Invited Review

Luteinizing hormone on Leydig cell structure and function

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Summary. The effects of luteinizing hormone (LH) and human chorionic gonadotrophic hormone (hCG) on Leydig cell structure and function are reviewed in this paper under two main headings; responses to LH and hCG stimulation and responses to LH deprivation. With acute LH stimulation, up to 2 hours following the LH injection, there was no change in the volume of a Leydig cell. However, Leydig cell peroxisomal volume and intraperoxisomal SCP_2 content showed a rapid and transient change. These changes can be considered to be specific because: i) no other Leydig cell organelle including smooth endoplasmic reticulum (SER) showed such a change, and ii) only the intraperoxisomal SCP_2 but not catalase (a marker enzyme for peroxisomes) showed such a change within 30 minutes of LH stimulation. As these changes occurred prior to the peak testosterone levels following this treatment, it is suggested that SCP_2 and peroxisomes may have an association with testosterone biosynthesis prior to cholesterol transport into mitochondria. With LH or hCG stimulation for longer periods, i.e. one day or more, the same morphological changes are produced in Leydig cells irrespective of the age of the species, dosage of LH or hCG, and with single or multiple doses. These changes include, Leydig cell hypertrophy and/or hyperplasia, increase in the cellular organelle content (mostly SER and mitochondria) and depletion of lipid droplets. In addition, a recent study showed that Leydig cell peroxisomal volume, SCP_2 content, the amount of intraperoxisomal SCP_2 and testosterone secretory capacity were also significantly increased in response to chronic LH treatment. The effects of LH deprivation by whatever means (e.g. hypophysectomy, with testosterone and 17ß-estradiol Silastic implants, LH antisera) on Leydig cell structure and function is generally described as opposite to those observed following LH or hCG stimulation. These include Leydig cell hypotrophy and hypoplasia, reductions in the cytoplasmic organelle content in general and specific reductions in SER and peroxisomal volumes, reductions in total catalase and SCP_2 in Leydig cells together with reductions in the intraperoxisomal SCP_2 content in Leydig cells and their testosterone secretory capacity.

Key words: Leydig cells, LH, Peroxisomes, Sterol carrier protein-2, Testosterone, Catalase, Stereology, Microscopy

Introduction

Leydig cells reside in the testis interstitium (see Fig. 1) and they are the main source of testosterone in the adult mammalian testis. Luteinizing hormone (LH) of the anterior pituitary regulates Leydig cells to produce testosterone; the latter hormone is required for many functions in the male reproductive tract at all stages of life, i.e. the embryonic, neonatal, pre-pubertal, pubertal and adult stages. In the adult mammalian male, testosterone is required for spermatogenesis, maintenance of accessory sex organ function, fertility and successful reproductive performance. In this article, changes in Leydig cell structure and function under conditions of LH stimulation and deprivation are reviewed. Emphasis is made on the average volume, ultrastructure, number per testis and testosterone secretory capacity. As human chorionic gonadotrophin (hCG) is used in many studies instead of LH to exert LH activity, those articles which describe changes in Leydig cells in response to hCG are also included in this review.

Response to luteinizing hormone (LH) or human chorionic gonadropin (hCG) stimulation

Acute stimulation

Following LH or hCG stimulation Leydig cells produce testosterone. Therefore, it is natural to study the structure and function of Leydig cells following LH/hCG stimulation by many investigators in this field of research. To the best of this author's knowledge, Christensen (1959) was the first to report on the response of Leydig cells to acute stimulation of rats with hCG. In

his study Christensen (1959) administered an intravenous injection of hCG (dosage not reported) to newborn and prepubertal albino rats. He reported that one half hour later, the fine structure of the interstitial cells did not change from those of age matching controls. Later, using stereological techniques, Nussdorfer et al. (1980) observed that the average Leydig cell volume and absolute volumes of many organelles such as smooth endoplasmic reticulum, mitochondria and peroxisomes were unchanged following two hours after a single injection of hCG (300 IU/kg). These findings were in agreement with those reported in Christensen's study (1959). Although transient morphological changes may have occurred in Leydig cells prior to two hours following hCG, these were not examined in these early studies (Christensen, 1959; Nussdorfer et al., 1980). Recently, Mendis-

Handagama et al. (1990a) performed a time course study on the effects of LH on the adult rat Leydig cells (Sprague Dawley strain); zero, half hour, one hour, and two hours following a single subcutaneous injection of 500 g of LH per rat. This study reported for the first time that although that there was no change in the average volume of a Leydig cell throughout this period as observed by Nussdorfer et al. (1980), there were other early LH-mediated structural changes in Leydig cells (Fig. 2). The volumes of peroxisomes and "negative bodies" (organelles which have been described as structurally identical to peroxisomes but do not contain catalase; Fig. 4) were increased three-fold ($P<0.01$) above the control values within one half hour, and remained significantly higher ($P<0.05$) than controls up to one hour after the LH injection (Fig. 2). However, the values for both "negative bodies" and peroxisomes were significantly lower at one hour than those at one half hour (Fig. 2). At two hours after the LH injection, the volume of peroxisomes per Leydig cell returned to control levels, which agrees with the findings of Nussdorfer et al. (1980) at this time point, but the volume of "negative bodies" per Leydig cell remained
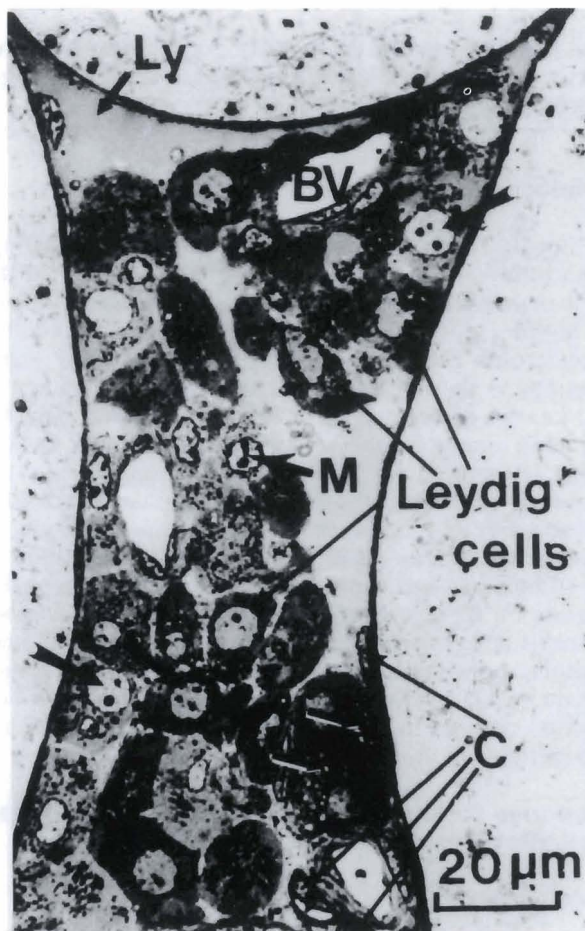

Fig. 1. This light micrograph shows the testis interstitium of an adult normal mouse. The Leydig cells appear as circular or polygonal profiles in section. The majority of Leydig cell nuclei appear as circular or oval profiles. Some nuclei have more than one nucleolus (large arrows). Two small arrows depict empty spaces in Leydig cell cytoplasm caused by the extraction of lipid droplets during tissue processing. BV: blood vessels; M: macrophages; Ly: lymphatic space; C: connective tissue cells. Bar: 20 μm (From J.Androl. 1990, 11, 539-547, with permission).
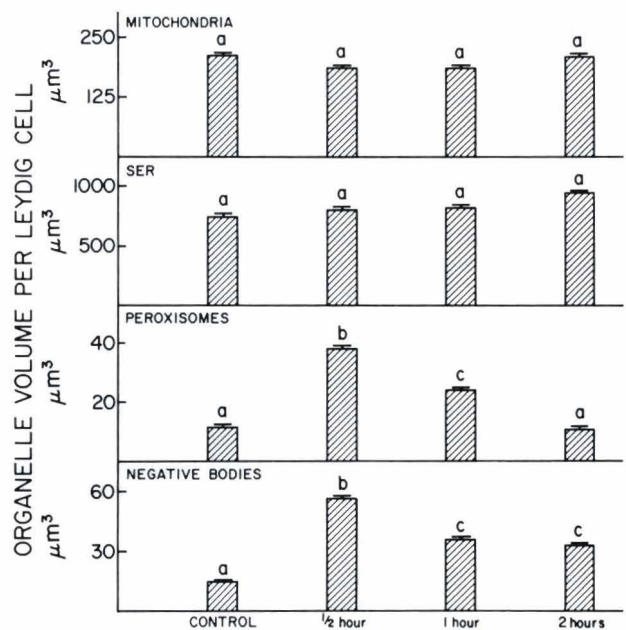

Fig. 2. This histogram shows organelle volumes per Leydig cell in control and LH- injected rats after one half, one and two hours. Different letters indicate statistically significant differences at $P<0.05$. The absolute volumes of mitochondria and smooth endoplasmic reticulum (SER) per Leydig cell were unchanged up to two hours after LH injection. In contrast, the absolute volumes of peroxisomes and negative bodies per Leydig cell increased three-fold above control levels one half hour after LH injection. The absolute volume of peroxisomes per Leydig cell reached control levels by two hours after LH injection. However, the absolute volume of negative bodies remained significantly higher than control levels, but significantly lower than the levels at one half hour after the LH injection. n=5 rats/group. Error bar: SEM. (From Endocrinology 1990, 127, 2947-2954, with permission).

elevated even at two hours (Fig. 2). These volume changes were not apparent in any other Leydig cell organelle including smooth endoplasmic reticulum, although latter organelle contains enzymes required for testosterone production by Leydig cells. Therefore, Mendis-Handagama et al. (1990a) considered that this acute effect of LH on Leydig cell peroxisomes is highly specific. Similarly, sterol carrier protein-2 (SCP_2) which binds cholesterol in 1:1 ratio (Vahouny et al., 1984) and has also been reported to be in high concentration in peroxisomes in Leydig cells of control rats (Mendis-Handagama et al., 1990b), increased rapidly and transiently one half hour after a LH injection (Figs. 4, 5). Such a change has not been observed in intraperoxisomal catalase in Leydig cells (catalase is considered as a marker enzyme for peroxisomes; Figs. 6, 7). Serum testosterone levels have been observed to rise transiently following LH or hCG injections (Hodgson and de Kretser, 1982; Mendis-Handagama et al., 1990b), but reach peak values after one and/or two hours following these treatments (Fig. 8, 5, 6), i.e. well after the occurrence of the peak volume of Leydig cell peroxisomes and intra-peroxisomal SCP_2 content. Therefore, these findings suggest a possibility of an association among peroxi-somes, SCP_2 and cholesterol

transport mechanisms during testosterone production following LH stimulation in Leydig cells.

Chronic stimulation

With regard to the Leydig cell structure and function in response to chronic LH or hCG, the same morphological changes are produced in many species, irrespective of the age of the animals tested (immature: Merkow et al., 1968; Chemes et al., 1976; Kuopio et al., 1989; sexually mature: Heller and Leach, 1971; Aoki et al., 1972; Christensen and Peacock, 1980; Nussdorfer et al., 1980; Mendis-Handagama et al., 1994), dosage of LH or hCG (such as 500 g of LH or 100 IU of hCG per rodent, 4 mg hCG per man), or whether one single dose or multiple doses were given for lengths of time varying from one day or more. Typically, there is hypertrophy of Leydig cells (Heller and Leach, 1971; Aoki and Massa, 1972; Christensen and Peacock, 1980; Nussdorfer et al., 1980; Mendis-Handagama et al., 1994) and increase in content of the cellular organelles involved in biosynthetic and secretory process of steroid hormones. Most commonly documented organelles are smooth endoplasmic reticulum and mitochondria (de Kretser, 1967; Merkow and Pardo, 1968; Merkow et al., 1968;
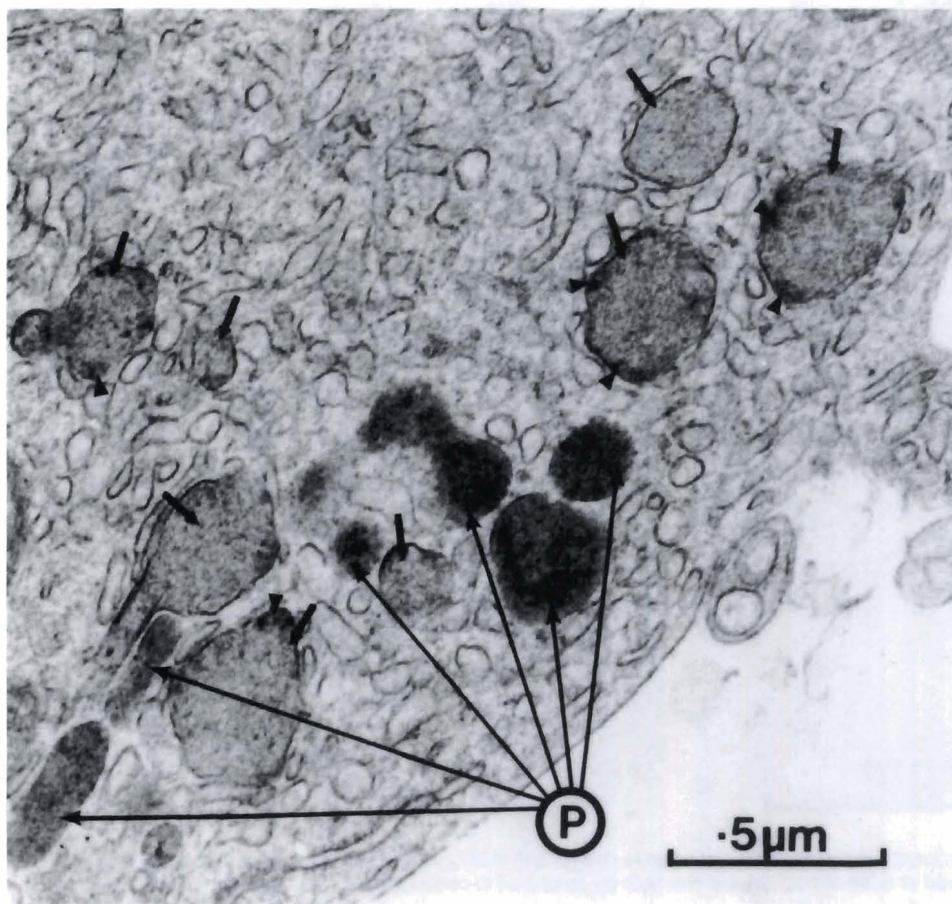

Fig. 3. Electron micrograph of Leydig cell cytoplasm one half hour after LH injection. Peroxisomes (P) are histochemically stained for catalase. The small arrows depict negative bodies and the arrow heads demonstrate edges of negative bodies stained for catalase. (From Endocrinology 1990, 127, 2947-2954, with permission).

Aoki et al., 1972; Nussdorfer et al., 1980). However, evidence is rapidly increasing that peroxisomes could also be added to this list (Nussdorfer et al., 1980; Mendis-Handagama et al., 1990a). Changes in size and shape of nuclei and mitochondria in Leydig cells are also reported in adult mice following hCG administration. Lipid droplet depletion in Leydig cells is observed in mice (Aoki, 1970; Russo and Sacerdote, 1971; Neaves 1978), rats (Nussdorfer et al., 1980), guinea pigs (Merkow and Pardo, 1968; Merkow et al., 1968) and

humans (de Kretser 1967; Heller and Leach, 1971). Latter observation is suggestive of using cholesterol stored in lipid droplets for steroid hormone production following LH stimulation.

Chronic LH treatment of adult rats for 14 days induced via subdermal implants of LH-filled Alzet miniosmotic pumps, which delivers 1 µg of LH per hour, has shown to produced a three-fold increase in the average volume of a Leydig cell compared to controls. However, the testosterone secretory capacity per Leydig
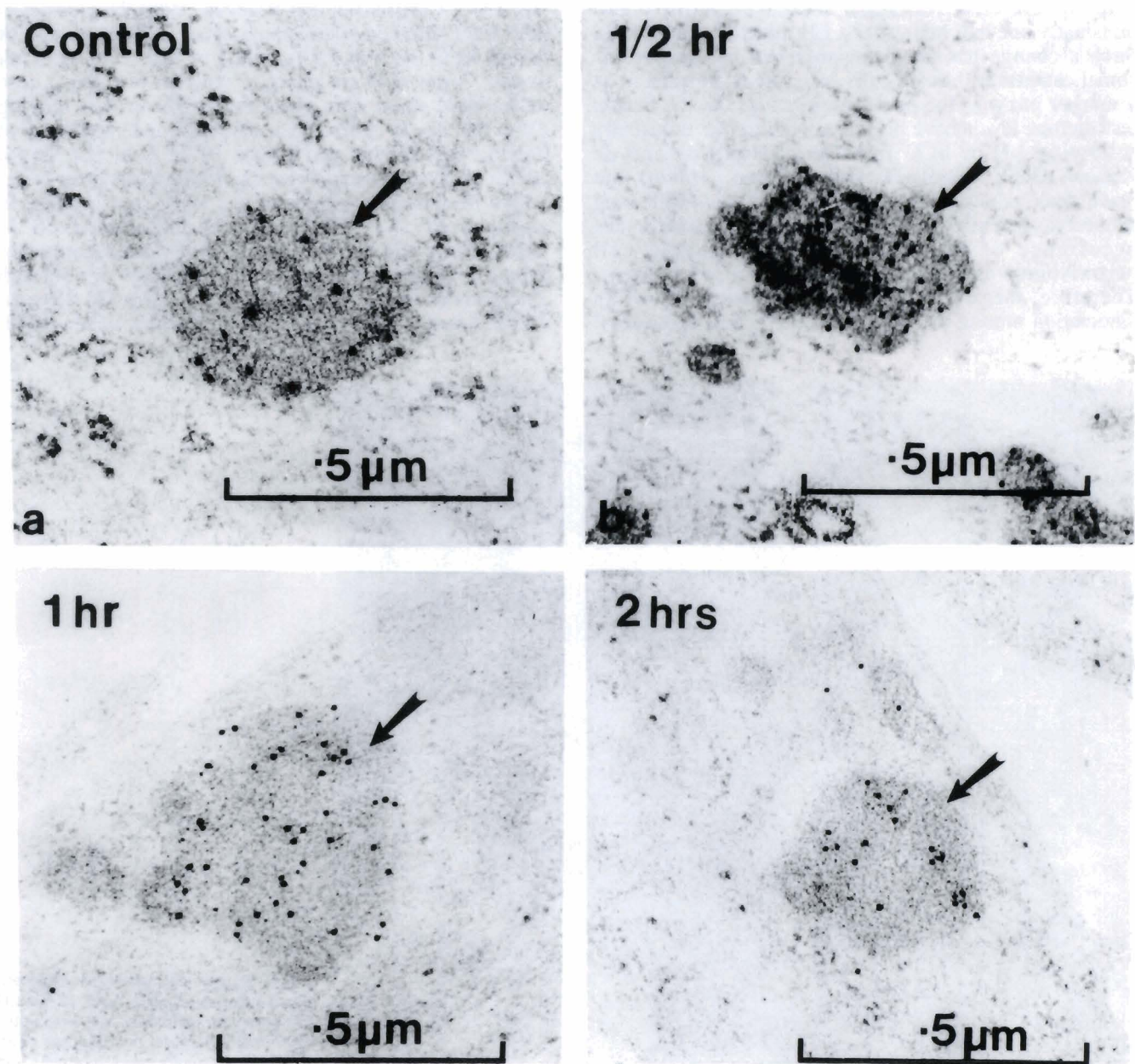

Fig. 4. Immunolocalization of SCP$_2$ (arrows) in Leydig cell peroxisomes of controls rats (a) and in LH injected rats after one half hour (b), one hour (c) and two hours (d). Increased labeling is observed at one half hour (b) and one hour (c) compared to control (a) and two hours (d). (From Endocrinology 1990, 127, 2947-2954, with permission)

GOLD PARTICLE DENSITY OVER LEYDIG
CELL PEROXISOMES FOR SCP$_2$ (no./μm^2)

CONTROL

½ hour

1 hour

2 hours

Control

1/2 hour

1 hour

2 hour

·5μm

cell in those rats was six-fold above control values (Mendis-Handagama et al., 1994). These results revealed that the magnitude of the increase in the testosterone secretory capacity per Leydig cell following chronic LH stimulation exceeds the degree of Leydig cell size-increase. Stereological and immunocytochemical electron microscopic investigations of these Leydig cells in chronically LH-stimulated rats have shown that the volume of peroxisomes per Leydig cell and the intraperoxisomal SCP$_2$ content in these Leydig cells are increased by two and three fold respectively, following
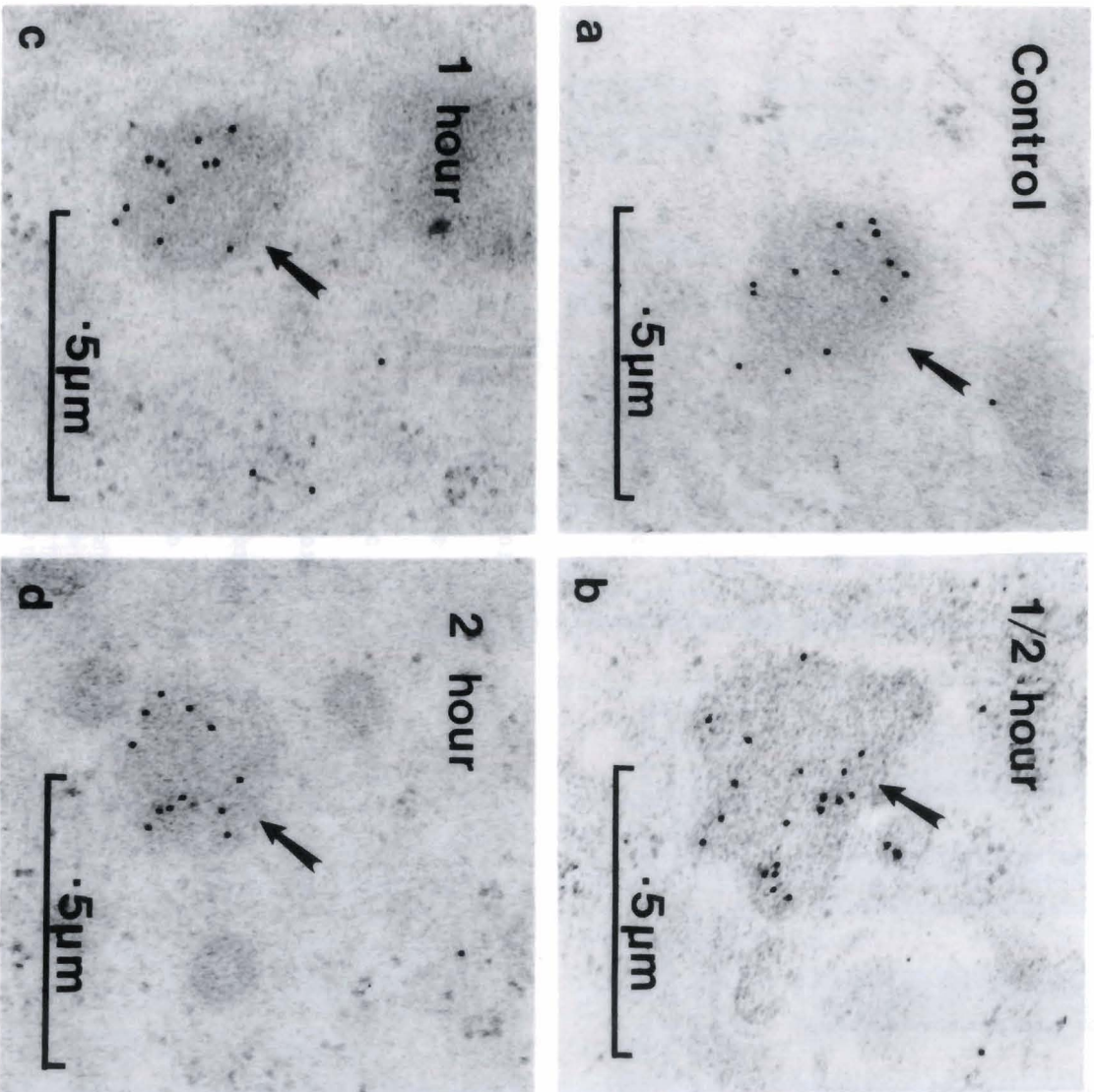
Fig. 5. This histogram shows the gold particle density (number per μm^2) over Leydig cell peroxisomes for SCP2 in control rats and LH-injected rats after one half hour, one hour and two hours. Different letters indicate statistically significant differences at P<0.05. n=5 rats/group. Error bar: SEM. (From Endocrinology 1990, 127:2947-2954, with permission).

Fig. 6. Immuno-localization of catalase (arrows) in Leydig cell peroxisomes of controls rats (**a**) and in LH injected rats after one half hour (**b**), one hour (**c**) and two hours (**d**). No difference is observed in gold labeling at all time points. (From Endocrinology, 1990, 127, 2947-2954, with permission)

chronic LH treatment (Mendis-Handgama et al., 1995a). By contrast, the intraperoxisomal catalase content has been observed to be significantly reduced in these chronically LH-treated rats, although catalase is a marker enzyme for peroxisomes. Taken together, it is evident that the chronic LH treatment of adult rats for 14 days results in the increase in the volume of SCP_2-rich peroxisomes in Leydig cells which are lower in catalase (Mendis-Handagama et al., 1995a). Furthermore, the latter study suggests that not only the peroxisome volume but also the intraperoxisomal SCP_2 content in Leydig cells is regulated by LH. These findings further support that peroxisomes and SCP_2 have a significant role in the steroidogenic function in Leydig cells.

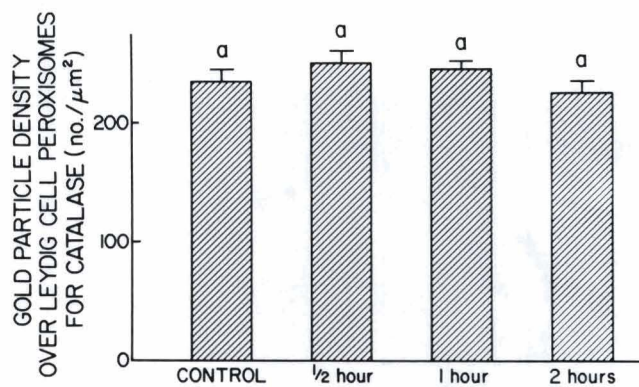Although acute LH stimulation does not alter the

Leydig cell number per testis, long-term LH or hCG stimulation increase the number of Leydig cells per testis. In immature rats, following hCG treatment (100 IU per rat per day) for 10 days, a four fold increase has been observed in Leydig cell number per testis (Chemes et al., 1976). In sexually mature rats, it is reported that hCG treatment (100 IU per rat per day) for five weeks resulted in a three fold increase in Leydig cell number per testis (Christensen and Peacock, 1980). In contrast, it is reported in normal men, prolonged treatment with hCG (4000 IU intramuscularly three times a week for six weeks or every second day for 16 weeks) does not alter Leydig cell number per testis (Heller and Leach, 1971). Although the exact reason for these differences in the responses of Leydig cell number per testis in the two species is not known, it is logical to suggest that these differences may be due to species differences. In addition, these variations in the Leydig cell numbers may be due to the differences in the morphometric techniques used and/or to the differences in the identification of Leydig cells by different investigators. The origin of the increased Leydig cell numbers in hCG treated rats

Fig. 7. This histogram shows the gold particle density (number per μm^2) over Leydig cell peroxisomes for catalase in control rats and LH-injected rats after one half hour, one hour and two hours. Gold particle density in peroxisomes at different time points is not significantly different among the groups. n=5 rats/group. Error bar: SEM. (From Endocrinology 1990, 127, 2947-2954, with permission).
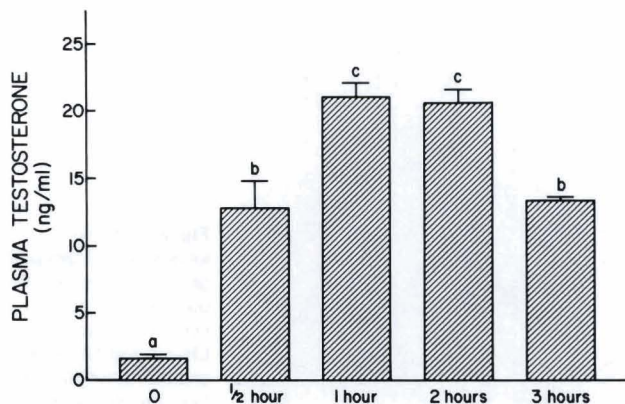

Fig. 8. Plasma testosterone levels of control and LH-injected rats after one half hour, one hour, two hours and three hours are shown in this histogram. Peak concentrations of plasma testosterone occur at one and two hours following the LH injection. n=5 or 6 rats per group. Error bar: SEM, (From The Leydig Cell, 1996, Chapter 34, pp 703-734, with permission).
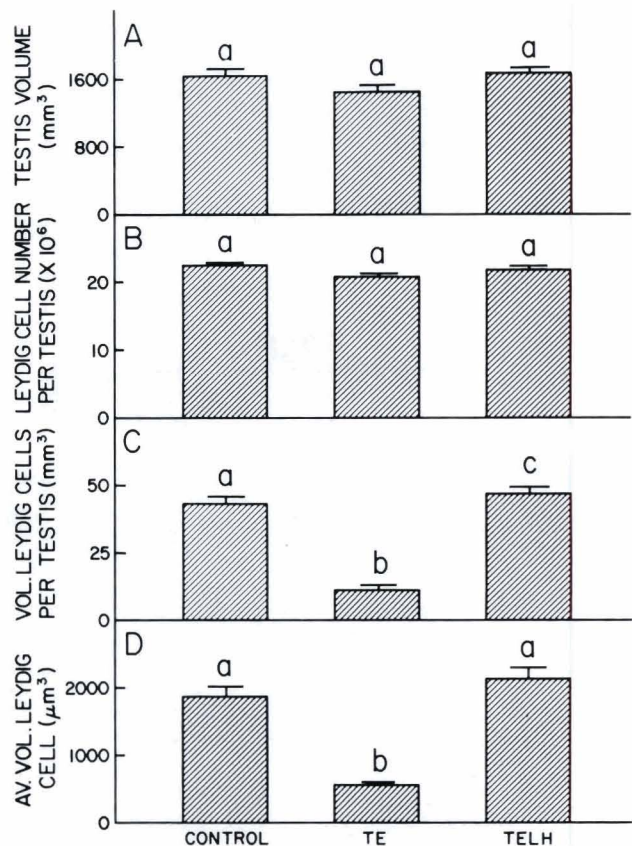

Fig. 9. Effects of TE implants (testosterone+estradiol) and TELH implants (testosterone+estradiol+LH) on testis volume, Leydig cell number per testis, volume of Leydig cells per testis and average volume of a Leydig cell in adult Sprague Dawley rats. n=5 rats/group. Error bar: SEM, (From Endocrinology, 1992, 131, 2839-2845, with permission).

appears to vary, depending on age. In the immature rat, the initial effect of hCG was suggested as to increase in the number of Leydig cells undergoing mitosis by a factor of 16. Subsequent to this, the effect of hCG was suggested as stimulation of precursor fibroblasts to differentiate into Leydig cells (Chemes et al., 1976).

Response to luteinizing hormone deprivation

Hypophysectomy (Christensen, 1959; Keeney and

Fig. 10. Electron micrograph of Leydig cells in the testis interstitium of a rat hypophysectomized for 36 days. Most Leydig cell nuclei (N) appear to be pleomorphic. Note that the lymphatic endothelium (arrows) surrounds the Leydig cells. This feature is not observed in normal rats. BV: blood vessels. (From The Leydig Cell, 1996, Chapter 34, pp 703-734, with permission).

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Ewing, 1990; Russell et al., 1992) and conditions or treatments that cause endogenous LH deprivation such as immunization with LH antisera (Wakabayashi and Tamaoki, 1966; Dym and Madhwaraj, 1977), and testosterone and estradiol Silastic implants (Ewing et al., 1983; Wing et al., 1984; Mendis-Handagama and Ewing, 1990; Mendis-Handagama et al., 1992), result in the alteration of Leydig cell structure and function. The
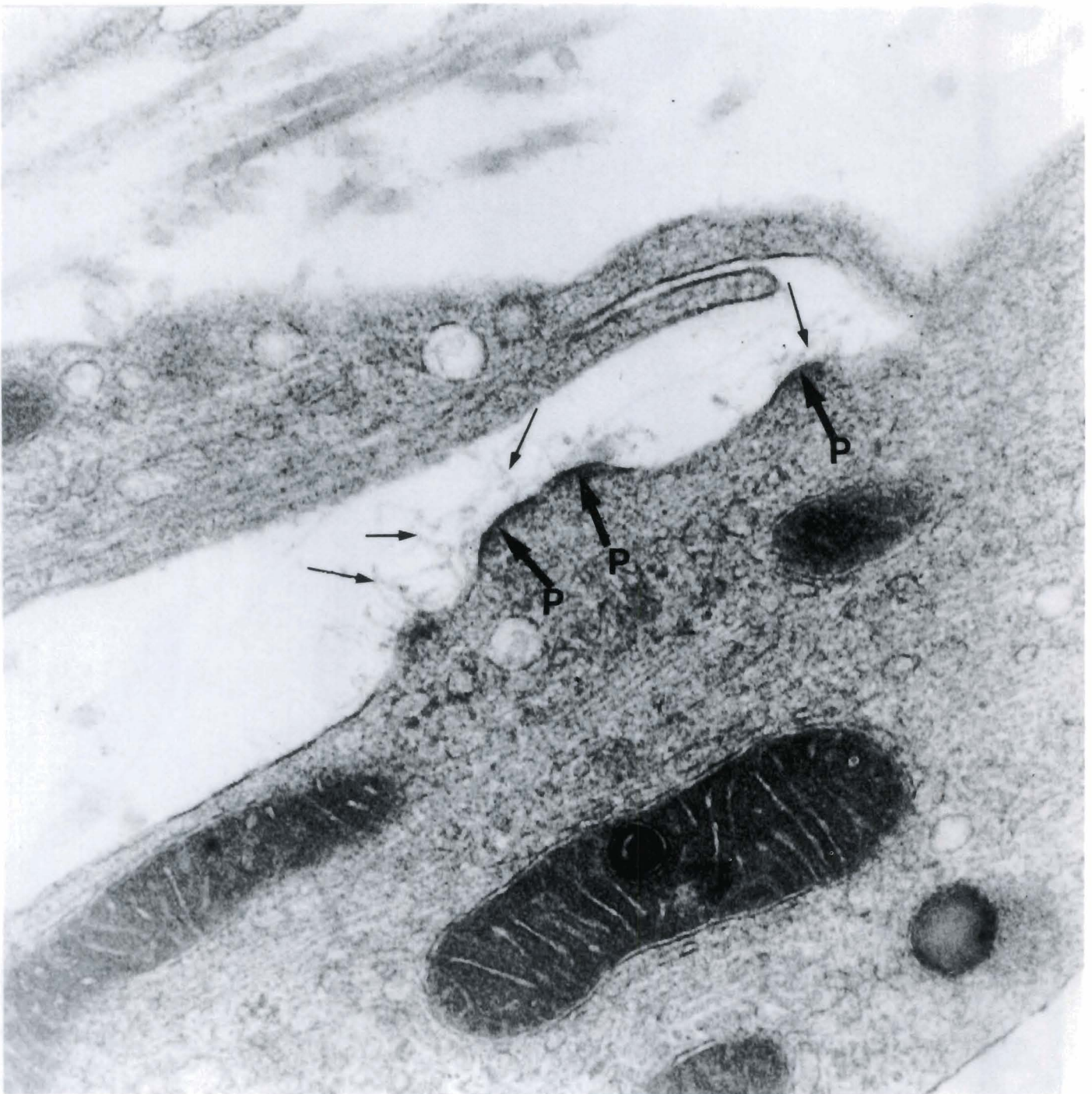

Fig. 11. Electron micrograph of a Leydig cell from a hypophysectomized rat of 36 days to demonstrate the presence of dense plaques (P) on the surface of the cell, which are associated with numerous extracellular fibrils (fine arrows). This feature is rarely observed in normal rat Leydig cells. (From The Leydig Cell, 1996, Chapter 34, pp 703-734, with permission).

effects of endogenous LH deprivation, by whatever means, on Leydig cell structure and function could be described generally as opposite to those observed following LH or hCG stimulation. To the best of this author's knowledge, Christensen was also the first to report changes in Leydig cells in response to LH deprivation, which was achieved in these rats via hypophysectomy (1959). According to this report (Christensen, 1959), five days (i.e. short term) following hypophysectomy in newborn and prepubertal albino rats, appearance of prominent intra-mitochondrial inclusions in the "interstitial cells" were the only change that was evident. However, 15 days following hypophysectomy (i.e. long term) Christensen (1959) reported that the
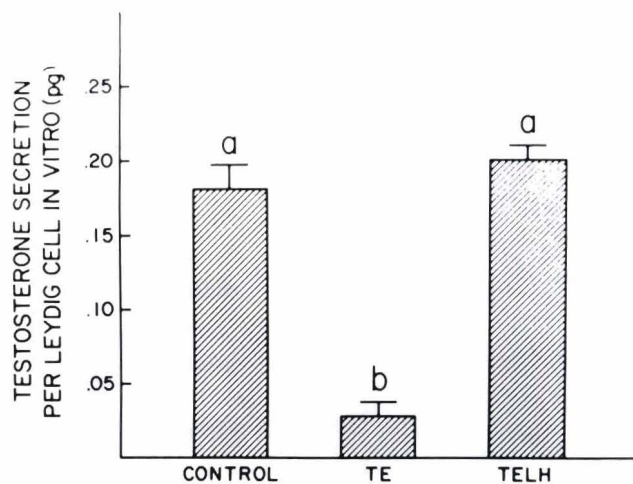

Fig. 12. LH-stimulated testosterone secretion per Leydig cell *in vitro* in control, TE (testosterone+estradiol implanted) and TELH (testosterone+estradiol+LH implanted) rats. Testosterone secretory capacity per Leydig cell is reduced to 1/7th of control values with TE treatment, but not with TELH treatment. Error bar: SEM. n=3 incubations per group. P<0.01. (From Endocrinology, 1992, 131, 2839-2845, with permission).

Leydig cells were definitely smaller, the mitochondria were reduced in size and showed very little internal structures, except for the presence of intra-mitochondrial inclusions. Later, many other studies on the effects of endogenous LH deprival in rats have shown that the average volume of a Leydig cell is reduced by this condition (Ewing et al., 1983; Irby et al., 1984; Wing et al., 1984; Kerr et al., 1986; Mendis-Handagama and Ewing, 1990), which confirmed Christensen's (1959) findings on Leydig cell size. Leydig cell cross-sectional area of adult rats (Sprague Dawley strain) is observed to be reduced by 29% within 24 hours of a single injection of anti-LH serum (Dym and Madhwaraj, 1977). Moreover, seven and eight days following subdermal implants of Silastic tubing filled with testosterone (2.5 cm) and 17 ß-estradiol (0.3 cm; Ewing et al., 1983; Wing et al., 1984; Mendis-Handagama et al., 1992) and six days following hypophysectomy (Russell et al., 1992) the average volume of a Leydig cell in adult Sprague Dawley rats are reduced to one-third of the control values. It has also been demonstrated that this effect of Leydig cell hypotrophy/atrophy in adult rats following endogenous LH deprivation could be prevented by simultaneous treatment or substitution with exogenous LH (Wing et al., 1984; Mendis-Handagama et al., 1992, Fig. 9).

Ultrastructural studies on these atrophic Leydig cells of LH-deprived rats have shown that pleomorphic nuclear profiles are more frequently observed in LH-deprived rats (Mendis-Handagama 1996; Fig. 10), especially during a treatment of long duration. By contrast, the majority of the nuclear profiles of control rats are not pleomorphic. It is interesting to document that in Sprague Dawley rats hypophysectomized for 36 days, lymphatic endothelium surrounds the Leydig cells, a feature that has not been previously reported (Fig. 10) and has not been observed in the testis interstitium of normal adult rats. Moreover, on the surfaces of these Leydig cells of these hypophysectomized rats, dense
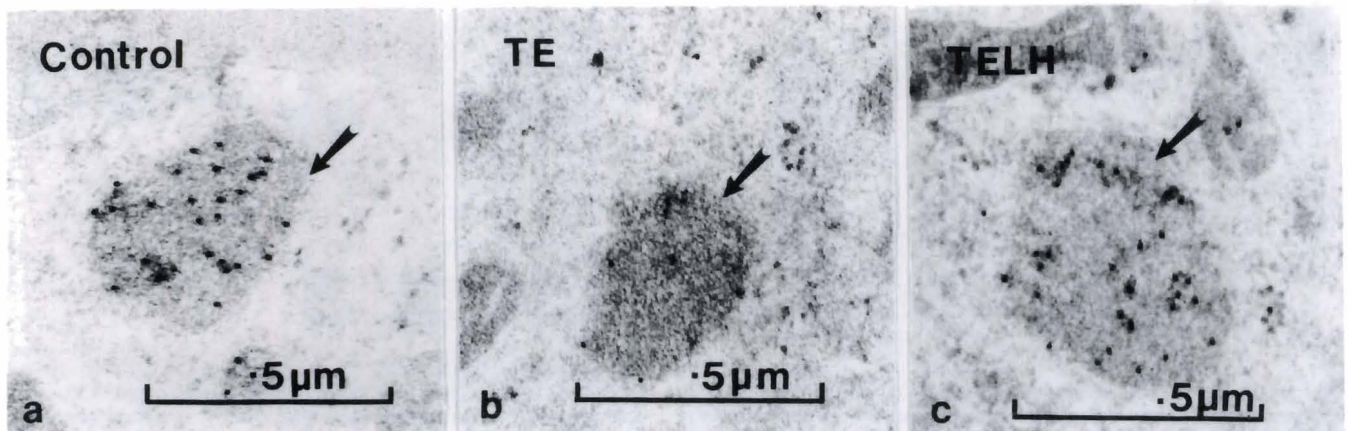

Fig.13. Representative peroxisomes from control, TE (testosterone + estradiol implanted) and TELH (testosterone+estradiol+LH implanted) rats immunolabeled for SCP$_2$. Intraperoxisomal SCP$_2$ content in Leydig cells is significantly reduced with TE treatment but not with TELH treatment. (From Endocrinology, 1992, 131, 2839-2845, with permission).

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plaques that are associated with numerous extracellular fibrils are frequently observed (Fig. 11). It is also reported that when gonadotrophin secretion was experimentally suppressed by hypophysectomy or testosterone implants (Irby et al., 1984; Kerr et al., 1986), the Leydig cells of the Australian wild bush rat *Rattus fuscipes* undergo atrophy. Moreover, large crystalloid inclusions appear in the cytoplasm of these Leydig cells. Because these crystalloids occur only in regressed Leydig cells that produce reduced amounts of testosterone, it is suggested that they may represent a storage form of steroid precursors and enzymes. However, additional studies are required to elucidate their precise function.

There is a positive and linear correlation between the average volume of a Leydig cell (size) and testosterone secretory capacity in hamster, rat and guinea pig testes perfused *in vitro* (Mendis-Handagama et al., 1988). This finding is also in agreement with Leydig cell size and testosterone secretory capacity following endogenous LH deprival as Leydig cell atrophy following endogenous LH deprival via what ever means is always associated with reduced testosterone secretory capacity of Leydig cells (Ewing et al., 1983; Wing et al., 1984; Klinefelter et al., 1987; Keeney et al., 1988; Mendis-Handagama et al., 1990a,b). Furthermore, the existence of a relationship between Leydig cell smooth endoplasmic reticulum and testosterone biosynthesis has been described in many qualitative (de Kretser, 1967; Merkow et al., 1968; Neaves, 1973) and quantitative (Ewing et al., 1983; Mendis-Handagama et al., 1988) ultra-structural studies. As conditions with endogenous LH deprivation cause Leydig cell hypotrophy/atrophy and reduced steroid secretory capacity, it is not surprising to observe reductions in Leydig cell organelle volumes and/or surface area. Reductions in surface area of smooth endoplasmic reticulum (Ewing et al., 1983;
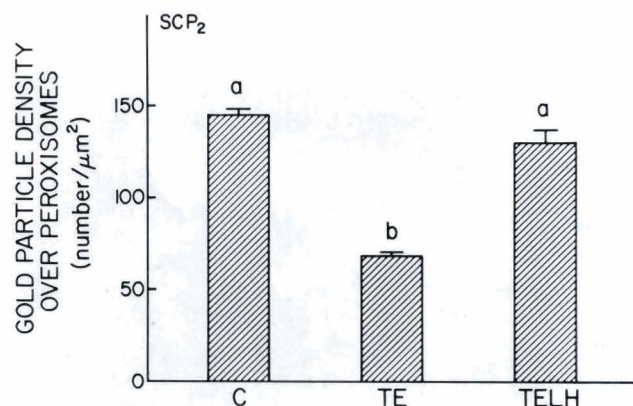
Wing et al., 1984) and rough endoplasmic reticulum (Ewing et al., 1983) per Leydig cell in adult rats (Sprague Dawley strain) have been reported following testosterone-estradiol treatment for seven days (Ewing et al., 1983) and six days following hypophysectomy (Russell et al., 1992). Moreover, volumes of smooth endoplasmic reticulum, rough endoplasmic reticulum, nucleus, mitochondria, and peroxisomes per Leydig cell are observed to be reduced with six and 28 days following hypophysectomy (Russell et al., 1992) and eight days following testosterone-estradiol treatment of adult rats (Wing et al., 1984). The volume of Golgi apparatus per Leydig cell is reduced following
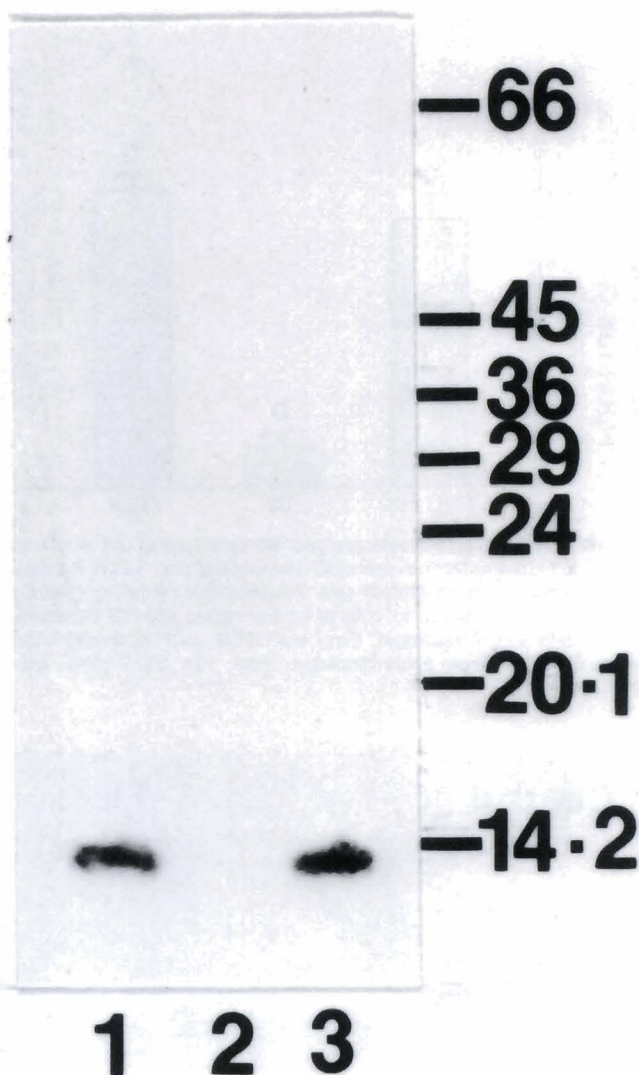

Fig. 14. Quantification of intraperoxisomal SCP$_2$ content in Leydig cells of control TE (testosterone+estradiol implanted) and TELH (testosterone +estradiol+LH implanted) rats showed that the intraperoxisomal SCP$_2$ content in Leydig cells is significantly reduced with TE treatment but not with TELH treatment. (From Endocrinology, 1992, 131, 2839-2845, with permission).

Fig. 15. Immunoblots for SCP$_2$ (14 kilodalton) in Leydig cells (.3x10^5/gp) of control TE-implanted and TELH-implanted rats (lanes 1, 2 and 3 respectively). These results show that SCP$_2$ in Leydig cells of TE-implanted rats is reduced to undetectable levels due to reduced volume of peroxisomes per Leydig cells, together with the reduced content of intraperoxisomal SCP2 in Leydig cells with TE treatment. (From Endocrinology, 1990, 127, 2847-2954, with permission).
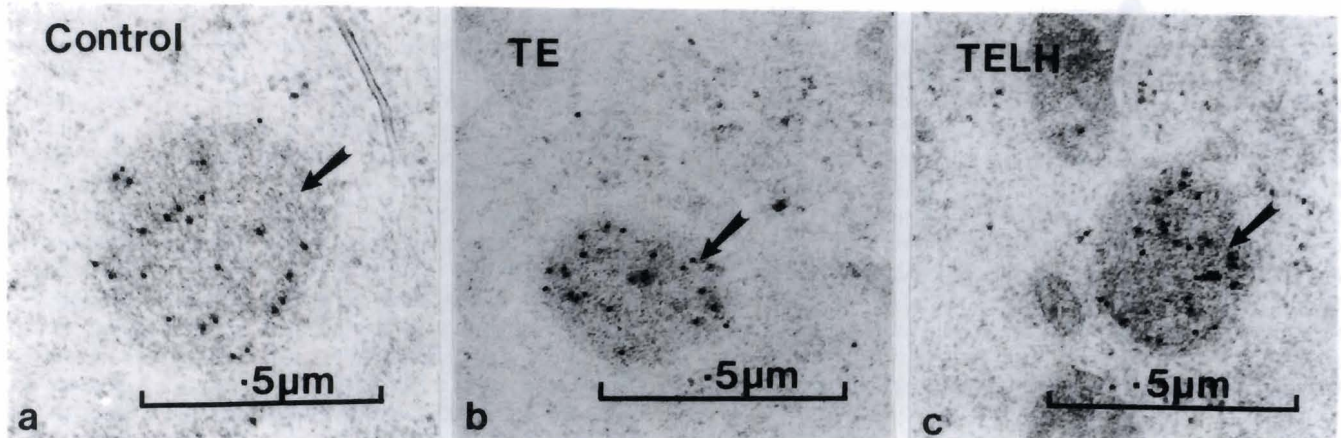
Fig. 16. Representative peroxisomes from control, TE (testosterone + estradiol implanted) and TELH (testosterone+estradiol+LH implanted) rats immunolabeled for catalase. Intraperoxisomal catalase content in Leydig cells appears to be similar in all treatment groups. (From Endocrinology, 1992, 131, 2839-2845, with permission).

Table 1. Volume of organelles per Leydig cell (μm^3) in control, TE and TELH rats.

ORGANELLE	CONTROL	TE	TELH	TE/CONTROL
SER	735 (60)	133 (8)*	767 (58)	1/6
Peroxisomes	11.91 (0.98)	2.02 (.03)*	13.99 (1.06)	1/6
Negative bodies	15.39 (1.26)	2.41 (.03)*	16.95 (1,29)	1/6
RER	18.56 (0.87)	4.19 (.02)*	21.49 (1.1)	1/4
Mitochondria	237 (19)	67 (4)*	234 (18)	1/3
Lysosomes	13.74 (1.1)	4.54 (.3)*	18.86 (1.43)	1/3
Nucleus	218 (7)	148 (9)*	234 (17)	2/3

Values are the mean, with the SE in parentheses (n=5 rats/group). *: significantly different at $P>0.01$. TE: LH-deprived rats (via testosterone and estradiol Silastic implants); TELH: TE rats exogeneously treated with LH. (From Endocrinology, 1992, 131, 2839-2845, with permission).

Fig. 17. Quantification of intraperoxisomal catalase content in Leydig cells of control TE (testosterone+estradiol implanted) and TELH (testosterone+estradiol+LH implanted) rats showed that the intraperoxisomal catalase content in Leydig cells is not different in the three treatment groups. (From Endocrinology, 1992, 131, 2839-2845, with permission)

hypophysectomy for six and 28 days (Russell et al., 1992), and the volumes of lysosomes and "negative bodies" per Leydig cell is reduced after eight days of testosterone-estradiol treatment (Mendis-Handagama et al., 1992). Moreover, reductions in surface area of many other organelles per Leydig cell in addition to smooth endoplasmic reticulum and rough endoplasmic reticulum, such as Golgi, annular gap junctions, inner and outer mitochondrial membrane per Leydig cell have been observed in six and 28 days after hypophysectomy (Russell et al., 1992). Again, it is also observed that these reductions in organelle volumes and testosterone secretory capacity in Leydig cells following short-term LH deprivation could be prevented by exogenous LH substitution (Table 1 and Fig. 9; Mendis-Handagama et al., 1992). By contrast, Ewing et al. (1983) did not observe any changes in the surface area of inner and outer mitochondrial membranes per Leydig cell following testosterone-estradiol treatment for seven days.

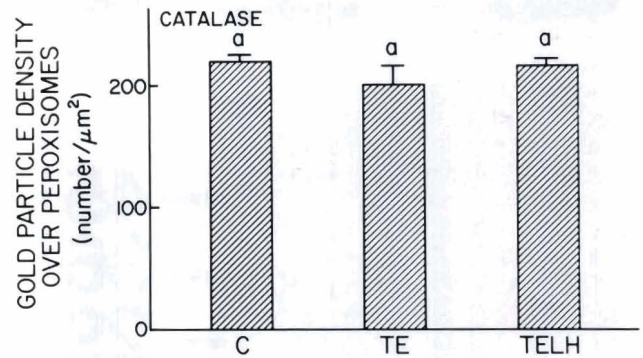An interesting feature to note in these studies is that some organelles appear to be more sensitive to endogenous LH deprivation than others. As stated earlier, Mendis-Handagama et al. (1992) showed that treatment of rats with testosterone-estradiol implants for eight days was able to induce atrophy of Leydig cells to one-third of control values. The stereological results in this study (Mendis-Handagama et al., 1992) demonstrated that all Leydig cell organelles were not reduced in volume to the same degree of Leydig cell atrophy in response to LH withdrawal; some organelles showed more LH-dependent than others. The volumes of organelles such as mitochondria and lysosomes per Leydig cell of testosterone-estradiol implanted rats were reduced to one-third of control values, similar to the degree of Leydig cell atrophy. By contrast, volumes of smooth endoplasmic reticulum, peroxisomes and "negative bodies" per Leydig cell were reduced to one-sixth of control values. Thus, the decrease in the volume of these

latter organelles was twice the overall reduction in average volume of a Leydig cell and that the magnitude of these volume reductions in smooth endoplasmic reticulum, peroxisomes and "negative bodies" per Leydig cell approximate the reduction in the LH-stimulated testosterone secretory capacity of these Leydig cells *in vitro* (Fig. 12). The greater reduction in the volume of smooth endoplasmic reticulum associated with reduced testosterone secretory capacity in Leydig cells of testosterone-estradiol implanted rats is in agreement with the fact that smooth endoplasmic reticulum contains the LH-regulated steroidogenic enzymes required to convert pregnenolone to testosterone (Tamaoki, 1973; Samuels et al., 1975). The

striking loss of volume in Leydig cell peroxisomes and its correlation with the reduction in testosterone secretion in LH-deprived rats is consistent with the observation of the increase in Leydig cell peroxisome volume after acute LH treatment (Mendis-Handagama et al., 1992). Again, these findings suggest that peroxisomes have a significant role in Leydig cell steroidogenesis. The "negative bodies" in Leydig cells, as mentioned earlier, are negative for catalase but otherwise morphologically identical to peroxisomes. Moreover, repeatedly it has been observed that these organelles respond similarly to peroxisomes with respect to the effects of LH stimulation and withdrawal (Mendis-Handagama et al., 1990a, 1992). Though not proven yet, these observations together with the recent findings on the existence of catalase negative peroxisomes in other tissue types (Roels and Cornelis, 1989; Oikawa and Novikoff, 1995) imply that "negative bodies" could be peroxisomes that are either immature or old and therefore, devoid of catalase as suggested by Mendis-Handagama et al. (1990a,b).

It is interesting to note that in adult rats, not only the peroxisome volume per Leydig cell, but also the intraperoxisomal content of SCP_2 show a specific reduction following eight days of endogenous LH deprivation achieved via testosterone-estradiol subdermal implants (Figs. 13, 14). Immunoblot analysis for SCP_2 has demonstrated that SCP_2 content reduced to undetectable levels in TE rats compared to control and TELH rats (Fig. 15). In comparison, no change has been observed in the content of intra-peroxisomal catalase in these Leydig cells (Figs. 16, 17; Mendis-Handagama et al., 1992) although total catalase content per Leydig cell
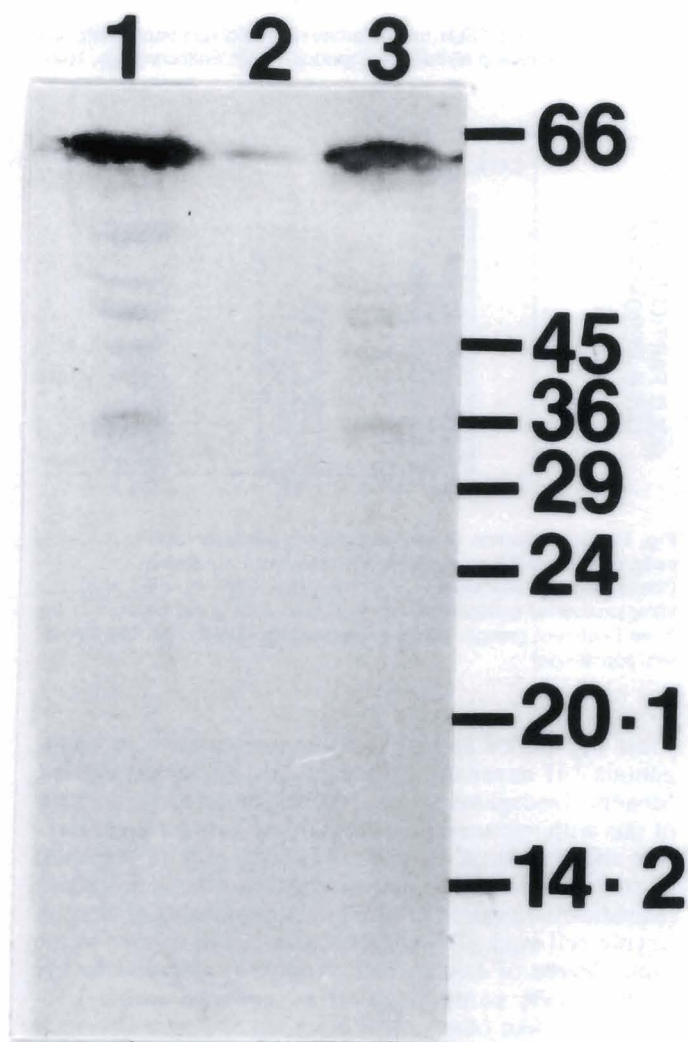

Fig. 18. Immunoblots for catalase (60 kilodaltons) in Leydig cells (.3x10⁵/gp) of control TE-implanted and TELH-implanted rats (lanes 1, 2 and 3 respectively). These results show that total catalase in Leydig cells of TE-implanted rats is reduced although the intraperoxisomal catalase was unchanged. This is due to a reduction in the volume of peroxisomes in Leydig cells in the TE-implanted rats. (From Endocrinology, 1990, 127, 2847-1954, with permission).
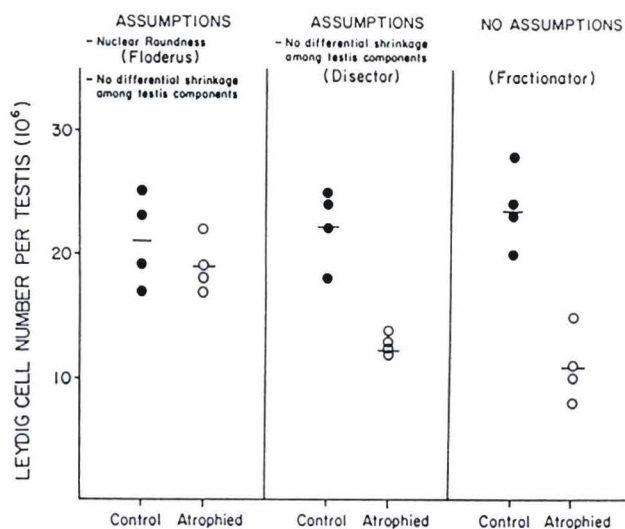

Fig. 19. Estimation of Leydig cell number per testis in control testes and atrophied testes of TE (testosterone+estradiol implanted for 16 weeks) rats obtained via the Floderus, disector and fractionator methods. A reduced number of Leydig cells per testis is obtained for atrophied testes with the use of the disector and the fractionator methods. (From Acta Stereol., 1992, 11(suppl), 495-500).

is reduced (Fig. 18). SCP_2 is known to bind cholesterol to 1:1 molar ratio (Vahouny et al., 1984) and has been shown to be important in the process of intracellular cholesterol transport mechanisms in the adrenal cortical cells (Vahouny et al., 1985) and luteal cells (McLean et al., 1989; Mendis-Handagama et al., 1995b). Therefore, based on these findings it is possible to hypothesize that the steroid secretory capacity of Leydig cells following endogenous LH deprivation is reduced at least in part because of a defect produced in the cholesterol transport mechanisms due to the reduced peroxisome volume and intraperoxisomal SCP_2 content.

It has been documented that in many studies on short term LH deprivation such as five days (Wing et al., 1984), six days (Russell et al., 1992) and eight days (Mendis-Handagama et al., 1992), no change is observed in Leydig cell number per testis (Fig. 6). Mendis-Handagama and Ewing (1990) showed that long-term LH deprivation for 16 weeks via testosterone-estradiol treatment causes a significant reduction ($P<0.05$) in the Leydig cell number per testis. Moreover, this study, which used the disector method (Sterio, 1984) revealed that the assumptions of nuclear shape, equal shrinkage and reference space in different species as well as in the same species with different treatments, introduce errors in the estimates of Leydig cell numbers. Although Keeney et al. (1988) could not show a statistically significant difference in Leydig cell number per testis between control and long-term LH-deprived rats (16 weeks) at $p<0.05$ level, a lower value has been observed in long-term LH-deprived rats for Leydig cell number per testis. According to Keeney and Ewing (1990), Leydig cell number per testis do not change significantly ($P<0.05$) following long term (16 weeks) hypophysectomy. Recent studies of Mendis-Handagama (Fig. 19; 1992a,b) using the fractionator method (Gundersen, 1986, 1988), which is a technique to quantify particles without any assumptions, confirmed that Leydig cell number per testes is reduced in adult rats following long-term LH-deprivation.

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