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Increase in Leydig Cell Number in Testes of Adult Rats Treated Chronically with an Excess of Human Chorionic Gonadotropin

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ABSTRACT

Leydig cell clusters in adult testes enlarge considerably under treatment with excess LH or hCG. However, it has been uncertain in the past whether this expansion involves an increase in the number of Leydig cells or merely an enlargement of the individual cells. We have used morphometric methods to investigate this question in the testes of rats treated chronically with hCG. Adult male Sprague-Dawley rats were injected s.c. daily with 100 IU hCG for up to 5 weeks. Testes were fixed by perfusion with Bouin's fixative, embedded in paraffin, sectioned longitudinally and stained for light microscopy with Mallory azan. To avoid counting macrophages, which are also numerous in the interstitial tissue, the animals were injected with trypan blue before sacrifice. The number of Leydig cells and their volume was determined by morphometric methods (Weibel and Bolender, 1973). Appropriate normal controls were included. The volume of Leydig cell clusters increased by a factor of 4.7 during the 5 weeks of hCG treatment. The number of Leydig cells (initially averaging 18.6 × 10^6/cm^3 testis) increased to 3 times the control value by 5 weeks of treatment ($P<0.001$), while the average volume of individual Leydig cells (initially $\sim$2200 μm^3) enlarged only 1.6 times. We conclude that chronic treatment with an excess of hCG increases the number of Leydig cells in the testes of adult rats and that this hyperplasia is more important than cell hypertrophy in the expansion of Leydig cell clusters.

INTRODUCTION

Leydig cell clusters in the adult testis undergo a striking increase in volume during chronic stimulation with excess LH or hCG. This change was noted in early work (Greep et al., 1936; Greep, 1937) and was generally assumed to result from an increase in the number of Leydig cells and in the size of the individual cells (for references see Christensen, 1975). Although there was some evidence that the number of Leydig cells in the rat testis did increase under these conditions (Schoen, 1964), the counting method was subject to criticism (Heller et al., 1971) and therefore could not be considered conclusive.

More recently, there have been indications that the volume increase in Leydig cell clusters under gonadotropin treatment is due primarily to an enlargement of the individual cells and that the number of cells remains relatively constant. After 16 weeks of hCG treatment (Heller and Leach, 1971), human testes showed larger Leydig cell clusters, but counts suggested that the cells had not become more numerous. Neaves (1973) came to the same conclusion in a study of seasonally-breeding rock hyraxes. Even though the average size of Leydig cell clusters increased by 150% during the breeding season, counts indicated little change in the number of Leydig cells at different times of the year. Results such as these have raised a serious question as to whether elevated LH/hCG levels are indeed capable of inducing Leydig cell proliferation in the adult testis.

The last decade has seen a phenomenal growth in the sophistication and use of quantitative techniques in microscopy. By these methods, usually termed "morphometry" or "stereology," it is now possible to derive three-dimensional data on number, volume, surface area or length by the analysis of two-dimensional light or electron micrographs

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(reviewed by Weibel and Bolender, 1973).

In the present study we have increased the size of Leydig cell clusters in adult male rats by daily injections of excess hCG for up to 5 weeks of treatment. Morphometric analysis of the resulting material at the light microscope level indicates a considerable increase in the number of Leydig cells, as well as an enlargement of the individual cells.

MATERIALS AND METHODS

This study involved 19 adult male Sprague-Dawley rats (West Jersey Biological Supply, Wenonah, NJ), all initially 200–250 g in weight. The animals were kept in a modern animal facility (temperature 23–25°C; lights on from 0600 to 1900 h), fed ad libitum with Layne Lab-Blox (Allied Mills, Inc., Chicago) and numbered individually for random selection over the course of the experiment. Three normal controls were utilized on Day 0. Experimental animals were injected s.c. daily with 100 IU hCG (Ayerst Laboratories) in 0.2 ml sterile saline. Three animals were killed each week at 1, 2, 3 or 4 weeks and 2 animals at 5 weeks of treatment. Two additional normal controls chosen on Day 0 were injected with saline and killed at 3 weeks. These controls did not differ significantly from the earlier ones in any parameter used in the study. Three days before use, each animal was injected i.p. with 1 ml/100 g BW of 3% trypan blue in saline to label macrophages, which are numberous in interstitial tissue of the rat testis and are easily mistaken for Leydig cells (Christensen and Gillim, 1969). Since the trypan blue was taken up significantly only by macrophages, it is unlikely that it affected the number or volume of Leydig cells during the 3 days.

One testis from each rat was prepared for light microscopy. After Nembutal anesthesia of the animal, a testis was fixed by perfusion (Christensen, 1965) with Bouin's fixative for 30 min. The volume of the perfused testis was measured by volume displacement. The testis was then cut into 3 vertical longitudinal slices, the middle slice including the mediastinum. After immersion fixation in Bouin's for another 1.5 h, the slices were dehydrated in ethanol, cleared and embedded in paraffin by routine methods. Several sections were cut at 7 μm from each slice and the sections mounted individually on slides. A randomly-chosen slide from each slice was numbered and stained with the Mallory azan staining method (Humason, 1962).

The counting procedures were designed to provide the information necessary for use in the morphometric equations described by Weibel and Bolender (1973). Point-counting methods were utilized to obtain the volume density, which is the volume of the Leydig cells/unit volume testis. If desired, the volume percentage can be derived by multiplying the volume density by 100. To determine the numerical density (N_V), or number of Leydig cells/unit volume testis, it was first necessary to count the number of Leydig cell nuclei/unit area testis on the section (N_A). The average nuclear diameter (D) and average section thickness (T) were measured as described below. The final numerical density was then calculated by the Floderus equation (Floderus, 1944): $N_V = N_A/(D + T - 2h)$, where h is a correction factor needed because nuclei that are barely included in the section are apt to be overlooked. For this study the value of h was assumed to be D/10. The average volume of the individual Leydig cell was determined by dividing the volume density by the numerical density.

The counts were carried out as follows. Sections were viewed with the 40X Neofluar objective of a Zeiss research microscope. The eyepiece was fitted with a quadratic grid (Scientific Products) containing 441 (21 × 21) intersections, utilized as counting points. For determination of Leydig cell volume density, the number of points falling on Leydig cells (cytoplasm or nucleus) was counted over the whole grid field. The number of "hits"/total number points yields the volume density. Counts for numerical density were made at the same time by counting all Leydig cell nuclei occurring within the total grid boundary (an area of 10^5 μm^2 on the specimen). Focusing was permitted. Leydig cells were easily distinguished from other cells of the interstitial tissue by their prominent nuclei with distinct peripheral heterochromatin and conspicuous nucleolus and by their reddish cytoplasm in these Mallory-azan preparations. Random fields were counted over the whole specimen, the position of successive fields being determined by steps on the vernier of the microscope stage, without viewing the specimen. The total number of grid fields counted for each section varied from 30 to 60. According to an equation given by Weibel and Bolender (1973, p. 262), we would have needed to count only ∼14 grid fields (6000 test points)/specimen to find the volume density with a relative error of <5% in our system. Data derived from the 3 sections from each animal were pooled and used in the equations to derive the desired values. The SEM in Table 1 is calculated from the average value for each rat.

Measurement of average nuclear diameter (D) was carried out with a 100X Zeiss apochromatic objective and an ocular micrometer, calibrated by means of a stage micrometer. It is often assumed that nuclear diameters can be measured accurately in sections only by analysis of the size distribution of nuclear profiles (Weibel and Bolender, 1973). To avoid this cumbersome procedure in the present study, nuclei were measured only if the optical middle of the nucleus, where the diameter was maximal, could be found at some focal level within the section. This approach should yield the actual diameter. Approximately 16 nuclei were measured/section. Since the nuclei were commonly somewhat oblong as seen in section, both the longest and shortest diameters were measured for each nucleus and the final diameter was considered to be the average of the two. The average nuclear diameter for the whole study was 6.37 ± 0.05 μm and no consistent difference was seen between values from different periods of treatment. The calculation of numerical density by the Floderus (1944) equation is accurate in the strictest sense only if the nuclei are spherical. Even though the nuclei in the present study were somewhat ellipsoidal, their eccentricity did not reach levels that would produce serious error (Haug, 1967; Fig. 3 of Bolender, 1978): 30% of the nuclei had axial ratios <1.1, 50% were <1.2, 70% were <1.3, 83% were <1.4 and 90% were <1.5.

Since section thickness (T) is also a critical factor

in determining Leydig cell numerical density, we felt it necessary to verify the thickness of the sections cut when the microtome was set for 7 μm. Such values are usually considered only approximate and might also differ from the final thickness of the deparaffinized section on the finished slide, where the nuclear counts are made. Our alternative measurement of section thickness was made by focusing on the upper and lower surface of the section while viewing with a 100X Zeiss apochromatic objective (which has an extremely shallow depth of focus, ∿0.5 μm) on a Zeiss Ultraphot II microscope. The fine focus on this instrument is calibrated in micrometers, allowing the difference in setting between the upper and lower surfaces to be read directly. The measurements were made in areas within the seminiferous tubule where the germ cells had finely granular cytoplasm, facilitating recognition of the 2 surfaces. Approximately 8 measurements were made on each slide and the average section thickness for the whole study was 6.8 ± 0.03 μm, which was used in the Floderus equation for all numerical density calculations.

We have measured the diameter of seminiferous tubules and the volume density of the interstitial tissue space throughout the treatment, to exclude the possibility that apparent changes in Leydig cell number might result actually from hCG-induced alterations in these other components of the testis (see Discussion and Table 1). The diameters of ∿18 randomly-selected seminiferous tubules were measured on each slide, using an ocular micrometer and a 10X objective. If the tubule was sectioned obliquely, the measurement was made across the narrowest dimension. The overall average diameter of seminiferous tubules throughout the study was 225 ± 4.4 μm and no consistent variation with treatment was discernible. The volume of the interstitial space (all space outside the seminiferous tubules) was measured by point-counting. The volume density of seminiferous tubules was then equal to 1 minus the interstitial volume density. Subtracting the Leydig cell volume density from that of the interstitial tissue as a whole yielded the "non-Leydig" interstitial volume density. None of these various values (Table 1) showed consistent changes with hCG treatment that could have produced the results described in this study.

RESULTS

Under chronic treatment with excess hCG, the cell clusters exhibited the expected increase in size, which is apparent when the interstitial tissue of a control (Figs. 1, 2) is compared with that of an animal after 5 weeks of treatment (Figs. 3, 4). In these light micrographs, the Leydig cells appear larger after treatment, but it is difficult to be certain whether the cells are more numerous, even though they appear to be.

The increase in the volume of Leydig cell clusters under hCG treatment is given quantitatively in Table 1 and is shown graphically in Fig. 5A. The volume of Leydig cells begins at 4.1% of testicular volume, rises gradually for the first 4 weeks and then more rapidly to reach a value of 19.4% at 5 weeks, without showing signs of reaching a plateau. The volume at 5 weeks is 4.7 times that of the control. Statistical analysis (Table 1) shows that the difference between the experimental and the control is significant at all time periods, with the possible exception of 3 weeks ($P<0.05$).

The change in Leydig cell number/unit volume testis over the 5 weeks of hCG treatment is given in Table 1 and Fig. 5B. Beginning with an average control value of 18.6×10^6 Leydig cells/cm^3 (∿1 g) testis, the value is significantly different from that of the control ($P<0.02$) by 1 week and continues its rise to reach 55.1×10^6 Leydig cells/cm^3 at 5 weeks, a 3-fold increase. The difference between experimentals and controls is significant at all time periods.

The change in average cell volume of individual Leydig cells over time is given in Table 1 and graphed in Fig. 5C. The average cell volume is initially 2199 μm^3 and by 5 weeks has increased by a factor of 1.6, reaching 3511 μm^3. None of the experimental means differs significantly from that of the control, except at 5 weeks, when the difference is significant at the $P<0.01$ level. The shape of this curve (Fig. 5C) must be considered with some reservation, because of the large SEM at 2 and 4 weeks. It should also be borne in mind that these cell volumes are derived by dividing the volume density (Fig. 5A) by the numerical density (Fig. 5B). Since the curve for numerical density in Fig. 5B has a slight rise at 3 weeks, the cell volume (Fig. 5C) has a corresponding dip that is somewhat exaggerated and probably not significant.

DISCUSSION

We have used modern techniques of morphometry to show in adult rats that the number of Leydig cells is significantly greater than that of the control ($P<0.02$) after 1 week of daily hCG treatment and that by 5 weeks the Leydig cell number has increased ∿3-fold over the control level ($P<0.001$). It appears that hyperplasia plays a more important role in the enlargement of Leydig clusters than does cell hypertrophy, since the 4.7-fold expansion of volume exhibited by the clusters at 5 weeks of treatment is comprised of a 3-fold increase of Leydig cell number and only a 1.6-fold growth in average cell size. Although these results have been obtained with hCG treatment, we assume they would also be true for LH, since these two

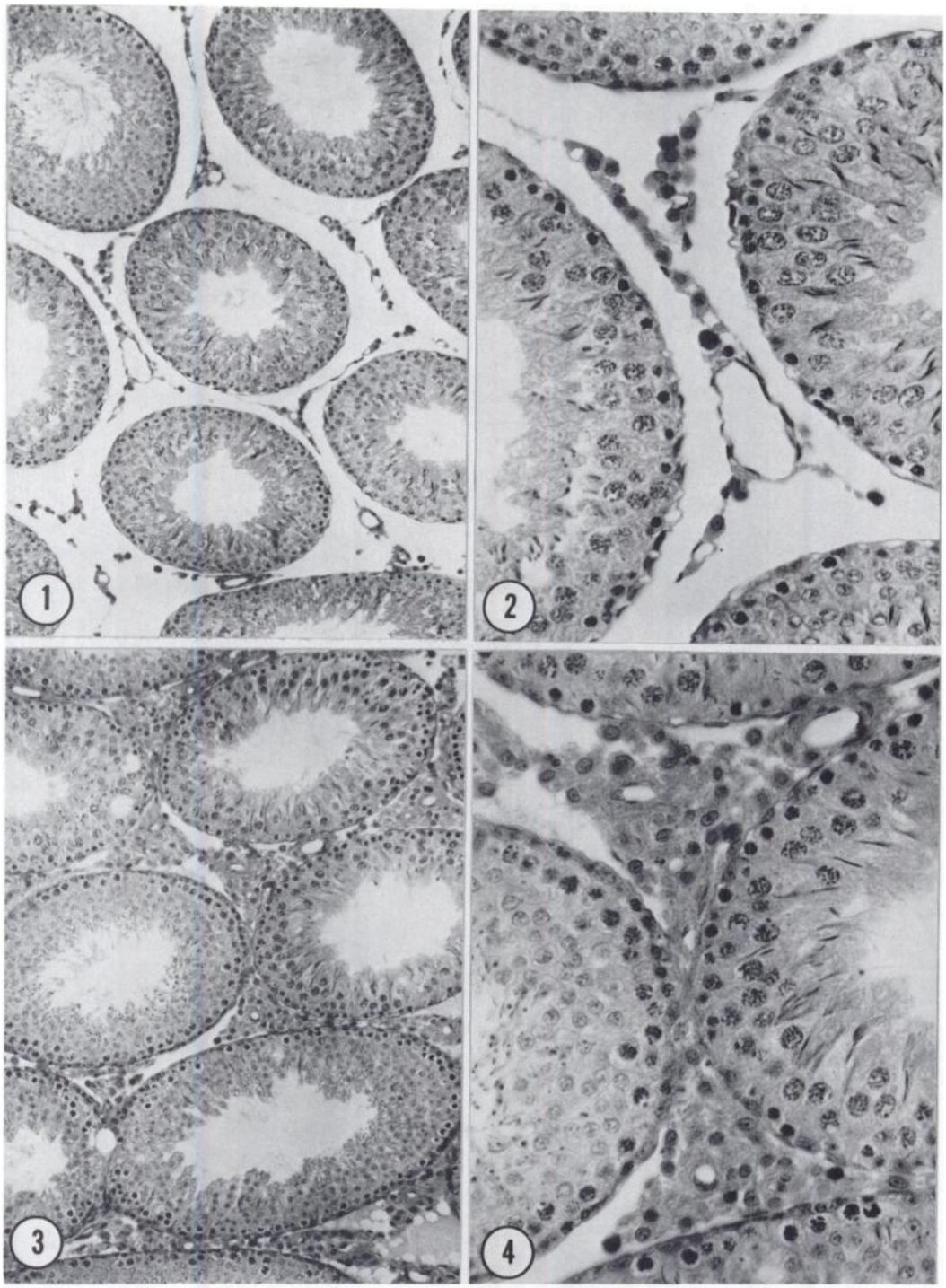

FIG. 1. Testis from a normal control rat, showing the usual sparse interstitial tissue. X152.

FIG. 2. Area of interstitial tissue from Fig. 1. The Leydig cells are of moderate size and are generally arranged in flattened clusters between the seminiferous tubules. X374.

FIG. 3. Testis from a rat that has received daily injections of hCG for 5 weeks. The interstitial tissue is abundant, compared with the normal control in Fig. 1. X152.

FIG. 4. Area of interstitial tissue from Fig. 3. The Leydig cells in this hCG treated testis are larger, showing more extensive cytoplasm than is seen in the control. The cells also appear to be more numerous, although it is difficult to be certain of this from inspection of the micrograph. X374.

TABLE 1. Morphometric data on the Leydig cells of adult rats treated with daily doses of 100 IU hCG for up to 5 weeks. Mean ± SEM.

hCG treatment (weeks)	Animals (n)	Testis volume (cm^3)	% Volume of Leydig cells in testis	Ratio to control (with significance level)	Leydig cell number ($\times 10^6$) per cm^3 testis	Ratio to control (with significance level)	Volume of individual Leydig cells (μm)^3	Ratio to control (with significance level)	% Volume of non-Leydig interstitial tissue	Diameter (μm) and % volume of seminiferous tubules in testes
0	5	1.66 ± 0.17	4.1 ± 0.43	–	18.6 ± 1.3	–	2199 ± 120.9	–	17 ± 3.0	235 ± 11.6 79% ± 2.8
1	3	1.83 ± 0.13	6.4 ± 0.29	1.6 (P<0.01)	25.1 ± 0.9	1.4 (P<0.02)	2559 ± 79.3	1.2 (NS)	21 ± 3.8	203 ± 5.7 73% ± 3.8
2	3	2.02 ± 0.30	7.8 ± 1.48	1.9 (P<0.05)	29.6 ± 2.1	1.6 (P<0.01)	2630 ± 432.5	1.2 (NS)	13 ± 0.6	224 ± 10.3 79% ± 1.2
3	3	1.45 ± 0.13	10.0 ± 0.87	2.4 (P<0.01)	40.4 ± 2.4	2.2 (P<0.001)	2461 ± 68.7	1.1 (NS)	12 ± 2.8	223 ± 4.7 78% ± 3.8
4	3	1.80[a]	12.4 ± 1.64	3.0 (P<0.001)	44.3 ± 5.3	2.4 (P<0.001)	2829 ± 300.8	1.3 (NS)	16 ± 1.2	228 ± 11.9 72% ± 0.3
5	2	1.55 ± 0.05	19.4 ± 2.86	4.7 (P<0.001)	55.1 ± 7.8	3.0 (P<0.001)	3511 ± 25.0	1.6 (P<0.01)	22 ± 2.5	234 ± 8.0 60% ± 5.5

[a] From 2 animals only, both with same testis volume.

hormones bind to the same receptor on the Leydig cell plasma membrane and have equivalent action (reviewed by Catt and Dufau, 1976). The only appreciable functional difference between these two hormones is the longer half-life of hCG in the plasma (Hsueh et al., 1977), but this would not be expected to affect the capacity of the hormones to induce hyperplasia.

There has been considerable controversy in the past about optimal methods for comparing the relative number of Leydig cells before and after a treatment. The earliest approach was simply to count the cells seen in several random microscopic fields. However, it was recognized that this method could be subject to serious

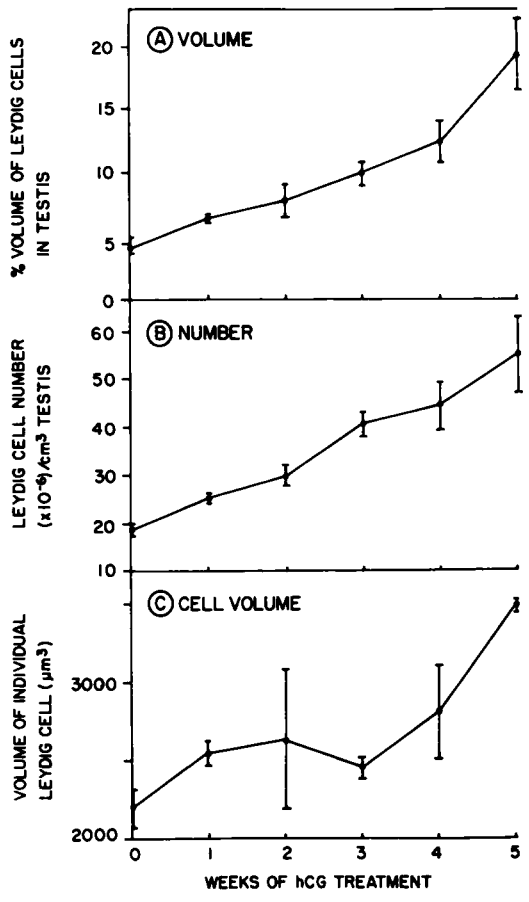

FIG. 5. Graphs showing the effect of daily hCG injections for up to 5 weeks. The treatment causes an increase in A) the percentage of testis volume occupied by Leydig cells; B) the number of Leydig cells in each cm^3 testis; and C) the volume of an average individual Leydig cell. These curves are based on the data presented in Table 1.

error. As a result, efforts were made to set up a reference standard within the testis as a basis for the Leydig cell counts. Clegg (1961, 1966), refining an earlier method of Sargent and McDonald (1948), established the seminiferous tubules as the internal reference and the resulting counts were expressed as Leydig cells/seminiferous tubule. This method was utilized by Schoen (1964) in the only other study that has dealt with the effect of chronic hCG treatment on Leydig cell numbers in the testes of adult rats. His paper was concerned primarily with the effect of x-irradiation on testicular steroidogenesis and gonadotropin response and it is therefore understandable that his description of Leydig cell increase escaped the notice of subsequent workers interested in the problem discussed in the present paper. As one of the controls in the study, adult rats received 100 IU of hCG daily for 14 days; it was reported that the number of Leydig cells doubled as a result of this treatment. Heller et al. (1971) later criticized this general method of counting Leydig cells and proposed an alternate method involving counts of both Leydig and Sertoli cell nucleoli, with the final data being expressed as a Leydig/Sertoli ratio. It would not, in fact, have been possible for Schoen (1964) to have used that method, since the level of histological preservation in his study (according to a statement in the text) was not adequate to allow the identification of Sertoli cells. Nevertheless, in spite of past questions on the methodology, the report by Schoen (1964) that the number of Leydig cells approximately doubled after 2 weeks of hCG treatment is confirmed in the results described here.

It is important to consider the validity of the approach utilized in the present study. The principal concern in the past has been that the treatment might induce changes in the volume of seminiferous tubules or interstitial tissue that could have a substantial effect on the Leydig cell counts. Another problem would arise if the Leydig nuclei increased in size during treatment; they would then be included in more sections and thus yield higher counts. We have given data in Table 1 to show that there are no consistent changes in the volume of seminiferous tubules, interstitial tissue, "non-Leydig" interstitial tissue, or in the diameters of seminiferous tubules that could offer an alternative explanation for our findings. We have also measured nuclear diameters throughout the study (see Materials and Methods) and find no consistent differences. However, the most compelling support for the approach used here lies in the fact that the numerical data have been obtained in terms of numerical density or the number of Leydig cells that occur in a cm^3 of testis tissue. If Leydig cells are uniformly distributed throughout the testis (as appears to be the case), then there are only two ways that the numerical density of the cells can become larger: 1) if the total number of Leydig cells in the testis increases; or 2) if the testis becomes smaller. In the latter case, the same number of cells would be packed into a smaller volume, so the cells/cm^3 tissue (numerical density) would be greater. In the present study, therefore, the only means by which the numerical density of the Leydig cells could increase by a factor of 3, without a corresponding increase in Leydig cell number, would be if 5 weeks of hCG treatment caused a 3-fold reduction in testis volume, which clearly did not occur (Table 1). We therefore conclude that the Leydig cells do indeed increase in number under chronic hCG treatment in the testes of adult rats.

The results of our study on the rat testis differ from those reported previously in humans and in the rock hyrax. Heller and Leach (1971) treated normal men with 4000 IU hCG 2 or 3 times a week for 6 or 16 weeks. Cell counts were made on biopsies, using the counting method of Heller et al. (1971). Although there was some variability in the response from one individual to another, the study led to the conclusion that the treatment increased the size of Leydig cells but did not produce any consistent increase in cell number. Neaves (1973) studied the striking changes in Leydig clusters over the seasonal cycle of rock hyraxes. Cell counts were made by measuring the total volume of Leydig cells/testis and then dividing that figure by the estimated volume of an average Leydig cell based on cell diameters. Although the size of the Leydig cell clusters expanded by a factor of 2.5 times as the animals came into the breeding season, the cell counts did not indicate an appreciable increase in the number of Leydig cells. The conclusion reached in those two papers thus differs from that of the present study and it is difficult to know whether this is due to the difference in species, in the level of circulating hormone, or in the methodology.

The results of this study indicate that daily doses of excess hCG continue to exert a hor-

monal effect throughout several weeks of treatment. The results of chronic administration appear to differ from the short term effects of excess LH/hCG. Within 2–3 days after a single injection of 100–500 IU hCG, the Leydig cells lose all detectable available LH/hCG receptors and no longer respond to hCG treatment by secreting testosterone (Sharpe, 1976; Hsueh et al., 1976; Haour and Saez, 1977). This "desensitization" is presumably a means of preventing overstimulation of Leydig cells by an acute rise in gonadotropin level. However, there is biochemical evidence that the Leydig cells are able to respond to elevated levels of hCG over a longer period. Mature rats treated 5 days with 100 IU hCG/day showed a 3.6-fold increase in testicular concentration of testosterone, compared with concentrations in normal controls (de Jong et al., 1974) and also exhibited a 2.5-fold increase in testosterone production by testicular homogenates in vitro (van de Vusse et al., 1975). In the study of Cusan et al. (1979), adult male rats injected twice a week with 10 IU hCG showed a marked reduction in available LH/hCG receptors/testis at 1 week of treatment, but by 4 weeks the binding of [^{125}I]-hCG/testis was back to control levels, where it remained when the study ended at 12 weeks. There was no significant change in the weight of the testis, seminal vesicle or ventral prostate over the 12 weeks of treatment. Similar findings have been reported by Risbridger and de Kretser (1979) in mature male rats injected daily with 10 IU or 100 IU hCG for up to 3 weeks. At both dosages there was an initial striking decrease in receptor concentration/testis at 1 week, but control levels were regained by 3 weeks of treatment. Plasma testosterone rose markedly by 1 week but was not significantly different from control values at 2 or 3 weeks. Zipf et al. (1978) administered 15 μg LH s.c. to mature rats twice daily for 10 days and found that serum testosterone, in response to a stimulatory dose of LH administered 2 h earlier, was elevated on Days 3, 5 and 7 and was more than twice the control concentration by 10 days. However, the concentration of LH/hCG receptors/testis, as measured by [^{125}I]-hCG binding, was reduced to ∿50% of control values throughout the course of treatment. It can be concluded from these various biochemical studies that chronic treatment of adult rat testes with excess hCG or LH provides a continuing stimulation of Leydig cell function. The findings of the present study are consistent with that conclusion in showing a continuous increase in the number of cells and in the size of individual cells during long term hCG treatment. This increase in cell number and size over the 5 weeks of treatment would also suggest that the rats did not build up a sufficient plasma concentration of antibodies against hCG to pose a problem for this study.

According to the biochemical studies cited in the previous paragraph, chronic excess LH/hCG results in a reduced number of LH/hCG receptors/testis at 1 week of treatment, but thereafter the concentration of receptors/testis gradually returns to the control level. There is an implication that the individual Leydig cells are able to restore their former receptor numbers in spite of the continued excess of hCG. However, the results of the present study suggest the alternative explanation that the Leydig cells may remain in a state of reduced receptor number (partial desensitization) throughout the extended treatment and that it is actually the increase in number of Leydig cells that restores the concentration of receptors/testis to the control value.

The physiological implications of the findings reported here are uncertain. If a long term rise in LH occurred physiologically, it might be anticipated that the increase in cell number would eventually enable the Leydig cell population to produce enough testosterone to come back into equilibrium with the hypothalamo-hypophyseal feedback system at the new level of demand. However, it is not obvious what circumstances would produce a chronic gonadotropin increase in a nonseasonal breeder such as the laboratory rat. It is unlikely that equilibrium could be attained with the high levels of hCG used in this study. The dosage was chosen to constitute a clear excess and also because this dose (100 IU/day) has been used routinely for decades in physiological and biochemical studies on male rats and is still commonly seen in the literature. The use of this dosage may thus permit our findings to be related more readily to previous work.

It is not clear what mechanism is responsible for the increase in Leydig cell numbers. During puberty, new Leydig cells arise by differentiation from fibroblast-like "stem" cells and also by division of mature Leydig cells (Christensen, 1975). In the adult testis the increase in Leydig cells under hormonal stimulus might occur by either of these mechanisms or by both. The present study did not include any

systematic search for dividing Leydig cells. Nevertheless, during the extensive counting procedures no dividing cells were noted in material from the first 3 weeks of treatment; division figures were seen on rare occasion in the fourth and fifth weeks. Further studies involving autoradiography, colchicine and other experimental approaches may reveal the source of new Leydig cells arising under hormone treatment in the testes of adult rats.

As a side issue, the data from normal controls in the present study allow an estimation of the rate of testosterone production by an average Leydig cell. Free and Tillson (1973) have reported that the testis of an adult rat normally secretes testosterone in vivo at a rate of ∿6.7 ng/g testis/min. We have shown here that there are ∿18.6 x 10^6 Leydig cells/cm^3 testis, a volume that weighs very nearly 1 g. An average individual Leydig cell would therefore secrete ∿0.5 pg testosterone/day, or ∿12,500 molecules of testosterone/second. These correlations are extended to the organelle level in a companion study from this laboratory (Mori and Christensen, 1980), involving EM morphometry of rat Leydig cells.

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