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# DUAL NATURE OF LEYDIG CELLS OF THE HUMAN TESTIS

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**SUMMARY**

• *This review is devoted to the human Ley dig cell, and systematizes published and own unpublished results from studies performed during the last decade. Ley dig cells are the main cell type in the testis that produce androgens which are important for the development of the male genital organs, secondary sex characteristics and behavior as well as for the processing and maintenance ofspertnatogenesis. A lot of information accumulated provides evidence that Ley dig cells of the human testis and the testis of some other species express or possess immunoreactivities for numerous marker substances characteristic for nerve andneuroendocrine cells. It is shown that human Leydig cells, beside of markers for steroidogenic activity, possess: neuronal markers, synoptic and storage vesicle proteins, neural cytoskeletal proteins, 5-hydroxytryptamine, enzymes involved in the synthesis ofcatecholatnines, neurohormones and/or their receptors, neuropeptides, calcium-binding proteins, cell adhesion molecules, glial cell antigens, components of the nitric oxide/cyclic guanosine monophosphate system, components of the renin/angiotensin system, and numerous growth factors and their receptors. These results provide new evidence for the neuroendocrine nature of Leydig cells. As consequence, t\vo main questions arise: (i) the origin of Leydig cells and (ii) their functional significance as neuroendocrine cells. The presumption that Leydig cells originate from mesenchymal-like cells of the mesonephros is the most common view in the literature. However, no data are provided concerning the origin of the stem cells from which the Leydig cell lineage develops. Mesenchyme comprises the embryonic connective tissue cells that may have mesodermal, ectoderrnal and neuroectodermal (neural crest) origin. In this relation and based on the recently established neuroendocrine feature, we speculate that Leydig stem cells may detach from unknown regions of the neural crest and migrate to the mesonephric and gonadal anlage at early stages of development. The functional significance of Leydig cells as neuroendocrine cells is also illustrated on the basis of the nitric oxide/cyclic guanosine monophosphate system. Accordingly, Leydig cells may regulate their steroidogenic activity by an intracrine or autocrine fashion. Furthermore, they are probably able to synchronize the activity of the cells in a Leydig cell cluster by a paracrine way. Leydig cells may influence the contractile activity of the smooth muscle cells ofbloodvessels, thus regulating the blood flow rate and the permeability for hormones and nutritive substances. Also, Leydig cells may regulate the contractile state ofperitubular myofibroblasts and myofibroblasts and smooth muscle cells of the tunica albuginea. Similarly, Leydig cells may communicate with Sertoli cells and germ cells of the seminiferous tubules. Leydig cells are a relatively stable, heterogeneous population of cells in the human testis which persists even in cases of impaired spermatogenesis,fibrosis and different pathological changes of the testis. This fact suggests that Leydig cells survive under unusual conditions due to precise regulatory systems which make them to a larger extent independent from the local homeostasis.*

## INTRODUCTION

• A large number of data has been accumulating during the last decades concerning the origin, nature, structural and functional features as well as the aging and pathological changes of the endocrine Leydig cells (LC) of different species (1, 2). Only rarely research was devoted to LC of the human testis. The endocrine function of LC is beyond doubt. Recently, results were obtained suggesting the neuroendocrine nature of LC (3-5). This newly described characteristics raised a number of questions concerning the true nature, the heterogeneity, the origin and the functional properties of LC. The present concise review deals primarily with the neuroendocrine qualities of the human LC and the consequences that follow from this fact.

## SHORT HISTORY OF THE LEYDIG CELL

• The history of the LC discovery has been recently systematized by Christensen (6). This topic was also object of previous publications (7-12). In 1850, a new cell type was described in the intertubular tissue of the testis of a number of mammalian species by Franz Leydig (13). In the human testis, these cells were described for the first time four years later by Kolliker (14). Until 1950, a many data have been published concerning the morphology and functional significance of humanLC (6, his Table 1). As Beissner (7) and Christensen (6, 8) reported, variable assumptions were proposed on their nature and functional features. The LC were considered as being connective tissue cells, cells associated with lymphatic vessels, neural ganglion cells, plasma cell-like structures, immature Sertoli cells, epithelial cells or embryonic epithelium.

**See Editorial on page!**

The first evidence for a possible endocrine role of the testis was provided by Berthold (15, 16). Reinke (17) and Regaud and Policard (18) tolerated the possibility that the testicular interstitial cells may be involved in internal secretion. Broad experimental studies performed by Ancel and Bouin (19) and Bouin and Ancel (20-22) provided indirect evidence for possible endocrine actions of the testis. Following a long period of controversial views (23-28), according to Christensen (6), the first direct evidence that LC are the main site of androgen production was provided by Wattenberg (29) who by means of a histochemical technique detected 3 6-hydroxysteroid dehydrogenase activity in LC of the rabbit testis. At the same time Christensen established androgen biosynthesis in mechanically separated interstitial tissue and seminiferous tubules from the rat testis, and in 1965 published their results providing the first direct biochemical evidence for isolated interstitial tissue as the main source of androgen secretion (30, see also 31). These studies have finished the long-lasting discussion about the steroidogenic activity of LC.

## MORPHOLOGY OF THE LEYDIG CELL

• Two updated reviews on LC structure have been recently published by Pudney (2) and Russell (32). There are distinct differences in the organization, number, shape and some properties of the LC in different species (2, 32). Previous excellent reviews provide detailed descriptions of the structural and functional features of human LC (8, 9, 33-44). In a recent review, Chemes (45) gives an up-to-date characterization of human LC and focuses on similarities and some important differences with the LC of other species. Human LC are distributed as single cells, small or larger groups within the loosely arranged interstitial tissue and often in the vicinity or around blood vessels. Rarely, rows of LC are seen to lie parallel to the border of the *lamina propria* (peritubular LC) or within the *lamina propria* (46,47), with preferential peripheral localization, near the outer sheath of connective tissue cells separating the *lamina propria* of the seminiferous tubules and the interstitial space (43, 48). Usually, individual LC or LC groups are surrounded by thin processes of "encapsulating fibroblasts" (45) or "covering cells" (49). The LC of adult human testis show well expressed morphological heterogeneity (see below). This is especially true for the testes of elderly men and testes of patients with reduced or impaired spermatogenesis (43, 50-52).

Light microscopically, human LC are differing in size, being polygonal, round, elongated or occasionally spindle shaped cells containing usually eccentrically situated round nuclei with prominent nucleoli and peripherally distributed heterochromatin (Fig.l). Numerous dark stained organelles and lipofuscin inclusions are seen perinuclearly. The cytoplasm consists of dark and light homogeneously stained areas. Some cells contain Reinke crystalloid, a structure found only in human LC (43, 45). Transmission electron microscopically, LC are distinguished by their abundant smooth endoplasmic reticulum, pleomorphic mitochondria with tubules and cristae as well as some lipid droplets (32,43,45). These cellular constituents are believed to be characteristic for the steroidogenic cells (Fig.2). In addition, human LC possess well developed Golgi complex and numerous lysosomes. Varying numbers of lipofuscin granules, peroxisomes, and crystalloid or paracristalline structures other than the Reinke ones could also be found within the cytoplasm of LC. Also, rough endoplasmic reticulum, ribosomes and cytoskeletal components, such as microtubules, actin and intermediate filaments, belong to the constant structures of LC. In the testis of some patients, LC possess differently long processes with varicosities. These cells lie in the intertubular space as small or large groups. Usually in their vicinity, polygonal or round LC are also located (Fig.3). In the processes of these LC, intermediate filaments and a variety of cytoplasmic vesicles

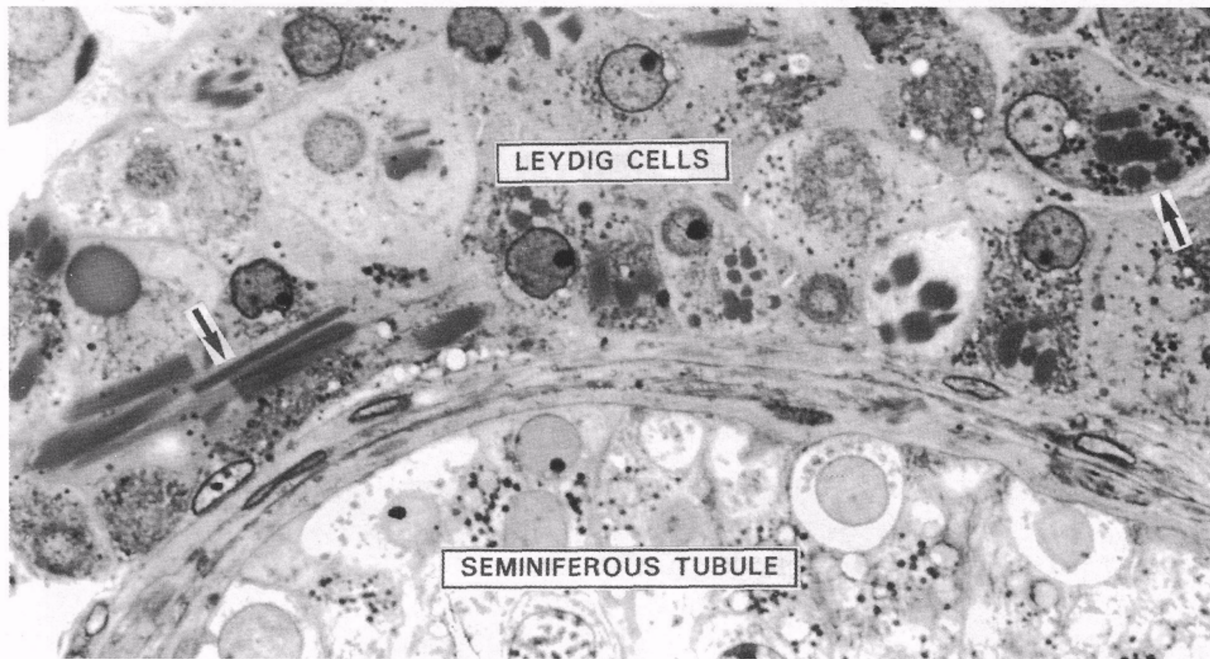



*Figure 1. Semithin section of a Leydig cell group in the vicinity of a seminiferous tubule. Note the different size, staining intensity, number of organdies, and Reinke crystalloids (arrows). Toluidine blue/Pyronin G stain.* * 7070.

(see below and 43) are characteristically distributed (Fig.2, 4-6). Between these processes and the body of adjacent LC, as well as between the plasma membranes of neighbouring LC, desmosome-like contacts and predominantly nexuses were established (32, 43, 45, 53). In the mouse testis, nexuses of LC contain the connexin 43, and carry signals that regulate their secretory activity (54).

**THE ENDOCRINE NATURE OF THE LEYDIG CELL**

• There is no doubt that LC represent the main cell type of the testis that is able to produce androgens from cholesterol (30, 31, 55-57 for review). The LC possess the machinery necessary for the binding and receptor-mediated endocytosis of low-density lipoproteins (LDL) and for *de novo* synthesis of cholesterol as well as for its transport towards the mitochondria where it will be converted to pregnenolone by the enzyme cholesterol side chain cleavage cytochrome P450 (P450scc), which is located on the matrix side of the inner mitochondrial membrane. In the LC, the lipid droplets are storage sites of cholesterol esters. The transport of cholesterol from the outer to the inner mitochondrial membrane is a rate-limiting step and is mediated by a steroidogenic acute regulatory protein (57). Three additional steps involving enzymes located in the LC cytoplasm continue the synthetic process and lead to formation of testosterone and metabolizing androgen precursors and biologically active androgens (58). At the same time these cells contain the enzyme aromatase that is responsible to convert testosterone to estrogens.

In LC of different species, enzyme histochemical and immunocytochemical studies have detected some of the enzymes involved in synthesis and metabolism of steroids such as 3B-hydroxysteroid dehydrogenase/$A^5$-$A^4$ isomerase (3B-HSD), 176-hydroxysteroid dehydrogenase (17JS-HSD), 1 lJ5-hydroxysteroid dehydrogenase (1 Ifi-HSD) as well as testosterone (59-65). Testosterone-like immunoreactivity is seen in human LC situated both inter- and peritubularly as well as in cells located within the *tunica albuginea* (Fig.7,8). Human LC also show 3B-HSD activity which is of lower staining intensity compared to the staining in rat testis. However, as for testosterone immunoreactivity (66), 3B-HSD activity varies considerably among the individual patients. This is consistent with biochemical and clinical results (67,68). Moreover, in the LC of adjacent areas as well as among the LC of a cluster, differences in the 3B-HSD activity are seen, reflecting probably differences in the functional activity of the individual LC or variants of their phenotype (see below).

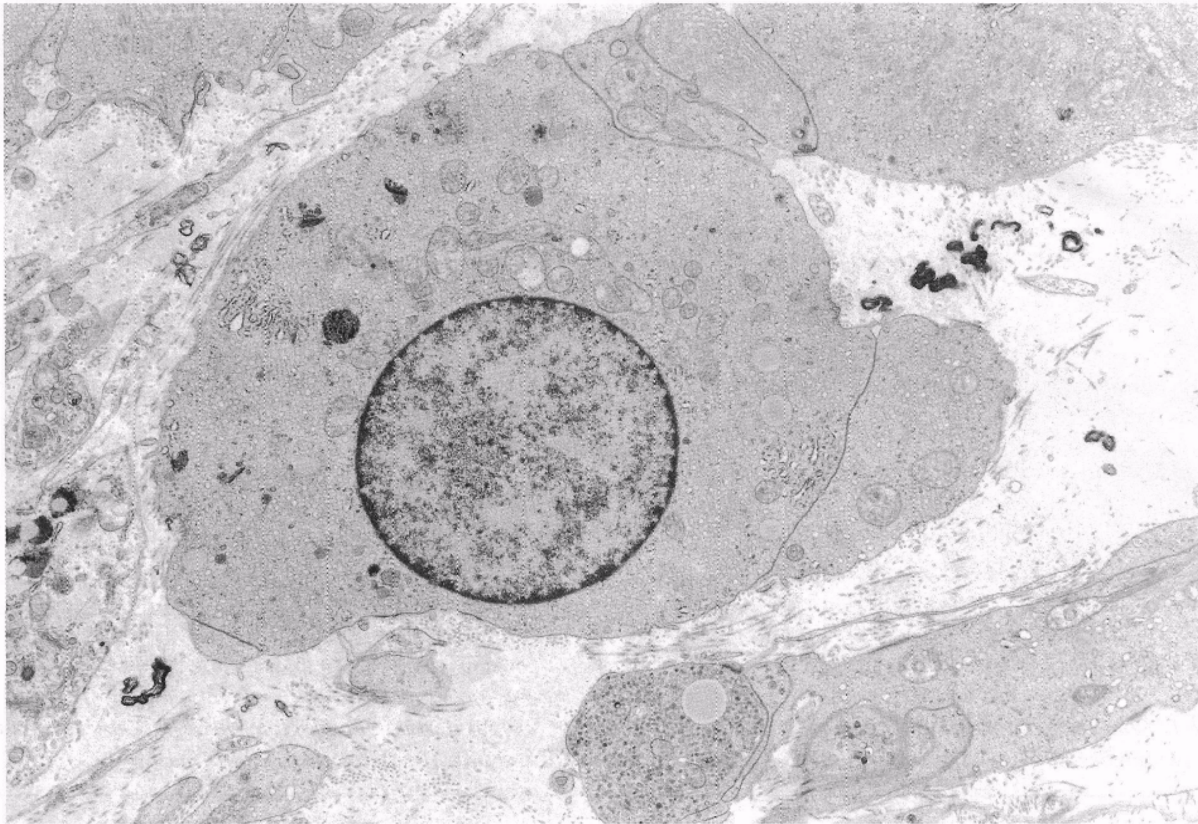


***Figure** 2. Transmission electron micrograph showing an ultrathin section through a Ley dig cell perikaryon and parts of somata and processes of adjacent Ley dig cells. Note the abundant smooth endoplasmic reticulum and the scanty rough endoplasmic reticulum. The nucleus is located eccentrically. Numerous mitochondria, different vesicles, lysosomes and a few lipid drops are visible. At the bottom, one Leydig cell process is seen that contains a large number of clear and dense-core vesicles. x 6000.*

Human LC exhibit also immunoreactivity for the LDL receptors (LDLR) (Fig.9) supporting the assumption that they can bind and internalize exogenous cholesterol for further utilization in the steroidogenic process. Furthermore, in LC of the human testis, androgen receptors, estrogen receptors and luteinizing hormone/human chorion gonadotropin (LH/hCG) receptors were also visualized immunocytochemically (see below).

The "classical" view considers the LC as the main source of production of androgens important for the development of the male genital tract, male secondary sex characteristics and behavior as well as of the processing and maintenance of steroidogenesis and spermatogenesis in the testis (69). According to this view, the testosterone synthesis and release is controlled by the hypophysial LH (or placental hCG during development). Both hormones act *via* specific LH/hCG receptors, the second messenger cyclic adenosine monophosphate (cAMP) and the protein kinase A. Testosterone released into the blood circulation inhibits by a feed back mechanism the expression of gonadotropin-releasing hormone (Gn-RH) in the hypothalamus, resulting in the cessation of LH secretion in the pituitary gland. In addition, testosterone directly inhibits the expression of LH by the hypophysis. By this way the regulatory circuit of the androgen secretion, in which the hypothalamus, the hypophysis and the testis are involved, appears closed (70, 71 for review).

## THE NEUROENDOCRINE NATURE OF THE LEYDIG CELL

• In the testis itself, some biologically active substances are produced which are also involved both in regulation of steroidogenesis and communication between testicular cells (58, 64, 69, 72, 73). It became evident that in addition to the classical control by the systemic hormones and steroids, local con-

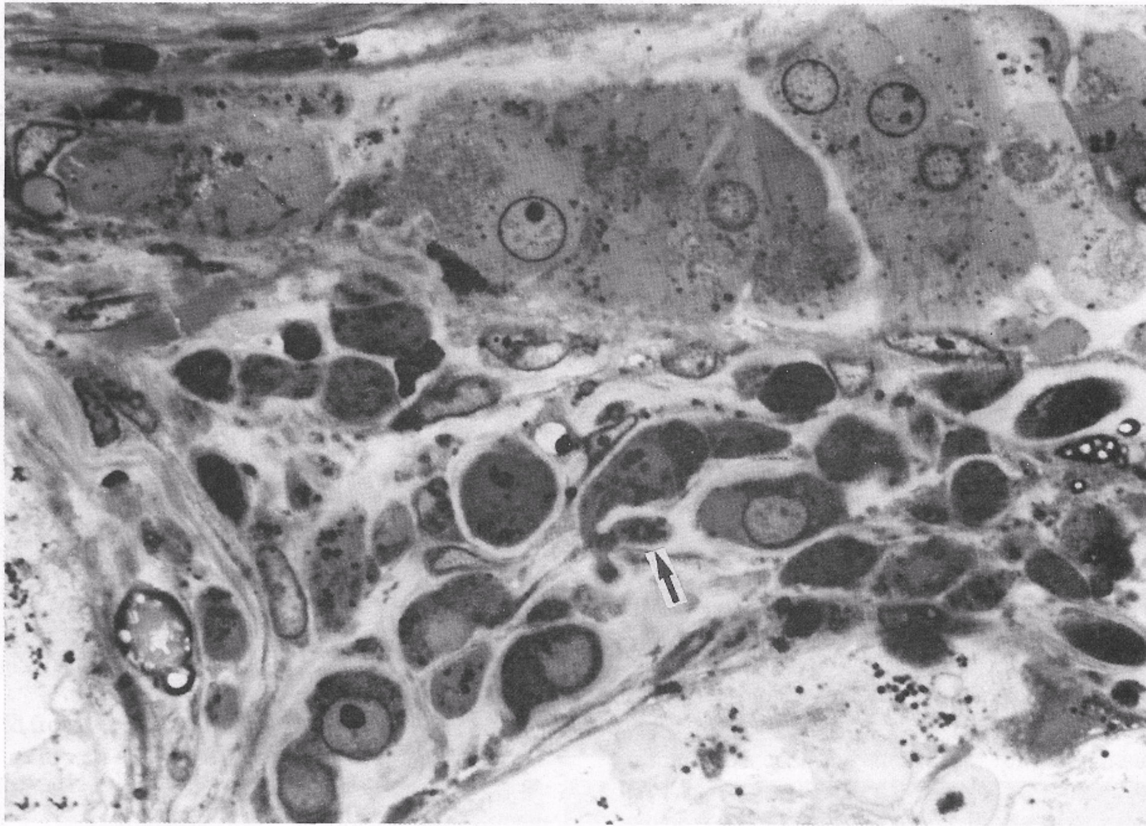


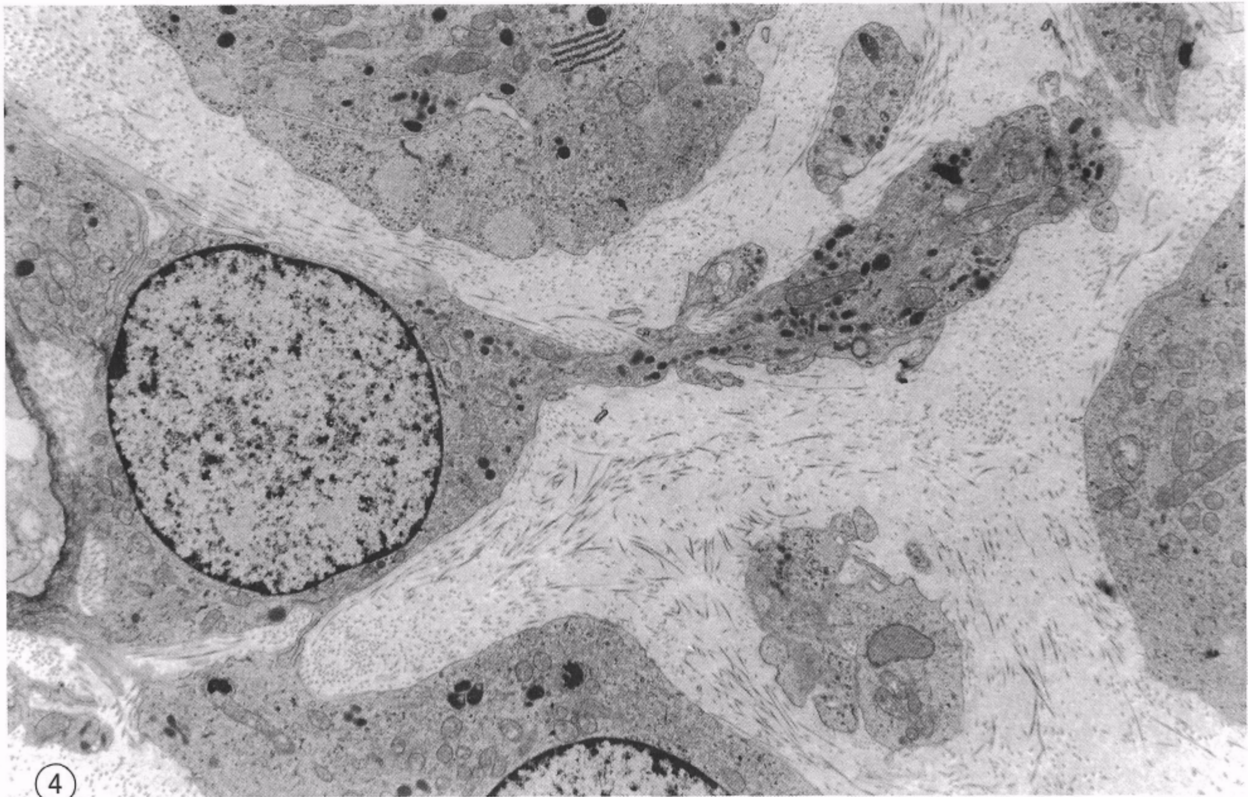
*Figure 3. Sewithin section of a I.evdig cell group composed of two cell types. The cells in the upper part' of the figure resemble those shown in Fig. 1, whereas the cells in the bottom part are smaller, densely stained and have partially branching processes (arrow). Toluidine blue/Pyronin G stain,* x *980.*

trol mechanisms of testicular functions are also of importance. It could be shown that some of the regulatory substances are produced by the different testicular cells themselves. Thus, increasing evidence accumulated that testicular functions may be subject to a local autocrine/paracrine regulation (74-81).

Additionally, pro-opiomelanocortin (POMC)-derived peptides, such as adrenocorticotropic hormone (ACTH), melanocyte stimulating hormone (MSH), 6-endorphin, (82-85) and methionine-enkephalin (met-Enk) were detected in LC of the rat testis (86) and it was suggested that these peptides may be involved by a paracrine or autocrine fashion in the regulation of testicular functions (87,88). Using immunocytochemical techniques met-Enk was also detected in human LC (4, 5). Although POMC-derivatives were found also in other cells and organs, these were the first neuroactive substances discovered in mammalian LC.

At this time it was established that a large number of biologically active substances other than the "classical" neurotransmitters are expressed in different neuronal populations in the central and peripheral nervous system (89-92). Depending on the kind of its release and the interaction with target cells, a neuroactive substance may play the role of hormone, neurotransmitter or neuromodulator. One of these substances, a member of the tachykinin family, the undecapeptide substance P (SP), was found to be associated with primary afferent neurons and to play an important role in the processing of autonomic and somatic sensory (nociceptive) information in the central and peripheral nervous system (93).

fn 1985, we started a study with the aim to establish whether the human LC possess immunoreactivity for the opioide peptide met-Enk. While opioide peptides have been detected in numerous organs (94), we decided to prove additionally the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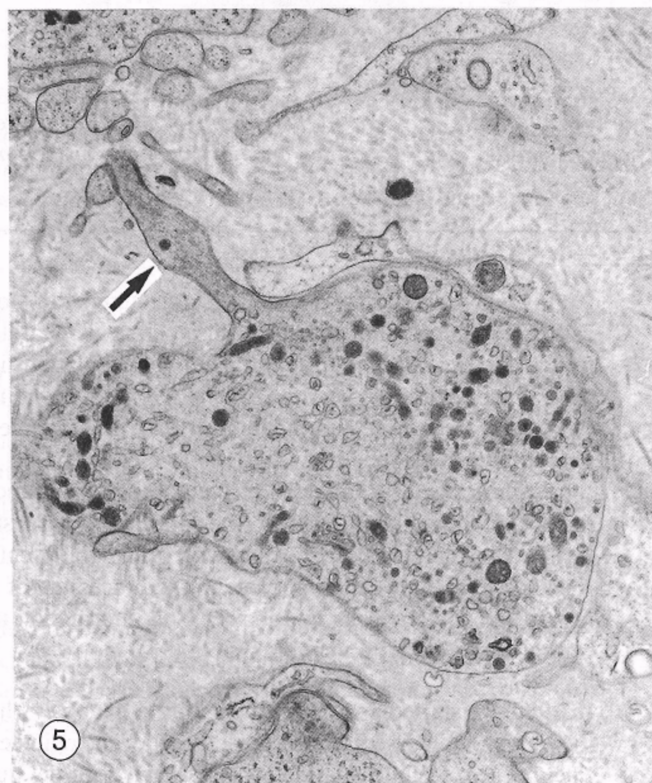
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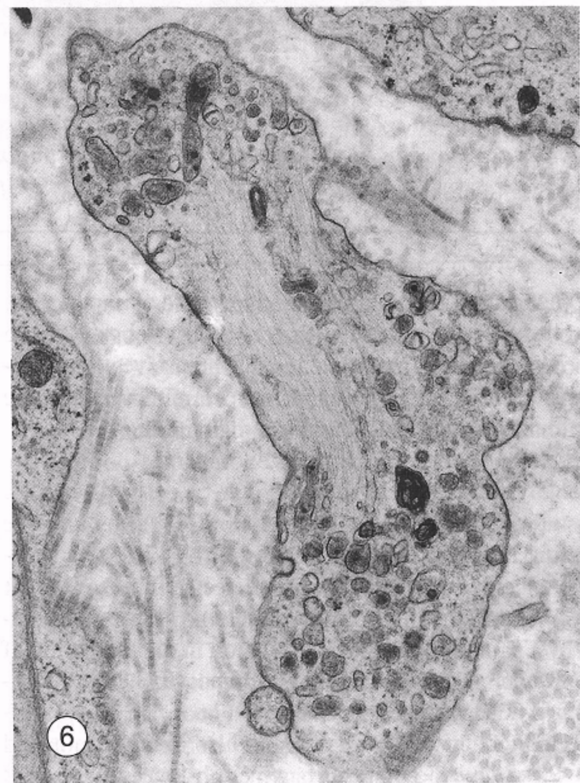
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localization of a neuropeptide confined only to neural structures. We chose SP, expecting to found it at least in nerve fibers located in the vicinity of LC. The results of this study confirmed that met-Enk immunoreactivity, as in the rat (86), was localized in human LC. SP-like immunoreactivity was not found as expected in nerve fibers. Immunoreactivity for SP in the human testis was seen evenly distributed within the LC cytoplasm, with distinct differences in the staining intensity. Thus, for the first time a substance, distributed only in structures that are developmentally related to the nervous system, was found in human LC (4, 5). As a consequence, this result raised two main questions. (/) whether LC possess neuroendocrine features, and (/';') whether LC are of neuroectodermal origin.

The next neuronal and neuroendocrine marker that was visualized in human LC was the glycolytic enzyme neuron specific enolase (NSE) (95). NSE is a hydrolase that is important for the conversion of 2-phosphoglycerate to phosphoenol pyruvate. In a later study, it was shown that a neuronal survival factor identified in the bovine brain is in fact identical to NSE (96). Subsequently, immunoreactivity for the growth-associated protein-43 (GAP-43) or neuromodulin was detected as a second neuronal marker (unpublished data) (Fig. 10).

Encouraged from the first results and having in mind that one individual neuronal marker may also appear in non-neuronal cells and a marker substance by itself cannot in general be accepted as sufficient evidence for the neuroendocrine phenorype of a cell, we started a wide scientific program with the aim to test the validity of the hypothesis about the neuroendocrine features of LC. Firstly, we established that the existence of neuropeptides is not only feature of human LC. We showed that SP, NSE and met-Enk immunoreactivity were also present in LC of mouse, rat, hamster, and guinea pig (97-99). Concerning the expression of SP and NSE there were some species dependent differences in LC of the animals studied. For example, we were not able to establish SP immunoreactivity in rat LC and NSE was seen also in Sertoli cells of the guinea pig testis only. Moreover, we obtained evidence that SP was really expressed in LC and not taken up from the extracellular space and accumulated in their cytoplasm. We found transcripts of the preprotachykinin-A mRNA in human and mouse, but not in rat and boar testes (100). In the same study, mRNA for both SP and neurokinin-A receptors were also established in human testis, suggesting the possibility that SP may act in an autocrine way on LC function. In a third study, we found that SP inhibits testosterone production in isolated adult hamster LC (98, 99) and, interestingly, that SP acts partially by modulating the binding capacity of LH receptors in LC (101). In addition, we established that both prepubertal and adult populations of LC in the hamster testis were SP-immunoreactive, suggesting that in these species no differences exist in the expression of SP during testis development (97-99). Also in the rat, no differences in the immunoreactivity for 36-HSD have been established between intertubular and peritubular LC that are considered to be different cell lineages (60). Interestingly, SP exerts different effects on the testosterone production by prepubertal and adult LC (97, 98, 102). This fact may be explained with differences in the structure of LH receptors in these cells (61). Another target for tachykinins seems to be the Sertoli cell. It has been established that tachykinins modulate the secretory activity of rat Sertoli cells (103) providing evidence for a paracrine role of SP and relative tachykinins in the testis.

In next two years, we found immunoreactivity for a number of markers (see below) and by electron microscopy, different clear and dense-core vesicles in human LC (Fig.2, 5-7) which may be responsible for the established synaptophysin and chromogranin A immunoreactivity (Fig. 11). These results suggested that human LC share a great similarity with cells of the diffuse neuroendocrine system (104, 105), or paraneurons (106) (see also 4, 98, 99, 102), and in 1993 we published a paper in which arguments were provided in favour of the neuroendocrine nature of LC (3). The neuroendocrine phenotype of LC remains relatively stable after disturbances of testicular functions (107) and even in LC tumors (108).

In addition to evidence for the steroidogenic activity (3J5-HSD, LDL receptor immunoreactivity [LDLR; Fig. 9]**, testosterone immunoreactivity [Fig. 7,8]**, androgen receptor immunoreactivity [AndrR]*, estrogen receptor immunoreactivity [EstrR]*, estrogen receptor-binding protein immunoreactivity [EstrR BP] *), we were able to establish the following partially neuroendocrine marker substances in the human LC:

Neuronal markers
- Neuron-specific enolase (NSE)
- Growth-associated protein-43 (GAP-43)
- Neuromodulin (Fig. 10)**

*Figure 4-6. Transmission electron micrographs of human Leydig cells. A Leydig cell with a long process having several short branches and numerous storage vesicles (Fig.4 ). Cross section of a Leydig cell process. Note the different vesicles and the filament bundle within the side branch (arrow)(Fig.5). Portion of a Leydig cell process containing variable storage and transport vesicles as well as a filament bundle (Fig.6). x 6000 (Fig.4), * 15 200 (Fig. 5, 6).*

**Synaptic and storage vesicle proteins**
- Synaptophysin (Syn)
- Chromogranin A + B (Chro A + B) (Fig. 11)

**Cytoskeletal proteins**
- Neurofilament protein 200 (NF-200; NF-H) (Fig.2)
- Neurofilament protein 160 (NF-160; NF-M) (Fig. 13)**
- Neurofilament protein 68 (NF-68; NF-L) (Fig. 14)**
- Microtubule-associated protein 2 (MAP2) (Fig. 15)

**Cell adhesion molecules**
- Neural cell adhesion molecule (N-CAM) (Fig. 16)
- Pan catherin (Cath) (Fig. 17)**

**Indoleamines**
- 5-Hydroxytryptamine (5-HT, serotonin) (Fig. 18)

**Enzymes involved in the synthesis of catecholamines**
- Tyrosine hydroxylase (TH)
- Aromatic L-amino acid decarboxylase (AAD)
- Dopamine-B-hydroxylase (DBH)
- Phenylethanolamine-N-methyltransferase (PMNT)**

**Neurohormones and/or their receptors**
- Growth hormone-releasing hormone (GHRH) (Fig. 19)**
- Corticotropin releasing hormone (CRH) (Fig.20)**
- ; • Luteinizing hormone-releasing hormone (LHRH, Gn-RH) (Fig. 21)**
- Luteinizing hormone receptor (LHR)**
- Gonadotropin-releasing hormone receptor (GnRHR)

**Neuropeptidcs and/or their receptors**
- SubstanceP (SP)
- SP receptor (SPR)
- Neurokinin A (NKA)
- Neurokinin A receptor (NKAR)
- Methionine-enkephalin (met-Enk)
- B-endorphin(fi-End)
- Neurotensin (NT) (Fig.22)**
- Neuropeptide tyrosine (NPY)**
- Vasoactive intestinal (poly) peptide (VIP)**
- Peptide histidine isoleucine (PHI)**
- a atrial natriuretic peptide (aANP)**
- Brain natriuretic peptide (BNP)**
- C type natriuretic peptide (CNP)**
- Big endothelin (big End)*
- Endothelin I (End I)*
- • Endothelin II (End II)*
- Endothelin receptor type A (EndRA)*
- Endothelin receptor type B (EndRB)*

**Glial cell antigens**
- Galactocerebroside (GalC) (Fig.23)**
- 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) (Fig.24)**
- Glial fibrillary acidic protein (GFAP) (Fig.25)**
- A2B5 antigen (A2B5) (Fig.26)**

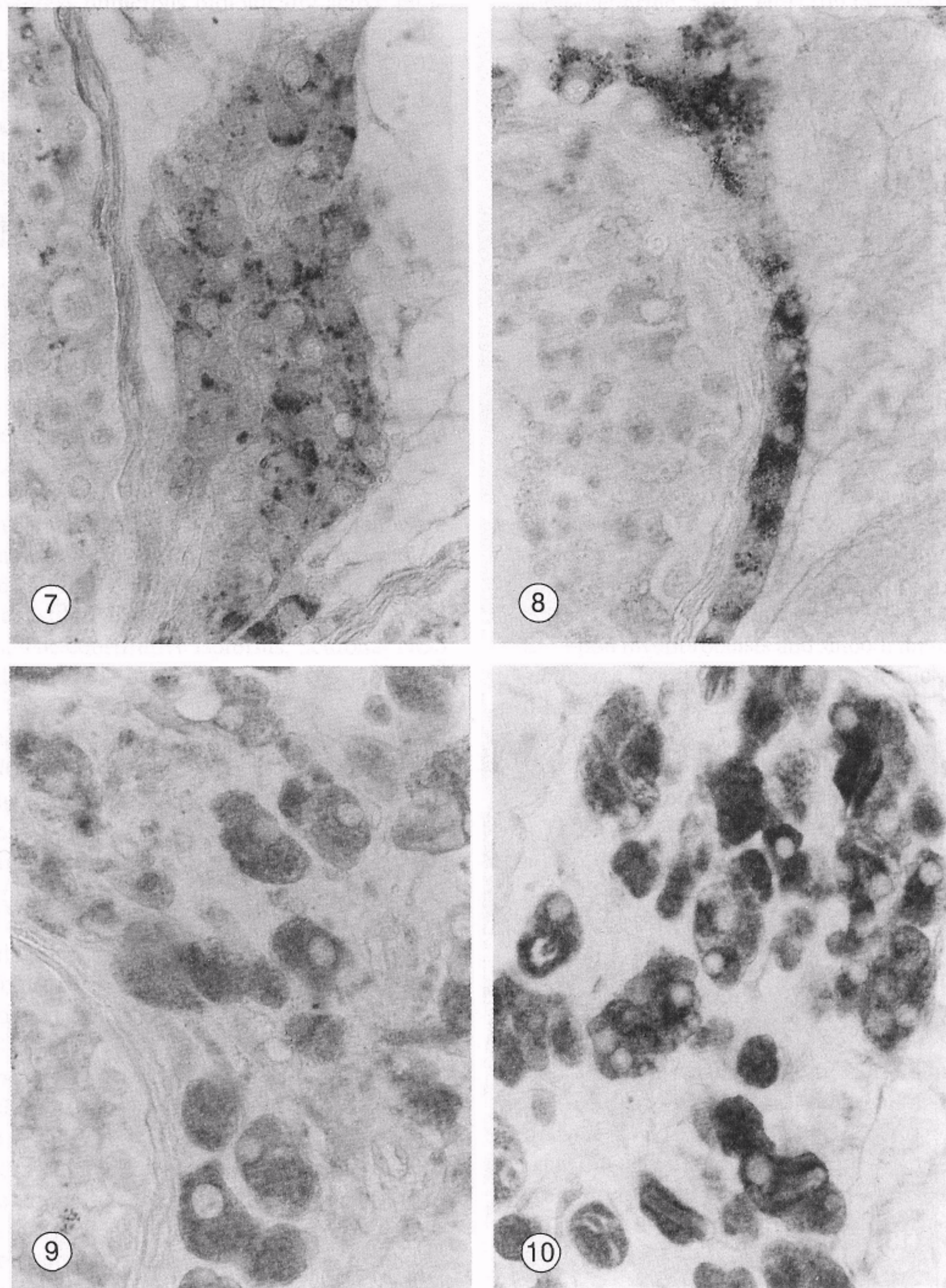
**Calcium-binding proteins**
- Protein S-100 (S-100) (Fig.27)
- Calmodulin (CaM)**
- Calbindin-D28 (CaB) (Fig.28)
- Parvalbumin (Pan')

**Components of the NO/cGMP system**
- Nitric oxide synthase, brain type (NOS-I) (Fig.29)
- Nitric oxide synthase, macrophage type (NOS-II)**
- Nitric oxide synthase, endothelial type (NOS-III)**
- Soluble guanylyl cyclase (sGC) (Fig. 30)
- Cyclic guanosine monophosphate (cGMP) (Fig.31)
- Aspartate (Asp)
- Glutamate (Glu)
- Calmodulin (CaM)
- $Ca^{2+}$/Calmodulin-dependent protein kinase II (Ca/CaM PKII)
- Superoxide dismutase (SOD) (Fig. 3 2)**

**Components of the renin/angiotensin system**
- Prorenin (proRen)*
- Renin(Ren)**
- Angiotensin I (Ang I) (Fig.33)*
- Angiotensin II (Ang II)*
- Angiotensin receptor type I (AngR I)*

**Growth factors and/or their receptors**
- Activin (Act)*
- Inhibin-a (Inh-a)**
- Transforming growth factor-fil (TGF-M)**
- Insulin-like growth factor-I (IGF-I)**
- Insulin-like growth factor-II (IGF-II)**
- Insulin-like growth factor-binding proteins 1, 2, 3, 4, 5 and 6 (IGFBP 1-6)**
- Epidermal growth factor receptor (EGFR)**
- Nerve growth factor receptor (NGFR) (Fig.34)**
- basic Fibroblast growth factor (bFGF) (Fig.35)**
- Vascular endothelial growth factor (VEGF)*
- Vascular endothelial growth factor receptor (VEGF R) *
- Endothelial cell growth factor (ECGF)*
- Platelet-derived growth factor-B (PDGF-B)*
- Platelet-derived growth factor-B receptor (PDGF-BR)*

* established by Dr Ergun alone or in collaboration with the authors of this review.
** new, unpublished results obtained by the authors of this review; for the remaining substances see 3-5, 95, 107-111.




*Figures 7-35 represent paraffin sections from human testes showing immunoreactivity for different antigens.*

***Figure** 7, **8.** Testosterone immunoreactivity in the cytoplasm ofintertubular and peritllbular Leydig cells. In some Sertoli cells positive granu/a are also seen, x 570.*

***Figure 9.** Immunoreactivity for low-density lipoprotein receptor. The Leydig cells show distinct differences in the staining intensity,* x 570.

***Figure 10.** Different growth-associated protein-43 immunoreactivity in the cytoplasm of Leydig cells,* x 570.

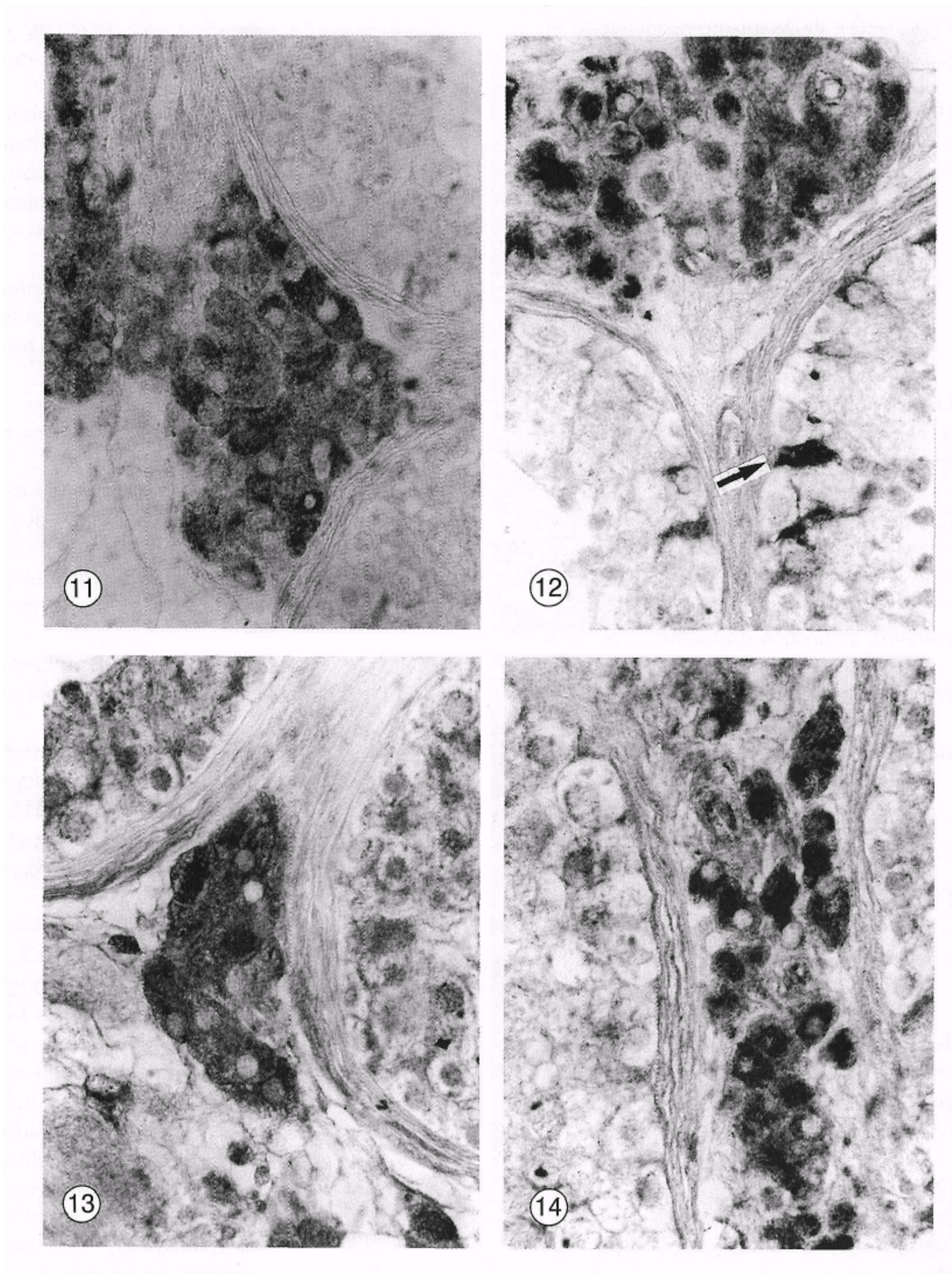



***Figure 11.*** *Chromogrcmin A immunoreactivity in the cytoplasm of human Leydig cells with fine granular appearance of the reaction precipitate.* x *570.*

***Figure 12.*** *Neurofilament protein-200 immunoreactivity in human Leydig cells. Note also the strong immunostaining in some Sertoli cells (arrow), x 570.*

***Figure 13.*** *Neurofilament protein-160 immunoreactivity in human Leydig cells,* x *570.*

***Figure 14.*** *Neurofilament protein-68 immunoreactivity in human Leydig cells,* x *570.*

Meanwhile, several papers were published showing that LC of different species express numerous biologically active molecules that may be involved in the regulation of LC functions, inculding testosterone secretion (58,63,64,67,73,97, 98,101, 102, 112-128). Examples include hormones such as arginine-vasopressin, oxytocin, GnRH, CRH, follicle stimulating hormone (FSH), prolactin, calcitonin, thyroid hormones, steroid hormones (androgens, estrogens, and glycocorticoids), growth factors and cytokines (bFGF, IGF-I, inhibins, activins, TGF-<x, TGF-B, EGF, PDGF, interleukin-1), some Sertoli cell factors as well as neuronal transmitters and regulatory peptides (catecholamines, 5-HT, melatonin, SP, POMC derivatives, natriuretic peptides, endothelins, angiotensin II, erythropoietin) were shown to act through different second messengers or directly on enzymes responsible for the synthesis of androgens. Some of these substances are produced by the LC themselves thus acting as autocrine regulators of their functions.

Recently, the main components of the NO/cGMP system were established in human LC (109, 110). Nitric oxide is a radical that is produced by a family of enzymes termed NOS. One inducible and two constitutive forms are expressed in different organs and tissues. The constitutive isoforms, neuronal NOS (NOS-I) and endothelial NOS (NOS-III), require $Ca^{2+}$/CaM and may be regulated by other factors such as protein kinase A, protein kinase C and $Ca^{2+}$/CaM PKII. The inducible or macrophage form of NOS (NOS-II) is $Ca^{24}$-insensitive and is activated by immunological or infectious stimuli and experimentally with substances such as lipopolysaccharide, interferon-y, interleukin-1 a, and TNF-a. We found moderate to strong imrnunoreactivity for NOS-I and moderate to low immunoreactivity for NOS-III and NOS-II, suggesting the possibility that LC are able to produce NO under different circumstances. This fact and the accumulation of the second messenger cGMP after treatment of isolated human LC with the NO donor sodium nitroprusside (110) suggest thatLC contain an active guanylyl cyclase which is the intracytoplasmatic receptor for NO. It was shown that NO inhibits testosterone secretion in rat LC (129, 130). Nitric oxide also directly inhibits the aromatase activity in ovarian granulosa-luteine cells, the enzyme responsible for the conversion of androgens to estrogens (131). In addition, we found that human LC showed imrnunoreactivity for molecules involved in the regulation of NOS-I activity, e.g. excitatory amino acids glutamate and aspartate, $Ca^{2+}$-binding protein CaM and $Ca^{2+}$/CaM PK II. Our unpublished results also showed that human LC exhibited imrnunoreactivity for both endothelial NOS-III and macrophage NOS-II. However, in contrast to Sertoli cells in which the NOS-III imrnunoreactivity was most intensive, the neuronal NOS-I clearly predominated in LC. Recent findings suggest that some human cells can express inducible NOS-II gene constitutively (132). It should be noted that NO mediates the action of numerous hormones (LH, LHRH, vasopressin, growth hormone) and neurotransmitters (SP, calcitonin-gene-related peptide [CGRP], acetylcholine, norepinephrine, 5-HT, etc) (109 for review; 133). Nitric oxide may also have cytotoxic properties if released in larger amounts and combining with superoxide anions (134). The produced peroxynitrite is a highly toxic molecule that may account for apoptosis and degeneration of Leydig, Sertoli and germ cells. However, both Leydig and Sertoli cells exhibit immunoreactivity for superoxide dismutase (unpublished data), suggesting that they are able to eliminate superoxide anions. Thus, a toxic effect of NO on these cells may be expected only at higher NO concentrations (135 for guinea pig LC).

Furthermore, LC-produced NO may (/) regulate the steroidogenic activity by an intracrine way, (») modulate the action of neuropeptides, hormones, growth factors and cytokines by an autocrine way, (//'/') synchronize the functional activity of neighbouring LC in a paracrine way, *(iv)* modulate the contractile activity of smooth muscle cells and vascular pericytes and regulate the blood flow rate and permeability of the vessels, and (v) influence the contraction state of perirubular myofibroblasts and contribute to the peristaltic activity of the seminiferous tubules (the same action may exert NO produced by the ectopic LC, upon myofibroblasts and smooth muscle cells in the *tunica albuginea,* contributing to the rhythmic contraction waves of the testicular capsule).

In contrast to the rat, human LC do not possess hemoxygenase-2 which is responsible for the production of an additional cellular messenger, namely carbon monoxide (CO) (110, 136), which also activates the sGC and leads to elevation of cGMP intracellular levels in the corresponding cells. It seems likely that CO is not effective in the regulation of human LC functions.

Recent findings provide evidence for the expression of $mRNA^{NPY}$ in cultured immature rat Leydig and Sertoli cells (137) and for the existence of a paracrine system for regulation of NPY gene in the testis. The levels of $mRNA^{NPY}$ increase after treatment with LH, FSH, interleukin-1 a, -lf>, forskolin (an activator of adenylyl cyclase), and phorbol 13-myristate 12-acetate (an activator of protein kinase C). Also, factors released from Sertoli and germ cells are involved in the regulation of NPY gene levels in LC. It is presumed that NPY may modulate testicular function. These results are also of particular interest because in nerves supplying the human ureter, tyrosine hydroxylase, NPY and VTP are colocalized with NOS (138). In adult human LC, we found a relatively low immunoreactivity for NPY, VIP and PHI. It seems likely that the expression of NPY reaches its peak in immature LC. - -

In human and rat LC, mRNA for thyrotropin releasing hormone (TRH), TRH gene and TRH itself as well as a partially inhibitory effect of TRH on their LH/hCG-induced testosterone secretion were recently established (139, 140). *In situ* hy-

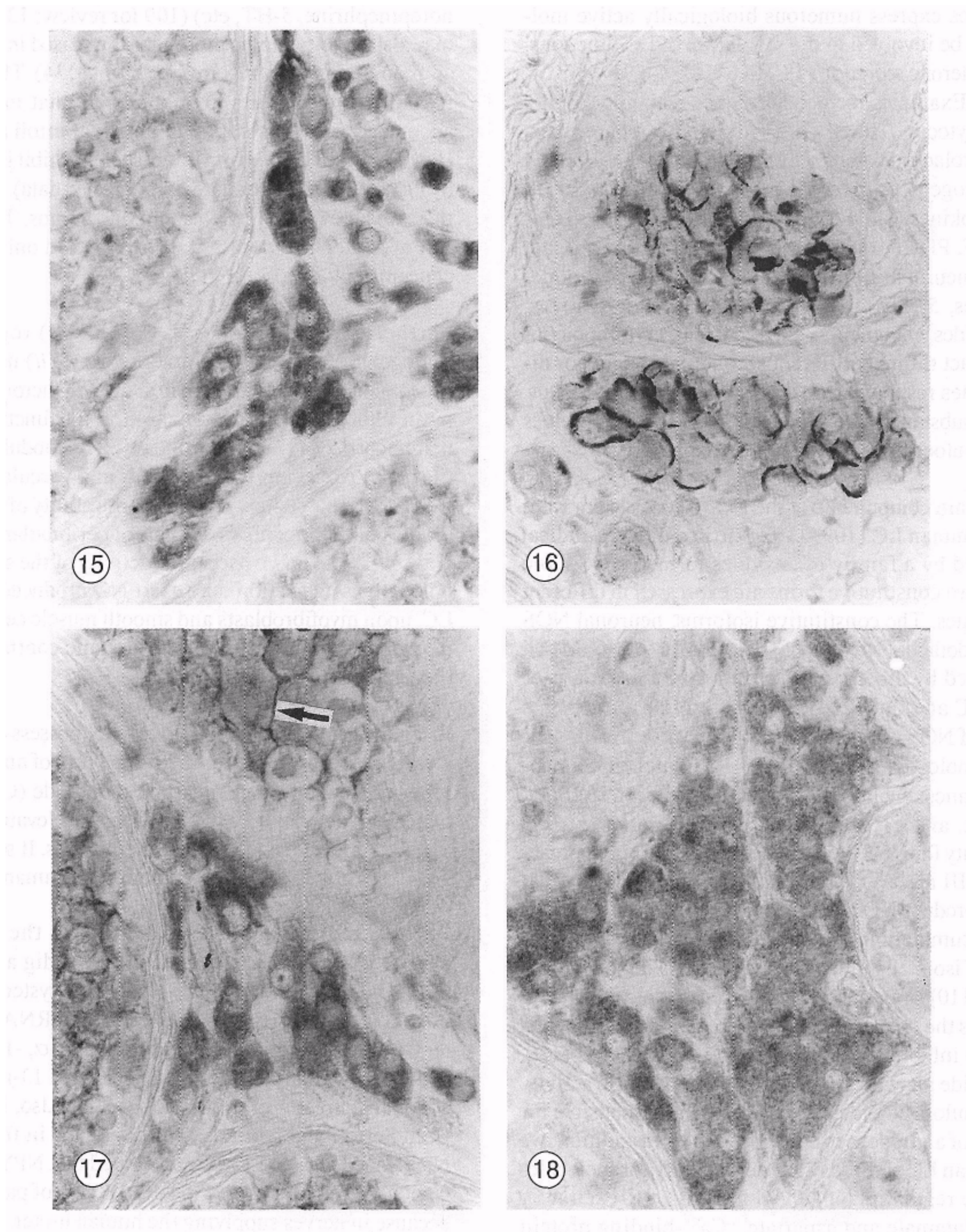



***Figure 15.*** *Microtubule-associated pro tein-2 immunoreactivity of human Leydig cells. Some Sertoli cells also show a low staining intensity.* * 570.

***Figure 16.*** *Neural cell adhesion molecule immunoreactivity on the surface of human Leydig cells clustered in two small groups.* x 570.

***Figure 17****. Immunoreactivity for pan-catherin within cytoplasm of human Leydig cells. Note that the reaction precipitate in the seminiferous tubules is located on the surface of Sertoli cells (arrow),* x 570.

*Figure 18. Serotonin immunoreactivity in a large Leydig cell group of human testis. *• 570.*

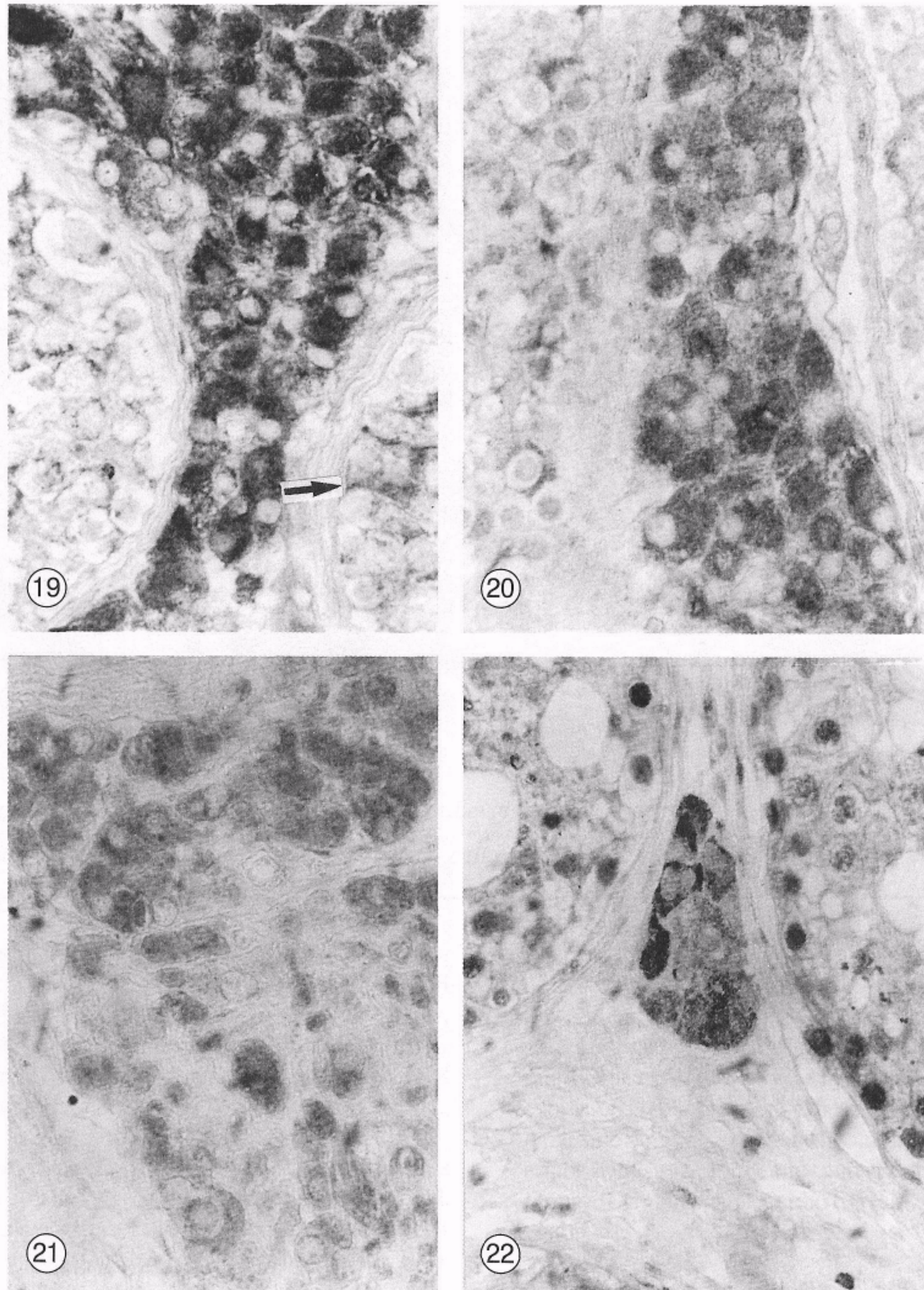



***Figure 19.*** *Growth hormone-releasing hormone imtnunoreactivity in human Leydig cells. The arrow points to positive Sertoli cell,* x 570.

***Figure 20.*** *Immunoreactivity for corticotmpin-releasing hormone in human Leydig cells, x 570.*

***Figure 21.*** *Luteinizing hormone-releasing hormone immunoreactivity in human Leydig cells,* x 570.

***Figure.*** 22. *Neurotensin immunoreactivity. Note the distinct differences in the staining intensity of individual Leydig cells in human testis.* x 570.

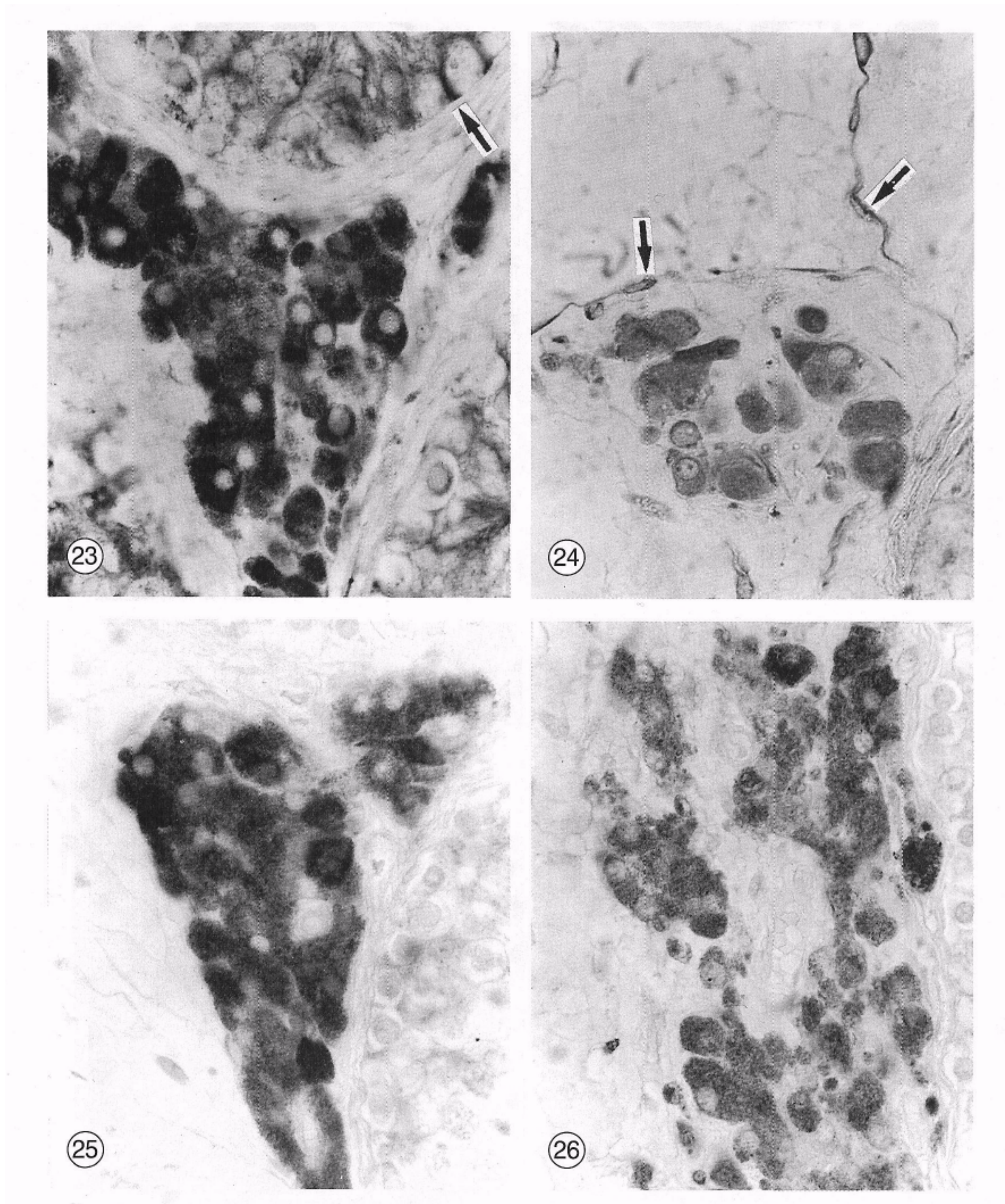



*Figure 23-26. Glial cell markers in Leydig cells of the human testis.*

***Figure** 23. Galactocerebroside immunoreactivity. Note also the intensive staining ofSertoli cells (arrow). * 570.*

***Figure** 24. 2 ',3'-cyclic nucleotide 3 '-phosphodiesterase immunoreactivity. Some interstitial "covering" cells also show a distinct immunoreactivity (arrows),* x *570.*

***Figure** 25. Glial fibrillary acidic protein immunoreactivity.* x J *70.*

***Figure 26.** A2B5 antigen immunoreactivity.* x *570.*

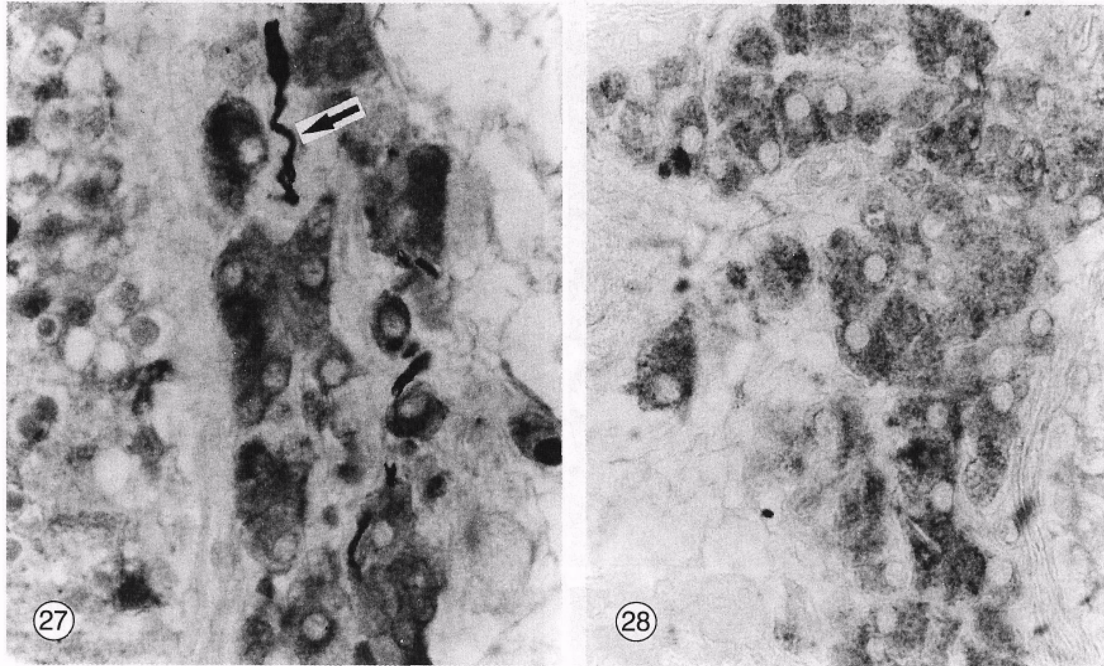


***Figure** 27. Immunoreactivity for S-100 protein in human Leydig cells and in nerve fibers in their vicinity (arrow),* x 570.

*Figure 28. Calbindin-D28 immunoreactivity in a Leydig cell group of the human testis.* x 570.

bridization studies showed that within the testis, TRH receptor mRNA was exclusively detected in LC (141). Recently, TRH immunoreactivity was found in LC of adult rats (142). Thus, to the modulatory hypothalamic neurohormones (GnRH, GHRH, POMC, and CRH) that influence LC functions, TRH has to be added as an autocrine regulator.

A new autocrine or paracrine regulator of LC function seems to be melatonin, an indole derivate secreted by the pineal gland. Recently, structural changes in the testis have been seen after melatonin treatment of immature rats and mice (143, 144). Melatonin binding sites were detected on rat LC (145) and it has been shown that rat testis possesses the enzymes necessary for the local production of melatonin (128). Recent results suggest that melatonin suppresses cAMP- and non-cAMP-stimulated testosterone production in rat LC *via* reducing cAMP production or in part by inhibiting 17-20 desmolase activity (127). However, prolonged exposure to melatonin results in sensitization of the LH-dependent adenylate cyclase activity.

There is evidence that natriuretic peptides may influence the testicular function *via* specific GC-A and to a lesser extent GC-B receptors (124,125,146-148). In a recent study, Middendorff *et al* (149) showed that one member of the natriuretic peptide family, the CNP, is produced by human LC and that these cells expressed GC-B receptors. These results suggest an autocrine/ paracrine action of CNP in the human testis. In addition, we established a moderate immunoreactivity for ANP and BNP in human LC, using particularly well-characterized antisera against these neuropeptides.

Another interesting fact is the occurrence of glial cell marker substances in LC of different species. The GFAP belongs to the group of intermediate filaments found in cells of neural origin (astrocytes) as well as in numerous cell types of non-neural tissues. GFAP was expressed in steroidogenic cells of the adrenal cortex and in hamster LC (150). In a number of other species including rat and human, these authors were not able to find any immunoreactivity for GFAP, showing clear species-dependent differences in the expression of this glial marker.

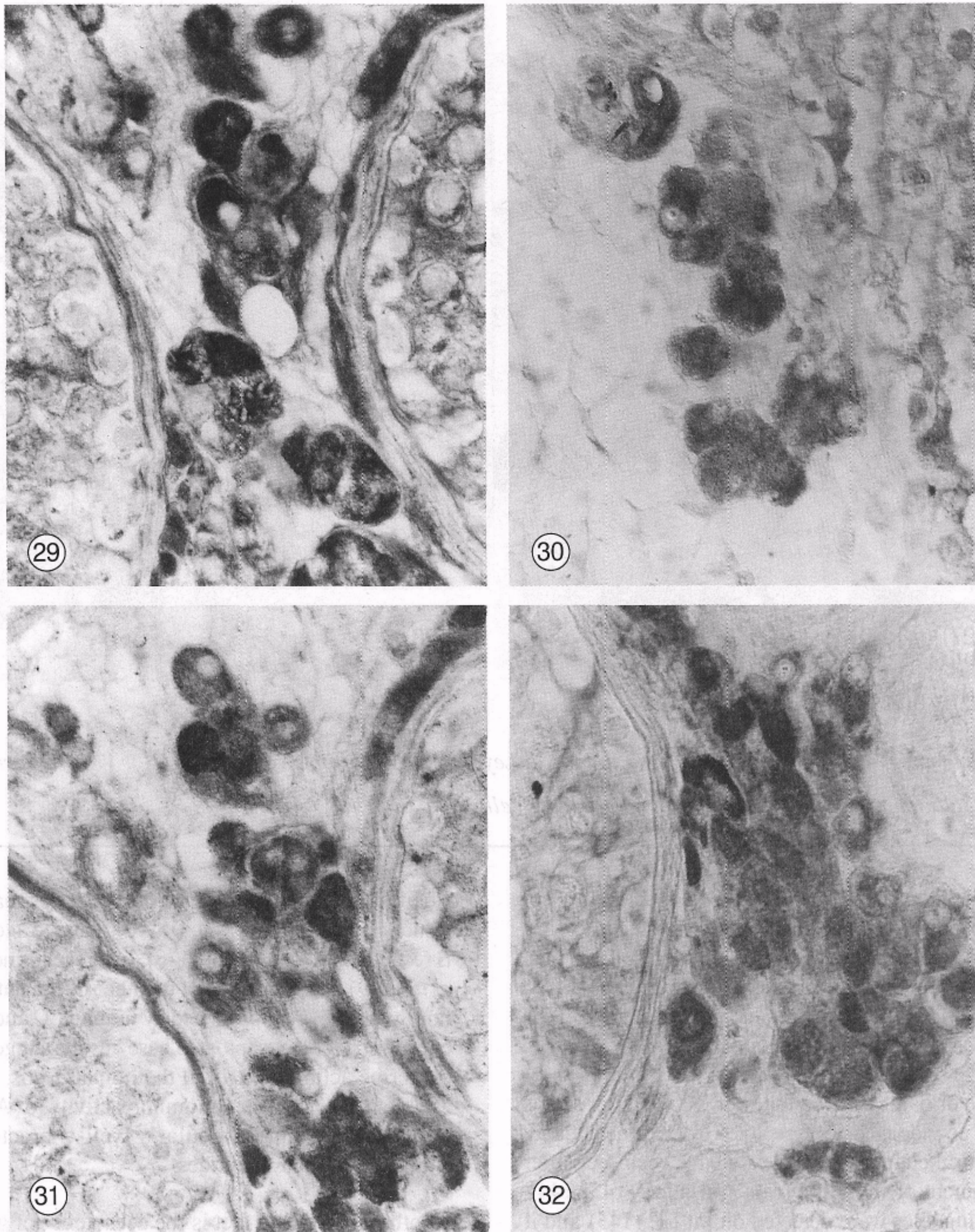



*Figure 29-32. Immunoreactivity for components of the nitric oxide/cGMP system in Leydig cells of the human testis.*

*Figure 29. Nitric oxide synthase-I immunoreactivity.* x 570.

*Figure 30. Soluble guanylyl cyclase immunoreactivity.* x 570.

*Figure 31. cGMP immunoreactivity in a serial section shown in Fig.29.* x 570.

*Figure 32. Superoxide dismutase immunoreactivity.* x 570.

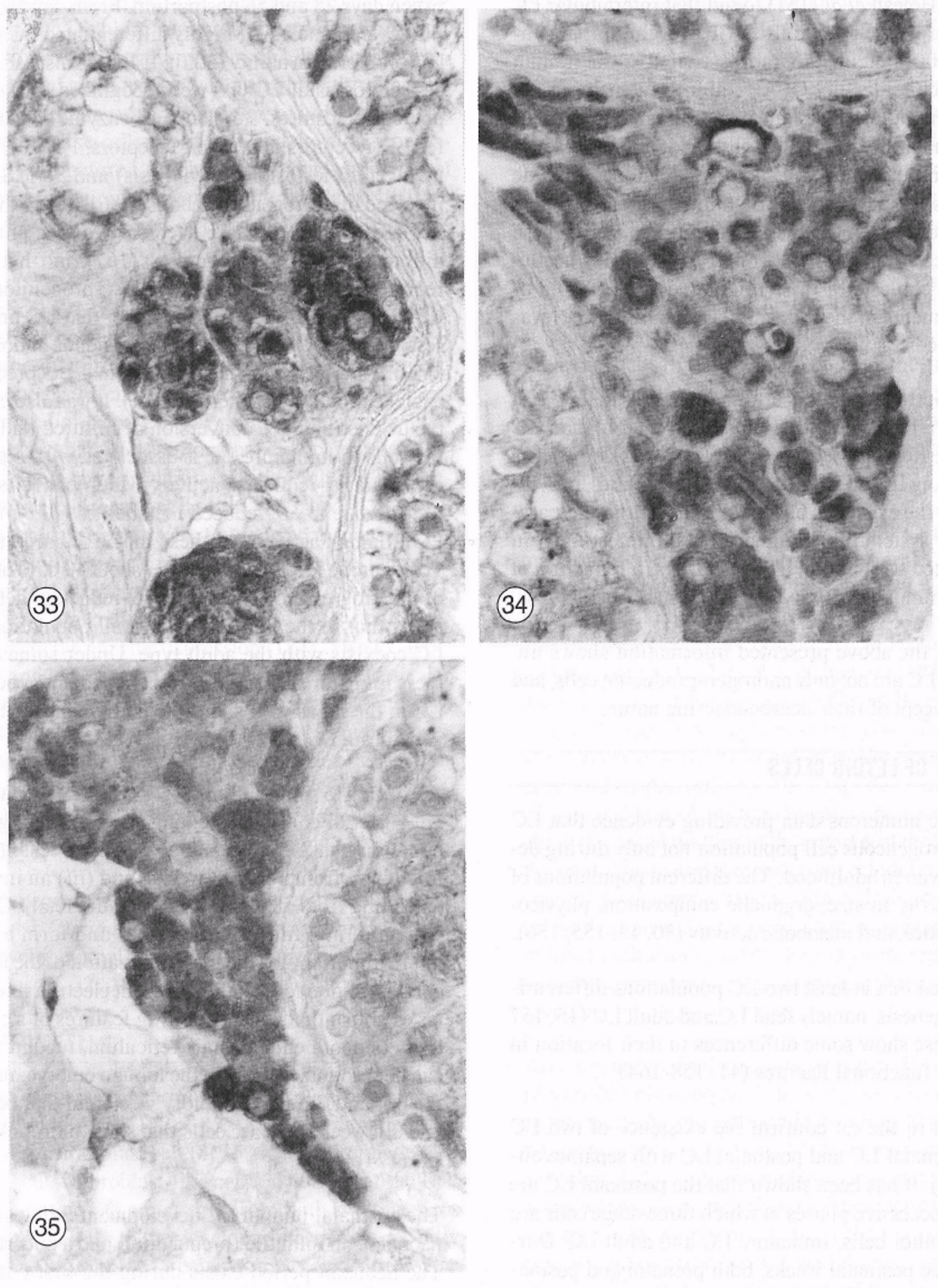



***Figure 33.*** *Angiotensin I immunoreactivity in a Leydig cell group of the human testis. * 570.*

***Figure 34.*** *Nerve growth factor receptor immunoreactivity in human Leydig cells,* x 570.

***Figure 35.*** *Basic fibroblast growth factor immunoreactivity in human Leydig cells,* x 570.

However, in a study on the barrier properties of blood vessels in the rat testis, Holesh *eta I* (151) found that intertubular LC adjacent to microvessels, exhibited GFAP, glutamine synthetase and S-100 protein immunoreactivity. In the human testis, we also found distinct GFAP staining intensity in LC by means of an immunocytochemical amplification technique (Fig.25). In an additional study, we observed immunoreactivity for other glial cell markers such as galactocerebroside (Fig.23), CNPase (Fig.24) and A2B5 antigen (Fig.26). There are no data on the possible functional significance of these marker substances. A possible role of GFAP in the intracellular transport of cholesterol in steroidogenesis (150) and of cytoskeletal filaments in the intracellular signaling (152) was recently suggested.

Despite some controversies concerning the renin-angiotensin system of human testis (121) it seems likely that human LC possess proRen, Ren (153), Ang I (Fig.33), Ang-converting enzyme (154), Ang II, and AngR I immunoreactivities, providing evidence for the existence of an additional autocrine/paracrine regulatory system in the human testis. Interestingly, in patients pretreated with antiandrogenic drugs, a reduction of angiotensin II immunoreactivity has been established (111).

Taken together, the above presented information shows unequivocally that LC are not only androgen-producing cells, and supports the concept of their neuroendocrine nature.

## HETEROGENEITY OF LEYD1S CELLS

• There are numerous data providing evidence that LC represent a heterogeneous cell population not only during development but even in adulthood. The different populations of LC exhibit diversity in size, organelle composition, physicochemical properties, and metabolic activity (30,43, 155,156).

It is well accepted that at least two LC populations differentiate during ontogenesis, namely fetal LC and adult LC (45,157 for review). These show some differences in their location in the testis and in functional features (41, 158-164).

Recent findings in the rat confirm the existence of two LC populations: prenatal LC and postnatal LC with separate ontogeny (61, 165). It has been shown that the postnatal LC are formed in t\vo successive phases in which three-stage cells are involved: progenitor cells, immature LC and adult LC. During the first three postnatal weeks, both prenatal and postnatal LC types coexist in the testis of several species. After that the prenatal LC undergo a gradual loss (157). Progenitors of LC resemble the stem cells (62) from which they are derived and could be seen in the rat testis between day 14 and 28 postpartum (166). They proliferate actively during this time. Then the progenitors start a phase of morphological differentiation and transform into immature LC that could be observed between days 28 and 56 postpartum. From day 56, the immature LC develop into adult, highly differentiated LC with extremely low proliferation ability (32,167,168). This differentiation process of postnatal LC was well recognized on the basis of morphological features, steroidogenic enzyme activity and mRNAs for LH receptors, androgen receptors. P450-17a (an enzyme involved in testosterone synthesis) and 3oc-HSD (an enzyme involved in androgens metabolism) (61,169). It was shown that LH and other factors are necessary for the proliferation and differentiation of LC progenitors (167), and that androgens are required for the differentiation of LC progenitors to immature LC (169). This is related to the fact that LC progenitors possess only few LH receptors but abundant androgen receptors (170). However, human mesenchymal LC precursors are capable to produce testosterone (171). It was also established that the hCG-stimulated conversion of cultured rat Leydig precursor cells to immature LC is associated with a progressive increase in 5a-reductase activity which converts the produced testosterone to 5a-reduced metabolites (172); thus immature LC although actively synthesizing do not secrete testosterone. Testosterone levels increase after about 40 days of age as a result of the progressive decrease of 5 a-reductase activity. It seems likely that in the adult testis a small population of immature LC coexists with the adult type. Under some circumstances these immature LC serve as reserve pool for generation of adult LC in the rat as well as for the permanent replacement of degenera ting LC (61).

According to some authors (157,162, 173) the human fetal LC are object of continuous changes. They undergo: (/') a differentiation phase from fetal age 8 to 14 weeks. (/';') a fetal mature phase from 14 to 18 weeks, and (/'//) an involution phase extending from 18 to 38 weeks. Mature fetal LC show specific structural and functional features and form a steroidogenic gland important for the masculinization of the foetus (157). In contrast to previous findings, recent electron microscopic studies establish that cells exhibiting features of steroidogenic activity (smooth endoplasmic reticulum, tubular mitochondria) can be recognized early in the human embryo, namely between the 6th and 7th postovulatory week and that between the 7th and 8th week there are cells that show morphological signs of LC (174).

The postnatal human LC development follows three stages: a neonatal, an infantile (prepubertal) and a pubertal period (45). The neonatal period exists during the first postnatal months and is characterized by numerous fetal type LC. At this period LC progressively increase in number and reach a maximum at the third postnatal month. After that and until the end of the first year LC rapidly regressed and a heterogeneous population of infantile LC, myofibroblasts and immature fibroblasts remained in the intertubulular space. The second period extends

between the first year of age and the recognition of the first pubertal signs. During this period, a LC population exists resembling dedifferentiated fetal LC and probably arising from fetal LC and/or *de novo* from Leydig precursor cells. The appearance of first pubertal signs marks the onset of the third, pubertal period that extends until complete sexual maturity is reached. Fibroblast-like cells proliferate and develop progressively to young and mature LC. The primary sites of origin of this population are the outer layers of the tubular wall from which mature LC migrate towards the intertubular space (45, 171).

The ontogenetical LC heterogeneity and the possibility that different types may coexist at definite developmental stages is probably reverberated in the results of some studies suggesting the existence of different adult LC populations. For example, after separation of rat LC on Percoll gradients, one light fraction with abundant hCG receptors that did not generate testosterone, and one heavier fraction lacking high LH/hCG affinity but producing testosterone, were established (175, 176). Although methodical reasons were found to be responsible for this "heterogeneity", recent findings in adult rats established two distinct subpopulations with different expression, of cytochrome P450IIAl.The authors explain these results with age-dependent shifts in the enzyme expression (177). Two LC types showing high and low capacity to secrete androgens were also proved applying a reverse haemolytic plaque assay (178). However, the light and dark cells in human testis may indicate differences in the regulation and/or function, rather than differences in LC steroidogenic capacity (179). Recently, distinct heterogeneity of human LC was demonstrated in connection with the main components of the NO/cGMP system (110).

The species-dependent differences in LC heterogeneity have to be emphasized. Obviously there are structural, biochemical, regulatory, developmental and functional differences of LC in different species (2, 32, 45, 61, 65, 100, 110, 180, 181).

## ORIGIN OF LEYDIG CELLS

• Current concepts concerning gonadal development presume mesenchymal (162, 173, 182-185), fibroblastic (37), peritubular myoid/fibroblast cell (8, 35, 45), macrophage (28, 186) or perivascular fibroblast-like cell (72) origin of the LC. Migration of putative LC precursors from both mesonephros and coelomic epithelium to the developing testis was recently described (185, 186, 188). Moreover, such a dual origin was proposed by these authors also for the Sertoli cells. Results on quail-chick chimeras recognized a mesonephric origin of LC (189). However, other results (190) argue against an early migration of cells from mesonephros into the testis. Recent findings using cultured mouse embryos provide further evidence for mesonephric contribution to peritubular myoid cells and other interstitial cells (191). This view is supported also by results of Mayerhofer *et al* (192) about cells exhibited N-CAM immunoreactivity at embryonic day 17 stretching from the mesonephros into the forming *rete testis* and continue into the testis itself. The interstitial cells are also immunoreactive for P450scc which gives the opportunity to distinguish them as LC. However, no information is presented by these authors of whether the cells located in the mesonephros or at the border between mesonephros and the gonadal anlage exhibit P450scc immunoreactivity.

Despite of these at a first glance controversial results, recent publications agree with the proposal that LC in rodent and human testes derive from undifferentiated mesonephric mesenchymal cells that migrate and invade the gonad at appropriate stage of early development. Obviously these cells comprise a mixed population of stem cells that gives also rise for peritubular myofibroblasts, interstitial fibroblasts and macrophages (61, 157, 174).

Regardless the general consensus that LC are of mesenchymal origin, "the ultimate embryonic origin of Leydig stem cells has not been established"(61, see also 45). As discussed above mesenchymal cells from two main sources, the mesonephros and the mesoderm, are considered to be the stem cells that undergo the LC lineage of development (185, 187). However, a third source of mesenchymal-like cells, namely the neural crest cells, has been neglected until now. There is a lot of information about the origin and fate of development of the neural crest and its derivatives (193-198). The neural crest consists of a population of pluripotent precursor cells that develops along the dorsal midline of the embryo, at the boundary between the neural plate and the epidermal ectoderm. At exact defined stage of development, neural crest cells segregate from the neural epithelium and migrate in a rostrocaudal pattern along well defined pathways towards their definite regions. During migration, neural crest cells change their shape, become elongated and elaborate long processes.

Arriving at the final position, neural crest cells generate cells of variable phenotypes. One group of cells become neural in nature (sensory and autonomic ganglia and glia cells of the peripheral nervous system, enteric neurons, adrenal gland medullar cells, and skin melanocytes) and others possess the morphological characteristics of mesenchymal cells. Depending on the location, neural crest cells may start the migratory process either prior (rostral neural crest) or after the closure (neural crest of the trunk region) of the neural tube (194).

The fact that LC of man and some rodents, in addition to their steroidogenic phenotype, exhibit numerous markers characteristic for paraneurons suggests that LC are neuroendocrine cells. Taking into account the pluripotent features and the wide dis-

tribution of neural crest cells throughout the organism as well as the narrow spatial relationships between the neural crest and the mesonephros/gonadal blastema, it could be presumed that neural crest cells migrate into these anlages and give rise not only to LC but also to peritubular myofibroblasts, fibroblasts of the intertubular space, vascular smooth muscle cells and probably to Sertoli cells (see below). The neural crest, the intermediate mesoderm, the pronephros, and the mesonephros develop during the third and fourth week of the gestational age. During this time massive migration of different cells and especially of neural crest cells has been observed. The crest progenitors for the suprarenal medulla, for example, invade the organ between the 15 stage embryo (33 gestational day) and the end of the embryonal period. At this time, approximately the 6th week of development (stage 15-17; 8-12 mm embryo), the initial signs of differentiation of the gonadal ridge at the ventral surface of the pronephric and metanephric ridges may be seen. The gonadal ridge develops below the suprarenal primordium and the pronephric structural components. During tins time narrow spatial and temporal interrelationships exist between the neural crest cells and the primordial structures of the genitourinary system. While sympathoadrenal progenitors migrate from the neural crest to the suprarenal medulla, similar cells may invade the developing pronephros and mesonephros and reach some days later the gonadal blastema.

Another organ that also receives its vegetative components from the neural crest is the gut. The enteric nervous system is derived from the vagal regions of the neural crest. The crest cells enter the gut near the pharynx and follow a rostrocaudal migratory way (198). Recent results provide evidence that the enteric neurons may derive from the sympathoadrenal lineage showing some characteristic features of sympathetic neurons and adrenal chromaffin cells (199). Cells from this lineage disperse throughout the body and are included in the diffuse neuroednocrine system according Pearse (104,105), or termed paraneurons by Fujita (106 for review). In some organs, the segregated neural crest cells having finished their migration regroup and generate cell clusters with well developed intercellular contacts. The LC also migrate to the testis and generate cell groups in the intertubular space (183). They are interconnected by gap and tight junctions and adhesion molecules such as N-C AM (3, 121, 192). In this connection, neural crest cells also show N-CAM immunoreactivity that during their migration disappears gradually and could be established again when they arrive at their final region and start to differentiate (200). The variable phenotype of LC, if assumed that their stem cells are neural crest cells, is not surprising. There is unequivocal evidence that neural crest cells comprise various progenitors, including the sublineage of sympathoadrenal, glial and melanogenic cells (193). This sublineage generates cells that produce both catecholaminergic and melanocyte populations. Similarly, in the rat retina a common progenitor for neurons and glia was established (201). The existence of pluripotent progenitors may explain the fact that LC exhibit both neuronal and glial markers. Thus, the possibility exists that pluripotent neural crest cells of mesenchymal shape may invade the gonadal anlage and serve as stem cells for the development of the somatic cell lineages of the testis. This presumption is supported by the fact that LC, peritubular myofibroblasts, connective tissue cells of the intertubular tissue, and Sertoli cells share some common antigenic features, suggesting their possible origin from a common progenitor. For example, both LC and Sertoli cells show immunoreactivity for NSE (98), oc-inhibin (202-204), the calcium-binding protein SPARC (205), vimentin (206) and NPY (138). Also, Sertoli cells share some similarities with nerve cells (207, 208). In Leydig and Sertoli cells, immunoreactivity for GAP-43, neurofilament proteins, MAP2, NOS, CaM, calbindin, S-100 protein, CNPase, GFAP, galactocerebroside, bFGF, TGF-B, Ang II, AngR I, big-End,End I, End II, EndRA, estrogen and androgen receptors has been established (3; unpublished data; also Ergiin's unpublished data). Moreover, LC and fibroblastic "covering" cells of the interstitial tissue (49) share common glial cell antigens such as galactocerebroside, CNPase, A2B5 and GFAP.

Neural crest-derived cells migrate also to the thymus (195,209) and under autocrine/paracrine influence of EGF differentiate to epithelial cells of a neuronal phenotype, expressing neurofilament proteins, SP and other tachykinins, NPY, somatostatin, specific neurotrophic factors (ciliary neurotrophic factor, interleukin-6), and cytokeratins (210). It seems likely that these cells undergo a process of epithelial-neural transition which proceeds under the influence of different growth factors, in comparison with the neural cell lineage of the gut, thyroid gland or pancreas.

Neural crest cells migrate along axons of prospective peripheral nerves and use them as guide in their movement towards the end region to be invaded. Ectopic LC located along peripheral nerves are an interesting phenomenon. These cells are situated either within the *tunica albuginea* or in regions located away from it. Ectopic LC are established as component of accessory suprarenal glands (Holstein, unpublished data) and along the spermatic cord (66,211-213). In the *tunica albuginea* and especially in the spermatic cord, ectopic LC and glomus cells and neurons are located in the vicinity or within peripheral nerve bundles (212). These findings support the idea for the possibility that neural crest cells use peripheral nerves as guiding structures during their migration towards the developing testis. It is probable that at the time of cessation of migration some LC progenitors proceed their differentiation at sites located away from the testis. It is likely that postpubertal LC progenitors also migrate to their end location using as guiding structures the *lamina propria* of seminiferous tubules and the vascular walls. This may explain the different sites of

occurrence of prepubertal and postpubertal LC established light and electron microscopically in testes of different mammals (45, 161).

The fact that for the immunocytochemical visualization of most of the substances found in LC, amplification procedures have to be applied, suggests a low quantity of these antigens. This may also suggest that they are not of functional importance for the LC. In the LC of some species, low-level functional transcripts which coexist with predominantly non-functional transcripts are established (214). Moreover, the functional variants are much less common, and detectable only with methods more sensitive than northern hybridization. Thus, sequence analysis and polymerase chain reaction assays are recommended if conclusions about their physiological significance be drawn (214, 215). However, the immunocytochemical visualization of some antigens as well as the expression of non-functional transcripts may be of importance for the establishment of the cell lineage. Hence, some of the substances detected may be considered rudimentary (or redundant, junk) products that could serve as marker substances which allow us to follow out the ontogenetic origin and development of the LC.

Are all LC of neural origin? This question cannot be answered precisely at the moment. The fact that LC represent a heterogeneous and dynamic cell population which may change its phenotype during normal and pathological conditions makes it difficult to distinguish different cell lineages. In the human and hamster, both the prepubertal and postpubertal LC exhibit some of the established neuroendocrine marker substances (97; unpublished data). These results suggest that these cell populations may originate from the same stem cell. As discussed above, progenitor cells may persist during the life of an individual species either in peritubular or perivascular location, or within the intertubular tissue as documented in the rat following degeneration of LC by the alkylating agent ethane-1,2-dimethyl sulphonate (EDS) and subsequent regeneration (45,65). Interestingly, the regenerated LC are not sensitive to repeated EDS applications. These cells show similarities with the immature adult type LC of the prepubertal testis (65). It seems likely that EDS inhibits steroidogenesis and increases intracellular levels of glutathione which leads to apoptotic LC death. However, EDS shows distinct species-dependent differences in its action. Therefore in the adult rat testis, progenitor cells persist which under some circumstances give rise to immature LC. Immature LC can proliferate for several weeks and differentiate to mature LC in which the proliferative activity ceases (65). The factor(s) responsible for the inhibition of LC proliferation is (are) not known yet. During the proliferation of immature LC, TGF-fi plays an inhibiting role. Human LC exhibit moderate to strong TGF-ft immunoreactivity, thus this growth factor may account for their very slow proliferative activity (32, 45,167,168). In adult human LC, only low to very low immunoreactivity for EGFR is found, which is in accordance with findings showing a more pronounced effect of EGF/TNF-a during the proliferative phases of LC development (65). Another candidate that may also contribute to the low proliferative activity of adult human LC may be NO. Recently, it was shown that the nerve growth factor (NGF) may induce the activity of a NOS isoform in proliferating neurons and the produced NO triggers a switch to growth arrest during differentiation of neuronal cells (216). As our unpublished data show, adult human LC like PC 12 cells possess NGFR immunoreactivity as well as immunoreactivity for the constitutive and inducible NOS isoforms. Colocalization of NGFR and NOS-I was already established in neurons of different regions of the central nervous system (217). Thus, NGF in addition to its specific function in the onset of meiosis (218) mediated by the Sertoli cells (219), together with NO, may contribute to the differentiation of the adult human LC.

## CONCLUSION

• The dual nature of LC comprises its endocrine and neuroendocrine features. Human LC are the main cell type in the testis that produce androgen hormones. Also, they are a cell type involved in the regulation of testicular functions in intracrine, autocrine and paracrine fashions. There is evidence that prepubertal and postpubertal LC show distinct similarities including their endocrine as well as neuroendocrine features. This suggests the possibility that both cell populations may originate from a common stem cell.

The accumulated information on the structural and biochemical composition of the LC supports the hypothesis that the Leydig stem cells are derivatives of the neuroectoderm and most probably of the neural crest. Human LC and LC of some vertebrates are immunoreactive for a number of neuronal, neuroendocrine and glial cell antigens. Future studies will confirm or reject this hypothesis. One difficult problem, in case that the neural crest hypothesis is valid, provides the determination of the neural crest region (head-vagal-pharyngeal or trunk) that contributes to the delivery of migrating stem cells which are responsible for the differentiation of the LC lineage. Another question is whether there is only one pluripotent stem cell type from which all known LC subtypes emerge and only the local homeostatic conditions in the testis are responsible for the generation of heterogeneous LC at a definite developmental stage. If so, which factors are responsible to keep in a latent form the progenitor cells which are important for the regeneration of died LC? Why do LC have an extremely low proliferative index and how does increase their number in cases of hyperplasia? Under normal circumstances no mitotic figures could be observed in human LC. Single mitotic figures (two in 87 biopsies) have been reported (220). However, these authors have not considered the possible existence of carcinoma

*in situ* cells within tubules located closely to the mitotic LC. Evidence was provided for single mitoses in LC situated in the vicinity of early germ cell tumors (221). These mitoses were explained with the possible action of mitogenic factors released by the tumor cells (221).

The LC behaves as a stable, nearly postmitotic cell type that is to a larger extent undependent of the functional activity of other structural components of the testis and of the spermatogenesis. This is not surprising because LC are not primarily important for spermatogenesis. This fact explains the persistence of LC in cases with impaired spermatogenesis, cryptorchidism, testicular atrophy, testicular tumors and other pathological changes. This behavior of the LC suggests that they possess very effective own regulatory mechanisms that ensure their survival under unusual conditions, including an ectopic position. It seems likely that under some circumstances LC may dedifferentiate. In these cases their neuroendocrine character becomes more prominent.

As shown in the present review, some of the biologically active substances detected in LC may be involved in different regulatory pathways subserving the regulation of LC functions or the communication between the somatic cell representatives of the testis. However, some of the substances of the LC may not have any functional significance and could be considered marker substances permitting further conclusions on their ectodermal neural origin and their relation to the remaining somatic and interstitial cells of the testis.

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