Inhibition of Spermatogenesis and Steroidogenesis During Long-Term Treatment with hCG in the Rat

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The effects of chronic treatment with hCG (100 I.U. s.c. every second day) on testicular morphology, LH receptor levels and concentration of steroid intermediates of the Δ_4 and Δ_5 biosynthetic pathways were studied in adult rats for periods extending from one to 12 weeks. Treatment with hCG causes a decrease in testis weight, a maximal inhibitory effect being observed after two and four weeks of treatment. At these time intervals, the loss of testis weight is accompanied by degenerative changes in most seminiferous tubules and hypertrophy of Leydig cells. Administration of hCG for one week leads also to an almost complete loss of LH binding sites and to a marked stimulation of the levels of testicular steroids of the Δ_4 and Δ_5 pathways, as well as to an increase in weights of secondary reproductive organs. The initial increment of testicular steroid levels is followed after two weeks of hCG administration by an apparent decrease of 17,20-desmolase activity suggested by a reduction in the levels of androst-5-ene-3β,17β-diol, androstenedione, testosterone, and 5α-dihydrotestosterone and an increase in the concentrations of pregnenolone, 17-OH-pregnenolone, progesterone and 17-OH-progesterone. Plasma and pituitary LH levels are maximally reduced at one and four weeks of treatment, respectively, while plasma and pituitary FSH levels are only slightly reduced after four weeks of hCG administration. The effects of hCG on all of the above-mentioned parameters, except for testicular morphology and testis weight, are completely reversible at the eight and 12 week intervals. This transiency in the effects of hCG is accompanied by a gradual increase in plasma levels of hCG antibodies. The present data show that chronic treatment of adult rats with hCG induces a marked degeneration of the seminiferous tubules and an inhibition of spermatogenesis that accompanies the well-known loss of testicular LH receptors and inhibition of the steroidogenic pathway.

Key words: hCG, spermatogenesis, steroidogenesis.

The concept that several peptide hormones can specifically regulate their homologous binding sites in target tissues is now well established (Kahn et al, 1973; Gavin et al, 1974; Lesnick and Roth, 1976). This hormonal regulation of receptor sites is characterized, in most cases, by an inverse relationship between the concentration of the circulating hormone and the tissue level of receptors. This negative regulation of the receptor population has been particularly well demonstrated in ovarian and testicular tissue after administration of exogenous oLH or hCG (Hsueh et al, 1976; Conti et al, 1976; Auclair et al, 1977a; Purvis et al, 1977; Chen and Payne, 1977; Haour and Saez, 1977; Sharpe, 1977; Kremers et al, 1977). Studies performed *in vitro* with Leydig cells or testicular subcellular preparations obtained after desensitization by hCG treatment have shown that the LH receptor loss is accompanied by an impairment of both the cyclic AMP and steroid responses to gonadotropin stimulation, along with the appearance of a blockage in the testicular steroidogenic pathway (Auclair et al, 1977a; Tsuruhara et al, 1977; Cigorraga et al, 1978; Chasalow, 1978; Chasalow et al, 1978).

Recently and somewhat surprisingly, it has been found that the administration of LHRH agonistic analogues can cause a marked reduction in testicular LH and prolactin receptor levels, accompanied by a decrease in the weights of the testis and secondary reproductive organs (Auclair et al, 1977a, b; Labrie et al, 1978; Bélanger et al, 1979a, 1980a, b; Cusan et al, 1979; Sandow et al,

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1977; Corbin, 1978; Rivier et al, 1979; Tcholakian et al, 1978; Catt et al, 1979; Huhtaniemi and Martikainen, 1978). The reduction in plasma and testicular androgen levels that follows treatment with LHRH agonists is associated with a blockage of the steroidogenic pathway at the level of 17-hydroxylase and 17,20-desmolase activities, which is similar to that found after hCG administration (Bélanger et al, 1979a, b; 1980a, b; Rivier et al, 1979; Dufau et al, 1979). Moreover, treatment with LHRH agonists leads to marked degenerative changes in the seminiferous tubules, with cessation of spermatogenesis after four to eight weeks of treatment (Rivier et al, 1979; Pelletier et al, 1978; Bélanger et al, 1979c).

Since our initial study has shown that daily or single injection of hCG led to a decrease in testicular weight similar to that observed after treatment with an LHRH agonist (Auclair et al, 1977a), it became of interest to study the effect of long-term treatment with hCG on spermatogenesis in the adult rat. The interest in this study is strengthened by the report that, in three patients, chronic treatment with hCG led to regressive changes in the seminiferous tubules and cessation of spermatogenesis (Maddock and Nelson, 1952). In the present study, we examine in detail the effect on testicular morphology of hCG administration for periods extending from one to 12 weeks and correlate these changes with the effect of the same treatment on LH receptor levels and the concentration of steroid metabolites of the Δ_4 and Δ_5 biosynthetic pathways.

Materials and Methods

Animals

Male Sprague-Dawley rats (obtained from Canadian Breeding Farms, St. Constant, Quebec), weighing 200 to 220 g upon arrival, were kept in a room with controlled temperature (22 C, ±1 C) and lighting (14 hours light to 10 hours darkness, lights on at 0500 hours) and were fed Purina Rat Chow and water *ad libitum*.

Treatment

Animals were injected subcutaneously with 100 I.U. of hCG (2910 I.U./mg; kindly supplied by Dr. J. P. Raynaud, Roussel-UCLAF, Romainville) or with the vehicle alone (0.1% gelatin to 0.9% NaCl), once every second day for periods extending from one to 12 weeks. At each time interval, animals were sacrificed by decapitation 48 hours after the last hCG injection. Organs and blood were then collected for radioimmunoassay of LH, FSH and steroids, radioreceptor assay of LH, and organ weight measurements. Each group consisted of eight animals.

LH/hCG Receptor Assay

Immediately after decapitation, the testes were weighed and kept at −20 C until assayed for ^{125}I-hCG binding as previously described (Auclair et al, 1977a).

Steroid Assays

Testicular pregnenolone (Preg), progesterone (Prog), 17-OH-progesterone (17-OH-Prog) androstenedione (Δ_4), testosterone (T), 5α-dihydrotestosterone (DHT) and 17β-estradiol (E_2) were measured as described after separation on LH-20 columns (Bélanger et al, 1979; 1980b; c). Androst-5-ene-3α,17β-diol (Δ_5-diol) and 17-OH-pregnenolone(17-OH-Preg) were also separated on LH-20 columns and were measured using antibodies developed against androst-5-ene-3β,17β-diol-15-0(carboxymethyl) oxyme-BSA and 17-OH-progesterone-3-0(carboxymethyl) oxyme-BSA, respectively. The limits of the assays are 5 to 2500 pg of Δ_5-diol per tube and 10 to 5000 pg of 17-OH-Preg per tube, respectively. The antiserum against Δ_5-diol was kindly supplied by Dr. K. M. Pirke, Max Plank, Germany. All other antibodies were developed in our laboratory. The inter- and intra-assay coefficients of variation for these assays are 10% and 8%, respectively.

Measurement of hCG Antibody Titers in Rat Plasma

Plasma obtained from individual rats at different time intervals during hCG treatment was used to measure the levels of hCG antibodies, using [^{125}I]-hCG as tracer. Plasma was serially diluted over a range of 250 to 50,000-fold with Tris-Mg buffer (0.1M Tris-HCl, 0.005M $MgCl_2$, pH 7.4). To each reaction tube were added 100 μl of diluted plasma, 100 μl of ^{125}I-labeled hCG (15,000 cpm, specific activity: 47.2 μCi/μg) and 300 μl of Tris-Mg buffer. Tubes were incubated at 23 C for 16 to 18 hours. Nonspecific binding was determined for each plasma dilution by incubation with an identical concentration of plasma obtained from saline-treated animals. The free ^{125}I-hCG was separated by adding to each tube 1 ml of a charcoal suspension (0.5% Norit A in Tris-Mg buffer and 0.2% BSA). After incubation for 30 minutes at 4 C, the mixture was centrifuged at 3000 rpm for 10 minutes. The radioactivity contained in the supernatant was measured in an LKB autogamma spectrometer with a counting efficiency of approximately 74%.

Pituitary and Plasma LH and FSH Assays

Anterior pituitaries were immediately homogenized in a glass-Teflon® homogenizer in 5 ml of PBS (0.05M Na_2HPO_4, 0.10 M NaCl, *p*H 7.4) buffer before centrifugation for 10 minutes at 2300 × g. The supernatant was kept at −20 C for RIA determinations. Levels of LH and FSH were measured by double-antibody radioimmunoassays using rat hormones and rabbit antisera kindly supplied by Dr. A. F. Parlow for the NIAMDD

Rat Pituitary Hormone Program, as described previously (Drouin and Labrie, 1976).

Morphological Studies

Testes from three to four rats from each experimental group (control and hCG-treated) were fixed by perfusion with Bouin's fluid. Testes were then post-fixed by immersion in the same fixative for two days before being embedded in paraffin. Cross sections were cut at 5 μm and stained with PAS hematoxylin. For each testis, ten sections obtained at different levels were carefully examined and the percentage of degenerative tubules was recorded. About 4000 tubule sections were counted for each testis.

Calculations

Radioimmunoassay data were analyzed using a program based on Model II of Rodbard and Lewald (Rodbard and Lewald, 1970). Statistical significance was assessed according to the multiple-range test of Duncan-Kramer (Kramer, 1956). All radioimmunoassay and radioreceptor assay data are presented as mean ± SEM of triplicate determinations.

Results

Figure 1A illustrates the time-course of changes in weights of reproductive organs in adult male rats treated every second day with 100 I.U. of hCG for 1, 2, 4, 8, or 12 weeks. A maximal inhibition of testicular weight was observed at two weeks (65% of control, $P < 0.01$) with a progressive return toward normal values at later time intervals. The decrease in testis weight remained significant ($P < 0.05$) at four (72% of control) and eight (80% of control) weeks, while the difference became nonsignificant at 12 weeks. The effects of hCG administration on seminal vesicle and ventral prostate weight were also transient. A maximal 50% stimulation of ventral prostate and seminal vesicle weight was observed one, two and four weeks after beginning hCG treatment, with a progressive decrease to 30 to 35% above control at eight and 12 weeks ($P < 0.01$, Fig. 1A).

Testicular LH receptor levels were almost completely suppressed after one week of hCG-

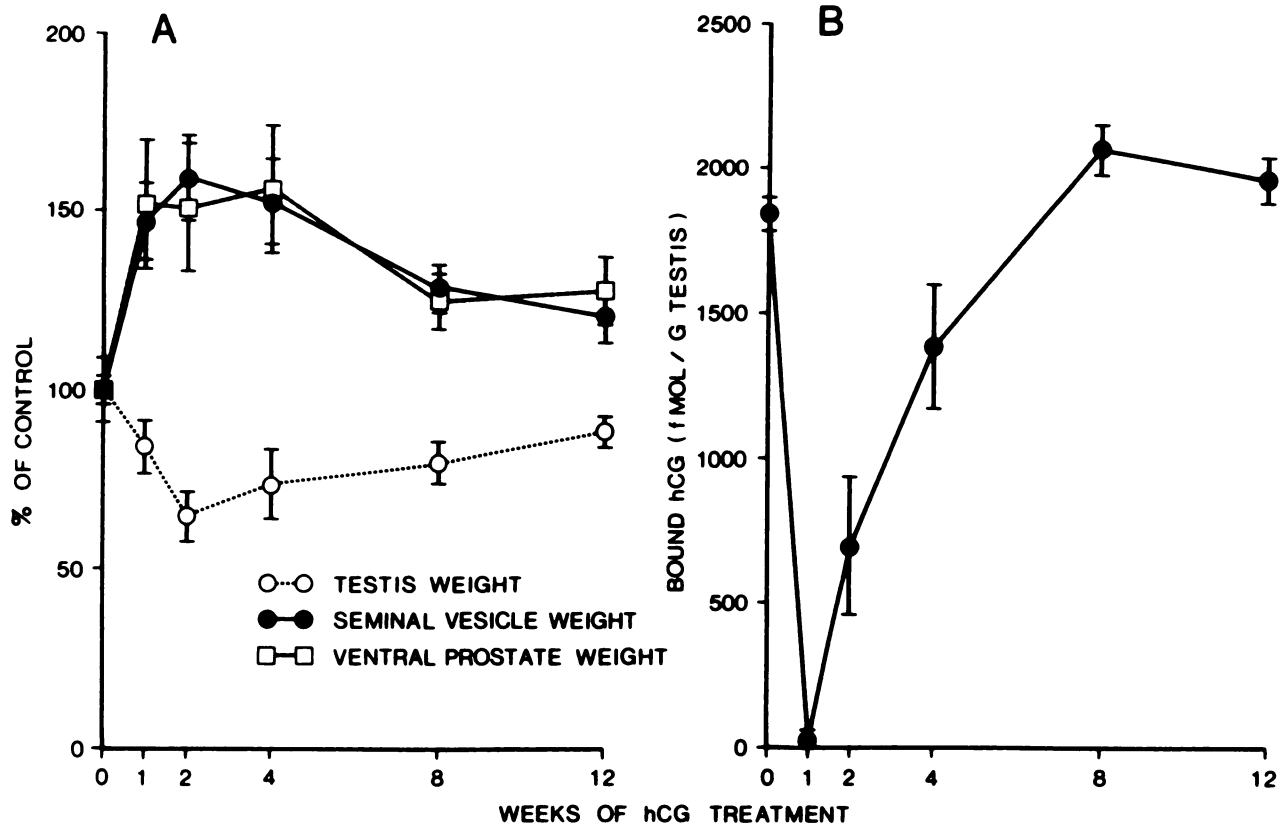

Fig. 1. Time-course of the effect of hCG administration (100 I.U. every second day for one to 12 weeks) on reproductive organ weights (A) and testicular LH/hCG receptor levels (B) in the adult rat. Eight animals were used in each group.

treatment (from 1841 ± 58 to 24.8 ± 8 fmoles/g testis; Fig. 1B). This receptor loss was short-lived and 100% recovery of LH receptors was seen at eight and 12 weeks. No effect of hCG administration was detected on testicular FSH and prolactin receptor levels (data not shown).

As illustrated in Figs. 2A and 2B, the inhibitory effect of hCG administration on plasma and pituitary LH levels also appeared transient. Plasma LH levels were rapidly reduced to 80% of control after one week of treatment, while pituitary LH content dropped more slowly to a 90% loss measured four weeks after the beginning of hCG administration. This marked inhibition was followed by a progressive increase to normal levels at eight weeks. A significant and smaller reduction in plasma and pituitary FSH concentrations was seen only at the end of the fourth week of treatment.

The effect of chronic hCG administration on testicular levels of the steroid intermediates of the Δ_4 and Δ_5 pathways leading to the production of testosterone is shown in Fig. 3. Following the first week of hCG treatment, the levels of all steroids were increased from 300 to 1200% of basal values (Fig. 3A). After two weeks of continuous hCG administration, the testicular concentrations of Preg, 17-OH-Preg, Prog and 17-OH-Prog were elevated to 8 to 30-fold above basal levels, while the levels of Δ^5-diol, T, DHT, and E_2 were increased to only 200 to 400% of control (Fig. 3B), thus suggesting a preferential inhibition of 17,20-desmolase activity.

At four weeks, the proportions of Δ_5-diol, Δ_4 and T were already markedly improved compared with the precursors, Preg, 17-OH-Preg, Prog and 17-OH-Prog (Fig. 3C). Essentially normal levels of testicular steroids were found at eight weeks (Fig. 3D).

This transiency of the inhibitory effects of hCG treatment on testicular LH receptor levels and androgen formation suggests the possibility that antibodies against hCG develop during the treatment and cause a progressive neutralization of the activity of the injected hormone. Measurements of plasma hCG antibody levels did in fact reveal a progressive formation of circulating hCG antibodies. The percentage of specifically bound ^{125}I-hCG at a 1/5000 dilution of plasma was 8.1 ± 3.6% and 25 ± 5.4% for animals treated for eight and 12 weeks, respectively. A typical antibody

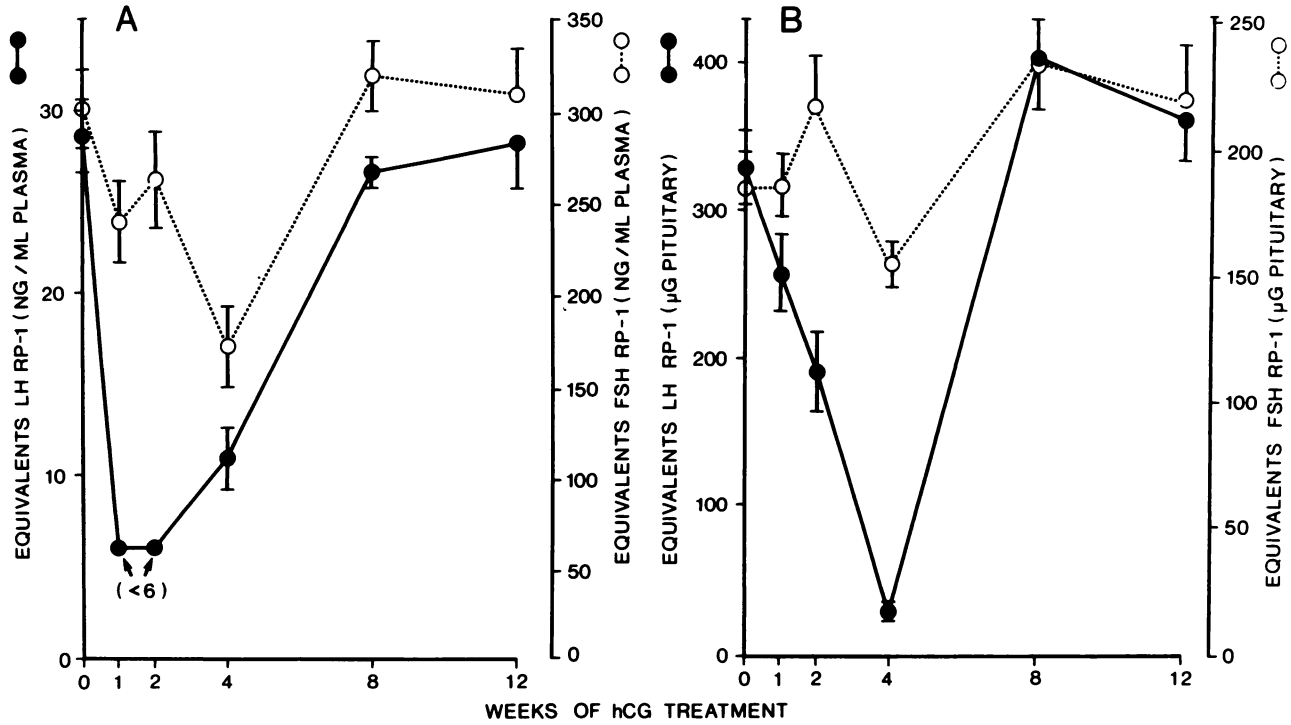

Fig. 2. Time-course of the effects of hCG administration (one to 12 weeks) on basal plasma levels (A) and pituitary content (B) of LH and FSH in adult male rats.

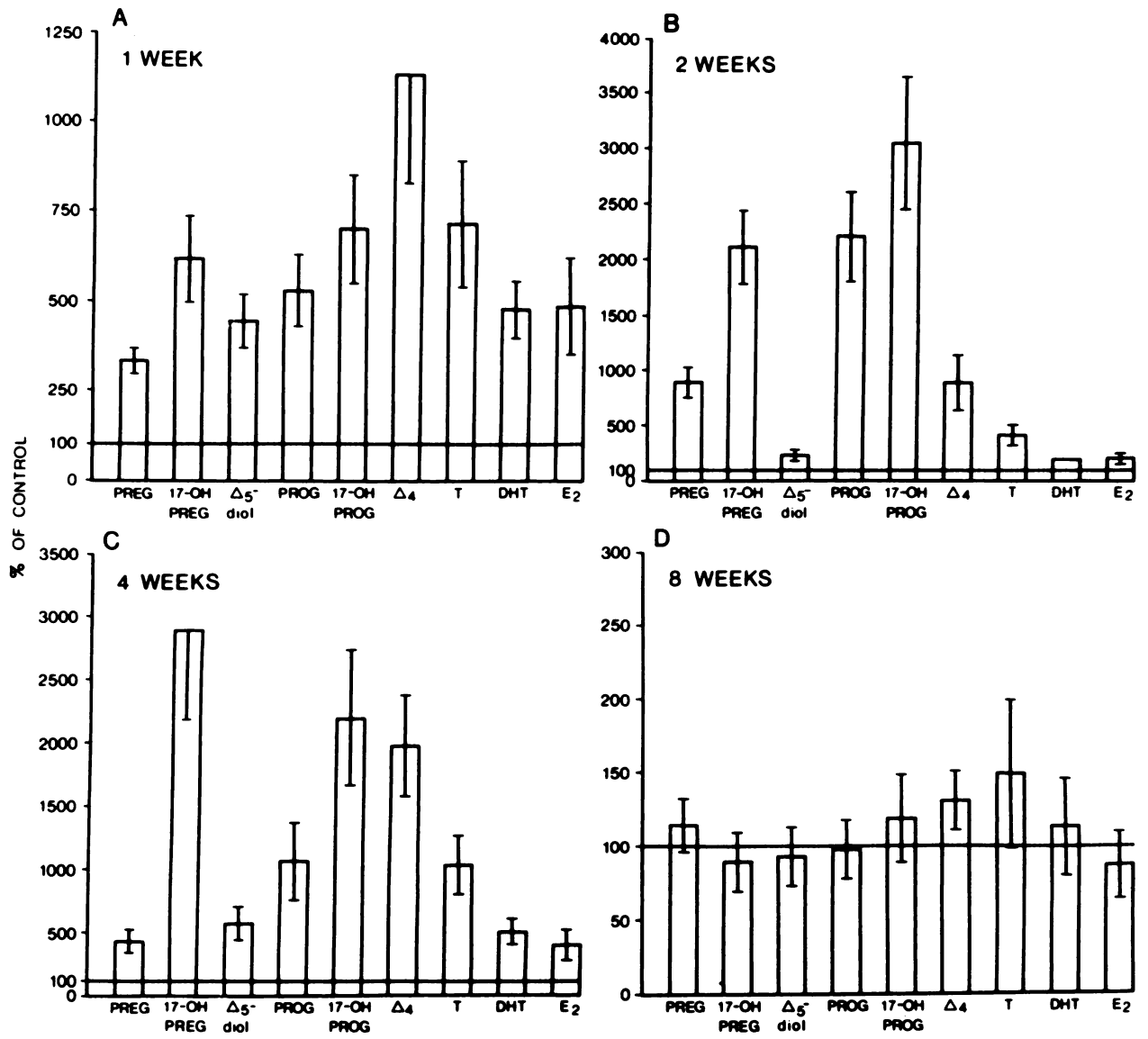

Fig. 3. Time-course of the effects of hCG administration (one to eight weeks) on testicular Δ_4 and Δ_5 pathway steroid metabolites in adult rats. Abbreviations: Pregnenolone (Preg); 17-OH-pregnenolone (17-OH-Preg); androst-5-ene-3β,17β-diol (Δ_5-diol); progesterone (Prog); 17-OH-progesterone (17-OH-Prog); androstenedione (Δ_4); testosterone (T); 5α-dihydrotestosterone (DHT) and 17β-estradiol (E_2). The animals were killed 48 hours after the last hCG injection.

dilution curve using plasma obtained from animals treated for 12 weeks with hCG is shown in Fig. 4, with 50% of total ^{125}I-hCG binding being observed at a plasma dilution of 1/8000. Although the antibodies to hCG have not been measured before eight weeks of treatment, it is very likely that they started to develop much earlier.

As mentioned above, the finding of decreased testis weight as early as one week after beginning hCG treatment (Auclair et al, 1977a) suggested the possibility of an inhibitory effect of such treatment on the seminiferous tubules. After one week of treatment, two major changes are observed in the hCG-treated animals: a marked hypertrophy and hyperplasia of the interstitial cells and degenerative changes in many seminiferous tubules (Fig. 5, Table 1). Whereas the hypertrophy of interstitial cells is quite uniform, degeneration of the seminiferous tubules is very heterogenous. In fact, tubules showing normal appearance are frequently observed in close proximity to degenerated tubules (Fig. 5).

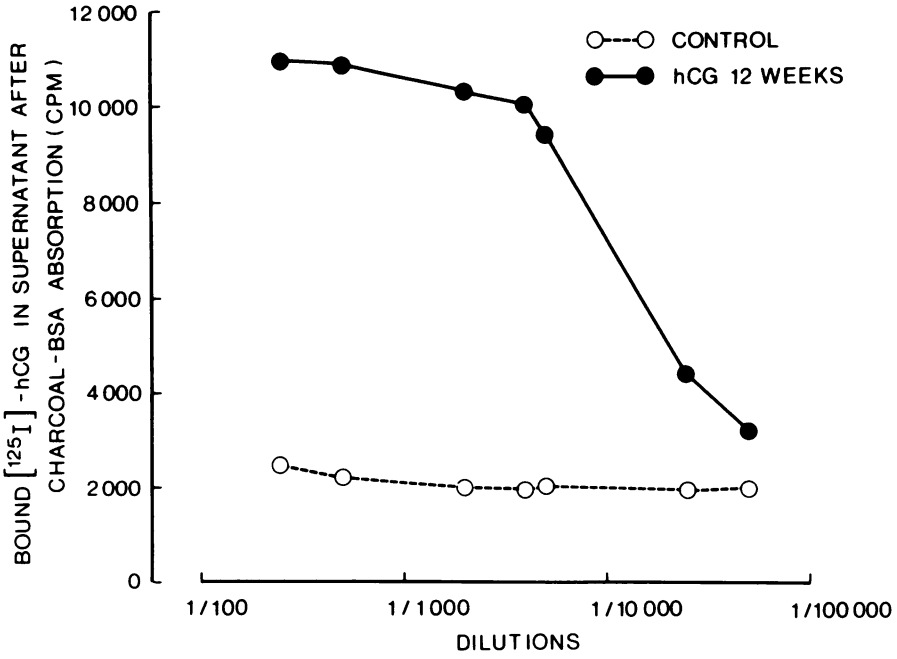

Fig. 4. Typical antibody dilution curve using serial dilutions of plasma obtained from animals treated for 12 weeks with hCG (100 I.U. every second day).

More dramatic changes are observed after two and four weeks of treatment, when almost all tubules exhibit signs of regression (Fig. 5, Table 1). In many tubules, both germinal and Sertoli cells have completely disappeared. In the most severe cases, the basement membrane is the only remaining element which can indicate the site of the original seminiferous tubule. At these two time intervals, the interstitial cells still appear hypertrophied and increased in number.

At the eight and 12 week time intervals, the histology of the testis shows a marked improvement. The interstitial cells are not observed to be hypertrophied and hyperplastic, and most tubules have a normal appearance (Fig. 5, Table 1). Only about 5 to 15% of tubules still show typical degenerative changes.

Discussion

The present study clearly shows that long-term treatment of adult rats with hCG can cause marked degenerative changes at the level of the seminiferous tubules and can inhibit spermatogenesis. The transiency of the inhibitory effect of hCG treatment is likely to be explained by the development of hCG antibodies that neutralize the activity of the injected gonadotropin. Although the exact time of appearance of these hCG antibodies has not been determined, it is likely that they appear in circulation prior to the recovery of LH receptors.

Several reports have shown that short-term *in vivo* administration of hCG to male rats can cause a dramatic loss in the number of testicular LH/hCG binding sites and a blockage in the steroidogenic pathway (Hsueh et al, 1976; Conti et al, 1976; Auclair et al, 1977a; Purvis et al, 1977; Chen and Payne, 1977; Haour and Saez, 1977; Sharpe, 1977; Kremers et al, 1977; Tsuruhara et al, 1977; Cigorraga et al, 1978; Chasalow, 1978; Chasalow et al, 1978). In gonadotropin-desensitized Leydig cells, the nature and extent of the steroidogenic blockage depends largely upon the dose and time of administration of hCG (Purvis et al, 1977; Haour and Saez, 1977; Cigorraga et al, 1978; Chasalow, 1978; Chasalow et al, 1978). The major steroidogenic block in desensitized cells appears to be located at the level of 17,20-desmolase activity, the site of conversion of 17-hydroxylated steroids to androgens, with secondary accumulation of the corresponding steroid precursors. After administration of higher doses of hCG, a lesion is observed in the early part of the biosynthetic pathway before Preg formation (Cigorraga et al, 1978). The decreased ratios of Δ_5-diol to 17-OH-Preg and Δ_4 to 17-OH-Prog observed in the present study after two weeks of treatment with hCG also indicate 17,20-desmolase as the major site of enzymatic blockage.

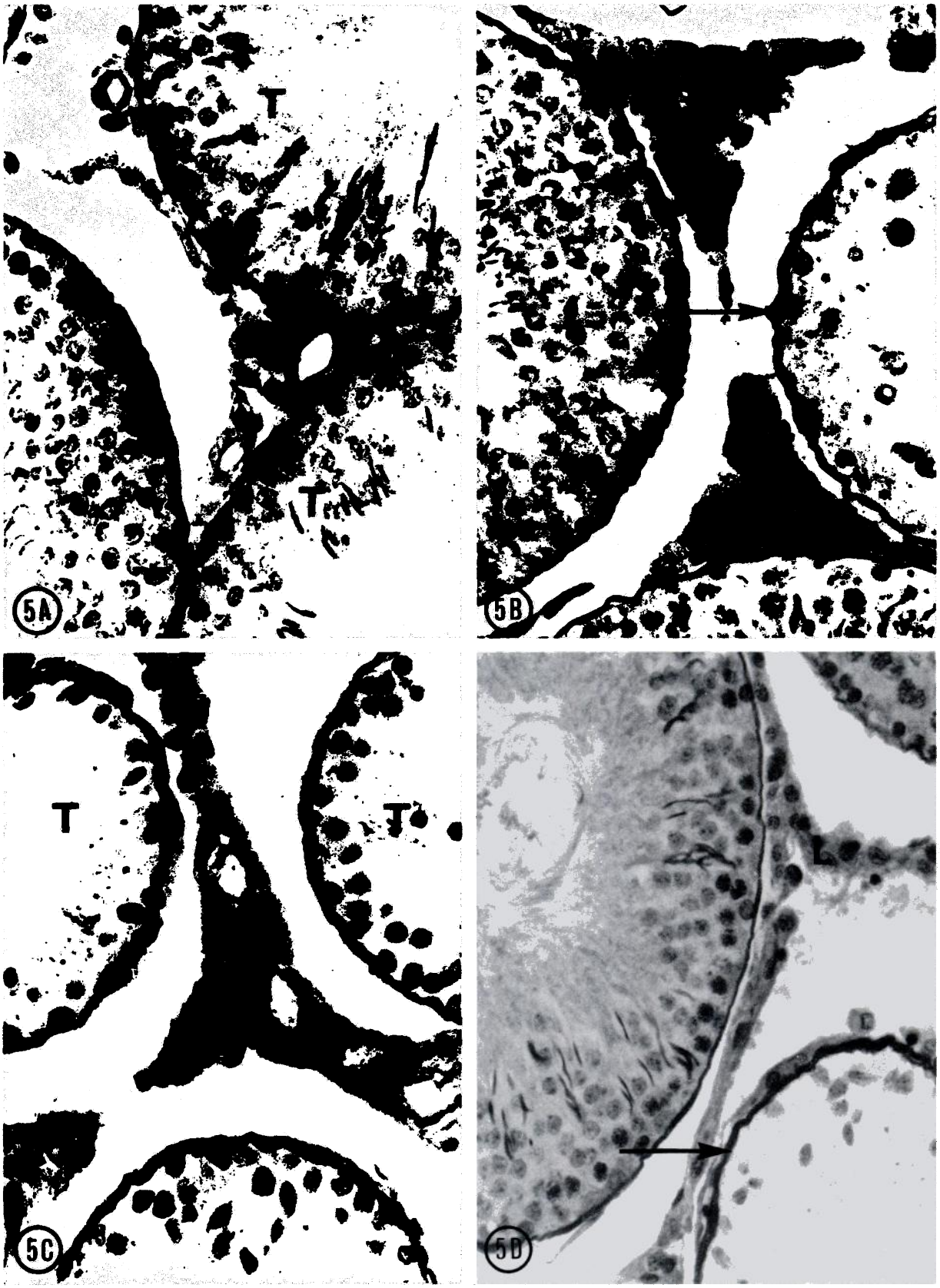

Fig. 5. Sections of rat testis at different time intervals after the beginning of hCG administration (×400). A. Control animal. Note the normal appearance of tubules (T) and Leydig cells (L). B. Two weeks of treatment. The Leydig cells (L) are hypertrophied. One tubule (→) shows degenerative changes. C. Four weeks of treatment. The Leydig cells (L) are still hypertrophied. The tubules (T) show signs of marked regression. D. Eight weeks of treatment. The Leydig cells (L) have a normal appearance. One tubule (→) is still degenerated, whereas another appears normal.

We have recently observed that three days after a single subcutaneous injection of 30 I.U. hCG to adult male rats, the response of testicular and plasma steroid levels to gonadotropin stimulation suggests a lesion at the level of both the 17-hydroxylase and 17,20-desmolase activities (Bélanger et al, 1980c). In a recent study (Chasalow, 1978; Chasalow et al, 1978), it was found that during the first hour after hCG administration, there was an increase in both 17-hydroxylase and 17,20-desmolase activities. At 2 hours, the activity of 17,20-desmolase was decreased but not that of 17-hydroxylase. However, at later time intervals, the activity of both enzymes was markedly decreased with minimal values at 12 hours and 48 hours and a return to normal at 96 hours. These changes are parallel to the changes of the steroidogenic response to dbcAMP. A similar block at these two steps of the steroidogenic pathway has been observed after treatment with LHRH agonists (Bélanger et al, 1980a). While it has been observed previously that the LHRH agonist-induced blockage of the steroidogenic pathway is reversible upon cessation of treatment (Rivier et al, 1979; Cusan and Pelletier, 1979), the present study shows the reversibility of the hCG-induced blockage. The gradual increase in plasma levels of hCG antibodies that neutralize the activity of the injected hormones is likely to explain the recovery of the testicular effects of hCG after eight and 12 weeks of administration of this gonadotropin.

The present observation of seminiferous tubule degeneration induced by hCG treatment, concomitant with a marked steroidogenic stimulation and Leydig cell hyperplasia, is somewhat paradoxical. The cytologic features accompanying Leydig cell hypertrophy after hCG stimulation have been described (Meskow et al, 1968; Black and Christensen, 1969). They are characterized by enlarged cells, along with increased number of mitochondria and hypertrophy of the smooth endoplasmic reticulum and of the Golgi complex, suggesting increased cellular activity and steroid production. The hypertrophy of Leydig cells is presumably a direct result of stimulation both by repetitive administration of hCG and by its relatively long half-life.

It is of great interest to analyze the present results simultaneously with those obtained in three patients treated chronically with hCG: seminiferous tubules were decreased in size and showed peritubular fibrosis, hyalinization of the basement membrane, cessation of spermatogenesis, as well as degeneration of germinal cells (Maddock and Nelson, 1952). These morphologic changes were accompanied by almost complete disappearance of spermatozoa from seminal fluid.

TABLE 1. Effect of Time of Administration of hCG (100 I.U. every second day) on the Degree of Seminiferous Tubule Degeneration in the Adult Rat*

Duration of hCG treatment (weeks)	Tubules showing signs of degeneration (%)
0	0–0.5
1	20–42
2	67–90
4	82–100
8	5–18
12	4–12

* Four animals per group.

The mechanisms responsible for seminiferous tubule damage and inhibition of spermatogenesis remain to be elucidated. However, they are likely to be related to the increased testicular level of some steroid metabolite(s) or to the altered ratio of testicular steroid concentrations occurring during hCG-induced desensitization. The steroids most likely involved are E_2 and Prog, Preg or their 17-hydroxy derivatives.

It is in fact well known that gonadotropins increase estrogen formation in man (Maddock and Nelson, 1952) and rat (Valladares et al, 1978; De Jong et al, 1973), and that hCG administration to male rats can cause an increased aromatization of T to E_2 (Valladares et al, 1978). Moreover, estrogens have been shown to cause decrease in testicular weight and testosterone production, probably through inhibition of 17-hydroxylase and 17,20-desmolase activities (Kremers et al, 1977; Bartke et al, 1977). In the present study, hCG administration caused a significant stimulation of testicular E_2 levels after one to four weeks of treatment. It is therefore possible that the increase in testicular E_2 levels can directly or indirectly cause seminiferous tubule lesions.

However, one of the most dramatic changes in testicular steroid level observed after treatment with hCG or LHRH agonists (Labrie et al, 1980; Bélanger et al, 1979c; Rivier et al, 1979; Bélanger et al, 1980a, b) is that of Prog. This steroid has been reported to have deleterious effects on the seminiferous tubules of intact male rats (Flick-

inger, 1977) and to maintain spermatogenesis in hypophysectomized animals (Steinberger et al, 1975; Harris and Bartke, 1975). Moreover, it should be mentioned that high doses of T have also been found to block spermatogenesis (Ewing et al, 1977). However, it is possible that the degenerative effects of LHRH agonists on the seminiferous tubules are due to both endogenous LH release (Auclair et al, 1977b) and a direct effect of the peptide on the testis (Hsueh and Erickson, 1979; Labrie et al, 1980).

The present data show that chronic hCG administration to adult male rats can cause marked seminiferous tubule damage and an almost complete inhibition of spermatogenesis. These changes are associated with the well-known loss of LH/hCG receptors and a partial impairment of the testicular steroid biosynthetic pathway, mainly indicative of decreased 17,20-desmolase activity. While the mechanisms responsible for the marked inhibitory effects of hCG on testicular functions remain to be elucidated, the inhibitory effects are reversible after eight to 12 weeks of treatment due to the development of antibodies to the exogenous gonadotropin.

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