Stimulation by Pituitary Gonadotropin of the Synthesis of Estrogen by Rat Ovary Slices *in vitro*.* (28527)

MASATAKA YUHARA,† ARTHUR I. COHEN‡ AND EDWARD H. FRIEDEN
(Introduced by P. Bernfeld)
Arthur G. Rotch Laboratory, The Boston Dispensary, Boston, Mass.

The effect of gonadotropic hormones upon synthesis of estrogen *in vitro* has been studied using ovaries of several species of animals(1-4). In the rat, the available data appear to be limited to the brief report by Stitch, Oakey and Eccles(5) that incubation of rat ovaries with ^{14}C-testosterone in the presence of chorionic gonadotropin leads to production of radioactive estradiol-17-β, as well as several other radioactive products.

The work reported here was undertaken to study the response of rat ovarian slices to pituitary gonadotropin *in vitro*, and to establish the conditions necessary for demonstrating increased production of estrogen in response to FSH.

Methods. Adult Wistar rats, weighing 190-250 g were used. In the majority of the experiments to be described, each animal received 40 I.U. of human chorionic gonadotropin§ daily for 3 days. On the fourth day, the ovaries were removed, sliced and preincubated at 38° in 3 ml of Krebs-Ringer bicarbonate buffer under 95% O_2-5% CO_2. Slices from 2 or more animals were pooled to provide 250 to 400 mg tissue per beaker. After 30 minutes preincubation, the slices were transferred to fresh medium containing the desired additions, and incubation continued for 3 hours under the same conditions. The tissue and medium were homogenized and extracted twice with 2 volumes of methylene chloride. The combined methylene chloride extracts were evaporated to dryness, the residue redissolved in petroleum ether, and the phenolic fraction extracted with 0.1 N NaOH. The aqueous layer was acidified, reextracted with petroleum ether, and the solvent evaporated. The residue was dissolved in a small amount of ethanol and taken up in sesame oil for bioassay.‖

The oily solution was injected into 21-day-old female mice, 5 animals being used for each dose level. One-third the total dose was injected daily for 3 days, the animals were killed on the fourth day, and the weights of the uteri were recorded. Three groups of animals receiving 0.00-0.06 μg of estradiol-17-β served as standards for each set of assays. The results are expressed as estradiol equivalent per gram wet tissue.

Armour hog FSH, 1 Armour Unit/mg, was used in most of the experiments. In a few instances as indicated in Table II, a more potent (2.5 ×) preparation (NIH-FSH-S-1) was used.¶

Results. The data (Tables I-III) demonstrate that addition of FSH to the medium used for incubation of rat ovary slices stimulated estrogen production under a variety of conditions. Table I indicates that, in an unsupplemented medium, prior treatment with HCG is a necessary prerequisite for demonstrating the stimulatory effect of FSH. However, when the incubation medium was supplemented with TPN, glucose-6-phosphate (glu-6-P) and androstenedione, there was little difference in response between ovaries of treated and those of untreated animals; a significant FSH effect was observed for both. Most of the animals used were treated with chorionic gonadotropin, however, since fewer animals were then required to provide the necessary amount of tissue.

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† Present address: Dept. of Animal Husbandry, Univ. of Okayama, Okayama, Japan.

‡ Present address: Abbott Laboratories, North Chicago, Ill.

§ Antuitrin-S, Parke, Davis and Co.

‖ No estrogenic activity could be detected when unfractionated extracts from incubations performed under a variety of conditions were assayed. Indeed, injection of such extracts usually resulted in uterine weights slightly, but consistently, smaller than those of oil-injected controls, suggesting the existence of some inhibitory substance in this material.

¶ Gift of Endocrine Study Section.

TABLE I. Effects of Pretreatment with HCG.

HCG*	TPN (5.4 μM)	Glu-6-P (15 μM)	FSH (mg)	Δ⁴-A†	Estradiol equivalents (μg/g/3 hr)
—	—	—	2.0	—	<.04
+	—	—	—	—	<.04
+	—	—	.5	—	.27
+	—	—	5.0	—	.37
—	+	+	—	100	.47
+	+	+	—	100	.52, .82
—	+	+	2.0	100	1.10
—	+	+	.2	100	1.00
+	+	+	2.0	100	1.14, 1.20

* Treated animals received 40 I.U. HCG daily for 3 days prior to experiment.
† Δ⁴-Androstene-3,17-dione.

Table II indicates that addition of TPNH-generating system (TPN + Glu-6-P) had little effect upon production of estrogen from endogenous precursors, but substantially increased estrogen production from androstenedione. Ryan(6) noted that TPNH was a necessary co-factor in conversion of androstenedione to estradiol by placental microsomes.

The data of Table III reveal that FSH stimulates estrogen synthesis whether or not TPNH, androstenedione, or both, are present. With added FSH, a TPNH requirement for estrogen synthesis from endogenous precursors also becomes apparent. With adequate amounts of FSH, androstenedione, and TPN, the amount of estrogen produced varied only slightly from one experiment to another. This figure (1.1-1.2 μg estradiol equivalents/g tissue/3 hr) may therefore represent the maximum estrogen-synthesizing capacity of the rat ovary under these conditions. The effects obtained with the Armour FSH preparation were duplicated by NIH FSH. Maximum stimulation occurs with FSH concentrations corresponding to 0.02-0.03 mg/ml of the latter.

When FSH is added to an incubation mixture containing TPN and Glu-6-P, but no androstenedione, the increment of estrogen produced amounts to about 0.5 μg estradiol equivalent (Table III, average of 2 experiments). With androstenedione (50-100 μg) present, the FSH-induced increment is also about 0.5 μg (from 0.65 to 1.15). These facts suggest that FSH has no effect upon the conversion of androstenedione to estrogen, but stimulates a reaction involving another, endogenous precursor. Hollander and Hollander(3) reported that addition of FSH *in vitro* provoked a substantial increase in

TABLE II. Effects of Addition of TPNH and Androstenedione.*

TPN (5.4 μM)	Glu-6-P (15 μM)	Δ⁴-A†	Estradiol equivalents (μg/g/3 hr)
—	—	—	.04
—	+	—	.04
+	+	—	.04
—	—	1000	.30‡
+	+	100	.82, .52

* All animals were treated with HCG (40 I.U./day for 3 days).
† Δ⁴-Androstene-3,17-dione.
‡ 2 mg Armour FSH was added to this incubation.

TABLE III. Effects of FSH Added *in vitro*.*

TPN (5.4 μM)	Glu-6-P (15 μM)	Δ⁴-A† (μg)	FSH (mg)	Estradiol equivalents (μg/g/3 hr)
—	—	—	—	.04
—	—, +	—	5.0	.26, .14
+	+	—	—	.04
+	+	—	5.0	.42
+	+	0	2.0	.58
+	+	50	2.0	1.14
+	+	250	2.0	1.14
+	+	1000	2.0	1.16
+	+	100	—	.52, .82
+	+	100	.2	1.00
+	+	100	.6	1.18
+	+	100	2.0	1.20
+	+	100	.04‡	.87
+	+	100	.08‡	.87
+	+	100	.16‡	1.08

* All animals received 120 I.U. of HCG.
† Δ⁴-Androstene-3,17-dione.
‡ NIH-FSH, assaying about 2.5 times the activity of Armour FSH.

the amount of ^{14}C-testosterone converted to estradiol by slices of ovaries from anestrus dogs. Whether the testosterone → estrogen conversion is also subject to FSH control in rat ovaries, or whether an earlier step in steroid metabolism is involved, requires further study.

Although our results have been expressed in terms of estradiol equivalents, the identity of the estrogenic substance(s) produced has not been established. However, when rat ovary slices were incubated with androstenedione-4-^{14}C, and the phenolic fraction chromatographed in a ligroin-propylene glycol-methanol system, one of the 7 radioactive peaks detected corresponded in position to estradiol-17-β.

Summary. Slices of ovaries from rats treated with chorionic gonadotropin produced biologically detectable amounts of estrogen when incubated under the proper conditions *in vitro*. FSH, TPN and Δ^4-androstene, 3,17-dione, in various combinations, enhanced estrogen production. In the presence of maximally effective concentrations of androstenedione and FSH, one gram of ovarian slices formed the biological equivalent of about 1.2 μg of estradiol-17-β in 3 hours.

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Recovery and Behavior of Hepatitis Virus from Swiss Mice Injected with Ascites Tumor.* (28528)

JOHN B. NELSON
The Rockefeller Institute, New York City

Weekly passage of an ascites tumor has been made in our laboratory in both Princeton (Pr) and Swiss mice as an index of contamination with murine hepatitis virus (MHV). Presence of the virus was indicated by an abnormal tumor response and appearance of necrotic lesions in the liver. Findings with Swiss mice are presented here as a supplement to an earlier report on Pr mice(1).

Materials and methods. The random-bred colony of Swiss mice at the Rockefeller Institute was begun in 1926 and operated for 32 years by conventional methods. In 1958 the breeders showed such a high rate of chronic respiratory disease (CRD) and were so heavily infested with mange mites and pinworms that a specific pathogen-free (SPF) sub-line was started as a replacement(2). The founding breeders were cesarian-derived and reared by foster mothers from our CRD-free colony of Pr mice.

Unless otherwise noted the experimental mice from the 2 Swiss and the Pr colonies were weanlings of either sex, 3 to 4 weeks old, (10-15 g), and were used in groups of 5. Intraperitoneal injections of both livers (ground) and ascitic fluids were usually made with .1 ml of approximately 10% suspensions in saline. The inoculum in nurslings and by other routes was .02 to .05 ml. Survivors were generally killed with ether on the 7th day and autopsied. Livers were examined with a dissecting microscope and ascitic fluids in a fresh state by phase contrast. Appearance of the fluid on the 7th day after injection was a valuable diagnostic aid in Swiss mice. The color was commonly blood-red in the absence of MHV and white or pale yellow in its presence. In the combined series ascitic fluid was injected first on one side of the abdomen and liver suspension

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