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Alterations of Testicular Function in the Unilaterally Cryptorchid Rat (41096)

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Abstract. Mature male Sprague–Dawley rats were either rendered unilaterally cryptorchid or used as intact controls. At 7, 14, 21, and 28 days postsurgery, five animals from each group were sacrificed, blood samples were collected, and the cryptorchid, eutopic, and intact control testes were removed. The cryptorchid testes weights decreased markedly below the controls while the weights of the eutopic testes did not vary from those of the controls. The levels of serum LH, FSH, and testosterone (T) did not significantly differ between the two groups throughout the study. The testicular content of T, expressed as nanograms per testis, decreased in the cryptorchid testes below control values from 30.6 ± 3.3 ng at 7 days to 10.8 ± 0.3 ng at 28 days while the content of the eutopic testes increased above controls from 71.1 ± 6.0 ng at 7 days to 131.7 ± 9.8 ng at 28 days. The cytoplasmic estrogen receptor level (^{3}H-E_2 bound/mg protein) of the cryptorchid testis increased threefold above control levels at 7 days and continued to rise to a sixfold increase above control levels at 28 days; the eutopic and control E_2R levels did not vary throughout the study. While these data demonstrate that the steroidogenic capacity of the cryptorchid testis was reduced, the increased E_2 binding capacity indicates that the viability of the Leydig cell population was not detrimentally altered during the experimental period. These data further suggest that as a result of unilateral cryptorchidism, a compensatory change occurred in the eutopic testis resulting in an increased steroidogenic capacity *in vivo*.

It is generally accepted that experimental cryptorchidism results in severe damage to the spermatogenic process and rapid degeneration of the seminiferous epithelium (1). Although it is currently assumed that the Sertoli cells are uneffected by cryptorchidism (2, 3), controversy still exists concerning the effects of cryptorchidism on Leydig cells; hypertrophy of Leydig cells (4) as well as little or no change in Leydig cell morphology (5, 6) have been reported. To add to this controversy, both an increase (7, 8) and a decrease (9, 10) in the capacity of the cryptorchid testis to produce steroids have been reported.

Conflicting data exist concerning the effects that cryptorchidism has on serum hormone levels. Serum gonadotropins were reported to be increased in the cryptorchid rat by some investigators (11, 12) while others reported little or no change (13). Serum testosterone (T) has also been reported as being increased (11) as well as unchanged (12, 13) as a result of cryptorchidism. To confound the issue, the above discrepant results were obtained from different cryptorchid animal models, animals of different ages, and varied duration of cryptorchidism.

The unilaterally cryptorchid animal offers a unique model for studying the effects of cryptorchidism on testicular function in that both the cryptorchid and eutopic testes are exposed to the same changes that might occur in the hormonal milieu as a result of the cryptorchidism. This animal model also allows the possible detection of any compensatory or detrimental changes which might occur in gonadal function. Indeed, a compensatory hyperplasia and hypertrophy of Leydig cells in the eutopic testis from the unilaterally cryptorchid rat have been reported (3). However, definitive results concerning unilaterally cryptorchid-induced alterations in the testis and in the hormonal levels of these animals are still lacking. We, therefore, have investigated the effects of unilateral cryptorchidism on testicular endocrine function and on the levels of serum gonadotropins and T.

Materials and Methods. *Animals.* The animals used in this study were adult male Sprague–Dawley rats (97 days of age) purchased from Hormone Assay Labs, Chi-

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cago, Illinois. They were housed in a 12-hr light–dark environment and provided with water and rat chow *ad libitum*. The animals were randomly divided into two groups classified as intact controls or animals which were made unilaterally cryptorchid. To render the animals unilaterally cryptorchid, the rats were anesthetized with sodium pentobarbital, a midline abdominal incision was made, and either the left or right testis was drawn through the inguinal canal and sutured to the abdominal wall after severing the gubernaculum (12, 14). Sham operations were performed on the control group. At 7, 14, 21, and 28 days after surgery, five rats from each group were anesthetized with ether and blood was drawn by cardiac puncture. The animals were then sacrificed and the cryptorchid, eutopic, and intact control testes were removed and used immediately or stored in liquid N_2 until utilized in subsequent studies. Sera were subsequently collected from the blood samples and stored at −20° until assayed.

Buffers and solutions. Radioimmunoassays (RIAs) were performed using PGB, phosphate-buffered saline with gelatin (0.15 *M* NaCl, 0.039 *M* NaH_2PO_4, 0.061 *M* Na_2HPO_4, and 0.1% Bloom 100 gelatin, pH 7.2). Aliquots of testicular tissue were frozen in medium 199, pH 7.4, with Hanks' salt and L-glutamine (GIBCO) with the additions of 0.35 g $NaHCO_3$/liter and 0.1% BSA (Pentex-Bovine Albumin, Fraction,V; Miles Laboratories, Inc.) for subsequent T determinations. Estrogen receptor assays were conducted using TE buffer (0.01 *M* Tris, 0.0015 *M* disodium EDTA, pH 7.4). A suspension of 0.3% Norit A charcoal and 0.03% dextran was prepared in TE buffer. [2,4,6,7-^{3}H]Estradiol-17β (E_2; 96.1 Ci/mmole) and [1,2,6,7-^{3}H]T (85 Ci/mmole) were purchased from Amersham–Searle and further purified by descending partition paper chromatography.

Serum hormone measurements. Serum LH and FSH RIAs were performed as described elsewhere (12). Pure rat LH (NIAMDD-Rat LH-I-5) and FSH (NIAMDD-Rat FSH-I-3) were iodinated using the Chloramine-T method (15). The LH values are expressed in terms of NIAMDD-rat LH-RP-1. The sensitivity of the LH RIA was 1.0 ng and the linear range of the standard curve was 1–100 ng. The antiserum to LH used was NIAMDD-anti-rat-LH serum-S-4 at a dilution of 1:40,000. FSH values are expressed in terms of NIAMDD-rat FSH-RP-1. The sensitivity of the FSH RIA was 10 ng and the linear range of the standard curve was 10–200 ng. The antiserum to FSH used was NIAMDD-anti-rat-FSH serum-S-9 at a dilution of 1:2,500. All gonadotropin determinations were obtained from a single assay and the intraassay coefficients of variation were found to be 3.8 and 4.4% for FSH and LH, respectively.

Serum T RIAs were performed as described elsewhere (12). Tracer quantities (1000 cpm) of [^{3}H]testosterone (85 Ci/mmole) were added to aliquots of sera to determine the percentage recoveries in the extraction procedure. Aliquots of sera (0.20 ml) were extracted twice with 10 ml of anhydrous diethyl ether. The samples were vortexed vigorously each time, frozen in a dry ice–acetone bath, and the organic phases were decanted and pooled for each sample. The ether was evaporated under N_2 gas and the extracts were brought into solution in PGB. Dilutions of these extracts were assayed for T by RIA. Recoveries of T extraction were 76–93%. The antiserum to T (R-181) was obtained from Radioassay Systems Laboratories, Inc., Carson, California. The antiserum has high specificity for T and relatively low cross-reactivity for dihydrotestosterone (6.6%), Δ^4-androstenedione (0.9%), and 5α-androstane-3β, 17β-diol (2.19%). Therefore, RIAs were performed directly on extracted samples. The antiserum was used at a final dilution of 1:170,000 which bound 50% of the [^{3}H]T. The sensitivity of the RIA was 9.5 pg and the linear range of the standard curve was 9.5–150 pg. Water blanks were extracted, assayed, and found to be below the sensitivity of the assay. The interassay and intraassay coefficients of variation were 15.5 and 12.3%, respectively.

Testicular testosterone content. Testicular T content was determined as described previously (12, 16). Aliquots of 100 mg (wet wt) of finely minced, decapsulated testes were placed in glass vials with 1.9 ml

of medium 199 buffer and frozen at −20° immediately. Samples were thawed and tracer [^{3}H]T was added to determine recoveries. The samples were homogenized in Kontes–Duall glass homogenizers, transferred to extraction tubes, and extracted twice with 10 ml of anhydrous diethyl ether. Dilutions of each extract were assayed in subsequent RIAs. Recoveries of testicular T were 73–97%. Medium 199 blanks were also run and found to be below the sensitivity of the assay.

Estrogen receptor assay. Cytosols were prepared from decapsulated testes and the specific binding of estradiol was assessed *in vitro* as previously described (17). Briefly, a constant amount of cytosol protein was added to tubes containing varying concentrations of [^{3}H]E_2; a second series of tubes contained, in addition, a 100-fold molar excess of unlabeled E_2 to determine nonspecific binding. The assays were performed for 18 hr at 4° and terminated by the addition of charcoal. The data obtained from these experiments were analyzed by the method of Scatchard (18). Protein concentrations were determined by the method of Lowry *et al.* (19) using bovine serum albumin as the standard.

Measurement of radioactivity and expression of data. Radioactivity in the gonadotropin RIAs was measured in a Beckman 310 gamma counter. Radioactivity in the steroid RIAs and receptor assay was determined by adding 10 ml of scintillation fluid containing 5 g of Permablend II (Packard) per liter of toluene. The samples were shaken to allow extraction of the [^{3}H]steroid into the toluene. Radioactivity was measured in a Beckman 250 liquid scintillation counter with a counting efficiency for tritium of 56%. Statistically significant differences between experimental and control groups were determined by Student's unpaired *t*-test and Duncan's multiple-range test. Statistical values are expressed as the mean ± the standard error (SEM).

Results. The observed changes in testicular weights during the experimental period are shown in Fig. 1. There was no difference noted between the testicular weights of the intact control and the eutopic testes throughout the study. However, the cryptorchid testes decreased in weight steadily from 0.65 ± 0.15 g at 7 days postsurgery to 0.28 ± 0.12 g at 28 days. This represents a statistically significant decrease in testis weight below controls at 7 days (56%, $P < 0.05$) through 28 days (83%, $P < 0.01$).

The levels of serum LH and FSH for the intact group over the course of this study ranged from 27 ± 6 to 62 ± 9 and 337 ± 31 to 523 ± 48 ng/ml, respectively. No statisti-

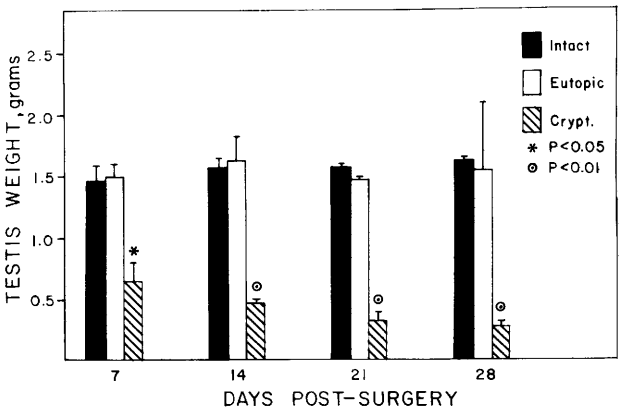

FIG. 1. Effects of experimental unilateral cryptochidism on testicular weights of mature rats. At 7, 14, 21, and 28 days postsurgery, control and experimental animals were sacrificed and the cryptorchid, eutopic, and intact control testes were removed. Each of the above values represents the mean ± SEM of five animals per group. Statistical analysis refers to a comparison between the experimental and control groups.

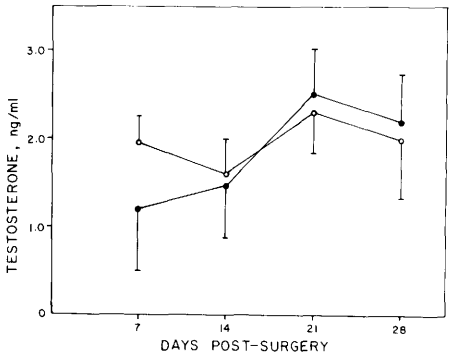

FIG. 2. Effect of experimental unilateral cryptorchidism on serum T levels of the mature rat. Serum T, expressed as ng/ml, was measured for intact (●) and unilaterally cryptorchid (○) animals at 7, 14, 21, and 28 days postsurgery. Each of the above values represents the mean ± SEM of five animals per group.

cal differences between control and experimental groups could be detected. Further, as illustrated in Fig. 2, no significant differences in the levels of serum T were demonstrated between the control and experimental groups.

The testicular content of T, expressed as nanograms per testis, is shown in Fig. 3. These data demonstrate a decrease in the T content of the cryptorchid testes from 30.6 ± 3.3 ng at 7 days to 10.8 ± 0.3 ng at 28 days. This decrease below controls was statistically significant throughout the study ($P < 0.01$). The data also indicate a compensatory increase in testicular T content of the eutopic testes above controls. Although an increase of 5% was not statistically significant at 14 days postsurgery, significant increases above controls of 35% at 7 days ($P < 0.01$), 30% at 21 days ($P < 0.05$), and 113% at 28 days ($P < 0.01$) were detected.

As depicted in Fig. 4, unilateral cryptorchidism resulted in a marked increase in the cytoplasmic E_2 binding capacity by the cryptorchid testes when compared with the controls. The cytoplasmic estrogen receptor (E_2R) levels of the cryptorchid testes, expressed as femtomoles of [^{3}H]E_2/mg cytosol protein, rose from 34.1 ± 1.8 at 7 days postsurgery to 69.4 ± 7.1 at 28 days. This represents a threefold increase above controls at 7 ($P < 0.01$) and 14 days ($P < 0.05$) and was further increased to fourfold at 21 days ($P < 0.05$) and sixfold at 28 days ($P < 0.01$). The cytoplasmic E_2R binding capacity of the eutopic testes was found to be equivalent to that of the controls at all time points.

Testicular content of cytoplasmic estradiol receptor, expressed as femtomoles of [^{3}H]estradiol bound/testis, is presented in Table I. These data reveal that estrogen binding capacity in the controls, although

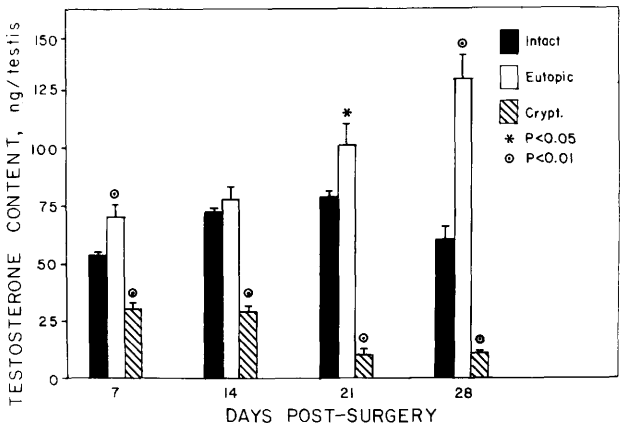

FIG. 3. Effects of experimental unilateral cryptorchidism on testicular T content of the mature rat. At 7, 14, 21, and 28 days postsurgery, control and experimental animals were sacrificed and the cryptorchid, eutopic, and intact control testes were removed. Testicular T content, expressed as ng/testis, was then measured for each testis. The above values represent the mean ± SEM for triplicate samples of tissue from each of five animals. Statistical analysis refers to a comparison between the experimental and the control groups.

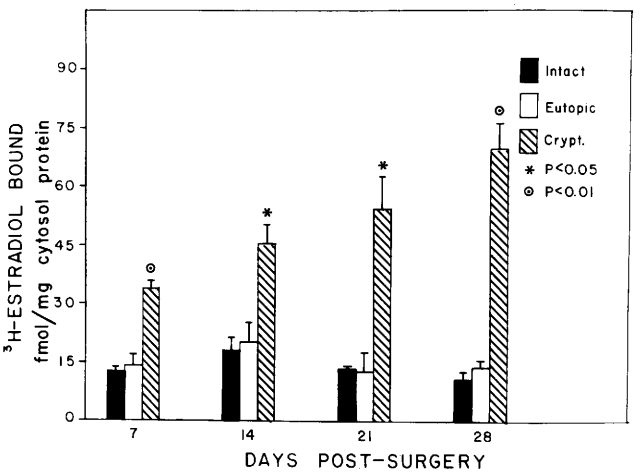

FIG. 4. Effects of experimental unilateral cryptorchidism on the levels of testicular cytoplasmic estrogen receptor. At 7, 14, 21, and 28 days postsurgery, experimental and control animals were sacrificed and the cryptorchid, eutopic, and intact control testes were removed. The cytoplasmic estradiol binding capacity, expressed as fmol [^{3}H]estradiol bound/mg testicular cytosol protein, was determined *in vitro* and analyzed by Scatchard plots as described under Materials and Methods. The data were corrected for nonspecific binding. Each of the above values represents the mean ± SEM from two experiments each utilizing pooled tissue from five animals. Statistical analysis refers to a comparison between the experimental and the control groups.

somewhat variable, did not change significantly throughout the study. Receptor content in both the eutopic and cryptorchid testes did not differ significantly from the mean control value. Using E_2R content as one of several possible cellular markers, it appears that Leydig cell number in the eutopic organ did not increase significantly. Further, these data suggest that Leydig cell number was not detrimentally affected in the cryptorchid testis.

Discussion. The data presented here indicate that the weights of the cryptorchid testes were markedly reduced as a result of unilateral cryptorchidism when compared with intact controls. This reduction in weight occurred rapidly, being evident as early as 7 days after the animals were made cryptorchid. The eutopic testes of these animals, however, did not reflect any weight change through 28 days when compared with intact controls. These results are in agreement with the observations of others (20, 21), who have shown a decrease in cryptorchid testicular weight due to a degeneration of the germinal epithelium.

In agreement with other investigators (22), our findings indicate that unilateral cryptorchidism did not result in any significant changes in serum levels of gonadotropins or T. Earlier, Clegg (3) had reported compensatory changes in the eutopic testis of the unilaterally cryptorchid rat including hyperplasia and hypertrophy of Leydig cells. It was postulated then that these changes were due to an increase in the secretion of gonadotropins. Although our results do not support Clegg's postulate of increased gonadotropin secretion, the possibility of compensatory changes in the

TABLE I. EFFECTS OF UNILATERAL CRYPTORCHIDISM ON CYTOPLASMIC ESTRADIOL BINDING CAPACITY, FEMTOMOLES OF [^{3}H-ESTRADIOL] BOUND PER TESTIS[a]

Day postsurgery	Control	Eutopic	Cryptorchid
7	336	431	453
14	560	668	449
21	441	494	377
28	369	453	418
Mean[b] ± SEM	426 ± 50	511 ± 53	424 ± 18

[a] The values represent the mean of five animals. The values were computed by using the mean values; estradiol bound/mg cytosol protein × mg cytosol protein/g tissue × g tissue/testis. A mean of 20.5 ± 0.5 mg cytosol protein/g tissue was obtained over a wide range of experiments.

[b] Refers to the mean of each group for the 28 days of study.

eutopic testis cannot be dismissed. Previously, we have shown that bilateral cryptorchidism resulted in an early increase in serum gonadotropins above controls (12). In agreement with our previous results and the present data, Gomes and Jain (22) have suggested that only bilateral cryptorchidism will result in an increase in gonadotropins and that the presence of one normal testis (unilateral cryptorchidism) is sufficient in preserving the normal feedback control of the gonadal–hypothalamic–pituitary axis. Therefore, while it can be suggested that the unaffected levels of serum gonadotropins and T resulting from unilateral cryptorchidism could be due to the normal capacity of the eutopic testis, it is also possible that the eutopic testis underwent a compensatory increase in its steroidogenic capacity and thus, maintained normal serum hormone levels.

In support of a possible compensatory change as a result of unilateral cryptorchidism, the data in Fig. 3 indicate that while the content of T per testis in the cryptorchid testes was reduced below controls throughout this study, the content of T per testis of the eutopic testes increased. By 28 days postsurgery, the eutopic testes contained twice as much T as the control testes and 13 times as much T as the cryptorchid testes. Since the weights of the control and eutopic testes did not differ, these results suggest that the *in vivo* steroidogenic capacity of the eutopic testis was dramatically increased as a result of unilateral cryptorchidism. This increased capacity could be due to several factors, including hypertrophy of Leydig cells, increased steroidal enzymatic activity, and increased sensitivity to LH. First, hypertrophy of Leydig cells in the eutopic testis of unilaterally experimental (3) and congenital cryptorchid (23) rats has been reported. However, a recent study (10) failed to confirm this finding. Second, increases in 17α-hydroxylase (20) and Δ^5-3β-hydroxysteroid dehydrogenase (24) were observed in the eutopic testis when compared with the contralateral cryptorchid testis. Because these enzyme activities were not compared with those of intact controls, it is difficult to determine if a compensatory increase in steroidogenic enzymes existed in the eutopic testis as a result of unilateral cryptorchidism. Lastly, it has been proposed recently that a compensatory mechanism in Leydig cells of the eutopic testis might involve increase in sensitivity to LH stimulation (10). It is conceivable, therefore, that unilateral cryptorchidism might increase the response of the eutopic testis to the normal circulating levels of LH through an increase in LH binding. This increased response would result in an increase in T production, thus compensating for the decreased testicular content of T exhibited by the cryptorchid testis. The decreased steroidogenic capacity of cryptorchid testis could be due to the increased temperature to which it was exposed (25).

Evidence which suggests that all functions of the Leydig cells were not affected by unilateral cryptorchidism is provided by the fact that the concentrations of the cytoplasmic E_2R in the cryptorchid testes were increased above controls at all time points. Since it has been demonstrated that the E_2R is localized in the interstitial tissue (26), this increase in binding capacity would reflect an increase in the Leydig cell proportion due to the loss of the germinal epithelium. Using E_2R as one parameter of cell function, this would suggest that little change had occurred in the viability of the Leydig cell population in the cryptorchid testis. Further, when the E_2 binding data were expressed per organ (Table I), no differences were detected among the various groups. This observation suggest that the actual number of Leydig cells per testis were not increased.

These data suggest that as a result of unilateral cryptorchidism a compensatory change occurred in the eutopic testis resulting in an increased steroidogenic capacity which allowed for a normal feedback control of the gonadal–hypothalamic–pituitary axis. This compensatory change was reflected in the normal serum gonadotropins and T levels and the increase in eutopic and decrease in cryptorchid testicular contents of T. Further, these data demonstrate that while the steroidogenic capacity of the cryptorchid testis was re-

duced, the E_2R binding capacity per testis did not change significantly throughout the study indicating that the Leydig cell population was not detrimentally altered.

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