Journal of Steroid Biochemistry, Vol. 10, pp. 669 to 675
Pergamon Press Ltd 1979. Printed in Great Britain

STEROIDOGENESIS IN HCG-RESPONSIVE LEYDIG CELL TUMOR VARIANTS*

A. LACROIX,† M. ASCOLI, D. PUETT,‡
and T. J. MCKENNA§
Departments of Medicine and Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232, U.S.A.

(*Received* 8 *July* 1978)

SUMMARY

Several steroids of the androgen biosynthetic pathway were measured in cell suspensions of two tumor variants originating from the gonadotropin-responsive murine M5480 Leydig cell tumor. In M5480A, the androgen-producing variant, testosterone and dihydrotestosterone were the main steroids produced basally; addition to HCG to the incubation medium resulted in increased testosterone but no change in dihydrotestosterone levels and the main steroid accumulating in the medium was progesterone. In M5480P, the progesterone-producing variant, progesterone and androstenedione were the main steroids produced, while testosterone and dihydrotestosterone production were negligible both basally and following stimulation with HCG. Progesterone levels increased more in M5480P than in M5480A with HCG stimulation. The steroid profiles observed in the M5480 tumor variants were contrasted with that of Leydig cell-enriched suspensions of normal mouse testes. Comparison of the ratios of progesterone to 17α-hydroxyprogesterone and of androstenedione to testosterone, in the two tumor variants with each other and with normal mouse testes, suggests the following alterations in steroidogenic enzymes activities: (a) a partial decrease in 17α-hydroxylase activity more marked in M5480P than in M5480A, (b) a decrease in 17β-hydroxysteroid dehydrogenase activity in M5480P. Finally, in the M5480A tumor variant, the absence of an increase in dihydrotestosterone levels with HCG stimulation suggests a decrease in 5α-reductase activity.

INTRODUCTION

The M5480 Leydig cell tumor, of spontaneous murine origin, was adapted for serial transplantation by Dr. W. F. Dunning of the Papanicolau Cancer Research Institute, Miami, FL. This tumor was shown to respond to LH stimulation *in vitro* by increased steroidogenesis [1]. The original tumors produced mainly testosterone and smaller amounts of progesterone; however, Moyle *et al* noted that, after several generations, the tumors produced mainly progesterone and 20α-dihydroprogesterone and only small quantities of testosterone [2]. Working with the same original tumor, in *in vivo* studies, Neaves showed that tumors maintained the testosterone levels of castrated recipient mice within normal levels, and that repeated injections of HCG produced a 5- to 6-fold increase in plasma testosterone [3]. Recently, we have identified two variants of the original M5480 tumor which differ in their steroid producing properties *in vitro* [4]. M5480A, the androgen-producing variant, was found to produce equal amounts of progesterone and testosterone under basal conditions; HCG stimulated the production of both steroids but relatively more of progesterone. M5480P, the progesterone-producing variant, was shown to produce mainly progesterone with very little testosterone both in the presence or in the absence of HCG.

The aim of the present study was to further characterize basal and gonadotropin-stimulated steroid biosynthesis in these two tumor variants and compare the results with similar studies in normal mouse Leydig cells. Several intermediate steroids of the androgen biosynthetic pathway were measured in cell suspensions incubated with and without HCG. Examination of the steroid profiles suggests decreased 17α-hydroxylase activity in both tumor variants, decreased 17β-hydroxysteroid dehydrogenase activity in M5480P, and impaired 5α-reductase activity after HCG stimulation in M5480A.

---

* This study was presented in part at the 60th Annual Meeting of the Endocrine Society, Miami, Florida, 1978.

† To whom reprint requests should be addressed. Present address: Medicine Branch, National Cancer Institute, National Institutes of Health, Building 10, Room 6B04, Bethesda, MD 20014, U.S.A.

‡ Recipient of a Research Career Development Award (No. AM00055).

§ Present address: St. Vincent's Hospital, Elm Park, Dublin 4, Ireland.

The following abbreviations and trivial names have been used: pregnenolone = 3β-hydroxy-5-pregnen-20-one; progesterone = 4-pregnen-3,20-dione; 17-hydroxyprogesterone = 17-hydroxy-4-pregnen-3-one; androstenedione = 4-androstene-3,17-dione; testosterone = 17β-hydroxy-4-androsten-3-one; dihydrotestosterone = 17β-hydroxy-5α-androstan-3-one; 20α-dihydroprogesterone = 20α-hydroxy-4-pregnen-3-one.

EXPERIMENTAL

Tumors. Generation 120 of M5480P was obtained from the Papanicolau Cancer Research Institute (Miami, Florida). Generation 176 of M5480A was generously provided by Dr. William B. Neaves (University of Texas Health Science Center at Dallas) who previously obtained generation 64 of the M5480 tumor from Dr. Dunning in 1973, and maintained it by serial transplantation every 14 days. In our laboratory, the tumors were transplanted either every 14 days (M5480A) or every 18 days (M5480P) into 7–9 week-old male C57B1/6J mice (Jackson Laboratory, Bar Harbor, Maine) by the method of Neaves[5].

Cell suspensions from Leydig cell tumors. Cell suspensions were prepared from 12–14 day old M5480A or 16–18 day old M5480P tumors as previously described [4]. Briefly, the cells were dispersed by forcing the minced tumors through a 30 mesh stainless steel grid into a petri dish containing Medium 199. After removal of the erythrocytes by hypotonic shock, the tumor cells were suspended in Medium 199 at final dilution of 2.5×10^6 cells/ml. Some tumors from generation 5–22 (M5480A) and 5–18 (M5480P) in our laboratory were used for the cell suspensions.

Cell suspensions from normal mouse testes. In each experiment, the testes of four adult C57B1/6J mice were removed after the animals were sacrificed by cervical dislocation. The testes were then dispersed as described by Van Damme *et al.*[6] except that Medium 199 was used for the cell suspensions. This preparation does not contain a homogeneous Leydig cell population, and thus no attempt was made to obtain cell numbers. Each ml of medium contained the cells dispersed from 50 mg of testicular tissue. In all cases, Medium 199 was supplemented with 1 mg/ml bovine serum albumin and 20 μg/ml gentamycin.

Incubation conditions. Two ml of normal or tumor cell suspensions were placed in 20 ml polyethylene vials; 100 μl of 10 mM sodium phosphate buffer, 0.15 M NaCl, 1 mg/ml bovine serum albumin, pH 7.4, was added to duplicate basal samples and 20 ng of purified HCG [7] in 100 μl of the same buffer was added to duplicate samples (HCG stimulated). This final concentration of 10 ng/ml of HCG has previously been shown to produce maximal stimulation of steroidogenesis in both Leydig cell tumor variants [4]. Incubations were performed at 37°C, under 95% O_2–5% CO_2, in a shaking water bath (60–80 rev./min), for either 60 or 120 min. At the end of the incubation, the contents of the vials were centrifuged at 1200 *g* for 10 min and the supernatants were stored frozen until steroid measurements were performed.

Steroid measurements. The following steroids were measured in the incubation medium using preparative chromatography and previously described specific radioimmunoassays: pregnenolone [8], progesterone and 17α-hydroxyprogesterone [9], androstenedione [10], testosterone and dihydrotestosterone [11]. The results reported represent the mean of values from duplicate incubation vials and are expressed in ng/ml of medium/60 min incubation. The steroid production increases linearly during the periods of incubations utilized [4], and thus allows this normalization of the data. Values under the sensitivities of the assays were assigned the lowest detectable value (0.1 or 0.2 ng/ml) for purposes of statistical analysis. These were performed using Student's paired *t*-test to compared steroid levels between basal and HCG stimulated cell suspensions; Student's *t*-test was used for other analysis.

RESULTS

Steroid levels in cell suspensions of M5480 tumor variants

A total of 7 experiments were performed using the M5480A tumors. The steroid levels in the cell suspension medium after incubation with or without HCG are given for each individual experiment in Table 1. When the M5480A cells were incubated under basal conditions, the steroid present in larger amount in the medium was testosterone; smaller amounts of dihydrotestosterone and progesterone were also present. Stimulation of the cells with HCG produced a significant increase in all steroids measured except pregnenolone and dihydrotestosterone. However, the main steroid measured in the medium after HCG stimulation was progesterone which increased 13-fold (mean), while testosterone increased only 2-fold (mean).

A total of 6 experiments were performed using the M5480P tumors and the results are presented in Table 2. After incubation in the basal state, the main steroids measured in M5480P were progesterone and androstenedione. The levels of testosterone and dihydrotestosterone were generally very low. Stimulation of the cells with HCG produced a significant increase of all steroids measured except 17α-hydroxyprogesterone. The main steroid accumulating in the medium with HCG stimulation was progesterone which increased about 50-fold (mean).

The mean level of each of the steroids measured in the M5480A and in the M5480P cell suspensions are compared in Fig. 1. It can be seen that progesterone levels were significantly higher after HCG stimulation in M5480P than in M5480A. Mean androstenedione levels were higher in M5480P than in M5480A, both basally and with HCG stimulation. Finally, testosterone and dihydrotestosterone levels were higher in M5480A than in M5480P under basal conditions and when stimulated by HCG.

Steroid levels in normal mouse testes cell suspensions

The steroid profile observed in the M5480 tumors was compared with that of normal mouse testes to ascertain if qualitative differences existed. The results of four different experiments with normal mouse testes are summarized in Table 3. Basally, testoster-

Table 1. Steroid levels in cell suspensions of the androgen-producing variant M5480A Leydig cell tumor incubated with or without (Basal) HCG†

	Pregnenolone		Progesterone		17-Hydroxy progesterone		Androstenedione		Testosterone		Dihydrotestosterone	
	Basal	HCG	Basal	HCG	Basal	HCG	Basal	HCG	Basal	HCG	Basal	HCG
1.‡	<0.1	7.1	1.5	27.0	1.1	5.1	0.9	3.4	6.3	13.5	5.8	4.5
2.	1.1	1.1	6.2	43.0	1.3	3.2	0.8	1.3	4.3	9.5	4.2	3.5
3.	0.7	1.8	0.8	36.8	0.3	4.1	1.2	4.0	5.4	15.4	0.9	2.9
4.	0.7	1.2	0.7	17.0	<0.2	1.3	0.4	1.4	2.6	5.0	0.4	0.6
5.	0.9	2.1	1.8	11.6	0.8	1.6	0.4	1.5	3.2	5.1	1.3	1.5
6.	0.2	0.8	4.0	44.5	1.4	6.7	0.8	1.0	10.3	14.7	4.1	4.3
7.	0.4	1.0	0.5	4.8	0.5	2.1	0.6	1.0	2.4	5.6	0.9	0.7
Mean ± S.E.M.	0.6 0.1	2.2 0.8	2.2 0.8	26.4 5.9	0.8 0.2	3.4 0.8	0.7 0.1	1.9 0.5	4.9 1.0	9.8 1.8	2.5 0.8	2.6 0.6
	N.S.		**		**		*		**		N.S.	

* $P < 0.025$.
** $P < 0.005$.
† HCG: 10 NG/ML. Results are expressed in ng/ml of medium/60 min of incubation with 2.5×10^6 cells.
‡ Individual experiment number.

Table 2. Steroid levels in cell suspensions of the progesterone-producing variant M5480P Leydig cell tumor incubated with or without (Basal) HCG†

	Pregnenolone		Progesterone		17-Hydroxy progesterone		Androstenedione		Testosterone		Dihydrotestosterone	
	Basal	HCG	Basal	HCG	Basal	HCG	Basal	HCG	Basal	HCG	Basal	HCG
1.‡	0.8	1.1	1.6	150.0	0.6	2.9	12.6	20.1	0.8	1.2	0.7	0.9
2.	—	—	1.9	85.0	<0.1	0.5	1.0	1.9	<0.1	0.2	0.1	<0.1
3.	0.2	1.1	6.2	114.0	0.6	0.7	1.3	1.7	<0.1	0.1	<0.1	<0.1
4.	0.8	1.5	0.9	106.0	1.3	4.2	9.2	17.0	0.9	2.5	0.3	0.5
5.	0.5	1.5	2.1	153.0	1.2	12.0	12.8	22.0	1.7	3.2	0.5	0.6
6.	<0.1	1.0	0.3	62.0	0.3	1.6	0.3	0.9	0.3	0.4	—	—
Mean ± S.E.M.	0.5	1.2	2.2	111.7	0.7	3.7	6.2	10.6	0.7	1.3	0.3	0.4
	0.1	0.1	0.8	14.5	0.2	1.8	2.4	4.1	0.3	0.5	0.1	0.2
		**		***		N.S		**		*		*

* $P < 0.05$.
** $P < 0.025$.
*** $P < 0.001$.
† HCG: 10 NG/ML. Results are expressed in ng/ml of medium/60 min of incubation with 2.5×10^6 cells.
‡ Individual experiment number.

Table 3. Steroid levels in cell suspensions of normal mouse testes incubated with or without (basal) HCG†

	Pregnenolone		Progesterone		17-Hydroxy progesterone		Androstenedione		Testosterone		Dihydrotestosterone	
	Basal	HCG	Basal	HCG	Basal	HCG	Basal	HCG	Basal	HCG	Basal	HCG
1.‡	0.4	0.3	0.3	0.5	0.3	1.4	0.4	34.3	4.3	152.5	—	—
2.	0.4	0.5	<0.2	0.4	<0.2	0.6	0.3	20.0	1.8	62.5	0.4	11.3
3.	<0.1	<0.1	<0.2	0.4	<0.2	0.6	0.4	19.3	1.8	47.3	0.4	8.2
4.	<0.1	<0.1	<0.2	0.3	<0.2	0.7	0.3	35.6	1.3	56.3	0.7	14.2
Mean ± S.E.M.	0.3	0.3	0.2	0.4	0.2	0.8	0.4	27.3	2.3	79.6	0.5	11.2
	0.1	0.1	0.1	0.1	0.1	0.2	0.1	4.4	0.6	24.5	0.1	0.7
		N.S.		*		*		**		*		**

* $P < 0.025$.
** $P < 0.005$.
† HCG: 10 NG/ML. Results are expressed in NG/ML of medium/60 min of incubation with cells dispersed from 50 mg of testes.
‡ Individual experiment number.

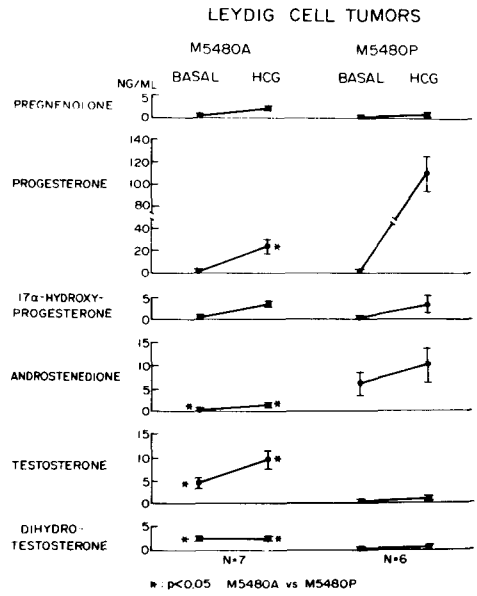

Fig. 1. Comparison of steroid levels (mean ± S.E.M.) in the androgen-producing variant (M5480A) versus in the progesterone-producing variant (M5480P) Leydig cell tumor; cell suspensions were incubated either in the absence (basal) or in the presence of HCG, 10 ng/ml. The results are expressed in ng of steroids/ml of medium/60 min incubation. N indicates the number of experiments in each group.

one was measured in small quantities in the cell suspension medium and the precursor levels were low. The addition of HCG during the incubation led to an accumulation mainly of testosterone, but also of androstenedione and dihydrotestosterone. In contrast with the tumor cells, HCG stimulation did not produce an accumulation of progesterone in the cell suspension medium of normal mouse testes.

Since no attempt was made to quantitate the number of Leydig cells in the suspensions of normal mouse testes, quantitative comparisons cannot be made with the steroid levels measured in the tumor cell suspensions. Qualitative comparisons can be made however by examination of the ratios between the steroids of the biosynthetic pathway in the three different cell preparations.

Progesterone/17-hydroxyprogesterone ratios in M5480 tumor variants and in normal mouse testes

The individual ratios of progesterone/17α-hydroxyprogesterone are shown in Fig. 2 for each experiment in the three different grroups of cell suspensions. Basally, the ratios are similar in the two tumor variants being greater than 1; in the normal mouse testes, the steroid levels were detectable only in one experiment with a ratio of 1. Following HCG stimulation, the progesterone/17α-hydroxyprogesterone ratios increased further in both tumor variants but more in M5480P tumor cells than in the M5480A tumor cells (mean ratios are 104.8 vs 8.1). In contrast, in the normal mouse testes, the progesterone/17α-hydroxyprogesterone ratio fell to less than 1 after HCG stimulation. Thus, qualitatively, the conversion of progesterone to 17α-hydroxyprogesterone is impaired in both M5480 tumor variants.

Androstenedione/testosterone ratios in M5480 tumor variants and in normal mouse testes

The ratios of androstenedione/testosterone, both basally and with HCG stimulation, were less than 1 in M5480A and in the normal mouse testes (Fig. 3). In contrast, in M5480P, both with and without HCG, the androstenedione/testosterone mean ratios were approximately 10. Thus, qualitatively, the

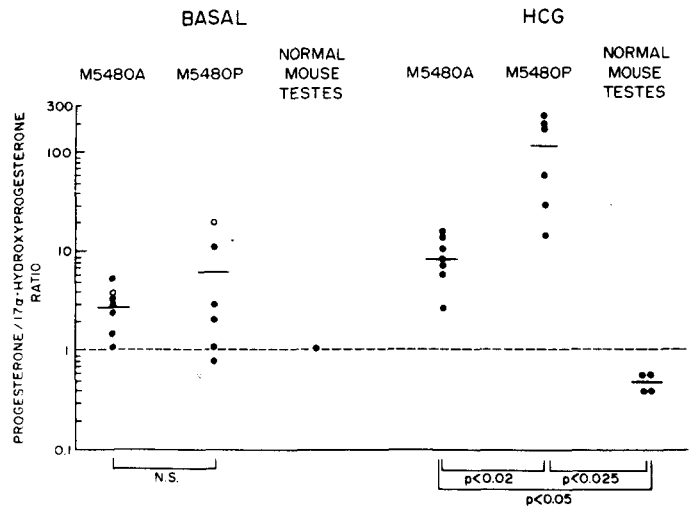

Fig. 2. Ratios of progesterone/17-hydroxyprogesterone in cell suspension medium of M5480A, of M5480P and of normal mouse testes incubated either with or without (basal) HCG, 10 ng/ml. The closed circles represent the ratios from individual experiments; the open circles indicate the minimum ratio calculated from experiments where the 17-hydroxyprogesterone level was <0.2 ng/ml. The bars indicate the mean ratios for each group. Note the semilogarithmic scale.

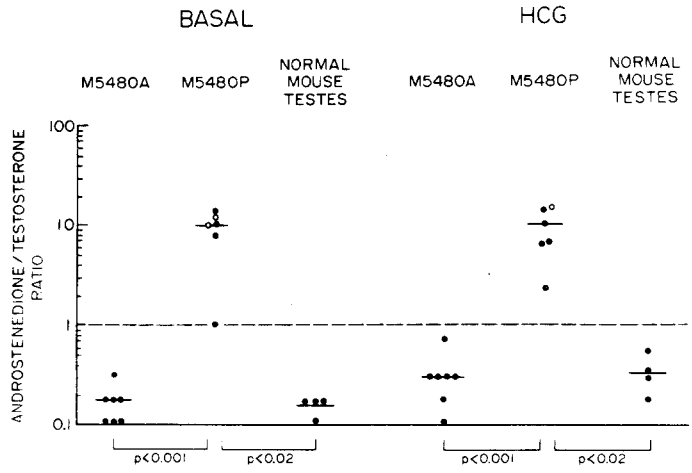

Fig. 3. Ratios of androstenedione/testosterone in cell suspension medium of M5480A, of M5480P, and of normal mouse testes incubated either with or without (basal) HCG, 10 ng/ml. The closed circles represent the ratios from individual experiments; the open circles indicate the minimum ratios calculated from experiments where the testosterone levels were <0.1 ng/ml. The bars indicate the mean ratios for each group. Note the semilogarithmic scale.

conversion of androstenedione to testosterone appears to be impaired in M5480P as compared to normal mouse testes and to M5480A tumors.

DISCUSSION

Our studies clearly show that the steroids produced by the two tumor variants of the same original M5480 Leydig cell tumor differ considerably. Using preparative chromatography and specific radioimmunoassays, this study confirms our previous report concerning the production of progesterone and testosterone in the M5480 tumor variants [4]. The most striking difference in the basal state is that the M5480P tumors produce mainly androstenedione while the M5480A tumors produce mainly testosterone. Dihydrotestosterone levels in the two tumor variants were found to parallel the testosterone levels in the basal state.

The tumors have been shown to contain specific LH and HCG receptors and to respond by increased steroidogenesis following gonadotropin stimulation [4, 12]. We have confirmed this stimulatory effect on progesterone and testosterone production and have extended it to other intermediates in the androgen biosynthetic pathway. The HCG stimulation accentuated further the differences in androstenedione and testosterone production observed between the tumor variants in the basal state; furthermore, following HCG stimulation, progesterone production predominated in both tumor variants, but was quantitatively greater in M5480P than in M5480A. Our findings in normal mouse testes were very similar to those of de la Torre *et al.*[13] as testosterone was the main steroid secreted both basally and with HCG stimulation, whereas progesterone levels remained low.

Examination of the progesterone/17α-hydroxyprogesterone ratios in the two tumor variants and in the normal mouse testes is compatible with a partial deficiency in 17α-hydroxylase activity in both tumor variants as compared to the normal mouse testes; this defect appears to be more severe in M5480P than in M5480A. Additional alterations in enzyme activity are also suggested by comparison of the androstenedione/testosterone ratios in the three cell preparations. The ratio of androstenedione to testosterone was equal or greater than 1 in M5480P whereas, in M5480A and normal mouse testes, it was smaller than 1. In M5480A as in the normal mouse testes, the conversion of androstenedione to testosterone appears to occur readily. In contrast, in M5480P, the accumulation of androstenedione, both basally and with HCG, suggests a decrease in 17β-hydroxysteroid dehydrogenase activity.

It is also of interest that dihydrotestosterone levels did not consistently increase after HCG stimulation in the tumor variants whereas it did in normal mouse testes in this study as well as in that of de la Torre *et al.*[13]. This finding suggests decreased 5α-reductase enzyme activity in the tumor cells following HCG stimulation. It is noteworthy that following HCG stimulation the progesterone levels increased to mean levels of 26.4 and 111.7 ng/ml respectively in M5480A and in M5480P but only to a mean level of 0.4 ng/ml in the normal mouse testes (Tables 1 and 3); since progesterone inhibits 5α-reductase activity [14], it is possible that following HCG stimulation the accumulation of progesterone inhibits 5α-reductase activity. In M5480P the limited substrate (testosterone) availability for the 5α-reductase could be responsible for the low dihydrotestosterone levels.

Alterations of the activity of steroidogenic enzymes have previously been observed in several steroid sec-

reting tumors. Accumulation of progesterone was noted in mouse interstitial cell tumors in tissue culture [15] and in cell suspensions [16]; accumulation of precursor steroids has also been documented commonly in adrenocortical cancer in mice [17] and in man [18, 19]. Accumulation of androstenedione with less testosterone production was noted in a transplantable mouse luteoma, but this pattern is normally found in ovarian stroma [20]. The loss in certain enzymes activity is often accompanied by a gain in others as recently reviewed [21]. The appearance of two variants which originate from the same initial tumor but differ by their steroid secretion is interesting, and similar observations were previously made in a mouse adrenal tumor after several transplantations [22]. The growth rate of the M5480 tumors also appears to have changed: initially Moyle *et al.*[2] reported that the tumors weighed 0.5–4.0 gm within 2–6 months of transplantation, whereas in our laboratory the tumors weighed 2 gm by 14 days (M5480A) and 1.4 gm by 18 days (M5480P) [4].

In summary, the steroid producing characteristics of two variants of M5480 Leydig cell tumors have been examined further. These studies suggest that in the androgen-producing variant 17α-hydroxylase activity is decreased, while in the progesterone-producing variant both 17α-hydroxylase and 17β-hydroxysteroid dehydrogenase activities are decreased. Also a functional decrease of 5α-reductase activity appears to be present in the androgen-producing variant under HCG stimulation.

Acknowledgements—It is a pleasure to thank Susan L. Douglas, Rebecca B. Miller, and Patricia L. Nix for their expert technical assistance. This work was supported by the following grants-in-aid from the USPHS: 5-R01-AM-05318, P30-HD-05797, AM15838 and CA23603. Partial support was also provided by the Dreyfus Foundation and the Vanderbilt University Research Council. A.L. was supported by a fellowship from the Medical Research Council of Canada.

REFERENCES

1. Moyle W. R. and Armstrong D. T.: Stimulation of testosterone biosynthesis by luteinizing hormone in transplantable mouse Leydig cell tumors. *Steroids* **15** (1970) 681–693.
2. Moyle W. R., Moudgal N. R. and Greep R. O.: Cessation of steroidogenesis in Leydig cell tumors after removal of luteinizing hormone and adenosine cyclic 3',5'-monophosphate. *J. biol. Chem.* **246** (1971) 4978–4982.
3. Neaves W. B.: Gonadotropin-induced proliferation of endoplasmic reticulum in an androgenic tumor and its relation to elevated plasma testosterone levels. *Cancer Res.* **35** (1975) 2663–2669.
4. Ascoli M. and Puett D.: Gonadotropin binding and stimulation of steroidogenesis in Leydig tumor cells. *Proc. natn. Acad. Sci. U.S.A.* **75** (1978) 99–102.
5. Neaves W. B.: Growth and composition of a transplantable murine Leydig cell tumor. *J. natn. Cancer Inst.* **55** (1975) 623–631.
6. Van Damme M.-P., Robertson D. M. and Diczfalusy E.: An improved *in vitro* bioassay method for measuring luteinizing hormone (LH) activity using mouse Leydig cell preparations. *Acta endocr. Corene.* **77** (1974) 655–671.
7. Holladay L. A. and Puett D.: Gonadotrophin and subunit conformation. *Archs. biochem. Biophys.* **171** (1975) 708–720.
8. DiPietro D. L., Brown R. D. and Strott C. A.: A pregnenolone radioimmunoassay utilizing a new fractionation technique for sheep antiserum. *J. clin. Endocr. Metab.* **35** (1972) 729–735.
9. Abraham G. E., Hopper K., Tulchinsky D., Swerdloff R. S. and Odell W. D.: Simultaneous measurement of plasma progesterone, 17-hydroxyprogesterone & estradiol-17β by radioimmunoassay. *Analyt. Lett.* **4** (1971) 325–335.
10. Abraham G. E. and Chakmakjian Z. H.: Serum steroid levels during the menstrual cycle in a bilaterally adrenalectomized woman. *J. clin. Endocr. Metab.* **37** (1973) 581–587.
11. Coyotupa J., Parlow A. F. and Abraham G. E.: Simultaneous radioimmunoassay of plasma testosterone and dihydrotestosterone. *Analys. Lett.* **5** (1972) 329–340.
12. Moudgal N. R., Moyle W. R. and Greep R. O.: Specific binding of luteinizing hormone to Leydig tumor cells. *J. biol. Chem.* **246** (1971) 4983–4986.
13. de la Torre B., Benagiano G. and Diczfalusy E.: Patyways of testosterone synthesis in decapsulated testes of mice. *Acta endocr., Corene* **81** (1976) 170–184.
14. Voigt W., Fernandez E. P. and Hsia S. L.: Transformation of testosterone into 17β-hydroxy-5α-androstan-3-one by microsomal preparations of human skin. *J. biol. Chem.* **245** (1970) 5594–5599.
15. Shin S.-I.: Studies on interstitial cells in tissue culture: Steroid biosynthesis in monolayers of mouse testicular interstitial cells. *Endocrinology* **81** (1967) 440–448.
16. Lucis O. J. and Lucis R.: Metabolism of steroids by transplantable mouse interstitial cell tumor. *Cancer Res.* **29** (1969) 1647–1652.
17. Dominguez O. V. and Huseby R. A.: *In vivo* and *in vitro* studies of steroid production by a luteoma and an adrenal cortical carcinoma in mice. *Endocrinology* **84** (1969) 1039–1047.
18. Lipsett M. B. and Wilson H.: Adrenocortical cancer: Steroid biosynthesis and metabolism evaluated by urinary metabolites. *J. clin. Endocr. Metab.* **22** (1962) 906–915.
19. McKenna T. J., Miller, R. B. and Liddle G. W.: Plasma pregnenolone and 17-OH-pregnenolone in patients with adrenal tumors, ACTH excess, or idiopathic hirsutism. *J. clin. Endocr. Metab.* **44** (1977) 231–236.
20. Roth L. M., Sternberg W. H., Huseby R. A., MacPhee A. A., Cole F. E. and Rice B. F.: Transplantable luteoma of the mouse. An ultrastructural and biochemical study. *Lab. Invest.* **27** (1972) 115–122.
21. Moyle W. R. and Greep R. O.: Steroid-secreting tumors as models in endocrinology. In *Hormones and Cancer* (Edited by K. W. McKerns). Academic Press, New York (1974) p. 329.
22. Block E. and Cohen A. I.: Steroid production *in vitro* by normal and adrenal tumor-bearing male mice. *J. natl. Cancer Inst.* **24** (1960) 97–107.