Effects of Follicle Stimulating Hormone (FSH) Upon Steroid Aromatization *in Vitro** (33380)

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Under the proper conditions, slices prepared from ovaries of HCG-treated rats respond to the addition of either FSH or Δ4-androstene-3, 17-dione by a significant increase in the amount of estrogen formed (1). However, the increment of estrogen formation in response to the addition of androstenedione was the same in the presence as in the absence of FSH; it was inferred, therefore, that estrogen synthesis by rat ovarian cells *in vitro* is stimulated by FSH at a locus prior to androstenedione in the biosynthetic sequence.

In order to verify this conclusion, and to extend our studies of the action of gonadotropins to other steroid substrates, an experimental procedure that avoids the necessity of time-consuming bioassays was obviously desirable. It has been found that the appearance of tritiated water in the medium when androstenedione-1,2-^{3}H or testosterone-1,2-^{3}H is incubated with rat ovary slices closely parallels aromatization, as judged by the formation of phenolic products. This report deals with the effects of FSH upon the aromatization of androstenedione, testosterone, and progesterone. It has been found that FSH has no effect upon the aromatization of any of the substrates used. One FSH preparation tested contained a contaminant that significantly inhibited the aromatization of androstenedione.

Methods. Adult female rats received 40–90 IU of HCG daily and were killed on the fourth day. The ovaries were removed, sliced, and the slices distributed randomly so that each incubation vessel contained 90–150 mg tissue. The slices were preincubated at 37° for 30 min in Krebs-Ringer bicarbonate solution containing 200 mg/100 ml glucose. The gas phase was 95% O_2–5% CO_2. At the end of the preincubation period, the slices were transferred to 2 ml of the same medium containing the radioactive steroid; 1 mg each of TPN and glucose-6-phosphate were also added except as indicated. The incubation was then continued under the same conditions for three hr. The contents of each vessel were then transferred to a flask, shell-frozen, and lyophilized, the distillate being collected in a trap immersed in dry ice-methyl cellosolve. Duplicate aliquots (0.10 ml) of each distillate were counted in 10 ml of Bray's solution (2) in a scintillation counter. Appropriate experiments indicated that the recovery of added tritiated water was quantitative (95–105%).

Androsterone-1,2-^{3}H, testosterone-1,2-^{3}H, and estradiol-4-^{14}C were purchased from New England Nuclear Corporation. Progesterone-1,2-^{3}H (as well as some samples of tritiated testosterone) were a gift of the Endocrine Study Section, NIH. Samples of the labeled steroids were checked for radiochemical purity by chromatography on paper; when significant heterogeneity was detected, the entire lot was rechromatographed before use. To verify the location of the label, samples of 1,2-tritiated steroids were incubated with 0.6 N NaOH for 20–24 hr, and the solution then lyophilized. From 46 to 51% of the tritium was recovered in the distillate.

Results. When androsterone-1,2-^{3}H was incubated for three hr in buffer alone, or in the presence of rat ovary slices that had been heated for five min at 100°, a negligible fraction of the original radioactivity was released into the medium as ^3H_2O (Table I). When the experiment was repeated with fresh tissue, a significant amount of radioactivity appeared in the distillate. This usually

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TABLE I. Aromatization of Androstenedione-1,2-^{3}H by Rat Tissue Slices.[a]

Tissue	Tissue wt. (mg)	Percentage of original counts recovered as ^3H_2O
None	—	0.22, 0.32
Ovary	154, 160	1.06, 0.54[b]
Ovary	154, 166	9.9, 9.9
Liver	99, 105	0.78, 0.81
Adrenal	33	2.4
Kidney	111, 112	6.6, 6.7

[a] The incubation medium contained 2.0–5.0 μCi (0.064–0.175 μg) labeled androstenedione. TPN and glucose-6-phosphate were omitted. Figures in the last column were corrected to 100 mg tissue.

[b] Slices heated 5 min at 100° before incubation.

amounted to 9–12% of the total for each 100 mg of tissue incubated, although occasionally, the (corrected) yield of tritiated water approached 20%. Under the same conditions, only small amounts (0.8–2.4%) of the radioactivity was rendered volatile after incubation of androstenedione-1,2-^{3}H with liver or adrenal slices, while the corresponding figure using rat kidney slices was 6.7%.

In order to validate the assumption that the amount of radioactive water formed during these experiments is a measure of the extent of aromatization of the substrate, androstenedione-1,2-^{3}H (0.6–1.5 μCi) was incubated with 100–300 mg rat ovary slices for periods of one to three hr in paired vessels. The contents of one vessel of each pair was frozen and lyophilized, and the tritium in the distillate determined. A measured amount (1.0 mg, 1.0 μCi) of estradiol-4-^{14}C was added to the second vessel, the slices and medium homogenized, and extracted five times with ethyl ether. The ether extract was extracted in turn with 1 *N* NaOH, the alkaline extract acidified with H_2SO_4, and re-extracted with benzene (phenolic fraction). The ^{3}H and ^{14}C contents of the phenolic fraction were determined. After corrections for losses during fractionation (based on ^{14}C recovery) and for the small amount of labeled androstenedione retained in the phenolic fraction, the ratio of volatile tritium to phenolic tritium was calculated. The results obtained in three experiments, each including a one- and three-hr incubation, are summarized in Table II. Although the calculated ratio (column 5) tends to increase somewhat between one and three hr, it appears to be reasonably constant (0.65 ± 0.05) for experiments in which the yield of tritiated water varied over a threefold range. (When testosterone-1,2-^{3}H was used as substrate, the ratio was 0.69 in each of two experiments with incubation times of one and three hr respectively.)

Table III summarizes the results that were obtained when FSH was added to incubations of rat ovary slices with tritiated androstenedione, progesterone, and testosterone. The first FSH preparation employed (Armour R-377201, 1 U/mg) showed significant inhibition of aromatization when present at a concentration of 0.5 mg/ml or greater. However, two other FSH samples (Armour Y-12610, 1.5 U/mg and NIH S-1, 2.5 U/mg) had no significant effect in biological-

TABLE II. Comparison of Radioactivity Recovered in Volatile and Phenolic Fractions.

Experiment no.	Wt. of tissue (mg)	Incubation time (hr)	Percentage of initial counts recovered[a]		Ratio
			Volatile fraction	Phenolic fraction	
1	116	1.0	3.07	5.00	0.61
		3.0	6.22	8.90	0.70
2	309	1.0	7.20	11.05	0.65
		3.0	9.30	12.5	0.74
3	314	1.0	4.75	8.50	0.56
		3.0	7.90	12.3	0.64

[a] Each figure is the average for duplicate experiments. Experiment 1 was performed with ovaries from untreated rats, experiments 2 and 3, with ovaries from HCG-treated animals.

TABLE III. Effects of FSH upon Aromatization of Tritiated Steroids.

Experiment no.	Substrate	Wt. of tissue (mg)	FSH added (mg)	Percentage of total counts recovered as ^3H_2O[a]
1	Androstenedione-1,2-^{3}H	100, 96	—	9.4, 10.6 (10.0)
	(5 μCi, 0.175 μg)	98, 106	0.20[b]	10.5, 8.5 (9.5)
		97, 96	1.00	6.5, 8.8 (7.7)
		101, 105	2.50	5.4, 5.6 (5.5)
2	Androstenedione-1,2-^{3}H	108, 104, 105	—	12.7, 11.6, 11.8 (12.0)
	(5 μCi, 0.175 μg)	103, 103	1.00[c]	12.3, 11.5 (11.9)
3	Androstenedione-1,2-^{3}H	100, 96	—	9.4, 10.6 (10.0)
	(5 μCi, 0.175 μg)	103	1.00[d]	10.2
4	Progesterone-1,2-^{3}H	103–109	—	3.51 ± 0.15 (4)
	(5 μCi, 0.052 μg)		1.00[b]	3.44 ± 0.15 (4)
5	Testosterone-1,2-^{3}H	102–108	—	16.0 ± 0.8 (5)
	(5.0 μCi, 0.040 μg)		1.00[b]	14.9 ± 0.6 (5)

[a] Figures in the last column are corrected to 100 mg tissue. For experiments 1, 2, and 3, the figures in parentheses are averages for the data shown; for experiments 4 and 5, the figures are averages ± SE for the number of experiments performed shown in parentheses.

[b] Armour FSH, lot 377201 (1 U/mg).

[c] Armour FSH, lot Y-12610 (1.5 U/mg).

[d] NIH FSH S-1 (2.5 U/mg).

ly equivalent amounts. At a concentration of 0.5 mg/ml, the first Armour sample slightly inhibited the aromatization of testosterone-1, 2-^{3}H and had no significant effect upon the aromatization of progesterone-1,2-^{3}H.

The omission of TPN and glucose-6-phosphate had no discernible effect upon the yield of tritiated water, whether or not FSH had been added.

Discussion. Although a significant increase in the amount of estrogen produced by rat ovaries *in vitro* can be effected by the addition of as little as 0.2 U/ml of FSH (1), considerably larger quantities of FSH failed to stimulate the aromatization of any of the substrates (progesterone, androstenedione, testosterone) tested. It would appear, therefore, that none of the reactions after progesterone in the biosynthetic sequence in the rat ovary are affected by FSH. In this respect, the ovary of the rat appears to differ from that of the dog, since Hollander and Hollander have reported that the addition of FSH to slices of anestrus dog ovaries increased the conversion of testosterone to estradiol (3). Our findings suggest that the effect of FSH in the rat is closely akin to that in the rabbit; the data obtained by Gospodarowicz (4) suggests that in rabbit follicles, FSH stimulates in early step in the sequence cholesterol ⟶ pregnenolone ⟶ dehydroepiandrosterone ⟶ androstenedione ⟶ estrogen.

The fact that inhibition of the aromatization of androstenedione was observed with only one of the three FSH preparations tested indicates that some component other than FSH was responsible for this effect. Efforts to identify the contaminant with one of the other pituitary hormones (LH, growth hormone, prolactin, ACTH, or syntocin) were unsuccessful, as was an attempt to duplicate the result with a crude extract of sheep anterior pituitary powder.

As measured by bioassay, the conversion of androstenedione to estrogen was significantly increased by the addition of glucose-6-phosphate and TPN (ref. 1). Omission of these cofactors, however, does not affect the yield of tritiated water from androstenedione-1,2-^{3}H. This indicates that the cofactor requirement is probably concerned with the relative amounts of estrone and estradiol produced.

Summary. Steroids labeled with ^{3}H in posi-

tions 1 and 2 were incubated with rat ovary slices, and the formation of tritiated water determined. During three hr at 37°, approximately 3.5, 10.0, and 15% of the total tritium present in progesterone-1,2-^{3}H, androstenedione-1,2-^{3}H, and testosterone-1,2-^{3}H respectively was converted to ^3H_2O by 100 mg of tissue. The addition of FSH had no effect upon the aromatization of any of the substrates tested, although one of the preparations tested contained a contaminant that significantly inhibited the aromatization of androstenedione.

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Radiation Responses of Embryonal and SV40 Transformed Hamster Cells in Culture* (33381)

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The mechanisms of virus–host cell interaction resulting in the transformation of normal cells to neoplastic cells has been examined in several systems. Elegant experiments with the SV40-3T3 mouse fibroblast system reported by Todaro and Green (1) demonstrated that active cell replication and DNA synthesis are required to fix the transforming event between virus and target cell. Stoker (2) observed that the frequency of transformation of polyoma virus was increased among BHK-21 hamster cells which have survived X-irradiation. Exposure of mouse fibroblast cells to radiomimetic thymidine analogues enhanced the efficiency of SV40 transformation fivefold (3). Borek and Sachs observed that 300 R of X-irradiation induced the *in vitro* transformation of hamster embryo cells when cultured on mouse cell feeder layers (4). Processes associated with cell replication were essential soon after irradiation to fix the transformed state (5). A hereditary and a physiological state of susceptibility to transformation appeared to exist in X-irradiated cells and mechanisms for repair of radiation damage ("error correction") directly controlled the development of the transformed state (6).

Recent studies in this laboratory demonstrated that exposure of primary hamster embryonic cells to 150 R of X-irradiation prior to infection produced a 15- to 50-fold increase in the number of transformed clones when compared with unirradiated controls (7). Radiation alone under these conditions did not produce transformed cells. Characterization of the radiation responses of normal embryonal cells in culture is fundamental to understanding the role of irradiation in sensitizing these cells to transformation by SV40 virus. The present report describes results obtained in the investigation of several parameters of radiation induced changes in normal hamster cells in culture. A parallel study was conducted on the radiation response of SV40 transformed isogenic hamster cells to define potential viral induced changes that may exist.

Materials and Methods. Preparation of embryonal cells. Hamster embryonic lung and kidney cells were prepared by a modification

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