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Control of Testicular Estrogen Synthesis[1]

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INTRODUCTION

The pivotal role played by the Sertoli cells in the process of spermatogenesis has been illustrated by a number of different approaches. Electron microscopic studies demonstrated that adjacent Sertoli cells formed specialized "tight" junctions, thereby forming the blood-testis barrier which effectively divides the tubule into basal and adluminal compartments. The Sertoli cell clearly provides mechanical support for the developing germ cells at all stages of differentiation. This cell has acquired additional functional significance from evidence showing that it may constitute a target cell for hormones within the seminiferous epithelium. The evidence for responsiveness to FSH has been amply reviewed in recent years (Means, 1975; Steinberger, 1975; Hansson et al., 1975; Fritz et al., 1975; Fritz, 1978) and includes the demonstration that FSH binds specifically to Sertoli cells, increases 3′-5′-AMP production, activates protein kinase and stimulates a variety of end responses (e.g. the production of androgen-binding protein (ABP), plasminogen activator and estrogen and the incorporation of ^{3}H-thymidine into DNA). FSH does not appear to influence directly any other cell types in the testis. The evidence that Sertoli cells but not germ cells are target cells for androgens stems from quite recent investigations: firstly, Sertoli cells contain androgen receptors, whereas the presence of specific androgen receptors in spermatocytes and spermatides appears unlikely. Secondly, androgens stimulate ABP production by Sertoli cell cultures, but a direct androgen action on germ cells has not been detected. The third line of evidence comes from the genetic studies of Lyon et al. (1975). These workers show that allophenic mice, which were a mosaic of Tfm/Y⟷+/Y cells, produced fertile sperm bearing the Tfm mutation on the X chromosome. It was postulated that the germ cells bearing the Tfm mutation may have developed normally in these animals because adjacent XY Sertoli cells contributed to a localized environment conducive to germ cell development. In addition, the X chromosome bearing the Tfm locus is normally activated no later than the early prophase of meiosis during spermatogenesis in mammals (Monesi, 1974), suggesting that specific androgen receptors are not synthesized after this stage of differentiation.

Since Sertoli cells appear to respond directly both to FSH and testosterone, it is clearly important to determine the nature of the responses to each hormone and to assess how these contribute to the environment in which the germ cells develop. In this review, we shall concentrate for the most part on one particular end response of Sertoli cells to FSH, namely, estrogen biosynthesis from testosterone.

EVIDENCE THAT THE TESTIS SECRETES ESTROGENS

In 1934, Zondek reported that a crude alcohol extract of horse testes exhibited estrogenic activity. Later, Beall (1940) showed that this estrogenic potency was due to the presence of estrone and estradiol at levels higher than in any other tissue which had been analyzed. These findings, which demonstrated the presence of estrogens in male tissues, destroyed the myth that these steroids were specific to the female.

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The ability of the testis to synthesize estrogens from 2 carbon precursors was demonstrated following the development of radioactive tracer techniques: Wotiz et al. (1955) showed that slices of embryonic testicular carcinoma synthesized progesterone, testosterone and estrogens from labelled acetate. Shortly afterwards, Rabinowitz (1956) reported that homogenates of testes from human, dog and cat incorporated labelled acetate into cholesterol, testosterone and 17β-estradiol. These studies suggested a common link in the biosynthesis of androgens and estrogens and it was not long before direct evidence of the conversion of testosterone to estradiol by stallion testis homogenates was obtained (Baggett et al., 1959). However, in several *in vitro* studies of testes from other species, estrogen biosynthesis was low or undetectable. In an attempt to determine how much of the total circulating estrogen was derived from the testis, the concentration of estrogen in the spermatic vein has been compared with the concentration in the peripheral plasma. Using this approach, Kelch et al. (1972) showed that the concentration of estradiol in the human spermatic vein was 50 times greater than that in peripheral plasma. From this, they calculated that the testicular secretion may account for about 25% of the total estrogen produced in men. Monkey and dog testes also secreted estradiol but in smaller amounts (Kelch et al., 1972). Similar measurements of the estradiol concentration in spermatic vein and peripheral plasma indicated that the rat testis secreted estradiol *in vivo* and contributed about 21% of the total circulating estrogen (de Jong et al., 1973).

Both Sertoli and interstitial cells have been implicated as sites of testicular estrogen biosynthesis, dating back to the demonstration of feminization in dog with Sertoli cell tumours (Zuckerman and McKeown, 1938) and in men with testicular tumours believed to be of interstitial cell origin (Hunt and Budd, 1939). De Jong et al. (1973, 1974) suggested that the seminiferous tubule rather than the interstitial cell compartment of the normal testis was the site of estrogen biosynthesis. In the first of these papers the authors noted that in intact rats given hCG, the increase in the level of testosterone in the testicular venous plasma was greater than that of estradiol. After 5 days of hCG treatment, the estradiol:testosterone ratio was further reduced. These observations suggested that the 2 steroids may be synthesized in different compartments of the testis (de Jong et al., 1973). In a subsequent study, estradiol and testosterone levels in total tissue and in seminiferous tubules and interstitial tissue isolated from intact adult rats, were measured before and after incubation for 3 h. Before incubation the concentration of estradiol was 9–15 times greater in interstitial tissue than in seminiferous tubules. The concentration of estradiol increased significantly during the incubation of total testis tissue and seminiferous tubules, whereas no increase occurred in interstitial tissue. The testosterone concentration, on the other hand, increased during the incubation of total testis tissue and interstitial tissue. These results are consistent with the view that the interstitial cells produce testosterone and that the seminiferous tubule is the site of estrogen biosynthesis *in vitro* (de Jong et al., 1974).

ESTROGEN BIOSYNTHESIS IN SERTOLI CELLS IN CULTURE

The development of methods for the isolation of Sertoli cells from rat testes and for the maintenance of these cells in primary culture (Dorrington et al., 1974, 1975) provided us with a direct means of determining if this cell type could synthesize estrogens. Secondly, it enabled us to investigate the role of gonadotrophins in the regulation of estrogen synthesis.

Sertoli cell aggregates, obtained from testes of 18- to 20-day-old rats by the combined enzymatic treatment described previously (Dorrington et al., 1975) were preincubated in a modified Eagle's Minimum Essential Medium at 32°C in the absence of hormones. After 24 h in culture, substrate testosterone (0.5 μM), together with test substances, was added. After varying periods of time in culture, medium was removed and extracted with diethyl ether. Estradiol and estrone contents of the ether extracts, as well as the ethanol extracts of cells before and after incubation, were determined by radioimmunoassay. Cells grown in medium containing testosterone alone synthesized low but detectable levels of estradiol; however, when 5 μg NIH-FSH/ml was added together with testosterone (0.5 μM), the amount of estradiol synthesized was dramatically increased (Fig. 1). A highly purified FSH preparation (G4-150C having an activity of 50 NIH-FSH-S1 units/mg) was also effective in stimulating estradiol synthesis. High concentrations of LH caused only a marginal stimulation (Fig. 1) which may have resulted from the low level of

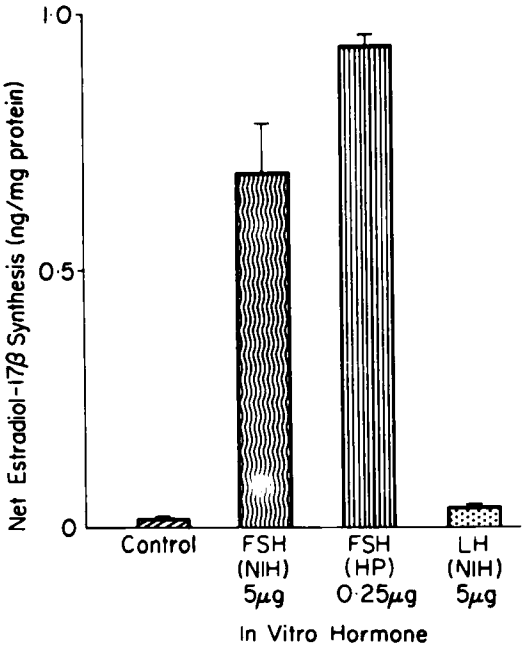

FIG. 1. Effect of NIH-FSH-S10, highly purified FSH (G4-150C, from Dr. H. Papkoff) and NIH-LH-S18 on estradiol synthesis by Sertoli cells from 18- to 20-day-old rats cultured for 48 h in medium containing 0.5 μM testosterone. Hormones were added at the beginning of the incubation period and again after 24 h (data from Dorrington and Armstrong, 1975).

FSH contamination in the LH preparation.

A comparison of the amount of estradiol vs. estrone synthesized by Sertoli cells (from 20-day-old rats) in the presence of testosterone as the aromatizable substrate is presented in Table 1. It is evident that estradiol predominated under the culture conditions employed both in the presence and absence of FSH. The amount of estradiol and estrone synthesized depended upon the concentration of FSH added. When the amount of estradiol synthesized was plotted against the log dose of NIH-FSH-S10, the response increased proportionally between 0.05 and 5 μg/ml, with a half-maximal effect at 0.25 μg/ml (Fig. 2).

TABLE 1. Effect of FSH (NIH-S10) on the synthesis of estradiol and estrone by Sertoli cells from 18 to 20-day old rats cultured for 24 h in the presence of 0.5 μM testosterone.

Treatment	Steroid synthesis (pg/mg protein)	
	Estradiol	Estrone
Control	113 ± 10	10 ± 3
0.5 μg FSH/ml	796 ± 32	93 ± 16
5.0 μg FSH/ml	1216 ± 32	122 ± 16

After 2 days in culture, the cell aggregates attached to the culture flask contained approximately 15% germ cells, as judged by electron microscopic examination. In order to rule out the germ cells as possible sources of estrogen, preparations from germ cell-depleted testes obtained from rats irradiated *in utero* have been studied. Pregnant rats were exposed to 125 rads of whole-body irradiation at 19 days of gestation, which resulted in a marked reduction in the number of germ cells in the seminiferous tubules of the male offspring. The amount of estradiol synthesized in response to FSH by cells prepared from testes of 20-day-old rats which had been irradiated *in utero* were similar (Dorrington et al., 1976a), indicating that the Sertoli cells were responsible for the estradiol synthesized and that their response to FSH was not modified by the absence of germ cells.

Sertoli cells from 20-day-old rats did not synthesize significant amounts of estradiol in response to FSH when incubated in the absence of exogenous steroid substrate, or in the presence of pregnenolone or progesterone (Dorrington and Armstrong, 1975; Dorrington et al., 1976b). The inability of Sertoli cells to convert pregnenolone and progesterone to a substrate for aromatization, suggests that the androgen precursor for estrogen biosynthesis *in vivo* is produced elsewhere in the testis, an interpreta-

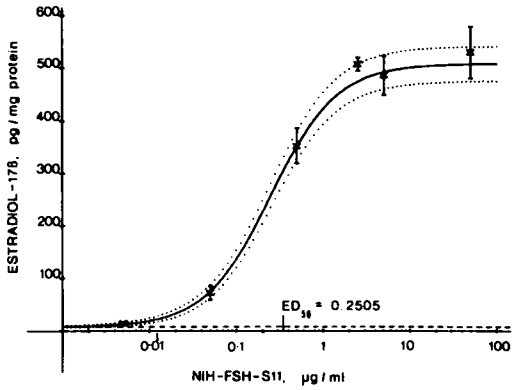

FIG. 2. Logarithmic dose-response curve for the effect of FSH (NIH-FSH-S11) on estradiol synthesis by Sertoli cells from 18- to 20-day-old rats cultured for 24 h in the presence of testosterone (0.5 μM). Each value is a mean ± SEM of 3–5 replicates.

tion in accord with other reports in the literature on the steroidogenic potential of isolated seminiferous tubules and interstitial tissue. Isolated seminiferous tubules did not synthesize testosterone from exogenous labelled cholesterol (Hall et al., 1969) or from endogenous cholesterol (Cooke et al., 1972), whereas interstitial tissue incubated under the same conditions synthesized androgen. Mitochondria from interstitial tissue could account for 92–97% of the pregnenolone and testosterone synthesized by mitochondria from whole testis tissue (van der Vusse et al., 1973).

In further investigations on the steroidogenic capacity of Sertoli cells, aggregates were prepared by the enzyme digestion procedure from tubules dissected from the testes of 35-day-old rats (an age at which testosterone production by the whole testis is high). This procedure ensures that the Sertoli cell preparations are free of contaminating Leydig cells. Sertoli cell aggregates prepared in this way and incubated immediately (without culture) had a limited capacity to metabolize ^{14}C-pregnenolone and no ^{14}C-testosterone was detected (Table 2). Furthermore, Sertoli cells from 33-day-old rats did not synthesize significant amounts of androgen when cultured in the presence or absence of gonadotrophins.

In contrast, cell suspensions enriched in Leydig cells synthesize testosterone. This synthesis is regulated by LH (Moyle and Ramachandran, 1973; Catt et al., 1974). The concentration of testosterone in the testicular lymph which bathes the seminiferous tubule is almost as high as that in the spermatic venous blood (Setchell, 1970) and there does not appear to be a barrier to the entry of testosterone and other steroids into the tubule (Parvinem et al., 1970). These observations suggest that both Leydig cells and Sertoli cells participate in the synthesis of estrogens. In this 2-cell type model (Fig. 3), it is proposed that testosterone formed by the Leydig cells under the influence of LH is transported through the lymph to the Sertoli cell, where it is converted to estrogen in the presence of FSH.

In addition to testosterone, various intermediates involved in the biosynthesis of estrogens (androstenedione, 19 OH-androstenedione and 19 OH-testosterone) have been tested as substrates (Fig. 4). Estradiol synthesis was stimulated by FSH in the presence of each of the C_{19} steroids, suggesting that FSH influences the conversion of androgens to estrogens, either directly or indirectly, at the aromatiza-

TABLE 2. Metabolism of [^{14}C]-pregnenolone by whole testis preparations and Sertoli cell-enriched preparations from 35-day-old rats.

	% Total radioactivity recovered from:	
	Whole testis	Sertoli cells
Pregnenolone*	63.0 ± 4.0	97.1 ± 1.0
Progesterone	7.9 ± 2.4	0.4 ± 0.2
17αOH progesterone	3.1 ± 0.6	0.2 ± 0.1
Androstenedione	3.2 ± 0.5	N.D.
Testosterone	1.3 ± 0.2	N.D.
Androstanediol	5.5 ± 1.6	N.D.
Androsterone	12.0 ± 2.2	N.D.

*Unmetabolized substrate.

Total testis tissue from 35-day-old rats (40 mg wet wt) and freshly prepared Sertoli cell-enriched aggregates (approximately 2 mg protein) obtained from isolated tubules by the enzyme digestion procedure (Dorrington et al., 1975) were incubated in 1 ml Krebs-Ringer bicarbonate buffer containing 1 mg glucose and 500 ng [^{14}C]-pregnenolone for 2 h at 32°C in an atmosphere of 95% O_2 and 5% CO_2. The results were expressed as the mean ± SEM of the percentage of the total radioactivity recovered after incubation of 2 mg protein. Three separate experiments were performed for each set of observations reported.

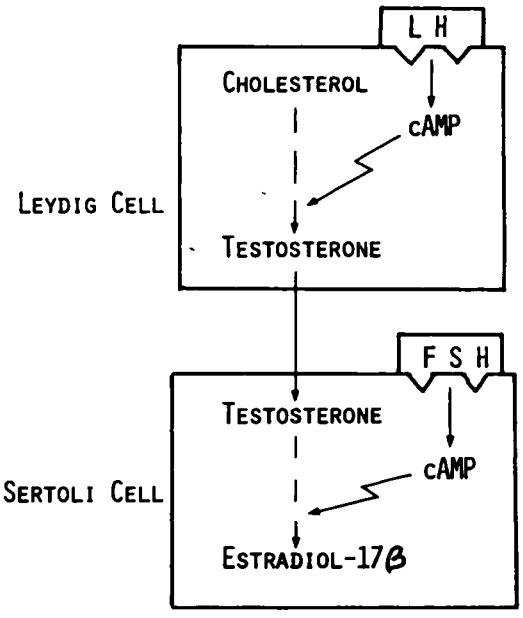

FIG. 3. Model of the 2 cell-2 gonadotrophin hypothesis for testicular estradiol synthesis.

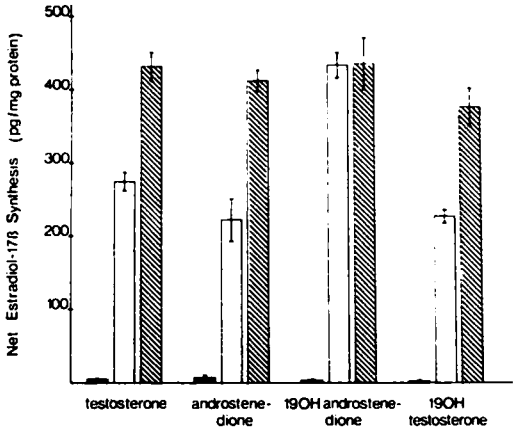

FIG. 4. Estradiol synthesis by Sertoli cells from 18- to 20-day-old rats cultured for 24 h in the presence of various substrates (0.5 μM). Solid bar, control; open bar, 5 μg NIH-FSH-S11/ml treated; cross-hatched bar, dibutyryl cyclic AMP (0.5 mM) treated. The results from a single experiment are presented and are the means ± SEM of 3 replicate cultures for each set of conditions. This experiment is representative of 3 similar experiments (data from Dorrington et al., 1976b).

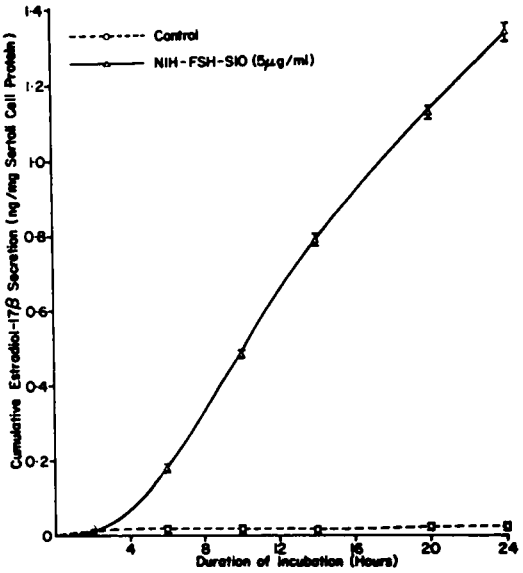

FIG. 5. Effects of FSH on cumulative secretion of estradiol throughout a 24 h incubation period in medium containing testosterone. Medium containing either testosterone (0.5 μM) alone (○) or testosterone and 5 μg NIH-FSH-S10/ml (△) was added at 0 time and replaced after 2, 6, 10, 14 and 20 h (data from Armstrong et al., 1975).

tion step (the conversion of 19-hydroxylated androgens to estrogens). Whether this is the only site in the biosynthetic pathway from testosterone to estrogens which is influenced by FSH cannot be determined from these experiments. The aromatization of androgens involves a series of closely integrated reactions and it is possible that FSH stimulates several steps in the overall process.

MECHANISM OF ACTION OF FSH

Since estradiol can be measured readily and accurately by radioimmunoassay, this steroid provides a useful end product for investigations on the mechanism of FSH action. When medium containing either testosterone alone or FSH and testosterone was collected at frequent intervals from cultures of immature Sertoli cells, no effect of FSH on the conversion of testosterone to estradiol was found during approximately the first 4 h of treatment. After this initial lag phase, estradiol synthesis was stimulated by FSH and persisted for at least 24 h (Fig. 5). We are particularly interested in the series of biochemical events which are triggered in Sertoli cells by FSH and which proceed during the 4 h lag period. FSH interacts with specific receptors on the plasma membranes of Sertoli cells. The number of FSH-binding sites/testis increases during the postnatal period when Sertoli cells are actively dividing. Maximum numbers are attained at about 15 days of age and remain constant up to 60 days of age (Fakunding et al., 1976). The amount of FSH bound to membranes prepared from tubules of Sertoli cell-enriched immature testes was directly related to the adenylate cyclase activity of the preparations (Means, 1975). Cyclic AMP is rapidly formed in response to the FSH stimulus in cultured Sertoli cells and is dose-dependent with a half maximal effect at 0.71 μg/ml (Fig. 6). In the latter experiments, Sertoli cells from 18- to 20-day-old rats were incubated in Krebs-Ringer bicarbonate buffer containing 1 mg glucose/ml and 0.5 mM MIX for 1 h. The minimal effective concentration of FSH under these conditions was approximately 0.05 μg/ml and 5 μg/ml produced a maximal response.

Dibutyryl cyclic AMP mimicked the *in vitro* effects of FSH on Sertoli cell functions, including an increase in the incorporation of [^{3}H]-leucine into protein and [^{3}H]-thymidine into DNA and the increased production of ABP and estrogen (Fritz et al., 1975). Eight-bromo-cyclic AMP also stimulated estrogen biosynthesis (Ta-

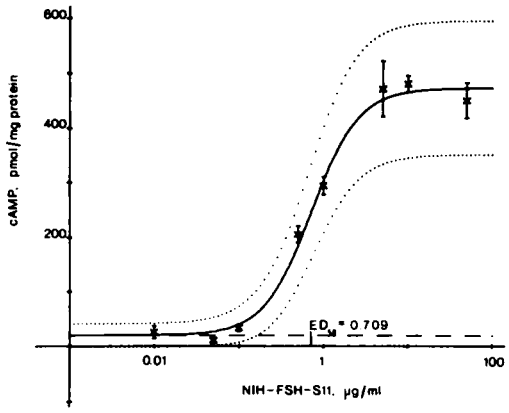

FIG. 6. Logarithmic dose-response curve for the effect of FSH (NIH-FSH-S11) on the levels of cyclic AMP in Sertoli cell preparations (from 18- to 20-day-old rats), when incubated after 48 h in culture in standard medium. All incubations were for 60 min at 32°C in Krebs-Ringer bicarbonate buffer containing 1 mg glucose/ml and 0.5 mM MIX. Each value is a mean ± SEM for 3–5 replicates.

ble 3). On the other hand, even high concentrations of cyclic AMP (5 mM) cause only a small increase in estrogen biosynthesis, probably associated with poor penetration of cyclic AMP into intact cells (Table 3). In an attempt to generate cyclic AMP intracellularly, cholera toxin was added to the Sertoli cell cultures. This toxin, which binds to Gm_1 gangliosides in the plasma membrane, activates adenylate cyclase in a wide variety of different tissues (Bennett et al., 1975). Cholera toxin effectively stimulated cyclic AMP production by Sertoli cells when incubated in the presence of 0.5 mM MIX and also stimulated estrogen synthesis from exogenous testosterone (Fig. 7, Table 3). Cholera toxin also duplicated the effects of FSH on other Sertoli cell functions (Fritz et al., 1976). Thus, FSH stimulates cyclic AMP production in immature Sertoli cells and cyclic AMP derivatives can stimulate estrogen biosynthesis. Whether cyclic AMP is an obligatory intermediate in FSH action remains unproven.

Protein synthesis is increased in the testes of immature or hypophysectomized mature rats following the injection of FSH (Means, 1975). We determined that the rate of incorporation of [^{3}H]-leucine into TCA-precipitable material was also increased in Sertoli cells cultured for 2 days in the presence of FSH (Dorrington et al., 1975). In order to determine if protein synthesis was required for the expression of the FSH effect on estrogen synthesis, we investigated the effects of puromycin on this process. After 30 min exposure of Sertoli cells to 5 × 10^{-6} M and

TABLE 3. Effects of various agents on estradiol synthesis by Sertoli cells from 18- to 20-day-old rats cultured for 24 h in medium containing 0.5 µM testosterone.

Treatment	Estradiol synthesis (pg/mg protein)
Control	25 ± 2
FSH (5 µg/ml)	231 ± 8
Dibutyryl cyclic AMP (0.1 mM)	152 ± 13
Dibutyryl cyclic AMP (0.5 mM)	354 ± 37
Cyclic AMP (5 mM)	48 ± 5
8-Br-cyclic AMP (0.5 mM)	250 ± 12
Choleratoxin (1 µg/ml)	198 ± 27

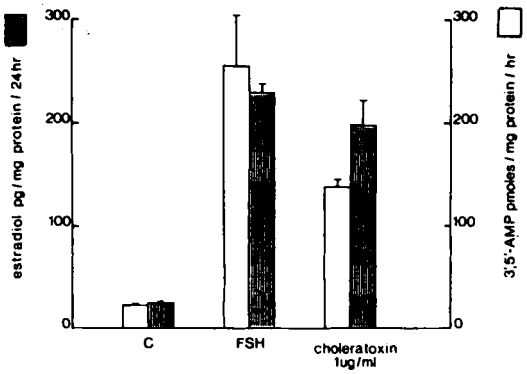

FIG. 7. Effects of FSH (5 µg NIH-FSH-S11/ml) and cholera toxin (1 µg/ml) on estradiol synthesis and on cyclic AMP production by Sertoli cells from 20-day-old rats. Estradiol synthesis: Cells were cultured in standard medium for 24 h after which testosterone (0.5 µM) alone, or testosterone and either FSH or cholera toxin, were added and the cells were cultured for another 24 h. Results shown are the means ± SEM of 3 replicate cultures in each of 2 experiments. Values in 'C' flasks (controls) indicate the amount of estradiol formed when cells were cultured in the presence of testosterone alone. Cyclic AMP production: After 48 h in culture in standard medium, the attached cells were removed from the culture flasks with trypsin. Soybean trypsin inhibitor was added and the cells were washed as described pr previously (Dorrington et al., 1975). The cells were incubated for 1 h in Krebs-Ringer bicarbonate buffer containing glucose (1 mg/ml) and 0.5 mM MIX, in the presence or absence of FSH and cholera toxin. Values are the mean ± S.E.M. for 3 replicate flasks from a single experiment and are representative of results from 2 experiments.

5 x 10^{-5} M puromycin, the incorporation of [^{3}H]-leucine into TCA-precipitable material was inhibited and the inhibition persisted for up to 24 h (Fig. 8). In the same experiment, cells were preincubated for 30 min with puromycin (5 x 10^{-6} M and 5 x 10^{-5} M) after which testosterone and FSH were added and the amounts of estrogen synthesized during the subsequent 24 h period were measured. Both concentrations of puromycin inhibited the effect of FSH on estradiol biosynthesis. The higher concentration of puromycin employed was more effective in inhibiting both protein and estrogen synthesis (Fig. 8).

In a subsequent series of experiments, testosterone alone or testosterone and FSH were added at 0 time and puromycin (5 x 10^{-5} M) was added at 0, 1, 2, 3, 4 or 6 h. All incubations were continued for 24 h and estradiol levels present in medium and cells were determined. Puromycin added at 0, 1 or 2 h after the addition of testosterone and FSH, abolished the FSH effect during the subsequent incubation period. When puromycin was added 3 h after the addition of hormones, some stimulation of estrogen synthesis by FSH was apparent. When the addition of puromycin was delayed until 4 or 6 h after exposure of the cells to FSH, no inhibition of hormone action was found (Fig. 9). The inability of puromycin to influence FSH-stimulated estrogen synthesis when added 4 h after FSH addition provides evidence that the cells are viable after exposure to this concentration of inhibitor. It is evident from these experiments that treatment of cells with FSH for 4 h prior to the addition of puromycin is essential in order to attain the maximal response. It is tempting to speculate that during the first 4 h of FSH action, there is the synthesis of sufficient protein required to permit elevated rates of conversion of testosterone to estradiol during the subsequent 20 h incubation period.

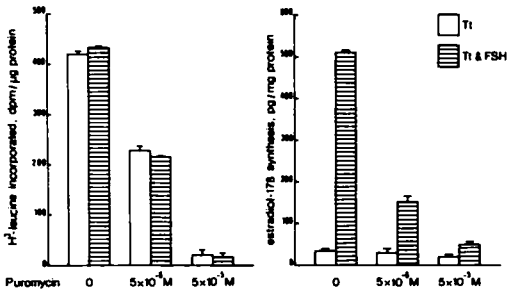

FIG. 8. Effect of puromycin on the incorporation of [^{3}H]-leucine into TCA-insoluble material and on estradiol synthesis by Sertoli cells isolated from 16-day-old rats. Leucine incorporation: Sertoli cell preparations were maintained in culture for 24 h, preincubated with puromycin for 30 min, after which testosterone alone (0.5 μM) or testosterone (0.5 h medium was removed and 2 ml Krebs-Ringer bicarbonate buffer containing 1 mg glucose/ml and 1/10th the amino acids in standard MEM and 1 μCi [^{3}H]-leucine/ml (final concentration of leucine 0.04 mM) were added. Incubations were terminated after 2 h by the addition of TCA (Dorrington et al., 1975). Estradiol synthesis: After 24 h in standard medium, cells were preincubated with puromycin for 30 min. Testosterone (0.5 μM) alone or testosterone and FSH (5 μg/ml) were then added and incubation continued for 24 h. Values are the mean ± SEM of three replicate cultures.

PHYSIOLOGICAL SIGNIFICANCE OF ESTROGEN SYNTHESIS BY SERTOLI CELLS

The data on estrogen biosynthesis presented above were obtained with Sertoli cell preparations isolated from testes of 16- to 20-day-old rats. Subsequently, estrogen biosynthesis in response to FSH was investigated in Sertoli cells from rats of different ages. Sertoli cells from 30-day-old (and 40-day-old) rats synthesized low levels of estradiol in the presence of testosterone and neither FSH nor dibutyryl cyclic AMP stimulated this process (Fig. 10),

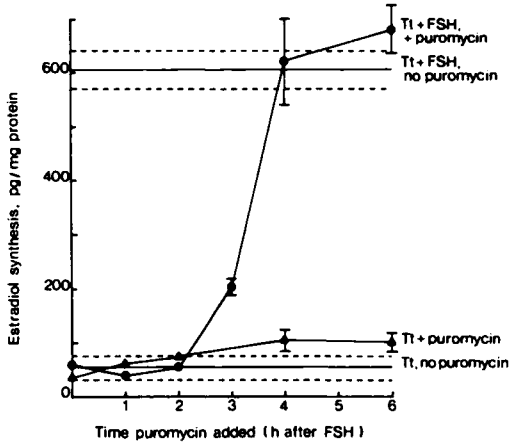

FIG. 9. Effect of puromycin added at different times on FSH-induced estradiol synthesis by Sertoli cells from 16-day-old rats. After 24 h in culture testosterone (0.5 μM) or testosterone and FSH (5 μg/ml) were added. At different times after the addition of hormones (i.e. 0, 1, 2, 3, 4, 5, or 6 h) puromycin (5 X 10^{-5} M) was added and all incubations allowed to proceed for 24 h. Values are the means ± SEM of three replicate flasks.

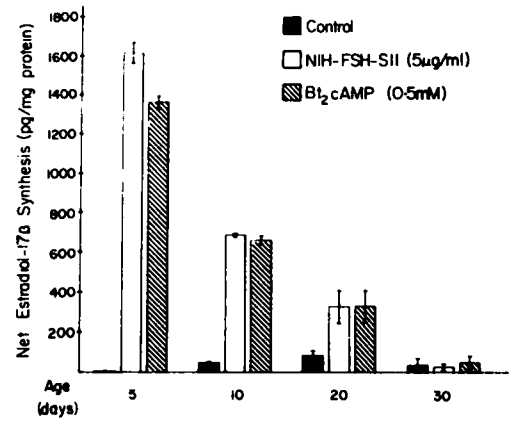

FIG. 10. Estradiol synthesis by Sertoli cells from rats of different ages, cultured for 24 h in the presence of 0.5 μM testosterone. Each bar represents the mean ± SEM of 3 replicates (data from Armstrong and Dorrington, 1977).

even in cells cultured in the presence of 0.5 mM MIX. The effects of FSH on estradiol synthesis by Sertoli cells prepared from rats of 5 and 10 days of age were much more dramatic (Fig. 10). The amount of estrone synthesized in response to FSH also decreased with increasing age of the animal from which the Sertoli cells were prepared (Fig. 11). If these observations can be extrapolated to the situation *in vivo*, then they indicate that estrogen synthesis is at a maximum before the first wave of spermatogenesis is initiated and raise the possibility that estrogen

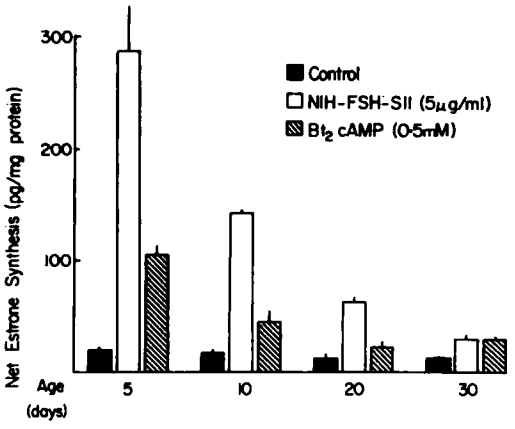

FIG. 11. Estrone synthesis by Sertoli cells from rats of different ages cultured for 24 h in the presence of 0.5 μM testosterone. Each bar represents the mean ± SEM of 3 replicates (data from Armstrong and Dorrington, 1977).

action may be important during prepubertal development. Possible functions of estrogen during this period include a local effect within the seminiferous tubule to establish the appropriate milieu for the initiation of spermatogenesis. A second possibility is an effect on the metabolism of interstitial cells. This hypothesis has received support from several lines of evidence: Stumpf (1969) found radioactivity concentrated in the nuclei of the interstitial cells of immature rat testes after the injection of ^{3}H-estradiol. Furthermore a specific estradiol receptor has been found in both the cytoplasmic and nuclear fractions of the rat testis interstitial tissue (Brinkmann et al., 1972; Mulder et al., 1973).

FSH has also been implicated in the control of maturation of Leydig cells. This stems from the work of Odell et al. (1973) which showed that FSH induced increased testicular responsiveness of immature rats to LH *in vivo*. Chen et al. (1976) reported that the number of hCG-binding sites and the testicular response to LH *in vivo*, as judged by testosterone production, were increased following treatment of hypophysectomized immature rats with FSH for several days. A direct action of FSH on Leydig cells seems unlikely since FSH binding to isolated Leydig cells is negligible (Catt, personal communication). It is possible that immature Sertoli cells, in response to FSH, secrete a factor which induces increased numbers of hCG receptors in Leydig cells. The administration of estradiol *in vivo* to hypophysectomized immature rats did not alter the number of hCG-binding sites of the testicular tissue. On the other hand, the enhanced testosterone production rate in response to LH following FSH treatment was suppressed in animals which had also received estrogen (Chen et al., 1977). On the basis of the combined information presented above, it seems possible that estrogen and other products synthesized by Sertoli cells and Leydig cells may be important in the control of prepubertal sexual maturation.

Whether estrogen continues to influence Leydig cell function in the adult animal is still not clear. Estrogen is secreted by the adult rat testis (de Jong et al., 1973) and estradiol receptors persist in mature Leydig cells (Mulder et al., 1973). Sertoli cells from 30- to 40-day-old rats synthesize low levels of estradiol and estrone even though their response to FSH is lost. Chowdhury et al. (1974) reported a marked drop in plasma and testicular concen-

trations of testosterone within 24 h after treatment of adult rats with estradiol benzoate. These workers were unable to detect a fall in the levels of LH and therefore suggested a direct inhibition of testosterone production by estradiol. The observation of Moger (1976) that estradiol benzoate treatment decreased the responsiveness of adult rat testes to exogenous LH *in vivo* supports this interpretation. Van Beurden et al. (1977) have recently re-examined the effects of estrogen on plasma and testicular levels of testosterone in intact and hypophysectomized rats. These workers found that testosterone was decreased in intact rats after the injection of estradiol, but concomitant with this effect, there was a decrease in plasma LH levels. Administration of estradiol to LH-treated, hypophysectomized rats failed to decrease testosterone secretion significantly. They concluded that the effects of exogenous estrogen on testosterone production in adult rats probably was secondary to the inhibition of LH secretion, rather than being due to a direct inhibitory action at the level of the Leydig cells. Whether endogenous estrogen of testicular origin plays a physiological role in regulation of gonadotropin secretion is still uncertain. Furthermore, the physiological significance of the presence of estradiol receptors in Leydig cells remains unknown.

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