Journal of Andrology, Vol. 9, No. 5, September/October 1988

The Regulation of the Proliferation and Differentiation of Rat Leydig Cell Precursor Cells After EDS Administration or Daily HCG Treatment

KATJA J. TEERDS,* DIRK G. DE ROOIJ,*
FOCKO F. G. ROMMERTS,‡ AND CEES J. G. WENSING†

From the Department of Cell Biology, Medical School, and the Department of Veterinary Anatomy, State University of Utrecht, and the Department of Biochemistry, Division of Chemical Endocrinology, Erasmus University Rotterdam, The Netherlands‡*

The proliferation and differentiation of possible Leydig cell precursors in adult rats were studied after destruction of the existing Leydig cells with EDS or after daily treatment with hCG. After 2 days with either treatment, a 12- to 16-fold increase in the number of [^{3}H]thymidine-incorporating interstitial cells was found. In the case of hCG treatment, this was probably due to the high plasma hCG levels. However, after EDS treatment, LH levels start to rise between days 1 and 3, suggesting a paracrine stimulation of the proliferation of interstitial cells.

After hCG treatment, a substantial increase in the numbers of Leydig cells was already found at day 2. It was concluded that hCG induced a rapid differentiation, without cell division, of existing precursor cells into recognizable Leydig cells. In rats treated with both EDS and hCG, new Leydig cells were not formed during the first 10 days. This indicates that EDS destroys not only mature Leydig cells but also those Leydig cell precursors that are able to differentiate rapidly into recognizable Leydig cells.

Key words: Leydig cell, Leydig cell precursor, LH, hCG, EDS, proliferation.

J Androl 1988; 9:343-351.

It has been demonstrated recently that the Leydig cell population in the adult rat testis has a great capacity for recovery after cell loss. This can be observed after administering ethane dimethyl sulfonate (EDS), which specifically destroys Leydig cells in the adult rat testis. At this time there are no indications that other testicular cells are affected directly by EDS (Kerr et al, 1985; Molenaar et al, 1985; Kerr and Donachie, 1986; Bartlett et al, 1986; Jackson et al, 1986a, 1986b; Morris et al, 1986; O'Leary et al, 1986). A complete repopulation of Leydig cells is established approximately 49 days following EDS administration. These new Leydig cells are derived from precursor cells that are not eliminated by EDS treatment (Jackson et al, 1986a; Molenaar et al, 1986).

The formation of new Leydig cells can also be induced by daily administration of high doses of human chorionic gonadotropin (hCG) to adult (Christensen and Peacock, 1980) and immature rats (Chemes et al, 1976). In the adult rat, a 1.4-fold increase in the number of Leydig cells has been observed after 1 week of daily hCG injections. This rise in the number of Leydig cells occurred much faster than after EDS administration. Following the administration of EDS,

Reprint requests: Katja J. Teerds, Ph.D., Department of Cell Biology, Medical School, State University of Utrecht, Nicolaas Beetsstraat 22, 3511 HG Utrecht, The Netherlands.

Submitted for publication October 12, 1987; revised version received February 22, 1988; accepted for publication February 22, 1988.

the new Leydig cells will arise from precursor cells that are triggered to differentiate and/or proliferate. This could also happen during hCG treatment, although in this case the Leydig cells that are present may also proliferate. The identity of the Leydig cell precursors and the regulatory mechanisms that govern their proliferation and differentiation are not known (Jackson et al, 1986a; Molenaar et al, 1986; Risbridger and de Kretser, 1986). The conditions for the development of a new population of Leydig cells after EDS or hCG treatment are different. After treatment with EDS, recognizable Leydig cells are absent and plasma LH levels rise slowly (Molenaar et al, 1986; Bartlett et al, 1986; Jackson et al, 1986b), whereas during the treatment of mature rats with hCG, plasma hCG levels are increased 15-fold from the first day onwards and Leydig cells remain present in the interstitial tissue.

In the present study, we have followed the time course of the stimulation of the proliferation and differentiation of possible Leydig cell precursors in mature rats following EDS administration and/or daily injections of hCG in order to investigate the process of Leydig cell formation in more detail.

Materials and Methods

Materials

EDS is not commercially available and was prepared as described by Rommerts et al (1985) from ethylene glycol and methane sulphonyl chloride. The compound was recrystallized twice from methylene chloride and was more than 99% pure as determined by nuclear magnetic resonance.

Human chorionic gonadotropin (hCG, Pregnyl) was obtained from Organon, Oss (The Netherlands). [^{3}H]Thymidine was obtained from The Radiochemical Centre, Amersham, England (specific activity 55 Ci/mM). Kodak NTB-2 dipping emulsion (Eastman/Kodak, Rochester, NY, USA) was used for autoradiography.

[^{3}H]Thymidine Labeling Studies

Three- to 5-month-old male Wistar rats in groups of four were used. EDS (30 mg/ml in DMSO:H_2O, 1:3 w/v) was administered in a single intraperitoneal injection (75 mg/kg body weight). Every day from 2 to 10 days after EDS administration, one group of rats received a [^{3}H]thymidine injection (1.5 μCi/g body weight). Other groups of rats received one injection of 100 IU hCG daily from 2 to 10 days and [^{3}H]thymidine was injected 24 hours after the last hCG injection. A group of control rats received only a single injection of [^{3}H]thymidine (1.5 μCi/g body weight). All rats were killed 2 hours after [^{3}H]thymidine administration. On the first day of treatment, another group of rats received a combination of EDS (75 mg/kg body weight) and hCG (100 IU) followed by one daily injection of 100 IU hCG during 6 consecutive days.

Each testis was fixed in 2% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 18 hours at 4 C. After this fixation, each testis was cut into two pieces and fixed for another 6 to 8 hours in 2% paraformaldehyde. After dehydration, the material was embedded in Technovit 7100 plastic (Kulzer & Co GmbH, Wehrheim, FRG), a glycol methacrylate. Five-μm sections were made at least 1 mm away from the edge where the testis was cut into two pieces to avoid damage to the interstitial compartment. The sections were stained by the PAS technique and subsequently coated with dipping emulsion. After 13 to 15 weeks of exposure at 4 C, the slides were developed and stained with Gill's hematoxylin (Polysciences Inc., Warrington, PA, USA). Tubular diameters were measured in several sections of variously treated rats. There appeared to be no significant difference in tubular diameters, showing that tissue shrinkage resulting from the fixation was the same for all animals treated.

Cell Counts

Nuclei of Leydig cells, macrophages, smooth muscle cells, and endothelial cells of the blood vessels were counted. Leydig cells were recognized by their spherical nucleus with a characteristic distribution of heterochromatin and blue-purple staining cytoplasm, whereas the macrophages were identified by their often irregularly shaped nucleus and slightly pink (PAS-positive) staining cytoplasm. Furthermore, nuclei from Sertoli cells were also counted when a nucleolus was present in the section studied. All interstitial cells that could not be identified as one of these cell types were counted together and considered to be possible Leydig cell precursors (henceforth called "precursor cells"). Cells were considered to be peritubular or perivascular in location when their nuclei were lying directly against the tubular or vascular wall.

The proliferative activity of the interstitial cells was estimated by counting the number of cells showing [^{3}H]thymidine incorporation (cells in S-phase) and the number of cells in mitosis. Cells in prophase, metaphase, and anaphase were scored as mitotic. Cell counts were performed in random testicular areas until 1000 Sertoli cells were scored. All cell numbers were expressed per 1000 Sertoli cells according to the method of Heller et al (1971), and these (relative) cell numbers were compared with each other.

Sections in which artifactual spaces were found between the seminiferous tubules and the interstitial tissue were excluded from the kinetic analysis. These spaces may arise locally from inadequately fixed testis tissue, or from mechanical disruption occurring during the tissue preparation.

Since Christensen and Peacock (1980) showed that the nuclear diameters of Leydig cells from control and hCG-treated rats were not significantly different, it was not necessary to apply corrections for changes in the nuclear diameter of Leydig cells during hCG treatment.

In the autoradiographs, the background was low in the sections studied. Cells were considered to be labeled when five or more silver grains were present above the nucleus.

The Students *t*-test for unpaired data was used for statistical analysis.

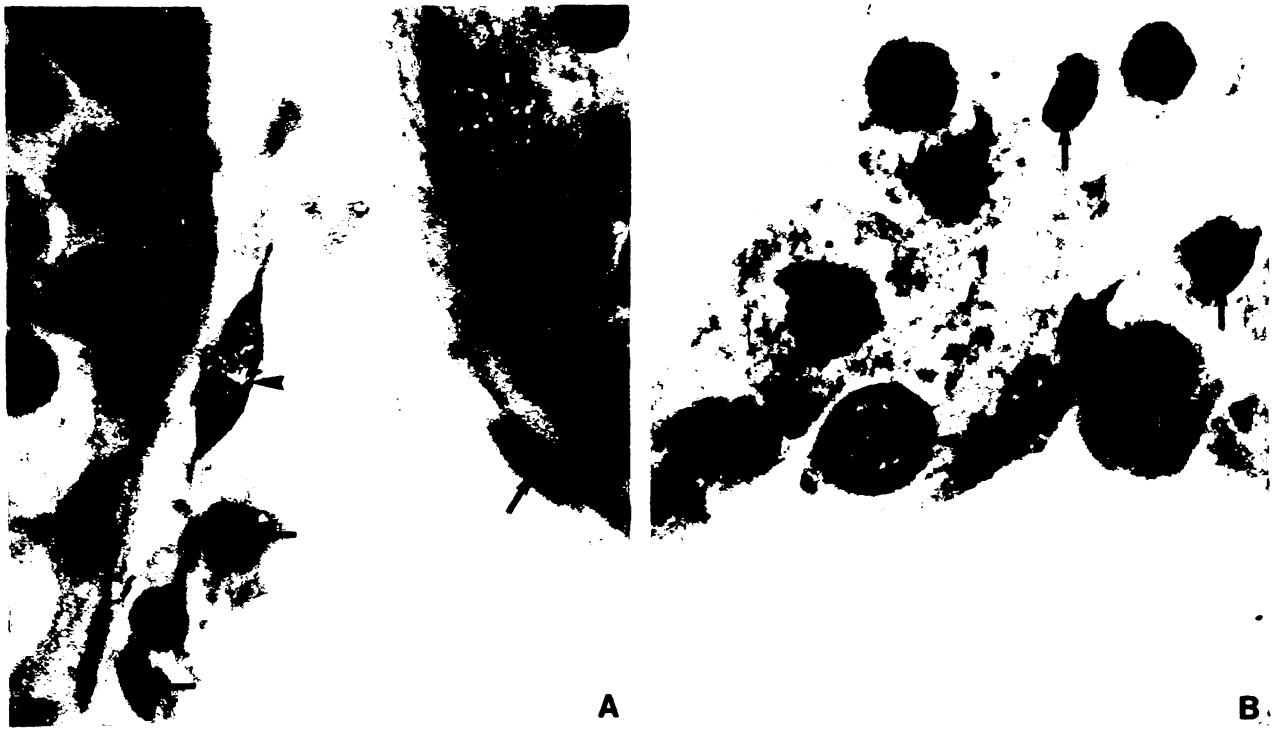

Fig. 1. Pictures were taken with the use of the brightfield-epipolarization technique. (A) Interstitial tissue 2 days following EDS administration. Some precursor cells were labeled (arrowhead), although unlabeled Leydig cell precursors were also present (arrows). Most Leydig cells had disappeared. (B) Interstitial tissue after eight injections of 100 IU hCG. Labeled (arrowhead) and unlabeled (asterisks) Leydig cells were observed. Unlabeled precursor cells (arrows) were also found (× 1517).

Results

Histology

The (labeled) Leydig cell precursor cells found after EDS administration often had a fibroblast-like appearance (Fig. 1A). At the light microscopic level there appeared to be no difference between the (labeled) precursor cells present after EDS administration or after hCG treatment (Fig. 1).

Occasionally, focal infiltration of monocytes and lymphocytes into the interstitial tissue as a result of EDS administration was observed, which has also been described by Kerr et al (1985). These cells often had small, irregularly shaped nuclei and sometimes a strong PAS-positive staining cytoplasm. They could be distinguished easily from the other interstitial cells. Areas in which these cells occurred were not included in the kinetic analysis.

As a result of the hCG treatment, testicular sections from three animals showed focal disruption of the seminiferous epithelium near the tunica albuginea, as has been described by van Vliet et al (1988). The damaged areas in these sections were excluded from the kinetic analysis presented in this study. No difference was found between the results in the affected or unaffected testis.

[^{3}H]Thymidine Incorporation of Leydig Cell Precursors and Leydig Cells

After administration of [^{3}H]thymidine to normal rats, the number of labeled Leydig cells was less than two per 1000 Sertoli cells, whereas the number of labeled Leydig cell precursors was found to be about seven per 1000 Sertoli cells (Figs. 2A, B).

A single injection of EDS caused a transient 16-fold increase in the number of labeled Leydig cell precursors 2 days after EDS administration, and from day 4 to day 8 the number of labeled precursor cells was at least 4 times higher than in the controls (Fig. 2A). A significant drop ($P < 0.01$) in the number of labeled precursor cells to a level 2-fold higher than in the controls occurred on day 9 after EDS administration. Since EDS had destroyed all Leydig cells and no new Leydig cells were formed during the first 10 days, [^{3}H]thymidine incorporation in Leydig cells could not be observed after EDS administration.

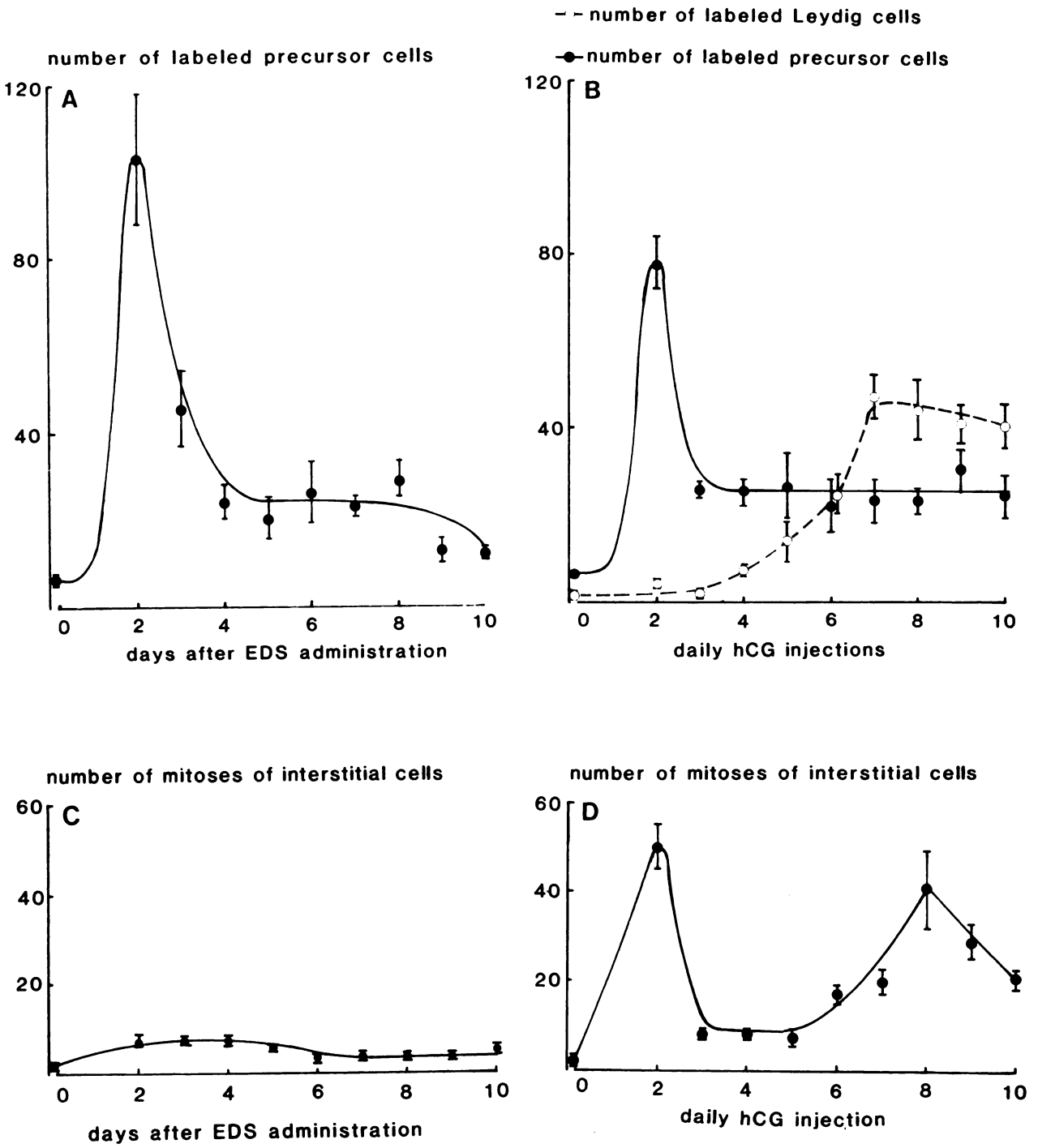

Fig. 2. (A,B). Number of labeled Leydig cell precursors during the first 10 days following EDS administration or 0 to 10 injections of 100 IU hCG and the number of labeled Leydig cells during daily hCG treatment, expressed per 1000 Sertoli cells. No labeled Leydig cells were observed after EDS administration. (C,D) Number of mitotic interstitial cells (probably Leydig cell precursors) during the first 10 days after EDS administration or daily treatment with 100 IU hCG.

In the rats treated with hCG for 2 days, the number of labeled Leydig cell precursors showed a transient 12-fold increase, and from day 4 to day 10 the number of labeled precursor cells was at least 4 times higher than in the controls (Fig. 2B). The significant drop in the number of labeled precursor cells that occurred 9 and 10 days after EDS administration was not observed in rats treated with hCG. During the first 3 days of hCG treatment, the labeling of the Leydig cells was low, similar to the controls (Fig. 2B). On the 4th day of the hCG treatment, the number of labeled Leydig cells started to increase, and a 45-fold stimulation was reached on the 7th day, which remained present until the 10th day (Figs. 1 and 2B).

A single dose of EDS gave similar patterns of [^{3}H]thymidine incorporation in precursor cells during the first 8 days compared with multiple injections of hCG, but the location of the labeled precursor cells in the interstitium was not entirely the same (Table 1). During daily injections of hCG the number of labeled precursor cells located perivascularly was twice as high as in rats treated with EDS and 4 times higher than in the controls. Labeled precursor cells in rats treated with EDS were predominantly located in the peritubular compartment. The total numbers of (labeled plus unlabeled) precursor cells located peritubularly, perivascularly, or elsewhere in the interstitium did not differ significantly after EDS administration or hCG treatment.

TABLE 1. Distribution of Labeled Precursor Cells after One EDS Injection or Daily HCG Treatment*

Treatment	% of labeled cells†		Not Peritubular or Perivascular
	Peritubular	Perivascular	
Control	42.2 ± 9.2	10.6 ± 6.1	47.2 ± 6.3
2 to 10 daily injections hCG	21.4 ± 2.0‡	43.1 ± 1.9‡	35.3 ± 2.0
2 to 10 days after EDS	59.0 ± 3.1§	22.8 ± 2.2§	18.0 ± 1.9‡§

*Numbers of peritubular and perivascularly located labeled precursor cells are expressed as percentage of the total number of labeled Leydig cell precursors.

†Means ± SEM were calculated from the data given in Fig. 2 and are expressed per 1000 Sertoli cells. The ratios calculated for each day did not differ significantly and were therefore taken together.

‡Significantly different from control ($P < 0.001$).

§Significantly different from two to 10 daily injections of hCG ($P < 0.001$).

Mitotic Activity of Leydig Cell Precursors and Leydig Cells

Following administration of EDS, a small but significant increase in the number of mitotic Leydig cell precursors was observed and a maximum was reached at day 2 (4-fold stimulation; Fig. 2C). After 5 or more days, the number of mitotic cells decreased, but remained higher than in the controls ($P < 0.001$).

A different pattern was found after daily injections of hCG. The number of mitotic interstitial cells increased transiently during the first 3 days of treatment (25-fold stimulation; Fig. 2D). A second transient increase of cells in mitosis was found around 8 days of hCG treatment (23-fold stimulation), which coincided with a rise in the number of labeled Leydig cells. During the intervening period of 3 to 5 days, the number of mitoses was increased only 4-fold.

Total Number of Precursor Cells and Leydig Cells

At 10 days after administration of EDS, the number of precursor cells (labeled and unlabeled) had increased by approximately 28% (Fig. 3A). Two or more days after a single injection of EDS, almost no Leydig cells could be detected in the interstitium.

After 10 days of hCG injections, the number of precursor cells had increased gradually by 27% (Fig. 3B). In contrast to this slowly developing rise in the number of precursor cells, hCG treatment caused a rapid increase in the number of recognizable Leydig cells (Fig. 3B). Already after two hCG injections, a 35% increase in the number of Leydig cells was observed, and a subsequent rise was found from day 6 onward. After 10 injections of hCG, the number of Leydig cells had increased by 78%.

Interstitial Cell Numbers After a Combination of EDS and hCG

To investigate whether the high plasma hCG levels after hCG treatment could accelerate the formation of morphologically recognizable Leydig cells after treatment with EDS, rats were treated with seven injections of hCG following one injection of EDS. At the end of this treatment, almost no Leydig cells could be detected (Table 2). The number of precursor cells present in the interstitium also was not significantly different from the number of precursor cells after EDS administration or hCG treatment alone.

Discussion

Our results provide an overall picture of the course

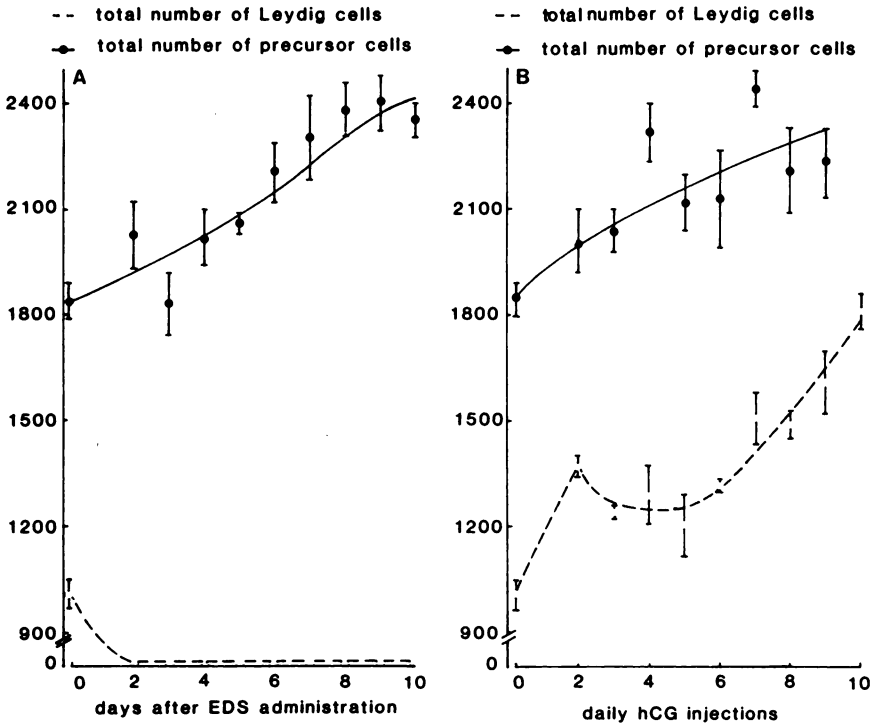

Fig. 3. (A,B). Total number of Leydig cell precursors (labeled + unlabeled) and total number of Leydig cells (labeled + unlabeled) during the first 10 days following EDS administration or daily treatment with 100 IU hCG, expressed per 1000 Sertoli cells. From 2 days after EDS administration onwards hardly any Leydig cells were observed in the interstitial tissue.

of the stimulation of Leydig cell precursor proliferative activity and differentiation after EDS administration or daily hCG treatment, using the incorporation of [^{3}H]thymidine and the number of mitotic cells as indicators of proliferative activity. In this study Leydig cell precursors have been defined as interstitial cells that cannot be otherwise identified as Leydig cells, macrophages, smooth muscle cells, or endothelial cells of the blood vessels.

After injection of [^{3}H]thymidine into normal adult rats, Leydig cells incorporating [^{3}H]thymidine or Leydig cells in mitosis were rare. Therefore, under normal conditions, the proliferative activity of Leydig cells and Leydig cell precursors in the adult testis is scanty, and the turnover of these cells is probably low. This agrees with other studies of the adult testis of the rat (Christensen, 1975), monkey (Fouquet and Raynaud, 1985) and man (Amat et al, 1986), which also reported that very few Leydig cells incorporated [^{3}H]thymidine or were in mitosis.

TABLE 2. Numbers of Leydig Cell Precursors and Leydig Cells at 7 Days after One Injection of EDS, Seven Injections of HCG, or a Combination of EDS Administration and Seven Injections of HCG.*

	EDS	hCG	EDS + hCG
Leydig cells	12 ± 2†	1461 ± 68	10 ± 4†
Precursor cells	2301 ± 117	2444 ± 58	2552 ± 41

*Mean ± SEM (N = 4) were in part calculated from the data given in Fig. 3 and are expressed per 1000 Sertoli cells.
†Significantly different from hCG treatment alone ($P<0.001$)

Our results show that Leydig cell precursors can be stimulated to proliferate by EDS administration and by hCG treatment. No significant difference was found between the numbers of labeled precursor cells after EDS administration or hCG treatment, although the location of the labeled precursor cells after EDS administration was mainly peritubular and after hCG treatment mainly perivascular. The position of the labeled precursor cells after EDS administration is consistent with recent results of Kerr et al (1987), who found that after EDS treatment the newly developed (fetal-type) Leydig cells were often located peritubularly.

The difference in the location of the labeled precursor cells after EDS and hCG treatment may be caused by the manner in which the stimulation occurs. During hCG treatment, the continuously high plasma hCG levels are probably stimulating the proliferation of precursor cells, although it cannot be completely excluded that some unknown local fac-

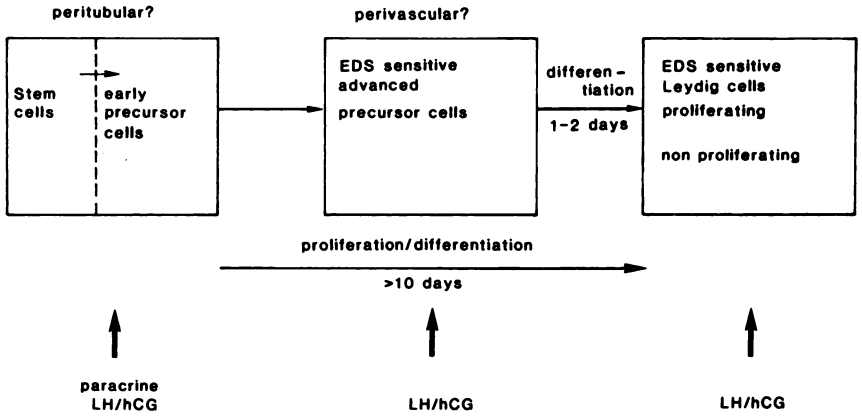

Fig. 4. Model for the regulation of the stimulation of Leydig cell and Leydig cell precursor proliferation and differentiation following EDS administration or daily hCG treatment. The proliferative activity of stem cells and early precursor cells is most likely stimulated by paracrine factor(s) and/or increasing levels of LH after EDS administration and continuously high plasma hCG levels during hCG treatment. As a result of the high plasma hCG levels, some advanced precursor cells are able to differentiate very rapidly into recognizable Leydig cells, whereas after EDS administration these advanced precursor cells are destroyed or inhibited. Furthermore, continuously high plasma hCG levels can stimulate the proliferative activity of recognizable Leydig cells.

tors also play a role. On the other hand, during the first days after EDS administration, plasma LH levels rise relatively slowly (Jackson and Morris, 1977; Morris, 1985; Bartlett et al, 1986; Molenaar et al, 1986), compared with the extremely high plasma hCG levels already present within a few hours after the first hCG injection. This indicates that, during the first days after EDS administration, other (paracrine) factors are most likely involved in the stimulation of the proliferative activity of the Leydig cell precursors (Fig. 4). A paracrine regulation of other aspects of Leydig cell development, such as differentiation and steroidogenic activities via as yet unknown, locally produced, factors, has already been described (Aoki and Fawcett, 1978; Rich et al, 1979; de Kretser, 1981; Sharpe et al, 1981; Sharpe, 1982, 1983; Bergh, 1982, 1983; de Kretser and Kerr, 1983; Kerr and Donachie, 1986). However, the mainly peritubular localization of labeled precursor cells after EDS administration might also be explained by preferential destruction of perivascularly located precursor cells.

The increase in the number of mitotic precursor cells 2 days after EDS administration was less than after two injections of hCG. This suggests that the stimulation of the proliferative activity of the precursor cells during hCG treatment takes place at an earlier time. Apparently during the first 2 days of hCG treatment, part of the cells stimulated to proliferate pass through both S-phase and mitosis, whereas at 2 days after EDS administration the first stimulated cells reached S-phase but not mitosis.

In addition to stimulating the proliferative activity of the precursor cells, hCG treatment also caused a rapid, 35% increase in the total number of recognizable Leydig cells after two injections. This increase must be caused by differentiation of precursor cells into morphologically recognizable Leydig cells because 24 hours after the first injection of hCG, the number of labeled Leydig cells was still at the control level (Teerds et al, 1986), and the number of Leydig cells was also still normal. Differentiation of precursor cells into recognizable Leydig cells occurs between 24 and 48 hours after the beginning of the hCG treatment. Furthermore, since the number of labeled precursor cells is still normal at 24 hours, it is not likely that these precursor cells also divide during the rapid process of differentiation. Therefore, we conclude that these new Leydig cells probably arise through differentiation of a specific class of precursor cells (advanced precursor cells) under the influence of high plasma hCG levels (Fig. 4).

Christensen and Peacock (1980) conclude from their study that it is not clear what mechanism is responsible for the increase in Leydig cell number during hCG treatment. The present study shows that both the proliferation of Leydig cells and the proliferation and differentiation of precursor cells into Leydig cells contribute to the increased numbers of Leydig cells during hCG treatment.

The presence of labeled Leydig cells after hCG treatment can be explained by the following mechanisms (Fig. 4): 1. as a result of the high plasma hCG levels, part of the Leydig cell population already present starts to proliferate after 4 days of treatment; 2. precursor cells acquire the morphologic characteristics of Leydig cells while they also continue to proliferate. Experiments are now in progress to investigate

which of these possibilities is correct.

It is interesting to compare these effects of hCG administration on the number of Leydig cells with reports of other authors on the steroidogenic activity of Leydig cells after hCG treatment. De Jong et al (1974) reported that a 3.6-fold increase in testicular concentrations of testosterone (T) was found after five daily injections of 100 IU hCG. This rise in T levels probably results not only from increased T production by the existing Leydig cells, but most likely also from the approximate 35% increase in the number of Leydig cells that we have shown occurs after hCG administration.

The absence of morphologically recognizable Leydig cells during the first 10 days after EDS administration indicates that the development of new Leydig cells takes more time under this regimen than during hCG treatment. Relatively low plasma LH levels during the first 2 to 3 days after EDS administration are not the reason for the retarded Leydig cell development (Bartlett et al, 1986; Molenaar et al, 1986) because, even after 7 days of combined EDS and hCG treatment, almost no Leydig cells were found. This absence indicates that the advanced precursor cells, capable of rapid differentiation into Leydig cells, are also killed by EDS, or else inhibited from differentiating (Fig. 4). To date, it has been assumed that EDS affects only morphologically recognizable Leydig cells (Kerr et al, 1985; Molenaar et al, 1985, 1986; Bartlett et al, 1986; Jackson et al, 1986a; Morris et al, 1986; O'Leary et al, 1986; Teerds et al, 1986).

The data derived from our study have led to the model presented in Fig. 4. As a result of the disappearance of Leydig cells after EDS administration and, concomitantly, the loss of the steroidogenic activity of the interstitial tissue, an as yet unknown paracrine factor is able to stimulate the proliferation of Leydig cell precursors, resulting in a rise in the number of labeled precursor cells. Under the influence of increasing levels of plasma gonadotropins (LH/hCG; Kerr et al, 1985; Bartlett et al, 1986; Jackson et al, 1986; Molenaar et al, 1986) and perhaps also some paracrine factor, these cells proliferate, leading to an increase in the total number of precursor cells (labeled plus unlabeled), which then differentiate into recognizable Leydig cells. In the hCG-treated rats, it was found that the high plasma hCG levels stimulated the proliferation of Leydig cell precursors. Furthermore, it became apparent that some precursor cells are able to differentiate very rapidly into recognizable Leydig cells. These advanced Leydig cell precursors are probably destroyed or inhibited by EDS because it takes 10 to 14 days for the first recognizable Leydig cells to be found following EDS administration, even when the initial plasma gonadotropin levels are kept high during combined EDS/hCG treatment. Under the influence of continuously high plasma hCG levels, recognizable Leydig cells also proliferate, thereby contributing to the increase of the Leydig cell population and probably to the rise in testicular steroid production.

In conclusion, the results indicate that several types of precursor cells under the influence of different regulatory mechanisms are involved in the proliferation and differentiation processes leading to the formation of recognizable Leydig cells after EDS administration or during hCG treatment. Experiments are now in progress to investigate the nature of the Leydig cell precursor cells and the role of one or more paracrine factors, as well as LH and/or hCG, in their differentiation into recognizable Leydig cells.

Acknowledgments

The authors wish to thank Mr. H. J. G. van der Kant for his skillful technical assistance. We are also grateful to Mr. A. N. van Rijn for making the drawings and the photographs. This work was supported by the Dutch Foundation for Medical Research (MEDIGON).

References

Amat P, Paniagua R, Nistal M, Martin A. Mitoses in adult human Leydig cells. Cell Tissue Res 1986; 243:219–221.

Aoki A, Fawcett DW. Is there a local feedback from the seminiferous tubules affecting activity of Leydig cells? Biol Reprod 1978; 19:144–158.

Bartlett JMS, Kerr JB, Sharpe RM. The effect of selective destruction and regeneration of rat Leydig cells on the intratesticular distribution of testosterone and morphology of the seminiferous epithelium. J Androl 1986; 7:240–253.

Bergh A. Local differences in Leydig cell morphology in the adult rat testis: evidence for a local control of Leydig cells by adjacent seminiferous tubules. Int J Androl 1982; 5:325–330.

Bergh A. Paracrine regulation of Leydig cells by the seminiferous tubules. Int J Androl 1983; 6:57–65.

Chemes HE, Rivarola MA, Bergada C. Effect of hCG on the interstitial cells and androgen production in the immature rat testis. J Reprod Fertil 1976; 46:279–282.

Christensen AK. Leydig cells. In: Hamilton DW, Greep RO, eds. Handbook of physiology; Vol. 5. Washington: American Physiological Society, 1975; 57–94.

Christensen AK, Peacock KC. Increase in Leydig cell number in the testis of adult rats treated chronically with an excess of human chorionic gonadotropin. Biol Reprod 1980; 22:383–391.

de Jong FH, Hey AH, van der Molen HJ. Oestradiol-17B and testosterone in rat testis tissue: effect of gonadotrophins, localization and production in vitro. J Endocrinol 1974; 60:409–419.

de Kretser DM. Sertoli cell–Leydig cell interaction in the regulation of testicular function. Int J Androl 1981; 5[Suppl]:11–17.

de Kretser DM, Kerr JB. The pituitary and the testis. In: de Kretser DM, Burger HG, Hudson B, eds. The effect of testicular damage on Sertoli and Leydig cell function. Berlin:

Springer-Verlag, 1983; 133–160.
Fouquet JP, Raynaud F. Renewal of Leydig cells in the neonate and adult monkey: a radioautographic study. Biol Cell 1985; 54:187–190.
Heller CG, Calli MF, Pearson JE, Leach DR. Method for quantification of Leydig cells in man. J Reprod Fertil 1971; 25:177–184.
Jackson CM, Morris ID. Gonadotrophin levels in male rats following impairment of Leydig cell function by ethylene dimethane sulphonate. Andrologia 1977; 9:29–35.
Jackson AE, O'Leary PO, Ayers MM, de Kretser DM. The effects of ethylene dimethane sulphonate (EDS) on rat Leydig cells: evidence to support a connective tissue origin of Leydig cells. Biol Reprod 1986a; 35:425–437.
Jackson NC, Jackson H, Shanks JH, Dixon JS, Lendon RG. Study using in-vivo binding of ^{125}I-labeled hCG, light and electron microscopy of the repopulation of rat Leydig cells after destruction due to administration of ethylene-1,2-dimethane sulphonate. J Reprod Fertil 1986b; 76:1–10.
Kerr JB, Donachie K, Rommerts FFG. Selective destruction and regeneration of rat Leydig cells in vivo. Cell Tissue Res 1985; 242:145–156.
Kerr JB, Donachie K. Regeneration of Leydig cells in unilaterally cryptorchid rats: evidence for stimulation by local testicular factors. Cell Tissue Res 1986; 245:649–655.
Kerr JB, Bartlett JMS, Donachie K, Sharpe RM. Origin of regenerating Leydig cells in the testis of the adult rat. An ultrastructural, morphometric and hormonal assay study. Cell Tissue Res 1987; 249:367–377.
Molenaar R, de Rooij DG, Rommerts FFG, Reuvers PJ, van der Molen HJ. Specific destruction of Leydig cells in mature rats after in vivo administration of ethane dimethyl sulfonate. Biol Reprod 1985; 33:1213–1222.
Molenaar R, de Rooij DG, Rommerts FFG, van der Molen HJ. Repopulation of Leydig cells in mature rats after selective destruction of the existent Leydig cell population with ethylene dimethane sulfonate (EDS) is dependent on LH and not FSH. Endocrinology 1986; 118:2546–2554.
Morris ID. Leydig cell resistance to the cytotoxic effect of ethylene dimethanesulphonate in the adult rat testis. J Endocrinol 1985; 105:311–316.
Morris ID, Phillips DM, Bardin CW. Ethylene dimethane sulphonate destroys Leydig cells in the rat testis. Endocrinology 1986; 118:709–719.
O'Leary P, Jackson AE, Averill S, de Kretser DM. The effects of ethane dimethyl sulphonate (EDS) on bilaterally cryptorchid rat testes. Mol Cell Endocrinol 1986; 45:183–190.
Rich KA, Kerr JB, de Kretser DM. Evidence for Leydig cell dysfunction in rats with seminiferous tubule damage. Mol Cell Endocrinol 1979; 13:123–135.
Risbridger GP, de Kretser DM. Percoll-gradient separation of Leydig cells from postnatal rat testes. J Reprod Fertil 1986; 76:331–338.
Rommerts FFG, Grootenhuis AJ, Hoogerbrugge JW, van der Molen HJ. Ethane dimethane sulfonate (EDS) specifically inhibits LH stimulated steroidogenesis in Leydig cells isolated from mature rats but not in cells from immature rats. Mol Cell Endocrinol 1985; 42:105–111.
Sharpe RM, Fraser HM, Cooper I, Rommerts FFG. Sertoli-Leydig cell communication via a LHRH-like factor. Nature 1981; 290:785–787.
Sharpe RM. The hormonal regulation of the Leydig cell. In: Finn CA, ed. Oxford reviews of reproductive biology 1982; 5:241–317.
Sharpe RM. Local control of testicular function. Q J Exp Physiol 1983; 68:265–287.
Teerds KJ, de Rooij DG, Molenaar R, Rommerts FFG, Wensing CJG. Specific cytotoxic effects of ethane dimethyl sulfonate (EDS) and repopulation of Leydig cells after depletion. In: Stefanini M, Conti M, Geremia R, Ziparo E, eds. Molecular and cellular endocrinology of the testis 1986; 173–183.
van Vliet J, Rommerts FFG, de Rooij DG, Buwalda G, Wensing CJG. Reduction of testicular blood flow and focal tissue degeneration following human chorionic gonadotrophin administration in the rat. J Endocrinol 1988, in press.