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COMPARATIVE EFFECTS OF MAMMALIAN GONADOTROPINS ON ANDROGEN FORMATION *IN VITRO* FROM MOUSE TESTIS INTERSTITIAL CELLS

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John Yuh-Lin Yu, Ling-Mei Wang and Mei-Lung Fei (1984) Comparative effects of mammalian gonadotropins on androgen formation *in vitro* from mouse testis interstitial cells. *Bull. Inst. Zool., Academia Sinica* **23**(1): 81-91. The purpose of this study was 1) to establish an optimized and validated *in vitro* bioassay of luteinizing hormone (LH) using dispersed mouse testis interstitial cell androgen production system; and 2) to compare the androgen formation activity by such assay of LH's/gonadotropins (GTH's) from various mammalian species. The testis interstitial cells from 7-8-wk old mice were prepared by mechanical dispersion technique. The effects on androgen formation of xanthine and continuous aeration during incubation as well as the variations in the cell numbers and incubation volume were compared. The standard incubation procedure was performed as follows: a total incubation volume of 0.22 ml containing 0.50×10^6 cells/tube incubated in Medium 199-xathine-heparin (pH, 7.40) with shaking at 100 cycles/min at 34°C for 4 hrs under continuous aeration of 95% O_2-5% CO_2. The androgen produced was assayed by radioimmunoassay using anti-testosterone serum. The mouse testis assay was highly sensitive and all purified pituitary LH's (ovine LH-S22, porcine LH-LER 786-3, bovine LH-LER 1716-2, rat LH-I5, and rat LH-RP1) and placental gonadotropins (HCG and PMSG) produced parallel dose-related androgen curves. The relative potencies of LH's assayed by the dispersed mouse testis interstitial cell system are generally in correspondence to the indicated biological potencies as previously determined *in vivo* by ovarian ascorbic acid depletion and ventral prostate weight assays. Ovine FSH-13 and porcine FSH-P1 also evoked dose-related androgen formation when higher amounts were assayed; this was likely due to the presence of minute amount of LH in the FSH preparations.

The androgen production activity of the testis interstitial cells from both rats and mice were also compared. The interstital cells of rats prepared by mechanical dispersion essentially did not produce androgen upon stimulation with ovine or porcine LH. In contrast, the mouse testis interstitial cells prepared by either collagenase or mechanical dispersion, were very responsive to LH stimulation in androgen formation; such activity was considerably higher than that of rat testis cells prepared by collagenase dispersion on basis of equal numbers of cells. The mouse assay thus provides a more convenient choice in preparation of testis interstitial cells for *in vitro* bioassay of LH.

The development of techniques in isolating and dispersing the interstitial cells from testes has been achieved in various species for *in vitro* studies of steroidogenesis in individual species *per se* or for use as a bioassay of gonadotropins from heterologous or homologous species (Callard and Ryan, 1977; Dufau

et al., 1976; Garfink *et al.*, 1978; Licht *et al.*, 1977; Maung and Follet, 1977; Van Damme *et al.*, 1974; Yu *et al.*, 1981). Among these animals, rat system has been studied most extensively, and has been used recently for the *in vitro* bioassay of LH's either in purified form or present in the blood from various mammalian species (Dufau *et al.*, 1974, 1976, 1977; Farmer *et al.*, 1977; Mendelson *et al.*, 1975; Moger, 1979; Solano *et al.*, 1979). In contrast, the mouse testis system has been relatively less studied. Previous investigations indicate that the dispersed mouse interstitial cells are also highly responsive to LH's from the rat and the primates (Ellinwood *et al.*, 1980; Steiner *et al.*, 1980; Van Damme *et al.*, 1974; Yu *et al.*, 1981). However, the androgen formation activities of LH's from other mammalian species were little investigated by the dispersed mouse testis assay.

The purpose of the present study was thus 1) to establish an optimized and validated *in vitro* bioassay of LH's using mouse testis interstitial cell androgen production system; and 2) to compare the androgen formation activity by such assay of LH's/GTH's from a variety of mammalian species. The information obtained from this study are useful for phylogenic comparison of intrinsic property of LH's, and for *in vitro* measurement of the biological activity of purified LH's/GTH's from various mammalian species. The data are also useful for further establishment of *in vitro* bioassay of LH's present in the blood as versus the radioimmunoassays.

MATERIALS AND METHODS

Hormones and Chemicals

Ovine LH-S22, ovine FSH-13, porcine FSH-P1, rat-LH-I5, bovine LH-LER 1716-2, and rat LH-RP1, were supplied from the National Institutes of Health, U.S.A. Procine LH-LER 786-3, was obtained from Dr. L.E. Reichert, Albany Medical College. PMSG (18.7 IU/mg), was purchased from China Chem. Co., Taipei. HCG (3360 IU/mg), testosterone, collagenase (Type I), bovine serum albumin (Fraction V), 5-methyl-isobutyl-xanthine, heparin, were from the Sigma Chem. Co. ^{3}H-Testosterone (1, 2, 6, 7-testosterone; 93.9 Curies/m mole), was purchased from the New England Nuclear. Testosterone antiserum, was a gift from Department of Laboratory Medicine, University of Washington, Seattle. Medium 199 (with Hanks salt and L-glutamine), was obtained from Grand Island Biological Co.

Animals

Mice (7-8 wk old), ICR, U.S.A., and Long Evans rats (7-8 wk old) were purchased from the National Laboratory Resources, Taipei. The animals were raised in a temperature controlled room (22±2°C), fed *ad libitum* with Purina Chow; the lighting schedule was 12 L : 12 D.

Preparation and incubation of interstitial cells from mouse testis for bioassay of purified LH/GTH

Standard procedure: The method in preparation of testicular interstitial cells was similar to that described previously (Yu *et al.*, 1981) which is a modified procedure reported by Dufau *et al.* (1976) and Van Damma *et al.* (1974). The animals were sacrificed by cervical dislocation; the testes were removed, weighed, and then placed in a plastic Petri dish containing 3 ml of aerated preincubation medium (Medium 199 with Hank's salts, L-glutamine and 25 mM HEPES, penicilin 10,000 units/100 ml, streptomycin 5 mg/100 ml, 0.1% bovine serum albumin, 10% sodium bicarbonate 1.0 ml/100 ml, pH 7.40). The testes were decapsulated and cut with surgical blade into small pieces; 4-8 testes were pooled for one run. The testes were then transfered to the Erlenmyer flask which contained the preincubation medium. The testicular pieces were gently dispersed for 15 min with a magnet stirrer surrounded by an ice-bath; the medium was repeatly withdrawn into a fire-polished Pasteur pipet over several minutes until homogeneous suspension was obtained. The cell suspension was then filtered through a fine

nylon mesh, and preincubated four one hour at 34°C with shaking at 50 cycles/min. The cell suspension was cooled in ice-water, and centrifuged at 6°C, 250 g for 10 min. Sedimented cells were suspended in incubation medium [preincubation medium+0.125 mM methyl-isobutyl-xanthine + sodium heparin (1 ml=20,000 USP units), 0.5 ml/100 ml]. Incubations were performed in polyethylene tube (13×100 mm) at 34°C, shaken at 100 cycles per min in a Dubnoff incubator. The total volume in an incubation tube was 220 μl, consisting of 100 μl cell suspension, 100 μl reference LH and 20 μl incubation medium. The numbers of total interstitial cells were counted in a hematocytometer under light microscope. The viability of cells was determined by trypan blue uptake technique. The total numbers of interstitial cells in an incubation tube was 0.5×10^6. At the end of 4 hr incubation, the tubes were placed in ice and 2.5 ml of 0.01 M PBS, pH 7.40, was added, and stored at −20°C until assays for androgen.

Establishment of optimized incubation conditions: The effects on androgen production of variations in incubation volume (0.22; 0.88 and 1.76 ml), and in the numbers of interstitial cells (0.35×10^6 and 0.70×10^6) as well as the effects of xanthine and continuous aeration during incubation were compared to establish the optimized and validated conditions for the *in vitro* bioassay of LH's using the mouse testis interstitial cells prepared by mechanical dispersion. Procine LH-LER 786-3 was used as a reference standard.

Comparisons of methods in preparation of testis interstitial cells from mice and rats—collagenase and mechanical dispersions

The androgen formation activity of the testis interstitial cells from mice and rats were compared with different preparations of the cells—the collagenase and mechanical dispersions. The procedure of mechanical dispersion was described in previous section. The collagenase dispersion procedure was essentially similar to that described by Dufau *et al.* (1976). Briefly, the decapsulated and sliced testes were incubated in preincubation medium (described in previous section) containing collagenase (Sigma, type I, 0.5 mg/ml) and bovine serum albumin, 1 mg/ml, for 20 min at 34°C with shaking at 100 cycles per min. The incubation tubes were then filled with cold preincubation medium, inverted gently several times, and the turbid supernatants were aspirated 5 min later. The residual tubules were rewashed once with the same procedure. The combined supernatants were then centrifuged at 200 g, 6°C, for 10 min to sediment the interstitial cells, which were finally resuspended in incubation medium (described in previous section). The viability and the numbers of total interstitial cells were examined as previously described.

The cells were then incubated with porcine LH in the incubation medium at 34°C for 4 hrs shaked at 100 cycles/min under continuous aeration of 95% O_2—5% CO_2. The incubated samples were stored at −20°C until assayed for androgen.

Radioimmunoassay of androgen

The androgen was determined throughout all experiments. The radioimmunoassay procedure for androgen was similar to that described previously (Yu *et al.*, 1981) which is a modification of Anderson *et al.* (1975) and Wingfield and Farner (1975). The modified procedure quantified total androgen, since a chromatographic separation of androgens was omited. Preliminary experiments on the quantification of androgen present in the incubation medium revealed that the androgen values were similar either with or without ether extraction procedure. Consequently, the radioimmunoassay of androgen was performed directly with the incubation medium without further ether extraction procedure. Aliquots of incubation medium, tritiated testosterone and anti-testosterone serum were added and then incubated for 20 hrs at 4°C. Dextran-coated charcol was used to separate the antibody-bound from the free steroid. Supernatant containing the bound labeled steroid was counted in a liquid scintillation spectrometer. The assay was sensitive to 10 pg of testosterone per assay tube.

The specificity of testosterone antiserum was described previously (Anderson *et al.*, 1975); it cross-reacted with dihydrotestosterone, androstenedione, and androstenediol at 90–, 12– and 11%, respectively relative to testosterone (100%), and had negligible cross-reactivities with other test steroids. The concentration of androgen in the sample was expressed as testosterone equivalent extrapolated from the standard curve. Previous study indicated that testosterone accounts for three quarters to four-fifth of total androgens in the incubation medium obtained from incubation of dispersed testis interstitial cells of mice at 6–8 wk old (Yu *et al.*, 1981).

RESULTS

Comparison of methods in preparation of testicular interstitial cells from mice and rats

The androgen formation capacity of dispersed interstitial cells from mouse and rat testes was compared following preparation by collagenase and mechanical dispersion techniques. As indicated in Fig. 1, the interstitial cells from mouse testis prepared by mechanical dissociation were very active in androgen production in response to LH stimulation; a dose-related androgen formation was produced by porcine LH-LER 786-3 ranging from 0.2 to 6.2 ng per tube. In contrast, the cells from rats prepared under indentical mechanical dispersion were essentially unresponsive to porcine LH in androgen formation.

The interstitial cells from the rat, following preparation with collagenase dispersion, however, produced a dose-related androgen response as stimulated by procine LH, although the androgen production activity was much less in comparison to the mouse testis prepared with identical collagenase dispersion procedures (Fig. 1). The androgen formation activity per unit number of total interstitial cells was thus much higher for the mouse testis.

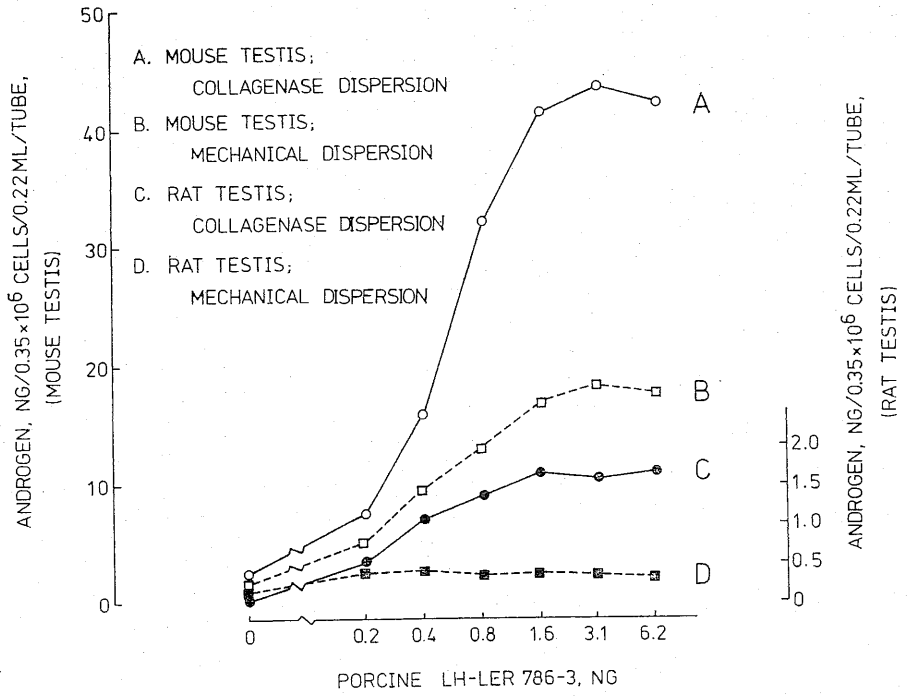

Fig. 1. Comparisons of androgen formation activity of the dispersed testis interstitial cells from mice and rats. The interstitial cells were prepared by either collagenase treatment or mechanical dispersion. The data represent the mean of two separate incubation experiments. See Materials and Methods for further details.

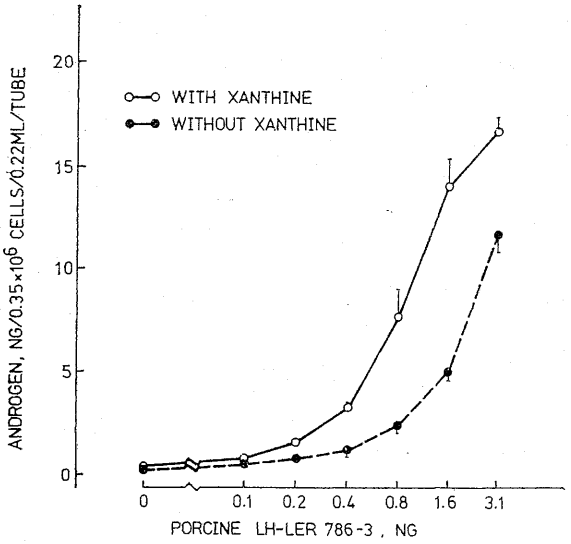

Fig. 2. The effect of 0.125 mM methyl-isobutylxanthine on androgen formation from dispersed mouse testis interstitial cells. The data are expressed as mean±SEM from three separate incubation experiments.

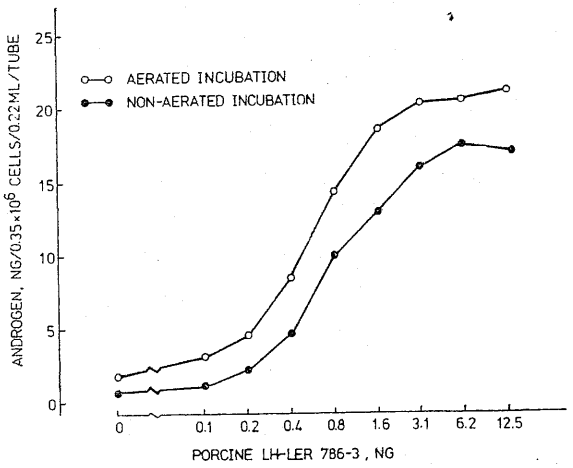

Fig. 3. The effect of continuous aeration during incubation on androgen production from dispersed mouse testis interstitial cells. The incubation medium was thoroughly aerated with 95% O_2–5% CO_2 before the start of incubation; during the 4-hr incubation period, one group of cells continuously received aeration while the other did not (with the tubes capped tightly). The data represent means from two separate incubation experiments.

The viability of the cells isolated by mechanical dispersion was generally, lower than that isolated by collagenase treatment; this fact was likely related to the lower activity of androgen formation observed in the cells prepared by mechanical dispersion.

Effects of xanthine and aeration on androgen production from dispersed mouse testis interstitial cells

Additions of 0.125 mM xanthine into incubation medium considerably increased the androgen formation by the mouse interstitial cells as illustrated by stimulation with various doses of porcine LH LER 786-3 ranging from 0.1 to 3.1 nanograms (Fig. 2). Xanthine, at concentration of 0.125 mM, was thus included in the incubation medium throughout the whole experiments.

An experiment was also conducted to demonstrate whether the continuous aeration of 95% O_2–5% CO_2 to the incubation medium during the 4 hr incubation period was essential for the optimal androgen formation. Prior to the initiation of incubation, all preincubation and incubation medium were thoroughly aerated; during the 4 hr incubation period, one group of cells continuously received the aeration while the other group of cells did not (with the tubes capped tightly). As shown in Fig. 3, the androgen formation from the cells receiving continuous aeration was approximately 30% higher than that from the cells without aeration. Although the continuous aeration did not produce a very profound effect on androgen formation, as compared to that without continuous aeration, the system of continuous aeration during incubation was used in this study.

Effects of incubation volume and cell numbers on androgen formation from dispersed mouse testis interstitial cells

The effect on androgen formation by the mouse testicular interstitial cells of variation in the volume of incubation medium with

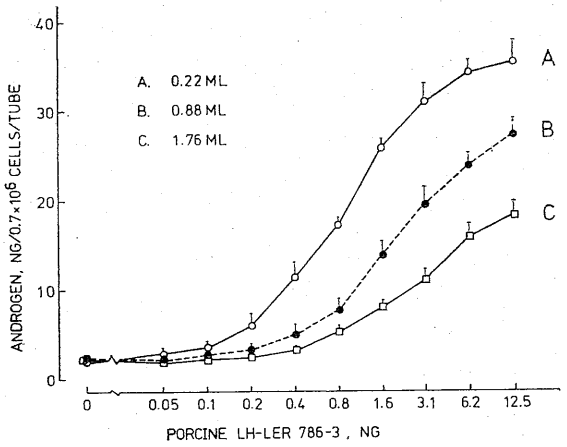

Fig. 4. Effects of volume of incubation medium on androgen production of the dispersed mouse interstitial cells. Three different volumes were selected, each of them contained identical numbers of interstitial cells. The data are expressed as mean±SEM from three experiments.

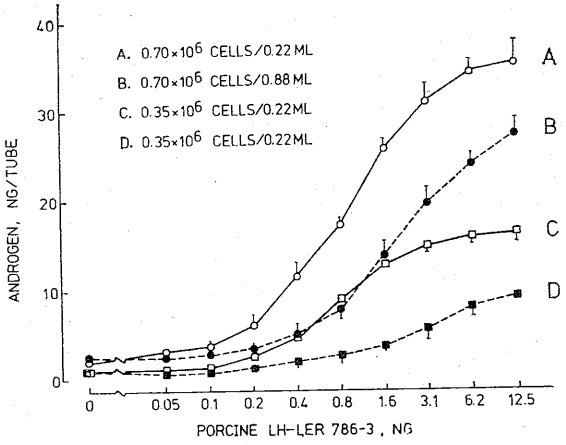

Fig. 5. Effects of variation in the numbers of cells on androgen formation from dispersed mouse testis interstitial cells. Two different numbers of cells were compared on two incubation volumes. The data are expressed as mean±SEM from three separate incubation experiments.

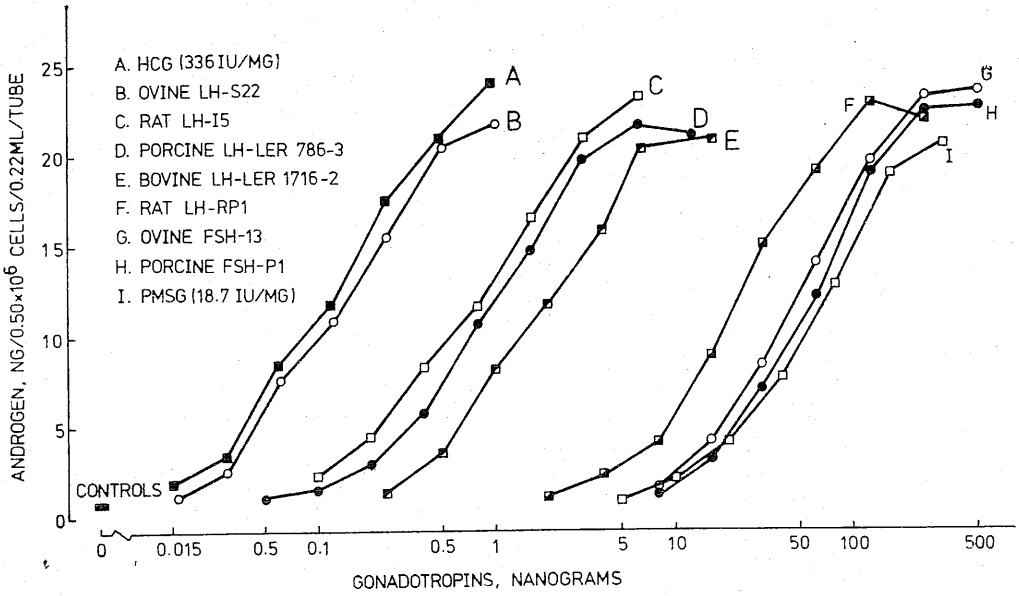

Fig. 6. Comparative effects of various mammalian pituitary LH's and placental gonadotropins on androgen formation from dispersed mouse testis interstitial cells. All hormone preparations assayed are expressed on weight basis. The data are the representative of a single experiment of duplicate incubations.

equal numbers of cells are shown in Fig. 4. As indicated, the androgen formation was increased with decreasing volumes of incubation medium. When the incubation volume was reduced from 1.76 ml to 0.88 ml, a decrease in ED_{50} was observed (from 2.6 to 1.6 ng of porcine LH-LER 786-3). Further decrease of ED_{50} was obtained when the incubation volume was reduced to 0.22 ml (to 0.9 ng porcine LH-LER 786-3). The effect of varying cell numbers was also studied at two different incubation volumes. As shown in Fig. 5, when the number of cells in a total incubation volume of 220 μl was varied from 0.35×10^6 cells to 0.70×10^6 cells, considerable increases in the androgen production were noted. Similar observation were noted when incubation volume was changed to 880 μl.

Comparisons of LH's/Gonadotropins from various vertebrate species on androgen porduction of dispersed mouse testis intertitial cells

The androgen formation activities of LH's/GTH's from various mammalian species were compared by the standard procedure of the mouse testis interstial cell androgen assay, as described in Materials and Methods. The purified LH/GTH were incubated with $0.50\times$

TABLE 1.
Comparisons of sensitivities and potencies of various mammalian gonadotropins in causing androgen production from dispersed interstitial cells of mouse testes using ovine LH-NIH-S22 as the reference standard[1].

Hormones	Numbers of incubation	Sensitivity[2] (ng)	Relative sensitivity[3]	Relative potency[4]	Linear range of doses (ng) to obtain regression for potency calculations
Ovine LH NIH-S22	10	0.021	1	1.0	0.03, 0.06, 0.12, 0.24, 0.48
HCG (3360IU/mg)	6	0.017	0.8	1.21	0.031, 0.062, 0.125, 0.25, 0.5
Rat LH NIAMDD-I5	2	0.13	6	0.15	0.2, 0.4, 0.8, 1.6, 3.1
Porcine LH LER 786-3	21	0.17	8	0.12	0.2, 0.4, 0.8, 1.6, 3.1
Bovine LH LER 1716-2	4	0.28	13	0.068	0.5, 1, 2, 4, 8
Rat LH NIAMDD-RP1	12	4.3	200	0.0059	8, 16, 31, 62, 125
Ovine FSH NIH-13	2	8.9	420	0.0028	16, 31, 62, 125, 250
Porcine FSH NIH-P1	3	9.0	430	0.0024	16, 31, 62, 125, 250
PMSG (18.7IU/mg)	1	9.3	440	0.0022	10, 20, 40, 80, 160

1. Incubation of testis cells was performed at 34°C for 4 hrs in Medium 199-xanthine-heparin. pH 7.4 in a Dubnoff shaking incubateor under continuously aeration of 95% O_2-5% CO_2.
2. The mean baseline androgen from interstitial cells not exposed to LH (controls) plus two standard deviations was the method used to extrpolate the sensitivity.
3. Caculated from sensitivity[2]: values of sensitivity obtained from all hormones assayed were divided by that of ovine-LH-S22.
4. The potency estimation between preparations of hormones was according to the statistical equations described by D. J. Finney, Statistical Method in Biological Assay, Hanfner Publishing Company, New York (1964).

10^6 cells in a total volume of 220 μl at 34°C under continuous aeration for 4 hrs. As indicated in Fig. 6, all pituitary LH preparations from mammalian species (ovine LH-S22, porcine LH-LER786-3, bovine LH-LER1716-2, rat LH-I5, and rat LH-RP1) as well as placental gonadotropins (HCG and PMSG) elicited dose-related androgen production. Among the purified LH's used in the present study, ovine LH-S22 was the most potent in stimulating androgen formation from the mouse testis cells. Concerning the placental gonadotropins, HCG was found to be extremely active in promoting androgen formation by the mouse testis. Based on the weight of the hormone preparations, HCG was slightly more potent than ovine LH-S22. The androgen formation activity of PMSG, expressed on the weight basis, was much less than was the HCG. With respect to FSH preparations, both ovine FSH and porcine FSH elicited a dose-related androgen production as well, although much larger amounts were needed. The ovine FSH-13 was approximately 5 times more potent than the porcine FSH-P1 under the present experimental conditions.

Comparisons of the potencies of the mammalian gonadotropins as assayed by the dispersed mouse testis interstitial cell system are presented in Table 1. The relative potencies were compared with ovine LH-S22 which being used as a reference standard in this regard.

DISCUSSION

The dispersed interstitial cells or isolated Leydig cells from rat testis are highly responsive to LH's from various mammalian species in terms of androgen formation; such cell preparations have subsequently been used for *in vitro* biological assay of LH's from various mammalian species including humans (Dufau *et al.*, 1974, 1976, 1977; Farmer *et al.*, 1977, Mendelson *et al.*, 1975; Moger, 1979; Solano *et al.*, 1979). The dispersed mouse testis interstitial cell system has also been used for *in vitro* bioassay of primate and rat LH's as well as the human chorionic gonadotropin (Ellinwood *et al.*, 1980; Romani *et al.*, 1977; Steiner *et al.*, 1980; Van Damme *et al.*, 1974, Yu *et al.*, 1981). It was indicated that the androgen formation activity of dispersed interstitial cells from the mouse testis are highly specific to the stimulations by LH's from rats and primates; other hormones evoke none or insignificant formation of androgen (Van Damme *et al.*, 1974). Nevertheless, the androgenic responses of dispersed mouse testis interstitial cells were relatively less studied as compared to the rat system. We have thus established the optimized and validated *in vitro* bioassay of LH's using dispersed mouse testis interstitial cell preparations and have compared the androgen formation activity of the dispersed mouse testicular interstitial cells in response to various mammalian LH's as well as the placental gonadotropins.

The results from the present study indicate that the mouse interstitial cell androgen assay provides a convenient tool for measuring the biological activity of pituitary LH preparations and placental gonadotropins from a variety of mammalian species. Such bioassay has unique advantages in terms of both sensitivity and the parallel dose-response curves obtained with all forms of mammalian LH's and the placental gonadotropins. The parallel standard curves obtained in the present study includes ovine, porcine, bovine, and rat LH's as well as HCG and PMSG. Such property of the mouse interstitial cell androgen assay consequently permits valid phylogenic comparisons of intrinsic biologic activity of gonadotropins among mammals. It can also be used as a convenient *in vitro* bioassay for estimating the biological potency of the purified LH's from a variety of mammalian species.

Differerent laboratories used different LH preparations for the *in vitro* bioassay; this renders some inconvenience for direct comparison of the sensitivity between rat and mouse testes assay systems (Dufau *et al.*, 1974, 1976; Farmer *et al.*, 1977; Moger, 1979; Solano *et al.*, 1979; Romani *et al.*, 1977; Van Damme *et al.*, 1974; Yu *et al.*, 1981). The observations from previous researchers and the findings from the present study indicate that the androgen

production activity of the dispersed testis interstitial cells from both rat and mice appear to be simliar in terms of sensitivity and specificity of the assays in response to LH's/GTH's from various mammalian species. Although both rat and mouse testis systems provide a convenient *in vitro* bioassay of LH's, the mouse assay system has certain advantages over the rat assay system. The results from the present study indicate that the mouse testicular interstitial cells prepared from either mechanical or collagenase dispersion is highly responsive to LH stimulation in androgen formation. In contrast, the androgen formation activity of rat testicular interstitial cells, following mechanical dispersion, is largely impaired. The mouse testis system is, thus, a more convenient assay in terms of the choice of the methods in preparation of the dispersed interstitial cells.

The relative potency of the purified pituitary LH's and placental gonadotropins as assayed by the dispersed mouse testis interstitial cell androgen production system demonstrated by the present study (Fig. 6 and Table 1), are generally in agreement with the indicated biological potency of the hormone preparations estimated by ovarian ascorbic acid depletion and ventral prostate weight assays (Dorfman and Shipley, 1956; Parlow, 1961). Both porcine and ovine FSH's exhibited the stimulating activity in androgen formation in this mouse testis assay, although the potencies were much lower than the mammalian LH's in general. It was indicated that both these FSH preparations contained some amounts of LH activity NIAMDD-NPA Hormones Distribution Program, 1978). It, thus, is likely that the androgen promoting activity of such FSH preparations, as observed in the present study, represents the minute amount of LH contained there-in, but not the character of the intrinic property of FSH *per se*. It was indicated also that in the rat testis interstitial cell system, high concentration of highly purified human FSH induces small amount of androgen formation; such activity is completely abolished by pre-incubation tn the presence of antiserum to human LH (Dufau *et al.*, 1976).

HCG was highly potent in eliciting the androgen production when assayed by the dispersed mouse tistis interstitial cell system. The biological activity of HCG preparation tested in such assay is 3,360 IU/mg. Highly purified preparation of HCG containing 12,000–18,000 IU/mg has been obtained (Mori, 1970; Sherwood and McShan, 1977). Our findings that the relatively much higher potency of HCG as compared to the purified pituitary LH's from subprimate mammalian species, on the weight basis of the hormone preparations, in evoking the androgen production *in vitro*, are in correspondence to the observations from the conventional *in vivo* bioassays by ovarian ascorbic acid depletion and ventral prostate weight methods (Dorfman and Shipley, 1956; Parlow, 1961). The lower androgen production activity of PMSG, expressed on the basis of weight, as observed in the present study, ascribed to the relatively low potency of the preparation used in the assay (18.7 IU/mg). It was demonstrated in the rat testis interstitial cell assay that the relative potency of PMSG containing 1,230 IU/mg was 0.091 in relative to ovine LH-S18 reference standard (=1.0) (Dufau *et al.*, 1976). Highly purified preparation of PMSG contaninining 16,000 IU/mg has been achieved (Sherwood and McShan, 1977). It thus appears that the androgen production activity of the highly purified PMSG (e.g. 16,000 IU/mg) is also relatively higher than many of the purified LH's from the pituitaries of mammalian species if assayed in both rat and mouse testis *in vitro*.

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多種哺乳類促性腺激素之雄性素生成能力之比較：小白鼠睪丸精間細胞離體培養

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本研究之目的：(1) 建立小白鼠睪丸精間細胞離體培養，以作爲促精間細胞激素之生物檢定；(2) 比較九種哺乳類之促精間細胞激素或促性腺激素之雄性素生成能力，觀察種別差異。以機械震盪法分離 7～8 週齡小白鼠睪丸之精間細胞測定適當培養條件：細胞數目，培養液容積，連續供應混合氣，及二氧嘌呤之影響。發現適當培養情況：每培養管含精間細胞數目爲 0.5×10^6，與各劑量之促精間細胞激素，一齊培養於 Medium 199-Xanthine-Heparin (pH 7.40), 34°C，連續震盪 (100次/秒)，及連續供應混合氣 (95% O_2-5% CO_2)，經 4 小時後，以放射免疫法直接測定培養液中，雄性素含量。

本法之敏感度極高，羊、猪、牛，與大白鼠之促精間細胞激素，以及人類絨毛性腺激素，孕馬血清性腺激素，均能引起相互平行，有劑量相關之雄性素生成。其相對生物活性與以活體生物檢定者極爲類似。大量之羊及猪之促濾胞激素亦引起有劑量相關之雄性素生成，此應是促濾胞激素製備時含有微量之促精間細胞激素引致之雄性素生成。本研究亦比較 7～8 週齡之大白鼠及小白鼠睪丸精間細胞離體培養之雄性素生成能力。結果顯示；以機械震盪法分離之大白鼠 (Rats) 睪丸精間細胞產生雄性素能力極差，而以酵素法分離者，其雄性產生能力亦遠低於小白鼠 (Mice) 睪丸精間細胞 (機械震盪或酵素分離法) 之雄性素產生能力。