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*Original Articles*

# Endocrine GEP-Cells in Primary Testicular Teratoma

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**Summary.** Differentiated teratomas frequently contain the apparent equivalent of gastrointestinal mucosa. 53 testicular teratomas were investigated for the incidence of entero-endocrine cells. Enterochromaffin(EC)-cells were demonstrated by formaldehyde induced fluorescence (FIF), while the other endocrine cells were identified by immunohistochemistry. 11 of 53 teratomas contained endocrine cells associated with the gastrointestinal epithelium. The most frequently found cell type was the EC-cell, followed by somatostatin-, glucagon- and pancreatic polypeptide-immunoreactive cells. The teratoma tissue blocks (20 of 53) also frequently exhibited normal testicular tissue which did not contain any EC-cell or other entero-endocrine cells.

The results are of interest in considering the cytogenesis of entero-endocrine cells and the histogenesis of testicular carcinoids, indicating that the entero-endocrine cells derive from the intestinal epithelium arising from undifferentiated stem cells. Furthermore, it seems probable that primary testicular carcinoids can develop from pre-existent teratomas by proliferation of their entero-endocrine cells.

**Key words:** Human – Immunohistochemistry – Testicular teratoma – EC-cells – Somatostatin – Glucagon – Pancreatic polypeptide.

Testicular teratoma containing epithelial formations resembling gastrointestinal mucosa have been repeatedly described (Teilum 1971). However it is uncertain whether these epithelial formations are also intermingled with highly differentiated cells like those of the gastro-entero-pancreatic (GEP) endocrine system. Ear-

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lier authors (Campbell 1959) were not been able to detect argentaffin cells in testicular teratomas containing intestinal epithelium but cells probably belonging to this group were observed by Azzopardi et al. (1961) in the epithelial lining of a teratoid mucous cyst. This was also confirmed by a case report of a testicular carcinoid (Wurster et al. 1976) and since the submission of this work Talerman et al. (1978) have shown that argentaffin cells occur in testicular teratoma.

The demonstration of endocrine GEP-cells in teratomas is of nosological interest and contributes both to understanding of the derivation of endocrine cells in the GEP-system and the histogenesis of testicular carcinoid. Pearse and Takor Takor (1976) considered these and other related endocrine cells to belong to the APUD-series (Pearse 1969) and thus to be derivatives of the neural crest. Other authors suggest that entero-endocrine cells develop in loco from undifferentiated stem cells (Cheng and Leblond 1974; Andrew 1974, 1976).

With regard to the histogenesis of testicular tumors, Collins and Pugh (1964) believe that carcinoids originate from the argentaffin cells, which so far have only been found in the prostate or in testicular teratoma. Brown and Richard (1969) consider the testicular carcinoids as monophyletical teratomas. Both opinions are based on the assumption that testicular carcinoids are derived from argentaffin cells. In the past decade argentaffin cells have been differentiated as a complex system of endocrine cells with multiple functions (see Grube and Forssmann 1980). The present investigation was therefore carried out to analyze testicular teratomas for the presence of endocrine GEP-cells. Their demonstration in testicular teratomas is made possible by the argentaffin (AA) reaction, formaldehyde-induced fluorescence (FIF) and the lead-haematoxylin-stain (LH) as well as specific immunohistochemical staining by immunofluorescence (IF) and immunoperoxidase (PAP). The study shows that various endocrine cell types are present in differentiated teratomas. A preliminary report has been published (Brodner et al. 1977).

## Material and Methods

Surgical specimens of 53 testicular teratomas were investigated. The tissue (the number of available blocks varied between 1 and 12, 4 blocks per case was the mean) was fixed in 10% formaldehyde and embedded in paraffin. In 20 cases the teratomas also contained adjacent normal testicular tissue, which was included in the investigation. 20 sequential serial sections of 5 µm were cut through the entire tumor material and investigated with the following methods:

*1. Light Microscopical Staining*

Some sections were stained with haematoxylin-eosin (Romeis 1968) for routine investigation. The argentaffin (AA) reaction of Masson and Fontana (Pearse 1972) was carried out for the demonstration of enterochromaffin-cells. Lead-haematoxylin-staining (Solcia et al. 1969) was used for the non-specific demonstration of endocrine cells.

*2. Formaldehyde Induced Fluorescence (FIF)*

This method, a modification of the Falck-Hillarp technique, was utilized for the specific demonstration of serotonin-containing cells (EC-cells). Immersion fixed tissue can also be used for serotonin fluorescence if the fixation is carried out with a formaldehyde solution only (Enerbäck 1973).

*3. Combined Histochemical Methods*

In order to demonstrate the histochemical staining properties of the GEP-cells and to reveal whether EC-cells also contain polypeptide hormones in addition to serotonin, consecutive sections were studied with IF, FIF, PAP, and AA reactions.

*4. Microscopy and Microphotography*

For fluorescence microscopy, a Leitz orthoplan microscope mounted with a surface illuminator and orthomat microscopic camera (automatic exposure control) was used. The filter combinations for the IF method were as follows: exciter filter UG 1, barrier filter K 430/K 460 or exciter filter BG 3+S 405, barrier filter K 470/K 490. For IF, the following set was used: exciter filter BG 12, barrier filter K 510/K 530 or exciter filter 2×KP 490 and barrier filter K 510, K 530. The sections were photographed with either DIN 27 for FIF and IF and with DIN 15 black and white film for the other staining methods.

The sections were mounted on albumin-glycerin coated slides, deparaffinized with xylol, and covered with Entelan$^{R}$ (Merck). The specificity of the formaldehyde induced fluorescence for serotonin was tested with sodium-hydroborate according to Corrodi et al. (1964) and Enerbäck (1973).

*5. Immunohistochemical Demonstration of GEP Hormones*

The slide-mounted sections were deparaffinized with xylol, rehydrated with a decreasing ethanol series and a phosphate-buffered physiological sodium chloride solution. Indirect immunohistochemical demonstration of the gastrointestinal polypeptide hormones was carried out with immunofluorescence (IF) according to Coons et al. (1955) and the peroxidase-anti-peroxidase (PAP) method according to Sternberger (1974).

For the IF method, the antisera were generally applied in a dilution of 1:50 or higher and for the PAP technique in a dilution of 1:2,000 or higher. Further details of the method and specificity controls are published elsewhere (Helmstaedter et al. 1977b).

Antisera against the following substances were used: synthetic bovine somatostatin, natural porcine glucagon, natural porcine secretin, synthetic human gastrin I, natural porcine cholecystokinin, natural bovine pancreatic polypeptide, synthetic porcine motilin, and synthetic human insulin. The antisera of the following groups were selected: HPP-antiserum from R.E. Chance and G.E. Feurle, somatostatin-, secretin-, glucagon-, cholecystokinin-, and gastrin-antiserum from G.E. Feurle, insulin-antiserum from R.L. Bautner and motilin-antiserum from N. Yanaihara. V. Mutt provided us with cholecystokinin. All antisera were raised in rabbits except the insulin-antiserum which was obtained from guinea pigs.

*6. Remark*

We are well aware that the immunohistochemical reaction is always expressed towards a certain amino acid sequence in one GEP-hormone which may occur in several molecular forms (family or related molecules). We have used the term, e.g., somatostatin cell although we know that the molecules demonstrated immunohistochemically may have identical or related amino acid sequences with respect to the molecule used for immunization. The term "somatostatin-like immunoreactive cells" would therefore be more accurate, but it is not used for simplification.

## Results

The 53 teratomas (classification see Table 1) were sectioned in sequence and investigated by all of the above mentioned methods. Endocrine GEP-cells containing histochemically demonstrable serotonin or polypeptide hormones were present only in those testicular teratomas which exhibit formations of columnar

epithelium, similar to the epithelia of the small intestine or colon. Twenty eight of the investigated teratomas contain such epithelia (4 differentiated teratomas = TD, Pugh 1975, and 24 partially differentiated teratomas = MTI, Pugh 1975). In this mucosa numerous goblet cells were found, exhibiting close similarity to colonic epithelium. In some cases, however epithelia with kinocilia resembling those in the early developing respiratory tract or intestinal epithelium were detected. The mucosa was generally rather smooth and the formation of elongated, occasionally ramifying glands with a large lumen were observed (Fig. 1).

In only 11 of the 28 epithelial-containing testicular teratomas were entero-endocrine cells detected (1 differentiated teratoma = TD, Pugh 1975, and 10 partially differentiated teratomas = MTI, Pugh 1975). These 11 teratomas exhibited in addition to epithelial tissue, connective tissue, bone, cartilage, and lymphatic tissue. Only one contained nervous elements which included ganglion cells and, thus, definitively neural crest or neural tube derived tissue.

The entero-endocrine cells, consistently seen within the epithelium, were not differentiated utilizing haematoxylin-eosin, nor detectable utilizing other morphological features with histological techniques (Fig. 1).

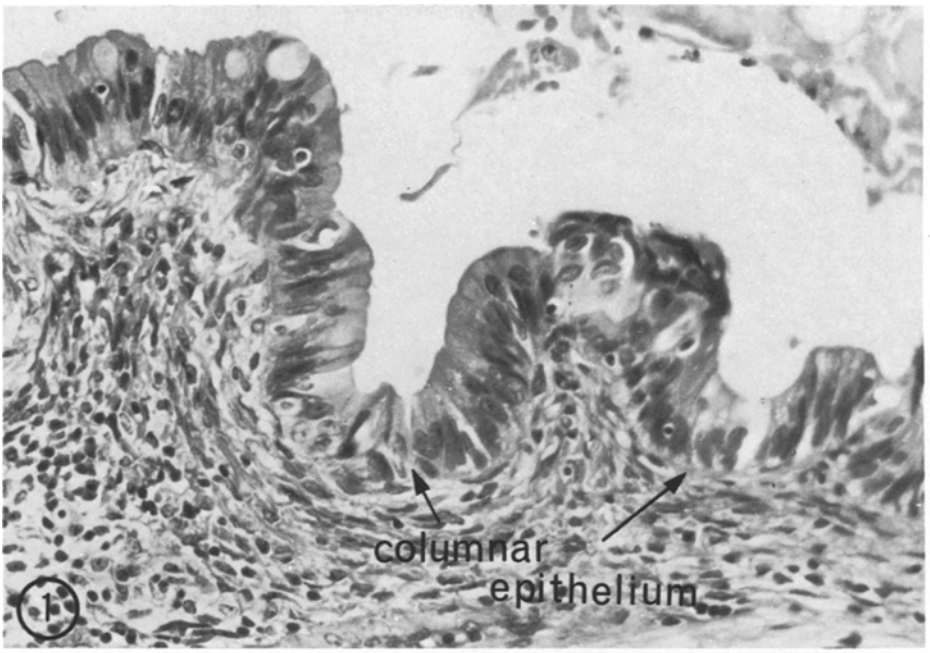
The distribution of the endocrine cell types in the 11 teratomas is summarized in Table 2. EC-cells occured in high frequency in terms of the total number of cases. This cell type was seen in 9 of 11 teratomas which contained intestinal mucosa with endocrine elements. The EC-cells were also observed as the most prevalent cell type in individual teratomas. We found a few EC-cells within kinocilia-containing epithelium and consider that these epithelial formations may correspond to respiratory mucosal elements, or to embryonic gastrointestinal epithelium which contains ciliated cells during the 9th to 10th week (Mathan et al. 1976). Fluorescence microscopy revealed that the EC-cell of the testicular teratoma exhibit features similar to those of normal intestinal epithelium. The serotonin specific fluorescence was particularly strong in the basal part of the EC-cells and the weakly fluorescent cell apex exhibits a connection with the luminal surface, forming a part of the brush border (Fig. 4). Using subsequent staining with the FIF and AA reaction, it can be seen that EC-cells of teratomas are also argentaffin (Fig. 2). With the combined methods of FIF-reaction and immunohistochemical PAP for polypeptide hormones the EC-cells of the terato-

**Fig. 1.** Area of a differentiated testicular teratoma showing *columnar epithelium* and lamina propria-like tissue. The epithelium shows some surface indentations and glandular formations. Hematoxylin-eosin. ×320

**Fig. 2.** Columnar epithelium in a differentiated teratoma stained by the Masson-Fontana reaction. An *argentaffin cell (arrow)* is observed. At this higher magnification the strong AA reactivity, mainly in the apiconuclear region of this endocrine GEP-cell, is evident. ×960

**Fig. 3.** Differentiated teratoma similar to that in Fig. 1 but stained with lead hematoxylin. An elongated, LH positive cell of an endocrine type is seen. ×700

**Fig. 4.** FIF reaction in a testicular teratoma showing two EC-stained cells. One cell cut through its axis shows a triangular shape, a connection with the glandular lumen, no stain of the nucleus, and a strong reaction in the basal part of the EC-cell. The other cell is obliquely cut. ×700


columnar
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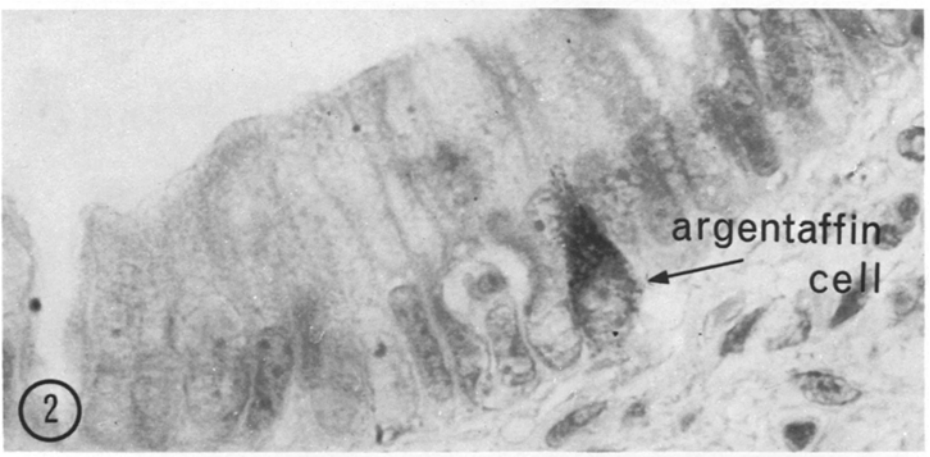
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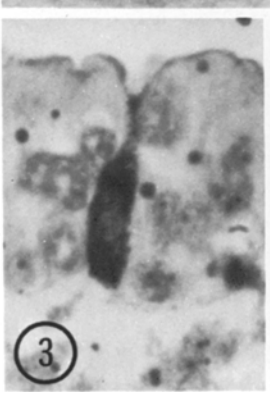
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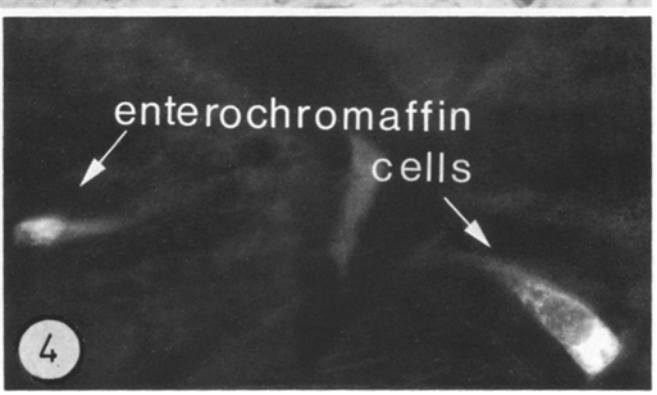
enterochromaffin
cells
4

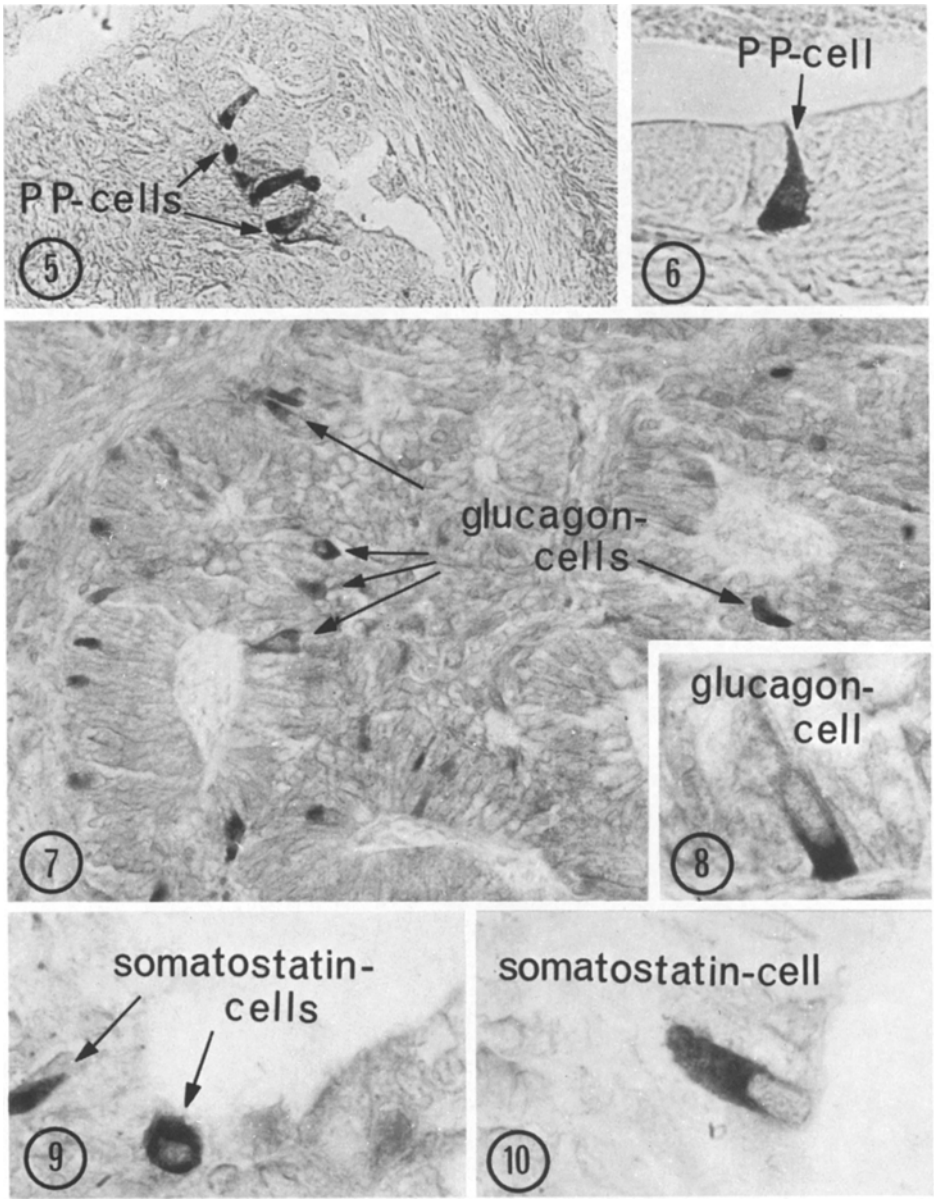


**Fig. 5.** Glandular epithelium of a differentiated teratoma stained for pancreatic polypeptide. Numerous cells stained by the PAP reaction are seen in the glandular formation. ×250

**Fig. 6.** *PP-cell* of another teratoma at higher magnification. Note the triangular shape and the connection with the luminal side of the epithelium. ×800

**Fig. 7.** Columnar epithelium of a differentiated teratoma stained for glucagon. Numerous cells exhibit immunoreactivity. ×300

**Fig. 8.** *Glucagon-cell* at higher magnification. Note the strong immunoreactivity at the basal part of the epithelium. ×800

**Fig. 9.** *Somatostatin-cells* of a teratoma. One cell is transversely sectioned. ×900

**Fig. 10.** *Somatostatin-cell* in transverse section. No staining of the nucleus is observed. ×900

**Table 1.** Classification of the investigated tumors

| | No of cases |
|---|---|
| Differentiated Teratoma = TD (Pugh 1975) | 4 |
| Partially differentiated Teratoma = MTI (Pugh 1975) | 26 |
| Undifferentiated Teratoma = MTU (Pugh 1975) | 15 |
| Embryonic Teratoma = yolk sac tumor (Teilum 1971) | 5 |
| Trophoblastic Teratoma = MTT (Pugh 1975) | 3 |
| Total | 53 |

**Table 2**

| Case-No. | EC-cells | Somato-statin | Glucagon | HPP | Teratomas differentiated (=TD) partially differentiated (=MTI) |
|---|---|---|---|---|---|
| 1 | + | + | + | + | TD |
| 7 | + | − | − | − | MTI |
| 8 | + | + | + | − | MTI |
| 10 | + | − | − | + | MTI |
| 13 | + | + | − | − | MTI |
| 14 | − | + | − | − | MTI |
| 21 | + | − | − | − | MTI |
| 27 | + | − | − | − | MTI |
| 42 | + | + | + | + | MTI |
| 43 | − | + | − | − | MTI |
| 48 | + | − | + | − | MTI |

mas are found to contain sertonin, but none of the polypeptide hormones sought in our study.

Among the antisera against the enterohormones used, those against somatostatin, pancreatic polypeptide, and glucagon showed a positive immunoreaction in various teratomas. In 8 of 11 teratomas containing EC-cells, polypeptide hormone-producing cells were also detected (Table 2). The somatostatin-cells were the most frequent, followed by glucagon and pancreatic polypeptide-immunoreactive cells (Fig. 5 to 10). The morphological features of these cells are similar to those already described in human gut epithelium. With antisera against gastrin, secretin, cholecystokinin, motilin and insulin, no immunoreactive cells were detected. In two teratomas all four endocrine cell types (EC-cells,

somatostatin-, glucagon-, and pancreatic polypeptide-cells) were present. Two teratomas contained only somatostatin-cells, while 7 showed a variable composition with two or three endocrine cell types (Table 2).

All endocrine GEP-cells identified in the teratomas by the IF method were also demonstrable using the PAP technique. The endocrine cells also stained with the LH stain, but only partially with the AA reaction (Figs. 2 and 3).

The normal testicular tissue around the 20 testicular teratomas contained no detectable endocrine GEP-cells with the methods used.

## Discussion

The results of this investigation show that entero-endocrine cells are present in certain testicular teratomas. The techniques used are suitable for the specific investigation of some of the entero-endocrine cells in tissue fixed for routine diagnosis. Using eight different antisera against entero-hormones, three antisera showed positive reactions with cells of the teratomas, i.e., somatostatin, glucagon, and pancreatic polypeptide. However, although no immunoreaction for the other GEP-hormones was observed, we cannot exclude the possibility that further endocrine cell types are present in testicular teratomas. The immunoreaction for many of the enterohormones requires special tissue preparation for immunohistochemistry. It has been shown that GEP-cells containing substance P (Pearse and Polak 1975) cannot be stained with routine-fixed tissue. Several authors have reported that routine-fixed tissue may be adequate for the immunohistochemical demonstration of other GEP-cells (McGuigan 1968; Piris and Whitehead 1974; Denk et al. 1976; Woodtli and Hedinger 1976).

The demonstration of three types of polypeptide hormone containing cells among the teratomas suggests that the epithelial formations are the equivalent of the midgut and hind-gut segments. The different gut segments in man and non-human primates contain different entero-endocrine cells in variable numbers (Forssmann and Ito 1976; Helmstaedter et al. 1977a). Those seen in the teratomas show a similar composition to that of the colon. Although EC-cells were found in teratomas, no demonstration of motilin cells was possible, but it is still a matter of controversy as to whether certain EC-cells also produce motilin (Pearse et al. 1974a; Polak et al. 1975; Larsson et al. 1975; Forssmann et al. 1976; Heitz et al. 1977; Helmstaedter et al. 1979).

All attempts to identify the endocrine cells by ultrastructural means were unsuccessful. It will therefore be necessary to use more material to obtain a complete immunohistochemical and ultrastructural analysis of endocrine GEP-cells in the differentiated tissue of teratomas.

Teratomas containing entero-endocrine cells are differentiated or partially differentiated (teratoma differentiated = TD or malignant teratoma intermediate = MTI, Pugh 1975). Besides epithelia, these teratomas contain other tissues, such as connective tissue, bone and cartilage. In most of the cases no nervous tissue was observed. The entero-endocrine cells were always found intermingled with epithelial cells and never seen in the lamina propria. These observations are of importance in considering the cytogenesis of the entero-endocrine cells.

Pearse and Takor Takor (1976) suggest that all entero-endocrine cell types and the other endocrine cells of the APUD series (Pearse 1969), derive from the neural crest. According to Pearse and Polak (1974), they migrate to the organs early embryonic stages where they subsequently remain and exert their endocrine functions. Other authors suggest that the migration of entero-endocrine cells to the gastrointestinal mucosa takes place at a later embryonic phase and that these cells derive from sprouts of the autonomic nervous system (see discussion of Osaka and Kobayashi 1976). However, origin of the endocrine GEP-cells from the neural crest is only credible if the cells develop in a phase when the three embryonic layers are differentiated and migration follows. This migration has not been demonstrated to date (Le Douarin and Teillet 1973; Pictet et al. 1976; Fontaine et al. 1977; Le Douarin 1978). In teratomas migration from neoplastic neural crest anlagen could not be demonstrated. Thus, GEP-cells may originate from neural elements or undifferentiated stem cells. It seems more probable that the entero-endocrine cells develop in loco from undifferentiated stem cells (Andrew 1974, 1976; Cheng and Leblond 1974).

The present investigation also gives some hints on the histogenesis of testicular carcinoids. Present literature distinguishes three groups of testicular carcinoids (see Wurster et al. 1976; Brodner and Wurster 1976; Talerman et al. 1978): (1) Carcinoids which are metastatic derivatives of a primary gastro-intestinal tumor, (2) carcinoids deriving from tissue of teratoid tumors, (3) carcinoids which develop primarily in testicular tissue, not associated with teratomas.

The enterochromaffin-cell may be the initial endocrine cell from which carcinoids originate. Thus, metastatic carcinoids may derive mainly from EC-cells of the gastro-intestinal tract where EC-cells are very frequent. According to our investigations, the second group of carcinoids may originate from the frequently occuring EC-cells in the differentiated tissue of differentiated (TD) or partially differentiated (MTI) teratomas and not from the testicular tissue, which does not seem to contain EC-cells (compare with the works of Simon et al. 1954; Berkheiser 1959; Sinnatamby et al. 1973). The existance of the third group of carcinoids, directly originating from testicular tissue, seems improbable because EC-cells are not present in normal testis. Collins and Pugh (1964) assert that enterochromaffin-cells occur regularly in testicular tissue. However, both in their analysis and in the literature, EC-cells have not been detected, in normal testicular tissue. In the male genital tract, EC-like cells have only been described in segments other than the testis, such as the prostate and urethra (Baumgarten et al. 1968; Hakanson et al. 1974; Aumüller et al. 1976). In our material no enterochromaffin-cells were found in testicular tissue surrounding the teratomas. In contrast other authors differentiate between primary carcinoids of the testis and a simplified teratoma (Kermarek and Duplay 1968) and a similar classification has been adopted for ovarian carcinoid tumors (Brown and Richart 1969). The primary testicular carcinoid may develop from a monophyletic differentiated teratoma and our results demonstrating an absence of EC-cells in normal testicular tissue compared with testicular teratoma, support this hypothesis. We suggest that the primary testicular carcinoids therefore originate from pre-existant teratomas.

Since EC-cells and entero-endocrine cells have been detected in teratomas,

they may be considered as carcinoid precursors (Pearse et al. 1974b). Further investigations are now being carried out to relate the possible origin of other polypeptide hormone-containing tumor cells from testicular teratoma. Indications of the existance of such carcinoids has been recently revealed by Yalla et al. (1974), who described the occurance of a testicular carcinoid with a Zollinger-Ellison-Syndrom related peptic ulcer.

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