast to the more usual mature teratomas.

Correspondence to: R. Hirose

Case report

A male infant, one of twins, weighing 2.476 kg, was delivered by cesarean section at 37 weeks of gestation. Neonatal physical examination revealed a fixed firm mass occupying almost the entire abdomen, as well as a huge mass in the sacrococcygeal region. Aside from low birth weight of 1.69 kg, the other twin, also a male, was found to be normal. The placenta showed no abnormal findings. At 30 weeks of gestation, antenatal ultrasonographic examination had demonstrated that the one twin had retroperitoneal and sacrococcygeal masses. Plain radiographs and computed tomography demonstrated two separate masses, one retroperitoneal and the other, sacrococcygeal, containing several skeletal parts but no vertebral column in either mass (Fig. 1a).

Exploratory laparotomy was performed on the first day of life. A huge ovoid mass covered with a transparent amnion-like membrane was encountered in the retroperitoneal region. After opening the membrane, serous yellow fluid was seen around the tumour which was easily enucleated except for a vascular pedicle and an intestine-like tubular organ connecting the tumour with the sacrococcygeal mass. The abdominal mass was removed after ligation and dissection in both pedicles. After a detailed evaluation of anorectal function, the sacrococcygeal mass was dissected out on the 27th day of life.

Pathological findings

The retroperitoneal and sacrococcygeal masses weighed 531 g and 222 g, and measured $12.0 \times 9.0 \times 10.0$ cm and $5.5 \times 7.0 \times 7.0$ cm, respectively. The retroperitoneal mass was covered with hair-bearing skin and showed four limb-like processes with digits bearing nails at their edges (Fig. 1b). A roentgenographic examination and subsequent section showed well-developed long bones with cartilagenous caps (Fig. 1c). The sacrococcygeal mass

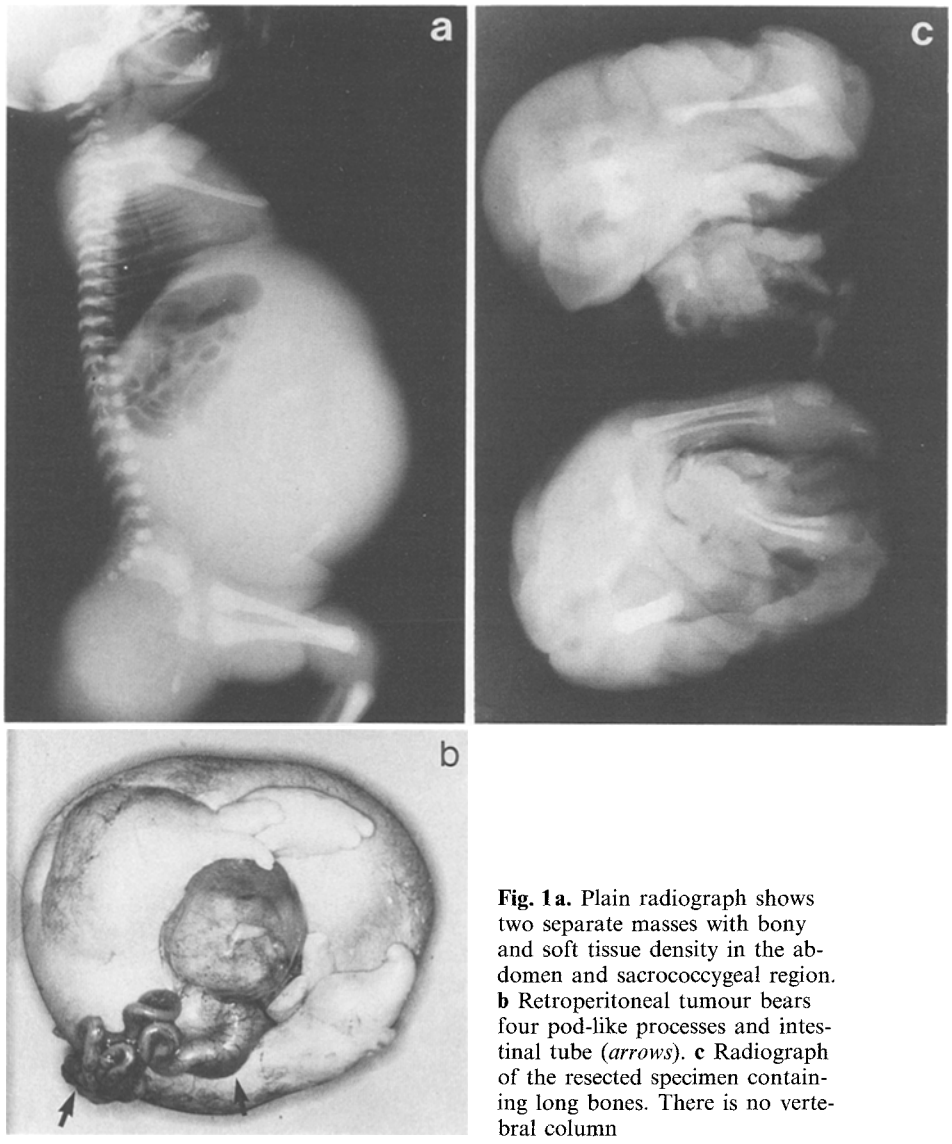


Fig. 1 a. Plain radiograph shows two separate masses with bony and soft tissue density in the abdomen and sacrococcygeal region. **b** Retroperitoneal tumour bears four pod-like processes and intestinal tube (*arrows*). **c** Radiograph of the resected specimen containing long bones. There is no vertebral column

Table 1. Gut tissue in mature teratomas

Case			Gut tissue					Other neural tissue
No.	Age/Sex	Site	Layers		Neuron		Endocrine cells	
			mucosa	muscle	ganglion	fibre		
Fetiform teratoma								
	Od/M	SC, RP	+	2 layers	++	++	+	+
1	4 m/F	temporal	+	1 layer	−	−	+	+
2	11 y/M	AM	+	1 layer	−	−	+	−
3	14 y/M	AM	+	1 layer	−	−	+	−
4	15 y/F	AM	+	−	−	−	+	+
5	3 m/M	RP	+	1 layer	−	−	+	+
6	8 m/F	SC	+	1 layer	−	+	+	+
7	1 d/F	SC	+	−	−	−	+	+
8	15 d/F	SC	+	1 layer	−	+	+	+
9	2 m/F	SC	+	−	−	−	+	+
10	5 m/M	testis	+	−	−	−	+	−

SC; sacrococcygeal; RP, retroperitoneal; AM, anterior mediastinal

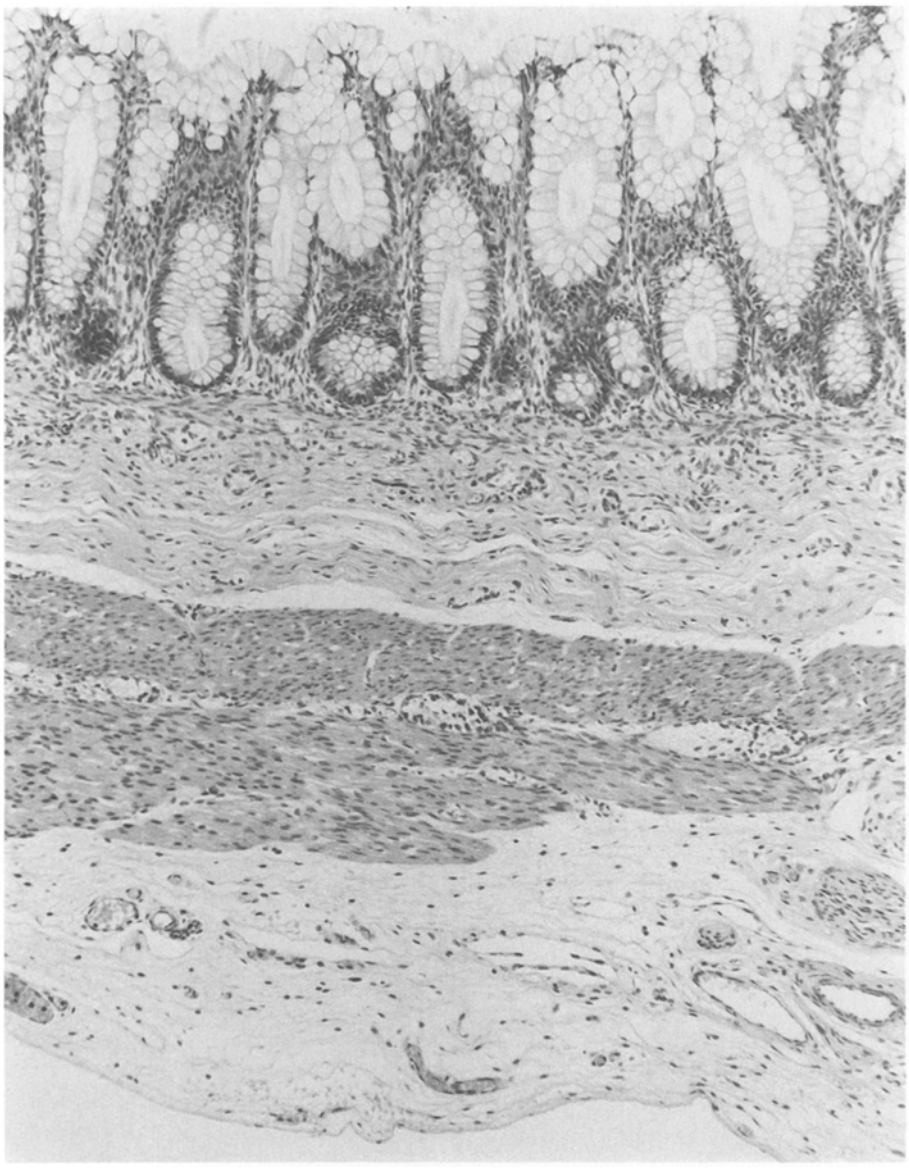


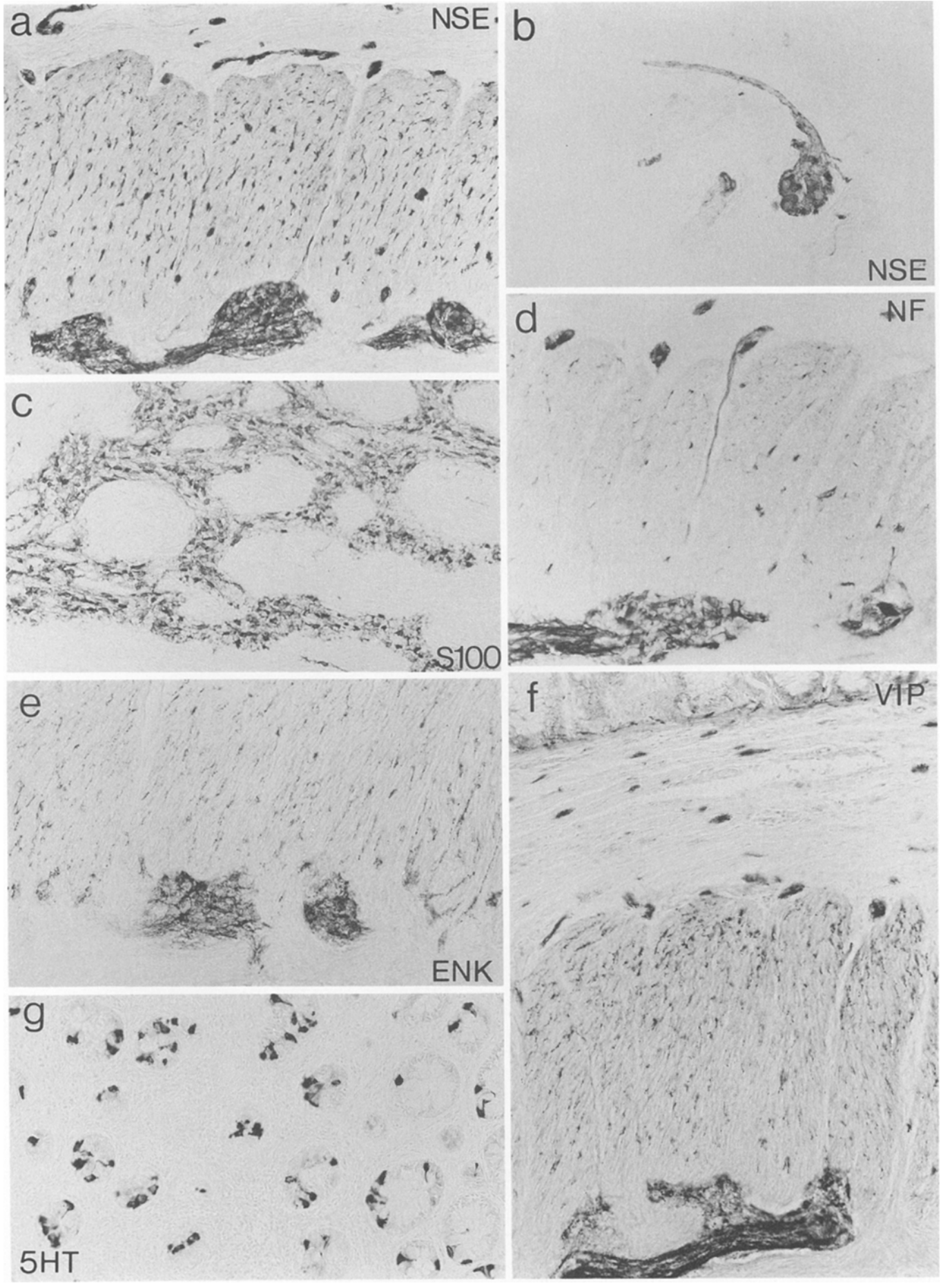
Fig. 2. Microscopic examination showed completely developed layers of intestinal wall. H & E, ×125

was also composed of bony, and fatty tissues, covered with skin. Inspite of a detailed exploration, no vertebral column was discernable in either mass. The intestine-like loop connecting the two masses was found to be vascularized from the retroperitoneal mass with complete mesentery-like structure.

Microscopic analysis revealed skin, bone with marrow, cartilage, adipose tissue, central nervous tissue including neuron and glia, pulmonary tissue, and gut tissue showing completely differentiated layers of large intestine; mucosa, submucosa, two muscle layers, and serosa (Fig. 2).

We examined 21 mature teratomas examined in our department over the last 10 years, 10 included enteric tissue components. These tumours were present in the temporal, anterior mediastinal, sacrococcygeal, retroperitoneal, and testicular regions (Table 1). Enteric tissues within these tumours showed variable differentiated layers. Four cases had only a mucosal layer, wheras 6 cases had mucosa and one layer of proper muscle (Fig. 5e). Gut components in the resected specimens were re-examined histologically and immunohistochemically.

The occurrence and distribution of neural and glial elements, and endocrine cells in gut tissue within the fetiform teratoma and the other usual teratomas were investigated by immunohistochemical staining. The intestinal tissue samples within the fetiform teratoma were prepared using two different procedures. One group of samples were fixed in 0.5% picric acid-4% paraformaldehyde in 0.05 M phosphate buffer (pH 7.3) for 1 h, and then soaked overnight with the same phosphate buffer containing 7% sucrose. Sections 16 μm thick were made on the cryostat. The other tissue samples were fixed in 10% formaldehyde and embedded in paraffin in the usual manner. Sections of 5 μm thickness were made for immunohistochemical examination. Tissue sections were incubated overnight with primary antisera at 4° C. We examined the distribution of neural and glial elements, using antisera for neuron specific enolase (NSE)


a
NSE
b
NSE
c
S100
d
NF
e
ENK
f
VIP
g
5HT

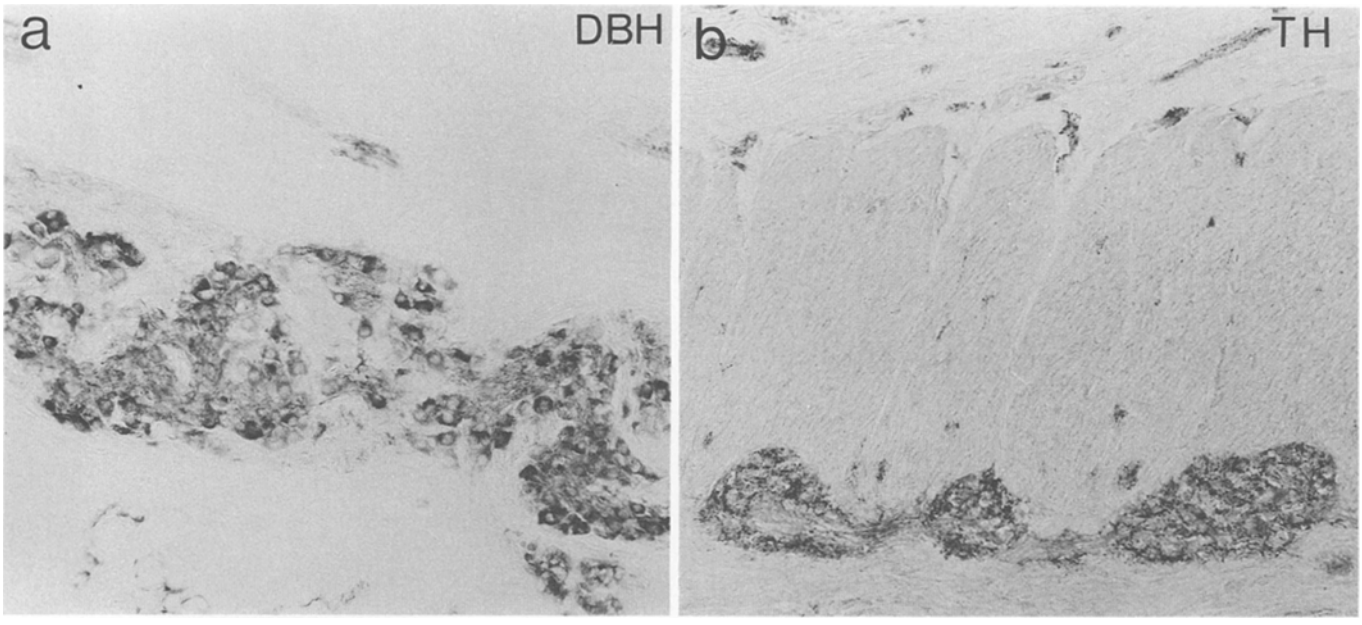


Fig. 4a, b. Immunohistochemical micrographs showing the distribution of catecholamine-producing enzymes in the gut tissue and mesentery of the fetiform teratoma, × 140. **a** Dopamine β-hydroxylase positive ganglia in the mesentery. **b** Tyrosine hydroxylase positive nerve fibres distributed in the myenteric plexus and other layers

Table 2. Characterizations of antibodies

Tissue antigen	Serum code	Dilution	Source
Neuron specific enolase	–	1:6000	(Taguchi et al. 1985)
Neurofilament (NF200 kD)	814342	1:2000	Boehringer Mannheim, FRG
Vasoactive intestinal peptide	R502	1:2500	(Yanaihara et al. 1977; Taguchi et al. 1983)
Substance P	2600642	1:2000	Miles Scientific, USA
Leu-Enkephalin	16044	1:400	IBL, Japan
Calcitonin gene-related peptide	RPN1842	1:3000	Amersham, UK
Somatostatin	64-714-1	1:1000	Miles Scientific, USA
Neuropeptide Y	R-844604	1:2300	Mllab, Sweden
S-100 protain	Z-311	1:1500	Dakopatts, Denmark
Chromogranin A	643	1:3000	Incstar, USA
5-hydroxytryptophan	–	1:4000	(Nada et al. 1984; Toyohara et al. 1985)
Doxamine β-hydroxylase	TE103	1:7500	Eugene Tech, USA
Tyrosine hydroxylase	TE101	1:5000	Eugene Tech, USA

and neurofilament (NF) as a general neural marker and S-100 protein as a glial marker. Antisera against dopamin β-hydroxylase (DBH) and tyrosine hydroxylase (TH) were used as marker for catecholaminergic nerves, and antisera for vasoactive intestinal polypeptide (VIP), substance-P, leucine-enkephalin (leu-ENK), somatostatin (SOM), neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), and glucagon (GLU) were used as markers of neuropeptides. In addition, anti-chromogranin-A (CGA) and anti-5-hydroxytryptophan (5HT) antisera were used for detecting epithelial endocrine cells. Characterization of the antisera used is summarized in Table 2. After being washed in phosphate buffered-saline, the prepared sections were stained by the immunoperoxidase technique of the biotin-streptavidin amplified system (Bio-Genex, Dublin, Calif., USA).

Fig. 3a–g. Immunohistochemical micrographs of neural and glial elements and endocrine cells in the gut tissue within the fetiform teratoma. (× 140) **a** Numerous neuron specific enolase (NSE)-immunoreactive nerves are distributed in the myenteric plexus and circular muscle layer. **b** NSE-positive neurons in the submucous ganglia. **c** Neurofilament (NF)-positive nerve cells and fibres are distributed in the myenteric and submucous plexuses. **d** Transverse section of the intestinal wall, shows a dense framework of myenteric plexus composed of a S100 protein-containing glial sheath. **e** Leucine-enkephalin immunoreactive nerve cells and fibres are distributed in the myenteric plexus and circular muscle layer. **f** Vasoactive intestinal peptide positive fibres are distributed in both ganglionated plexuses and circular muscle layer. **g** Numerous 5-hydroxytryptophan (5HT)-immunoreactive endocrine cells are seen in the mucosal epithelium

Results

In the gut tissue of the fetiform teratoma, numerous nerve fibres, with a relatively small number of intramural ganglion cells, formed a well-developed enteric nervous meshwork. NSE-immunoreactivity was localized in the nerve cells and the fibres forming submucous (Fig. 3a)

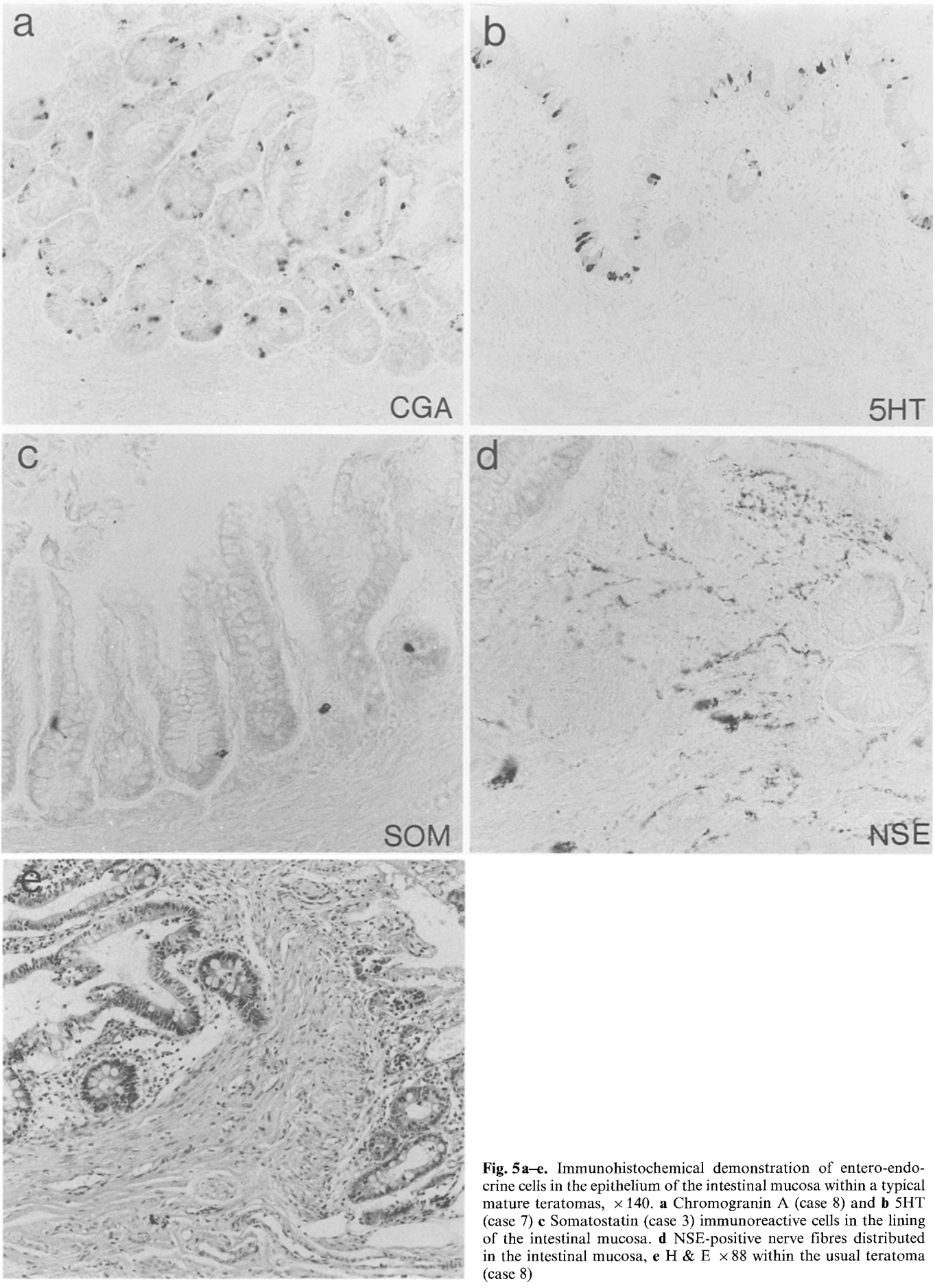


Fig. 5a–e. Immunohistochemical demonstration of entero-endocrine cells in the epithelium of the intestinal mucosa within a typical mature teratomas, ×140. **a** Chromogranin A (case 8) and **b** 5HT (case 7) **c** Somatostatin (case 3) immunoreactive cells in the lining of the intestinal mucosa. **d** NSE-positive nerve fibres distributed in the intestinal mucosa, **e** H & E ×88 within the usual teratoma (case 8)

and myenteric ganglionated plexuses (Fig. 3b). NSE-containing nerve fibres were distributed throughout all the layers of the intestinal tube (Fig. 3b). NF-immunoreactivity was also seen in the nerve cells and fibres. (Fig. 3c). Glial sheaths containing S100-protein were also distributed in all layers and formed a dense framework of supportive cells (Fig. 3d). Examination of immunoreactivity for neuropeptides revealed: VIP, substance-P, leu-ENK, SOM, NPY, and CGRP, except for GLU, in the nerves forming these networks (Fig. 3e, f). Some ganglia and nerve fasciculi, immunopositive for DBH and TH, were recognized in the mesentery, which projected nerve bundles to the intestinal wall (Fig. 4a). A large number of DBH- and TH-positive nerve fibres were recognized in all layers (Fig. 4b). Many epithelial endocrine cells immunopositive for CGA, 5HT, GLU, SOM and NPY were recognized in the lining of the mucosa (Fig. 3g).

Epithelial endocrine cells immunoreactive for CGA, 5HT, SOM, or GLU were encountered in every examined gut component within the mature teratoma, irrespective of the differentiation of intestinal layers (Fig. 5a–c). Epithelial endocrine cells were even encountered in the lining of intestinal mucosa with no muscular layer. However, there were no enteric ganglia in the gut components within the usual mature teratomas. Furthermore, most of them lacked intramural neural or glial elements. Two however, did contain scattered nerve fibres beside the mucosa (Fig. 5d, e).

Discussion

In the present study, immunohistochemical evaluation demonstrated an enteric nervous network in our fetiform teratoma which resembled a normal enteric nervous system. It was composed of several kinds of neuropeptide and amine-containing nerves and a dense glial sheath. Furthermore, the intramural neural network seen was composed of both so-called "intrinsic" nerves from intramural ganglia, and "extrinsic" nerves from DBH and TH-positive mesenteric ganglia, which mimicked sympathetic nerves. In teratomas, we meet with mixtures of tissues; mature and immature, of bewildering variety and complexity. Intestinal epithelium with smooth muscle coats exhibiting a varied degree of differentiation are also frequently recognized in teratomas, however, enteric ganglia are rarely encountered. In spite of the frequent presence of both gut tissue and neurons within a teratoid tumour, only a few cases have been cited in literature describing intramural neural elements in teratomas or fetus in fetu (Willis 1935; Heifetz et al. 1988). Heifetz et al. (1988) reported the case of a fetus in fetu containing intestinal structure, which bore no ganglion cells but had nerve bundles in the subserosal layer. Only one case, demonstrated by Willis (1935) bore well-differentiated intestinal tissue including nerve plexuses, which was poor in neurons and rich in glial elements.

It is well-known that enteric and sympathetic ganglia are of neural crest origin (Nawer 1956). Intramural ganglia are derived from neuroblasts which migrate into the gastrointestinal tract during the early stage of gestation (Okamoto and Ueda 1967; LeDouarin 1973; Gershon et al. 1980; Serbedzia et al. 1991). These undifferentiated migrants from the neural crest colonize the gut and acquire their mature phenotype within the enteric microenvironment (Epstein et al. 1980; Gershon et al. 1980). Gut components in the other mature teratomas lacked intramural ganglia and, furthermore, most contained no neural or glial elements. The intestinal tissue in these mature teratomas, and the fetiform teratoma lacking enteric nerves reported by Heifetz et al. (1988) contained no, or only one layer of the proper muscle. Thus, development of the intestinal layers which play an important role in determining the migratory pathway and microenvironment inducing colonization and phenotypic expression of precursor cells, must be present to form an intramural nervous system. The presence of enteric ganglionated plexuses composed of several phenotypes of neurons suggests a highly co-ordinated inductive process in the formation of this tissue.

The endocrine cells of the gut epithelium have been suggested to originate in the neural crest (Pearse 1969; Pictet et al. 1976), because they possess the same metabolic pattern and synthetic, storage, and secretion mechanisms as cells of the amine precursor uptake and decarboxylation (APUD) System (Pearse 1969). Other authors argue against this hypothesis and support the view that they are of endodermal derivation, as supported by several embryological studies (Andrew 1974, 1983; Fontaine and LeDouarin 1977; LeDouarin 1978). In the present study, however, amine or peptide-containing epithelial endocrine cells were encountered in the epithelium of gut tissue within the usual mature teratomas and in the fetiform lesion, irrespective of the differentiation of intestinal layers or the occurrence of neural elements in the contiguous tissues. These findings suggest that epithelial endocrine cells have a different origin from enteric neural elements and the same origin as intestinal epithelium, that being endodermal.

Fetus in fetu is an extremely rare condition and a term initially described as a form of incomplete twinning, in which a malformed parasitic twin is found in the body of its partner (Lewis 1961). Many cases have been reported, with mass lesions showing various degree of fetiformity. The pathogenesis of this disease, however, is still controversial, as fetus in fetu and teratoma form an anatomical continuum with no clear point of separation (Afshar et al. 1982; Stephens et al. 1989). Some authors believe that fetus in fetu is an inclusion of a monozygotic diamniotic twin (Lord 1956; Willis 1958; Lewis 1961), whereas others believe that fetus in fetu is a remarkably complex, well-differentiated, highly organized teratoma (Gross and Clatworthy 1951; Heifetz et al. 1988). The presence of a vertebral axis and an appropriate arrangement of other organs or limbs with respect to the axis, indicating embryonic development beyond the primitive streak stage, has been adopted as a landmark to differentiate fetus in fetu from well-differentiated teratoma (Willis 1958). In this communication, as our case lacked an unequivocal vertebral column, we chose the term fetiform teratoma. Several authors have argued against Willis' arbitraty criteria as they exclude

numerous invertebrate teratomas showing more organization than the usual teratomas reported (Wollin and Ozonoff 1961; Heifetz et al. 1988; Eng et al. 1989; Ouimet and Russo 1989), and his facts are also based on the assumption that a vertebral column representative of axiation does not occur in teratomas. The present case, which showed an extreme degree of fetiformity including encapsulation by a fluid-filled amnion-like membrane and suspension of the mass by a vascular pedicle resembling an umbilical cord, also strongly supports the theory that the fetus in fetu is a higher form of differentiation of teratoma.

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