Journal of Steroid Biochemistry, 1975, Vol. 6, pp. 1459–1465. Pergamon Press. Printed in Great Britain

# ANDROGEN BINDING SITES IN TESTIS CELL FRACTIONS AS MEASURED BY A NUCLEAR EXCHANGE ASSAY

BARBARA M. SANBORN,* ANNA STEINBERGER,* MARVIN L. MEISTRICH† and EMIL STEINBERGER*

Department of Reproductive Biology and Endocrinology, The University of Texas Medical School at Houston, Texas, 77025* and Section of Experimental Radiotherapy, The University of Texas Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77025†, U.S.A.

(*Received* 17 *June* 1975)

SUMMARY

A nuclear androgen exchange assay was previously developed in this laboratory for use in the rat testis. Further studies have shown that the dependence of exchange upon hormonal pretreatment was concentration dependent in the range of 2–60 μg/ml under *in vitro* conditions. The order of specificity was testosterone ≫ progesterone, estradiol ≫ cortisol, no hormone. Hormone-dependent exchange activity was found to persist in the testes of 0–70 day hypophysectomized adult (100–170 day old) and 0–24 day hypophysectomized immature (35–59 day old) animals. The exchange activity per mg DNA was greater in freshly isolated Sertoli cell preparations from long term hypophysectomized rat testes than in comparable total testes. Exchange activity was also observed in subfractions of germ cell preparations, achieved by use of an elutriator rotor. The highest concentration of activity was associated with the most mature cell types. These data demonstrate that androgen exchange activity exists in both Sertoli cell and germ cell fractions and suggest that the mechanism of action of androgens in the testis may involve interaction with more than one cell type.

## INTRODUCTION

The rat testis has been shown to contain two types of androphilic proteins. The first to be described, termed androgen binding protein or ABP, is formed by Sertoli cells in the seminiferous tubules and is secreted into the epididymis; its concentration is affected by FSH and androgens [1–6].

The second androphilic macromolecule was detected following *in vivo* injection of [$^3$H]-testosterone into immature hypophysectomized rats and had the properties of an intracellular receptor—a slow dissociation rate constant, heat instability, and a 6–8S sedimentation coefficient [7]. Cytoplasmic and nuclear androgen receptors have also been detected in adult hypophysectomized rats [8, 9]. In addition, macromolecular species which bind testosterone have been reported to exist in nonflagellate germ cells, and testosterone uptake has been observed in epididymal sperm [10].

Study of testicular androgen receptors is made difficult by the high endogenous concentration of testosterone. Therefore, an attempt was made to adapt the nuclear exchange assay of Anderson *et al.* [11] for use in the testis. This method relies in principle on the interaction of unlabeled hormone, endogenous or exogenous, with a specific cytoplasmic receptor. The steroid–receptor complex migrates to the nucleus and binds to an acceptor site. The nuclei are then isolated and labeled hormone is exchanged for unlabeled hormone under conditions where the nuclear-bound receptor complex is stable.

Testicular androgen exchange activity was shown to be androgen-sensitive, to persist for up to 21 days after hypophysectomy in the adult animal, and to be present in both minced, washed tubules and in the corresponding germ cell fractions [12].* The present study explores in further detail certain variables of the exchange assay procedure itself and the cellular location of the exchange activity within the seminiferous tubules.

## MATERIALS AND METHODS

*Experimental animals and chemicals.* Hypophysectomized rats (Sprague–Dawley) were obtained from Hormone Assay Laboratories. Intact animals (90–160 days of age) were either of the Sprague–Dawley or Long-Evans strains. [1α,2α-$^3$H]-testosterone (New England Nuclear, 40–50 Ci/mmol) was 98% pure. Unlabeled steroids were obtained from Steraloids and were used without further purification.

*Pretreatment with steroid* in vitro. Perfused, decapsulated testes (gently teased or minced, depending upon the cell types studied) were incubated for 1 h at 33° in 0·01 M Tris–Cl (pH 7·4), 0·85% saline (Tris–saline), 0·1% glucose, 2–4% dimethylsulfoxide in the absence or presence of 100–200 μg/ml testosterone as

* Portions of this work were presented at the Second Annual NIH Workshop on the Testis, Chapel Hill, North Carolina, April 2–4, 1975.

indicated. Subsequently, the tissue was washed repeatedly with cold Tris–saline and homogenized.

*Nuclear exchange assay.* The tissue was homogenized in 0·32 M sucrose, 3 mM $MgCl_2$, 0·01 M Tris-Cl pH 7·4 (Buffer A). The homogenate was filtered through cotton gauze, centrifuged (15 min, 800 *g*), and washed three times by resuspension and centrifugation. The resulting crude nuclear pellet was suspended by gentle homogenization in Buffer A. Aliquots (0·5 ml, containing 15–40 μg DNA) were pipetted into tubes containing 0·1 ml $^3$H-testosterone (22 nM final concentration) or into tubes containing [$^3$H]-testosterone plus unlabeled testosterone (11 μM) for assessment of non-specific binding and incubated for 45 min at 25°C. The reaction mixtures were diluted with cold 3 mM $MgCl_2$, 0·01 M Tris–Cl pH 7·4, the tubes centrifuged (10 min, 800 *g*), and the resulting pellets washed three times. Radioactivity was extracted with 2 × 1·5 ml ether–chloroform (4:1 v/v), evaporated to dryness, and counted in ScintiPrep 1/toluene (Fisher). Data points represent specific binding, defined as the difference between total and non-specific counts determined in triplicate or quadruplicate, unless otherwise indicated. The nuclear suspension contained predominantly intact nuclei with little clumping, cytoplasmic membrane fragments, and cellular debris. The DNA content/tube [13] varied by less than 12%. Under the assay conditions, the nuclear exchange activity was a linear function of the concentration of DNA added [12].

*Germ cell fractionation.* For studies involving mixed germ cell fractions, the seminiferous tubules of the perfused, decapsulated testes were gently teased apart and washed copiously on a 125 μm stainless steel grid with a forceful stream of Tris–saline to remove most of the interstitial cells [14]. The tubules were then finely minced and the freed germ cells washed through the grid with buffer. The germ cell fractions contained primarily spermatocytes and spermatids, a number of spermatogonia and occasional interstitial and Sertoli cells.

For studies involving further fractionation of the germ cells, the gently teased tissue was treated with 0·25% trypsin for 20 min at 33° after preincubation with testosterone. The tubules were then washed as described above. This procedure assured virtually complete removal of the interstitial cells [14]. The tubules were minced and washed thoroughly with Dulbecco's phosphate-buffered saline (PBS) plus 0·1% glucose. The intratubular cells were then treated with trypsin (0·1%) and deoxyribonuclease (20 μg/ml) for 10 min at 31°. Following the addition of fetal calf serum to inhibit the action of trypsin, the cells were centrifuged, resuspended in PBS containing 2 μg/ml purified deoxyribonuclease (Worthington), 0·02% soybean trypsin inhibitor, and 5 mM 2-napthol-6,8-disulfonic acid (NDA) and were passed through a 25 μm nylon screen.

Cell separation was performed by velocity sedimentation in an elutriator rotor (Beckman Instruments) as described by Grabske *et al.* [15] with the following modifications. To minimize clumping, 5 mM NDA was added to the buffer (0·5% bovine serum albumin in PBS). The cell suspension, containing $10^9$ cells in 100 ml, was pumped into the chamber through the peristaltic pump, eliminating the need for

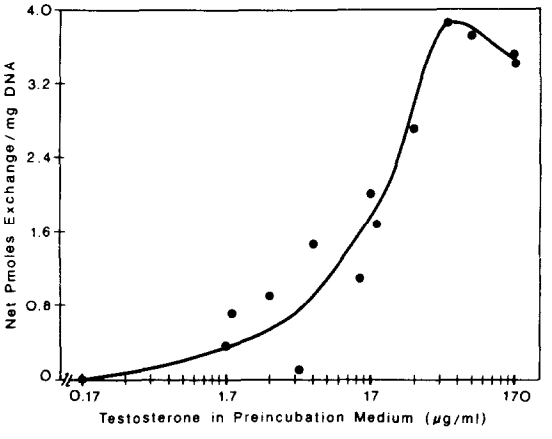


Fig. 1. The effect of preincubation of germ cell fractions with varying concentrations of testosterone on the nuclear exchange activity. Germ cell fractions from adult Long Evans rats (75–110 days of age) were prepared [14], resuspended in the incubation medium, and dispensed into vials containing testosterone dissolved in dimethylsulfoxide (3·4% final concentration). After preincubation (1 h, 33°), the cells were washed with Tris–saline prior to homogenization and subsequent nuclear exchange assay. Data represent a composite from four experiments. Net exchange is the difference between total exchange at any point and exchange in a comparable germ cell fraction preincubated in dimethylsulfoxide alone. Testosterone concentrations are plotted on a log scale.

TABLE 1

EXPERIMENTAL CONDITIONS FOR VELOCITY SEDIMENTATION SEPARATION OF RAT TESTICULAR CELLS BY CENTRIFUGAL ELUTRIATION

| Fraction | Volume Collected (ml) | Flow Rate (ml/min) | Rotor Speed (rpm) | Cell Type Most Enriched |
|---|---|---|---|---|
| 1 | 180 | 38 | 2600 | – |
| 2 | 100 | 38 | 1300 | Pachytene Spermatocytes |
| 3 | 240 | 9.9 | 3540 | Spermatozoa |
| 4 | 150 | 24 | 3540 | Late Spermatids |
| 5 | 150 | 31 | 2260 | Round Spermatids |

TABLE 2

EFFECT OF PRETREATMENT WITH STEROID ON NUCLEAR EXCHANGE ACTIVITY

A germ cell fraction was prepared from the testes of a 74 day old rat as described in Methods. Aliquots of cells were incubated with 19 μg/ml of the steroid indicated in 4% dimethylsulfoxide for 1 h at 33°. The cells were then washed and processed as described for the nuclear exchange assay.

| Steroid | Exchange Activity | |
|---|---|---|
| | Fmoles/mg DNA | % of Testosterone |
| Testosterone | 969 | 100% |
| Testosterone (190 μg/ml) | 705 | 73 |
| Progesterone | 570 | 59 |
| Estradiol - 17β | 464 | 48 |
| Cortisol | 36 | 4 |
| None | 6 | 1 |

the inconvenient mixing chamber. The conditions used for obtaining cell fractions are outlined in Table 1. The smaller cells were passed through the chamber in Fraction 1, leaving the larger cells in the chamber. These were washed out of the chamber in Fraction 2. Fraction 1 was then reintroduced into the chamber and separated by elutriation into three more fractions. The resulting cell suspensions were used to prepare nuclear pellets which were subjected to the exchange procedure.

*Sertoli cell preparation.* Sertoli cells were isolated subsequent to preincubation of the perfused, decapsulated testes [6, 16]. The minced tissue was digested with 0·25% collagenase (Difco) in Hank's balanced salt solution for 1 h and rinsed extensively on a 75 μm stainless steel grid with Tris–saline. The resulting Sertoli cell preparations were then processed for the exchange assay. In some cases, aliquots of the final nuclear suspensions were pelleted, fixed with Bouin's solution, embedded in paraffin, sectioned, and stained with PAS-hematoxylin. Examination of the sections revealed that the preparations contained roughly 60% Sertoli cell nuclei. Approximately 80% of the nuclei were free of surrounding cytoplasm.

## RESULTS

*Effect of pretreatment with steroids.* Previous studies had indicated that the testicular exchange was increased by pretreatment with testosterone, *in vivo* or *in vitro* [12]. The concentration dependence of the *in vitro* preincubation step is shown in Fig. 1. The exchange activity was increased by prior treatment with 2–60 μg/ml testosterone. Optimal exchange was observed at about 57 μg/ml.

In Table 2 are expressed the relative ability of a number of steroids to promote the increase in exchange activity. While testosterone was by far the most effective, pretreatment with both progesterone and estradiol increased the exchange activity substantially over incubation in buffer alone.

*Target organ specificity of exchange.* The exchange activity was compared in a variety of rat tissues. As shown in Fig. 2, epididymal sperm showed the highest exchange activity/mg DNA, while activity was virtually absent in spleen. Other experiments have shown that exchange in the testicular germ cells was increased by preincubation with testosterone (Table 2) but that exchange in the prostate from an intact adult animal was similar following preincubation in the absence or presence of testosterone (data not shown).

*Effect of hypophysectomy on exchange activity.* Testicular androgen exchange activity had been shown to persist for at least 21 days after hypophysectomy in mature animals [12]. This suggested cellular localization in Sertoli cells or early germ cells. Additional experiments revealed measurable exchange activity in testes of rats hypophysectomized for more than 21 days, using both immature and mature animals (Table 3).

To further ascertain whether the exchange activity was associated with the Sertoli cells themselves, the activities in freshly isolated Sertoli cell preparations [6, 16] were compared with those in corresponding whole testes from long-term hypophysectomized rats. As can be seen in Table 3, the exchange activities/mg DNA in the Sertoli cell preparations were equal to or greater than those in comparable whole testes. In all cases activity was increased by pretreatment with testosterone.

*Exchange activity in fractionated germ cells.* Previous work had suggested that a significant amount of nuclear exchange activity in the testis was associated with the germ cell fraction [12]. Therefore, germinal elements were separated into enriched fractions in a Beckman elutriator rotor [15]. The results of one such experiment are summarized in Table 4. The exchange sites were reasonably stable throughout the separation procedure subsequent to preincubation with the unlabeled hormone (4 h) as judged by the recovery of exchange sites in the eluted fractions (82%

TABLE 3

EXCHANGE ACTIVITY IN INTACT TESTES AND SERTOLI CELL FRACTIONS AT INTERVALS FOLLOWING HYPOPHYSECTOMY

Adult animals were 100 days of age at hypophysectomy; immature animals were 35 days old at surgery. Preincubations were carried out *in vitro* as described in Methods. Data represent specific binding with individual points measured in triplicate or quadruplicate. Multiple values represent determinations on separate preparations. ND = no detectable difference between total and nonspecific exchange.

| Days after Hypophysectomy | | Pretreatment | Fmoles Exchange / mg DNA | |
|---|---|---|---|---|
| | | | Intact Testis | Sertoli cells |
| Mature | 0 | –T | 99,88 | – |
| | | +T | 200 | – |
| | 6 | +T | 680 | – |
| | 7 | +T | 130,310 | – |
| | 8 | +T | 400 | – |
| | 19 | –T | 27 | – |
| | | +T | 260,190,180 | – |
| | 20 | +T | 150 | – |
| | 32 | –T | – | 48 |
| | | +T | – | 230 |
| | 42 | –T | 17 | 44 |
| | | +T | 28,30 | 91,97 |
| | 70 | –T | 170 | 240 |
| | | +T | 707 | 1160 |
| Immature | 0 | +T | 99 | – |
| | 20 | +T | 78,62 | – |
| | 28 | –T | 26 | – |
| | | +T | 70 | – |
| | 70 | –T | ND | – |
| | | +T | ND | – |

TABLE 4

NUCLEAR EXCHANGE IN ENRICHED GERM CELL FRACTIONS

Testes from eight 100 day old Sprague-Dawley rats were perfused, decapsulated, gently spread, and incubated with testosterone in 20 ml of buffer as described in Methods except that 50 mg trypin (Difco) was added during the final 20 min. The tubules were then washed, minced finely, and the liberated germ cells washed thoroughly with Dulbecco's phosphate-buffered saline plus 0.1% glucose (14). The germ cells were then treated with trypsin and deoxyribonuclease and spun through the elutriator rotor (Beckman) as described by Grabske *et al.*, (15). The resulting fractions were used to prepare nuclear pellets which were subjected to the exchange procedure employing DNA concentrations previously shown to be in the linear range of the assay (12). Cell and nuclei counts were determined using a hemocytometer and phase contrast microscopy. Nuclei were also counted after staining with methylene blue. Total exchange values were corrected for the recovery of nuclei. Counts may include cytoplasmic fragments detached from late spermatids, contributing to the low DNA recoveries in the late spermatid fraction. The DNA content of the sample saved for assay prior to loading onto the rotor may be low due to residual deoxyribonuclease activity. Note that the total number of sites in this sample was essentially the same as in the initial fraction.

| Fraction | Nuclei/Cells % Recovery | μg DNA/ $10^6$ Nuclei | Fmoles Exchange per | | | |
|---|---|---|---|---|---|---|
| | | | Mg DNA | $10^6$ Nuclei | Total Fraction | |
| Initial germ cell fraction | 52 | 8.1 | 380 | 3.0 | 3300 | |
| Cells loaded on elutriator | 47 | 3.7 | 920 | 3.3 | 3900 | |
| Pachytene spermatocytes | 75 | 9.6 | 250 | 2.4 | 140 | |
| Round spermatids | 47 | 4.0 | 210 | 0.8 | 480 | |
| Late spermatids | 37 | 1.6 | 980 | 1.5 | 690 | 82% |
| Sperm, sperm heads | 31 | 3.3 | 2800 | 9.3 | 1900 | |

TABLE 5

CELLULAR COMPOSITION OF FRACTIONS OF RAT TESTICULAR CELLS SEPARATED BY CENTRIFUGAL ELUTRIATION

Numbers represent the percent of each cell type based on differential counts of 500-750 cells on PAS-hematoxylin stained smears. (m) refers to a predominance of multinucleate cells which probably arise as a result of coalescence of cells through cytoplasmic bridges during cell suspension preparation. (*) Residual bodies (cytoplasmic fragments containing ribonuclear protein aggregates) detached from late state spermatids are found in the nuclear fraction as isolated and are included in the cell counts (27).

| Cell Type | Elutriator Fraction | | | | |
|---|---|---|---|---|---|
| | Pachytene Spermatocytes | Round Spermatids | Late Spermatids | Spermatozoa | Cells Loaded |
| Spermatogonia and primary spermatocytes (to zygotene) | 2(m) | 1 | <1 | 0 | <1 |
| Spermatocytes (pachytenes, some in meiotic division, some secondary spermatocytes) | 34 | <1 | 0 | 0 | 3 |
| Early spermatids (Steps 1-11) | 39(m) | 51 | 11 | 0 | 29 |
| Elongating spermatids (Steps 12-15, with cytoplasm but no flagella) | 10(m) | 34 | 21 | 0 | 18 |
| Sperm heads (Nuclei of Step 12-19 Spermatids) | 2 | 4 | 15 | 32 | 12 |
| Spermatozoa (Spermatids with flagella, Steps 16-19, some 12-15) | <1 | <1 | 12 | 68 | 25 |
| Residual bodies* | 2 | 7 | 38 | <1 | 9 |
| Sertoli cells | 10 | 0 | 0 | 0 | 2 |
| Leydig cells | 0 | <1 | 0 | 0 | <1 |
| Unknowns, macrophages, fibroblasts, and degenerating cells | <1 | 2 | 3 | 0 | 2 |

of the activity in the initial suspension); cell recovery from the rotor was quantitative. The fractions obtained were enriched in (a) pachytene spermatocytes; (b) round spermatids and early elongating spermatids; (c) late spermatids and residual bodies; and (d) intact spermatozoa and sperm heads, according to the criteria of Meistrich *et al.* [17]. Cytological examinations of the fractions were performed and the results are presented in Table 5. As can be seen in Table 4, the majority of the exchangeable sites expressed as per cent of the total was found to be located in the most mature cell types. Specific binding approached 70% of total binding in these fractions (data not shown). Expressed per nucleus or per DNA, the concentration of exchangeable sites exhibited striking cell specificity and represented an enrichment compared to the total cell suspension. The results were consistent in two separate experiments, indicating that the exchange sites were located either in the nuclei of the spermatozoa or were associated with contaminating structures such as sperm tails or residual bodies. Exchange activity in a nuclear pellet prepared after sonication (1 min, microprobe, Heat Systems Ultrasonics) of a total germ cell fraction and containing only sperm heads (nuclei of spermatids in Steps 12–19) was increased relative to the total fraction (2·86 vs 1·21 pmol/mg DNA, respectively).

## DISCUSSION

Androgen nuclear exchange activity in the testis has been further characterized in this paper. The androgen dependence of the pretreatment *in vitro* was marked and was related to the concentration of the steroid added to the medium (Fig. 1). The range of effective concentrations (2–60 μg/ml) was above the concentration estimated to be present in testis fluid (32 ng/ml, [18]). The hormone concentration in the microenvironment of the seminiferous tubule may be somewhat higher [19]. Even taking this into account, it appears that if the exchange is to be physiologically meaningful, there must be a mechanism operating in the environment of the tubule which lowers the effective concentration requirement relative to the situation with washed germ cells. Exchange could also be increased by treatment with 1 mg of testosterone administered *in vivo* 1 h prior to sacrifice [12], a dose which restores testicular fluid levels of testosterone [18] and qualitatively maintains spermatogenesis [20].

The hormone dependence of the pretreatment reaction was not entirely specific for testosterone (Table 2). Progesterone and estradiol were at least partially effective in this regard. In contrast, in the exchange reaction itself, testosterone and progesterone competed about equally while estradiol was ineffective [12]. Evidence from the literature points to a possible interaction of progestogens with components of the androgen-responsive systems in a number of tissues. Progesterone and synthetic progestogens such as cyproterone acetate (which is also a potent antiandrogen) are reasonably potent inhibitors of androgen uptake by prostate [21, 22]. More recently, Mowszowicz *et al.*[23] have postulated that certain proges-

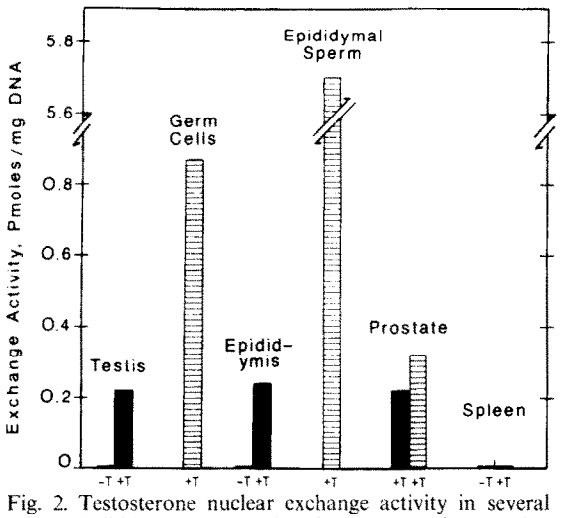


Fig. 2. Testosterone nuclear exchange activity in several organs. Solid bars represent data from an experiment using an 111 day old rat; striped bars represent data from a 73 day old rat. Whole tissues were minced and preincubated as described elsewhere in 3·4% dimethylsulfoxide (−T) or solvent plus 170 μg/ml testosterone (+T) at a tissue medium ratio of approximately ½ prior to processing for the exchange assay. Epididymal sperm were obtained by washing minced epididimydes on a 125 μm stainless steel grid.

togens (but not progesterone itself) exert "synandrogenic" effects by interacting with kidney androgen receptors at sites other than the androgen binding site. Galena *et al.*[10] found more uptake of progesterone than of androgens in testicular germ cell fractions and also observed competition for [$^3$H]-progesterone uptake by testosterone.

The following evidence is consistent with the hypothesis that some nuclear exchange activity is located in the Sertoli cells of the seminiferous tubules. [A]. Measurable binding/mg DNA remains in testes and seminiferous tubules from adult rats hypophysectomized for 6–70 days (Table 3). The binding is 2–10 fold greater in tissue preincubated with testosterone than in tissue incubated without hormone (Table 3). The exchange specificity does not change with duration of hypophysectomy (data not shown). [B]. Measurable binding occurs/mg DNA in testes from immature rats hypophysectomized for 20–28 days (Table 3). [C]. Specific binding is still present in tubules freed of interstitial cells by extensive washing and minced to remove the bulk of the germinal cells [12]. [D]. Finally, Sertoli cells isolated from rat testes show specific exchange per testis and per mg DNA equal to or greater than that in corresponding whole testes from long term hypophysectomized rats (Table 3).

Nuclear exchange activity appears also to be present in the germ cells. Substantial exchange activity with the same specificity as observed for whole testis was found in germ cells isolated from intact adult rat testes [12]. This activity was present in highest concentration in the more mature cell types (Table 4) and was enriched in the head region of the elongated spermatids as shown by the persistence of exchange after sonication which disrupts cells, flagella, and less stable nuclei. Since the androgen dependent processes in germ cell maturation include the meiotic division [24], one might expect to find receptors in the spermatocytes. The contamination of the pachytene fraction with spermatids was sufficiently high (Table 5) as to preclude any statements about the existence of nuclear exchange in the spermatocytes themselves. The role of the exchange activity in spermatid maturation remains to be investigated. The presence of a high level of exchange activity in epididymal spermatozoa (Fig. 2) is consistent with the finding in the testis and suggests a possible direct interaction of androgen with spermatozoa themselves.

At this point, it is not possible to equate nuclear exchange activity in the testis with the presence of nuclear-bound receptor. The fact that preincubation with exogenous steroid results in significantly greater exchange levels under conditions where the uptake of 0·4M KCl extractable labeled androgen is increased (Sanborn, unpublished observations) is certainly consistent with this interpretation. The present attempts also cannot be considered as quantitative estimates of exchange activity since experimental aspects such as the stability of receptors during preincubation and the saturability of the exchange phenomenon are still being evaluated. The data do, however, give useful information, on a relative scale, about the ability of testes components to exchange nuclear bound androgen.

The data reported here are also consistent with the findings of several workers concerning androgen receptors studied by direct labeling methods. Hansson *et al.*[7] reported that the intracellular androgen receptors were of tubular origin and persisted for up to 24 days following hypophysectomy in immature rats. Mulder *et al.*[8, 9] found testicular nuclear uptake into 0·4M KCl extractable material 3–20 days after hypophysectomy in adult animals. These data are consistent with the hypothesis that the receptors are located in the Sertoli cells or young germ cells. Galena *et al.* [10], on the other hand, reported macromolecules which bound androgens in non-flagellate germ cells from intact adult rats. They also found androgen uptake by epididymal sperm, a finding supported by the data in this paper but not by those of others [25, 26].

In the light of all of this evidence, it is highly probable that androgen receptors exist in both Sertoli cells and in components of the germ cell fraction. The study of the location and control of androgen responsiveness, *i.e.* androgen receptor concentration, will be of great importance in understanding hormonal influences in the testis.

*Acknowledgements*—The authors gratefully acknowledge the assistance of Dr. A. K. Chowdhury in the morphological examination of the germ cell nuclei and thank him and Dr. J. S. H. Elkington for helpful discussions. They also thank Ms. H. Kuo, C. Williamson, R. Rodriguez and

P. Trostle for valuable assistance in various aspects of this work. This work was supported by Contract NO1-HD3-2782, NICHHD (BMS), Grant NIH-1-PO1-HDO8338 (ES), and grants from the Population Council and NCI, Grant CA06294 (MLM).

## REFERENCES

1. Hansson V., Trygstad O., French F. S., McLean W. S., Smith A. A., Tindall D. J., Weddington S. C., Petrusz P., Nayfeh S. N. and Ritzen E. M.: *Nature Lond.* **250** (1974) 387–391.
2. Vernon R. G., Kopec B. and Fritz I. B.: *Molec. cell. Endocr.* **1** (1975) 167–187.
3. Steinberger E., Steinberger A. and Sanborn B.: In *Physiology and Genetics of Reproduction* Part A, (Edited by E. M. Coutinho and F. Fuchs). Plenum Press, New York (1974) pp. 163–181.
4. Sanborn B. M., Elkington J. S. H., Tcholakian R. K., Chowdhury M. and Steinberger E.: *Endocrinology* **96** (1975) 304–312.
5. Elkington J. S. H., Sanborn B. M. and Steinberger E.: *Molec. cell. Endocr.* **2** (1975) 157–170.
6. Steinberger A., Heindel J. J., Lindsey J. N., Elkington J. S. H., Sanborn B. M. and Steinberger E.: *Endocr. Research Commun.* (1975) in press.
7. Hansson V., McLean W. S., Smith A. A., Tindall D. J., Weddington S. C., Nayfeh S. N. and French F. S.: *Steroids* **23** (1974) 823–832.
8. Mulder E., Peters M. J., van Beurden W. M. O. and van der Molen H. J.: *FEBS Lett.* **47** (1974) 209–211.
9. Mulder E., Peters M. J., de Vries J. and van der Molen H. J.: *Molec. cell. Endocr.* **2** (1975) 171–182.
10. Galena H. J., Pillai A. K. and Terner C.: *J. Endocr.* **63** (1974) 223–237.
11. Anderson J., Clark J. H. and Peck E. J. Jr.: *Biochem. J.* **126** (1972) 561–567.
12. Sanborn B. M. and Steinberger E.: *Endocr. Research Commun.* 1975, in press.
13. Burton K.: In *Methods in Enzymology* (Edited by L. Grossman and K. Moldave). Academic Press, New York, Vol. 13 (1968) pp. 163–166.
14. Steinberger A.: In *Methods in Enzymology* (Edited by B. O. O'Malley and J. G. Hardman). Academic Press, New York, Vol. 39, Part D (1975) pp. 283–296.
15. Grabske R. J., Lake S., Gladhill B. L. and Meistrich M. L.: *Cell. Physiol.* **86** (1975) 177–190.
16. Steinberger A., Heindel J. J., Lindsey J. N., Elkington J. S. H., Sanborn B. M. and Steinberger E.: Second Annual NIH Workshop on the Testis. Chapel Hill, N.C., April 2–4 (1975) in press.
17. Meistrich M. L., Bruce W. R. and Clermont Y.: *Expl. Cell Res.* **79** (1973) 213–227.
18. Harris M. E. and Bartke A.: *Endocrinology* **95** (1974) 701–706.
19. Setchell B. P. and Waites G. M. H.: In *Handbook of Physiology* (Edited by D. W. Hamilton and R. O. Greep). American Physiological Society, Washington D.C., Vol. 5 (1975) pp. 143–172.
20. Ludwig D. J.: *Endocrinology* **46** (1950) 453–481.
21. Fang S., Anderson K. M. and Liao S.: *J. biol. Chem.* **244** (1969) 6584–6595.
22. Mangan F. R. and Mainwaring W. I. P.: *Gynec. Invest.* **2** (1971/1972) 300–304.
23. Mowszowicz I., Bieber D. E., Chung K. W., Bullock L. P. and Bardin C. W.: *Endocrinology* **95** (1974) 1589–1599.
24. Steinberger E.: *Physiol. Rev.* **51** (1971) 1–22.
25. Blaquier J. A.: *Biochem. biophys. Res. Commun.* **45** (1971) 1076–1082.
26. Tindall D. J., French F. S. and Nayfeh S. N.: *Biochem. biophys. Res. Commun.* **49** (1972) 1391–1397.
27. Platz R. D., Grimes S. R., Meistrich M. L. and Hnilica L. S.: *J. biol. Chem.* **250** (1975) 5791–5800.